# THE FINE STRUCTURE OF BONE CELLS

H. ROBERT DUDLEY, M.D.,* and DAVID SPIRO, M.D.

From the Department of Pathology, Harvard Medical School, and the Edwin S. Webster Memorial Laboratory of the Department of Pathology, Massachusetts General Hospital, Boston. Dr. Spiro's present address is Department of Pathology, Columbia University, College of Physicians and Surgeons, New York.

## ABSTRACT

An electron microscopic study of Araldite-embedded, undecalcified human woven and chick lamellar bone is presented. The fine structure of the cells of bone in their normal milieu is described. Active osteoblasts possess abundant granular endoplasmic reticulum, numerous small vesicles, and a few secretion droplets. Their long cytoplasmic processes penetrate the osteoid. The transition of osteoblasts into osteoid osteocytes and then into osteocytes is traced and found to involve a progressive reduction of cytoplasmic organelles. Adjoining the osteocytes and their processes is a layer of amorphous material which is interposed between the cell surfaces and the bone walls of their respective cavities. Osteoclasts contain numerous non-membrane-associated ribosomes, abundant mitochondria, and little granular endoplasmic reticulum, thus differing markedly from other bone cells. The brush border is a complex of cytoplasmic processes adjacent to a resorption zone in bone. No unmineralized collagen is seen at resorption sites and it appears that collagen is removed before or at the time of mineral solution. All bone surfaces are covered by cells, some of which lack distinctive qualities and are designated endosteal lining cells. The structure of osteoid, bone, and early mineralization sites is illustrated and discussed.

The ultrastructure of bone has not been extensively studied owing to the many technical obstacles. Some of the structural features of osteoblasts and osteoclasts have been described as they appeared in embryonic fowl and in kittens (7, 23). Previous studies have shown that the bone lesions in patients with polyostotic fibrous dysplasia contain coarse woven bone in small trabeculae and that this tissue can be cut easily without prior decalcification (12). The purpose of this paper is to present results of a study of the ultrastructure of bone cells as they appear in coarse fiber bone and in newly formed lamellar cancellous bone.

## MATERIALS AND METHODS

Osseous tissues obtained from four biopsies of three patients with polyostotic fibrous dysplasia were selected to allow study of coarse woven bone. Cancellous bone specimens from the sternum of ten-week-old white Leghorn chicks were selected for study of newly formed lamellar bone.

The fresh specimens were immediately placed in buffered 1 per cent osmium tetroxide with sucrose added at 4°C (2), and while immersed they were cut into smaller pieces. After 4 hours' fixation at 4°C, dehydration at room temperature was accomplished in 50, 70, 90, and 95 per cent acetone solutions for ½ hour each, followed by three changes, 1 hour each, of absolute acetone. Infiltration in an Araldite medium with the proportions of 5 ml resin, 5 ml hardener, and 0.2 ml dibutyl phthalate was carried out at room temperature for 18 hours (26). Two changes of the infiltrating medium for 2 additional hours each were followed by embedding in an Araldite mixture identical with the infiltrating mixture except that it contained also 0.35 ml of accelerator. After the specimens had remained for 2 to 5 days in an oven at 60°C, thick sections of approxi-

* Deceased.



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mately 2 $\mu$ were cut using glass knives and a Porter-Blum microtome. These sections were examined under a phase contrast microscope and the block was further trimmed for the desired area. Thin sections were cut with a diamond knife. The grids used were covered with a lightly carbon-coated Formvar film. An RCA EMU 3B electron microscope was used for most of the study, although a Siemens Elmiskop I was used occasionally. In order to enhance the contrast of collagen fibers in this Araldite-embedded material, the sections were "stained" with aqueous and ethyl alcoholic solutions of potassium permanganate, uranyl acetate, and phosphotungstic acid. A limitation of the staining methods under a variety of pH and ionic conditions was the fact that they all produced some degree of demineralization as compared with unstained sections from the same block.

## RESULTS

The major difference between coarse woven bone and lamellar bone resides in the arrangement of the bundles of mineralized collagen fibers. The

*Abbreviations Used in the Figures*

*AM*, amorphous material
*B*, bone
*Bl*, osteoblast
*C*, cisternal dilatation
*Cl*, osteoclast
*D*, dense secretory droplet
*ELC*, endosteal lining cell
*ER*, endoplasmic reticulum
*G*, Golgi
*M*, mitochondria
*N*, nucleus
*NP*, nuclear pore
*O*, osteoid
*Oc*, osteocyte
*OOc*, osteoid osteocyte
*P*, cell process
*R*, ribosome
*s*, disrupted cell surface membrane
*V*, large vacuole
*v*, vesicle

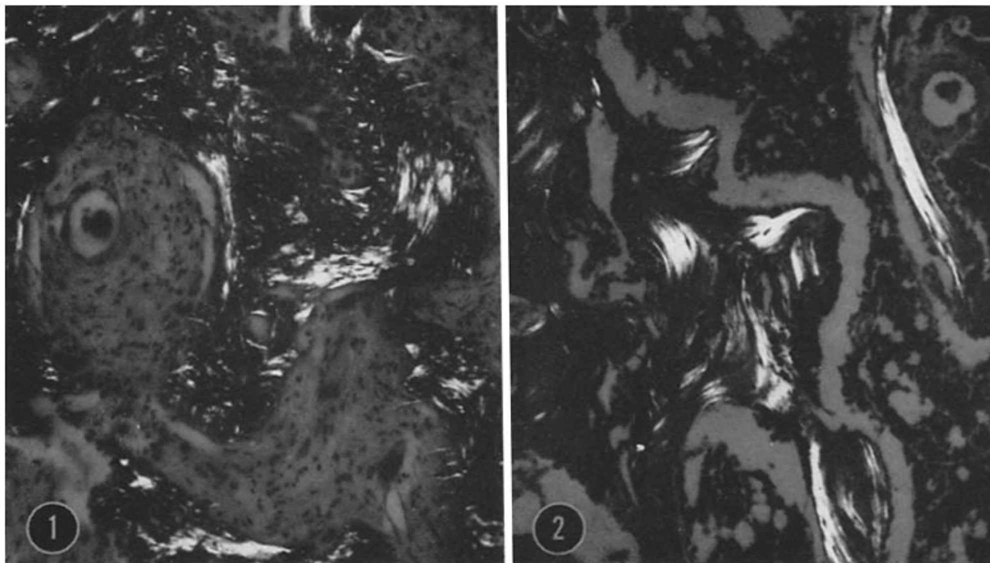


FIGURE 1

In the coarse woven bone illustrated, fiber bundles in the decalcified trabeculae are not in parallel strata but form a tweed-like pattern. Photomicrograph × 120, partially polarized.

FIGURE 2

The newly formed lamellar bone trabeculae contain fiber bundles in strata. Photomicrograph × 120, partially polarized.



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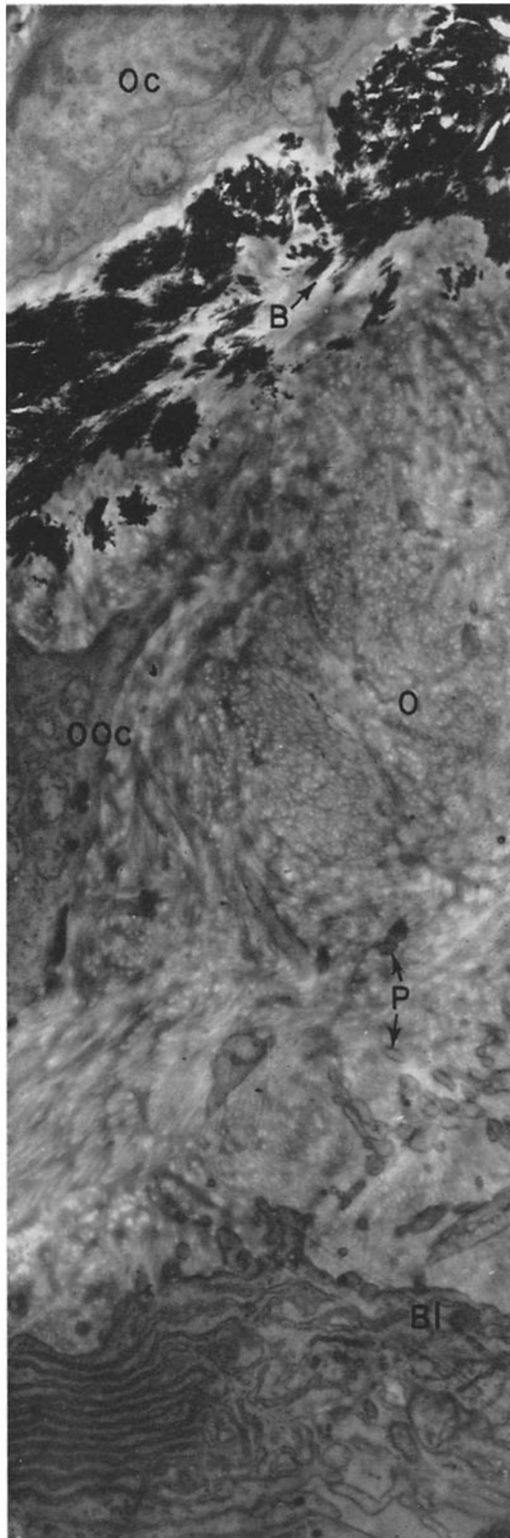


coarse woven bone trabeculae are composed of bundles of mineralized collagen of irregular size which are randomly arranged (Fig. 1). In the lamellar bone, however, the trabeculae are made up of small bundles of mineralized collagen which run in strata and which tend to change direction in successive strata (Fig. 2). Another difference between the two types of bone is that the osteocytes of lamellar bone are fusiform with the long diameter parallel to the axis of the lamella, whereas the osteocytes in woven bone are more rounded.

The relatively great density of bone mineral allows easy distinction between zones of mineralized collagen and unmineralized osteoid (Fig. 3). The cells associated with these different layers will be described separately, but no distinction will be made with respect to tissue source since no cytologic differences are manifest.

*Osteoblasts*

Osteoblasts are numerous on both types of bone and no essential ultrastructural difference is found. The osteoblast is polarized in that the surface nearest bone has many cytoplasmic processes which may extend deep into the abundant subjacent collagen fibers of the osteoid (Figs. 3 and 6). The opposite surface of the cell has relatively few processes and the cell surface rests in a moderately dense amorphous matrix containing only a few small collagen fibers (Fig. 4).

