

CARTILAGE RESORPTION IN THE TIBIAL EPIPHYSEAL PLATE OF GROWING RATS

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ABSTRACT

An electron microscopic study of the tibial epiphyseal plates of growing rats reveals that the resorption of unmineralized and mineralized cartilage occurs by two different mechanisms. During resorption the unmineralized transverse cartilaginous walls between chondrocytes are invaded by capillary sprouts. At the resorption zone, numerous cytoplasmic processes derived primarily from the perivascular cells and, to a lesser extent, from the endothelial cells of the sprouts penetrate and appear to lyse the unmineralized transverse cartilaginous walls. Hydrolases released from the degenerating chondrocytes and/or capillary sprouts may also participate in this process. The second resorption mechanism involves the mineralized longitudinal cartilaginous septa. Resorption of these septa is mediated by chondroclasts whose fine structure is identical with that of osteoclasts. The active surface of the chondroclasts has a ruffled border. The surface membrane of the chondroclasts is relatively smooth on either side of the ruffled border and lies in direct apposition with the underlying mineralized cartilage. This observation suggests that the microenvironment in the zone of resorption may be maintained by the neighboring unruffled surfaces of the chondroclasts, which thus seal off and segregate the active portions of these cells.

INTRODUCTION

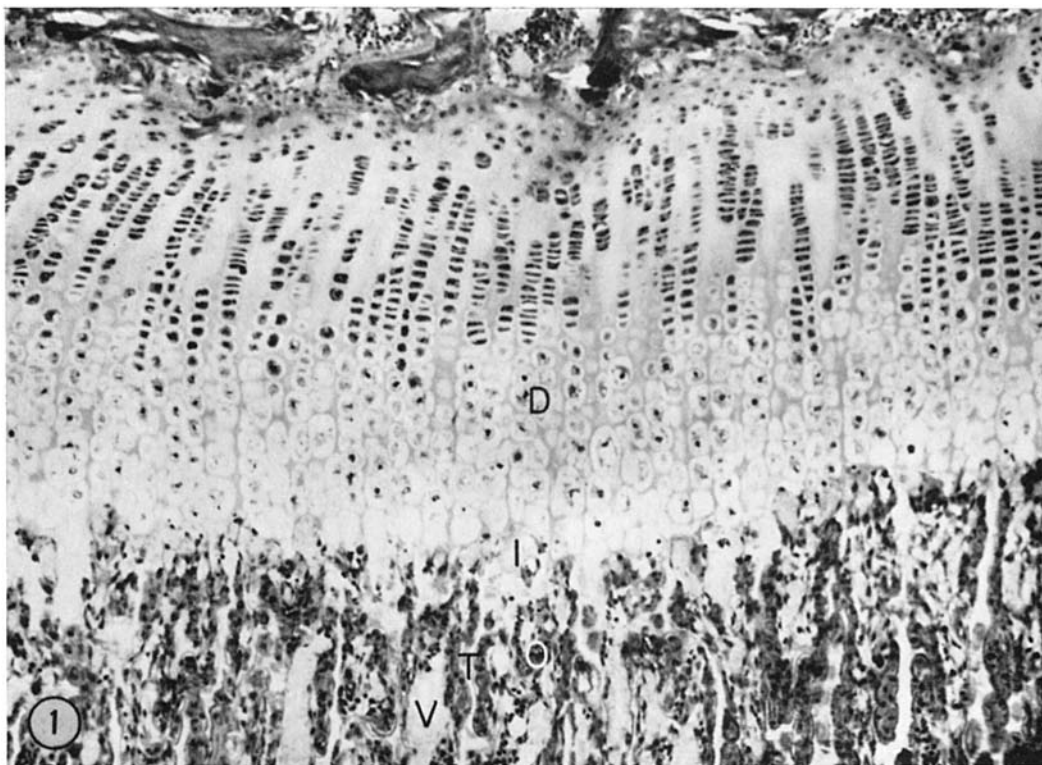
While the fine structural features of bone resorption are well defined (1-4) the ultrastructural features of cartilage resorption remain unclear. Cameron has suggested that the mineralized cartilage in the epiphyseal plate is eroded by capillaries (5). On the other hand Anderson and Parker have stated that demineralization of the cartilage in the growth plate must occur before the matrix is resorbed by such vessels (6).

In this study the resorption of cartilage in the tibial epiphyseal plate of growing rats has been investigated by electron microscopy. The present observations indicate that the resorption of unmineralized and mineralized cartilage occurs by two different mechanisms. The resorption of un-

mineralized cartilage is mediated by capillaries while the erosion of mineralized cartilage is mediated by chondroclasts.

MATERIALS AND METHODS

Eight rats weighing 100-125 g were used in this study. The proximal ends of the tibiae were excised from anesthetized animals, quickly split in the sagittal plane, and immersed immediately in 6.25% cacodylate- or phosphate-buffered glutaraldehyde (pH 7.6). The growth cartilage and adjacent portions of the metaphysis were excised from these strips under a dissecting microscope and trimmed into blocks measuring approximately 3-4 mm in length and 1 mm in thickness. The trimmed blocks were further fixed in glutaraldehyde for 3 hr, rinsed several times



FIGURES 1 and 2 are light micrographs of the epiphyseal plate, and Figs. 3-12 are electron micrographs of the zone of vascular ingrowth. The figures are mounted in such a manner that the proximal epiphysis is located at the top of each figure. The illustrations utilized are micrographs of unstained sections.

FIGURE 1 The zone of vascular invasion (*I*) lies between columns of degenerating chondrocytes (*D*) and the metaphyseal zone of ossification (*O*). The vessels (*V*) in the metaphysis are separated by primary trabeculae (*T*). $4\ \mu$ thick, undecalcified, methacrylate-embedded section, stained by the Goldner technique. $\times 100$.

with the buffer solutions, and stored overnight in the cold. The blocks of tissue were subsequently postfixed in 2% Veronal-acetate-buffered osmium tetroxide (pH 7.4) with added sucrose for 4 hr, dehydrated in acetone, and embedded in Araldite, or dehydrated in alcohol and embedded in Epon. The blocks of tissue were so oriented in the embedding capsules that longitudinal planes presented. $2\ \mu$ thick sections of the plastic embedded material were examined under the phase contrast microscope. The blocks were oriented in the microtome such that the thin sections cut with the diamond knife represented almost perfect longitudinal planes. Demineralization of the tissues in the knife boat was minimized by using distilled water whose pH had been adjusted to 7.0 by the addition of dilute sodium hydroxide, and also by removing the thin sections from the knife boat as quickly as possible (7).

