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Ultrastructural Aspects of Chondrodystrophia Calcificans Congenita (Syndrome of Conradi-Hünermann)

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Summary. An ultrastructural study of chondrodystrophia calcificans congenita is reported. Foci of initial calcification of cartilage are characterized by coexistence of three different types of crystals, probably due to abnormal proteoglycan composition of cartilage matrix. The calcification process in chondrodystrophia calcificans congenita is apparently not related to 'matrix vesicles' as it is in normal cartilage.

Key words: Chondrodystrophia calcificans congenita – Cartilage calcification – Ultrastructure of cartilage.

Chondrodystrophia calcificans congenita (C.C.C.) (Hünermann, 1931) is a rare bone disease observed for the first time in 1914 by Conradi. Its main trait is the appearance of numerous small foci of calcification in cartilaginous bones early in life. There are more than one hundred case reports in the literature (Spranger et al., 1970; Zieger and Conter, 1973). The main features are a rhizomelic micromelia in association with facial-bone anomalies causing hypertelorism, saddle nose, macro- or microcephalia, wide fontanelles, ogival palate, micrognathia, etc. Rib agenesis, syndactylism, congenital dislocation of the hip, club foot, spina bifida and scoliosis have also been associated with C.C.C.

In X-rays an irregular calcification is evident, mainly in carpal and tarsal bones, with involvement of epiphyses of long and possibly flat bones. Typically their radiopacity is nor uniform, but shows little spots giving the picture of stippled epiphyses. Occasional spotty calcification of joint cartilages and paraarticular tissues and even laringeal cartilages has been recorded (Caffey, 1973).

Combined extraskeletal lesions involve eye (bilateral cataract, optical-nerve atrophy), skin (ichthyosis, dyskeratosis), heart (congenital malformations), urinary tract (congenital anomalies), and nervous system (mental retardation, muscular spasms).

Given the wide clinical variability of the disease, Jeune et al. (1963) identify three forms of C.C.C.:

1. A major form: diffuse skeletal lesions with severe micromelia, together with ocular, cutaneous and cardiovascular anomalies leading to precocious death from congenital debility or infection.

2. An intermediate form: diffuse skeletal involvement with mild micromelia, irrelevant extraskeletal anomalies and a better prognosis.

3. A minor form: skeletal lesions limited to the hands and feet, consistent with good health.

No specific biochemical finding has been reported to be associated with or characteristic of C.C.C., so that the diagnosis is grounded on clinical and radiological data.

Case Report

O.A., 14d. baby, born to unrelated parents at term of the first, uneventful pregnancy. The father (31 y) is in good health, the mother (33y) has had no previous miscarriage, and did not ingest any drug during this pregnancy. Weight at birth was 3200 gr. From the first day of life the baby was cyanotic and he was promptly taken to the Bambino Gesù Pediatric Hospital in Rome.

On physical examination the baby appeared to be a rhizomelic dwarf, in very serious condition, with cyanosis of the face and legs. The skin looked thick, hair and eyebrows were sparse and dry. Hypertelorism without eye abnormalities, saddle nose, macroglossia, ogival palate vault and short neck were found. There were no apparent abnormalities of the shoulder girdle, ribs, or spine. Anthropometric data: body lenght 44.5 cm; head circumference 36 cm.; bregmatic fontanelle 4×4 cm.; occipital fontanelle 2.5×3 cm.; parietal sutures still open; upper limbs: arm 5 cm., forearm 6.2 cm.; lower limbs: thigh 7 cm., leg 9 cm. There was severe limitation of active and passive limb movements because of strong muscle hypertonia and spasm. C.N.S. examination revealed feeble crying and weak osteotendineous reflexes. No abnormalities were found in the lungs, heart, or abdomen.

All routine analyses were normal; there was no atypical aminoaciduria. Mucopolysaccharides were not found in the urine. Kariotype: 46 xy. There were no abnormal findings on ophthalmological examination.

X-Ray Examination. Skull of normal shape and size, with regular ossification of all bones. Metopic and bregmatic fontanelles wide. Mild hypertelorism. On lateral view, macroglossia and small calcifications of laryngeal cartilages were evident.

Along the spine, including the sacrum (Fig. 1) numerous small spot-like calcifications were evident in spinous and transverse apophyses. The upper limbs were short and stumpy, in particular the humerus.

Shortening of lower limbs involved both femora and tibiae. By maintaining their normal length, the fibulae extended upwards beyond the tibiae, thus resembling an achondroplastic pattern. Small calcifications of paraosteal tissues were evident at the level of lower humeral and upper femoral metaphyses.

The lower femoral and upper tibial metaphyses appeared enlarged and shortened. Several calcified nodules were also evident in the lower epiphyses of the right tibia. In both hands small calcifications were visible in carpal bones (right and left capitate and left hamate). Spot-like calcifications were evident in the proximal epiphyses of metacarpal bones which in turn appeared shortened. There was hypoplasia of the terminal phalanges. X-ray of the feet showed numerous small calcified foci in the talus, calcaneum, and cuboid and cuneiform bones.

