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Phagocytosis of Bone Collagen by Osteoclasts in Two Cases of Pycnodysostosis

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Summary. Electron microscopic examination of bone biopsies obtained from two patients suffering from pycnodysostosis revealed that osteoclasts contained (sometimes large) cytoplasmic vacuoles filled with bone collagen fibrils. These vacuoles stained positive for acid phosphatase activity, thereby suggesting that bone matrix had been phagocytosed and subsequently exposed to hydrolytic enzymes of the lysosomal apparatus. Collagen-containing vacuoles were not observed in osteoclasts of individuals not suffering from this disease.

Key words: Osteoclasts — Bone resorption — Collagen degradation — Phagocytosis — Pycnodysostosis.

There is still no general consensus in the literature as to the sequence of events leading to the resorption of bone. Some authors are of the opinion that osteoclasts have the capacity to degrade both bone mineral and bone matrix [1–4]. Heersche [5], however, put forward the view that the activities of osteoclasts are restricted to the removal of bone mineral and that the organic matrix is degraded by neighboring mononuclear cells. Sakamoto and Sakamoto [6] proposed that bone collagen is degraded by collagenase produced by osteoblasts and osteocytes.

Here we present evidence lending support to the view that osteoclasts have the capacity to degrade both the mineral component of bone and its matrix. The evidence is based on electron microscopic observations of bone biopsies obtained from two patients suffering from pycnodysostosis, a rare osteopetrosislike bone disease of unknown etiology [7, 8] characterized by the following clinical symptoms: a short stature, failure of closure of the cranial sutures, open fontanelles, obtuse mandibular angles, partial or total aplasia of the terminal phalanges, generalized increased roentgenographic density of the skeleton, and predisposition to bone fractures [9, 10].

Several studies dealing with the pathology of this disease have appeared in the literature [11–14]. The present study is the first report on the fine structure of osteoclasts in pycnodysostotic patients.

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Materials and Methods

Bone biopsies were obtained from four patients, an 18-month-old boy (patient A), a 35-year-old woman (patient B), and two 2-year-old girls (patients C and D). Whereas patients A and B exhibited skeletal deformities typical of pycnodysostosis [7–10], patients C and D did not show clinical symptoms of this disease. The latter patients were treated for congenital hip dislocation and served as control.

The skull of both patients A and B was relatively large and their anterior fontanelle persisted. Arms and legs were undersized, while the site of implantation of the thumbs was more proximal than normal. In patient A, retrognathia was observed and the mandibular angles measured about 180°. In patient B, the mandibular angles were only slightly increased. In both patients, neither the liver nor the spleen was enlarged and no signs of anemia were observed. X-ray investigations of the entire skeleton showed generalized hyperdense and sclerotic bone without diffuse thickening of the spongiosa. Partial aplasia of some of the terminal phalanges was seen in patient A while in patient B all terminal phalanges were absent.

Biopsies from the iliac crest of patients A, C, and D were fixed in either 4% paraformaldehyde and 1% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4) or 2.5% glutaraldehyde in 0.1 M sodium-cacodylate buffer (pH 7.4). Part of the material was demineralized in 0.1 M EDTA and 2.5% glutaraldehyde in cacodylate buffer (pH 7.4). Following postfixation in 1% OsO_4 for one h at 4°C, the tissues were dehydrated through an ethanol

