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Achondrogenesis: Report on a Case, with Particular Reference to Ultrastructure and Histochemistry

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Summary. This paper gives the main findings related to the case of a female newborn with achondrogenesis. The most important abnormalities affected the hyaline cartilage. This was characterized by absence of seriated chondrocytes, low levels of glycoproteins and acid glycosaminoglycans, and exceedingly low amounts of collagen fibrils which, in any case, had an abnormal structure. Almost no endochondral ossification was observed, and abortive attempts at calcification were only occasionally found in the cytoplasm of degenerated chondrocytes. Membranous and periosteal ossification, on the other hand, were normal. The ossified areas were separated from the uncalcified cartilage by the interposition of a connective, periosteum-like strip. The only ossification in the vertebral bodies occurred along thin invaginations of the perichondrium; intervertebral disks were not developed.

These data suggest that achondrogenesis is a skeletal disorder essentially derived from a disturbed development of hyaline cartilage, and involving the persistence of features typical of primitive mesenchymal tissue.

Key words: Achondrogenesis — Ossification — Cartilage Pathology — Skeletal Disorders.

Achondrogenesis was first described as long ago as 1936 by Parenti, but its rarity is such that its pathology is still not completely understood. As stressed recently by McKusick (1972), the confusion often felt about the disease is mainly due to the possibility of finding forms of the disorder which differ considerably in severity and extent of skeletal involvement. The confusion felt is aggravated by the fact that only a few of the investigations on achondrogenesis have been accompanied by a complete pathological study. Very few histological and histochemical investigations are, in fact, available (Remagen *et al.*, 1971), and no ultrastructural findings are reported in the literature.

We have recently had the opportunity of studying a newborn whose death was due to a skeletal disorder consistent with the diagnosis of achondrogenesis (type I of McKusick). The present paper reports the main clinical, radiological and pathological data pertinent to this study, and gives special emphasis to the histochemical and ultrastructural findings.

Case Report

Female newborn, 1st child of a healthy woman of 26. Normal delivery after a 32-week, uncomplicated pregnancy. The mother was Rh-negative, but was not isoimmunized; the 28-year-old father was healthy. Both had normal karyogram. Their pedigree pattern was unimportant and there was no history of consanguineity. Four months after delivery, the mother had a new pregnancy which ended in abortion 21 weeks after the beginning of ame-

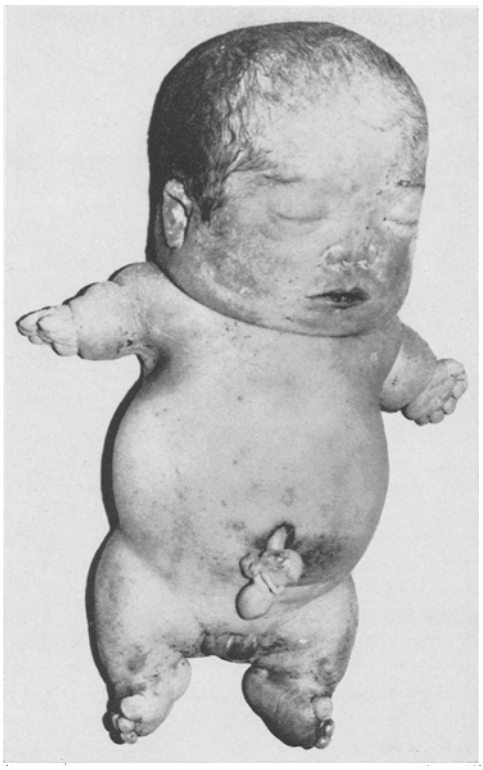


Fig. 1. Front view, showing short, deformed limbs, large head, abnormally short torax, and protruding abdomen

norrhea. The fetus was soaked, but it was possible to establish that the histological characteristics of his skeleton were normal.

Physical Examination. The baby had severe micromelia and only gasping respirations. She died 15 minutes after delivery. Her body weight was 1080 g and her height 24 cm. Severe skeletal deformities were evident (Fig. 1). The head was disproportionately large, with a circumference of 28 cm. The neck was short, so that the head seemed to rest directly on the shoulders. The abdomen protruded and was almost spherical. The extremities were extremely short; the arms measured 5 cm from shoulders to finger-tips and the legs 7 cm from hip to toe. Fingers and toes were both shorter than normal and no phalanx subdivision was evident in them.