Osteoblasts may have structural modifications of their cell surfaces at sites of contiguity. The commonest finding is for the apposed cell surfaces to show no alteration and for the surfaces to be separated by extracellular spaces of variable widths (Fig. 5). However, occasionally the cell surfaces are very closely apposed and are dense without apparent thickening (Fig. 4). These localized dense zones are approximately 1 $\mu$ long and are sharply delineated from the less dense cell surface with which they are in continuity. Another modification is a desmosome-like structure characterized by a zone, 0.1 to 0.3 $\mu$ long, where contiguous cell

FIGURE 3

Electron micrograph showing the relationships of an osteoblast (*Bl*), of an osteoid osteocyte (*OOc*), and of an osteocyte (*Oc*) to their respective environments. The thick layer of osteoid (*O*) is penetrated by osteoblast processes (*P*) and is much less dense than the mineralized osteoid (*B*) which surrounds the osteocyte. × 8,000.



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surfaces appear thickened and dense to electrons (Fig. 4). The interspace between these adjacent dense membranes is very narrow, and dense material is accumulated on the internal aspect of each membrane. Resolution of any further structure was not attempted. Often cell surfaces of adjacent osteoblasts are reflected around a bundle of collagen fibers, and cell processes penetrate the bundle (Figs. 5 and 6).

The most striking characteristic of osteoblast cytoplasm is the abundant endoplasmic reticulum (ER). The paired membranes may be free of small, dense particles, but most of them have these granules on the outer surface (Fig. 4). The granular endoplasmic reticulum can be followed in continuity for several micra and there are frequent anastomoses between adjacent paired membrane systems. The paired membranes enclose a lumen which varies in size from several hundred angstrom units to the dimensions of large cisternae (Fig. 4). The lumen contents are uniform, amorphous, and more dense to electrons than the surrounding cytoplasm. Continuity between the nuclear envelope and the granular ER is frequently seen (Fig. 5).

Many vesicles approximately 200 to 400 A in diameter are noted throughout the cell. They are intimately associated with the granular ER (Fig. 4). Their single membrane is free of granules, and the contents of their lumens are denser than the surrounding cytoplasm.

Moderate numbers of ovoid mitochondria having abundant cristae are noted in the cytoplasms. The outer mitochondrial membrane and the adjacent granular ER are often in very close apposition but definite continuity between them is not demonstrated (Fig. 6).

Smooth surfaced membranes in lamellae and small vacuoles are interpreted as Golgi apparatus (Fig. 8). Membrane-limited secretion-type droplets are found. The membranes surrounding these droplets are often very dense and have smooth outer surfaces with irregularly serrated internal surfaces (Figs. 5 to 7). In addition to the above described organelles, the cytoplasm contains occasional small, dense particles which are probably non-membrane-associated ribosomes.

The nucleus is not distinctive and has gentle folds or deep indentations in the nuclear envelope. One or more nucleoli composed of compactly grouped small particles are seen. Nuclear pores are often large (Fig. 7).

### *Osteoid*

Osteoid layers vary greatly in thickness in the tissues examined but uniformly contain unmineralized collagen fibers with a 640 A repeating period plus a number of interperiod bands (Fig. 8). The individual fibers vary in size, measuring approximately 200 A in diameter near the osteoblast cell surface while most fibers measure approximately 600 A in deeper loci (Fig. 8). Although osteoblast processes are more numerous in the superficial osteoid, cell processes are encountered also in the deepest layers (Figs. 3 and 6). The transition zone between osteoid and mineralized osteoid, bone, is relatively abrupt (Figs. 3 and 10). There is no gradient of mineralization in these zones. The collagen fibers in the osteoid contain no mineral, whereas the collagen fibers in the adjacent bone contain abundant mineral (Figs. 3 and 10). Very rarely, however, partially mineralized collagen fibers are present in the osteoid zone. Here small, dense mineral particles are seen along the surface of the collagen fibers as well as within the collagen fibers when the latter are sectioned transversely (Fig. 9).

In osteoid, one frequently encounters a cell that is surrounded on all surfaces by osteoid (Fig. 3). This cell appears to be a transition between an osteoblast and an osteocyte and is here called an

FIGURE 4

Stratified osteoblastic cells show increasing cytoplasmic complexity as the osteoid (*O*) is approached. Many osteoblast processes (*P*) penetrate the osteoid on one cell surface, and collagen fibers are absent on the other. There are cisternal dilations (*C*) of the lumens enclosed by granular endoplasmic reticulum (*ER*), and several desmosome-like structures between contiguous cells (arrows). Double arrows point to another type of differentiated cell surface composed of two dense cell membranes separated by a narrow extracellular space of uniform width. Numerous small vesicles (*v*) in the osteoblast (*Bl*) are widely dispersed. Stained with potassium permanganate. × 11,000.



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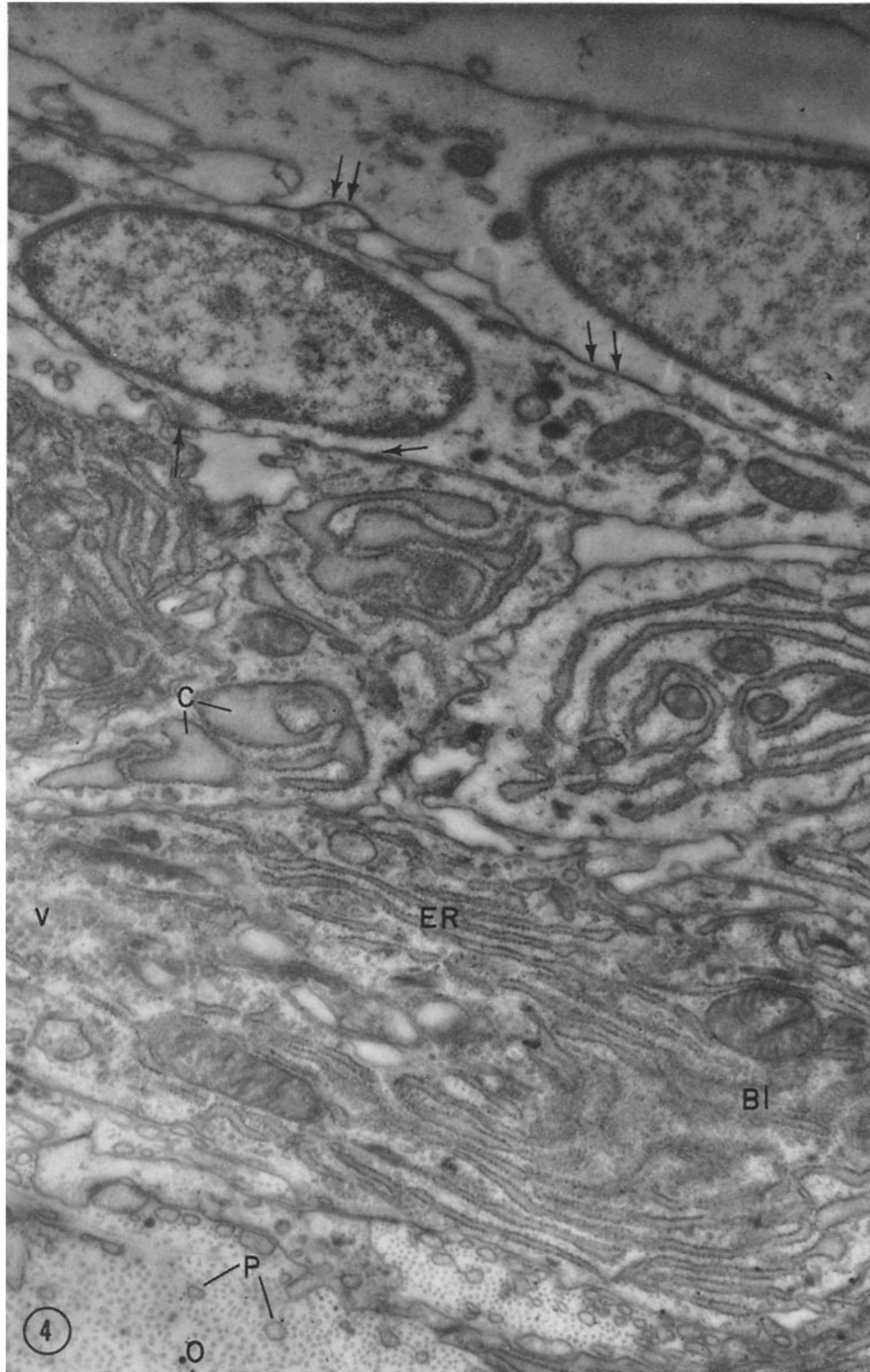




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osteoid osteocyte. It closely resembles an osteoblast with respect to cytoplasmic organelles but it has fewer of them. Its long cytoplasmic processes branch very little, some of them extending toward the osteoid surface and others extending toward or into the mineralized zone (Fig. 10).

*Osteocytes*

Osteocytes embedded in mineralized bone occupy lacunae (Figs. 11 and 12). The cell surface does not rest on the mineralized collagen but is separated from it by a layer of structureless material of moderate density (Fig. 12). The osteocytes have cytoplasmic processes which extend into the bone canaliculi, and these processes are also separated from the mineralized collagen by an amorphous matrix.