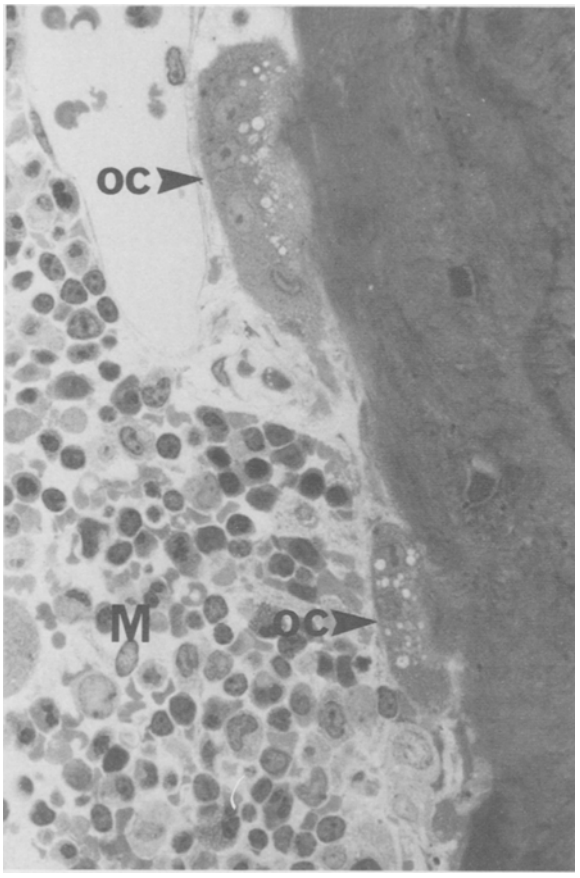

Fig. 1. Light micrograph of osteoclasts (*OC*) adjacent to bone (demineralized); *M*: marrow; methylene blue. ×600.

series and embedded in LX-112. Semithin sections of 1 μm were obtained with glass knives and stained with methylene blue. Ultrathin sections were cut with a diamond knife, stained with uranyl-acetate and lead-citrate and examined in a Philips EM 300 electron microscope.

Biopsies from patient B were obtained from the lower jaw during surgical treatment of a fracture of the alveolar process. The material was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). For demonstration of acid phosphatase activity several bone fragments of patient B were demineralized in 0.1 M EDTA in 0.1 M sodium cacodylate buffer (pH 7.4) at 4°C during 5 days and incubated in a medium containing beta-glycerophosphate at pH 5.0, according to the method described by Barka and Anderson [15]. Postfixation was carried out in 1% OsO_4 in the presence of 0.05 M potassium ferrocyanide [16]. The specimens were then dehydrated and embedded in LX-112. Control specimens were incubated in the absence of substratum. Ultrathin sections were examined in the electron microscope with or without being counter-stained with uranyl and lead.

Results

In the biopsies from the two pycnodysostotic patients, multinucleated giant cells were seen bordering some of the bone trabeculae (Fig. 1). These cells were often associated with small excavations in the bone surface, resembling Howship's lacunae. In the electron microscope the multinucleated cells exhibited a ruffled border, a clear zone, a circumnuclear Golgi-complex, many mitochondria and vacuoles in their cytoplasm (Figs. 2A, B, 3A, 4A, B), thereby fulfilling the morphological criteria of osteoclasts [17, 18]. Along the ruffled border of these cells relatively wide areas of bone that were apparently free of mineral crystals were seen (Figs. 2B, 4A, B). These areas measured about 4 μm in width. In connection herewith it is worth mentioning that we often observed small vesicles in the cytoplasm of the osteoclasts measuring about 0.4 μm in diameter. These vesicles contained crystal-like structures (Fig. 2C) which disappeared following treatment of tissue sections with 0.1 M EDTA for 30 min.

A striking observation in the biopsies of patients A and B but not in those of the control patients C and D was that the cytoplasm of the osteoclasts contained vacuoles filled with cross-banded structures that could not be distinguished from bone collagen fibrils (Figs 2D, E, 3B, 4C, 5). These vacuoles were observed in 57 out of 62 osteoclasts. The intracellular localization of the collagen-containing vacuoles was proved in a study of serial sections (Fig. 5). In many of these vacuoles, the fibrils were surrounded by electron-dense material (Figs 2D, E, 3B, 4C, 5). In sections of non-demineralized material these vacuoles apparently did not contain mineral crystals (Figs 2D, E, 4C, 5). Some of the collagen-containing vacuoles appeared to have a rather large and irregular shape (Figs 2D, 6A).

Examination of ultrathin sections from material incubated for acid phosphatase activity revealed the presence of electron-dense lead precipitate in almost all collagen-containing vacuoles (Figs 6A, B, C). Control specimens incubated in the absence of substratum were negative (Fig. 6D). Precipitate was sporadically seen between the infoldings of the ruffled border [cf. Lucht, 19].

