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Electron Microscopic Study of Chondroid Tissue in the Cat Mandible

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Summary. This paper deals with electron microscopic appearance of chondroid tissue. Samples from eight cat mandibles were studied without decalcification. The ultrastructural characteristics of the chondroid tissue cells are common with young osteocytes. The interterritorial matrix of chondroid tissue is mineralized, being constituted of large collagen fibrils and calcospherites. The compositions of these parts of the chondroid tissue matrix and of bony matrix are similar but they are two different tissues. The pericellular matrix of the chondroid tissue consists of finely branched filaments, thin collagen fibrils, and an abundant ground substance. It resembles a cartilage matrix and contains type II collagen which is not present in bony matrix.

Key words: Chondroid tissue — Mandible — EM.

Chondroid tissue is the calcified tissue intermediate between bone and cartilage and is principally observed in the cranio-facial region during fetal life [1, 2].

Histological and microradiographic examinations of human chondroid tissue clearly show that this tissue cannot be considered either bone or cartilage. In undecalcified samples it appears as a tissue in which the matrix is mineralized, except for the pericellular area. The limit between the mineralized and nonmineralized pericellular areas is very irregular and the uneven border is due to small granular aggregations of mineral [3]. The degree of mineralization of chondroid tissue is high, comparable to woven bone, but clearly different when compared to enamel, calcified cartilage, dentin, and lamellar bone [4].

As we demonstrated using indirect immunofluorescence and immunoperoxydase techniques, chondroid tissue matrix contains type II collagen in the pericellular area and a great amount of collagen type I distributed in all the matrix [5]. It is then different from bone which doesn't contain collagen type II and from hyaline cartilage in which type I collagen is not present. In fact, collagen type I is only formed in the perichondrium, in the fibrocartilage, and during the formation of endochondral bone.

However, some questions remain unanswered. Are chondroid tissue cells similar to osteocytes or to chondrocytes? Is chondroid tissue a fibrocartilage which also contains collagen type I and II? Thus electron microscopic investigations were undertaken in which particular attention was paid to the fine structure of chondroid tissue cells and matrix.

Material and Methods

Eight 10-day-old kittens were perfused with NaCl (0.9%) followed by glutaraldehyde (2.5%) in cacodylate buffer (0.05 M, pH 7.2). The mandibles were dissected, and the samples taken from the symphyseal mandibular region were immersed in 4% glutaraldehyde in the same cacodylate buffer for 12 hours at 4°C. After washing with cacodylate buffer containing 0.1 M sucrose and after postfixation in osmium tetroxide (1%) in cacodylate buffer (0.1 M, pH 7.4), the samples were initially stained with saturated uranyl acetate solution (2%) for 12 hours. Fixed and dehydrated samples were embedded in Epon (Serva, Germany) according to the technique described by Luft [6]. Semithin and ultrathin sections were performed on ultramicrotome type Ultracut (Reichert-Jung, Austria). Semithin sections stained with toluidine blue were used for light microscope examination. Ultrathin sec-

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tions of about 900 Å were stained with 2% uranyl acetate for 20 minutes and lead citrate for 5 minutes. A Zeiss (EM9A, Germany) transmission electron microscope was used.

Results

Electron microscopic examination of undecalcified chondroid tissue shows that it consists of ovoid cells interspaced by an abundant extracellular matrix which can be divided into two different areas: a pericellular, unmineralized matrix and an interstitial mineralized matrix (Fig. 1). The cells have a small number of short microvilli and a few processes, the base of which is seen in Figures 1 and 2. They generally have an eccentrically placed nucleus, ovoid or cup-shaped, with a prominent nucleolus and a heterochromatin that is marginated at the nuclear membrane. As shown in Figure 2, the cytoplasm is relatively large, rich in rough endoplasmic reticulum which can have extremely dilated cisternae. The mitochondria are randomly distributed and the Golgi apparatus is well developed. Lysosomes are also present in the chondroid tissue cells and a few lipid droplets are occasionally seen. The endoplasmic reticulum is sometimes filled with a granular material having a more electron-dense appearance than that of intracellular matrix (Fig. 3). Numerous smooth or coated vesicles (Fig. 7: CV) are seen in the cytoplasm, and the plasma membrane at the sites of invagination is coated on its outer surface (Figs. 2 and 3: CS). Intracellular microfilaments (Figs. 3 and 4: IM) are prominent and sometimes disposed in bundles, particularly in the cell processes. The cells adhere to the neighboring cells by means of gap-junctions (GJ) visible at the extremities of cell processes or between uncompletely differentiated cells (Fig. 3).