Post-mortem x-ray examination (Fig. 2) showed a relatively large, normally ossified skull with normal relationship between the calvarium and the basal and facial bones. The calvarium was, however, thin, especially at the level of the parietals. Very small ossification centers were visible in the vertebral bodies. They appeared as dots in frontal projection and as thin lines in lateral projection. The vertebral arches and spines were more evident than the bodies, but the posterior portion of the arches was uncalcified. The ribs were thin and short; they were also swollen and irregular at the costo-chondral junctions. The clavicles were normal, while the scapulae were small and irregular. An irregular zone of ossification was present in the ilia; its lower border was arched.

Another ossified area, probably belonging to the pubic bones, was hardly visible on both sides. The tubular bones of the arms were visible as short and very irregular shaft-segments in which the normal shapes of the humerus, radius and ulna were not recognizable. Only two small centers of ossification, probably belonging to the carpus, were visible in the hands.

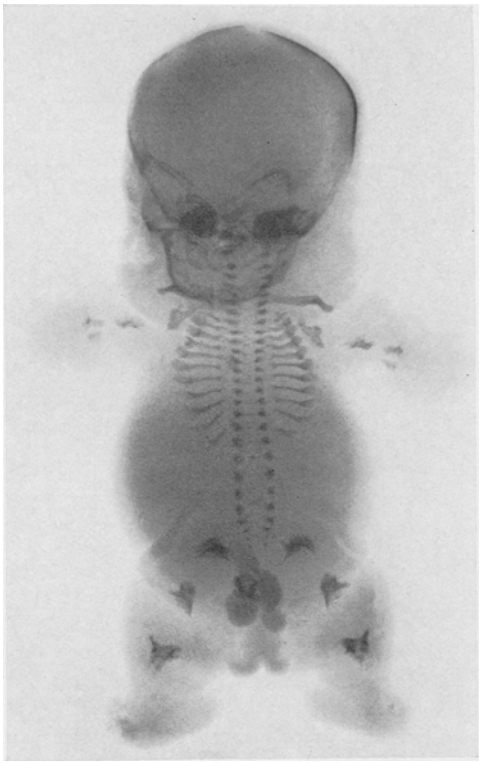


Fig. 2. Front roentgenograms. Note almost complete absence of calcification in vertebral bodies

The femurs and the tibiae appeared as irregular bone segments, while the fibula and the ossification centers of the feet were not recognizable.

Post-mortem Examination (autopsy n° 44353). Only major necroscopic features are reported. Lung, heart, liver and spleen were congested. The lungs were atelectatic. A few haemorrhagic petechiae were visible in the pleurae. The brain was congested and oedematous. The calvarium and the base of the skull did not appear to be grossly deformed. Dissected long bones of arms and legs appeared as irregularly lobulated, slightly elongated cartilaginous segments.

Microscopic Examination. Osseous and cartilaginous specimens were taken from calvarium, base of skull, clavicles, ribs (costo-chondral junctions), vertebrae, and dissected humeri, femurs and tibiae. Other specimens were taken from lungs, myocardium, aorta, liver, spleen, kidneys, pancreas, adrenals, ileum, thymus, and skin. All the specimens were fixed in 4% formalin buffered at pH 7.2 with phosphate buffer. Small fragments were taken from the femurs and ribs, and post-fixed with 1% OsO_4 buffered at pH 7.2, as above. These small fragments were embedded in Araldite and used for electron microscopy. The other specimens were embedded in paraffin. Those including calcified areas were previously decalcified with 2% formic acid.

Sections from paraffin blocks were stained with haematoxylin-eosin for routine examination. They were also stained by one of the following methods: a) Mallory method for collagen; b) silver nitrate—methenamine for argyrophilic structures; c) aldehyde-fucsin for elastic fibers; d) periodic acid—Schiff (PAS) for glycoproteins; e) 1% Alcian blue, pH 1.8, and colloidal iron (Mowry, 1958), pH 1.8, for acid glycosaminoglycans; f) 1% Alcian blue in the

