

## CHONDROITIN 4- AND 6-SULFATE IN GROWTH AND ARTICULAR CARTILAGE OF YOUNG AND ADULT HUMANS

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The absolute concentrations of chondroitin 4- and 6-sulfate are compared in articular and ossification cartilages from normal humans. In newborn humans, the absolute concentration of chondroitin 4-sulfate decreases nearly three folds from the deeper ossification cartilage to the articular surface, whereas that of chondroitin 6-sulfate does not change. Adult epiphyseal cartilage, where the ossification process has finished, contains exclusively chondroitin 6-sulfate. The chondroitin sulfate extracts from fetal cartilages contains a significant amount of non-sulfated disaccharides units, which are absent in adult and newborn cartilages. These observations suggest that chondroitin 4-sulfate is an important component in the ossification process.

Among the macromolecular components of cartilages, the proteoglycans and their constituent glycosaminoglycan chains are known to be related to the tensile strength and resilience of articular cartilages, and are known to be involved in ossification process that occurs in the growth cartilages.

Variations in the concentration of cartilaginous glycosaminoglycans under normal and pathological conditions have been reported by several groups. For examples, changes in the relative proportions of chondroitin 4-sulfate and chondroitin 6-sulfate in epiphyseal cartilages with aging (P. A. S. Mourão et al., 1976, *Biochim. Biophys. Acta*, 428: 19-26; P. A. S. Mourão, 1988, *Arthritis Rheum.*, 31: 1028-1033; R. C. Harab & P. A. S. Mourão, 1989, *Biochim. Biophys. Acta*, 992: 237-240) and arthrosis (Y. M. Michelacci et al., 1979, *Connect. Tissue Res.*, 7: 29-36). The molecular weight of chondroitin sulfate obtained from articular surfaces differs from ossification cartilages (I. L. Jones & R. Lemperg, 1975, *Biochim. Biophys. Acta*, 392: 310-318) and also the concentration of keratan sulfate in articular cartilage increases with aging.

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This report details the variation of the distribution of glycosaminoglycans in different models of normal cartilages in term of their relative and absolute concentration.

Normal cartilages from adult and newborn humans were carefully separated from the adherent tissues. The glycosaminoglycans were extracted from cartilage as previously described for another type of tissue (R. M. Albano & P. A. S. Mourão, 1986, *J. Biol. Chem.*, 261: 758-765). In brief, the dried cartilage (10 mg) was suspended in 1 ml of 0.1 M sodium acetate buffer (pH 5.5) contain 1 mg papain, 5 mM EDTA and 5 mM cysteine, and incubated at 60°C during 24 h. The incubation mixture was then centrifuged (3000X G for 10 min at room temperature) and another 1 mg of papain in 1 ml of the same buffer was added to the pellet to incubation for another 24 h. The clear supernatant of the two extractions was joined and completely precipitate by the addition of 10% cetylpyridinium chloride (CPC). The CPC-precipitated glycosaminoglycans were dissolved in a 2 M NaCl solution and then re-precipitated by the addition of 2 volumes of 95% ethanol and maintained at 10°C for 24 h. The precipitate formed was collected by centrifugation (3000X G for 15 min at room temperature), vacuum dried and dissolved in 0.5 ml of distilled water. Approximately 95% or more of the total cartilaginous glycosaminoglycans were extracted by this method.

The glycosaminoglycans were identified by agarose gel electrophoresis and degradation with specific mucopolysaccharidases (C. P. Dietrich & S. M. C. Dietrich, 1976, *Anal. Biochem.*, 70: 645-647). Only one band appears on the agarose gel and this band has the same mobility as the standard chondroitin 4/6 sulfate (data not shown).