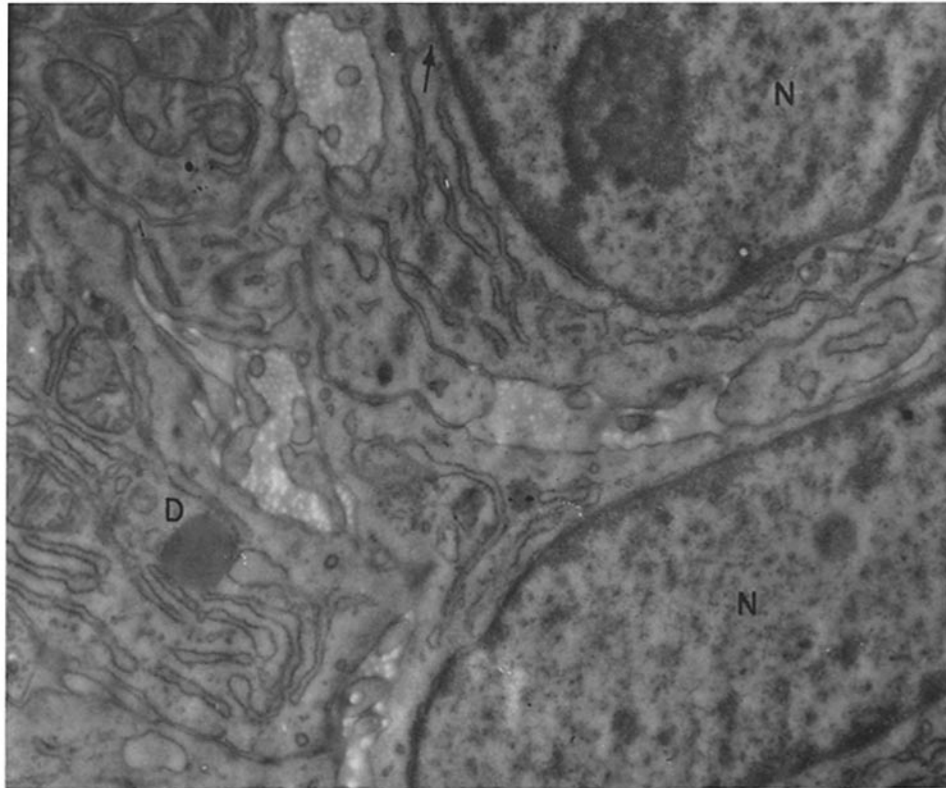


**Figure 5**

Portions of four osteoblasts are separated in part by bundles of collagen in which cell processes are embedded. A dense secretion-type droplet (*D*) is shown in one cell. Continuity between the nuclear envelope and the granular ER is seen (arrow). Continuity between smooth ER and the cell surface membrane is seen in the center of the picture. × 14,000.

The mineralized collagen fibers are aggregated into bundles. Fiber-bone bundles intersect each other randomly and lamellar bundles are arranged in strata. Bone crystals deposited selectively on the fibers in periodic orientation of approximately 640 A form prominent striations transverse to the long axis of the fibers (Figs. 11 and 14). Adjacent fibers, closely apposed, often have the striations in register so that a given period site can be followed for several fiber widths (Figs. 10, 11, 14, and 15).

The osteocyte has a single nucleus, the membrane of which exhibits nuclear pores, and one or two nucleoli. Granular endoplasmic reticulum is present but is less abundant than in the osteoid osteocytes. There are irregular dilatations in the granular endoplasmic reticulum which contain moderately dense amorphous material. Such



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dilatations may measure up to several micra in the chick. Parallel membranous lamellae and clusters of small vacuoles together, interpreted as Golgi, are present. The mitochondria, which are few in number, are rounded and swollen and have relatively few cristae. Small vesicles similar to those seen in the osteoblasts are randomly dispersed in the cytoplasm. Occasional round secretion-type droplets are also found and many contain a number of very dense particles.

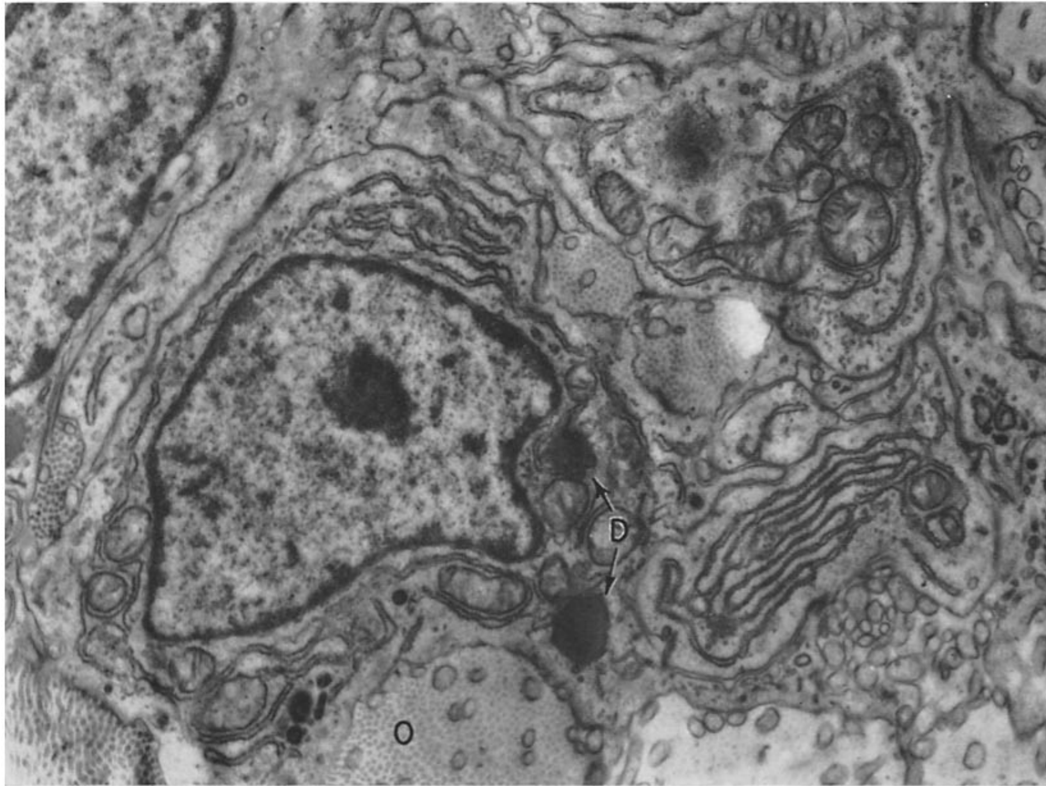


FIGURE 6

Electron micrograph showing osteoblasts with numerous processes embedded in osteoid (*O*) and in the intercellular bundles of collagen. Two large secretion-type droplete (*D*) are seen in one cell. One per cent phosphotungstic acid. × 10,000.

### *Osteoclasts*

Osteoclasts appear as multinucleated cells in intimate relationship to a mineralized bone surface (Fig. 13). Their nuclei are usually in that part of the cell remote from the bone. The nuclei have no distinctive features. Usually one prominent nucleolus and one or more smaller aggregates of small dense particles are seen. Nuclear pores are not prominent. The cytoplasmic constituents differ markedly from those observed in other bone cells. The mitochondria are extremely numerous, have abundant cristae, and are globular or ovoid. They are especially numerous in regions of the cell near bone. There is a paucity of granular endoplasmic reticulum. The cytoplasm, at lower magnification, is quite dense. At higher magnification, much of the density appears to be due to large numbers of small dense particles, approximately 100 to 200 A, interpreted as non-membrane-associated ribosomes. These are often in closely packed clusters (Figs. 14 and 15).

The bone-osteoclast interface is a very complex zone and presents several patterns. In the first, the bone surface is concave and the osteoclast is present in the concavity of the Howship's lacunae



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(Fig. 13). The surface of the osteoclast forms numerous long cytoplasmic processes which subdivide into smaller ones (Fig. 13). Some of these are in contact with the mineralized bone. In regions where the processes are not in contact with the bone surface and in the spaces between the processes, there is amorphous material of moderate density (Fig. 15). There are deep and irregular osteoclast there is an irregular zone of altered mineralized bone. This resorption zone consists of isolated fragments of mineralized collagen as well as free apatite crystals occurring both individually and in clumps. Irregular mineralized masses are seen in an amorphous matrix and may come into contact with an osteoclast process or become separated from the underlying bone and

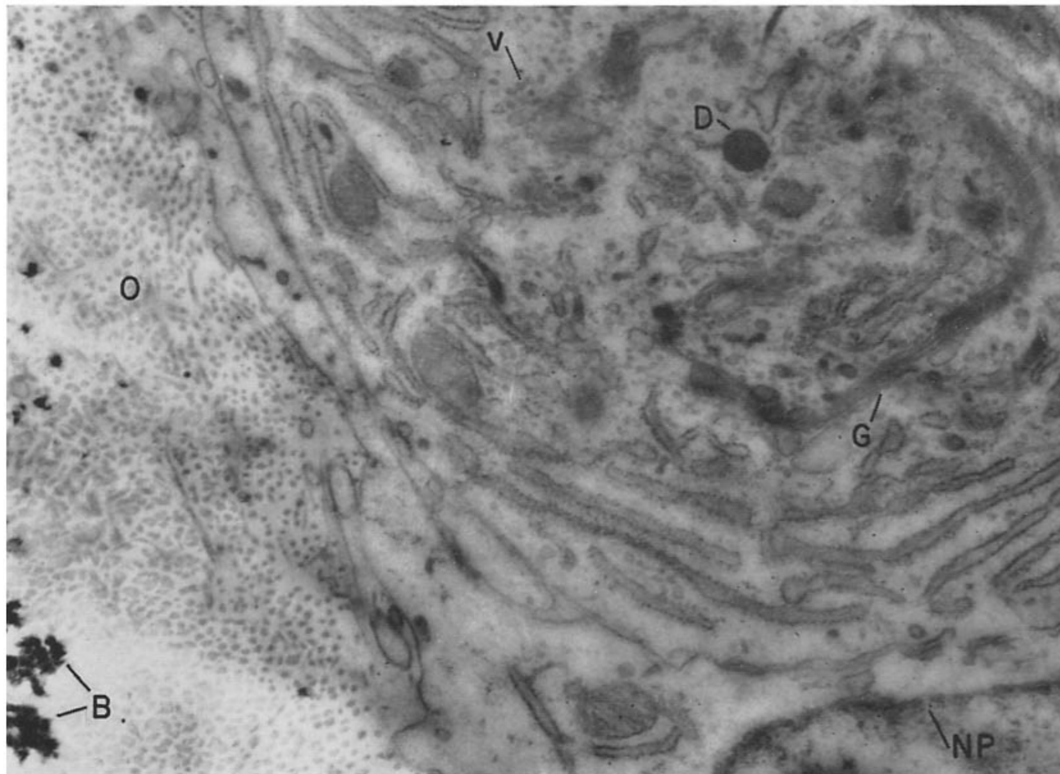


FIGURE 7

An osteoblast-osteoid junction. Nuclear pores (*NP*), Golgi material (*G*), secretory droplets (*D*), and dispersed small vesicles (*v*) are shown. The clumps of mineral (*B*) in the deeper osteoid zone may be artifactitious in contour, since this specimen was stained and staining causes irregular demineralization. Uranyl acetate. × 12,000.

invaginations into the cell of the extracellular space between the cell processes. These invaginations appear to end blindly and often form dilated canals or vacuoles when sectioned transversely. Serial sections, however, reveal that these vacuoles are continuous with the cell surface and their contents are continuous, therefore, with the extracellular space.