Behaviour. On the ninth day a sudden rise of temperature and dyspnoea were accompanied by signs of chest infection. In spite of prompt treatment the baby died on June 6, 1975.

Autopsy Findings. (N°. 74–75 on 6.9.75) Rhizomelic dwarfism in male newborn with prominent saddle nose (body length 44.5 cm.). Bilateral bronchopneumonia. Urolithiasis with bilateral hydronephrosis and suppurative pyelonephritis. Bilateral cryptorchidism. Organ weights in gr.: thymus 2, heart 20, right lung 42, left lung 28, liver 120, spleen 8, adrenals 6.

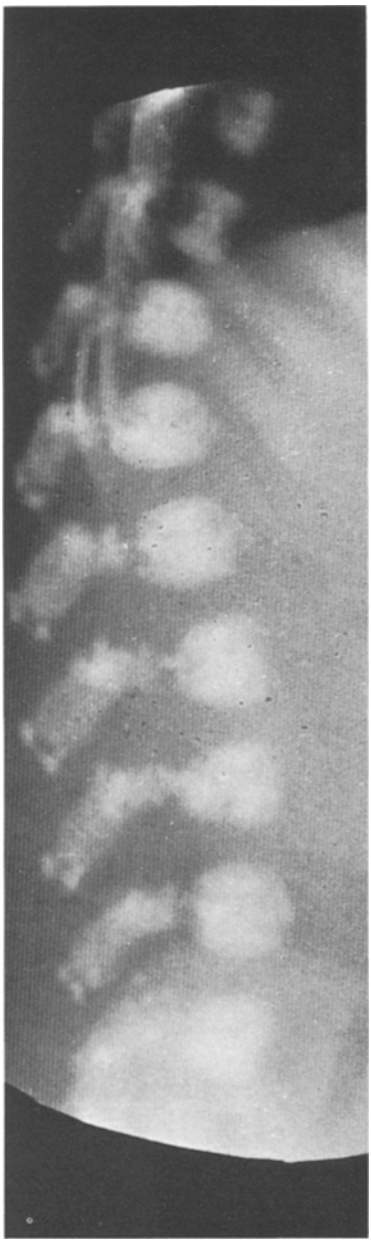

Fig. 1. Chondrodystrophia calcificans congenita. Lateral view of lumbosacral tract of the spine. Small calcification foci in transverse and spinous apophyses

Results

Histological examination was carried out on various organs including the skeleton (vertebrae, tarsal bones, and upper femoral epiphyses). In both lungs bronchopneumonic foci were found, hemorrhagic in type. Suppurative pyelonephritis