The degree of demineralization occurring as a

consequence of staining (1) was assessed by examination of unstained thin sections. Similar sections stained with uranyl acetate and lead citrate, lead hydroxide, or 2% phosphotungstic acid in water were also examined under a Siemens Elmiskop I electron microscope. Blocks of tissue containing the epiphyseal plate and adjacent portions of the metaphysis were also fixed in 10% neutral buffered formalin and embedded in methacrylate. $4\ \mu$ thick sections of this material were cut on a Jung microtome 1120, stained by the Goldner technique as well as by Kruttsay's modification of the von Kossa method, and examined under the ordinary light microscope (8, 9).

OBSERVATIONS

Light Microscope Observations

The histological features of the epiphyseal plate have been well described by Dodds and other

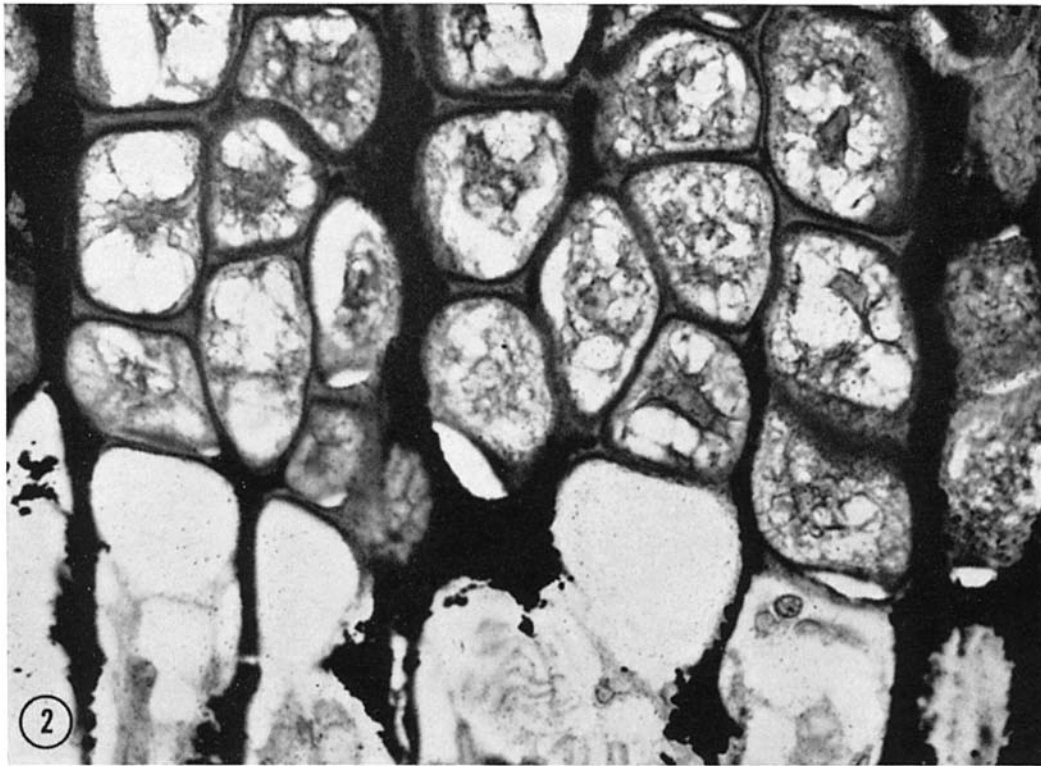


FIGURE 2 Note the deposition of mineral within some of the longitudinal septa between the chondrocytes. The transverse walls between cartilage cells, the superficial portions of the longitudinal septa, and the capsules surrounding the chondrocytes are unmineralized. Methacrylate-embedded section, stained by modified von Kossa method. $\times 750$.

workers (10–13), and will only be summarized here. The chondrocytes in the growth cartilage are arranged in longitudinal columns (Fig. 1). The cartilage cells that lie adjacent to the zone of vascular ingrowth display degenerative changes (Figs. 1 and 2). The cartilaginous matrix between degenerating chondrocytes exhibits a specific pattern of mineralization. About two-thirds of the longitudinal septa are partially or completely mineralized, while the remainder are essentially unmineralized (Fig. 2). The capsule surrounding individual cartilage cells is never mineralized. Since the transverse walls between cartilage cells and the superficial portions of the longitudinal septa are composed of capsular material, these are also unmineralized. Numerous elongated distended capillaries, which are oriented in the longitudinal axis of the bone, penetrate the growth cartilage (Figs. 1 and 2). In the metaphysis these vessels are separated by primary trabeculae, which are fewer

in number than the longitudinal septa, the number of primary trabeculae being approximately one-half of the number of longitudinal septa between the columns of cartilage cells (Fig. 1).

Electron Microscopic Observations

The zone of vascular ingrowth is subdivided into compartments by the remaining longitudinal cartilaginous septa (Fig. 3). These compartments are occupied by single, more or less centrally located blood vessels that resemble the capillary sprouts described in granulation tissue (14,15). The endothelium lining these vessels is discontinuous and contains numerous cisternae of rough-surfaced endoplasmic reticulum and clusters of ribosomes not associated with membranes, prominent Golgi complexes, and occasional dense bodies. The basement membrane between endothelial and perivascular cells is barely discernible and frequently absent. The perivascular cells form a sin-

gle incomplete layer around the endothelium (Fig. 3). The pericytes resemble the endothelial cells, but dense bodies are more numerous within their cytoplasm (Figs. 3 and 5). The lumina of the sprouts are filled with electron-opaque material derived from plasma (Figs. 3, 5, 7, and 12) and contain numerous erythrocytes, platelets, and other hematogenous elements. Similar hematogenous cells may be found emigrating through the discontinuities of the endothelium as well as extravascularly admixed with material derived from plasma (Figs. 3 and 11).

The lacunae adjacent to the region of vascular ingrowth contain chondrocytes that exhibit varying degrees of degeneration, such as dissolution of surface membranes and loss of cytoplasmic organelles and nuclei (Fig. 4) (16-19). The unbanded collagen fibrils in the central portions of the longitudinal septa tend to be oriented parallel to the long axis of the bone (Figs. 3 and 4). These fibrils are more randomly arranged in the superficial portions of the longitudinal septa and the transverse walls (Fig. 4). The latter regions correspond with the capsules seen by light microscopy.