Between unaltered bone and the overlying lie between cell processes (Fig. 16). Numerous needle-shaped crystals randomly oriented are also found (Fig. 17). Some of the crystals are found in the vacuoles that are not in continuity with the surface of the osteoclast in the particular plane of section. No unmineralized collagen fibers are found at the osteoclast-bone interface.

In a second type of osteoclast-bone interface the surface of the cell apposed to bone is relatively

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smooth. Fewer cell processes are found (Fig. 18) in such locations, and the underlying bone shows minimal evidence of resorption.

Another variation in the structure of the osteoclast is represented by cells in which the mitochondria are greatly swollen and have very little internal structure (Fig. 19). Concomitantly, the cytoplasm has lost its density to electrons and contains few if any ribosomes. The bone surface underlying such an osteoclast again shows minimal evidence of resorption. The osteoclast cell surface away from the bone is relatively smooth with only occasional short cytoplasmic processes.

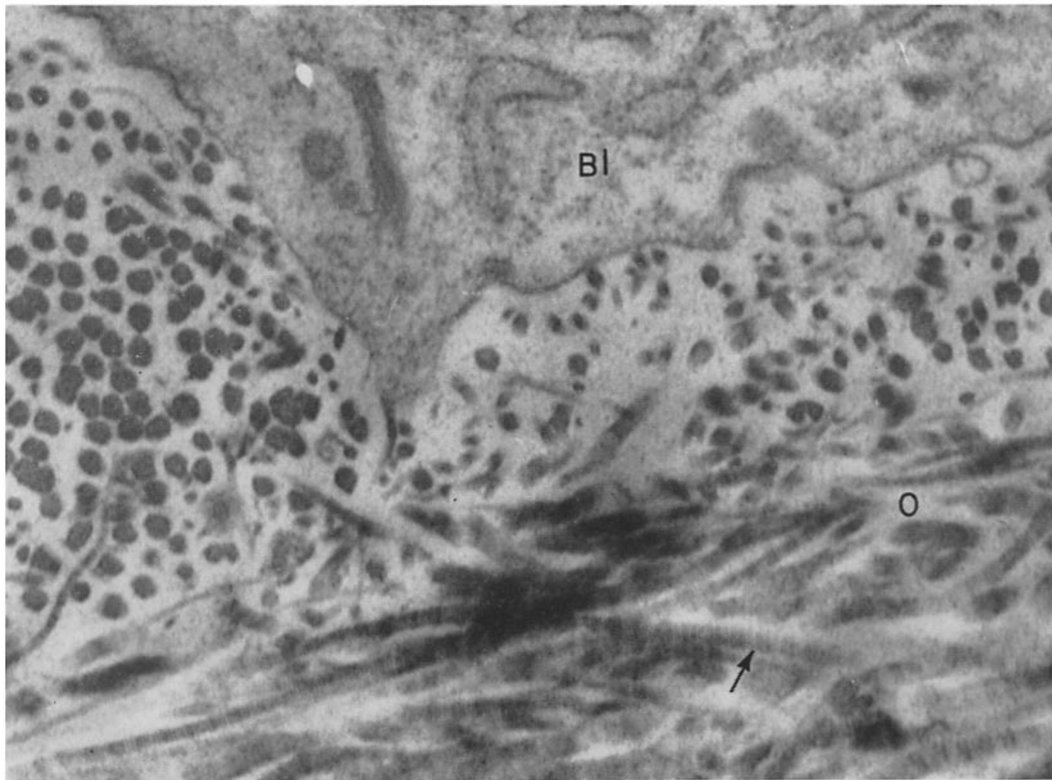


FIGURE 8

Osteoblast-osteoid junction showing smaller collagen fibers immediately adjacent to the cell surface and larger fibers in the deeper layers. The fibers have 640 A repeating period with interperiod bands (arrow). Uranyl acetate. × 52,000.

### *Endosteal Lining Cell*

No bone surface is found to be free of cells (Fig. 20). Cells lacking the distinctive qualities of those described above are found on bone surfaces where there is neither osteoid synthesis nor bone resorption (Figs. 13 and 21). The cell surfaces are separated from the smooth underlying bone by a very thin layer of amorphous matrix, and in some foci the surfaces appeared to be in contact with the bone. These cells are considered to be endosteal lining cells. Their cytoplasmic constituents are sparse and include mitochondria, granular and smooth surfaced endoplasmic reticulum, and small numbers of vesicles.

## DISCUSSION

The fine structure of the several components of osseous tissue does not differ significantly whether the tissues examined are coarse fiber bone or rapidly forming lamellar bone. Species variations



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have not yet been observed in studies dealing with fowl fetal bone (6), kittens (5, 23), rats (1), human infants (18, 19), and in this study with ten-week-old chicks and adolescent children.

Osteoblasts which appear to preside over fibrogenesis are functionally and structurally polarized in that abundant collagen fibers and interfiber substance, together constituting osteoid, are found matrix. The mitochondria are moderately numerous and may be in very intimate relationship to the granular ER (Fig. 6). Numerous small membrane-limited vesicles are found dispersed in the cytoplasm, not just in proximity to the parallel arrays of smooth membranes interpreted as Golgi material. These small vesicles have smooth membranes and are similar to those described by

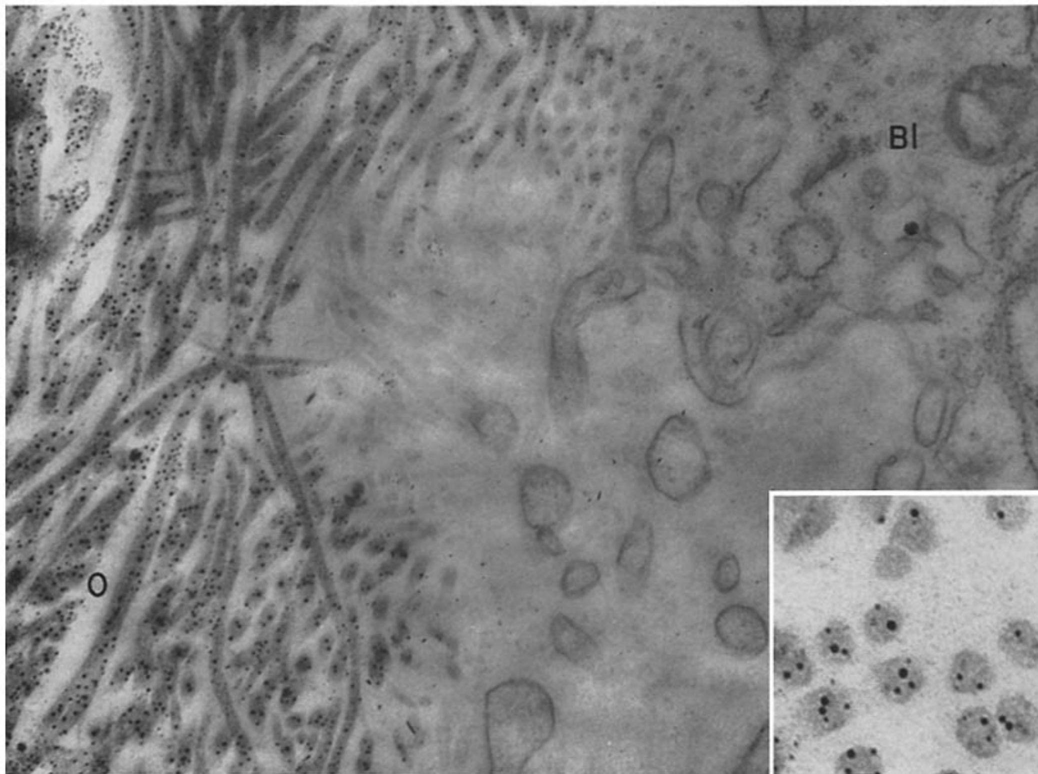


FIGURE 9

Osteoblast-osteoid junction showing small dense particles on and in (insert) collagen fibers. The particles are on fibers in both the deep and superficial zones of osteoid but are not seen on cell processes. No stain. × 28,000; insert × 74,000.

on one surface, while only a few small collagen fibers in an abundant amorphous interfiber substance are seen on the surface remote from bone. Osteoblasts contain abundant granular endoplasmic reticulum (9, 23), in keeping with their active role in the synthesis of osteoid. The paired membranes enclose and delineate a lumen which varies greatly in diameter (8). The lumen contents are amorphous and of low density, but this density is higher than that of the cytoplasmic Porter and Pappas (17) in fibroblasts and interpreted by these authors as probably being part of the Golgi apparatus. The contents of the vesicles are amorphous and of low density, but this density is greater than that of the cytoplasmic matrix. The vesicles may be closely associated with the granular ER.

Large secretory-like vacuoles in the osteoblasts and in their cellular derivatives contain moderately osmiophilic amorphous material enclosed by



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limiting membranes of still greater density. They may represent the large, dense granules described by Fitton-Jackson and Randall (8), who presented evidence to support the concept that these granules contained a non-sulphated amino polysaccharide.

Modifications of contiguous osteoblast cell surfaces are seen to consist of desmosome-like structures which are similar to those described by Fitton-Jackson and Randall (8). Another modification consists of a localized increased density of contiguous membranes which are closely apposed but not thickened. The modifications are similar to those present in the intercalated discs of the myocardium (25).