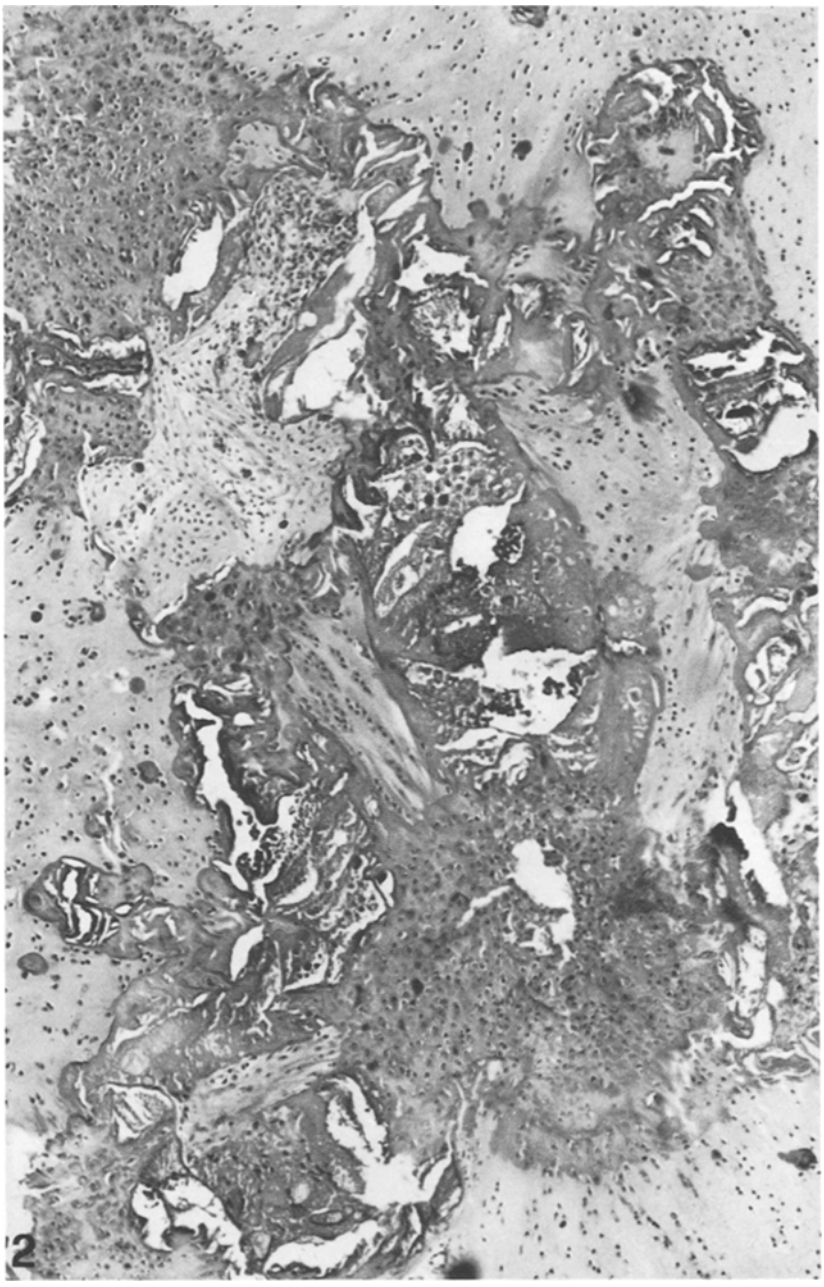

Fig. 2. Chondrodystrophia calcificans congenita. First cuneiform bone. Area of calcified cartilage showing pseudocystic spaces. H.E. × 80

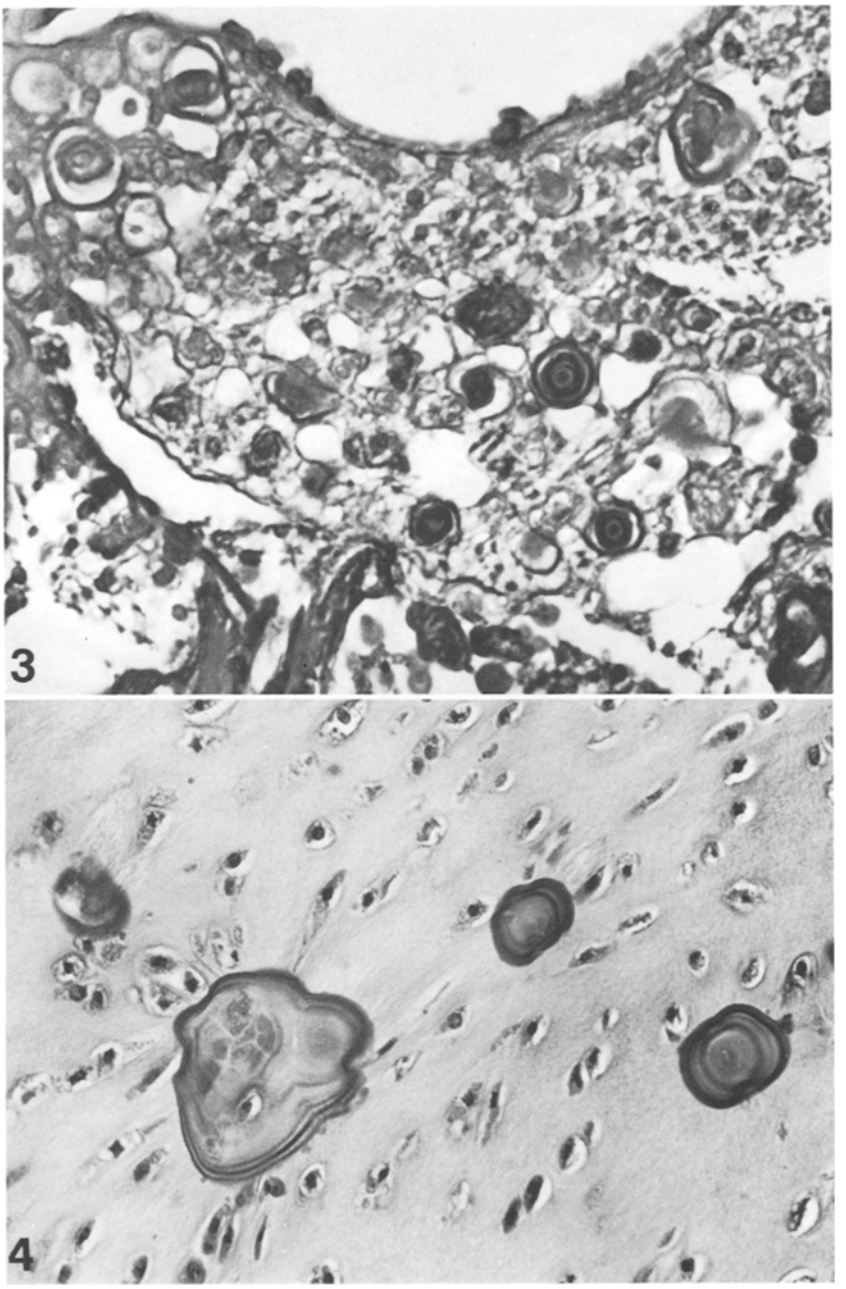

Fig. 3. Detail of Figure 2 at higher mag. Concentrically stratified calcospherites in calcified areas. H.E. × 500

