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CALCIFICATION OF AGING ARTICULAR CARTILAGE IN MAN

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Calcification of the articular cartilage was studied ultrastructurally using normal femoral heads obtained from necropsies of persons ranging in age from 11 months to 80 years. Mineral crystals which appeared during the initial stages of deposition were morphologically divided into two types. Type A crystals were slender, twisted and curved, measuring from 100 nm to 360 nm in length. Type B crystals were short, needle-like and slightly curved, measuring from 30 nm to 160 nm in length. Type A crystals were found mainly in the developing epiphysis during childhood. Type B crystals were generally found in the calcified zone of adult articular cartilage. Both types of crystals initially appeared in close proximity to extracellular membrane-invested electron dense particles called "matrix vesicles", and gradually increased in number to form calcified cartilage matrix. The morphological differences between type A and B crystals might be caused by biochemical alterations of the cartilage matrices and/or biomechanical changes in the joints of children and adults.

Key words: articular cartilage; calcification; matrix vesicles; mineral crystals; ultrastructural study

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In early childhood, the epiphysis of the long bones in humans is composed of hyaline cartilage containing a vascular network. Several months after birth, the secondary center of ossification, in which endochondral bone formation occurs, appears in close proximity to the vascular network in the center of the epiphysis (Wilsman & Van Sickle 1970), and extends continuously outward until only a thin layer of cartilage is retained at the articular surface. Endochondral bone formation is characterized by a gradual calcification of the cartilage matrix adjacent to the hypertrophic or degenerating cartilage cells (Bloom & Bloom 1940). In adult articular cartilage, a prominent basophilic line or "tidemark" (Fawns & Landells 1953) runs transversely from the base of the articular cartilage to the surface of the joint and eventually becomes the underlying calcified zone. The tidemark amd the calcified zone firmly connect the non-calcified cartilage layer to the subchondral bone (Redler & Mow 1975). However, the differences in the mode of calcium deposition between the developing epiphysis in childhood and the permanent articular cartilage in adulthood have not been clarified.

The present paper aims to study the mode of calcification of articular cartilage in children and adults using femoral heads obtained at necropsy.

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MATERIALS AND METHODS

Articular cartilages were obtained from the weight-bearing portion of femoral heads taken from 23 necropsies of persons ranging in age from 11 months to 80 years. These femoral heads were selected from patients who had pathologically normal hip joints. Complete cross sections of the articular cartilage, including subchondral bone, were cut into small pieces perpendicularly to the joint surface. For light microscopic observation, specimens were fixed in neutral buffered formalin and the sections were stained with hematoxylin and eosin, von Kossa, alcian blue and safranin-O fast-green-iron hematoxylin. Specimens for electron microscopy were not decalcified. Samples were immediately fixed in 1.4 per cent glutaraldehyde in 0.1 M Sorensen's phosphate buffer, and post-fixed in buffered 1 per cent osmium tetroxide for 2 hours. After dehydration in increasing concentrations of alcohol, the specimens were infiltrated with and embedded in Epon 812. Relatively thick sections were stained with alkaline toluidine blue for general light microscopic observations. Ultrathin sections were cut on a Porter-Blum MT-1 ultramicrotome using glass knives. The sections were stained either doubly or triply with uranyl acetate, lead citrate and phosphotungstic acid and examined with a Hitachi H-500 electron microscope at an accelerating voltage of 75 kv. For assessment of the quantity and quality of mineral deposits, the thin sections were coated with carbon for analysis by a JEM 100-B with an Ortec intregated electron probe X-ray microanalyzer.

RESULTS

Light microscopic observations

The articular cartilages and underlying subchondral bone were well preserved and showed no pathological abnormalities.

In children up to 12 years of age, the secondary center of ossification appeared in the epiphysis and displayed endochondral bone formation. It was characterized by a central ossified area with peripheral hypertrophic or degenerating cartilage cells embedded in a wide zone of basophilic cartilage matrix. In addition, island-like aggregations of basophilic granules were frequently found in the outer zone of the peripheral hypertrophic cartilage cells and in close proximity to colonies of cartilage cells (Figure 1A).