The collagen fibers in osteoid are not of uniform diameter. Small fibers measuring 200 A are found near the surface of the osteoblast and in the most superficial layers of the osteoid, as well as in the bundles of fibers between contiguous osteoblasts. In each of these areas there are also larger fibers measuring up to 600 A in diameter. In the deeper layers of osteoid, however, the fibers are uniformly large. This variation in fiber size was shown in fowl fetal bone (8), in kittens (23), and in rats (1). The gradient of fiber diameters suggests that lateral aggregation occurs extracellularly and that the tropocollagen units for this growth are in a dispersed form in the interfiber substance. This agrees with previous interpretations (8, 17). The presence of abundant osteoblast cell processes in those zones where this lateral aggregation occurs extracellularly permits the suggestion that osteoblasts not only elaborate the precursors of collagen and the interfiber substance but also preside over

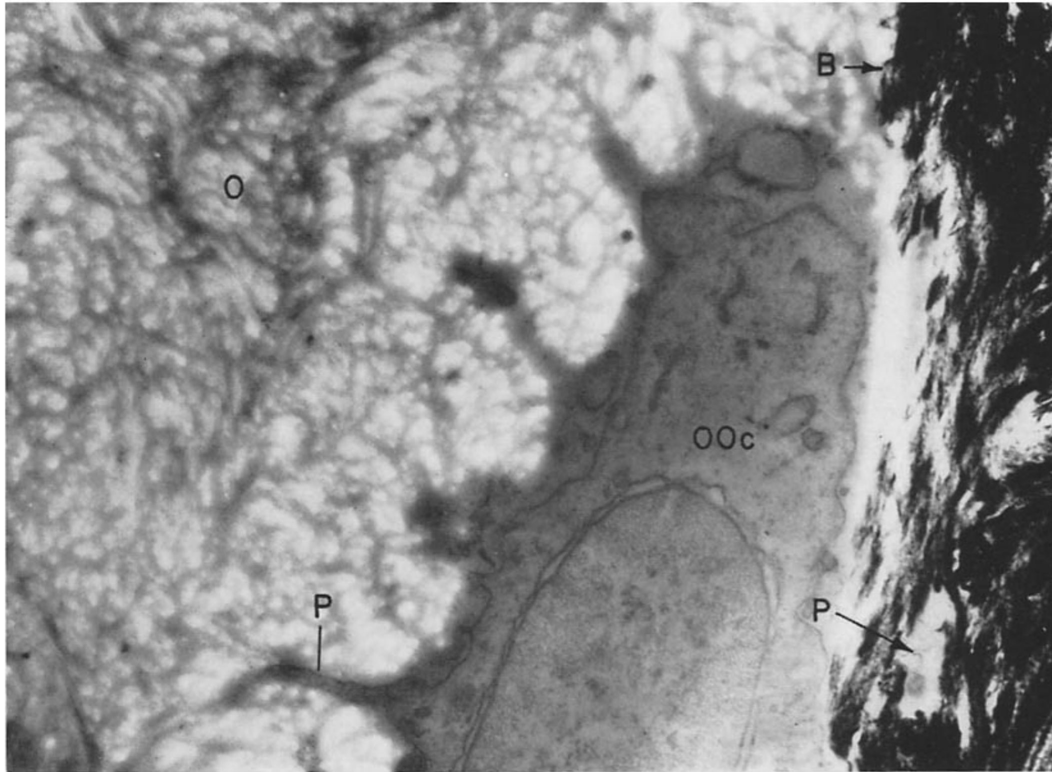


FIGURE 10

Electron micrograph showing bone (*B*), osteoid (*O*), and a cell partially surrounded by both substances. This cell is designated an osteoid osteocyte (*OOc*) and has processes (*P*) that extend into both the bone and the osteoid. In the bone the periods of the adjacent mineralized collagen fibers are in register for several fiber widths. × 20,000.

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the state of the polymerization, perhaps by altering hydration and electrolyte concentration. It is known that polyelectrolyte concentrations affect the aggregation state of macromolecules of collagen (22).

Osteoid osteocytes having qualitatively the same cytoplasmic constituents as osteoblasts, with high degrees of polymerization the mucopolysaccharides of bone and osteoid gave a pale pink stain with the PAS reaction, whereas with the lower polymerization states observable in zones of resorption and in certain zones in osteoid there was an intense pink stain (13). In forming bone, the zone of deep staining is at the junction of

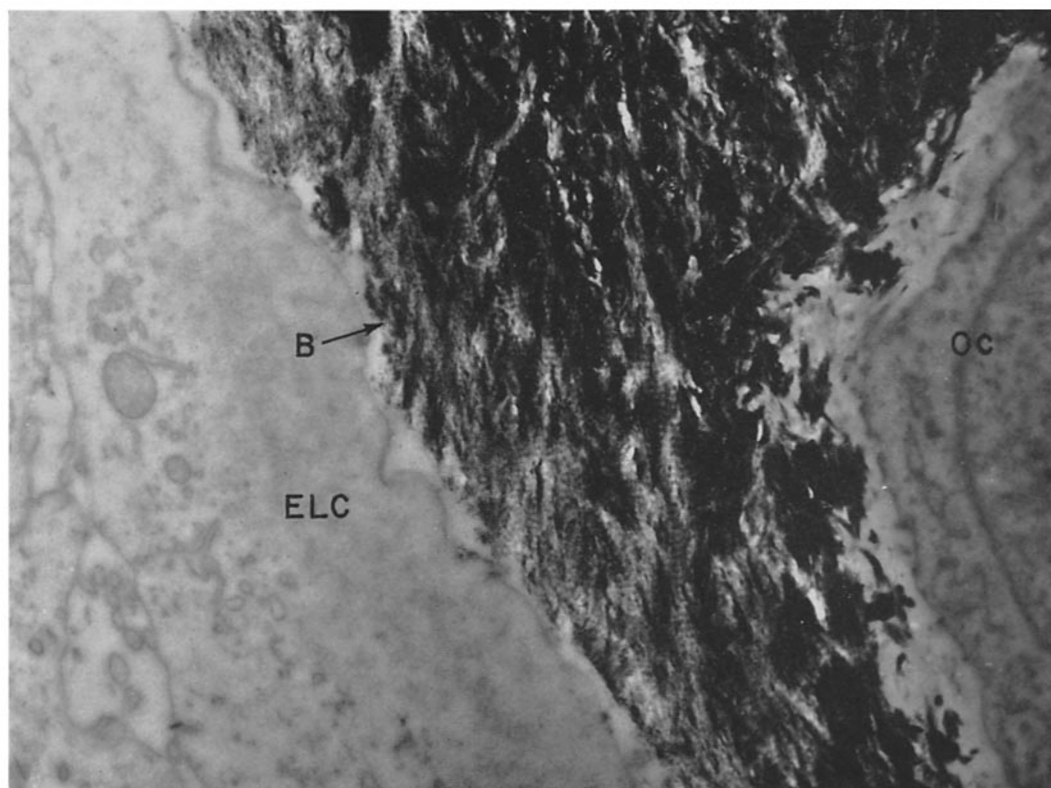


FIGURE 11

An osteocyte (*Oc*), bone (*B*), and an endosteal lining cell (*ELC*) are illustrated. The osteocyte has a layer of amorphous material interposed between the cell surface and the bone. The periods of the adjacent mineralized collagen fibers are in register though not invariably so. The endosteal lining cell has sparse cytoplasmic organelles, and the underlying bone has neither osteoid nor evidence of resorption. $\times$ 14,000.

though fewer of each, also have cell processes that penetrate osteoid in those regions where collagen fiber diameters are large but where little or no mineralization has yet occurred. These cells, in company with the deeper cell processes of osteoblasts, may affect the state of polymerization of acid mucopolysaccharides in the interfiber substance of osteoid. Heller-Steinberg concluded that mineralized and unmineralized collagen at the depth where osteoid osteocytes and deep osteoblast cell processes are most numerous. The importance of the state of polymerization of acid mucopolysaccharides in the mineralization process has been emphasized recently by Glimcher (10).

Osteocytes in lacunae and their cell processes in osseous canaliculi appear to be a further simplifi-



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cation of osteoblasts. Their swollen mitochondria may reflect their more precarious nutritional state. Neither the surface of the cell nor that of the cell processes touches the mineralized collagen walls in their respective cavities. A layer of non-fibrillar material of moderate density separates the cell surfaces from the mineralized wall. This layer is osteocytes stain intensely with the PAS reaction, suggesting chemical similarity. Lipp (16) suggested that the material filling the finer canaliculi acts as a metabolic conduit by depolymerization and repolymerization under enzymatic control. The source of this material may be related to the secretion-type droplets in the osteocytes. The

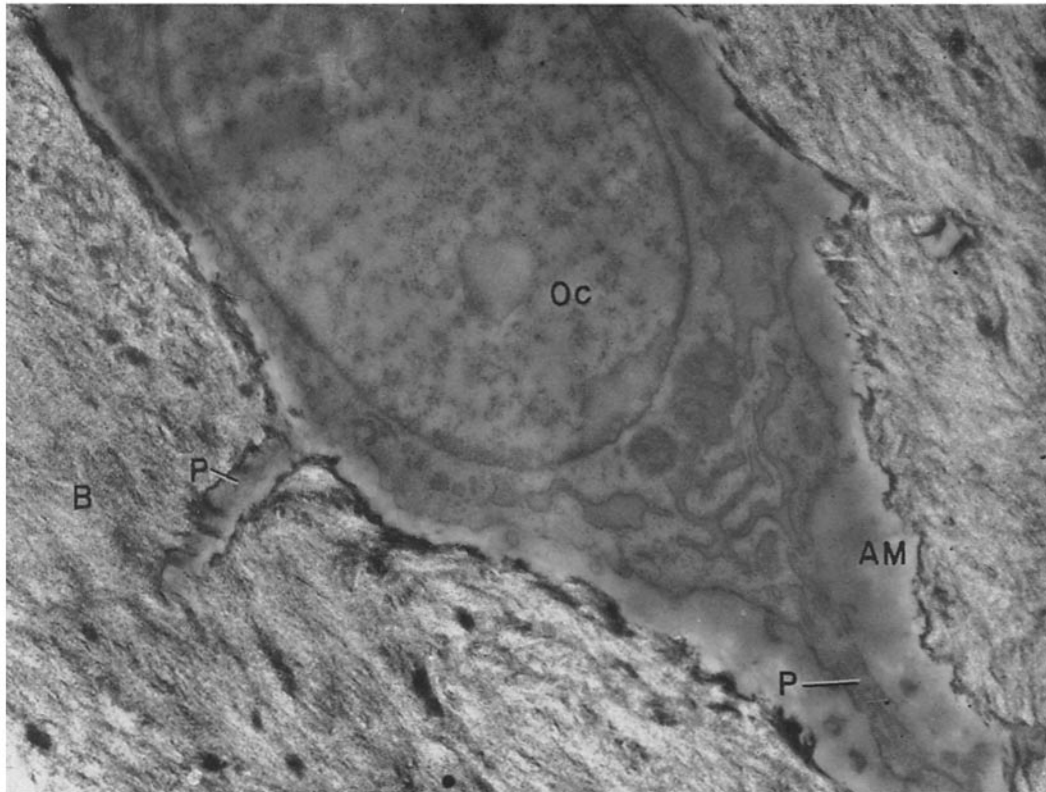


FIGURE 12

An osteocyte (*Oc*) has its cell surface and processes (*P*) separated from the surrounding bone (*B*) by a layer of amorphous material (*AM*). The osteocyte has cisternal dilatations of the lumens enclosed by granular ER. × 16,000.

the *Grenzscheide* or limiting membrane which Rutishauser and Kind showed to be rich in lipids and mucopolysaccharides (21). The lipid component shown by them may be derived from the cell membrane. This sheath of amorphous material appears to be analogous to the sheath of Neumann about each odontoblast process in dentine, which Wislocki *et al.* showed to contain an acid mucopolysaccharide (27). Both the sheath of Neumann and the amorphous material about the extracellular material may aid in maintaining the integrity of osteocytes and their processes in bone.

