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An ultrastructural study of articular chondrocalcinosis in cases of knee osteoarthritis

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Summary. A study was made by transmission electron microscopy of tissue specimens (cartilage, meniscus and synovial membrane) taken from 5 knees presenting radiological and anatomical signs of articular chondrocalcinosis and osteoarthritis. It was part of a broader study which included analysis of the same specimens by macroscopy and light microscopy as well as by X-ray diffraction of the mineral deposits.

In cartilage and meniscus juxta-cellular calcium pyrophosphate dihydrate (CPPD) crystals of variable sizes were observed in the extracellular organic matrix, independent of the collagen fibrils. They occured mainly in the superficial and middle zones but could also be seen intermingled with the apatite crystals in the cartilage calcified zone. In synovial membrane most of the CPPD crystals were extracellular but some of them could be seen in cytoplasmic phagocytic vacuoles.

These observations are in agreement with those in the literature regarding the ultrastructural picture of chondrocalcinosis and support the thesis that the crystals originate in the cartilage and are phagocytized in the synovial membrane.

Although the results of the present study do not provide direct evidence of a relationship between chondrocalcinosis and osteoarthritis, the data of the ultrastructural investigation appear nevertheless of great interest as a complement to the data furnished by light microscopy.

Key words: Articular chondrocalcinosis – CPPD disease – Knee osteoarthritis – Cartilage – Meniscus – Synovial membrane

Pathological calcium deposits in the synovial joints consist almost exclusively of calcium pyrophosphate dihydrate (CPPD) crystals (Lagier et al. 1966; Lagier 1981; Boivin et al. 1981 b) and present a highly characteristic radio-

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logical image due to their arrangement in the cartilage, fibrocartilage and synovial membrane. This condition is therefore known as "articular chondrocalcinosis" and is considered to be the manifestation of a "CPPD disease" (McCarty 1979a) which has also occasionally been observed in extraarticular situation (Lagier 1981).

The objective approach made possible by anatomopathology provides both an adequate interpretation of the radiological images of advanced stages as well as valuable data concerning the early histological stages. These data should be obtained mainly by means of ultrastructural study, which, whenever possible, should be accompanied by biochemical investigation. The results of such a study should be considered with reference to the site from which the sample was taken as established by previous macroscopic and light microscopical examination.

This approach was used in the present study of the excised tissue from five patients who had undergone surgery for knee osteoarthritis.

Material and methods

1. Clinical history of the subjects

Case 1 (left knee surgical specimen – T. 2104/80). Eighty-one year-old woman who for many years had suffered from polyarticular pain and osteoarthritis of the left knee (swelling and pain upon weight pressure).

Case 2 (left knee surgical specimen – T. 11036/80). Eighty-four year-old man who for many years had suffered from osteoarthritis of shoulders, hips and knees (medial erosion in the left knee).

Case 3 (right knee surgical specimen – T. 3180/77). Eighty year-old woman with osteoarthritis of both knees (medial erosion in the right knee).

Case 4 (right knee surgical specimen – T. 11273/80). Eighty-one year-old woman with osteoarthritis of both knees.

Case 5 (right knee surgical specimen – T. 5522/80). Thirty-four year-old man with post-traumatic osteoarthritis of the right knee and no other arthropathy. At age 20, the right medial meniscus was excised after locking during running. At age 22, a plasty of medial lateral ligament was performed.

In each of these cases, radiological signs of mineral deposits in the knee menisci indicating articular chondrocalcinosis had been observed (Fig. 1). Available radiological data were insufficient for detection of calcification in other joints except in case 4, where it was observed in the articular disks of both distal radio-ulnar joints. Unilateral total knee replacement was performed in each of the first 4 cases, in which arthrotomy had shown eburnation of the femur and tibial surfaces of the medial compartment. Case 5 was treated by tibial osteotomy after a lateral arthrotomy showed remodelling of the corresponding cartilage surfaces and meniscus.

2. Histological methods

In all the surgical specimens mineral deposits were seen macroscopically, at least by stereomicroscope. Where there were no deposits, cartilage and fibrocartilage appeared normal and synovial membrane was slightly hyperplastic.

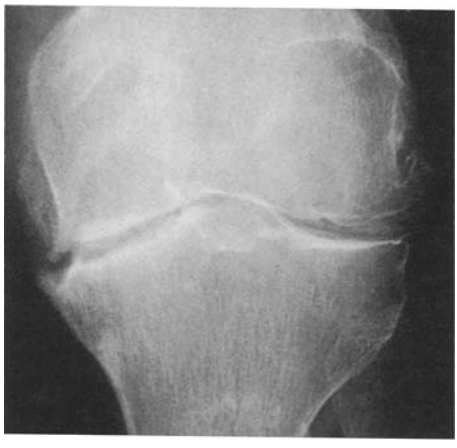

Fig. 1. Frontal X-ray of left knee (June 2, 80) (case 1)

The samples used for histological study were taken from cartilage, meniscus and synovium in cases 1 and 2, from cartilage and synovium in cases 3 and 4, and from meniscus and synovium in case 5. Except those intended for X-ray diffraction, the samples were immediately fixed and then studied by light microscopy, microradiography and transmission electron microscopy.

X-ray diffraction was performed in each case and for each kind of tissue examined, on unfixed mineral material that had been pulverized in a mortar. This was studied by means of either Guinier or Chesley cameras (Ni-filtered Cu Kα radiation, at 40 kV and 24 mA; 7 h exposure for the Guinier and 10 h for the Chesley). Certain areas on the sections prepared for microradiography were also used for an analysis by means of the Chesley camera (100 µm collimator).

Light microscopy was performed on the excised material that had been fixed in 10% neutral formalin and embedded in paraffin (using either hematoxylin-eosin-stained sections or unstained sections which were examined by polarized light after the paraffin had been completely removed in a 56° C oven). Light microscopy was also performed on unstained sections prepared for microradiography.