Two-thirds of the longitudinal septa that lie adjacent to the zone of vascular ingrowth contain large aggregates of electron-opaque crystals which measure from 300 Å to 1200 Å in length and about 50 Å in the other dimension. These hydroxyapatite crystals are not found in the superficial portions of the longitudinal septa (Fig. 4). The transverse walls between individual chondrocytes in the cell columns also lack mineral (Figs. 3 and 4). However, wedge-shaped spurs of mineral may occasionally protrude from the mineralized longitudinal septa into the adjacent transverse walls for short distances (3-4 μ) (Fig. 4). The degree of mineralization within the longitudinal walls increases progressively as the region of vascular invasion is approached.

Numerous branching, elongated cytoplasmic processes of the perivascular cells which interdigitate with less numerous but similar processes of the endothelial cells penetrate the areas where the unmineralized cartilage is being resorbed (Figs. 5 and 6). Many processes, cut in various planes of section and seemingly detached from their cells of origin, are also frequently seen in these areas. The cytoplasmic processes of the perivascular and endothelial cells frequently surround or partially enclose remnants of the cartilage matrix (Fig. 6). These processes have a high electron opacity, contain large numbers of filaments measuring about 60 Å in diameter, and are devoid of cytoplasmic organelles. The surfaces of the processes are frequently coated with electron-opaque material which is partially globular and partially filamentous in appearance. Similar material is also seen free within the matrix near the processes. This form of resorption occurs in the transverse wall immediately adjacent to the tip of the sprouts as well as in the superficial unmineralized portions of the longitudinal septa that confine the compartment containing the vessel.

The resorption of mineralized matrix begins one lacuna distal to the tips of the capillary sprouts and is always associated with a chondroclast (Fig. 10). The chondroclasts (Fig. 7) are morphologically identical with osteoclasts (20-22), and the removal of the mineralized cartilage occurs in the manner described by previous investigators (1-4). In addition to the cytological features enumerated by other workers (1-4, 21, 22) the chondroclasts and osteoclasts present in the metaphysis contain many vesicles exhibiting oval, rounded, and elongated profiles (Figs. 8-11). These vesicles are most numerous near the peripheral portions of the cells (Figs. 9-11) and contain granular material of varying electron opacity (Figs. 8 and 9). A relatively clear zone may be present between the

FIGURE 3 The unmineralized transverse wall (*W*) separates the compartment containing the advancing capillary sprout from the degenerated chondrocyte in its lacuna. The longitudinal septa show different degrees of mineralization. Large numbers of erythrocytes and electron-dense material are seen in the lumen of the sprout. Several erythrocytes and electron-dense material are also seen outside this vessel. Two macrophages (*M*) are present between the perivascular cell (*P*) and the longitudinal septum in the right-hand portion of the figure. The perivascular cells contain several dense bodies. A chondroclast (*C*) is situated between the endothelium (*E*) and the longitudinal septum near the lower right-hand margin of the figure. Note the longitudinal orientation of the collagen fibrils in this septum. $\times 4000$.



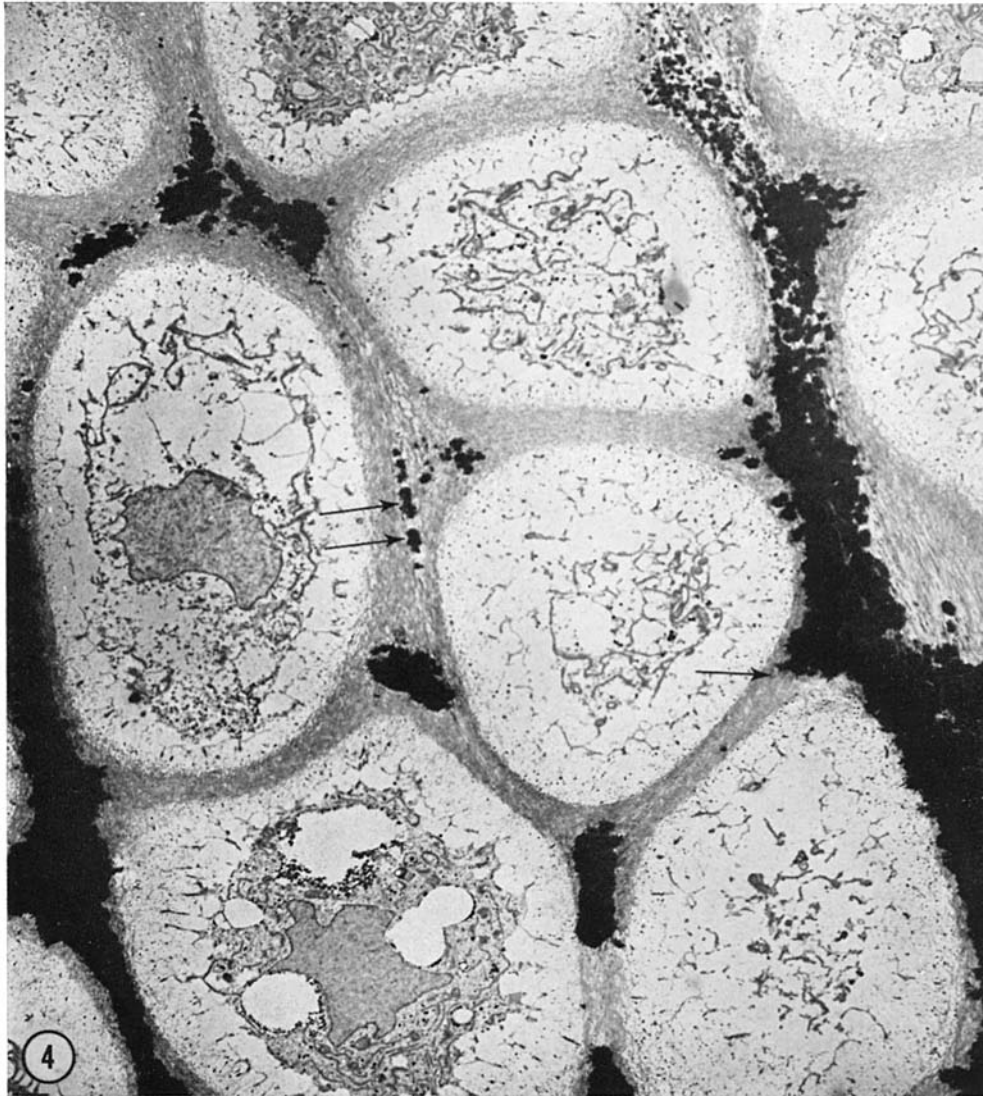


FIGURE 4 Columns of degenerating chondrocytes overlie the zone of vascular invasion. Note the lack of mineral in the transverse walls as well as in the superficial portions of the longitudinal septa. A mineralized spur (arrow) projects into the transverse wall from the adjacent mineralized septum. Small aggregates of mineral (double arrows) are seen within another septum. $\times 3000$.

