Biochemical Composition of Rat Costal Cartilage Prior to and During Calcification[1] (36618)

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A tissue which could be collected in quantity prior to, during and after its calcification would permit the *in vivo* investigation of biochemical events accompanying these stages in calcification. Alcock and Reid (1) presented data which showed that costal cartilage in the rat commenced calcification at a well-defined body weight of 35–45 g. Percentage ash in dry cartilage increased from 3.5% at 15–40 g body weight to 58.7% at 174 g body weight. Alcock and Reid suggested that rat costal cartilage might serve as an *in vivo* model for investigation of various problems related to calcification.

This paper reports data on the inorganic composition of costal cartilage from 8 to 49 day old (20–200 g body weight) rats which differs in some aspects from that previously reported (1). In addition analyses for collagen, hexosamine and uronic acid permit correlation of changes in the mineral phase with changes in the organic portion of costal cartilage during its development.

Methods. Thoracic cages were removed from groups of 10–14 Holtzman strain rats of 8–49 days of age. The animals through 30 days of age were of mixed sex as present at birth while the animals at 42 and 49 days of age were male rats only. No differences between sexes in the components analyzed were evident through 30 days of age. In most groups animals from 3 or 4 litters were included. Costal cartilage was separated from each cage approximately 1 mm distal to the chondrocostal junction and pooled in the groups of 10–14 animals of each age. Histologic examination confirmed that only cartilage was included in the samples. The pooled samples were reduced to fine pieces with scissors and defatted and dried by passage through acetone. Each pooled sample contained from 0.25 to 1.0 g dry fat-free tissue. Ash was determined by heating 100 mg of each pooled tissue to constant weight at 500°. Calcium and magnesium were determined on solutions of the ash by atomic absorption spectrometry (2). Phosphorus was estimated on an additional aliquot of ash by the Fiske and SubbaRow procedure (3). Five milligram samples of pooled cartilage were analyzed for hydroxyproline (4), uronic acid (5) and hexosamine (6). Collagen was calculated by multiplying hydroxyproline content by 7.46 (7).

Results. The ash content of the cartilage (Table I) began to increase from 8.7% after 20 days of age (44 g body wt) and was 28.9% at 49 days of age (201 g body wt). At 4 months of age cartilage from a single male and a single female rat contained 34.2 and 39.3% ash, respectively. Rib bone from 4 month old rats contained 60–65% ash. Percentage calcium in the ash (Table I) and in whole dry fat-free cartilage (Fig. 1c), however, increased after 16 days of age (36.0 g body wt). In contrast, the phosphorus contents of the ash (Table I) and of whole cartilage (Fig. 1c) did not increase consistently until after 18 days of age. The molar Ca/P ratios did not exceed 1.2 at any experimental period and thus did not approach that of hydroxyapatite which is 1.67. The increment of added calcium and phosphorus accumulated from 16 to 30 and from 14 to 49 days gave Ca/P molar ratios of 1.18 and 1.25, respectively. Therefore the additional mineral deposited after rapid mineralization began also failed to demonstrate a Ca/P

[1] This work was supported in part by a grant from the Graduate School, University of Minnesota.

TABLE I. Body Weight, Ash and Magnesium Contents of Costal Cartilage and Calcium and Phosphorus Contents of Costal Cartilage Ash of Rats at Various Ages.

Age of rats (days)	Wt of rats (g)	Cartilage-% Ash	Ash-% Ca	Ash-% P	Cartilage magnesium (mg/g)
8	20.2	10.4	5.1	8.8	1.2
10	27.3	11.5	4.4	5.2	1.0
12	28.0	7.5	6.2	6.7	1.0
14	30.7	8.0	6.3	8.6	
16	36.0	7.6	6.2	6.2	1.2
18	38.7	6.7	9.6	7.9	1.2
20	44.6	8.7	15.5	9.9	1.3
22	51.2	12.3	19.6	13.3	1.4
26	75.5	14.5	22.6	14.9	1.6
30	90.2	16.0	27.2	20.0	1.7
42	171.8	25.1	26.1	18.9	1.8
49	200.5	28.9	33.3	22.9	2.3

ratio similar to that of hydroxyapatite. Increased cartilage magnesium (Table I) was also noted beginning with the 20 and 22 day experimental periods.

The quantities of collagen, hexosamine and uronic acid in costal cartilage demonstrated different relationships to animal age. The amount of collagen (Fig. 1a) increased consistently from 8 to 16 days and then began to decrease sharply after 16 days as the ash content increased (Table I). However, a further drop in cartilage collagen from 30 to 49 days, a period during which the ash content of the tissue continued to increase was not observed. Cartilage hexosamine (Fig. 1b) was stable until the 20 day experimental period, decreased from 20 through 30 days animal age and then increased at the last two experimental periods. In contrast the amount of uronic acid found in the cartilage (Fig. 1b) decreased throughout the experiment until the last experimental period when an increase occurred.