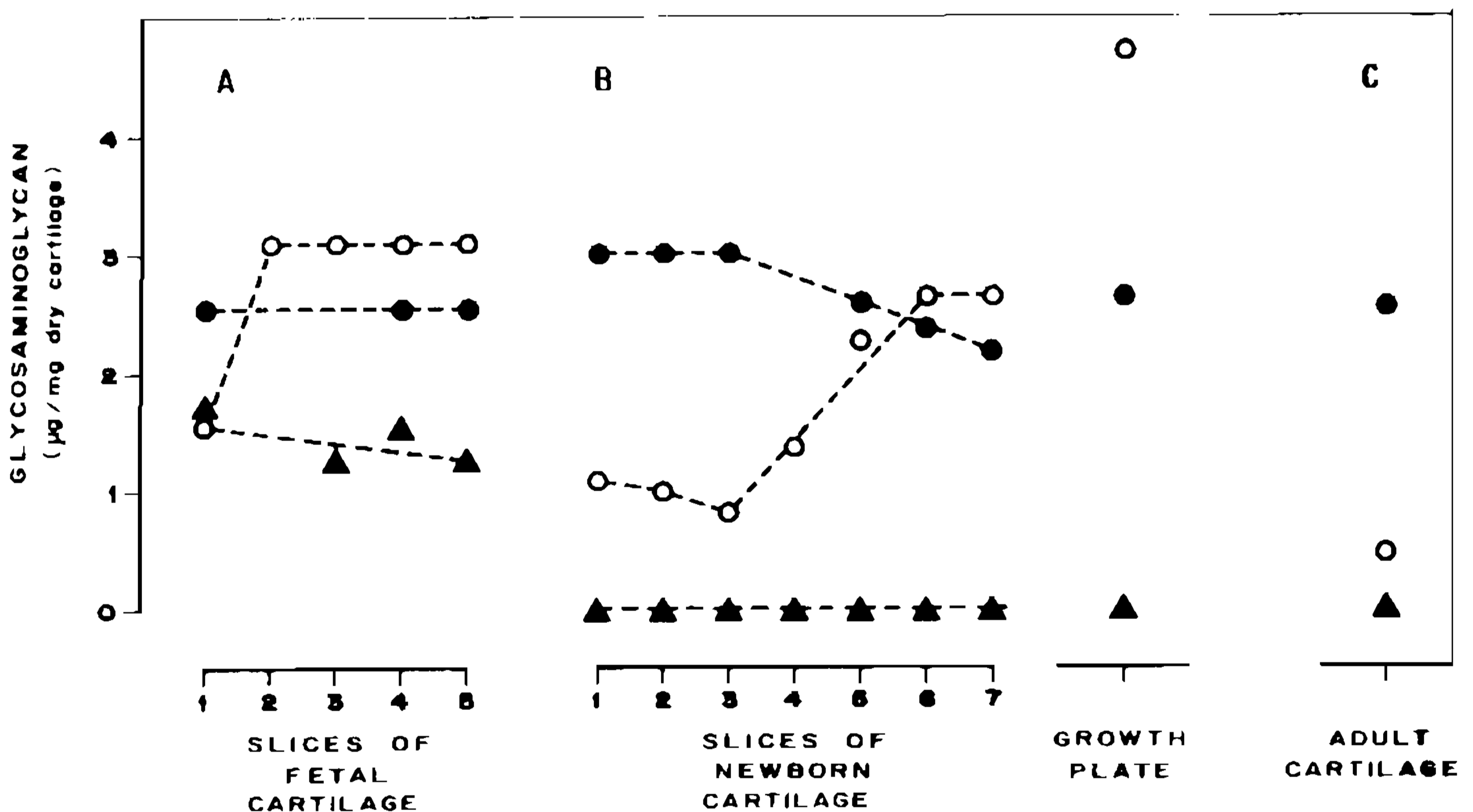
Based on the relative proportions of disaccharides formed by chondroitinase AC (H. Saito et al.,

1968, *J. Biol. Chem.*, 243: 1536-1542) and on the hexuronic acid contents, it is possible to calculate the absolute concentrations of chondroitin 4- and 6-sulfate in the various types of cartilage. In order to distinguish the articular and ossification cartilage in newborns humans, serial slices of cartilage were cut from the articular surface of femur and tibial epiphyses to the cartilage-metaphysis bone junction. The growth plate of newborns are carefully cut and analysed too. The chondroitin 4-sulfate content decreases with age while the chondroitin 6-sulfate remains constant. Analysis of the the slices shows that there is a marked increase in the chondroitin 4-sulfate content from the articular surface to the cartilage-bone junction, while chondroitin 6-sulfate absolute concentration remains the same (Fig). In agreement with previous works, the chondroitin sulfate extracted from fetal cartilages contains a significant amount of non-sulfated disaccharides units, which are absent in adult and newborn cartilages.

Our observation that the concentration of

4-sulfate units in the chondroitin chains increases in the ossification cartilage agrees with other studies, which showed an increase in the relative and absolute concentration of chondroitin 4-sulfate in growth cartilage of several mammals. Furthermore, the relative amount of chondroitin 4-sulfate increases in tumoral and arthrosic cartilages in which calcification commonly occurs. In addition, phylogenetic studies described by M. B. Mathews (1966, *Clin. Orthop.*, 48: 267-283) have shown that cartilage from bony fish contains more chondroitin 4-sulfate than cartilage from cartilaginous fish. In other studies, M. B. Mathews & J. E. Glacov (1966, *J. Clin. Invest.*, 45: 1103-1111) have also shown that calcified skull cartilage contains more chondroitin 4-sulfate than does uncalcified skull cartilage.

The results presented here indicate that chondroitin 4-sulfate is related to the ossification process, whereas chondroitin 6-sulfate seems to be related to the integrity of articular surfaces.



Absolute concentrations of chondroitin 4-sulfate (-O-), chondroitin 6-sulfate (-●-) and desulfated chondroitin (-Δ-) in cartilages from fetal (A), newborn (B) and adult (C) femoral epiphysis. In A and B, serial slices of cartilage were obtained from the articular surface (1) in the direction of cartilage-metaphysis bone junction (5 or 7). In addition, the growth plate cartilage of the newborn epiphysis was analyzed separately in the experiment of B. The glycosaminoglycans were extracted from the cartilage and identified as described in the text.