single limiting membrane of these organelles and their finely granular contents. The vesicles are frequently seen near the large peripheral cytoplasmic vacuoles of the chondroclasts (Figs. 9-11). Some of the vesicles and saccules of the Golgi apparatus are partially filled with similar electron-opaque material (Fig. 8). Numerous crystals measuring up to 2500 Å in length and 50 Å in

the other dimension are present between the microvilli of the ruffled borders in tissues processed with phosphate buffer (Figs. 10 and 11). On the other hand, such crystals are not seen in the resorption zones of tissues processed in cacodylate buffer. On either side of the ruffled border the surface membrane of the chondroclast is relatively smooth and lies in close contact with the somewhat

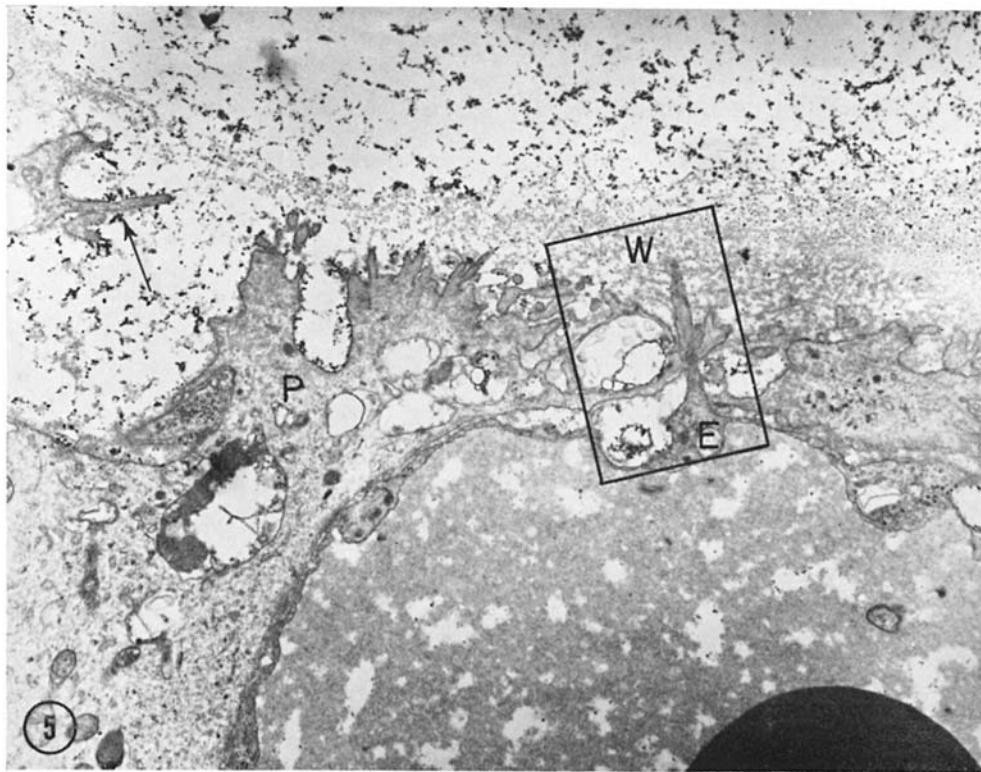


FIGURE 5 Numerous electron-opaque interdigitating processes of the perivascular (*P*) and endothelial (*E*) cells penetrate this transverse wall (*W*). Note the decrease in the thickness as well as the decreased number of collagen fibrils within this wall. Several large vacuoles containing amorphous debris are seen in the cytoplasm adjacent to the processes. Several isolated processes (arrow) are also present in the upper left-hand portion of the figure. The area enclosed within the rectangle is shown at higher magnification in the following figure. $\times 10,000$.

irregularly contoured surface of the underlying mineralized cartilage (Figs. 10 and 11). Those portions of the cytoplasm adjacent to the smooth surfaces of the chondroclasts, and previously termed clear zones (4, 21), are devoid of cytoplasmic organelles and have a uniform appearance.

Monocytes and/or macrophages, as well as blood vessels, are frequently found near those surfaces of the chondroclasts that are not in contact with the mineralized cartilage (Figs. 7 and 11). Small aggregates of hydroxyapatite crystals are also found occasionally within cytoplasmic vacuoles of the perivascular and/or endothelial cells in regions of mineralized cartilage that are undergoing resorption (Fig. 12).

DISCUSSION

There is considerable disagreement in the literature as regards the pattern of mineral deposition within the epiphyseal plate (5, 6, 23, 24). The present observations are in agreement with those workers who have stated that the transverse walls are not mineralized (12, 20, 25). The apparent occurrence of mineral occasionally within the transverse walls appears to be the result of the imperfect longitudinal orientation of the cell columns and their consequent oblique sectioning. The present observations also indicate that there is a superficial layer of unmineralized cartilage matrix between the lacunar surfaces and the mineralized cartilage in the deeper portions of the longitudinal septa.

The resorption of the two different types of cartilage found in the epiphyseal plate, namely unmineralized and mineralized, occurs by two different mechanisms. The first involves the resorption of unmineralized cartilage by the capillary sprouts while dissolution of the latter appears to be mediated by chondroclasts. Numerous processes derived from the walls of the sprouts are invariably found in the region where the unmineralized cartilage is undergoing resorption. Whether the dissolution of this cartilage results from direct penetration by these cytoplasmic processes, or from the release of enzymes or other constituents from the perivascular and/or endothelial cells, is not known. Dense bodies resembling lysosomes have been noted in both the perivascular and endothelial cells of the sprouts. These lysosomes may play a role in the dissolution of cartilage since lysosomal enzymes have been previously implicated in this regard (26-30).

It is well established that degeneration of the overlying chondrocytes in the cell columns occurs concomitantly with the invasion of the lacunae by the sprouts. It has also been shown that cartilage cells release hydrolases that cause dissolution of the cartilaginous ground substance (28, 30). Such a mechanism could contribute to the resorption of cartilage.