Discussion

The observations presented in this paper indicate that in case of pycnodysostosis, osteoclasts differ in various respects from those in normal individuals. A relatively wide area of bone matrix free of mineral crystals was seen along the ruffled border of the cells. Such areas were not seen in sites where the osteoclasts were not in contact with the bone surface. This observation, together with the pres-

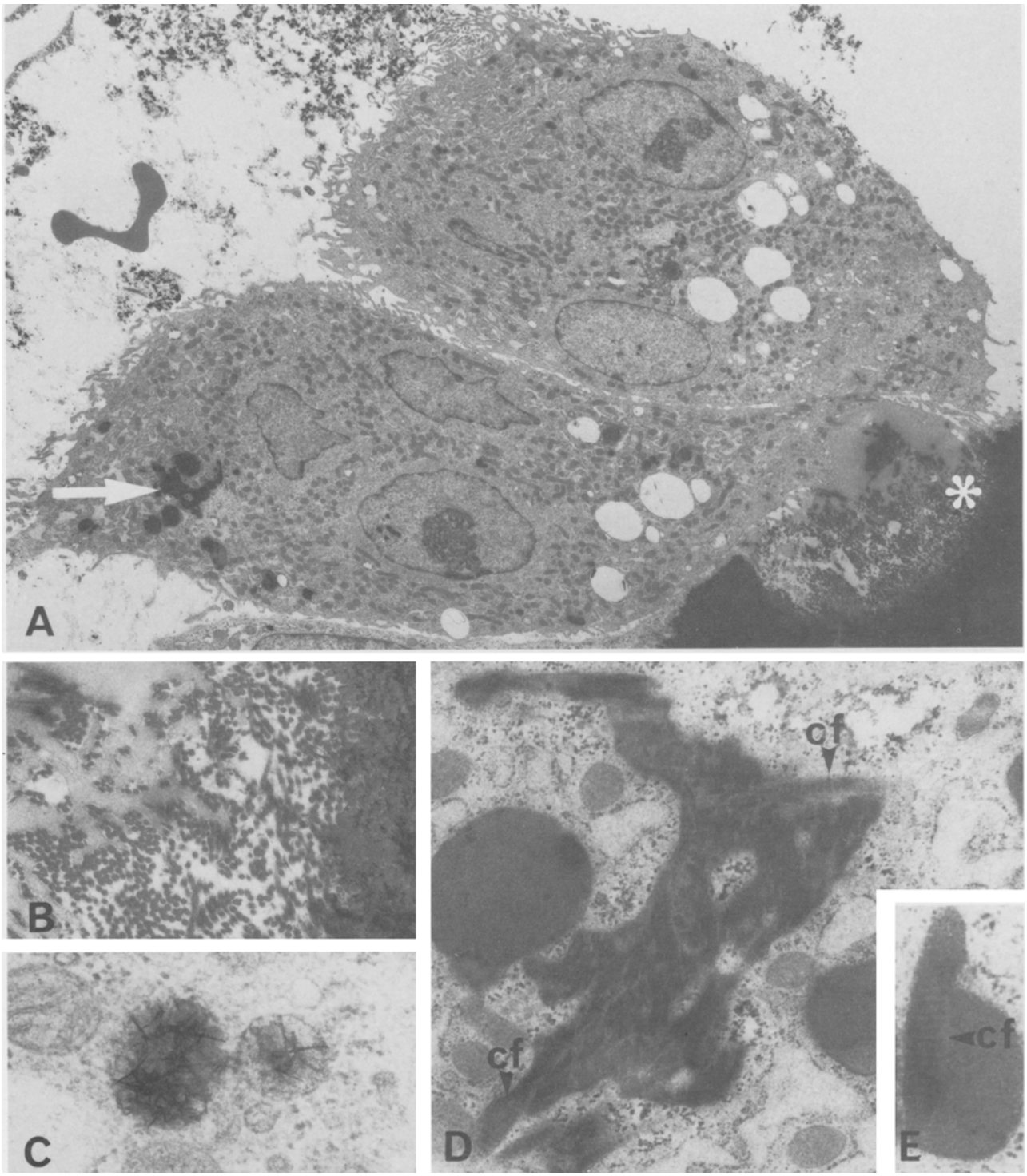

Fig. 2A. Osteoclast associated with small Howship's lacuna (not demineralized). ×3,200. **B, C, D, E.** High power electron micrographs of same osteoclast. **B.** The bone lining the lacuna is free of mineral crystals (see *asterisk* in **Fig. 2A**). ×16,400. **C.** Cytoplasmic vacuoles containing mineral crystals. ×51,000. **D, E.** Cytoplasmic vacuoles enclosing collagen fibrils (see *arrow* in **Fig. 2A**). Note the presence of electron-dense material surrounding cross-banded collagen fibrils and the absence of mineral crystals. *cf:* collagen fibril. **D.** ×22,000. **E.** ×31,000.