The osteoclast differs markedly from all other bone cells. The cytoplasm of this cell is characterized by great numbers of mitochondria and numerous non-membrane-associated ribosomes which are often in clusters. The latter are probably responsible for the basophilia of these cells, since there is very sparse granular endoplasmic reticulum. Despite the high content of acid phosphatase



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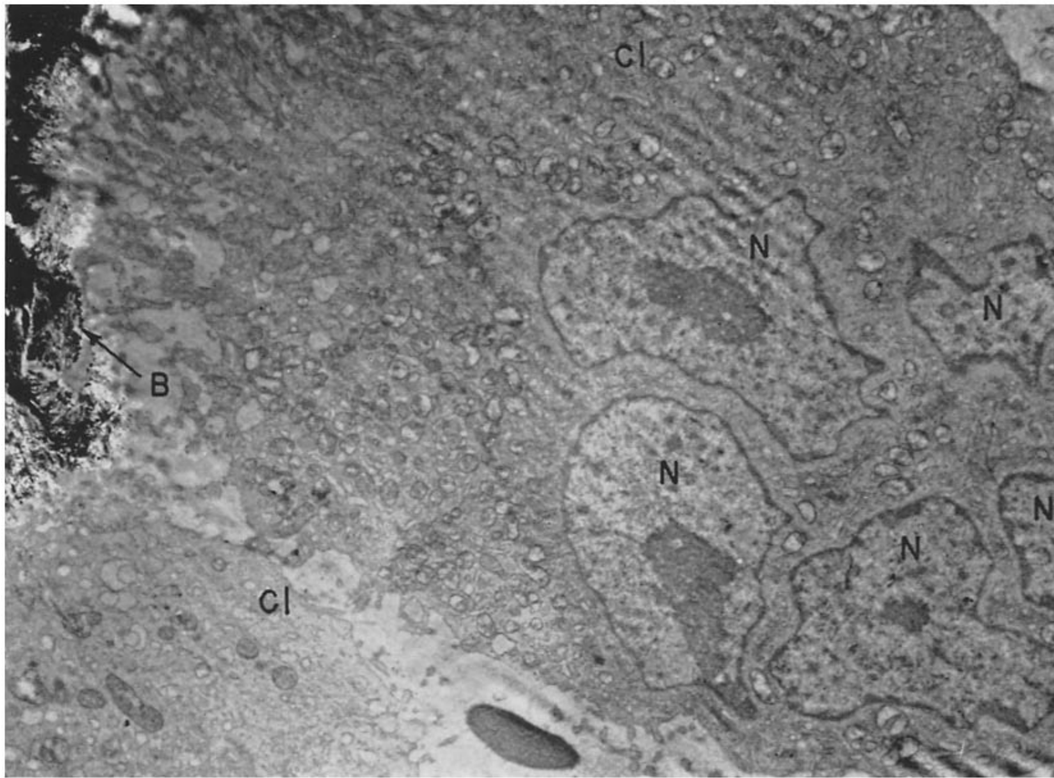


FIGURE 13

An osteoclast (*Cl*) in a concavity or Howship's lacuna in bone (*B*). A small part of a second osteoclast (*Cl*) is also seen below. The multiple nuclei (*N*) are usually in that part of the cell remote from the bone. Large numbers of mitochondria and vacuoles lie between the zone of complex cytoplasmic processes and the region containing the nuclei. × 6,000.

in osteoclasts (3), there are only rare dense bodies suggesting lysosomes (4). The cell surface near bone undergoing active resorption is composed of a complex series of cytoplasmic processes which unquestionably corresponds to the brush border of Kölliker. Kölliker thought that the brush border was part of the osteoclast (14), and subsequent workers including Kroon (15), Scott and Pease (23), and Hancox (11) agreed with his conclusion. The expression "ruffled border" of the osteoclast was used by Scott and Pease (23), who also noted inorganic crystals between the cytoplasmic processes. They concluded that the crystals gain access "into the cytoplasm" of the cell by means of the deep invaginations of the extracellular space between the processes. In the material examined in this study, the continuity between extracellular spaces and crystal-containing lacunae is shown, and in no instance did crystals enter the cytoplasm of the osteoclast. Some of the crystals appear to be

FIGURE 14

The osteoclast-bone junction shows the numerous cytoplasmic processes overlying bone. In the osteoclast, clusters of ribosomes (*R*) are dispersed between numerous mitochondria (*M*) and vacuoles (*V*). Bone fragments and individual apatite crystals are seen in the amorphous material between osteoclast processes. No unmineralized collagen fibers are seen. × 40,000.



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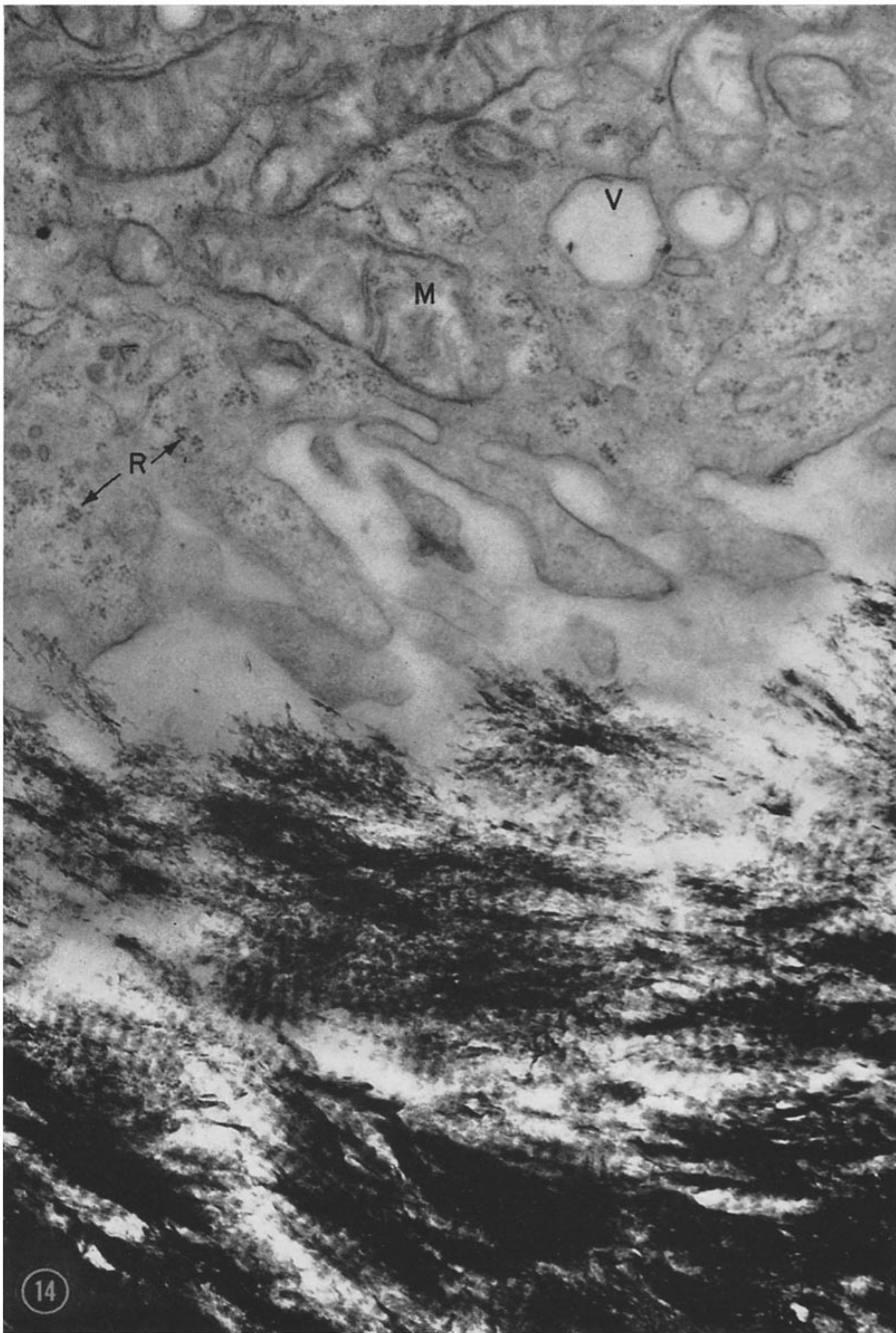




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in membrane-limited vacuoles within the cell, but serial sections show these vacuoles to be tangentially sectioned invaginations of extracellular spaces.

In the region of bone resorption, the osseous surface exhibits a narrow zone of partial resorption in which there are fewer mineralized collagen fibers than in the subjacent intact bone. Furthermore, the surface of such a resorption zone may contain clusters of unoriented crystals or fragments of bone still showing periodic structure. Scott and Pease described this zone as having a "fringed appearance" (23). Unmineralized collagen fibers were not seen in the regions of active bone resorption. It would appear, therefore, that bone resorption involves the solution of collagen and interfiber substance either as a primary step or simultaneously with crystal solution. The presence of free unoriented crystals suggests that at least some collagen is solubilized prior to solution of all mineral. The process of deaggregation of collagen fibers may depend on the ability of the osteoclast either to alter the ionic environment or to synthesize a collagenase. The ribosome content and abundant mitochondria found in osteoclasts suggest that these cells are capable of extensive synthetic activity.