Microradiography was used only on an osteocartilaginous sample from the tibial plateau of case 1; it was fixed in ethyl alcohol, embedded in methyl-methacrylate using xylol as intermediary solvent. The samples were cut into sections with a low-speed saw, ground manually to a thickness of 50 µm and then microradiographed (Boivin and Baud 1983).

Transmission electron microscopy was performed in each case and for each of the tissues studied, on at least 4 fragments of about 1 mm^3 prepared under a stereomicroscope, prefixed for 2–4 h at 4° C (5% glutaraldehyde buffered with 0.1 M sodium cacodylate; final pH 7.2) (Sabatini et al. 1963), washed at 4° C (0.2 M solution of saccharose in a 0.1 M sodium cacodylate buffer pH 7.2), postfixed for 2 h at 4° C (1% solution of osmium tetroxide in 0.1 M sodium cacodylate buffer). The fragments were then dehydrated in ethyl alcohol baths of increasing concentrations and embedded (with propylene oxide as intermediary solvent) either in epon B (Luft 1961) or in ERL 4206 (Spurr 1969). Ultrathin sections were cut with a Porter Blum – I ultramicrotome equipped with a diamond knife then either stained with lead citrate (Venable and Coggeshall 1965) or left unstained and examined by means of a Philips EM 200 electron microscope.

Results

In all of the samples examined by X-ray diffraction, the mineral deposits were found to be composed only of CPPD crystals, for the most part in

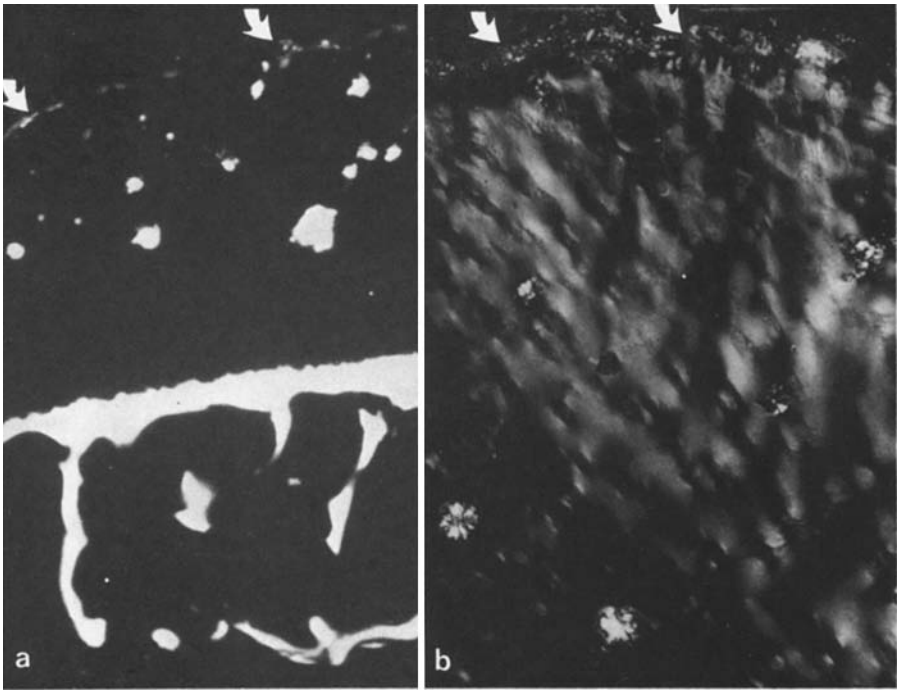

Fig. 2a, b. Topographical histological aspect of chondrocalcinosis in articular cartilage (case 1). **a** Microradiograph of the left tibial plateau (×20). Below, cancellous bone trabeculae and bone cortex flanked by an indented basal plate of calcified cartilage. Above, articular cartilage with mineral deposits on the surface (*arrows*) and – in the form of spots – in the outer half. **b** Microphotograph under polarized light of the outer half of the articular cartilage (section used for the above microradiograph – HE × 100). Crystal deposits on the surface (*arrows*) and as clusters between collagen fibers of the extracellular organic matrix

triclinic form but also in monoclinic form. No other kinds of mineral substance were detected.

For all 5 cases, light and electron microscopical data were the same for each type of tissue studied. The CPPD crystals presented in light microscopy as rod-like images with positive birefringence. They could often be seen in situ by electron microscopy in the form of electrondense bodies that were very variable in length, thickness and shape. The size of the bodies ranged from about 0.2×0.1 μm to 8×4 μm. However, the crystals were sometimes dislodged in the course of preparation of the sections, either during cutting or staining; in that case, their localization could only be detected by means of the clefts they had made in the organic matrix.

Changes of articular cartilage

Most of the mineral deposits were observed on a sharply eroded cartilage surface (Fig. 2a). Some were also be seen at a deeper level mainly in tangential and transitional zones in the form of isolated or confluent spotty deposits