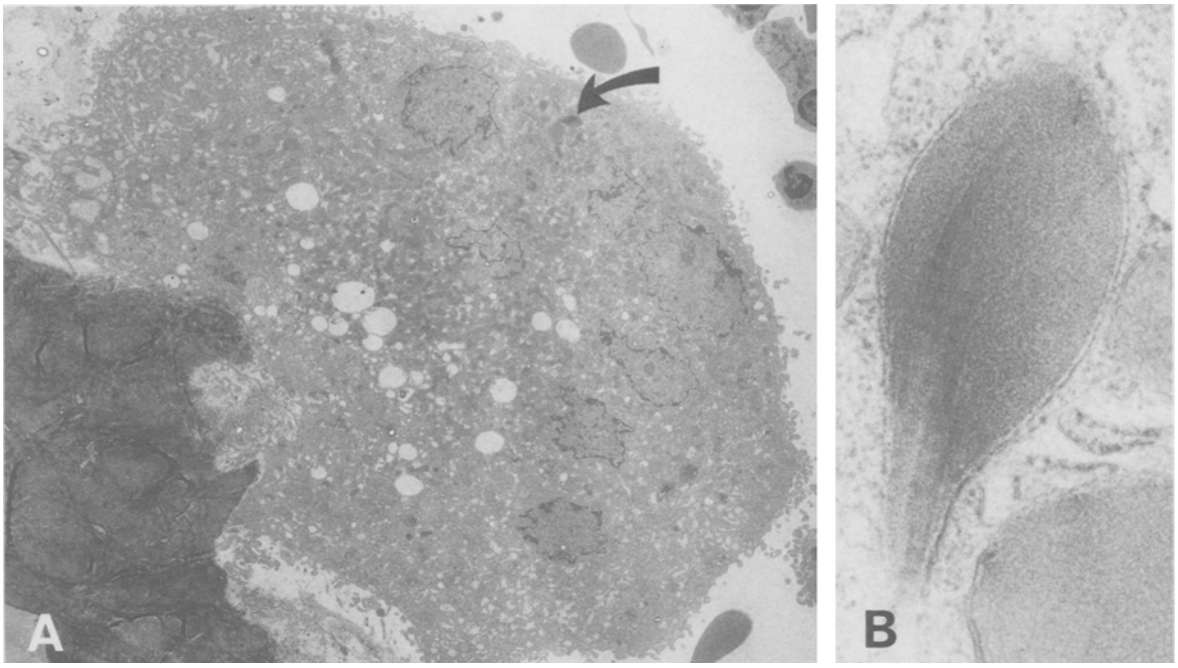

Fig. 3A. Osteoclast associated with Howship's lacuna (demineralized). ×2,500. **B.** Higher magnification of collagen-containing vacuole indicated in **Fig. 3A** (*arrow*). ×50,000.

