Morphological study of articular cartilage in pyrophosphate arthropathy

(Chondrocalcinosis articularis or calcium pyrophosphate dihydrate crystal deposition disease)

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Clinical reports of patients with calcifications of articular structures and crystal synovitis have recently appeared with increasing frequency. The crystals have been identified as calcium pyrophosphate dihydrate (CPPD), and the disease has consequently been called CPPD crystal deposition disease (McCarty, 1966), pyrophosphate arthropathy (Russell, Bisaz, Fleisch, Currey, Rubinstein, Dietz, Boussina, Micheli, and Fallet, 1970), or pyrophosphate synovitis (Bjelle and Sundén, 1971). The earlier designations, articular chondrocalcinosis (Žitňan and Sit'aj, 1963) and pseudogout (McCarty, Kohn, and Faires, 1962), are also still in common use. A number of metabolic diseases have been found among certain of these patients, and CPPD deposition disease has in these cases been considered 'secondary' (*cf.* McCarty, 1966). Among the 'primary' cases, a number of patients with a hereditary type of the disease are found.

Some reports on the pathological morphology of the articular cartilage have been presented (McCarty Gatter, and Hughes, 1963; Žitňan, Sit'aj, Hüttl, Škrovina, Hanic, Marković, and Trnavská, 1963; de Sèze, Fressinaud, Besson, Mazabraud, and Mitrovic, 1963; Bundens, Brighton, and Weitzman, 1965; Lagier and Ott, 1969; Reginato, Valenzuela, Martinez, Passano, and Daza, 1970), but the results are contradictory.

The aim of the present study was to investigate the morphology of articular cartilage from patients with 'familial' CPPD deposition disease of varying severity.

Material

Articular cartilage was used from the knee joints of five patients, four of whom were related. In the family of the fifth patient several cases of pyrophosphate deposition disease had been diagnosed. The mean age of the patients was 46 years (range 39–53). None was receiving pharmacotherapy at the time of biopsy and none had been treated with steroids, generally or locally. Calcifications of menisci and articular cartilage were seen on clinical radiographs from every patient all of whom had previously had synovitis in the knee joint operated upon. Biopsies were taken by the method of Brighton (1967) from the knee joints of four patients. In the case of the fifth patient, the biopsy was taken from the inner side of the medial condyle of the lower femoral epiphysis at synovectomy. The specimens were square-shaped, reaching down to the bone, the dimensions of the cartilage surface being about 0.5×1 cm.

Methods

The biopsy specimens were treated in four ways:

- (1) A section approximately 200μ thick was cut with a razor blade directly from each of the fresh specimens. These sections were surveyed for calcifications macroscopically and further investigated by light microscopy and radiographs. Micro-x-ray diffractions of the calcifications were performed according to the method of Bjelle and Sundström (1969).
- (2) Fixation of other sections from each biopsy specimen in phosphate-buffered formaldehyde (pH 7.2) was performed for 24 hrs. These pieces were about 3 mm. thick generally going down to and including bone. After they had been embedded in paraffin, 15μ sections were cut

and used for routine histology, applying haematoxylin and eosin, Alcian blue, toluidine blue, and Masson's stain.

(3) Unstained sections were used for polarized light microscopy (fixation as above; decalcification for 8 days in a 3 per cent solution of EDTA in phosphate buffer (pH 7.2)). The same histological methods were applied as above.

(4) Contact microradiography, using ultrasoft x rays (8–12 Å), has been used to correlate biophysical information and morphological structures on a cellular level (Engström and Lindström, 1950; Engström and Lundberg, 1957). In the present study, this technique was applied to correlate the distribution of dry mass concentration with cartilage microscopic structures, and to localize crystal precipitations.

Sections 5 μ thick taken from undecalcified and decalcified (EDTA) specimens, were prepared by the method of Bergendahl and Engfeldt (1970). The sections were microradiographed at 1.5 kV and 1.0 mA for 50 min., the sample being placed at a distance of about 55 mm. from the focal spot, which was about 0.1 mm. in diameter. Fine-grained photographic emulsion (Kodak Maximum Resolution Plate) was used.

(5) Sections, about 1 mm. in thickness, were fixed for 24 hrs in a 0.02 M phosphate buffer (pH 7.2) containing 0.15 M NaCl, 2 per cent. paraformaldehyde, 2 per cent. glutaraldehyde and 0.5 per cent. cetylpyridinium chloride. After washing in the buffer vehicle for 1 hr, postfixation with osmium tetroxide (in the same buffer) for half an hour was performed, and finally the sections were embedded in Epon. For orientation in light microscopy, 1 μ sections, stained with toluidine blue in borax, were used, the first ones including all layers (the surface to the deep layer close to the bone). Subsequent ultrathin sections were taken from selected areas.