The matrix of chondroid tissue consists of ground substance, fibrils, and other structures which are related to the mineralization, such as hydroxyapatite crystals and calcospherites. In the interstitial part of the matrix which becomes mineralized and which is located furthest from the cells (Fig. 5), collagen fibers are randomly arranged and are principally formed by 750 Å diameter fibrils. Each fibril presents the characteristic 640 Å-axial pattern. The osmiophilic granules (OG) which are seen in the matrix have the same aspect as the calcospherites observed in the calcified cartilage [7], the dentin, and the rapid-growing woven bone [8]. As previously discussed [5], the same structures as in calcospherites [8] can be found in these osmiophilic granules (Fig. 6): a peripheral or marginal zone (★), an intermediate zone (fine arrow), and in the central region, an initial calcification locus (thick arrow). In the interterritorial matrix, which is formed by interwoven bundles of thick collagen fibers, a few matrix vesicles (MV) [5] as well as cell processes (CP) can also be found (Fig. 5).

The principal characteristic of the pericellular matrix is its heterogeneity (Fig. 7). The pericellular matrix contains a delicate network of extracellular microfilaments (EM), small granules (*), and collagenous fibrils interspersed in abundant ground substance. The diameter of the fine collagen fibrils varies from 70–200 Å (arrows); large collagen fibrils (750 Å), as in the interterritorial matrix, can also be observed.

Discussion

Despite many histological observations of different kinds of tissues intermediate between bone and cartilage, very little detailed information about their fine structure is available. One of these tissues is called "chondroid bone." It is most often described, by histological and electron microscopic examination [9, 10], as a tissue with cells very similar to chondrocytes and located in a bonelike ma-

Fig. 1. Electron micrograph of chondroid tissue from the symphyseal mandibular region of a 10-day-old kitten (×5,600). Undecalcified sample.

Fig. 2. Electron micrograph of a chondroid tissue cell (×12,600). Undecalcified sample.

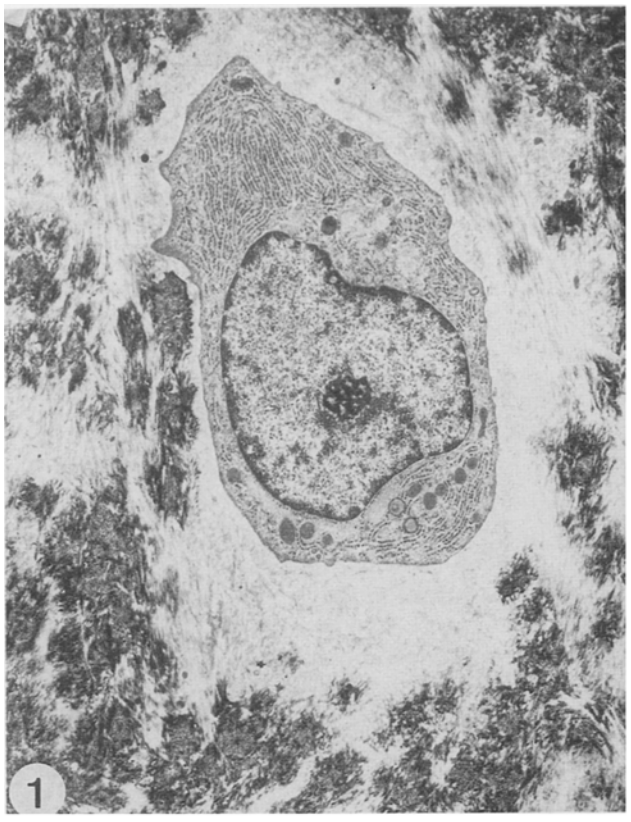
Fig. 3. Higher magnification of Figure 2. IM: intracellular microfilaments; GJ: gap junction; CS: coated surface of the cell membrane (×21,000).