In adults ranging from 26 to 80 years of age, cartilage cells, arranged perpendicularly to the articular surface, were found deep within the articular cartilage. A distinct basophilic line or "tidemark", running transversely to the articular surface, divided the innermost part of the articular cartilage into calcified and overlying non-calcified zones. Just above the tidemark, a large number of small basophilic granules were observed which continued into the underlying basophilic cartilage matrix (Figure 1B). The basophilic granules and basophilic cartilage matrix observed in children and adults showed a strong positive reaction when subjected to von Kossa staining.

Electron microscopic observations

Mode of crystalline deposition. In children, basophilic granules appeared ultrastructurally

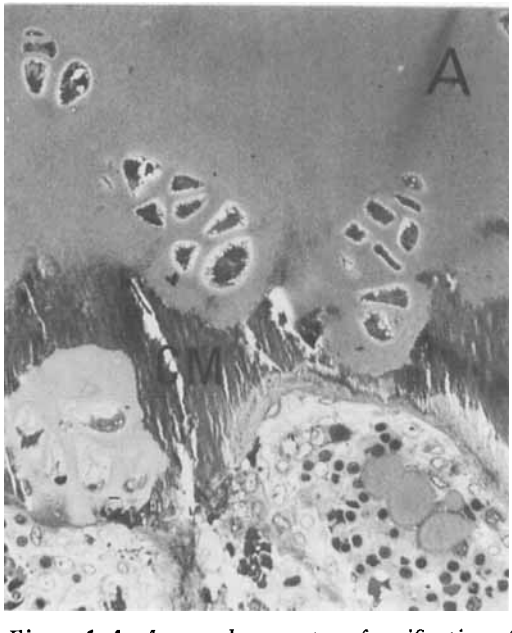

Figure 1 A. A secondary center of ossification of an 11-month-old child. A wide basophilic calcified matrix (CM) is found between upper hyalinous cartilage and lower bone marrow (× 150).

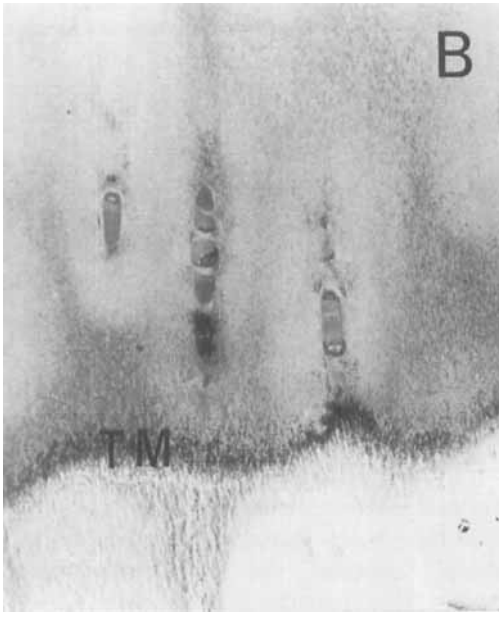

Figure 1 B. A tidemark (TM) of an adult articular cartilage running transversely between the upper non-calcified and lower calcified zones (× 150).

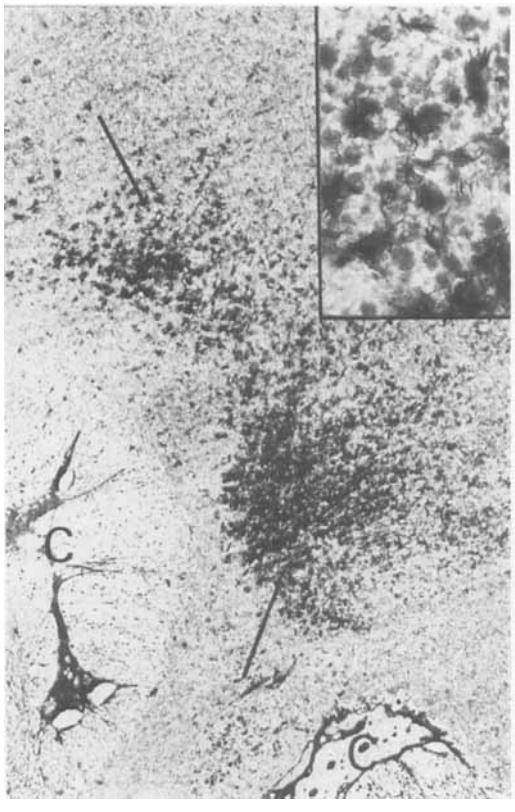

Figure 2. A secondary center of ossification of a 2-year-old child. Focal aggregations of electron dense crystalline clusters (arrows) are seen in the vicinity of the hypertrophic cartilage cells (C) (× 2,900). Inset shows the crystalline deposits of the perilacunar matrix. (× 29,000).