The occasional clusters of mineral within cytoplasmic vacuoles of perivascular and/or endothelial cells are probably remnants of mineralized cartilage that has been previously eroded by chondroclasts. In contrast to a previous interpretation that macrophages play a significant role in the resorption of mineralized cartilage (6), mineral-containing vacuoles have not been observed in this study in cells with morphological characteristics typical of macrophages.

The resorption of mineralized cartilage is restricted to the region of the ruffled border of the chondroclasts. On either side of the ruffled border the surface membrane is smooth and lies in contact with the underlying unchanged mineralized cartilage. The smooth portions of the plasma mem-

brane may maintain the microenvironment in the zone of resorption by sealing off and segregating the resorption zones. The cytological and functional differences between the smooth and ruffled portions of the surface membranes of chondroclasts and osteoclasts probably account for the scalloping noticed by light microscopy in regions where both cartilage and bone are undergoing active resorption.

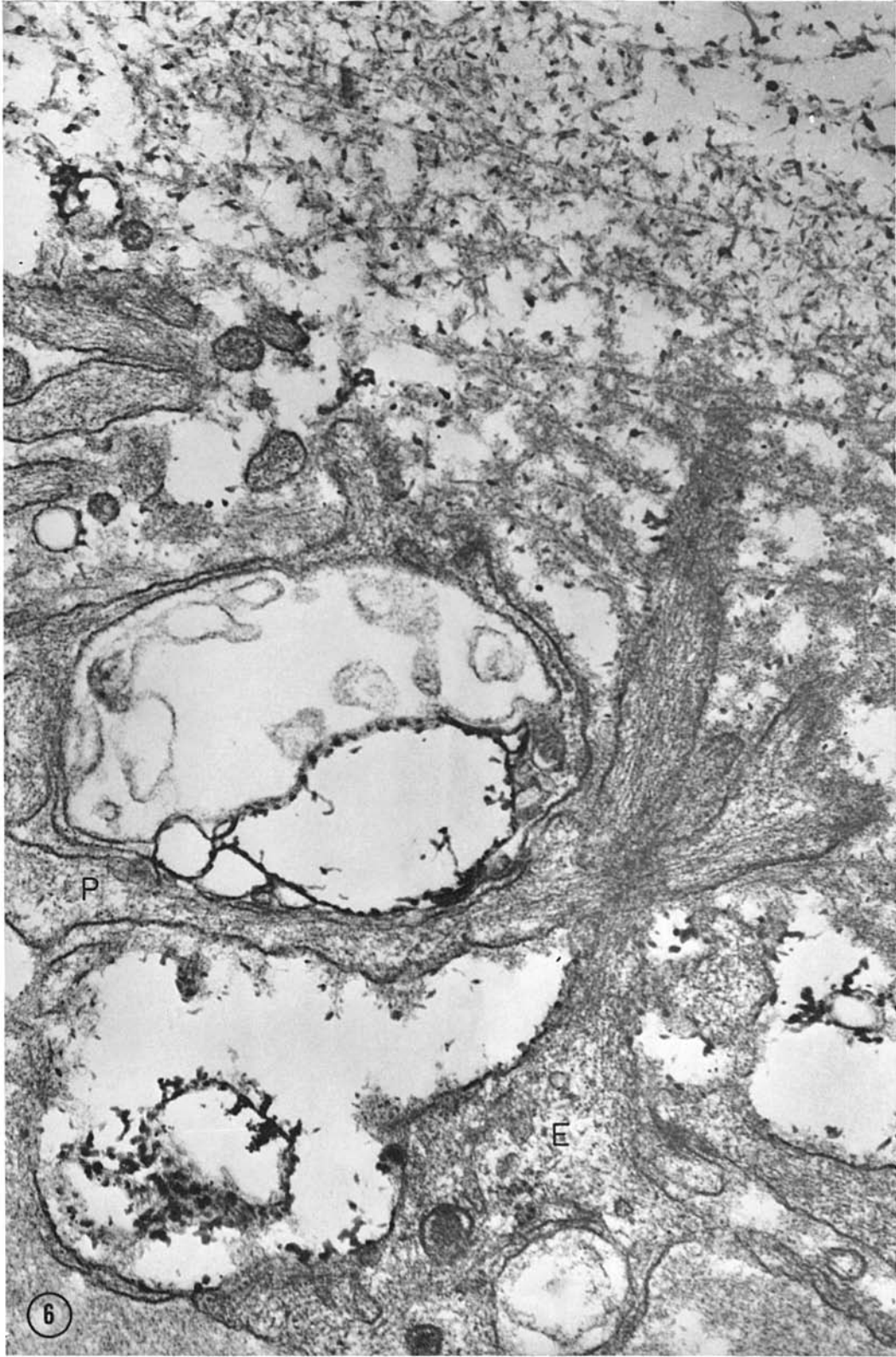
Numerous vesicles containing electron-opaque material have been observed in the cytoplasm of the chondroclasts. These structures resemble the lysosomal granules of leukocytes (31) and macrophages (32). The apparent association of these structures with the prominent Golgi complexes of the chondroclasts is in accord with evidence indicating that the Golgi apparatus is implicated in the formation of lysosomes (33, 34). Proof of the lysosomal nature of cytoplasmic particles depends on cytochemical staining. However, the presence of such structures within both chondroclasts and osteoclasts is in complete accord with the demonstration of acid phosphatase in these cells by histochemical techniques (35) and would provide a morphological basis for observations indicating that proteases released from lysosomes play a role in the dissolution of mineralized tissues (36, 37).

Since crystals noted between the folds of the ruffled border in the tissues fixed with the phosphate buffer are not seen in tissue processed with the cacodylate buffer it seems likely that they are artifacts of preparation. Such crystals, which have been previously described by other workers in phosphate-buffered tissues (38), may be composed of calcium phosphate that has formed by the interaction between calcium ions in high concentration in the areas of bone and cartilage resorption and the phosphate ions of the buffer.

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FIGURE 6 The processes of the perivascular (*P*) and endothelial (*E*) cells contain numerous filaments. These processes enclose globular and filamentous material which is also present in the overlying matrix. Similar material adheres to the surface membranes of the processes. The decrease in the number of collagen fibrils within the matrix is readily apparent. $\times 52,000$.



