Virchows Arch. A Path. Anat. and Histol. 373, 167–175 (1977)

Virchows Archiv A

Case Report

Histologic, Microradiographic and Electron Microscopic Investigations of Bone Tissue in a Case of Craniodiaphyseal Dysplasia*

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Summary. The results of histologic, microradiographic and electron microscopic investigations carried out on two bone biopsies pertaining to a case of craniodiaphyseal dysplasia are reported. They show that the affected skeletal segments are chiefly characterized by enhancement of bone volume, and defective calcification of the bone matrix. Moreover, interstitial calcification of skeletal muscle has been found.

Key words: Craniodiaphyseal dysplasia – Skeletal pathology.

Introduction

Craniodiaphyseal dysplasia is characterized by dysplasic changes involving cranial, facial and long bones, by early and severe modification of the form of the face, by nasal obstruction and by possible compression of cranial nerves (Joseph et al., 1958).

The disorder is similar to the Camurati-Engelmann's syndrome (Camurati, 1922; Engelmann, 1929); however, in this syndrome craniofacial bones are only slightly involved while their abnormal development is the most prominent feature in craniodiaphyseal dysplasia (Maroteaux, 1974). It is possible that variants of Camurati-Engelmann's syndrome characterized by leontiasis ossea (Vilaseca et al., 1954) should actually be considered as cases of craniodiaphyseal dysplasia, which disease shows many similarities with the so-called craniometa-

* This investigation has been partly supported by grants of the Italian National Research Council

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physeal dysplasia. This can be differentiated since it usually spares diaphysis and pelvis (Maroteaux, 1974; McKusick, 1972).

Recently, we have had the opportunity of studying two biopsies, one of the *skull* and the other of the *pelvis*, of a case whose clinical and radiological characteristics, initially considered to be compatible with the diagnosis of Camurati-Engelmann's syndrome (Menichini et al., 1972), subsequently appeared to be consistent with the diagnosis of craniodiaphyseal dysplasia (Menichini et al., 1976). The present report deals with the histologic, microradiographic and electron microscopic studies of these biopsies.

Case Report

The clinical and radiological features of this case have previously been reported when the patient was 1-year-old (Menichini et al., 1972); at that time, he was considered to be affected by Camurati-Engelmann's syndrome, since the involvement of his craniofacial bones was limited. The development of the disease with age and findings showing that this boy had to be considered to be a case of *craniodiaphyseal dysplasia* have recently been described (Menichini et al., 1976). Thus only the most representative and significant data are reported in this paper.

T.M., 5 years old was hospitalized for progressive increase of the circumference of his skull (up to 65 cm), prominence of frontal and parietal bones, and severe modification of his face. This showed evident broadening of the nasal bridge due to osseous wings extending to the zygoma bilaterally; moreover, hypertelorism was present and the maxillo-zygomatic regions were prominent.

The abnormal development of the craniofacial bones induced complete obstruction of the nasal cavities (only mouth breathing was possible) and of the lacrymal chanals (with consequent chronic inflammation). Moreover, the patient was affected by right hypoacusia. Vision in his left eye was greatly reduced.

X-Ray Examination. The thickness of the calvarium was increased being from 2 to 2.5 cm in frontal and parietal areas. The coronal suture was fused. Both the vault and the base of the skull were sclerotic, the sella turcica was greatly reduced, the facial bones sclerotic and of homogeneous x-ray density. The diaphyses of the long bones, especially those of femurs and tibiae, were enlarged, slightly curved, their x-ray density was enhanced. The pelvic bones were thickened and dense. The phalanges were almost of cylindrical or conical shape and their radiopacity was irregularly increased.

Laboratory Findings. Calcium and phosphate concentration in blood and urine was normal; hydroxyproline levels and alkaline phosphatase activity in the urine were elevated; excretion of cyclic AMP in urine was also elevated.