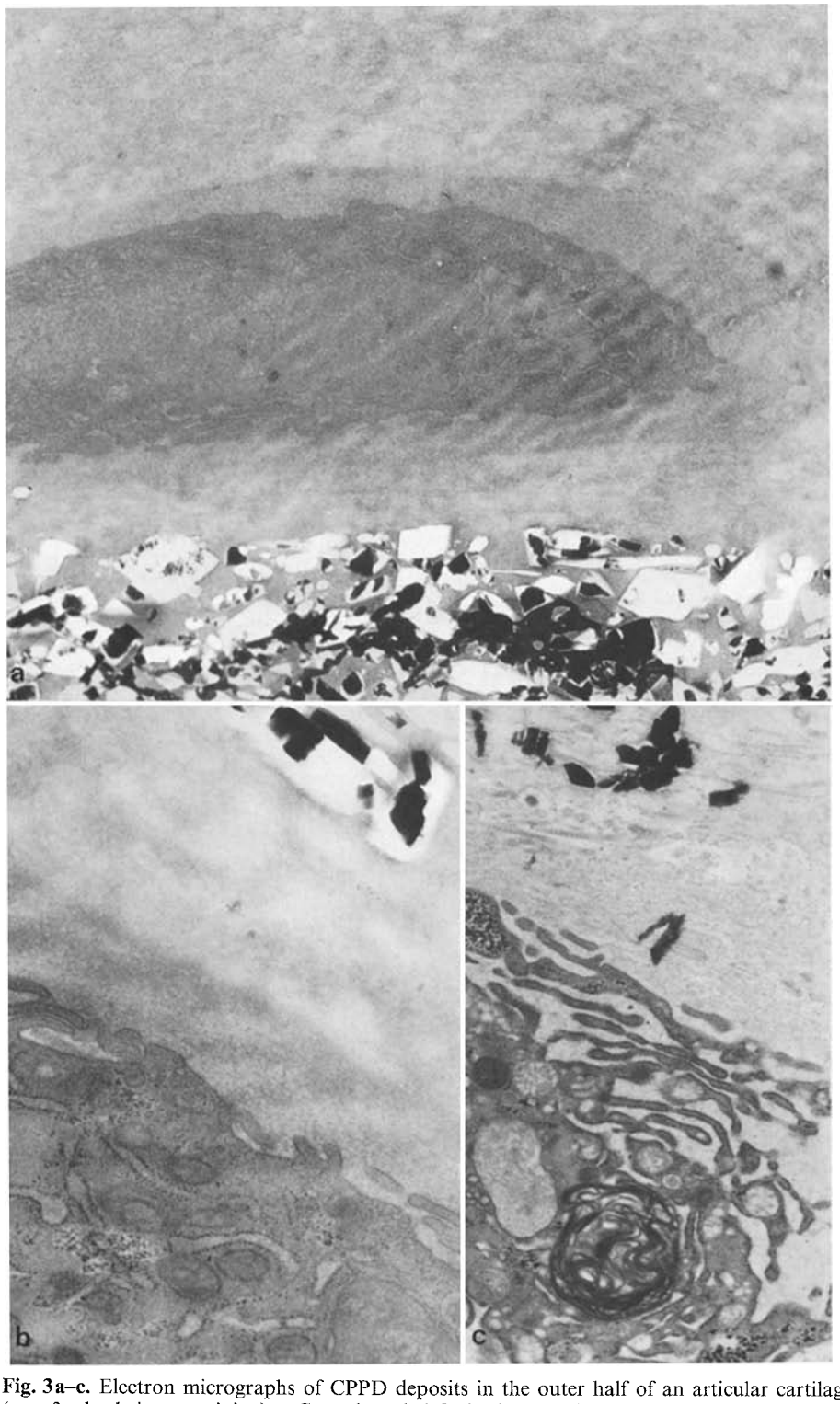

Fig. 3a–c. Electron micrographs of CPPD deposits in the outer half of an articular cartilage (case 3 – lead citrate staining). **a** Crystals and clefts in the organic matrix facing an unmodified cell (×9,000). **b** Some crystals and their clefts in the organic matrix near a secreting cell (endoplasmic reticulum; mitochondria in the cytoplasm) (×24,800). **c** Some crystals in the organic matrix (intermingled with collagen fibrils near a damaged cell (myelin body and glycogen granules in the cytoplasm) (×13,500)