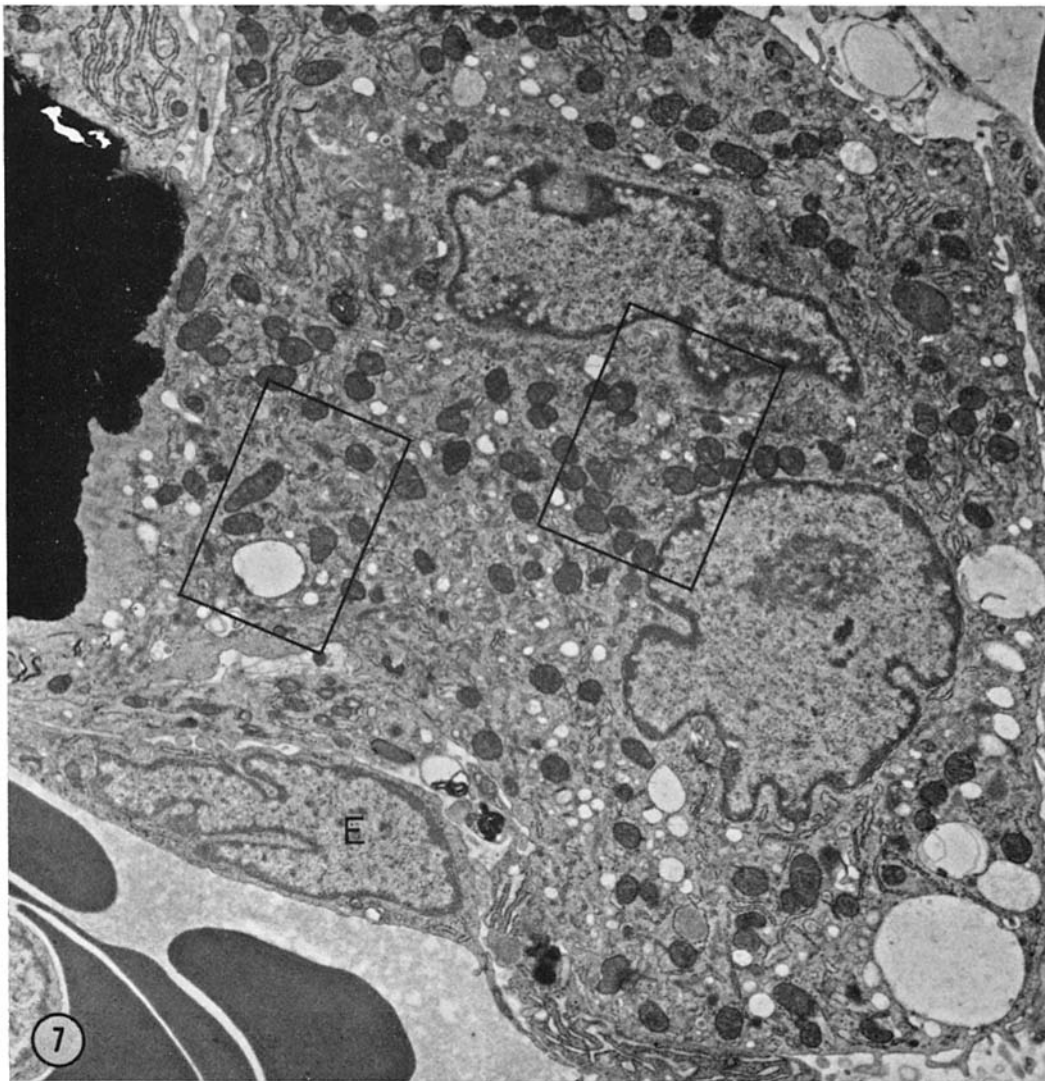


FIGURE 7 A multinucleated chondroclast lies in apposition with a mineralized longitudinal septum. The ruffled border is not included in this plane of section. The relatively smooth portion of the surface membrane is closely applied to the underlying mineralized cartilage. The endothelium (*E*) of a blood vessel abuts on another surface of this chondroclast. The areas enclosed within the rectangles are shown at higher magnifications in Figs. 8 and 9. $\times 7000$.