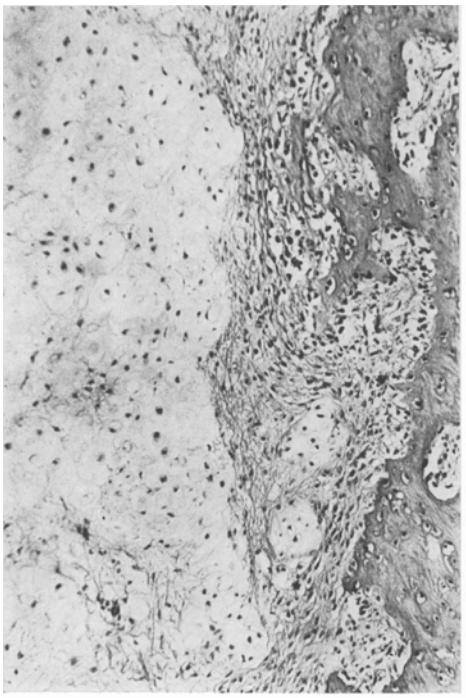


Fig. 3

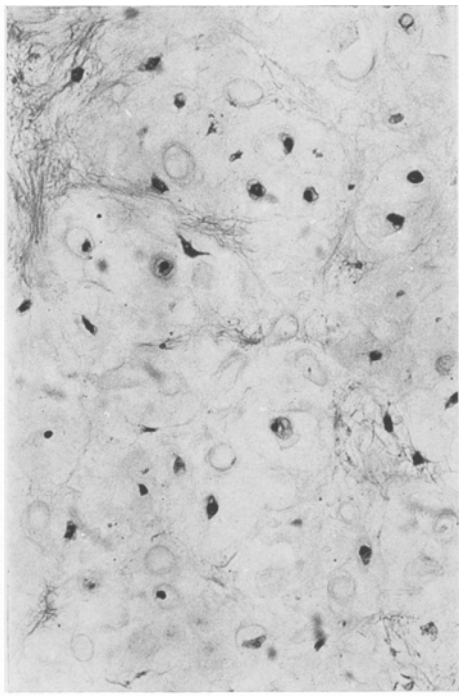


Fig. 4

Fig. 3. Section of a costo-chondral junction. The cartilage is separated from the osseous trabeculae by a connective strip which shows osteogenetic activity. No seriated cartilage is present. Haematoxylin-eosin, × 100

Fig. 4. Detail of costal cartilage. Haematoxylin-eosin, × 270

presence of 0.4 and 0.9 M $MgCl_2$ (Scott and Dorling, 1965), for differentiating between chondroitin and keratan sulphate; g) colloidal iron after hyaluronidase digestion, with the same aim as in *f*. Hyaluronidase (bovine texticular hyaluronidase, Sigma Chemical Company) was used at the concentration of 1 mg/1 ml in 0.1 N phosphate buffer, pH 5.4, at 37° C for 12 hours. Control sections were left in the same buffer for the same period of time and at the same temperature. Sections for electron microscopy were examined unstained and after staining with uranyl acetate and lead citrate.

Results of Optical Microscopy. The process of *endochondral ossification* was completely abnormal. In all the skeletal segments studied—these included the base of the skull, despite the lack of grossly abnormal features in it—no seriated cartilage was visible (Figs. 3, 5, 7). Traces of seriation could only be made out in the epiphyses of the clavicle and, to a much lower degree, in those of the femur. The limit between the uncalcified cartilage and osseous trabeculae was a thin rim of connective tissue which was a direct continuation of the periosteum and the perichondrium (Figs. 3, 5, 7). This connective strip showed osteogenetic activity. The osseous trabeculae appeared to derive from the periosteum in the cortical portion of each bone segment, and from the thin periosteum-like connective strip, without the intervention of cartilage calcification, in the central portion. The lack of cartilage calcification was also shown

clearly by the absence of axial cartilaginous cores along the osseous trabeculae. The connective strip was only interrupted in the base of the skull and in the femurs, in both of which a few areas of cartilage calcification were present. Traces of seriation were visible in cartilage in the epiphyses of the clavicles, but the cell columns were short and irregular. In all bones the osseous trabeculae—whether originating in the periosteum or in the periosteum-like strip—appeared normal and surrounded by active osteoblasts and a few osteoclasts. The bone marrow spaces contained abundant haemopoietic tissue.

The most significant abnormality was found in the structure of the cartilage. This consisted of roundish, faintly stained chondrocytes which had a small nucleus and a barely visible cytoplasm (Fig. 4). The single chondrocytes were contained within roundish lacunae whose boundary was made apparent by its slight basophilia and the presence of very thin fibrils. The surrounding matrix appeared almost unstained in sections stained with haematoxylin and eosin (Fig. 4). Irregular areas containing blood vessels and very thin fibrils were visible in the cartilage. The cells in these areas had either a fusiform, fibroblast-like shape or a star-like shape.

The histologic abnormalities in cartilage structure and endochondral ossification reported above were accompanied by changes in the general morphology of many skeletal segments. The long bones of the limbs were deformed and only the central part of their diaphyses was ossified. The costo-chondral junctions were unusually large and the limit between cartilage and bone was curved, with the concavity facing the cartilage (Fig. 5). The vertebral bodies were highly abnormal. They contained no well-defined ossification centers. A thin strip of connective tissue penetrated from the perichondrium into the vertebral body, and traces of ossification similar to membranous ossification could be detected at various points along it (Fig. 6). Another thin connective strip penetrated irregularly into the vertebral cartilage. This was probably evidence of an abortive attempt to form the intervertebral disks, which were not recognizable (Fig. 6).