Fig. 4. Chondrodystrophia calcificans congenita. Cartilage with initial deposition of calcium salts giving the appearance of concentric rings. Inside lacunae are evident chondrocytes with pyknotic nuclei. H.E. × 500

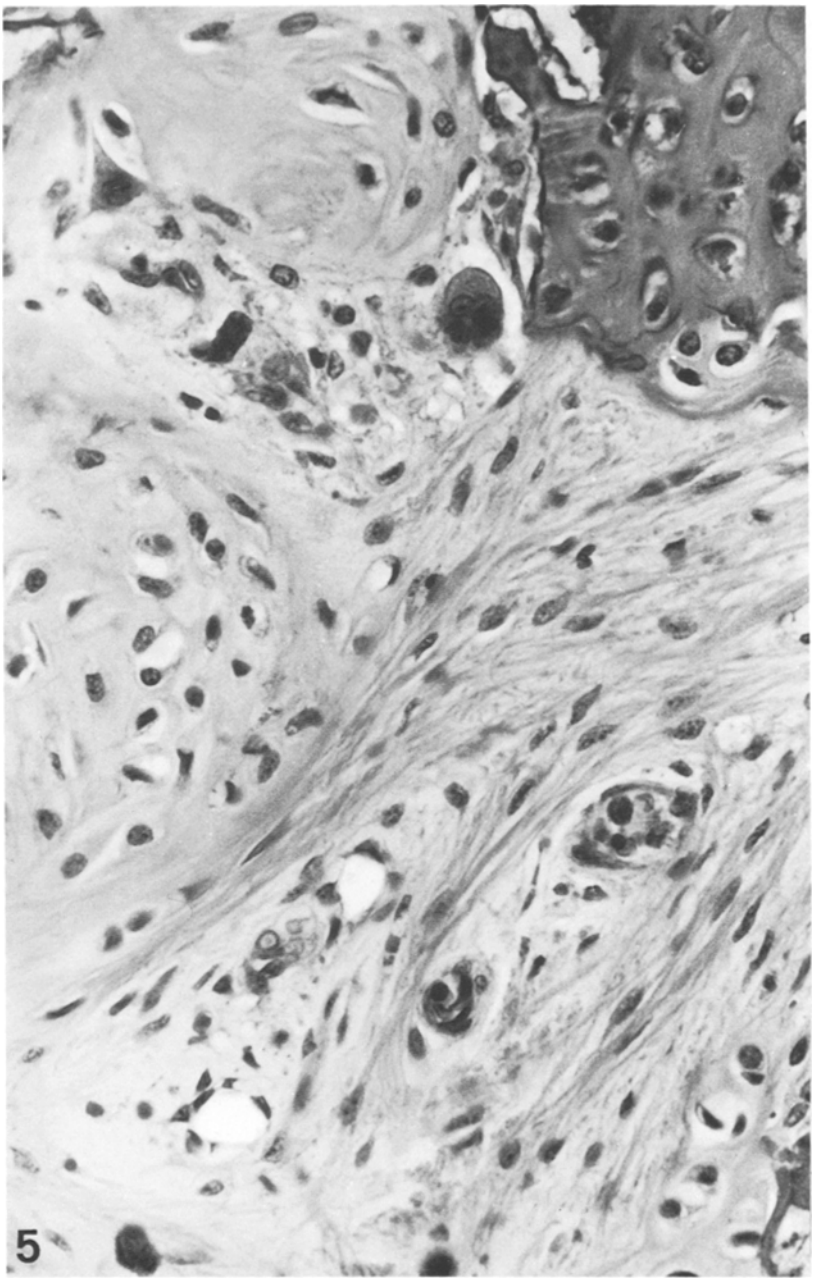

Fig. 5. Chondrodystrophia calcificans congenita. Fibrous aspect of calcified area. A giant multinucleated cell is present near the right upper corner. H.E. × 500