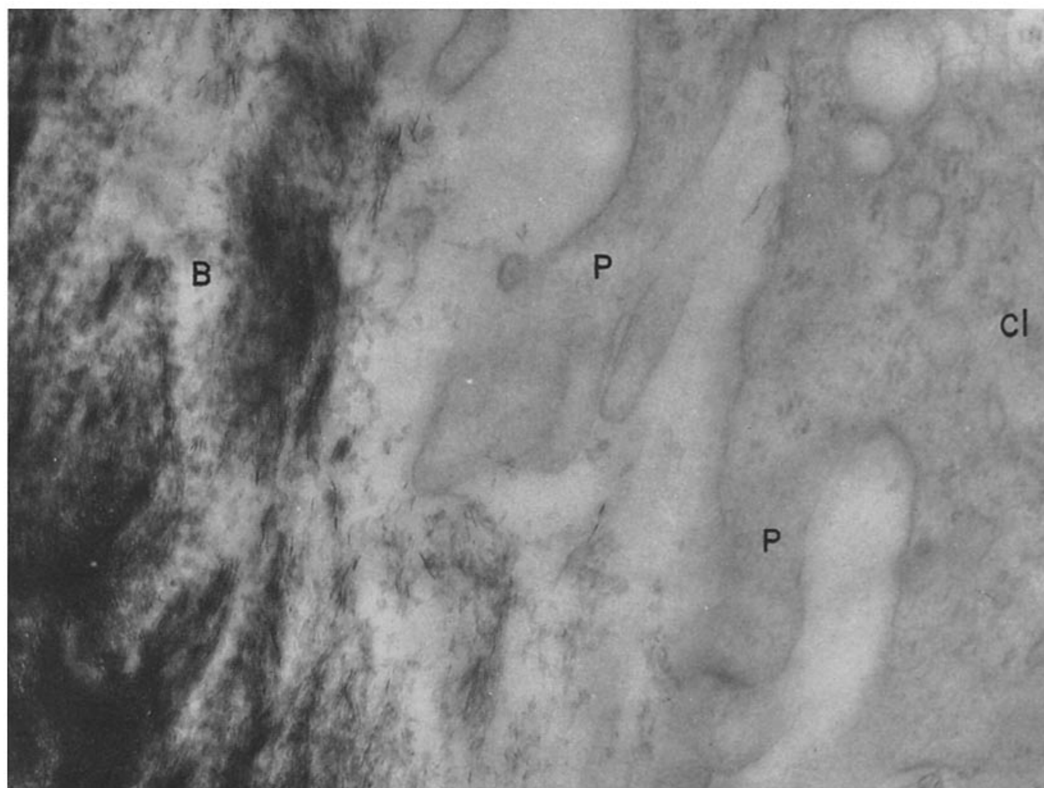


FIGURE 15

Osteoclast-bone junction showing the individual apatite crystals and fragments of bone that lie in the amorphous matrix between osteoclast processes (*P*). No unmineralized collagen is seen. Note the arrays of non-membrane-associated ribosomes. × 140,000.

In some areas, osteoclasts showed a relatively smooth cell surface near bone which also lacked a well developed resorption zone. These are thought to be inactive osteoclasts. Degenerating osteoclasts show a reduction of cytoplasmic organelles including the ribosomes. Loss of the latter probably explains the acidophilia of such cells.



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This study sheds no light on the genesis of osteoclasts. No cells which can be interpreted as developing osteoclasts were seen. The marked cytologic differences between osteoblasts and their derivatives on the one hand and osteoclasts on the other suggest that they are not related unless through a more primitive precursor of both. There is a morphologic relationship between osteoblasts, suggests that they are relatively inactive and function as endosteal lining cells.

When collagen fibers are mineralized, a 640 A axial periodicity is prominent (5, 9, 18, 19). Further, dense bands in the interband region can be seen (5) and are often evident in the material examined. The bands on contiguous mineralized collagen fibers were often, though not invariably,

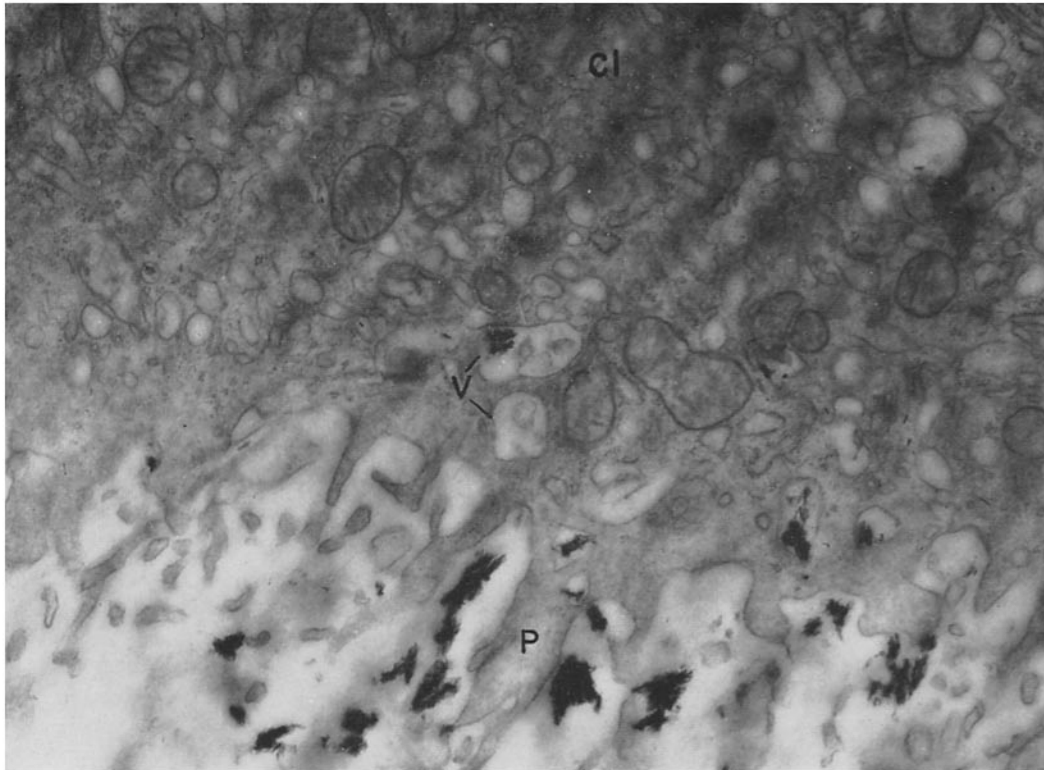


FIGURE 16

The osteoclast (*Cl*) has fragments of mineral between cytoplasmic processes (*P*) and in vacuoles (*V*). In serial sections, the lumens of the vacuoles are continuous with the amorphous material between the cell processes and thus represent invagination of extracellular spaces into the osteoclast. × 21,000.

osteoid osteocytes, and osteocytes in that the latter two represent progressive simplifications of the osteoblast.

The surfaces of all bone tissue examined were covered by cells. In some regions the cells were not associated with underlying osteoid and were usually separated from mineralized bone by a thin layer of amorphous matrix. The relative paucity of cytoplasmic organelles in these cells

in register, as has been shown by other authors (5, 9, 18, 19). At higher magnifications, individual crystals appeared rod-like and measured 40 to 50 A in width. The several dense mineral bands seen on the underlying collagen could not be well correlated with the position of the individual crystals, though the latter were parallel to the axis of the fiber.

Early mineralization of collagen has been illus-



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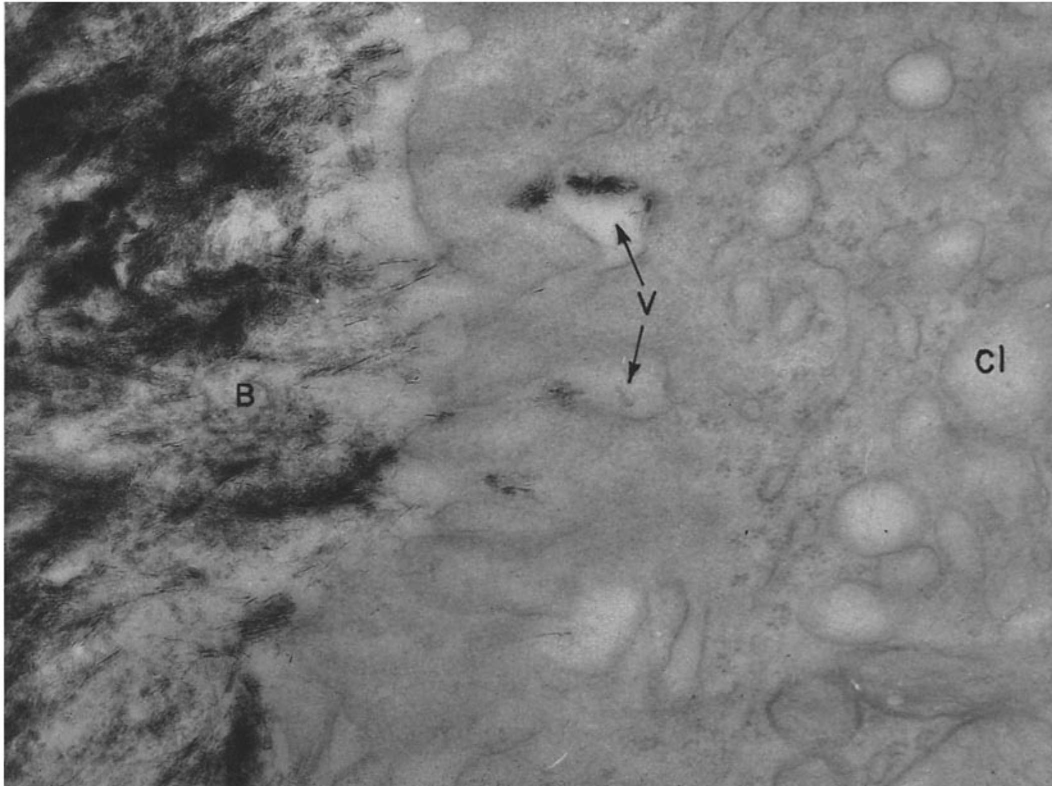


FIGURE 17

Osteoclast-bone junction showing individual apatite crystals in the extracellular amorphous material (*V*) which is invaginated into the osteoclast. No unmineralized collagen fibers are seen. × 120,000.