FIG. 1 Radiograph (dental x -ray film) of a cartilage section, around 200 μ thickness. The calcifications are seen as white dots in the intermediate layer of the cartilage between the faintly outlined cartilage surface (arrows) and the bone trabeculae, which are sharply demarcated in the lower part of the specimen.

Results

Some calcifications could be located macroscopically, but the amount and the localization of the calcifications in each specimen were determined more accurately from a radiograph (Fig. 1). The amount of calcifications was variable and in one of the patients, only few and small radiodense areas could be observed. However, calcifications were sufficient in all patients to give the typical micro- x -ray diffractograms of CPPD crystals (Fig. 2).

In all patients calcifications were located in the intermediate layer and no accumulation of CPPD crystals could be found in the surface layer (Fig. 3). In one of the patients calcifications were also observed in the deep layer but were never seen involving the bone (Fig. 4).

The calcifications usually appeared as distinctly outlined areas (Figs 3 and 4), but small more irregularly delineated calcified areas were also encountered. These latter areas were not so heavily calcified having more preserved matrix in undecalcified sections (Fig. 5). Sometimes, areas of dense calcifications were numerous and lying close to each other, but a delineation of the different areas was generally seen (Figs 4 and 6). In some places, however, the border between the different areas had disappeared. The matrix, immediately adjacent to and delineating areas with heavy calcification usually had a higher stainability with all dyes (haematoxylin and eosin, toluidine blue, Alcian blue and Masson's stain) than the surrounding matrix (Figs 5 and 6).

Unstained sections were examined in polarized light. The same distribution of birefringent material was found as expected from stained sections. Circular

FIG. 2 Micro x -ray diffractogram from the calcification, seen in Fig. 1, showing the 'fingerprint' of calcium pyrophosphate dihydrate.

FIG. 3 Photomicrograph of section of undecalcified articular cartilage from patient with pyrophosphate synovitis. The calcifications are typically situated in the middle of the cartilage (the intermediate layer) as delineated rounded areas. The surrounding cartilage of the intermediate layer or in the surface (left side of Figure) and deep layers (right side of Figure) have an overall 'normal' appearance. Decalcified section. Alcian blue. $\times 46$

FIG. 4 Heavy crystal deposits, some of which are seen close to the underlying 'tidemark layer' but not involving this layer or the bone (right side of Figure). Undecalcified section. Masson's stain. $\times 30$

FIG. 5 Calcifications, less distinctly outlined than in Fig. 4. No consistent relationship can be established between the chondrocytes and the mineral deposits. Decalcified specimen. Alcian blue. $\times 150$

FIG. 6 Section from same patient as shown in Figs 4 and 5. A thin zone surrounding the calcifications exhibit a higher stainability. Despite the close localization of the rounded areas of calcification, they are often separated by the thin zone of more intensely stained matrix. No obvious sign of degeneration of cells adjacent to the mineral deposits. Masson's stain. $\times 60$

patterns of birefringent material (spheruliths) were found within the large clusters, both in the centre and also constituting part of the border of the cluster (Fig. 7). At higher magnification, crystals were seen which had the same appearance and birefringence (Fig. 8) as those found in the synovial fluid. In ultrasoft microradiographs, the material with high dry mass concentration showed a similar distribution as that of birefringent material in subsequent sections, and thus was interpreted as being due to crystals (Fig. 9). Most crystals were found in clusters, generally densely packed in the centre. At the periphery, the crystals were often more scattered and with a radiating orientation (Figs 9 and 10). Sometimes these peripheral crystals were densely packed

and layered parallel to the border with a thin, adjacent zone of matrix having a higher dry mass concentration than the surrounding cartilage tissue. This was not found around borders of radiating crystals. Generally, no clear-cut relation between the crystal deposits and the chondrocytes was encountered. However, crystals from large clusters were occasionally seen radiating into a cell territory and also into a cell, apparently without respecting any pericellular zone or cellular membrane (Fig. 10, arrow).

In undecalcified ultrathin sections observed with the electron microscope, the crystals were also generally found in clusters. They also, however, appeared scattered in the matrix (Fig. 11). In the periphery of the clusters the crystals were often found

FIG. 8 Same section as Fig. 7 in polarized light at higher magnification. The crystals are of the same shape as those found in synovial fluid. $\times 600$

FIG. 7 Undecalcified section in polarized light. Within the clusters, several spheruliths are discerned as crescents. $\times 300$

FIG. 9 Ultrasoft microradiograph of undecalcified, unstained section. The areas of higher dry mass concentration have the same distribution as in sections observed in polarized light in Fig. 7. $\times 150$

FIG. 10 The same as Fig. 9. Crystals radiating into the matrix and also into a cell. At sites (arrow) crystals are parallel to the border of the cluster. $\times 150$

FIG. 12 High magnification of a few small CPPD crystals, showing 'bubbled' appearance of crystals disintegrating in the electron beam. Undecalcified. Non-contrasted section. $\times 75,000$

FIG. 11 Ultrastructure of crystals at the periphery of a cluster. The crystals are radiating from the centre of the cluster. The delineation is less sharply marked than in light microscopy. Undecalcified. Non-contrasted section. $\times 12,000$

radiating from the centre into the matrix more diffusely than expected from light microscopy (Fig. 11). At high magnifications, the crystals were seen to deteriorate in the electron beam (Fig. 12).