Membranous ossification of calvarium and clavicle appeared to be normal. The calvarium was thin, but neither osteogenetic activity nor the structure of the osseous trabeculae were appreciably different from those found in normal subjects of the same age.

The *lungs* were atelectatic and congested. The morphology of the bronchial cartilage was the same as that described for the cartilage of the long bones. The bronchial mucus glands were unusually large. A few of the large elastic pulmonary arteries had an irregular internal outline apparently as a result of segmental thickenings of their wall. A similar localized thickening was found in the toracic *aorta*, where the thickened part appeared vacuolated and showed irregularities in the arrangement of its structures. A rather high degree of haemopoiesis was found in the *liver*. No significant changes were found in the other organs.

Histochemical results, too, showed that the cartilage structure was abnormal, but revealed no abnormalities in the osseous tissue. The cartilage matrix was slightly stained by the PAS method. Only a very thin border, containing a few fibrils, was deeply stained around the chondrocytes (Fig. 7). The chondrocytes were easier to recognize than in haematoxylin-eosin stained sections, chiefly because of the PAS-positivity of this border. Cytoplasmic PAS-positive material was only rarely observed.

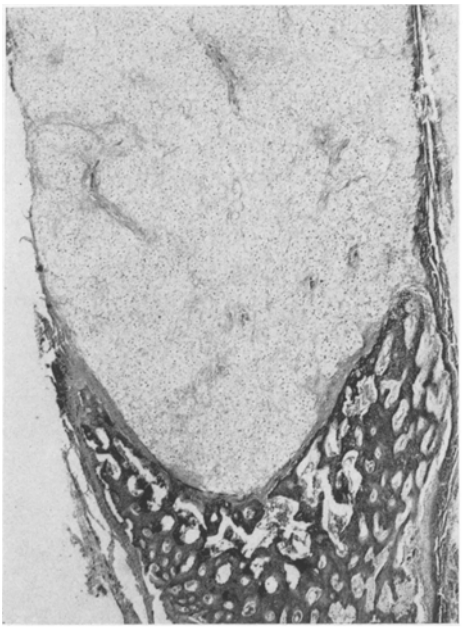


Fig. 5

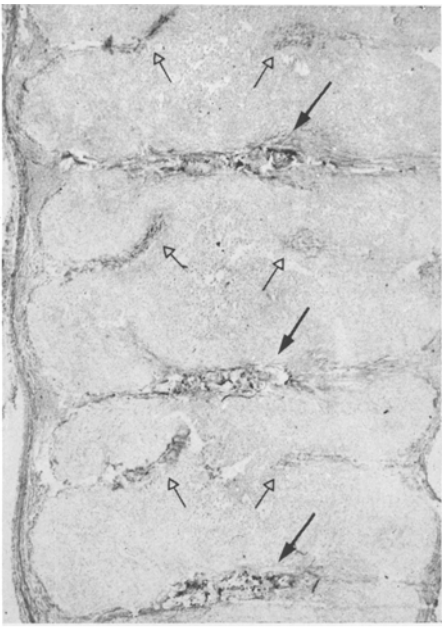


Fig. 6

Fig. 5. Section of costo-chondral junction. The limit between cartilage and bone is curved and is occupied by a connective strip. Haematoxylin-eosin, × 16

Fig. 6. Section of vertebral bodies. Black arrows point to connective strips which penetrate from the perichondrium into the vertebral bodies. Calcification irregularly occurs along these strips. White arrows point to other connective strips which seem to correspond to abortive intervertebral disks. PAS, × 10

After Alcian blue and colloidal iron staining, the intercellular matrix was slightly but diffusely stained. After hyaluronidase digestion, only the borders of the cellular lacunae were still stained by colloidal iron; these borders were also stained by Alcian blue in 0.9 M $MgCl_2$. After treatment with aldehyde-fucsin, the cartilage matrix was very deeply stained, appeared vacuolated, and had a fibrillar appearance. After silver nitrate staining, the matrix was almost unstained, the borders of the chondrocyte lacunae were argyrophilic, and the matrix appeared to be crossed irregularly by thin bundles of argyrophilic fibrils, which often surrounded small blood vessels. These fibrils were also stained by the Mallory method.

The bronchial cartilage had the same histochemical properties as the skeletal cartilage. The bronchial mucus glands were deeply stained by Alcian blue, colloidal iron, and aldehyde-fucsin. This last method showed clearly that both in the large elastic pulmonary arteries and in the aorta, the focal thickenings of the vessel wall contained elastic fibers which were disorganized, irregularly arranged, and of variable thickness (Fig. 8 a). The thickened areas were not argyrophilic (Fig. 8 b). The vacuoles present in the aortic thickening contained small amounts of colloidal iron and Alcian blue positive material.