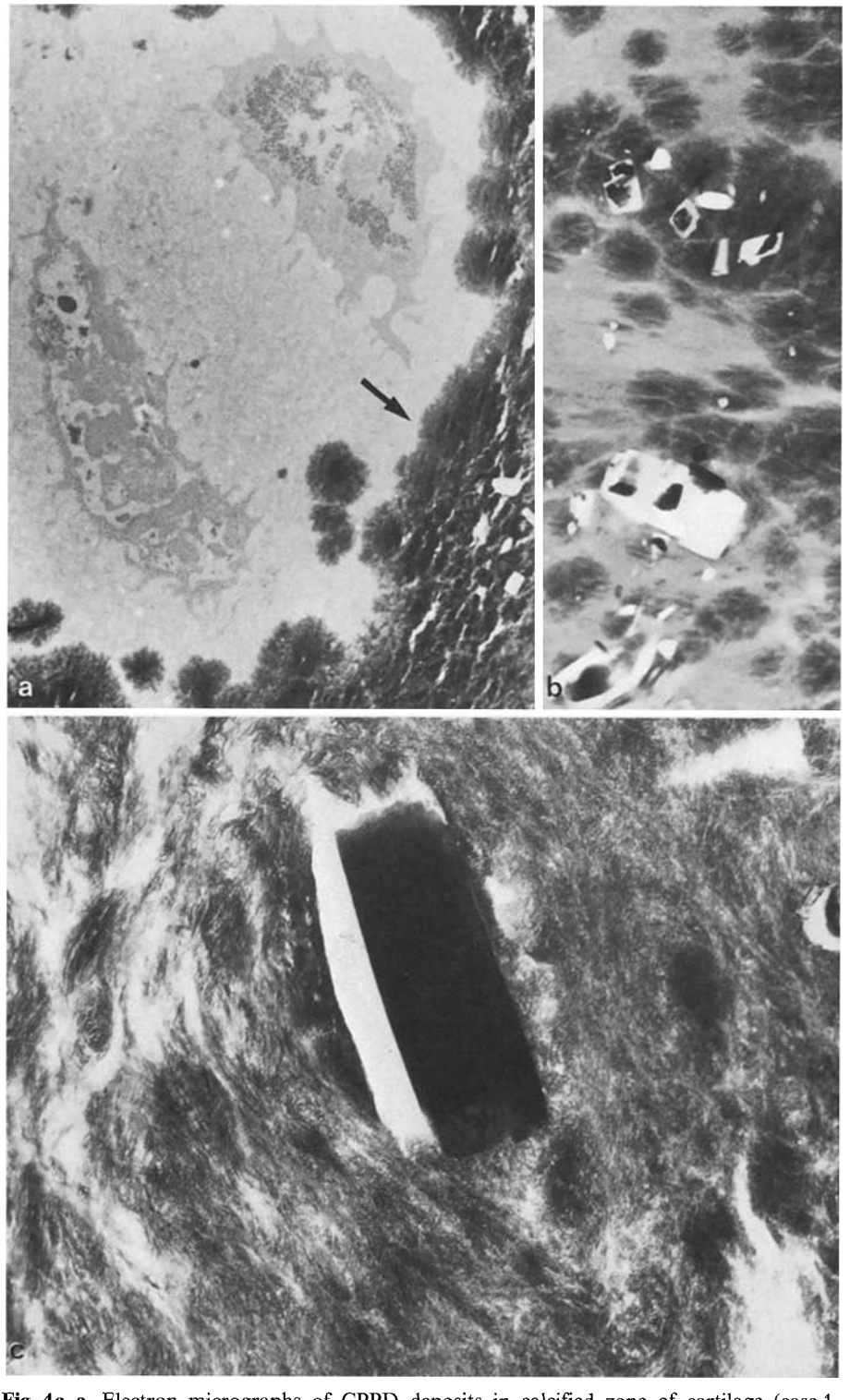

Fig. 4a–c. Electron micrographs of CPPD deposits in calcified zone of cartilage (case 1 – lead citrate staining). **a** Apatite crystal deposits near two cells. The *arrow* indicates a region with CPPD crystal clefts (×7,500). **b** Detail of an area near that of (**a**) in which some clefts contain CPPD crystals (×6,800). **c** CPPD crystal slightly displaced from its cleft and surrounded by apatite crystals (×30,600)

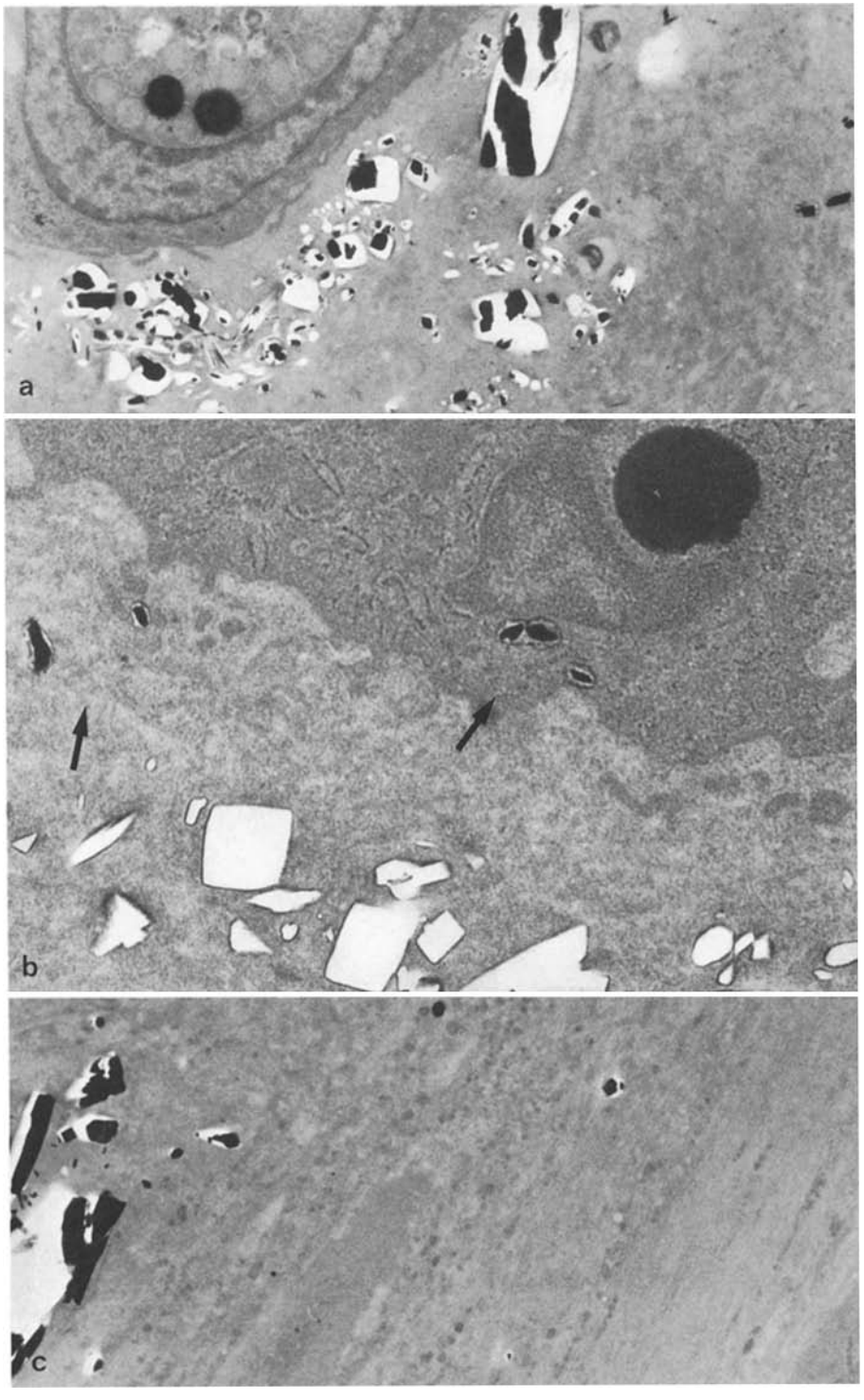

Fig. 5a–c. Electron micrographs of CPPD deposits in a meniscus. **a** Crystals (and/or fragments of crystals) and their clefts in the extracellular organic matrix near a cell. In the cytoplasm, two electron-dense masses presumed to be lipid droplets (case 1 – lead citrate staining – × 5,700). **b** Crystal clefts in the organic matrix near a cell whose cytoplasm contains an electron-dense mass presumed to be a lipid droplet. Small crystal particles (and their clefts) can be seen in extracellular matrix (*arrow*) as well as in cytoplasm (*arrow*) thus indicating phagocytosis (case 1 – lead citrate staining – × 15,500). **c** Crystals (and/or fragments of crystals) and their clefts in an extracellular organic matrix containing collagen fibrils and matrix vesicles (case 4 – unstained section – × 11,500)