Microscopic Examination. Two bone biopsies have been studied. The first was taken from the *calvarium* (posterior zone of frontal bone) during a surgical operation carried out to enlarge the optical foramina. It was a cylinder of compact bone measuring about 1.5 cm in length. The second biopsy was taken about 8 months later from the *iliac crest.*

Both biopsies were immediately fixed in 4% paraformaldehyde buffered to pH 7.2 with phosphate buffer. Longitudinal sections about 1 mm thick were obtained from them when they were still soaked in formalin. Part of these sections were reduced to a thickness of about 75 μ by grinding and were used for microradiography. The remaining sections were reduced to small specimens, which were post-fixed with 1% osmium tetroxide buffered as above, and were embedded in Araldite without decalcification. Semithin (about 1 μ thick) and ultrathin (about 800 Å thick) sections were obtained from these pieces. The former were examined under the light microscope either after staining with Azure II – Methylene blue and with von Kossa method, or after decalcification by flotation on 2% formic acid and staining with periodic acid – Schiff (PAS) and with Alcian blue at pH 1.8. The ultrathin sections were examined under the electron microscope either unstained or after staining with uranyl acetate and lead citrate.

Results

Microradiographic Results. The cranial and pelvic biopsies had the same microradiographic appearance. They consisted of thick osseous trabeculae, separated by thin vascular and bone marrow spaces. The trabeculae were irregularly oriented in the pelvis, but showed a preferential orientation in the skull (Fig. 1). Here three layers of trabeculae were recognizable: an outer, thin layer whose trabeculae were oriented tangentially to the cranial surface; an intermediate, thin layer whose trabeculae were obliquely oriented in respect to those of the

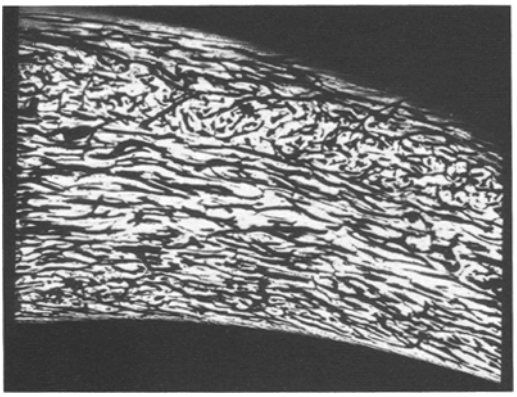

Fig. 1. Microradiography of a section of the skull biopsy. The bone tissue consists of thick trabeculae which are collected in three layers: an outer and an inner layer of trabeculae oriented tangentially to the skull surface and an intermediate layer of obliquely oriented trabeculae. ×2.2