As a rule, ultrastructural investigations have been restricted to the intercellular matrix of the cartilage, because specimens for electron microscopy could only be obtained 24 hours after death, by when autolytic processes had presumably occurred in cells. A few cellular findings of particular interest have, however, been recorded.

Electron Microscopic Results. The fine structure of the cartilage matrix was fairly uniform. It consisted of very small fibrils, whose thickness ranged between 120 and 380 Å. Their length was not measurable, because of their irregular

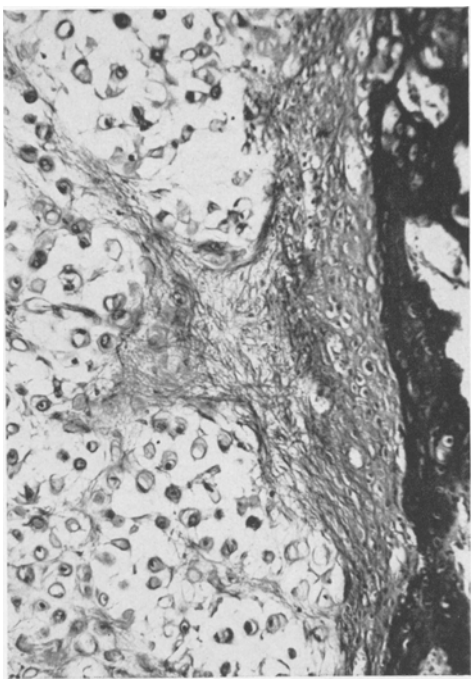


Fig. 7. Detail of the junction between cartilage and bone in a femur. Section stained with PAS. The osseous trabeculae are deeply stained. They are in contact with a connective strip. The cartilage matrix is unstained. × 115

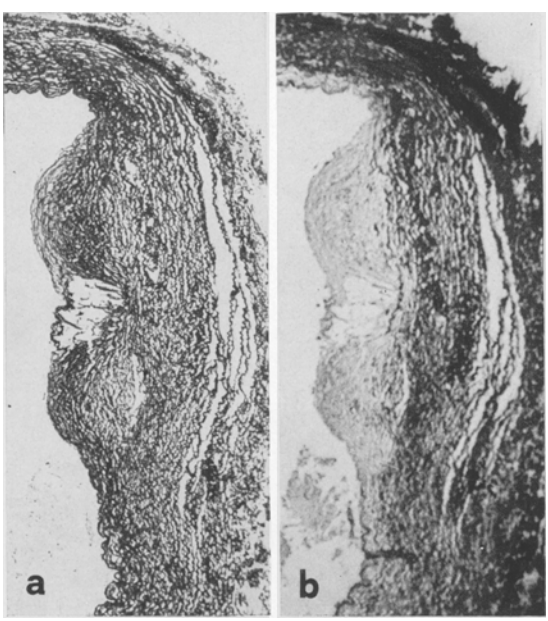
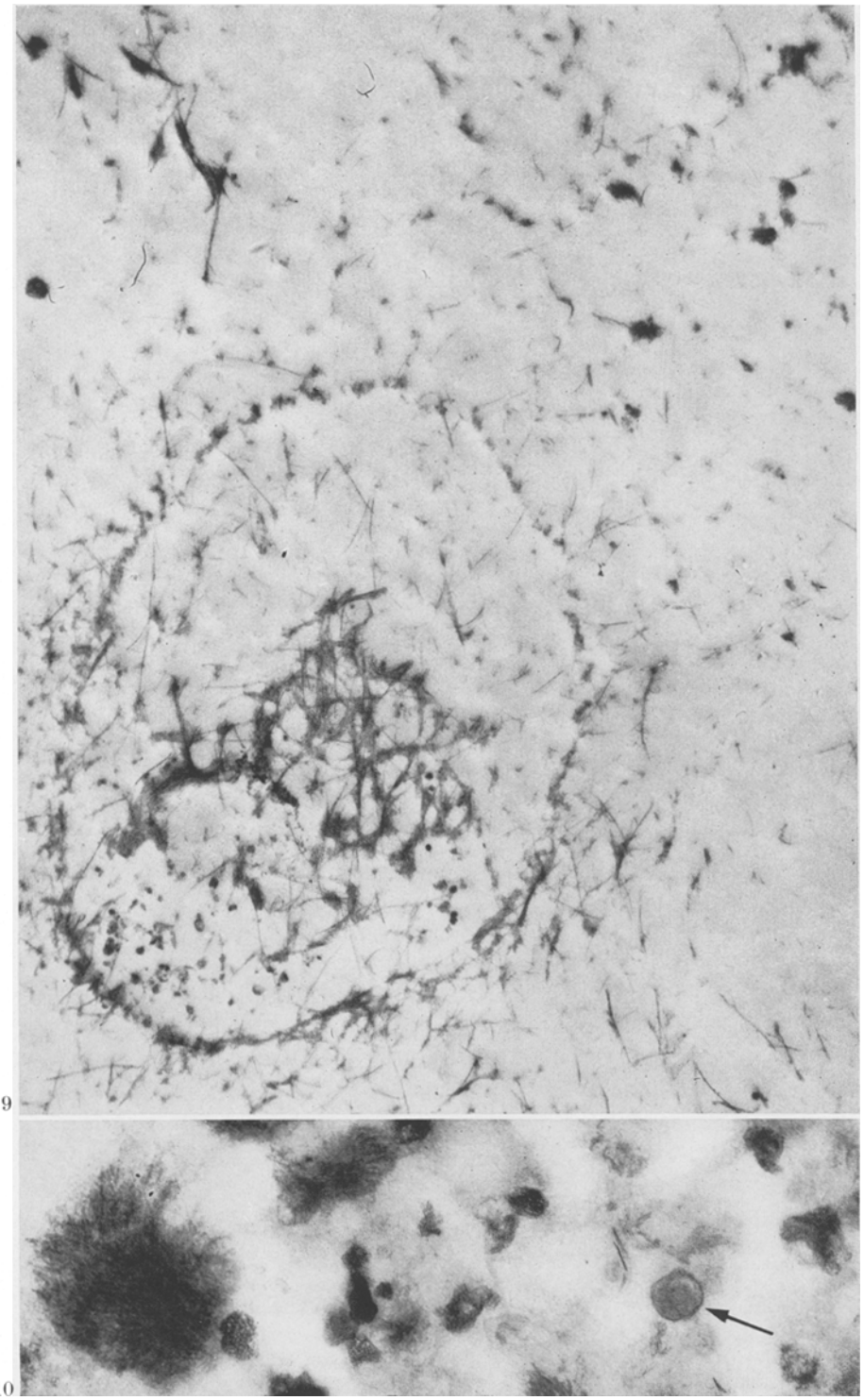