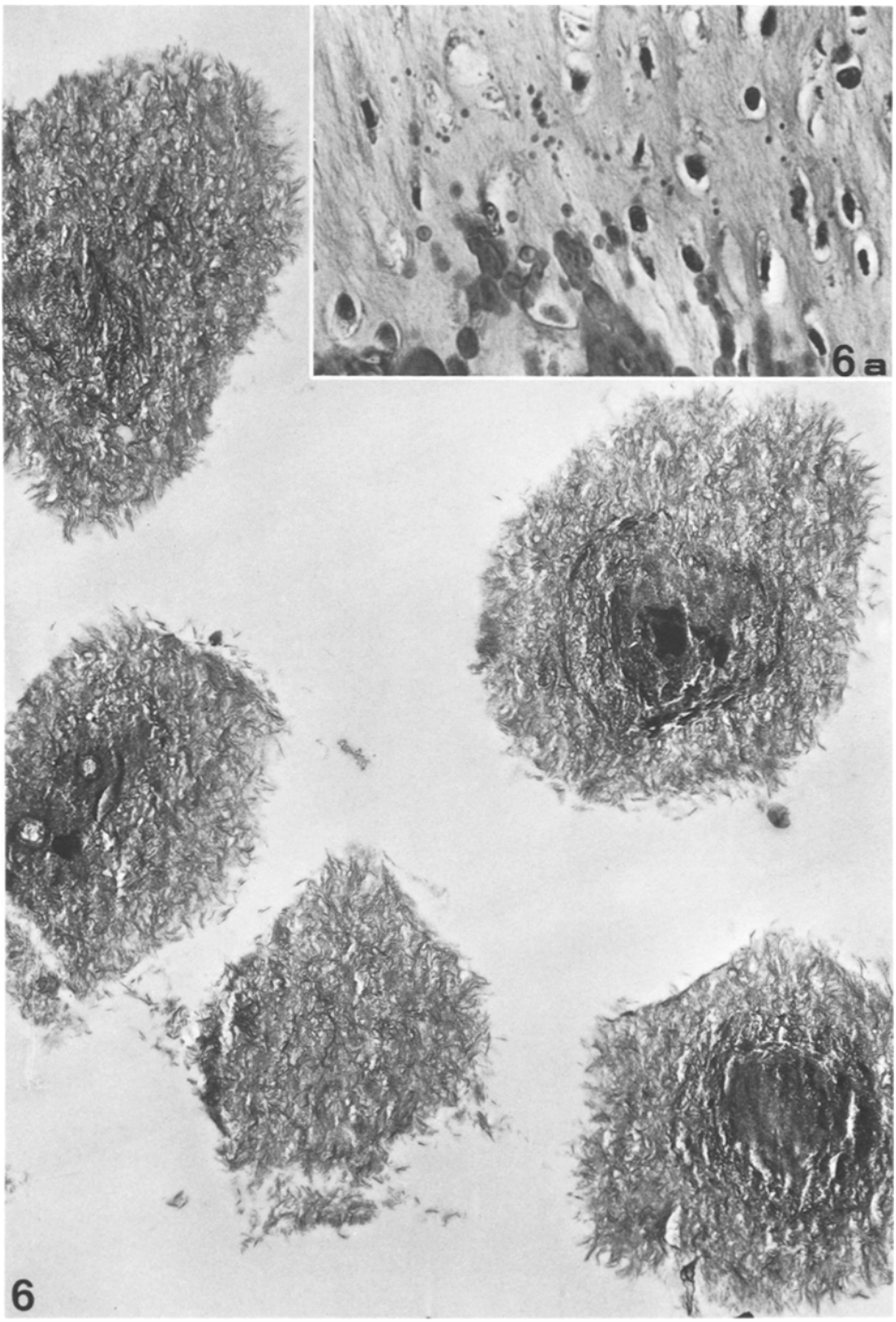

Fig. 6. Chondrodystrophia calcificans congenita. Electron micrograph showing areas of focal calcification in cartilage. Unstained. ×15,000. **a** Same finding as shown in light microscopy. H.E. ×500