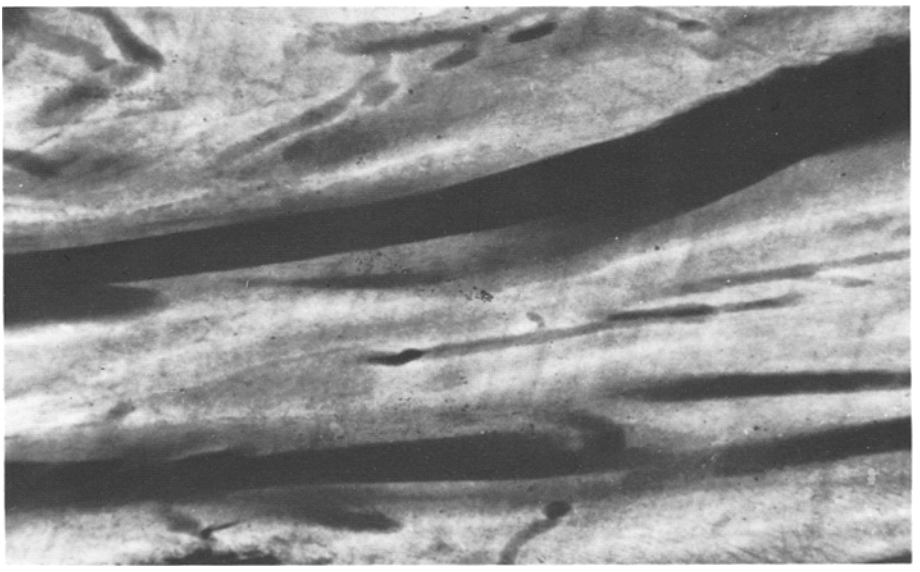

Fig. 2. Detail of a microradiography of the pelvic biopsy. Note the low degree of calcification of the bone matrix around vascular spaces which are similar to haversian channels. ×125

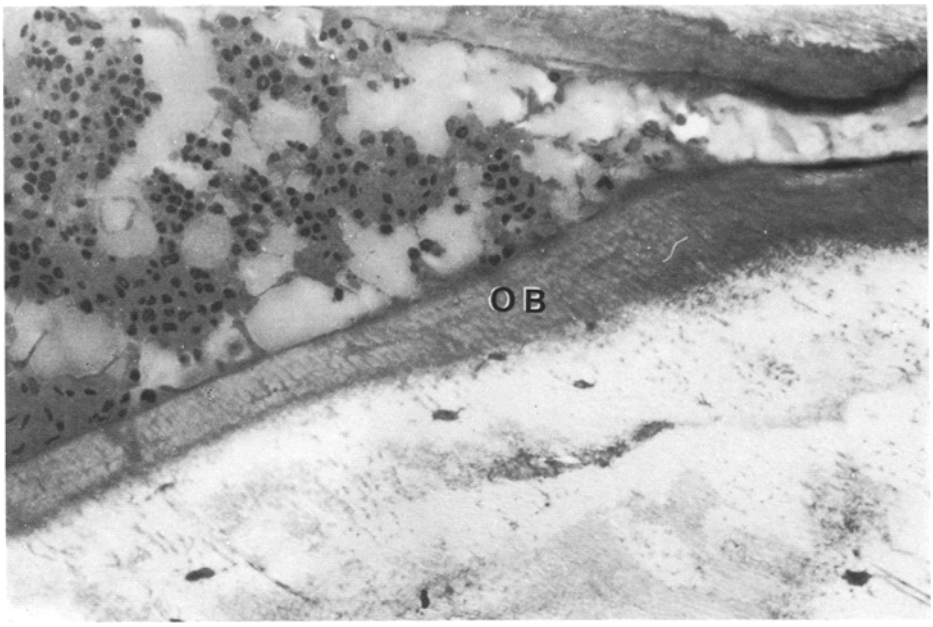

Fig. 3. Detail of a thin, undecalcified section of the skull biopsy stained with Azure II – Methylene blue. Part of a wide trabecula is visible below: it is in contact with a thick osteoid border (OB). Fat and hemopoietic cells are present in the bone marrow space. × 320