Fig. 4. Electron micrograph of the cytoplasma of chondroid tissue cell. IM: intracellular microfilaments (×38,000).

Fig. 5. Electron micrograph of chondroid tissue. Undecalcified sample. Interterritorial area undergoing mineralization. OG: osmiophilic granules similar to calcospherites; MV: matrix vesicle; CP: cellular process (×19,200).

Fig. 6. Electron micrograph of osmiophilic granule in chondroid tissue (×57,000). ★: peripheral zone; thick arrow: initial calcification locus; fine arrow: intermediate zone.

Fig. 7. Electron micrograph of chondroid tissue. Pericellular matrix with abundant ground substance. EM: extracellular microfilaments; CV: coated vesicles; *: granules or transversal sectioned microfilaments; thick arrows: fine collagen fibrils (×57,000).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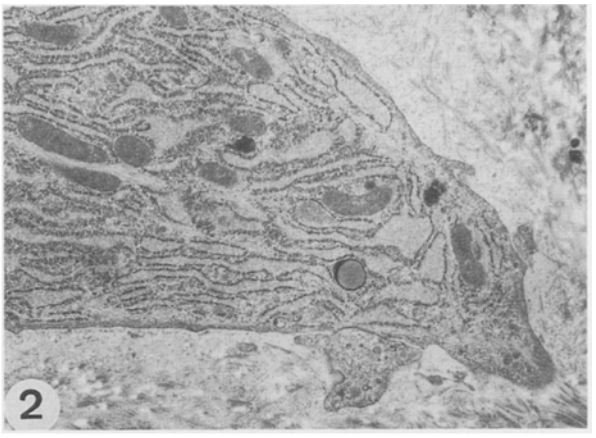
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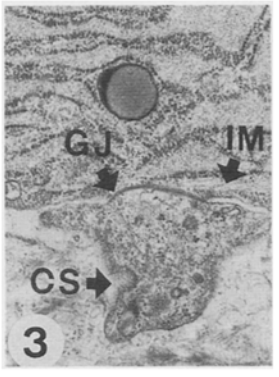
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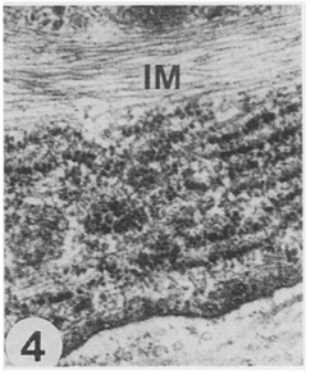
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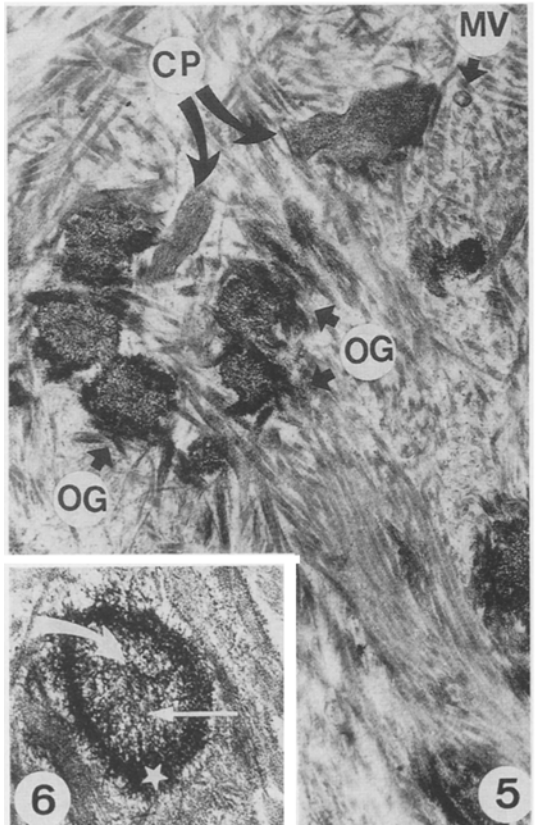
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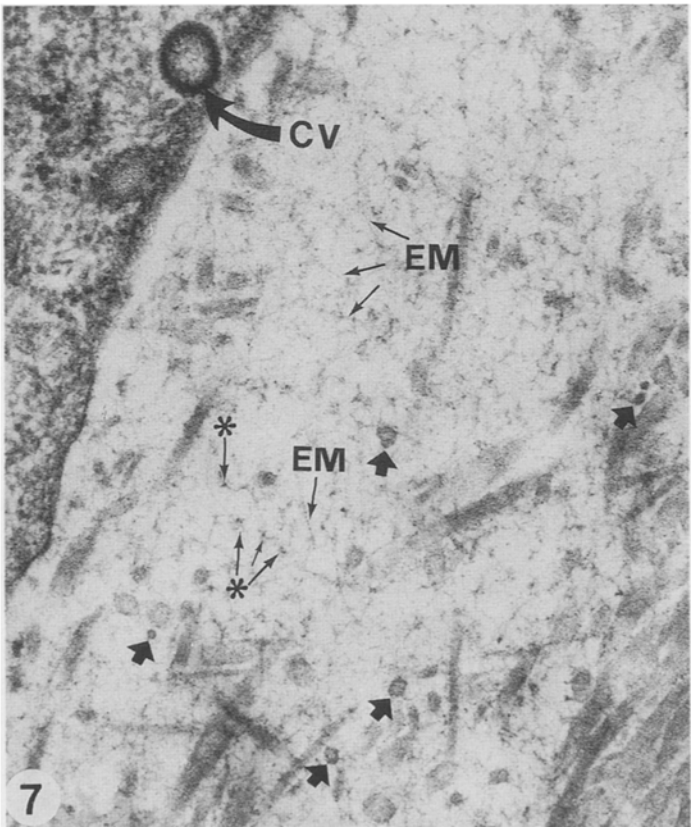
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7

