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Differences in Collagen Metabolism between Normal and Osteoarthritic Human Articular Cartilage

Abstract. *Normal human articular cartilage synthesizes only one type of α chain, which exhibits the chromatographic behavior of the α_1(II) chains described for chick and bovine cartilage. Osteoarthritic cartilage, on the other hand, synthesizes in addition a collagen containing α_2 chains and β components. The different structural features of the two types of collagen may account for some of the functional defects of osteoarthritic cartilage.*

Osteoarthritis is a degenerative disease affecting articular cartilage. The early changes are associated with a loss of proteoglycans, while the collagen concentration remains constant (*1*). Cartilage destruction seems to begin in areas exposed to maximum mechanical stress leading to eburnation or complete loss of the hyaline tissue. Many investigators have focused on the metabolic changes that accompany osteoarthritis, particularly on the synthesis and turnover of glycosaminoglycans.

Recently it has been shown that collagen from chick cartilage (*2*, *3*) and mammalian cartilage (*4*) differ significantly from that of other tissues. Cartilage collagen is assembled from three identical α chains identified as α_1(II) to distinguish them from the homologous chain of skin, bone, and other adult tissues or α_1(I). The most significant differences between these two types of collagens reside in hydroxylysine and glycosidically bound carbohydrates, since the α_1(II) chains contain four- to fivefold more of these residues than the α_1(I).

The fact that this unique type of collagen is found in articular cartilage suggests that its particular configuration may be essential for the structural integrity of this tissue. This highly glycosylated structure may be the most suitable in establishing an adequate collagen-proteoglycan complex. An alteration of the fibrous network may affect this interaction and lead to a poor functional unit unable to resist stress.

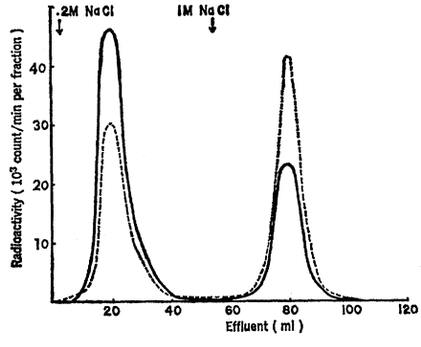

Fig. 1. Profiles of biosynthetically labeled cartilage extracts as they are eluted from a DEAE-cellulose column. After 50 ml of 0.05*M* tris buffer, *p*H 7.5, containing 0.2*M* NaCl was circulated, the salt concentration was raised to 1*M* to elute the proteoglycans. The solid line corresponds to a normal specimen, and the dotted line corresponds to osteoarthritic cartilage.

In an attempt to begin looking at this problem, we have compared the nature of the collagen synthesized by normal human cartilage to that synthesized by cartilagenous tissue proximal to areas of osteoarthritic degeneration.

Osteoarthritic cartilage was obtained primarily from human subjects undergoing surgical resection of the femoral head during replacement with prosthetic devices. Samples were taken from the areas surrounding the denuded area where cartilage was being actively eroded. Normal cartilage was obtained from autopsy or after corrective surgery not complicated by osteoarthritis.

Cartilage slices were incubated in 10 ml of Dulbecco's phosphate-buffered solution (GIBCO) containing 30 μc of L-[2,3-^{3}H]proline (specific activity, 29.8 c/mmole). Incubations were carried out at 37°C for 4 hours with gentle shaking under air. At the end of this period, the tissue slices were removed, rinsed with saline, and homogenized with a VirTis-45 homogenizer in 0.45*M* NaCl adjusted to *p*H 7.0 with 0.02*M* sodium phosphate. The homogenate was kept in a shaker at 4°C for 24 hours and then centrifuged. The supernatant was dialyzed first against distilled water and then 0.05*M* tris buffer, *p*H 7.5, in 0.2*M* NaCl.

The dialyzed extracts were chromatographed on a diethylaminoethyl (DEAE)-cellulose column (0.6 by 30 cm) and eluted with 0.05*M* tris · HCl buffer in 0.2*M* NaCl (*3*). After 50 to 60 ml of effluent volume was collected, the NaCl concentration was raised to 1*M*, and the elution was continued. The flow rate was kept at 25 ml/hour, and 2-ml fractions were collected.

The radioactive material that eluted with the initial buffer was collected, lyophilized, dissolved in 0.06*M* sodium acetate buffer, *p*H 4.8, and dialyzed exhaustively against the same buffer.

The radioactive fraction recovered from the DEAE-cellulose column was mixed with 2 to 3 mg of purified acid-soluble collagen from rat skin, heated at 40°C for 15 minutes, and chromatographed on carboxymethyl (CM) cellulose with the use of a linear gradient between 0 and 1.0*M* NaCl (50 ml each) (*5*). A small column (0.8 by 5 cm) equilibrated with 0.06*M* sodium acetate buffer, *p*H 4.8 at 40°C, was used. The flow rate was maintained at 10 to 12 ml/hour and 1-ml fractions were collected, read at 230 nm, and counted in a Beckman liquid scintillation counter.