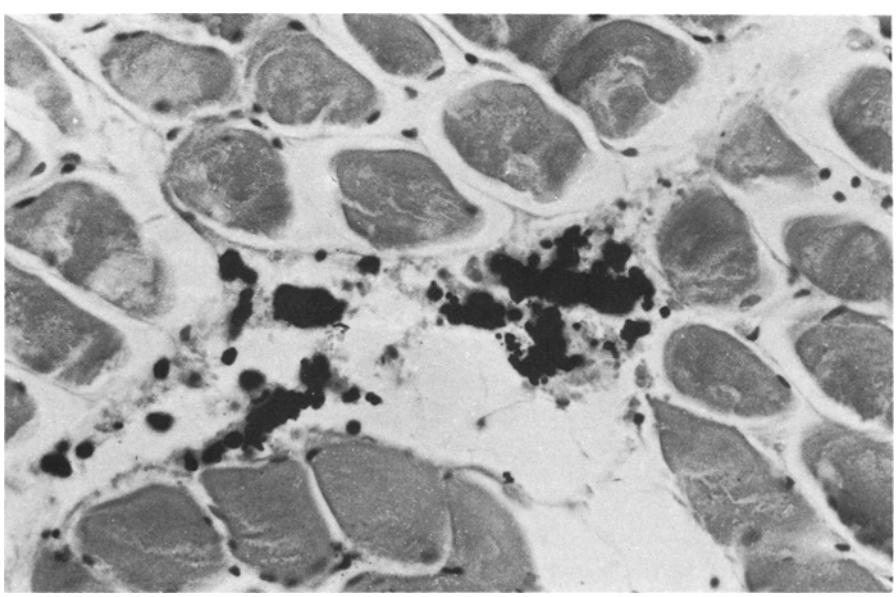

Fig. 4. Area of calcification in interstice of skeletal muscle. Von Kossa, × 320

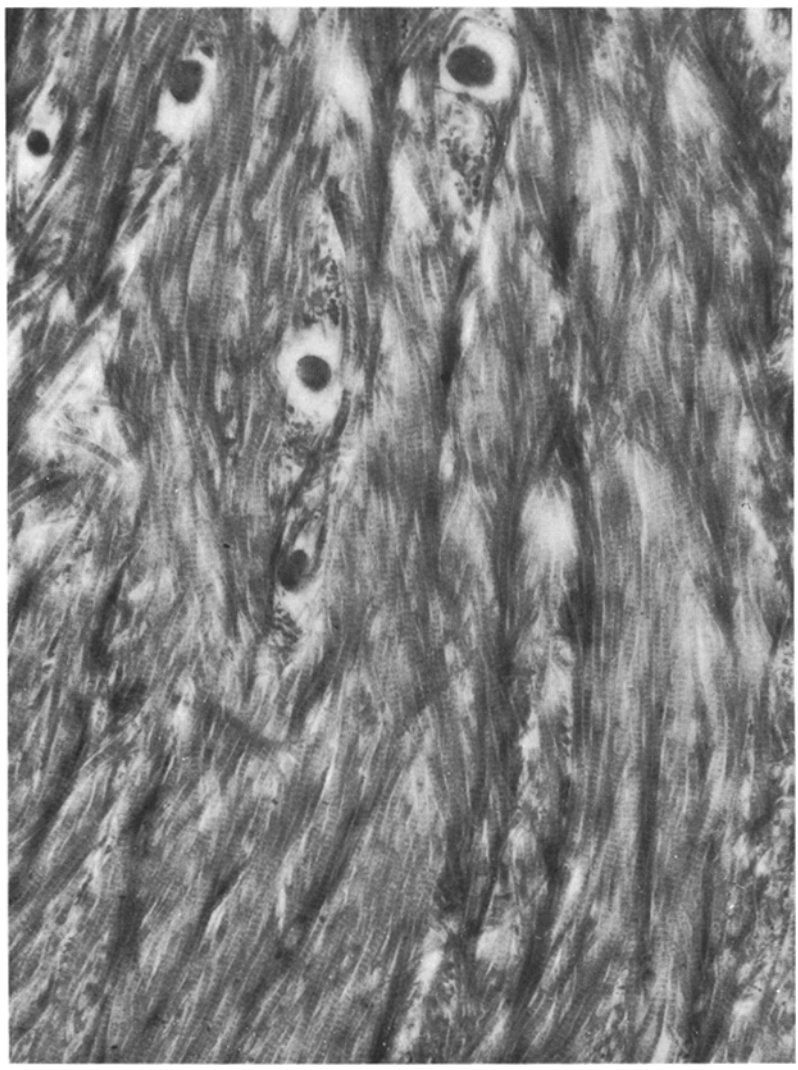

Fig. 5. Electron micrograph of osteoid tissue: note the presence of roundish, electron-dense bodies between collagen fibrils and the complete lack of calcification. Uranyl acetate and lead citrate, $\times 16{,}000$

outer and inner layers; and an internal, thick layer whose trabeculae showed the same tangential orientation as that of the outer layer.