as electron dense crystalline clusters which were focally aggregated in the cartilage matrix among hypertrophic or degenerating cartilage cells (Figure 2). They were composed of needle-shaped crystalline clusters and appeared in close proximity to membrane-invested round or oval electron dense particles located chiefly in the perilacunar matrix (Figure 2-inset). In an electron dense cartilage matrix, the crystalline clusters were diffusely deposited in the cartilage matrix with the exception of the lacunar matrix.

In adults, these electron dense crystalline clusters appeared in cartilage matrix deep within the articular cartilage. Just above the tidemark, they were largely found in the perilacunar and interlacunar matrix (Figure 3). These crystalline deposits were also close to the membrane-invested electron dense particles (Figure 3-inset). In the tidemark and underlying calcified zone of the articular cartilage, electron dense crystalline clusters gradually increased in number forming the electron dense cartilage matrix, where collagen fibrils run perpendicularly to the articular surface.

The electron dense particles of the perilacunar matrix were larger, more irregular in shape and more abundant than those of the interlacunar matrix. Occasionally, electron dense crystals were found in the form of large, polyhedral or round electron dense masses which varied in size.

Fine structure of crystals. Morphologically, electron dense crystals in the initial stages of deposition were divided into types A and B. Type A crystals were found mainly in the

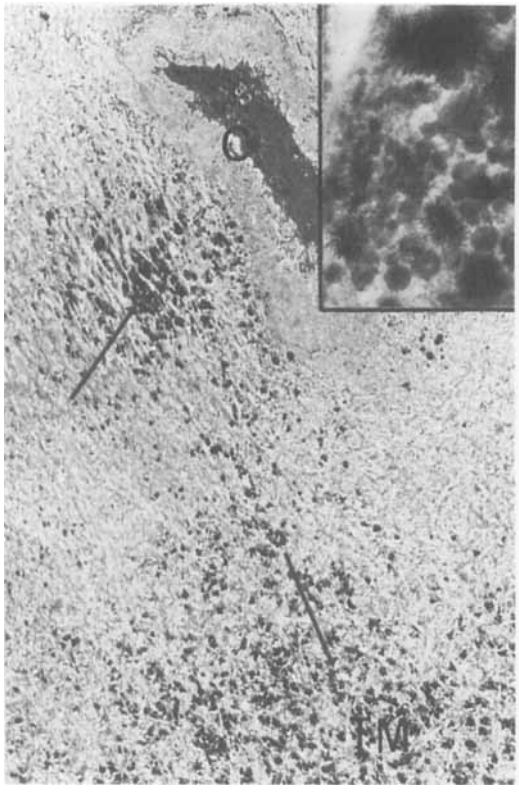

Figure 3. Electron dense crystalline deposits (arrows) of an adult articular cartilage. They appear not only in the vicinity of the cartilage cells (C) but also in the interlacunar matrix near the tidemark (TM) (× 2900). Inset shows the crystals around the perilacunar matrix (× 29,000).

cartilage of children from 11 months to 10 years of age and were characteristically slender, twisted and curved, measuring from 100 nm to 360 nm in length and approximately 10 nm in width. They tended to form clumped crystalline clusters (Figure 4). Type B crystals were short and needle-like and measured from 30 nm to 160 nm in length and approximately 5 nm in width. They were found mainly in adults from 26 to 80 years of age and had a tendency to form sunburst shaped crystalline clusters (Figure 5).