Another change was also found in the cartilage matrix (Fig. 13). It was seen as irregularly delineated areas with staining weaker than that of normal cartilage, using the same dyes as above. These changes were especially apparent in one patient with

few and small calcifications and were visible on clinical and specimen radiographs. In other patients, with heavier crystal deposits, the areas of weakly-stained matrix were smaller and not so easily recognized. Cells involved in these areas were usually surrounded by a thin border of matrix, frequently seen as continuous extension of seemingly unchanged matrix, making this formation look like a small peninsula at the end of an isthmus (Fig. 13, arrow).

FIG. 13 Areas of faint staining are seen in lower intermediate or upper part of deep layer. Cells within these areas are generally surrounded by a thin border of seemingly unchanged matrix (arrow). Undecalcified section from the cartilage of a patient with small calcifications. Toluidine blue. $\times 170$

In ultrasoft microradiographs of these areas, a higher dry mass concentration was found in both undecalcified and decalcified sections (Fig. 14). This type of change was not seen immediately surrounding calcifications but was also found in the intermediate layer. The parts of matrix without calcifications or the changes encountered above did not display any morphological alterations compared with normal cartilage.

Areas of calcifications appeared in intercellular matrix without any consistent apparent relation to the chondrocytes (Figs 3 to 6 and 10). It was not possible to decide if degenerative changes of the cells were present or not. Morphological signs of osteoarthritis were not found in any of the specimens, either in the surface layer or in the deep layer and underlying bone. It should be mentioned, however, that all specimens were taken from sites not usually affected by degenerative joint disease (Figs 3 and 4).

Discussion

The peculiar localization of precipitates of inorganic crystals not found in normal mineralization or in pathological calcifications in other parts of the body suggests that a localized alteration might be responsible. A detailed study of the distribution of the crystals and of the possible relationship between crystals and normal or altered cartilage structures in well-defined clinical materials are thus of great interest. The identification of CPPD crystals on a micro-scale level is vital in this context.

The contradictory findings between earlier studies regarding the localization of calcifications and changes in the structure of articular cartilage in pyrophosphate arthropathy might partly be explained by differences in the age of the patients and in the sites of the biopsies, and possibly also by the lack of crystallographic identification of the calcifications. Furthermore, the correlation between pyrophosphate crystal precipitations and the changes of osteoarthritis has

FIG. 14 Same kind of altered areas as shown in Fig. 7. Cells are seen surrounded by a thin zone of normally stained matrix attached to the surrounding normally stained matrix by a thin stalk. Ultrasoft microradiograph of undecalcified section. $\times 110$

not been clarified in relation to the factors encountered above.

In the present study the patients were younger than those in earlier studies (de Sèze and others, 1963; McCarty and others, 1963; Lagier and Ott, 1969; Reginato and others, 1970) with one exception (Bundens and others, 1965). A micro-x-ray diffraction procedure was applied (Bjelle and Sundström, 1969) by which the minimum diameter of the calcification required for analysis is 50μ (equal to the diameter of the collimator) and the thickness of the specimen is largely determined by the sectioning method. This technique allowed crystallographic identification of several crystal precipitates in the same specimen. In none of the patients were changes of osteoarthritis seen roentgenologically. The biopsy technique applied did not allow inspection of the whole joint surface and all specimens were taken from non-weight-bearing sites.

In two investigations (McCarty and others, 1963; Reginato and others, 1970), in which large areas of the cartilage were inspected, no degenerative changes were encountered in the non-weight-bearing sites. In the only published study of a cartilage biopsy specimen from a young person affected by CPPD deposition disease, no osteoarthrotic changes at all were reported (Bundens and others, 1965). In some investigations (de Sèze and others, 1963; Lagier and Ott, 1969), the patients were elderly (70 to 89 years) and both the extensive calcifications on the surface of the articular cartilage and the advanced osteoarthritis might be explained by the age. X-ray diffraction was not performed on the surface calcifications in these studies and the possibility cannot be excluded that they were due to crystals other than

CPPD. In the present study no calcifications were found at the articular surface.