The microradiographic density varied from low to very low in the peripheral part of the trabeculae, but was variable but usually elevated in their axial portions (Fig. 2).

Histological Results. Under the light microscope the structure of the cranial biopsy was similar to that of the biopsy taken from the iliac crest. In both

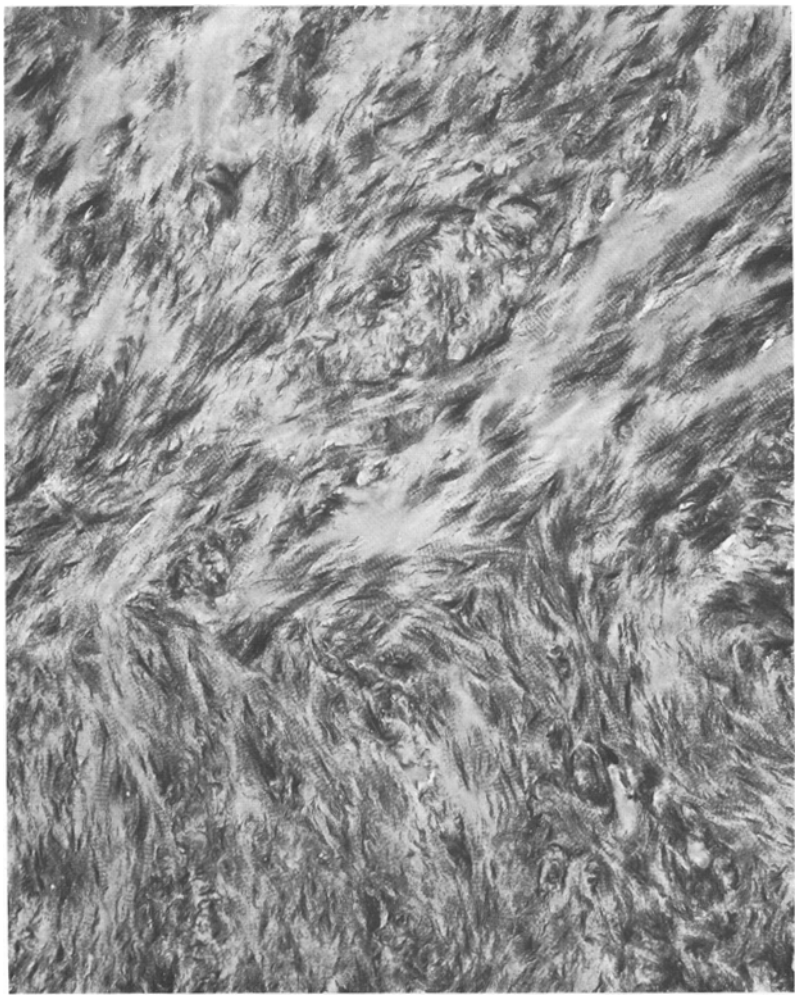

Fig. 6. Electron micrograph of incompletely calcified matrix: most of the inorganic substance forms bands related to the periodicity of the collagen fibrils; many areas are uncalcified. Unstained, $\times 10{,}000$

sites, the single sections consisted of osseous trabeculae of variable thickness. However, they were often very thick, sometimes resembling compact bone. These trabeculae contained variable numbers of osteocytes, many of which had enlarged and irregularly shaped lacunae. Almost invariably, the borders of the trabeculae were in contact with very thick borders of completely uncalcified osteoid (Fig. 3). The junction between this tissue and the calcified matrix was very sharp in many cases, while in other cases a calcification front was recognizable. The uncalcified osteoid sometimes contained osteocyte-like cells, and was occasionally in contact with roundish osteoblasts; more frequently, it was in contact with thin, elongated cells and with fibroblasts. These sometimes

occupied the vascular spaces which appeared fibrotic, but usually these spaces contained hemopoietic cells and blood vessels.