Both type A and B crystals were almost always found within or in close apposition to the surface of the extracellular membrane-

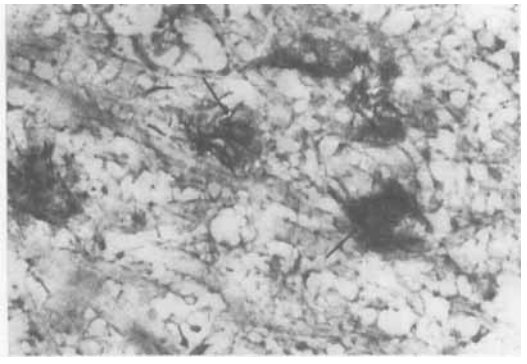

Figure 4. Type A crystals (arrows) in a child. They are slender, twisted and curved, measuring from 100 nm to 360 nm in length (× 48,000).

invested electron dense particles. However, when the crystals increased in population and formed clusters, the membrane-invested electron dense particles disappeared.

Electron probe X-ray microanalysis. A point analysis of types A and B crystals indicated that the integral counts peaked at 3.69 Kev and 2.01 Kev which corresponded with calcium and phosphate, respectively. In one child, analysis of an area of sparse deposition, which corresponded to an initial stage of deposition, indicated the phosphate peak was higher than that of calcium (Figure 6A). An analysis of the electron dense cartilage matrix demonstrated the calcium peak was more

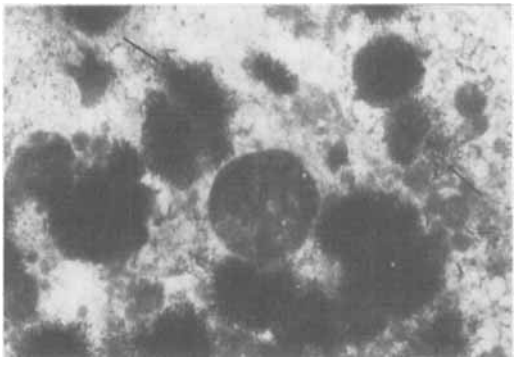

Figure 5. Type B crystals (arrows) in an adult. They are short, needle-like and slightly curved, measuring from 30 nm to 160 nm in length (× 48,000).

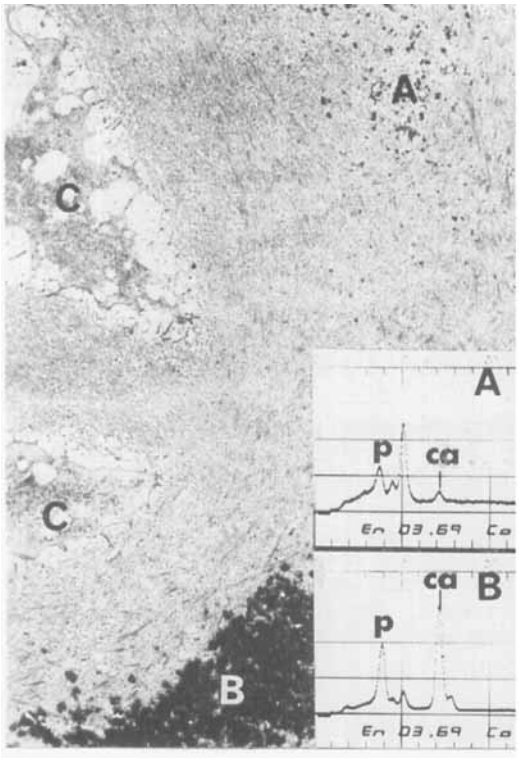

Figure 6. A point X-ray microanalysis from a child (× 4800). A, taken from an area of sparse crystalline deposits, shows that the phosphate peak is higher than that of calcium. B, taken from an area where the crystalline clusters are diffusely deposited around degenerating cartilage cells (C), shows the calcium peak is more prominent and separated into K_α and K_β.