trix and thus different from the tissue described here. Hall [11] demonstrated that secondary cartilage of a paralyzed embryonic chick can transform into chondroid bone; he believes that this tissue is formed by entrapped chondroblasts and chondrocytes which have a matrix production similar to that of an osteoblast. In this case, the change in cell activity is a true metaplasia according to the definition of Beresford [12], and this change depends on biomechanical stimulation [13]. Fracture healing is another example of biomechanical influence on bone differentiation. It occurs by intramembranous bone formation in an immobilized fracture, while endochondral ossification occurs in a noncompletely immobilized one [14]; a fibroblastic differentiation is observed in nonunion fractures [15]. We believe also that the differentiation of osteo-progenitor cells can produce not only bone and cartilage but other tissues as well, such as the chondroid tissue described here.

Histological observation shows that chondroid tissue is an intermediate between bone and cartilage. Following our observations, the cells of the chondroid tissue are separated by a matrix whose calcification spares the pericellular zone and is granular [3]. Chondroid tissue is a relatively highly mineralized tissue clearly different when compared to the mineral content of enamel, dentin, lamellar bone and calcified cartilage, evaluated by histophotometric measurements [4]. Examination of decalcified samples of chondroid tissue clearly shows a difference between lacunae arrangement and toluidine or methylene blue stain affinity of bone, cartilage and chondroid tissue [16]. The chondroid tissue differs from other connective tissues, whether calcified or not, by the composition of the organic part of its matrix. As we have already shown using indirect immunofluorescence and immunoperoxydase techniques, it contains type I and II collagen [5]. Immunological studies thus confirm that chondroid tissue is certainly not bone or cartilage since the bony matrix consists of collagen types I and V [17] and the cartilaginous matrix consists of type II [18], type (1α, 2α, 3α) [19], type IX or M-collagen disulphide-linked [20], and type X [21, 22].