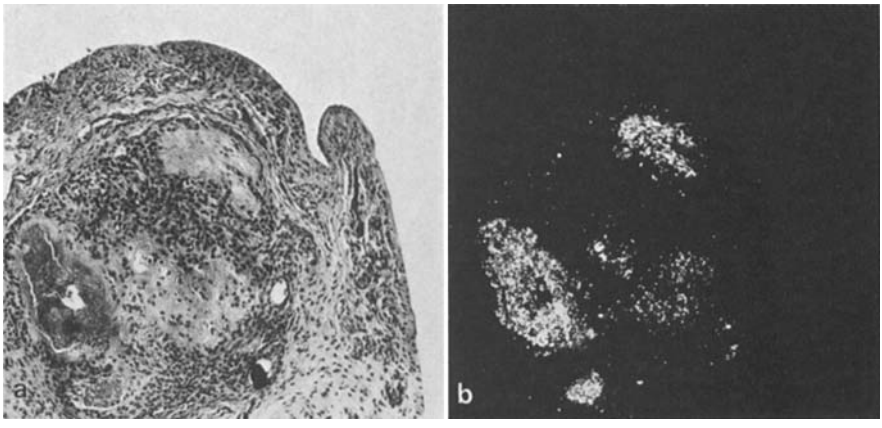

Fig. 6a, b. Topographical histological aspect of chondrocalcinosis in synovial membrane (case 1, ×70). Under a hyperplastic intima, histiocytic proliferation and necrotic areas containing most of the crystal deposits. **a** HE by ordinary light. **b** By polarized light, unstained section of the same region as in **(a)**

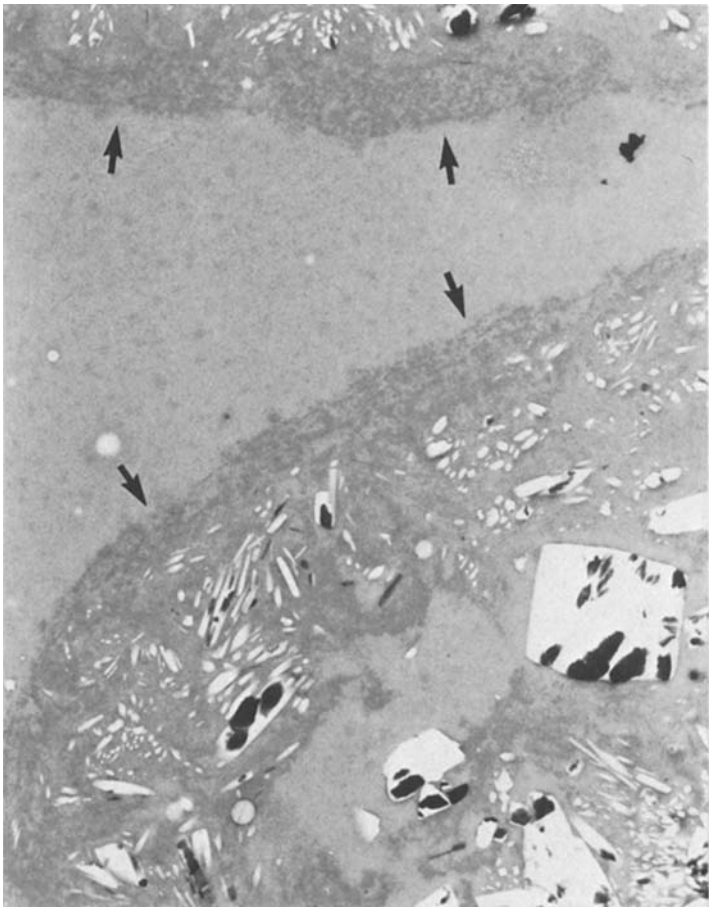

Fig. 7. Electron micrograph of CPPD deposits and their clefts in a synovial membrane whose surface is covered by a layer of exudative material (*arrows*) facing the articular cavity (case 5 – lead citrate staining – ×8,100)