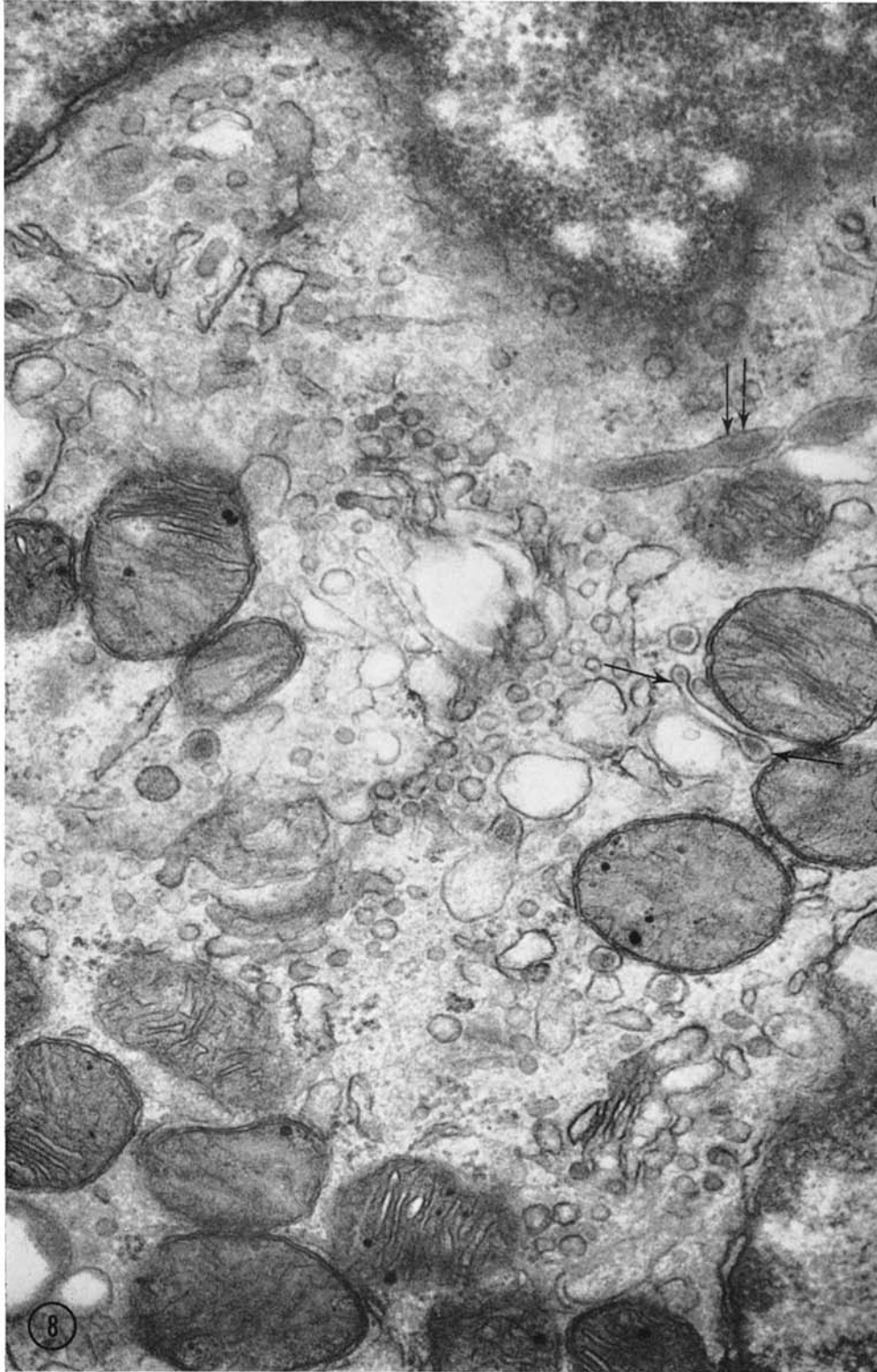


FIGURE 8 Vacuoles and saccules (single arrows) of the Golgi apparatus are partially filled with electron-dense material. A vacuole exhibiting elongated profiles (double arrows) contains similar material. $\times 51,000$.



FIGURE 9 Many vacuoles exhibit oval, rounded, and elongated profiles and contain granular electron-opaque material. $\times 55,000$.

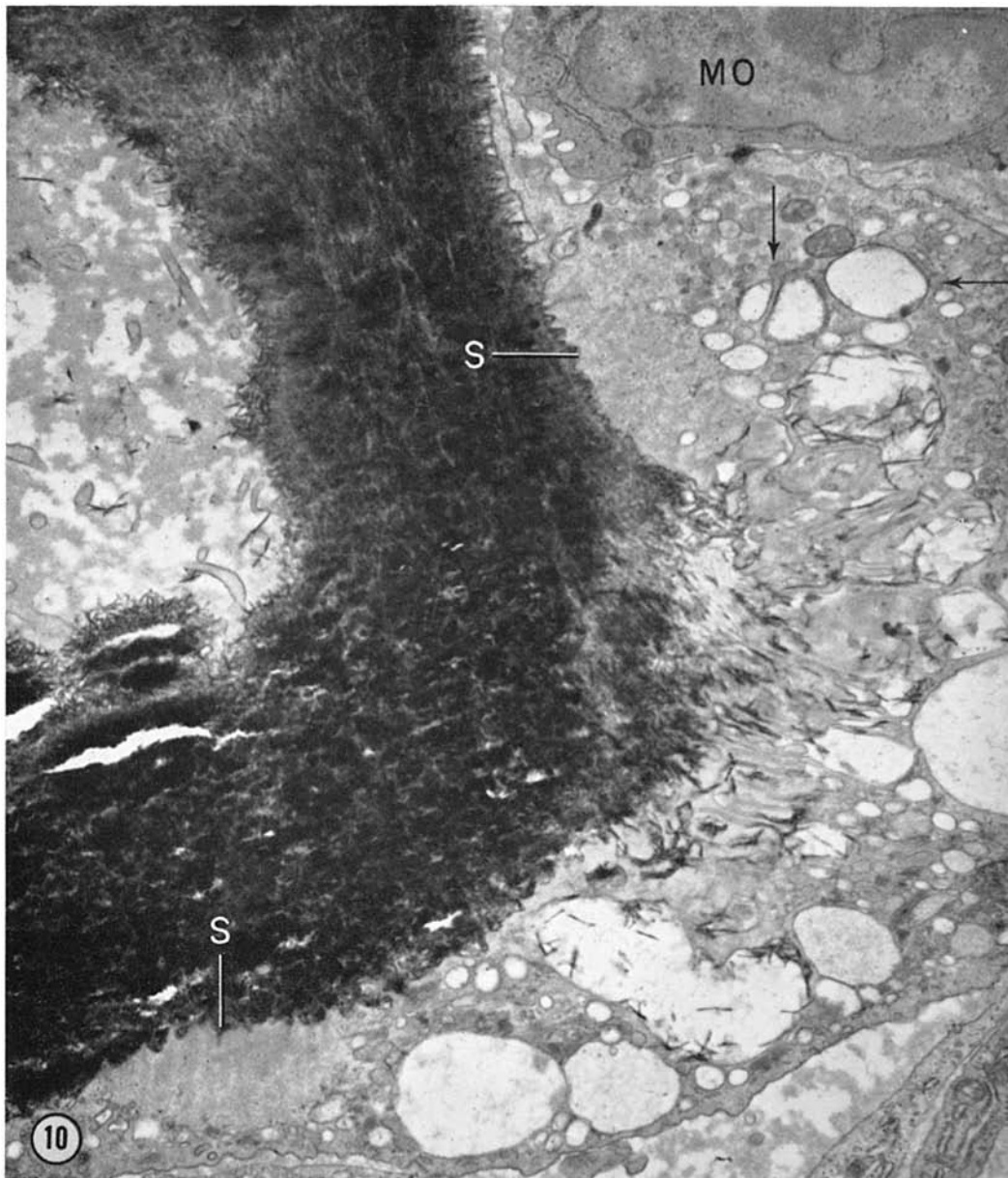


FIGURE 10 A chondroclast lies adjacent to a mineralized longitudinal septum. The smooth portions (*S*) of the cell surface membrane, which are in contact with the underlying mineralized tissue, lie on either side of the ruffled border. Numerous elongated crystals are seen within the microvilli of the ruffled border. Large empty vacuoles and numerous small vacuoles that are filled with material of varying electron opacity are seen in the adjacent cytoplasm. Some of these smaller vacuoles are found near the limiting membranes of the large vacuoles (arrows). A portion of a monocyte (*MO*) is seen in the upper right-hand portion of the figure. $\times 13,000$.