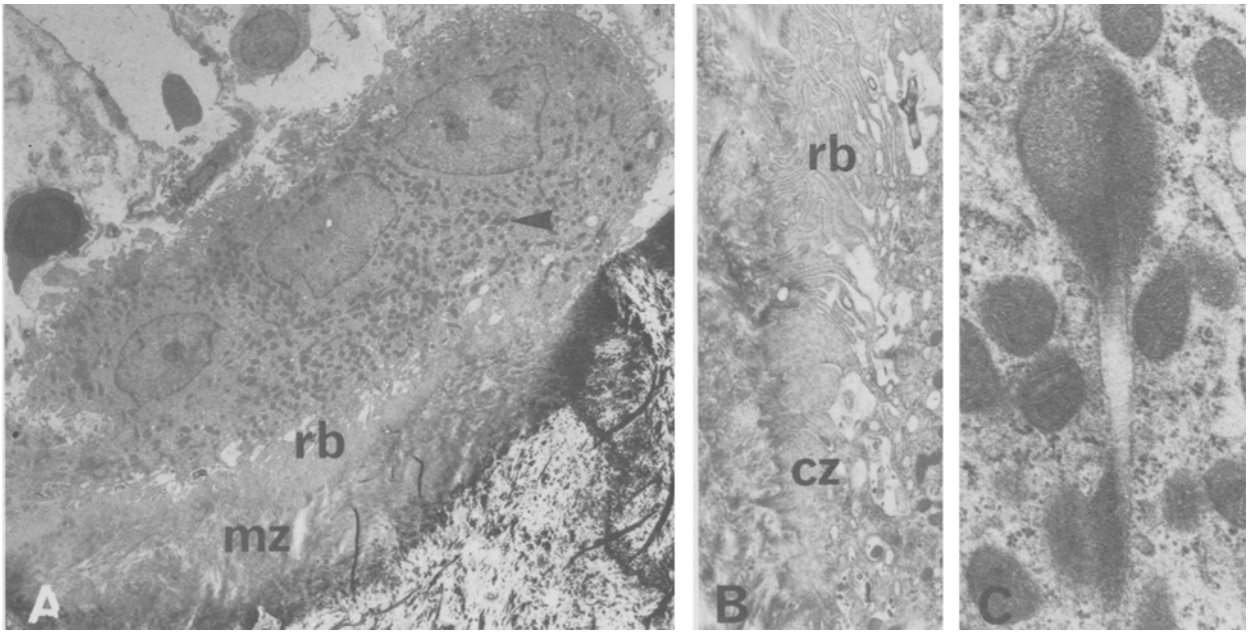

Fig. 4A. Osteoclast associated with Howship's lacuna. *Arrowhead* indicates collagen-containing vacuole shown at higher magnification in **Fig. 4C.** Note the mineral-free zone (*mz*). **B.** High power electron micrograph of ruffled border (**rb**) and clear zone (**cz**) (not demineralized). **C.** High power electron micrograph of collagen-containing vacuole shown in **Fig. 4A** (*arrow*). *rb:* ruffled border. **A** ×2,300; **B** ×8,600; **C** ×30,000.

ence of cytoplasmic vesicles containing mineral crystal-like structures is in line with the view that the bone surface is exposed to a local demineralizing activity of the osteoclasts [20] prior to the removal of matrix components.

The capacity of osteoclasts to phagocytose has previously been demonstrated for substances like mineral crystals [1], lanthanum [21], ferritine, and thorium-dioxide [22]. However, the occurrence of intracytoplasmic vacuoles enclosing bone collagen