After decalcification, the bone matrix was homogeneously although slightly PAS-positive, while was practically unstained by Alcian blue. Many trabeculae showed irregular borders, apparently because of the presence of Howship's lacunae, but the osteoclasts were few and scattered.

A small fragment of *skeletal* muscle was present in the pelvic biopsy. The von Kossa method showed the presence of irregularly calcified areas in the interstitium (Fig. 4).

Electron Microscopic Results. The electron microscopic results confirmed the variable degree of calcification of the bone matrix. The osteoid tissue was usually completely uncalcified (Fig. 5). It consisted of collagen fibrils having a thickness of about 700 Å and a periodic banding of about 640 Å. These fibrils were irregularly oriented and only occasionally formed lamellar structures. Roundish, electron-dense bodies surrounded by a clear space were scattered between the collagen fibrils. A few of these bodies appeared surrounded by a membrane. Incompletely calcified areas showed roundish and elongated islands of calcification consisting of a few needle-shaped crystals oriented in the same direction as that of the collagen fibrils, and of finely granular material forming electron-dense bands which were closely related to the periodic banding of the fibrils (Fig. 6). These islands of calcification were separated by the interposition of areas of variable extent which completely lacked inorganic substance.

Not all of the trabeculae showed defective calcification; many of them appeared completely calcified and their inorganic substance consisted of bundles of needle-shaped crystals oriented along the axis of the collagen fibrils and more or less completely masking the underlying structures.

The cells placed along the osteoid borders were usually spindle-shaped. They resembled fibroblasts rather than osteoblasts, and frequently had very few cytoplasmic organelles and a reduced rough ergastoplasmic reticulum.

Discussion

The morphological changes of the bone tissue found in the present case were typical. The overproduction of bone and the striking increase in volume on one hand and the low degree of calcification of the matrix and the presence of thick osteoid borders on the other hand, constituted an easily recognizable microscopic picture.

The lesions were of the same type in the membranous bone of the calvarium and in the endochondral bone of the pelvis, showing that the bone changes were not dependent on the type of bone or skeletal segment. In both sites, osteosclerosis was evident. It was comparable to that reported in cases of Camurati-Engelmann's syndrome (De Sèze and Grivaux, 1950; Griffiths, 1956).

It is impossible at present to say whether the defect of the calcification process is primary and responsible for the whole disease, or if the primary defect is in the structure and composition of the organic matrix. Alternatively

some unknown disturbance of the delicate and complex mechanism which leads to bone formation and calcification may be responsible. In this regard it has recently been shown that bone calcification is in some way promoted and probably regulated by roundish, electron-dense bodies of cellular origin (Bernard and Pease, 1969; Bonucci, 1971; Anderson, 1973) similar to the matrix vesicles of the calcifying cartilage (Anderson, 1967, 1969; Bonucci, 1967, 1970, 1971). In the present case, many roundish bodies similar to matrix vesicles were present between the collagen fibrils of the osteoid tissue, which seemed normal in this respect. However, these bodies were not calcified. The fine structure of the collagen fibrils did not seem abnormal and the stainability of the matrix with PAS and Alcian blue was the same as that found in normal bone.

All these findings suggest that the overdevelopment and defective calcification of the bone matrix observed were not dependent on intrinsic abnormalities of the matrix itself. However, the study of more cases is necessary before drawing conclusions, in particular about the state and function of the cells. The interstitial calcification found in the small fragment of voluntary muscle attached to the pelvic biopsy could be of great significance especially as an index of a possible generalized abnormality of calcium and phosphate metabolism and/or of connective tissue conformation. It suggests the necessity of extending future studies of cases of craniodiaphyseal dysplasia to extraskeletal tissues.

Acknowledgements. The technical assistance of Miss Giuliana Silvestrini and Mr. L. Virgilii is gratefully acknowledged.

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Received November 5, 1976