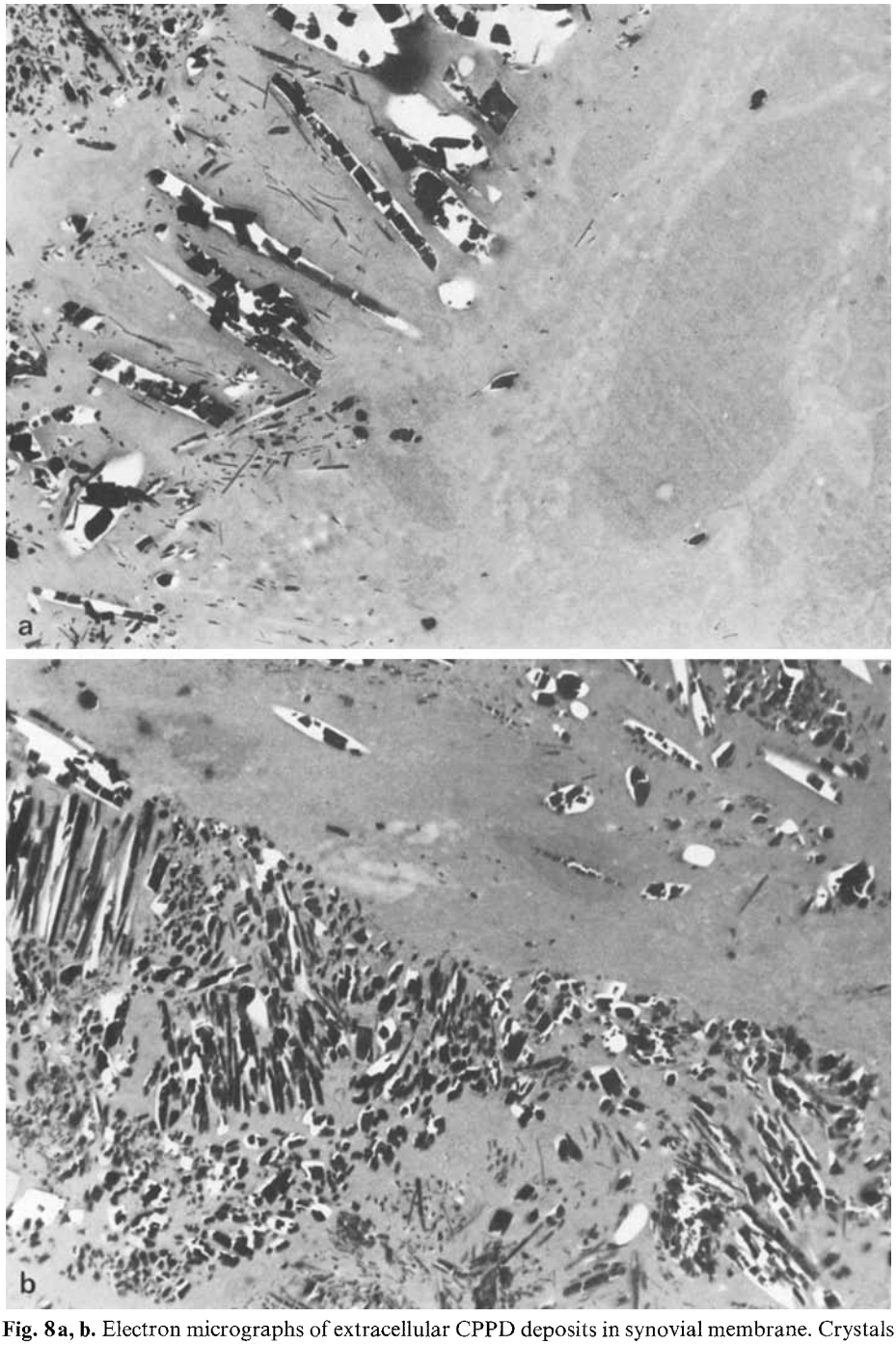

Fig. 8a, b. Electron micrographs of extracellular CPPD deposits in synovial membrane. Crystals of various sizes and shapes and their clefts (lead citrate staining). **a** Crystals near cells (case 3, ×4,700). **b** Crystals partly in parallel arrangement (case 1, ×2,500)

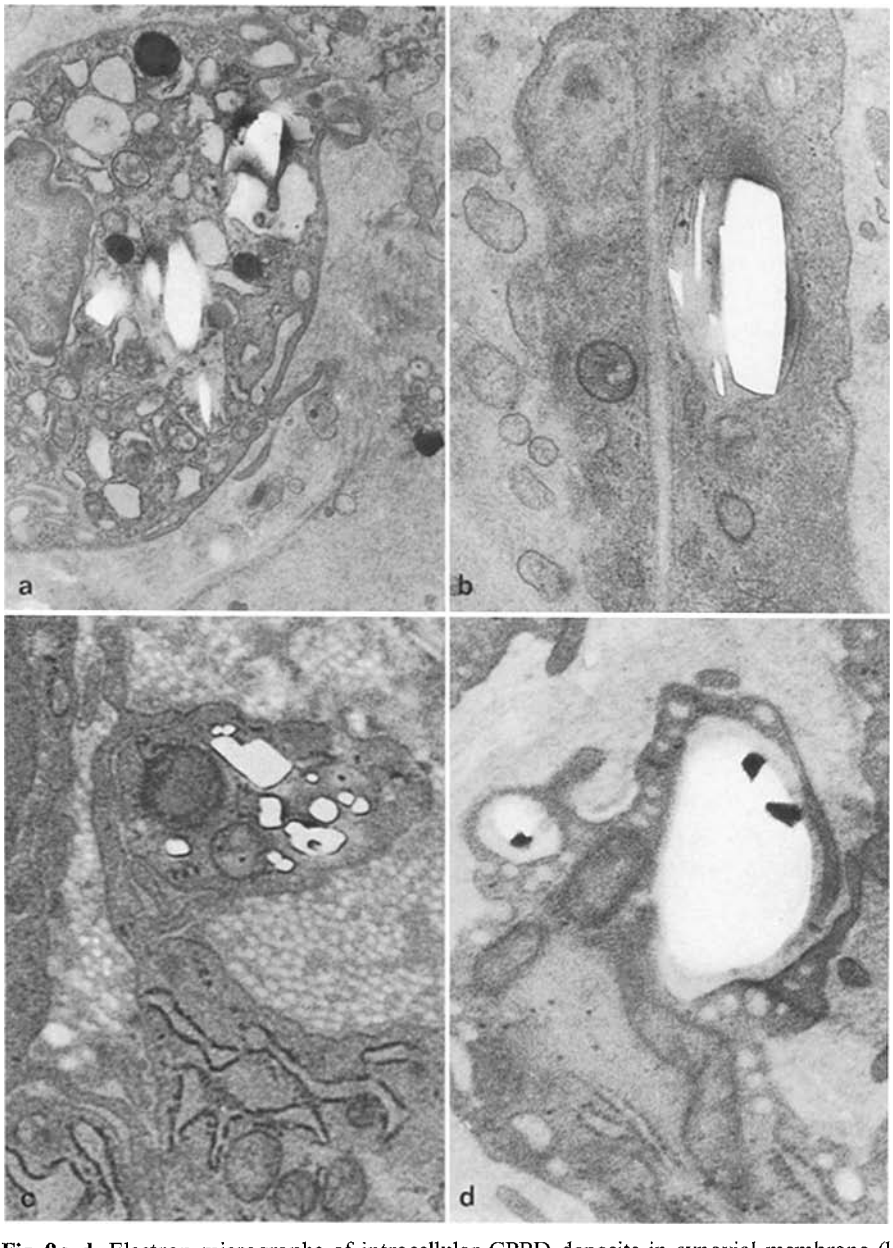

Fig. 9a–d. Electron micrographs of intracellular CPPD deposits in synovial membrane (lead citrate staining). **a** Crystal clefts in cytoplasm containing endoplasmic reticulum, mitochondria and electron-dense bodies presumed to be lipid droplets or lysosomes (case 1, ×10,600). **b** Intracytoplasmic crystal clefts within a phagocytic vacuole delimited by a membrane (case 1, ×24,300). **c** Crystal clefts in a fold of cytoplasm which elsewhere contains endoplasmic reticulum and mitochondria. Outside the cells, transversal sections of collagen fibrils (case 1, ×20,300). **d** Cleft in extracellular organic matrix containing debris of crystals and surrounded by two thin cytoplasmic folds (case 3, ×7,800)

in a matrix that appeared normal in other respects by light microscopy (Fig. 2b). The crystals were rod-shaped and sometimes orientated as star-like spherulites.