Fig. 8 a and b. Serial sections of the thickened part of the aortic wall. a) Aldehyde-fucsin; b) silver nitrate. The thickening contains disorganized elastic fibers and is vacuolated. It does not contain argyrophilic fibrils. × 45



Figs. 9 and 10

orientation (Fig. 9). Most appeared to be very short. Large, seemingly empty areas separated these fibrils, which were only occasionally grouped in small bundles. This happened mainly around chondrocytes, in the very same areas where lacunar borders were visible under the optical microscope. In this position, the fibrils formed a complete circle, often enclosing a chondrocyte; at other times they enclosed an empty lacunar space or a space containing an irregular network of fibrils (Fig. 9).

The fibrils forming these pericellular or perilacunar bundles hardly ever showed periodic banding. Those found elsewhere in the matrix only occasionally showed periodic banding, the interval being about 250 Å. Many fibrils were in contact with, and apparently coated by, an amorphous material.

A few chondrocytes, showing varying degrees of degeneration, contained intracytoplasmic foci of calcification (Fig. 10). This was the only calcification of cartilage found in this case, apart from a few restricted areas in which granular, apparently normal calcification was detectable. In both sites the calcified foci consisted of roundish clusters of intrinsically electron-dense, elongated crystals. A number of these crystals were occasionally related to roundish, electron-dense bodies, some of them surrounded by a membrane. In this case, the crystals were often curved and followed the outer border of these bodies (Fig. 10).

Discussion

The clinical, radiological and morphological data found in the present case are all consistent with the diagnosis of achondrogenesis and are similar to those previously reported as characteristic of this skeletal disorder (Fraccaro, 1952; Houston *et al.*, 1972; Jimenez *et al.*, 1973; Langer *et al.*, 1969; Parenti, 1936; Remagen *et al.*, 1971; Saldino, 1971; Xanthakos and Rejent, 1973).

The most severe skeletal abnormalities were the "immaturity" of the hyaline cartilage and the almost complete absence of endochondral ossification. Membranous ossification, on the other hand, was completely normal. Periosteal ossification did not seem qualitatively abnormal, but it was restricted to small, irregular segments which corresponded approximately to the central zone of the diaphyses and of some flat bones. Ossification was not limited to the cortical zone of these bones. A thin, periosteum-like layer, not unlike the "periosteal strip" found in achondroplastic subjects (Jaffe, 1972), usually divided the abnormal, uncalcified cartilage matrix from the ossified axial area. The bone trabeculae were formed on the side of this layer which faced the bone in a way reminiscent of membranous ossification.