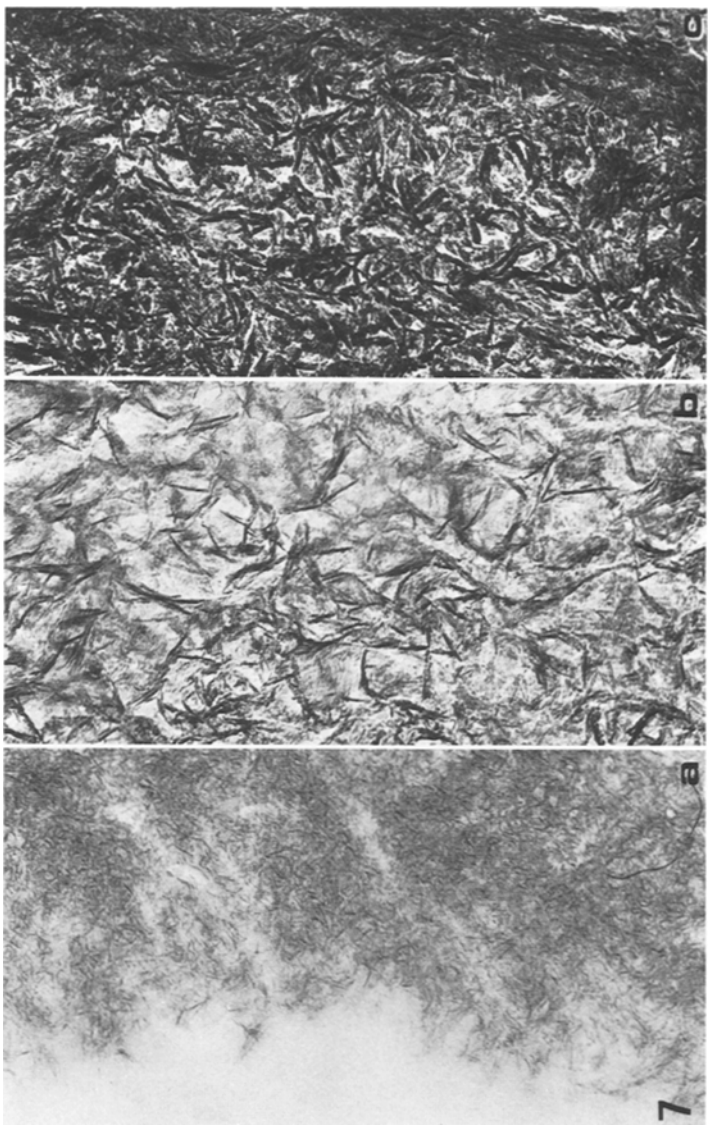

Fig. 7a–c. Chondrodystrophia calcificans congenita. Electron micrograph showing the different crystalline patterns in focal areas of cartilage calcification. In **a**, the crystals are thin, apparently short, and closely collected together; in **b**, the crystals are needleshaped and loosely arranged; in **c**, the crystals are thick and closely packed. Unstained ×60,000

was seen in both kidneys. There were no significant lesions in liver, skin, or adrenals. The thyroid and parathyroids retained their foetal histological pattern.

In the cartilaginous parts of tarsal and metatarsal bones there existed multiple widespread foci where the cartilaginous matrix was rarefied and partially calcified (Fig. 2). In these areas pseudocystic spaces were seen containing concentrically stratified calcospherites (Fig. 3). In areas of scanty calcification chondrocytes seemed shrunken with pyknotic nuclei (Fig. 4). Beyond the limits of the calcified areas small round dots of calcification appeared scattered or radially oriented (Fig. 6a). Occasionally such cartilaginous calcified areas were adjacent