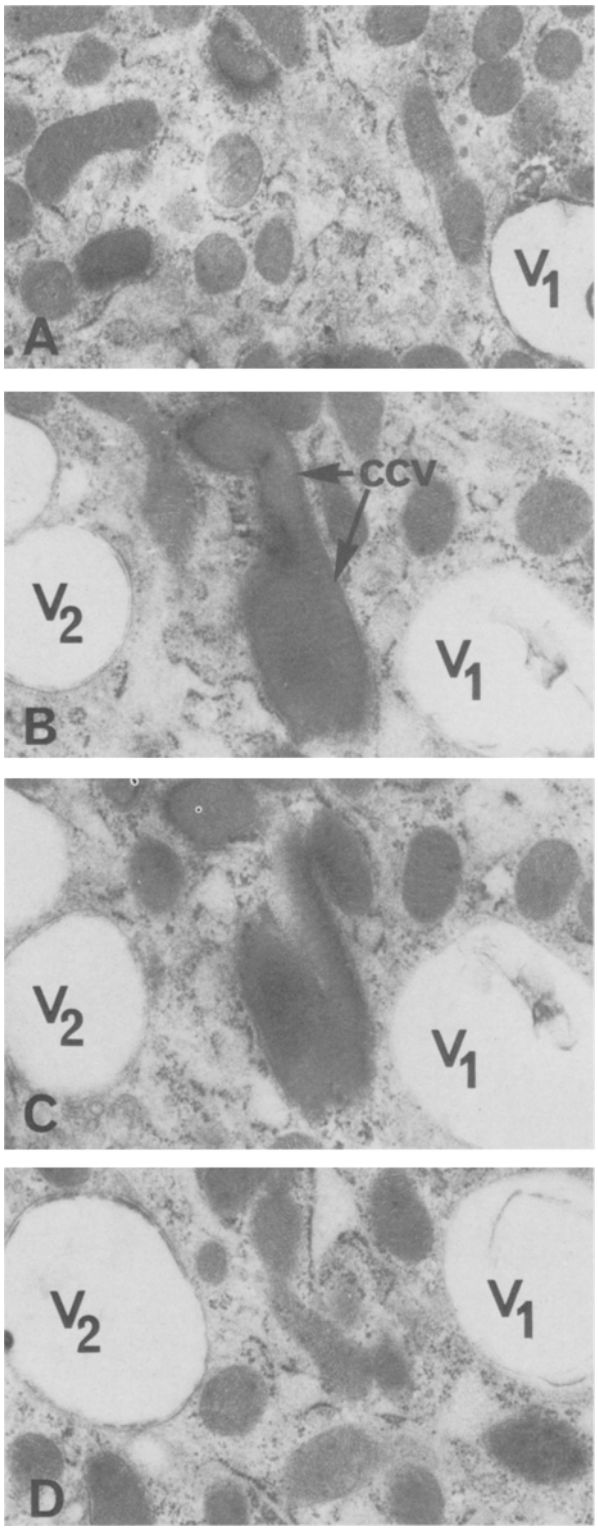

Fig. 5. Electron micrographs of a collagen-containing vacuole (*ccv*) followed in serial sections of the osteoclast shown in **Fig. 2.** Micrographs were obtained from a series of 40 consecutive sections; **Fig. 5A** was taken from section no. 15, **5B** from no. 17, **C** from no. 18, and **D** from no. 20. V_1 and V_2 represent cytoplasmic vacuoles (not demineralized). ×24,000.

fibrils has not been reported before in the literature for osteoclasts. Collagen fibrils enclosed in cytoplasmic vacuoles are often seen in other cell types, such as fibroblasts and macrophages. With regard to the latter cell types, several lines of evidence indicate that following the uptake of collagen from the extracellular space the fibrils are digested by lysosomal enzymes (reviewed by Melcher and Chan) [23].

On the basis of our observations we propose that in the case of pycnodysostosis, similar degradative processes may occur in osteoclasts. This together with the observation of mineral-containing vacuoles in one and the same osteoclast lends support to the view (1–4) that bone mineral as well as bone collagen may be degraded by one and the same cell. The occurrence of two sets of cytoplasmic vacuoles, one containing mineral crystals and the other containing collagenous fibrils, would seem to indicate that in case of pycnodysostosis the two major components of bone are degraded in separate parts of the vacuolar system. Whether collagen is taken up by the osteoclasts as large bone matrix fragments or as single fibrils that are subsequently piled up in large vacuoles, is not clear.

Our observations indicate the potential capacity of osteoclasts to phagocytose the collagenous matrix of bone. Since this capacity does not seem to be expressed in bone of normal individuals [2, 5, 17, the present study], it seems likely that in the normal situation bone collagen is broken down in a somewhat different fashion: in the extracellular space under the influence of collagenase [4, 24] and/or other proteolytic enzymes [4]. In the light of this possibility it is tempting to suggest that in pycnodysostosis the extracellular pathway of collagen breakdown is inhibited. This could possibly be due to matrix defects or to defects in the enzyme machinery of the osteoclasts. Alternatively, our observations would fit in with the hypothesis that degradation of bone collagen normally occurs in the vacuolar apparatus of the osteoclast, but at such a high rate that the chance of observing collagen-containing vacuoles in the cytoplasm is extremely small. If the latter view holds true, the intracytoplasmic accumulation of collagen fibrils in osteoclasts would point to a disturbance in the intracellular pathway of collagen breakdown.