Fig. 9. Electron micrograph, showing the fine structure of the cartilage. The intercellular matrix contains irregularly oriented collagenous fibrils of variable length and thickness. Some of them form a roundish outline which seems to correspond to a border of a cell lacuna. Within this border an irregular network of fibrils is visible. Uranyl acetate and lead citrate, × 11000

Fig. 10. Electron micrograph, showing clusters of intrinsically electron dense crystals found within a cellular lacuna. In stained sections, these clusters appear to be mixed with remnants of degenerated chondrocyte. The arrow points to a roundish structure whose border is outlined by electron dense, probably inorganic material. Unstained, × 41000

The morphology of the cartilage was sharply differentiated from that of normal cartilage. The chondrocytes were not collected in isogenic groups but were irregularly distributed throughout the cartilage matrix. This, and the fact that they were mixed with fibroblast-like cells and star-shaped cells, gave the cartilage its immature appearance. The matrix produced by these chondrocytes was both morphologically and histochemically abnormal. It contained an exceedingly low number of fibrils, and only a few of them could be identified as collagen fibrils by means of their periodic banding. There was no connection between most fibrils, so that there was no trace of the typical collagen network visible in normal cartilage under the electron microscope (Cameron, 1963; Revel and Hay, 1963; Robinson and Cameron, 1956; Scott and Pease, 1956; Takuma, 1960). A thin layer of fibrils was, however, visible around chondrocytes; the fibrils then looked like the border of a cell lacuna. This fibrillar border contained glycoproteins, as shown by its PAS-positivity, and acid glycosaminoglycans which, being stainable with Alcian blue in the presence of 0.9 M $MgCl_2$ and being resistant to hyaluronidase digestion, very probably consisted of keratan sulphate. As previously reported by Remagen *et al.* (1971), the intercellular matrix contained a low amount of glycoproteins and acid proteoglycans. Moreover, its stainability with Alcian blue and with colloidal iron was greatly reduced by 0.9 M $MgCl_2$ and by hyaluronidase digestion, respectively, thus showing that the intercellular matrix mainly contained chondroitin sulphate.

The reduction in the amount of proteoglycans was also visible under the electron microscope. Ultrastructural histochemistry has, in fact, shown that cartilage acid proteoglycans consist mainly of roundish granules and star-like, electron dense granules (both 100–150 Å across) connected by thin filaments to the collagen fibrils (Anderson and Sajdera, 1971; Bonucci, 1971 b; Campo and Phillips, 1973; Khan and Overton, 1970; Luft, 1965; Matukas *et al.*, 1967; Smith, 1970). These granules were not visible in the cartilage matrix in the present case.

Electron microscopy showed that, although virtually no cartilage calcification had occurred, abortive attempts to calcify were occasionally visible. These appeared as intracellular accumulation of inorganic crystals, some of which were related to electron-dense, roundish bodies. These bodies were probably of the same type as those found extracellularly in normal cartilage and recently named "calcifying globules" (Bonucci, 1971a).

The abnormal development of the cartilage and the lack of endochondral ossification were the cause of the severe alterations seen in the skeleton. Both clinically and radiologically it could be seen that these alterations had occurred mainly in the limbs and the vertebral column. The almost complete lack of calcification in the vertebral bodies was one of the most important lesions in this case. It seems to be the most characteristic alteration which occurs in achondrogenesis, differentiating it from other chondrodystrophic conditions, such as thanatophoric dwarfism and homozygous achondroplasia (Houston *et al.*, 1972; Langer *et al.*, 1969; Saldino, 1971). Histologically, the vertebral column was completely abnormal. No ossification centers were visible in the vertebral bodies, and the ossification process only occurred along thin connective strips which seemed to be invaginations of the perichondrium within the cartilage. These strips looked very like the "periosteal strips" found between cartilage and ossified

areas in the long bones of the limbs and in the ribs. The lack of true ossification centers was accompanied by incomplete division of the vertebral bodies and by the absence of intervertebral disks, which were replaced by thin connective septa running irregularly through the vertebral cartilage. Remagen *et al.* (1970) have suggested that these septa are due to pathological development of the persisting primary division of the sclerotoms.

The observation that in the present case the bronchial cartilage showed the same histological and histochemical structures as the cartilage of the skeleton was particularly interesting. It suggests that the dystrophic condition typical of achondrogenesis applies to all the hyaline cartilage and is not restricted to cartilage which normally calcifies.

The changes found in the wall of the large elastic pulmonary arteries and in the aorta are not easy to interpret at present. Investigations on new cases of achondrogenesis will determine whether they were findings specific to this case or are to be considered typical features of the pathology of achondrogenesis. The fact that the elastic fibers of other tissues (skin, for instance) were normal in appearance seems to favour the first of these two possibilities.