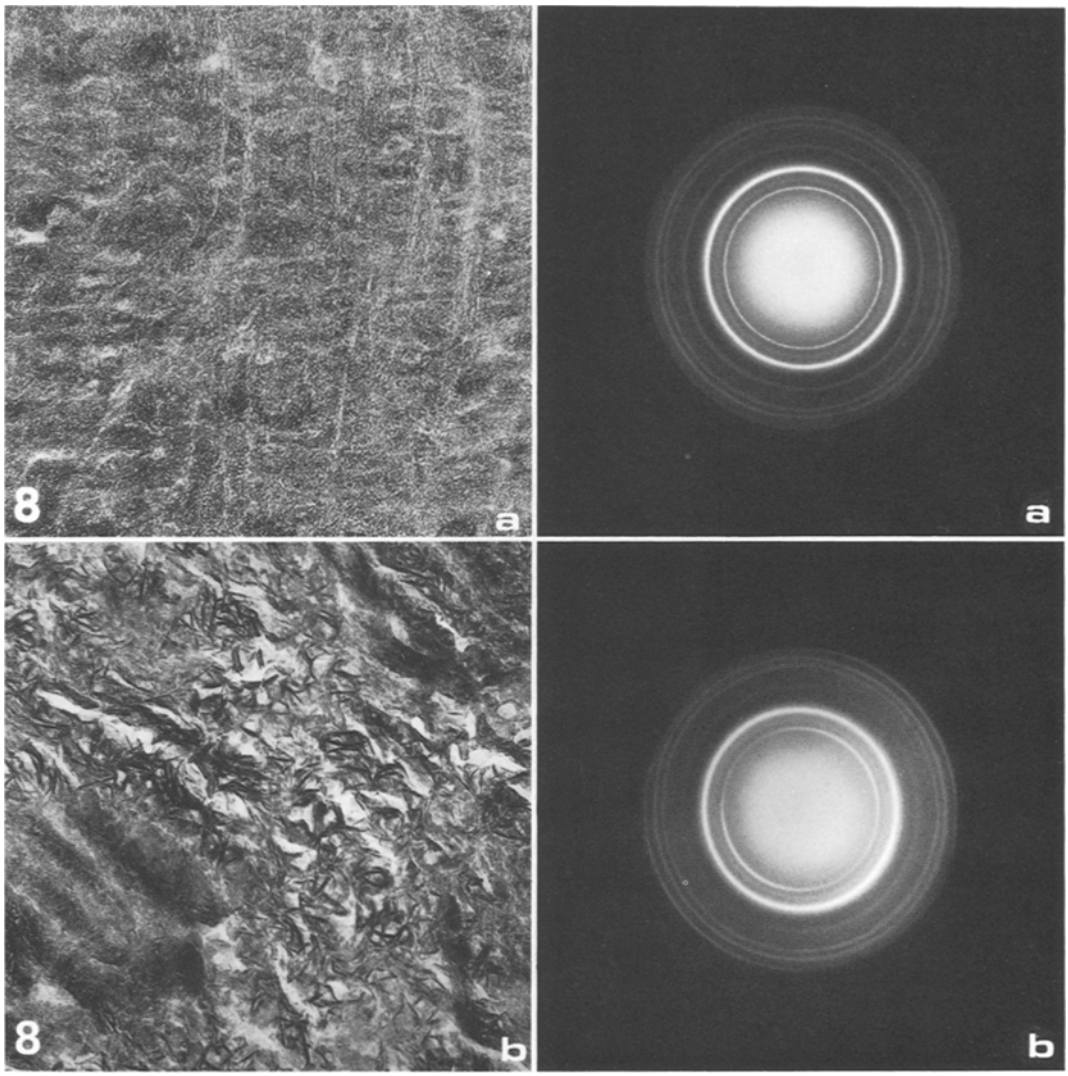

Fig. 8. Chondrodystrophia calcificans congenita. Electron microdiffractograms of focal areas of calcification containing (**a**) very thin crystals like those shown in Fig. 7a and (**b**) thick crystals like those shown in Fig. 7b. No changes of the diffraction rings are visible. × 35,000

to cellular connective tissue zones containing multinucleated osteoclastic-like giant cells (Fig. 5). These may have represented ossification centers of tarsal bones. There were no abnormal findings in laryngeal cartilages. The proliferating cartilage of the proximal femoral epiphysis appeared hypoplastic.

To examine the abnormal calcification of cartilage, small specimens were taken from tarsal bones for electronmicroscope investigation. They were fixed in formaldehyde, postfixed in osmium tetroxide and embedded in Araldite. Semithin sections (about 1 μ thick) were stained with Azure II and Methylene

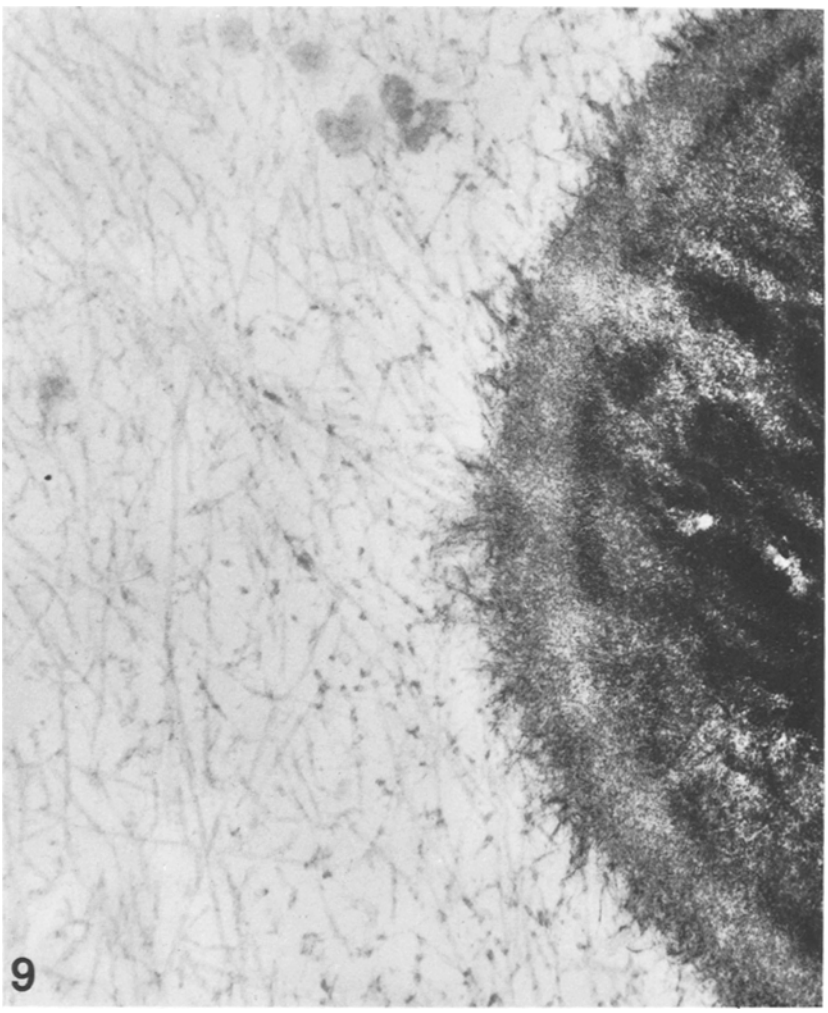

Fig. 9. Detail of a focal area of cartilage calcification in a section stained with uranyl acetate and lead citrate. × 56,000

blue, and with von Kossa method for calcium phosphate. Ultrathin sections (about 700 Å thick) were examined both unstained and after staining with uranyl acetate and lead citrate.

In selected areas, electron diffraction of calcified zones was carried out, using untreated sections and operating at 80 kV and at a magnification of 11,000 in a Siemens Elmiskop 1A electron microscope equipped with a field-limiting diaphragm of 50 μm.

Findings similar to those observed in sections from paraffin-embedded blocks were found in semithin sections. In particular, areas of calcification of variable size were scattered through the cartilage matrix. Some of them were in close relationship with chondrocytes, but the majority were not related to cells.