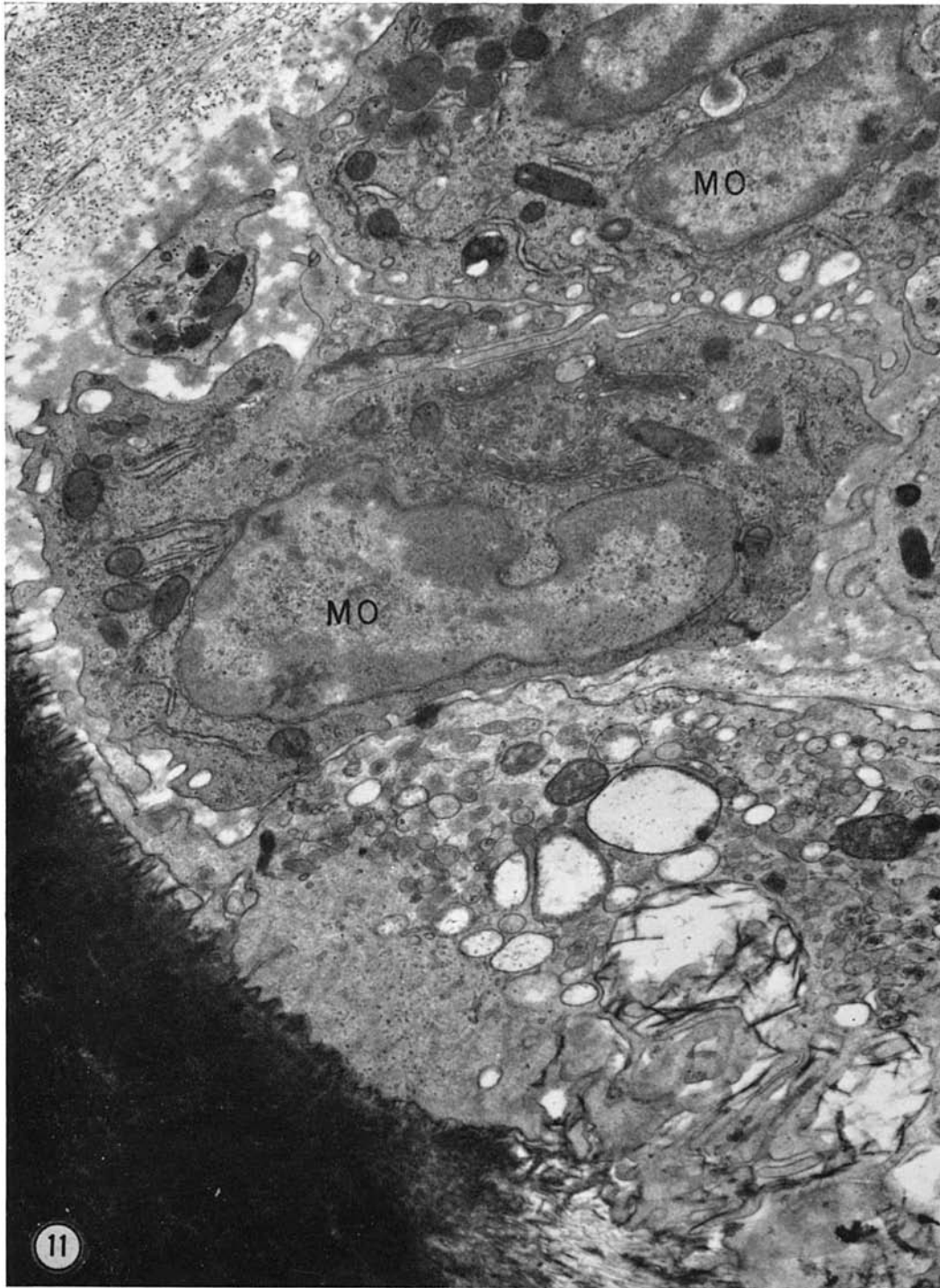


FIGURE 11 Similar to Fig. 10. Two monocytes (*MO*) are seen near that surface of the chondroclast which does not face the mineralized septum. $\times 19,000$.

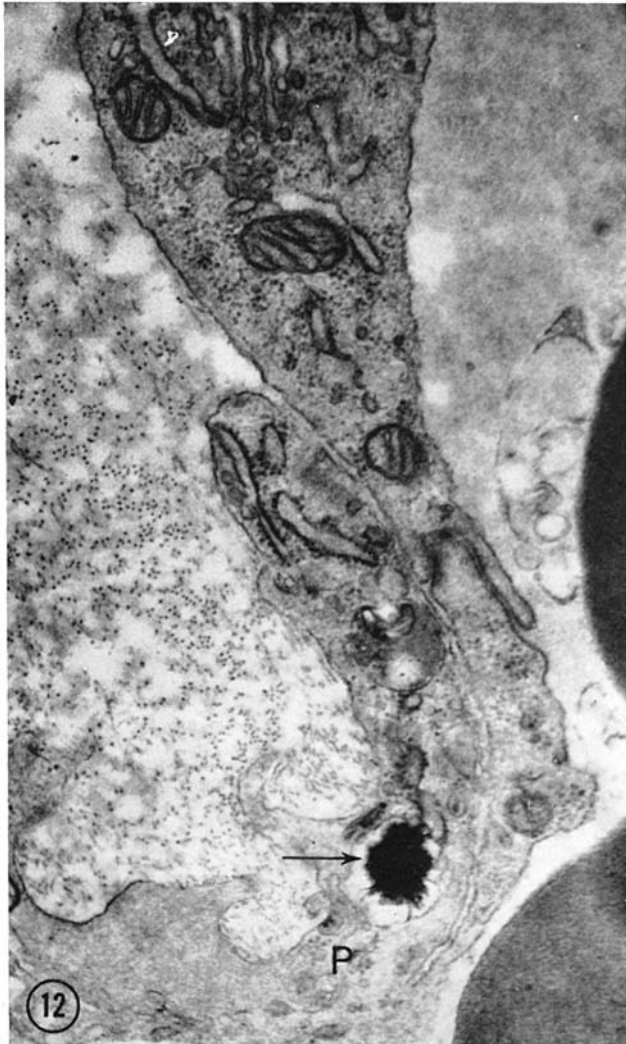


FIGURE 12 An aggregate of hydroxyapatite crystals is noted within the cytoplasmic vacuoles of a perivascular cell (P). $\times 60,000$.

For References See Page 290.

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