From a clinical point of view, the correlation between pyrophosphate arthropathy and osteoarthritis has been a matter of debate (Currey and others, 1966). The mean age of the patients in clinical reports of pyrophosphate arthropathy has usually been high, and the frequent finding of osteoarthritis in pyrophosphate arthropathy in aged individuals might be due to simultaneous phenomena with neither causative nor secondary relationships.

The development of early calcifications and the progression of the disease has been described as a result of 'focal metaplastic alterations' in the surface layer with secondary reparative processes from the periphery of the cartilage surface (Žitňan and Sit'aj, 1963). Chronic synovitis might produce an ingrowing granulation tissue unspecifically and also degenerative changes in the cartilage, but it has not been encountered in the other studies. In one of the patients in the present series, synovectomy was performed and the synovial lining was inspected. However, no granulation tissue (pannus) was observed at the margins of the cartilage, either visually at the operation or histologically on a cartilage biopsy from the inner margin of the femoral condyle. The observation by Žitňan and Sit'aj (1963) was made in cartilage from first metatarsal joints operated upon for hallux valgus, and the changes encountered might be due to an arthritic process of another kind.

In some studies, the first deposits were supposed to occur in the immediate surrounding of the cells (McCarty and others, 1963; Bundens and others, 1965) and to occupy certain chondrocytes (Lagier and Ott, 1969). This could not be confirmed in the present study, nor could degeneration of cells, adjacent to small calcifications, be observed. This was also confirmed on cells observed electronmicroscopically but a more extensive study with ultrathin serial sectioning is required to clarify the chondrocyte ultrastructure in this disease.

The accumulation of stains at the border of some calcified areas could be an artefact, since such accumulation frequently takes place at section borders. However, it has been stated by several investigators of other calcification processes, that a concentration both of acid and of basic dyes takes place immediately adjacent to calcified areas. These areas correspond to areas of increased dry mass concentration observed in ultrasoft microradiography while in the areas of radial growth of the crystal precipitates no increased dry mass concentration was observed.

In light microscopy, the crystals seemed to occur only in delineated areas, giving the balloon-like demarcated characteristic structure, when large and dense deposits were found. The spherulith figures

observed in polarized light indicate that the large calcified areas are non-homogenous and contain several nucleation centres. The distribution of these spheruliths did not seem to correspond to the chondrocyte distribution at undecalcified sites of the same layer. Ultrastructurally and in ultrasoft microradiographs further evidence for this was found. The ultrastructure further revealed that the crystals appear more scattered than observed in light microscopy and that the size of the crystals varied considerably.

'Degenerative areas without calcifications' were found in the intermediate layer by de Sèze and others (1963), but have not been recorded by other investigators. These changes appear to be similar to the 'areas of low stainability', described in the present study. It is tempting to suggest that these matrix alterations, occurring in the same layer of the articular cartilage subjected to CPPD deposits, are specific for this disease and that they might constitute signs of an altered metabolism of the cartilage.

All patients in the present study had pyrophosphate arthropathy of the hereditary type, which might have an entirely different pathophysiological mechanism from the non-hereditary and/or the secondary type of disease. This study has indicated the presence of one primary structural change of cartilage matrix in pyrophosphate arthropathy. This observation is of further interest since pyrophosphate is produced during the breakdown of energy-rich triphosphates and is released in a great number of synthetic reactions. A defect in pyrophosphate metabolism might thus result in changes in the cartilage matrix by inhibition of synthesis, and possibly also in the formation of pyrophosphate crystals.

Summary

A morphological study was performed on biopsy specimens of articular cartilage taken from the lower femoral epiphysis of five patients afflicted by the hereditary type of pyrophosphate arthropathy (calcium pyrophosphate dihydrate (CPPD) crystal deposition disease) of varying severity. The techniques applied were light microscopical staining, polarized light microscopy of unstained sections, ultrasoft microradiography and electronmicroscopy of ultrathin, undecalcified sections. Deposits of CPPD crystals were identified in all specimens by micro-x-ray diffraction. The calcifications generally appeared as delineated rounded areas, located in the intermediate layer. Spheruliths were frequently seen within the clusters. They were never seen involving the cartilage surface, the 'tide-mark' layer or the bone. The surrounding cartilage tissue displayed few alterations, but at certain sites a thin zone of matrix immediately adjacent to dense calcifications showed a higher stainability and also an increased dry mass

concentration compared to that of surrounding normal cartilage. However, irregularly outlined areas with low stainability were also observed; these were located in the intermediate layer and were especially noticeable in one patient with only a few small calcifications. Calcifications were not found in those areas but an increased dry mass concentration was encountered. In undecalcified ultrathin sections studied by electron microscopy, most crystals were found in clusters corresponding to the calcified areas seen by light microscopy. Dispersed localization outside the clusters was frequently encountered. No relationship between small (early?) calcifications and chondrocytes was found with any of the techniques applied in the present study.

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