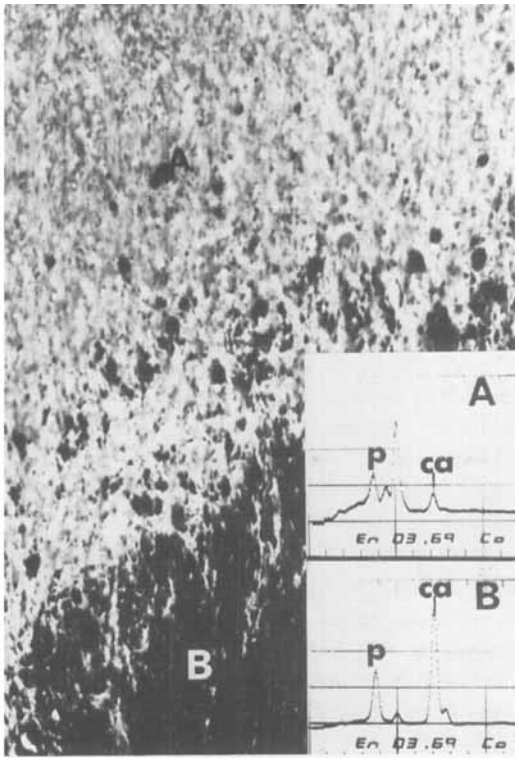

Figure 7. A point X-ray microanalysis from an adult (× 6000). The detected peaks are similar to those of a child; A is from the crystalline clusters just above the tidemark and B is from the tidemark.

prominent than that of phosphate and was characteristically separated into K_α and K_β (Figure 6B). A point analysis of an adult articular cartilage revealed a pattern similar to that of a child (Figure 7A and B). The mineral composition, however, was indistinguishable between type A and B crystals.

DISCUSSION

In the present study, the mode of calcification of human articular cartilage at different ages was documented at the fine structural level. The mineral crystals in the cartilage matrix were morphologically divided into types A and B. Type A crystals, found mainly in children, formed clumped crystalline clusters among hypertrophic and degenerating cartilage cells. Type b crystals, found mainly in adults, were smaller than type A, and formed sunburst shaped crystalline clusters. Morphological differences between mineral crystals were observed by Bosman et al. (1977) who reported the coexistence of three different types of crystals during the initial stages of calcification of cartilage in the pathological condition of chondrodystrophia calcificans congenita. However, the fine structure and distribution of the crystals observed in the present study differed from those described by Bosman et al. The morphological differences between mineral

crystals in the cartilage of children and adults may depend upon the chemical alteration of the ground substances of the cartilage matrix, including proteoglycans (Mourão et al. 1976) and/or collagen (Gay et al. 1976, von der Mark & von der Mark 1977). Moreover, the biomechanical roles of the joints in childhood may differ from those in adulthood; the former has the characteristics of epiphyseal development and remodelling while the latter, completely ossified, may serve as a resistance to biomechanical stresses of the joint. Such biochemical and biomechanical changes in articular cartilage between children and adults may be responsible for the morphological differences in the mineral crystals of the cartilage.

During the initial stages of mineral deposition in the cartilage matrix, the mineral crystals appeared closely related to the extracellular membrane-invested electron dense particles the morphological features of which resembled "matrix vesicles" or "calcifyin globules"as previously reported by Anderson (1969) and Bonucci (1970), respectively. It has been widely accepted that the matrix vesicles serve as the initial site of calcium deposition in various calcifying tissues such as bone (Bonucci 1971), dentine (Bernard 1972), cartilage (Bonucci & Dearden 1976), and in pathological conditions (Schajowicz et al. 1974, Williams et al. 1976). Ali & Evans (1973) demonstrated that the matrix vesicles incorporated calcium and phosphate to form hydroxyapatite. Arsenis (1972) and Brighton & Hunt (1974) postulated that mitochondria may participate in the initial process of calcification and that the membrane-invested particles could be released from the cytoplasm to form matrix vesicles. In the present study, however, the mineral crystals were deposited in a mass composed of irregular electron dense particles of various sizes. They are presumed to be derived from cell debris or degenerated cartilage cells.

By using a non-dispersive electron probe X-ray microanalyzer, type A and type B crystals were found to be identically made up of calcium and phosphate complexes, probably hydroxyapatite. Based on mineral composition alone, however, type A crystals could not be distinguished from type B crystals. Such zonal differences in the calcium/phosphate ratio may be due not only to changes in concentration of the mineral components but also to alterations in the proteoglycans of the cartilage matrix.

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