Figure 1 shows the elution profiles from a DEAE-cellulose column of the

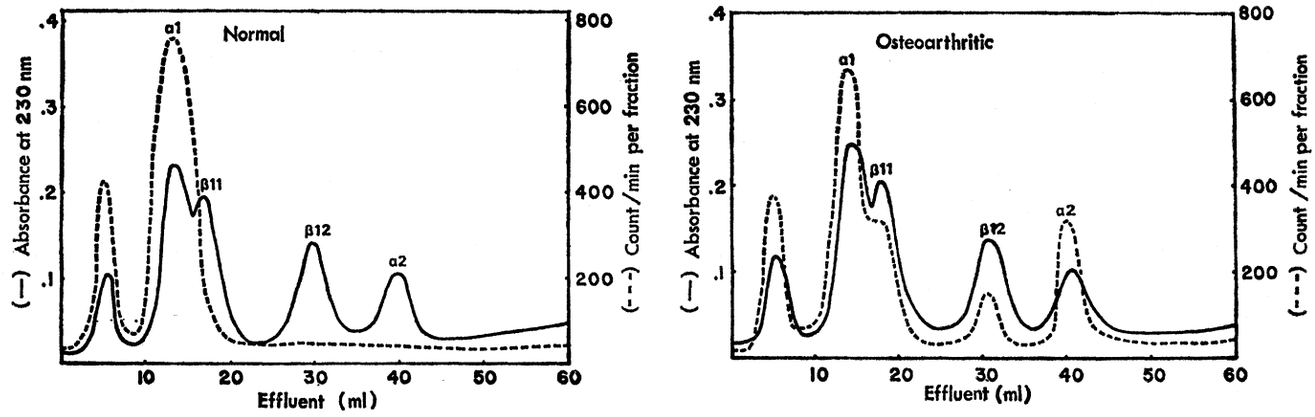

Fig. 2. The material in the first peak from the DEAE cellulose was applied to a carboxymethyl-cellulose column, and eluted as described in the text. Carrier acid-soluble collagen from rat skin was added, and its elution profile, monitored at 230 nm, is plotted as a reference (solid line). The chromatogram on the left corresponds to normal cartilage; the one on the right to osteoarthritic cartilage. The dotted lines represent the radioactivity in the effluent.

L-[^{3}H]proline-labeled cartilage extract obtained as described. The solid line represents normal cartilage from the head of the humerus of a 12-year-old male, removed surgically; the dotted line shows osteoarthritic cartilage from a 50-year-old woman who underwent hip replacement. This preliminary chromatographic procedure to separate collagen from the proteoglycans was necessary for the subsequent efficient separation of the collagen chains.

The incorporation of L-[2,3-^{3}H]proline into collagen (first peak) and into proteoglycans (second peak) varied from one preparation to another. In general, the ratio of synthesis of collagen to the proteoglycan backbone was higher in newborn cartilage and cartilage from normal subjects of various ages and lower in osteoarthritic cartilage.

The collagen-rich fraction was lyophilized, dissolved in 0.06*M* sodium acetate buffer, *p*H 4.8, denatured by heating, and placed on a CM-cellulose column. Carrier collagen, extracted from rat skin with 0.5*M* acetic acid and purified (*6*) was always added to help resolve the radioactive components (Fig. 2). Whereas the normal articular cartilage gives rise to a peak which corresponds to the α_1 chains of collagen, the cartilage from the osteoarthritic joint synthesizes, in addition, significant amounts of α_2 chains together with the dimeric cross-linked components β_{11} and β_{12}. Analysis of the radioactivity under the α_1 and α_2 chain peaks showed that it consists of proline and hydroxyproline in ratios normally found in these types of collagen (*4, 5*).

Our experiment demonstrates a metabolic abnormality in osteoarthritic cartilage. A tissue which exclusively synthesizes $[\alpha_1(II)]_3$ collagen is now synthesizing a more ubiquitous type of collagen containing α_2 chains.

Some recent experimental findings may help to interpret our observations. Chick chondrocytes in culture maintain the characteristic morphology of cartilage and actively synthetize glycosaminoglycans. A change in the growth media will cause these cells to readily acquire fibroblastic features and stop synthesizing chondroitin sulfate (*8*). It has also been shown that articular cartilage from young rabbits will synthesize exclusively cartilage type collagen $[\alpha_1(II)]_3$ when maintained in organ culture. When the isolated chondrocytes are incubated under similar conditions, they synthesize both α_1 and α_2 chains (*9*). These experiments suggest that the environment may modify cellular expression, and raise the question of what factors may be most intimately involved in the initiation and perpetuation of degenerative joint disease.

The mechanism which underlies such a change in metabolic patterns remains unknown. Although the high levels of lysosomal enzymes encountered in this disease may play a significant role in this connection (*10*), since osteoarthritic cartilage is histologically heterogeneous, it becomes mandatory to find out which cells are secreting which collagen, and, in particular, to ascertain the origin of the cells that are now producing the skin-bone type collagen. Are these cells chondroblasts that have altered their differentiation pattern or are they cells that have moved in from the subchondral area? As tissue-specific collagens are becoming more and more apparent, one can postulate that cartilage, which in its normal state contains a highly glycosylated collagen of the $[\alpha_1(II)]_3$ type requires such a specific structure for adequate interactions with the proteoglycans. Substitution by a different collagen, such as the $[\alpha_1(I)]_2$-$(\alpha_2)_1$ type, much less glycosylated and which can generate a different fiber organization, could give rise to a functional defect as seen in osteoarthritis.

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