Discussion. Calcification was observed to begin in costal cartilage at approximately the same age (20 days) and body weight (44 g) as previously reported (1). However, the subsequent rate of accumulation of ash, calcium and phosphorus was much slower than found in the earlier study. In the present study, ash, calcium and phosphorus were not similar to that of rib bone even at 4 months of age. In contrast, Alcock and Reid (1) observed an ash content of costal cartilage from rats with body weight similar to the above which was only slightly less than that of rib bone. The apparent differences between the analytical results from the 2 studies can not be completely explained by variations in analytical methodology even though total cartilage phosphorus was determined in the present study and only inorganic phosphorus in the earlier investigation. Perhaps there was a subtile difference between the samples collected in this study and in the previous study. The failure to approach a molar Ca/P ratio of 1.67 for total cartilage mineral and for additional mineral accumulated during rapid mineralization indicated the presence of large quantities of nonhydroxyapatite mineral in the cartilage. Thus, although mineralization began at a distinct age and animal weight, the failure to obtain complete and rapid calcification and the failure to achieve a molar Ca/P ratio similar to hydroxyapatite suggest that rat costal cartilage may be of only limited usefulness for studying the process of calcification.

The analytical results of this study demonstrate the complexity of biochemical changes which occur during development of rat costal cartilage. An increased calcium content was observed before increases in either ash or phosphorus were evident. An increase in the calcium content of cartilage and of cartilage ash slightly prior to increased phosphorus

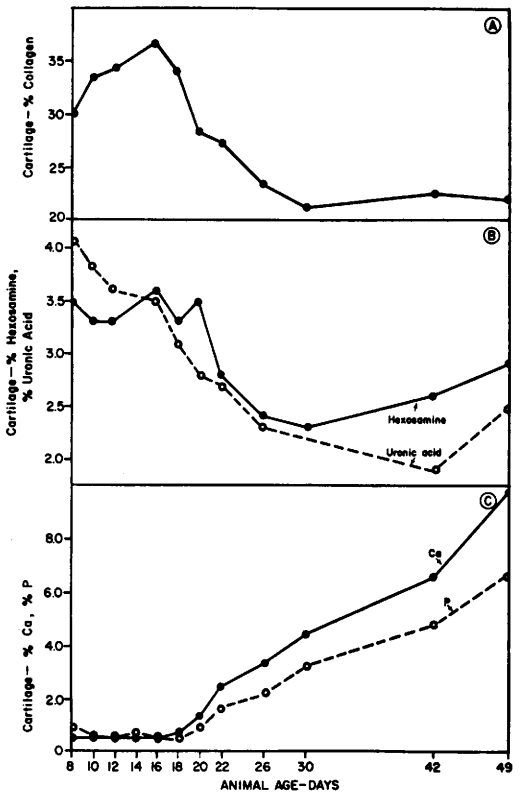

FIG. 1. Collagen, hexosamine, uronic acid, calcium and phosphorus of costal cartilage from rats of various ages. Each point represents a pooled sample of cartilage from 10 to 14 animals.

content was also recorded in an experiment preliminary to the present experiment. However, detailed studies will be required to confirm and to establish the significance of this observation.

Collagen content did not maintain a simple inverse relationship to ash content. The ash content failed to change appreciably from 12 through 20 days of age. However, collagen content increased from 12 to 16 days and then decreased from 16 to 18 and from 18 to 20 days. In addition collagen content remained constant from 30 to 49 days, a period during which ash content increased. Collagen and ash together represented only 40.4 and 50.7% of dry fat-free cartilage at 8 and 49 days of age, respectively. A portion of the remaining cartilage is composed of glycoproteins and protein–polysaccharides. However, even when these substances and the cellular content are considered, a significant fraction of the cartilage remains undefined. Little attention has been devoted to this undefined material.

Hexosamine and uronic acid represent carbohydrate components of glycoproteins, glycosaminoglycans and protein–polysaccharides of cartilage. Since the quantities of these 2 substances follow different patterns of change with development of the cartilage, there must not only be a change in the total quantity of these materials, but also a change in structure or relative content of the various carbohydrate containing substances during cartilage development.

Summary. The ash, calcium and phosphorus contents of rat costal cartilage began to increase subsequent to 20, 16 and 18 days of age, respectively. However, at termination of the experiment (49 days of age), the quantities of the above 3 substances in costal cartilage were still much less than in rib bone. The amounts of collagen, hexosamine and uronic acid in rat costal cartilage had different relationships to animal age. No consistent relationships were observed among changes in the quantities of organic and inorganic cartilage components.

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Received Jan. 26, 1972. P.S.E.B.M., 1972, Vol. 140.