Excluding all pathological findings, only fibrocartilage contains concomitantly type I and II collagen but histological and microradiographical aspects of fibrocartilage are different from those of chondroid tissue. Moreover, the electron microscopic observations (Figs. 1 and 2) show that chondroid tissue cells have more similarities with osteoblasts than with chondrocytes.

The ultrastructural data presented in this study indicate, as our previous histological and immunological studies have shown, that chondroid tissue has some common characteristics with cartilage and bone. The ultrastructural characteristics of bone are not fundamentally different from those of chondroid tissue [23]. Indeed, a classically described osteoblast has a large ovoid nucleus and contains a well-developed Golgi zone, an extensive granular endoplasmic reticulum, an abundant number of mitochondria, ribosomes and also coated vesicles such as we find in chondroid tissue cells (Figs. 2 and 3). Its plasma membrane presents progressively lengthening processes (Figs. 2 and 5) with a morphological development similar to the processes of osteocytes. The collagen compositions of bone matrix and of the part of the matrix that becomes mineralized in chondroid tissue are identical in morphologic appearance in that they are principally constituted by large collagen fibrils; as in all bone tissues, moreover, the matrix shows calcospherites as in woven bone. The common characteristics between chondroid tissue and bone are thus osteoblastlike cells (Figs. 1, 2, 3 and 4), cell processes, and cell junctions (Figs. 1, 2 and 3) as well as a similar interterritorial matrix composition (Fig. 5) and calcospheritelike elements (Figs. 5 and 6). However, only the first two characteristics are strictly specific to bone ultrastructure. Indeed, gap junctions are observed in bone tissue, during chondrogenesis [24] and scarce cell contacts are detectable in fully developed cartilage [25]. As for the large collagen fibrils, they are also observed in fibrocartilage [23] and in cement [26]. Calcospherites are also seen in calcified cartilage and dentin. Calcospherites were described in "rapid growing woven bone" and were found in certain pathological bone tissues, particularly in lathyrism [8]. However, the concept of "rapid growing woven bone" is not a precise one and is often used without any measurements of calcification rate. It is almost described in developing bone. Thus, one can then ask if there is a morphological relationship between "rapid growing woven bone" and chondroid tissue.

Some criteria established by Bernard and Pease [27] for woven bone formation are present in chondroid tissue, such as the growth of hydroxyapatite into spheroidal nodules similar to calcospherites and the subsequent fusion of these nodules. However, the presence of type II collagen in chondroid tissue [5] is not compatible with the hypothesis that chondroid tissue is woven bone or another type of bony tissue.

A comparison between chondroid tissue and cartilage shows that chondroid tissue cells and chondrocytes (Figs. 1 and 2) are different, but the peri-

cellular part of the chondroid tissue matrix can be compared with cartilage and with mandibular secondary cartilage of the symphyseal region or of the alveolar region [2]. The pericellular chondroid tissue matrix contains microfilaments, matrix granules or transversally sectioned microfilaments, and thin collagen fibrils. All the elements found in the pericellular chondroid tissue matrix (Fig. 7) are present in variable quantities and probably depend on degree of cell differentiation and localization of the cells, which can be situated near or far from the collagen bundles which join the two hemimandibles of the symphyseal suture. Moreover, the chondroid tissue also contains type II collagen as cartilage.

The interconnecting network of finely branched filaments in the pericellular area of chondroid tissue (Fig. 7) resembles a cartilage matrix; this characteristic cannot be used as a mean of identification because it can also be seen in the pericellular area of osteocytes. It is usually a very thin layer [28] and is constituted of thin fibrils with a diameter varying from 112–292 Å, according to Knese [9].

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