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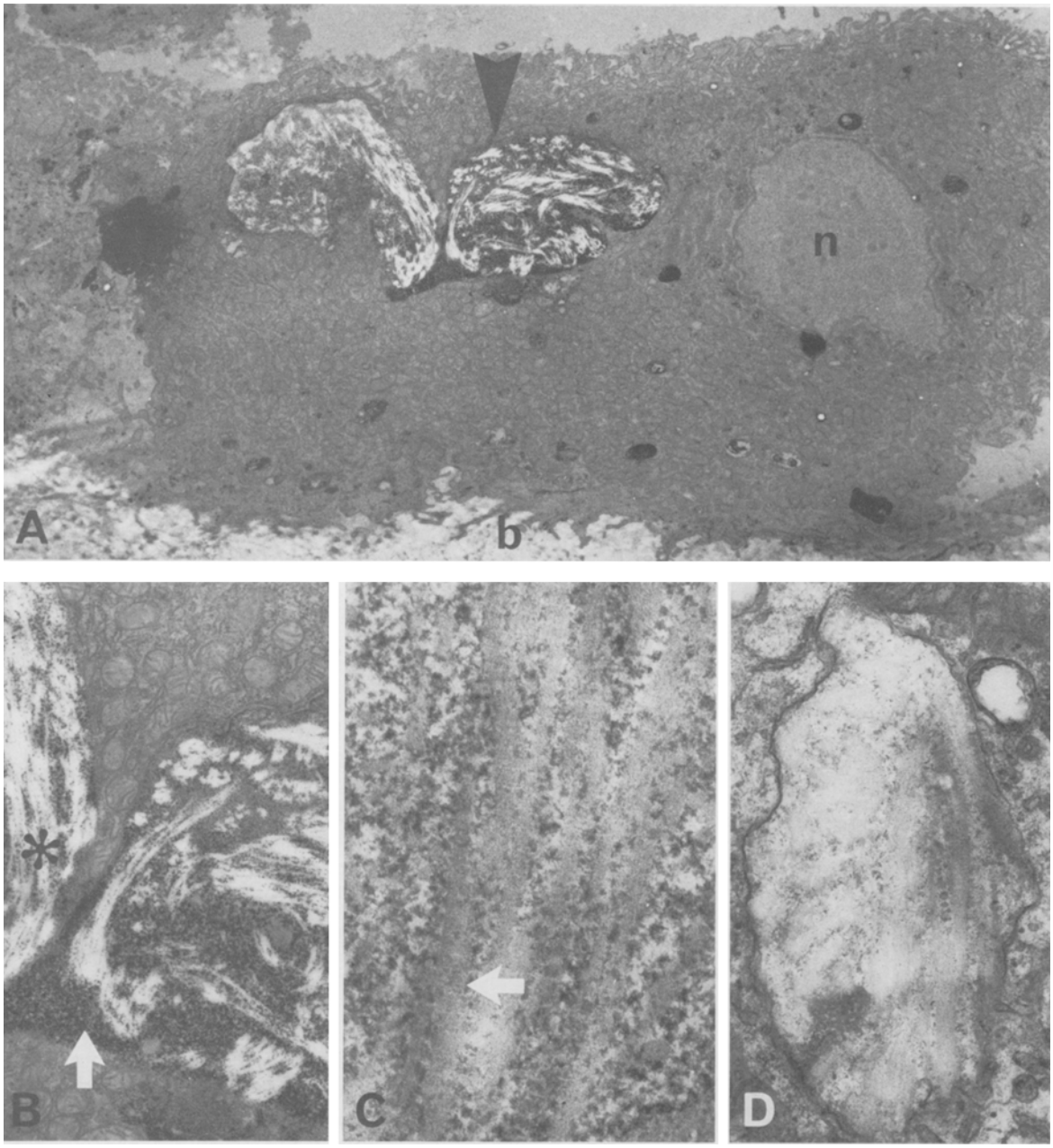

Fig. 6A. Low power electron micrograph of an osteoclast after staining for acid phosphatase activity. Note the presence of a large collagen-containing vacuole (*arrowhead*). *b:* bone, *n:* nucleus (demineralized). ×3,800. **B.** Higher magnification of vacuole indicated in **Fig. 6A.** Note the presence of lead precipitate (*arrow*), indicating acid phosphatase activity. ×12,600. **C.** Part of the same vacuole (see *asterisk* in **Fig. 6B**) in an adjacent section revealing the cross-banded pattern of collagen fibrils (*arrow*) after counter-staining with uranyl and lead. ×58,000. **D.** Collagen-containing vacuole of osteoclast incubated in the absence of substratum; counter-stained with uranyl and lead. ×27,000.

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