By electron microscopy they were seen to be juxtacellular in what appeared to be an otherwise normal matrix and their occurrence did not seem related to the matrix vesicles which can also be observed in normal cartilage. The crystals were never observed within the cells; they could be seen between the collagen fibrils but not attached to them. Although the neighbouring cells were generally normal in aspect (Fig. 3a, b), some necrotic cells were noted that did not seem to be related to the proximity of the deposits even in the superficial region (Fig. 3c).

Deeper in the radial zone, no crystals were observed, either by light or electron microscopy. In the calcified zone below the tide mark, however, some crystals were observed but only by electron microscopy. These were either partially or entirely surrounded by apatite crystals which are normal deposits in this area (Fig. 4).

Changes of menisci

Crystal deposits were observed mainly on the surface but small deeper clusters were also present; either within the fibrocartilage which appeared otherwise normal by light microscopy or in degenerated and remodelled regions.

As seen by electron microscopy, these crystals were of similar sizes to those seen in cartilage deposits. They were also extracellular appearing in an otherwise normal matrix and often near the cells (Fig. 5a, b). One cell was observed, however, whose cytoplasm contained small electron-dense particles that can be presumed to be phagocytized crystal fragments (Fig. 5b). In two cases an area with numerous collagen fibrils was also observed and contained numerous electron-dense matrix vesicles and some crystals or crystal fragments (Fig. 5c).

Changes of the synovial membrane

Synovial membrane presented signs of chronic inflammation and showed crystal deposits in a more or less advanced stage, either mingled with histiocytes and giant cells of an active reaction or in necrotic areas (Fig. 6).

By electron microscopy, an abundance of CPPD crystals was observed in extracellular matrix of the synovial membrane (Figs. 7, 8). Their shape was usually elongated and their length and thickness were very variable. They were frequently parallely orientated and were often found near the synovial cells. However, crystal particles and/or crystal clefts were also seen in the cytoplasm (Fig. 9a–c), in which case they were localized in vacuoles of which the membrane could be identified (Fig. 9b), indicating phagocytic activity (Fig. 9d).

Discussion

In the present study the observations made by electron microscopy confirm a previous impression given by light microscopy regarding articular chon-

drocalcinosis; the findings seem to indicate that crystals originate in the cartilage and fibrocartilage while phagocytosis appears to be an essential activity of the synovial membrane. These conclusions are analogous to those reported in the literature, in particular by McCarty (1979b).

I. Characteristics of the mineral deposits

In all of the cases studied, which had been chosen because of radiological and histological evidence of meniscal calcification of the "articular chondrocalcinosis" type, the knee mineral deposits consisted only of CPPD crystals (predominantly triclinic form but also monoclinic form). This observation was in agreement with personal experience involving crystallographic study of more than 50 cases of articular chondrocalcinosis (Lagier et al. 1966; Lagier 1981; Boivin et al. 1981b) as well as with numerous data reported in the literature (McCarty 1979a and b). The data obtained by polarized light microscopy were analogous.

For the purposes of transmission electron microscopy the criterion for classifying crystals as CPPD crystals was the similarity in appearance and size of the distinctive electron-dense images to those described as CPPD crystals in the literature (Reginato et al. 1974; Bjelle and Sundström 1975; Schumacher 1968 and 1976). It was not possible to identify them by electron diffraction, a technique which destroys the CPPD crystals. These crystals were embedded in the organic matrix, thus forming clefts which evoke the presence of crystals even after they have been dislodged. The variability in length, thickness and shape might be due in part to differences in orientation but also possibly to degrees in development and/or to deterioration of some crystals. While cutting by microtome seems to explain the transverse segmentation presented by the larger crystals, such an artefact cannot explain smaller crystals whose integrity is indicated by their presence in their own individual clefts. Some small rounded or cuboid images (especially in phagocytic vacuoles) are perhaps due to erosion of previously larger crystals; this hypothesis would need further investigations particularly in the light of the studies concerning in vivo CPPD solubility and clearance (McCarty et al. 1979a and b). Some small electron-dense images might presumably correspond to the "ultramicrocrystals" observed by Bjelle et al. (1980) in synovial fluid and which were not identifiable by light microscopy.

II. Relationship between CPPD crystals and joint tissues

a) On the cartilage, the mineral deposits appear essentially as interrupted areas on the surface (this interruption explains their occasional absence in biopsy material (Bjelle 1972)) and in clusters essentially seen in the tangen tial and intermediary zones. They are in the extracellular matrix but appear unconnected to the collagen fibrils or to matrix vesicles; these observations confirm those of the literature (Schumacher 1968; Reginato et al. 1974; Bjelle and Sundström 1975).

For various reasons we think that these modifications are not secondary

to a significant deterioration of the cartilage matrix; a) most of the CPPD deposits are not accompanied by cartilage degeneration and are observed in cartilage that is revealed to be otherwise normal by both macroscopic and conventional histological examination (Lagier and Ott 1969; Lagier 1981) – b) considered within the context of a wide range of pathological tissue calcifications, dystrophic calcification secondary to prior tissue deterioration is essentially composed of apatite crystals (Lagier et al. 1966; Lagier 1981; Boivin et al. 1981 b).