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References

Anderson, H. C., Sajdera, S. W.: The fine structure of bovine nasal cartilage. Extraction as a technique to study proteoglycans and collagen in cartilage matrix. J. Cell Biol. **49**, 650–663 (1971)

Bonucci, E.: The locus of initial calcification in cartilage and bone. Clin. Orthop. **78**, 108–139 (1971a)

Bonucci, E.: Problemi attuali attinenti all'istochimica di talune matrici calcificanti normali e patologiche. Riv. Istoch. Norm. Pat. **17**, 153–234 (1971b)

Cameron, D. A.: The fine structure of bone and calcified cartilage. Clin. Orthop. **26**, 199–228 (1963)

Campo, R. D., Philips, S. J.: Electron microscopic visualization of proteoglycans and collagen in bovine costal cartilage. Calcif. Tiss. Res. **13**, 83–92 (1973)

Fraccaro, M.: Contributo allo studio delle malattie del mesenchima osteopoietico. L'acondrogenesi. Folia hered. path. **1**, 190–205 (1952)

Houston, C. S., Awen, C. F., Kent, H. P.: Fatal neonatal dwarfism. J. Canad. Ass. Radiol. **23**, 45–61 (1972)

Jaffe, H. L.: Metabolic, degenerative, and inflammatory diseases of bone and joints, p. 193–206. München: Urban & Schwarzenberg 1972

Jimenez, R. B., Holmes, L. B., Kaiser, J. S., Weber, A. L.: Achondrogenesis. Pediatrics **51**, 1087–1090 (1973)

Khan, T. A., Overton, J.: Lanthanum staining of developing chick cartilage and reaggregating cells. J. Cell Biol. **44**, 433–438 (1970)

Langer, L. O. Jr., Spranger, J. W., Greinacher, I., Herdman, R. C.: Thanatophoric dwarfism. A condition confused with achondroplasia in the neonate, with brief comments on achondrogenesis and homozygous achondroplasia. Radiology **92**, 285–303 (1969)

Luft, J. H.: The fine structure of hyaline cartilage matrix following ruthenium red fixative and staining. J. Cell Biol. **27**, 61A (abstr.) (1965)

Matukas, V. J., Panner, B. J., Orbison, J. L.: Studies on ultrastructural identification and distribution of protein-polysaccharide in cartilage matrix. J. Cell Biol. **32**, 365–377 (1967)

McKusick, V. A.: Heritable disorders of connective tissue, 4th ed., p. 759–762. Saint Louis: The C. V. Mosby Company 1972

Mowry, R. W.: Improved procedure for the staining of acidic polysaccharides by Müller's colloidal (hydrous) ferric oxide and its combination with the Feulgen and the periodic acid—Schiff reactions. Leb. Invest. **7**, 566–576 (1958)

Parenti, G. C.: La anosteogenesi. Pathologica **28**, 447–462 (1936)

Remagen, W., Hienz, H. A., Wiedmann, H.-R.: Zum Problem der Frühentwicklung der Wirbelsäule. Z. Anat. Entwickl.-Gesch. **131**, 39–44 (1970)

Remagen, W., Hienz, H. A., Wiedmann, H.-R.: Zur Achondrogenesis. Verh. dtsch. Ges. Path. **55**, 510–513 (1971)

Revel, J.-P., Hay, E. D.: An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. Z. Zellforsch. **61**, 110–144 (1963)

Robinson, R. A., Cameron, D. A.: Electron microscopy of cartilage and bone matrix at the distal epiphyseal line of the femur in the newborn infant. J. biophys. biochem. Cytol. **2**, 253–260 (1956)

Saldino, R. M.: Lethal short-limbed dwarfism: achondrogenesis and thanatophoric dwarfism. Amer. J. Roentgenol. **112**, 185–187 (1971)

Scott, B. L., Pease, D. C.: Electron microscopy of the epiphyseal apparatus. Anat. Rec. **126**, 465–495 (1956)

Scott, J. E., Dorling, J.: Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian blue in salt solutions. Histochemie **5**, 221–233 (1965)

Smith, J. W.: The disposition of proteinpolysaccharide in the epiphyseal plate cartilage of the young rabbit. J. Cell Sci. **6**, 843–864 (1970)

Takuma, S.: Electron microscopy of the developing cartilaginous epiphysis. Arch. oral Biol. **2**, 111–119 (1960)

Xanthakos, U. F., Rejent, M. M.: Achondrogenesis: case report and review of the literature. J. Pediat. **82**, 658–663 (1973)

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