In unstained ultrathin sections, the calcified areas appeared to be of variable size. The smallest of them (Fig. 6) consisted of roundish aggregates of needle-shaped, intrinsically electron-dense crystalline structures. Characteristically, the central zones of these aggregates were more compact and electron-dense than the peripheral zones (Figs. 6, 9). The widest calcified areas had an irregular shape, but they too showed zones of variable density.

In both small and large calcified areas, different types of crystals were recognizable. In some areas, usually in those at the periphery of the calcified zones, the crystals were very thin (about 15 Å thick) and appeared as filament-like structures rather than as true crystals (Fig. 7a). Most of them were irregularly oriented; a few were collected in elongated bundles looking like calcified collagen fibrils. In other areas, the crystals were both needle- and filament-like and had a thickness of about 35 Å (Fig. 7b). In still other areas, usually in those forming the central part of the calcified zones, the crystals appeared needle-like and had a thickness of about 60 Å (Fig. 7c). They were irregularly oriented and often formed a network-like structure whose meshes contained an amorphous, intrinsically electron-dense material. The electron diffractograms obtained from these three types of crystalline structures did not differ as far as the number and diameter of the diffraction rings were concerned (Fig. 8). However, these rings were sharper and more intense in the diffractograms obtained from central parts of the calcified zones, where the thickest crystals were placed, than in those obtained from the zones where crystals of intermediate thickness were present, and from the superficial zones where the thinnest crystals were found.

Staining with uranium and lead showed that most of the calcified areas were in relationship to thin (about 250 Å thick) collagen fibrils (Fig. 9). In a few cases, however, the inorganic substance was in contact with, and was also placed over, thick (about 1800 Å), irregularly oriented fibrils which sometimes formed areas of asbestos-like degeneration. Often, crystals protruded from the edge of the calcified matrix towards the uncalcified part (Figs. 6, 7, 9).

A few electron-dense, roundish bodies were sometimes present in the matrix surrounding the calcified areas (Fig. 9).

Discussion

The clinical, radiological, and histological findings show that the patient was affected by chondrodystrophia calcificans congenita (syndrome of Conradi-Hünermann). For the first time, the focal calcified areas irregularly scattered through the cartilage matrix which are typical of this condition have been studies with the electron microscope.

The most interesting ultrastructural finding was the coexistence of different types of crystals in the same calcified areas. The central zones of these areas were more electron-dense than were the peripheral parts. Moreover, they frequently contained crystals thicker than those of the intermediate zones which, in turn, had crystals thicker than those of the superficial zones. In spite of these differences, all these crystals gave electron diffractograms of the same

type, although the diffraction rings were sharper and better defined where the crystals were thicker. On the basis of these findings alone, it is difficult to explain how these different crystalline structures are formed. Since electron diffractograms show that the various types of crystals have the same chemical structure, it is suggested that the crystals of the central zone may be thicker than those at the surface because they have time for incremental growth.

On the other hand, it is possible that the organic matrix of the central zones has a chemical composition and, consequently, nucleating properties different from those of the matrix of the superficial areas. In this respect, it is worth recalling that, on the basis of histochemical findings, it has been suggested that the formation of focal calcification in chondrodystrophia calcificans congenita is due to abnormal proteoglycan composition of the cartilage matrix (Tasker et al., 1971; Zieger and Conter, 1973).

Bearing in mind the fundamental role glycoproteins and acid proteoglycans play in calcification (Bowness, 1968; Kobayashi, 1971; Irving, 1973), this suggestion seems tenable. In this regard, our investigation does not furnish any additional evidence because we have not been able to study the matrix of the central core of the calcified areas. On the other hand, while the uncalcified matrix did not look abnormal under the electron microscope, areas of fibrillation looking like those of "asbestos" degeneration were sometimes found and these were often undergoing calcification. Although these areas were too few to explain the formation of all of the focal areas of calcification found in the present case, they are not without significance especially if it is considered that asbestos degeneration occurs in aging cartilage (Amprino and Bairati, 1933; Dearden et al., 1974; Bonucci, 1975) and our case is a neonate.

Besides asbestos degeneration, the cartilage showed areas of cysti-like modification, some of which were more or less completely calcified. These findings suggest that, as previously reported (Zieger and Conter, 1973) regressive changes in the catilage matrix are responsible for the development of focal calcification.

It would be interesting to ascertain the role the cells may have in this process; unfortunately, in our autopsy material, autolytic processes had modified the chondrocyte structure to such an extent that the cells could not be studied under the electron microscope to any advantage.

Small electron-dense, roundish bodies were sometimes present near the calcified areas. These bodies, which were easily recognizable as "matrix vesicles" (Anderson, 1967, 1969; Bonucci, 1967, 1970, 1971) were not so numerous as they are in normal calcifying cartilage. None of them was calcified or calcifying, and we have not been able to establish whether the initiation of the calcification process in chondrodystrophia calcificans congenita is related to them as it is in normal cartilage and bone (Bonucci, 1975).

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