Favorable conditions for these deposits are possibly reflected in some staining modifications reported in the cartilage matrix (Bjelle 1972 and 1981 a, b). However, it seems likely that the cells play a still undefined role in the formation of CPPD since the crystals appear essentially in their vicinity. It is interesting to note that apatite crystal deposits which make up the normal mineral substance of cartilage calcified zone also seem to be related to the proximity of the cells.

The greater density of deposits on the surface as compared with the underlying zones can not be explained by migration of these deposits since there is no renewal of cartilage tissue analogous to that of stratified epithelium; mitoses can not be found in normal adult cartilage (Stockwell and Meachim 1973). It seems possible that these topographical characteristics – and the absence of crystals deeper in the radial zone – can be explained by gradients of impregnation of the cartilage by nutrients diffusing from the synovial fluid which is related to a well-known physiological process (Maroudas 1968; McKibbin 1973; Mankin and Radin 1979). The presence of CPPD crystals in the calcified zone (Boivin et al. 1981 a) might possibly be due to its impregnation by substances coming from the subchondral vessels, although this route of nutrition is still under discussion. In fact, inorganic pyrophosphate has been found in crystal-deprived synovial fluid of patients with chondrocalcinosis (Russell et al. 1970).

Thus it seems possible that CPPD crystal deposits are – in certain subjects with a particular terrain (Nuki et al. 1978; Lust et al. 1981) and depending on local nutritional conditions in the cartilage – the result of a process similar to that which has been produced in vitro. Combination of inorganic pyrophosphate and $CaCl_2$ in solution gives rise to calcium pyrophosphate in tri- and monoclinic crystals if the incubation period is sufficiently long (Hearn and Russell 1980). Combination of sodium pyrophosphate and $CaCl_2$ in gels lead to CPPD crystals in tri- and monoclinic forms (Pritzker et al. 1978).

b) The same morphological characteristics were observed in the meniscus as in the articular cartilage and the same hypotheses as to their origin are suggested.

However, areas rich in matrix vesicles were observed that were neither directly associated with apatite crystals, as they usually are (Ali 1977) nor with CPPD crystals. One can presume that these vesicles play no role in the formation of CPPD since the crystals observed elsewhere in meniscus and in articular cartilage were not near matrix vesicles. Further research

would be needed to determine the nature of the cuboid deposits observed in the vicinity of vesicles. Are they similar to unidentified cuboid crystals described by Ali and Griffiths (1981) in "arthritic articular cartilage"? Are they the result of CPPD crystal erosion? Do numerous vesicles reflect an evolution toward a state of secondary tissue deterioration in meniscus as well as – possibly – in articular cartilage? Such an evolution seems suggested by the presence of crystal particles phagocytosis, which was not observed in articular cartilage cells. Further studies are needed to define the relationship between the changes seen by electron microscopy and those seen in "degenerated" meniscus by light microscopy.

c) In the synovial membrane, crystal phagocytosis is an important phenomenon whose existence was strongly suggested by light microscopy and demonstrated by electron microscopy as already reported in other papers (Schumacher 1968 and 1976; Kariya et al. 1970). This explains the well known synovitis of articular chondrocalcinosis (McCarty et al. 1966).

Most of the crystals, however, are extracellular, often grouped more or less parallel and sometimes in spherolites. Further studies are needed to establish the origin of these extracellular crystals, to determine whether they come directly from the cartilage and menisci propelled by intraarticular currents and lymphatic drainage, whether some originate in the synovium itself or were released from necrosed phagocytic cells. The latter hypothesis, however, can be excluded a priori in certain cases, in view of the difference of size between the larger extracellular crystals and the smaller intracellular crystals. Further studies are also needed to compare these synovial changes with CPPD clearance as investigated by McCarty et al. (1979a and b).

III. Relationship between chondrocalcinosis and osteoarthritis

In order to obtain articular tissue fixed under the conditions necessary for electron microscopy, we chose, for the present study, joints requiring surgery, i.e., 5 cases of knee osteoarthritis, 4 of which concerned aged patients with a clinical picture evoking more or less generalized osteoarthritis. It is of interest to examine the results of this study in the light of possible information they could provide regarding the relationship between chondrocalcinosis and osteoarthritis.

Indeed, although most anatomico-pathological observations of CPPD crystal deposits are made in joints which appear otherwise normal (Lagier 1981), osteoarthritis and articular chondrocalcinosis are rather frequently observed together in the same patient, usually in cases of generalized osteoarthritis. Entire cartilage destruction in such affected joints can explain the possible mixture in synovial fluid of both CPPD crystals and hydroxyapatite crystals without bony structure (Dieppe 1978). These clinical observations are to be compared with data showing organic pyrophosphate release from osteoarthritis cartilage incubate (Howell et al. 1976).

Our observations were made on cartilage and meniscus tissue samples not affected locally by the joint deterioration or remodelling (as determined

by macroscopy and light microscopy). Only the synovium showed active remodelling that could be attributed to both chondrocalcinosis and osteoarthritis. The results of the present study were not significantly different from those reported in the literature for electron microscopy studies made on chondrocalcinosis joints whom only some of which were also affected with osteoarthritis. They suggest the 3 following remarks.

a) In a joint affected by osteoarthritis and chondrocalcinosis, the undeteriorated part of the cartilage shows no obvious ultrastructural difference from analogous tissues of a joint affected with chondrocalcinosis alone.

b) Although the crystal deposits seem to induce erosion of the organic matrix and the fibers of the cartilage or the meniscus, electron microscopy showed that they were not directly responsible for necrosis of the neighbouring cells.

c) Electron microscopy demonstrates crystal phagocytosis in synovial membrane, which explains the clinical signs of inflammation and might possibly play a partial role in the histogenesis of osteoarthrotic remodelling.

It would seem therefore that electron microscopy can make only a partial contribution – although admittedly sometimes an invaluable one – to an understanding of the relationship between chondrocalcinosis and osteoarthritis. The use of these elements is only valid, however, if it is the complement of a rigorous morphological study by both macroscopy and light microscopy.

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