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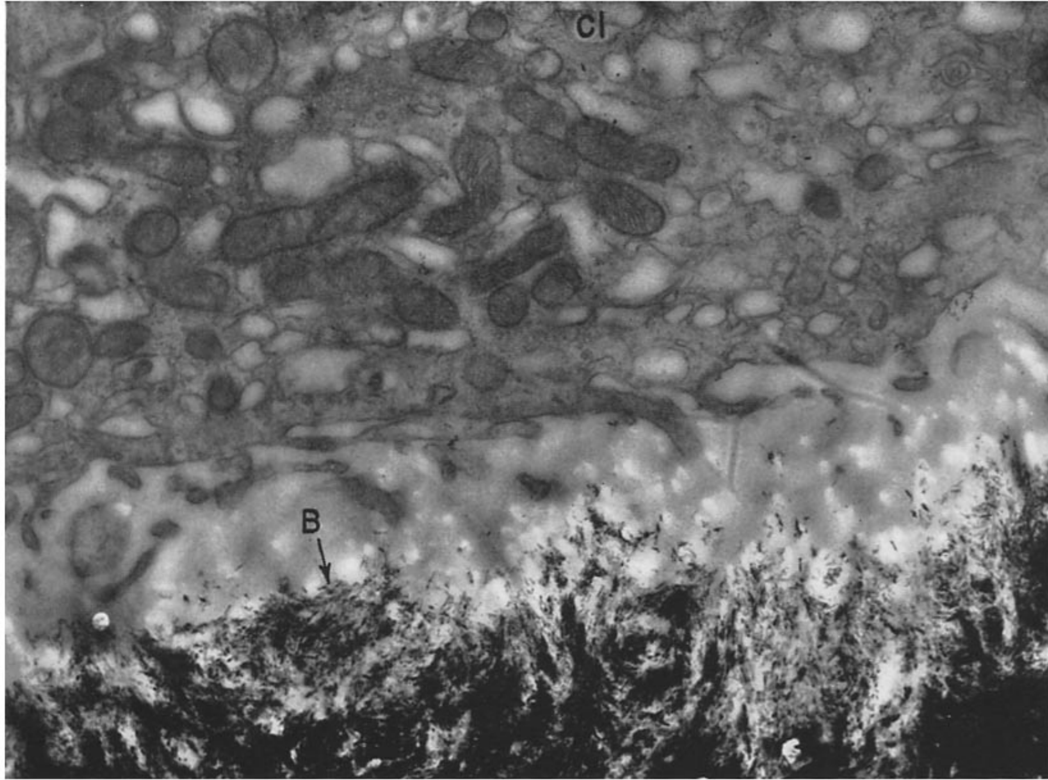


FIGURE 18

The osteoclast (*Cl*) has fewer cytopalsmic processes than are seen in Fig. 16, but a resorption zone on the bone (*B*) surface is apparent. × 41,000.

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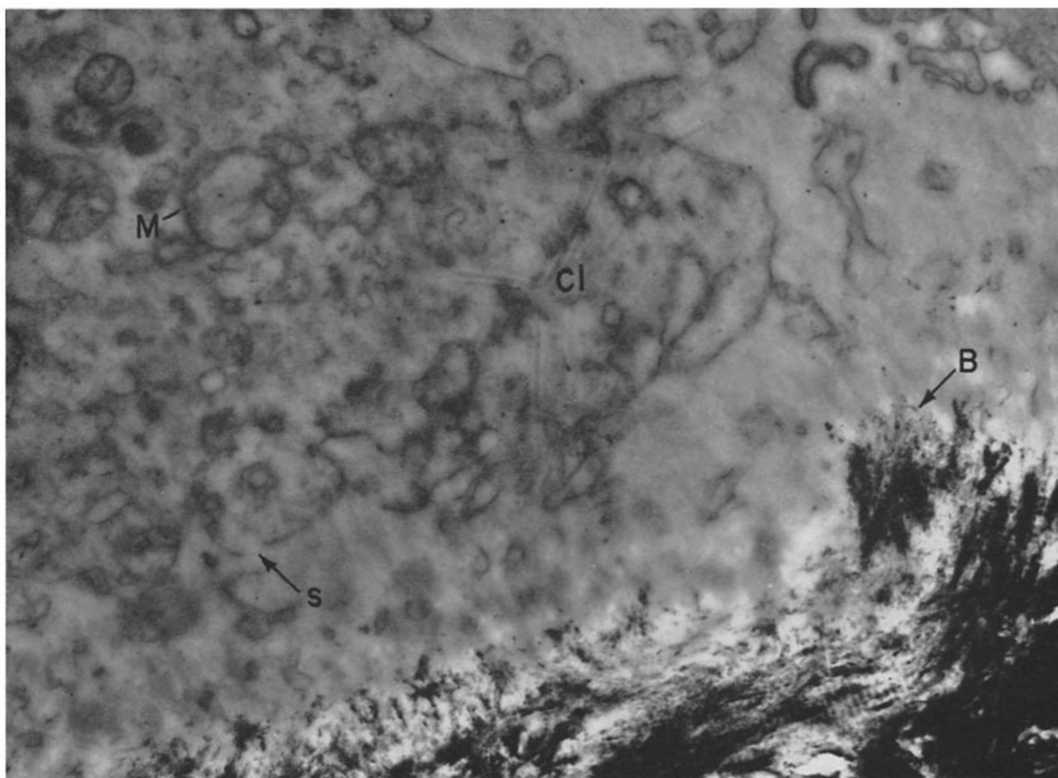


FIGURE 19

The osteoclast (*Cl*) illustrated shows swollen mitochondria (*M*), disintegration of cell surfaces (*s*), and loss of ribosomes. The underlying bone (*B*) shows a resorption zone. Adjacent cells were well preserved. × 19,500.

trated by Robinson and Watson (20), Sheldon and Robinson (24), and Fitton-Jackson and Randall (8) as occurring as small rounded crystals, usually more than one, on specific sites on native collagen fibrils. Glimcher (10) showed mineral deposits which give an electron diffraction pattern of apatite having diameters of 20 to 150 A at regular intervals along the transverse axis of reconstituted collagen fibrils in a manner corresponding to the intraperiod structure of the fibril. He suggested that the primary nucleation and early growth centers occur once per axial period. In this study occasional fields showed a pattern suggestive of that published by the aforementioned authors (Fig. 9). Small rounded mineral masses in and on collagen fibrils were seen; in transverse sections most of the deposits appeared to be within the fibrils. The mineral deposits occurred not just in the deeper osteoid layers where they might be expected, but diffusely throughout the osteoid and on small fibrils remote from the osteoid surface. When other sections from the same block were examined, this phenomenon was not seen. These deposits can be interpreted as sublimation

FIGURE 20

An osteoclast (*Cl*) is shown at a site of junction with an osteoblast (*Bl*). The resorption zone underlying both cells suggests recent withdrawal of the osteoclast from that surface of bone now occupied by the osteoblast. Note the characteristic cytoplasmic constituents of each cell. × 23,000.



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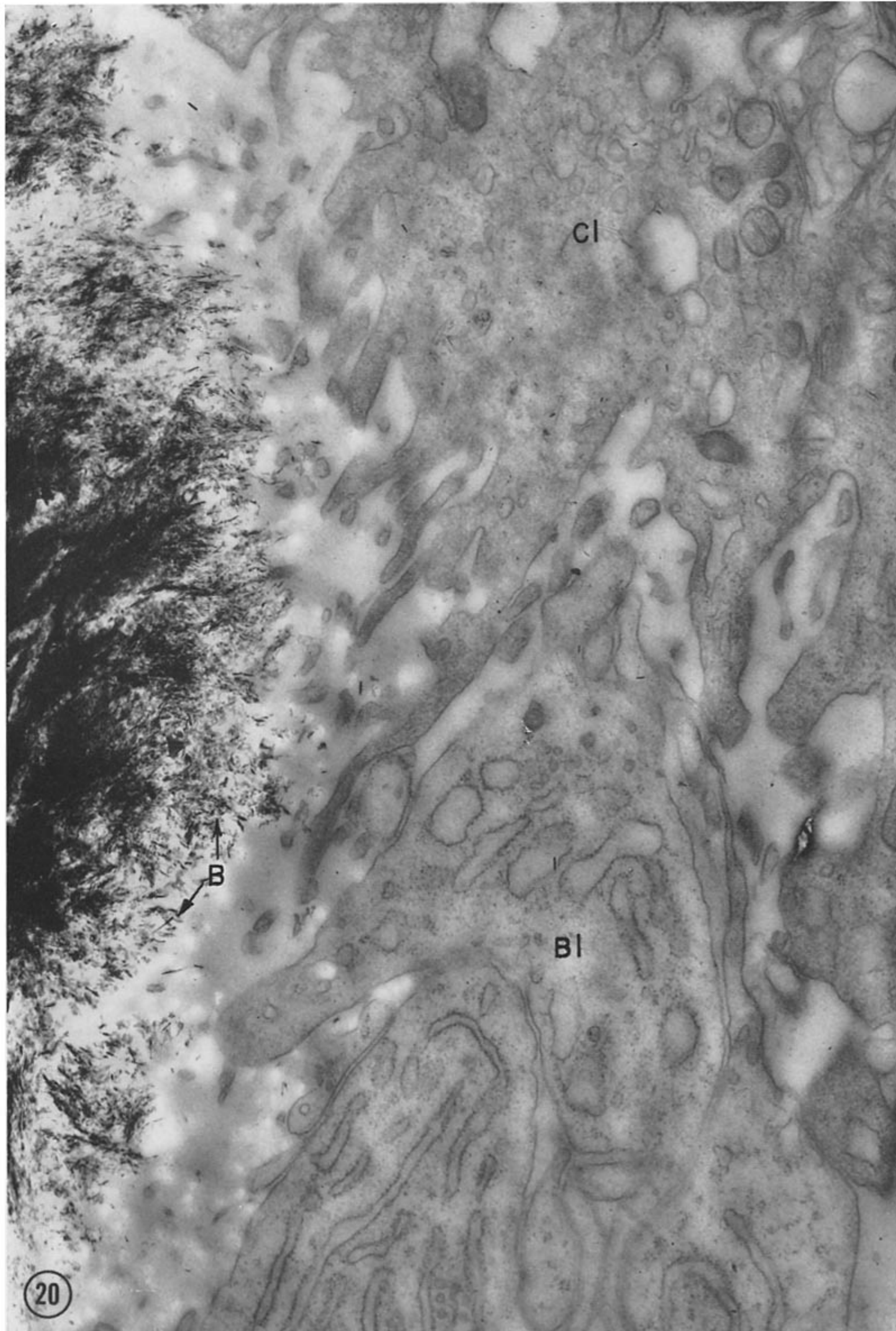


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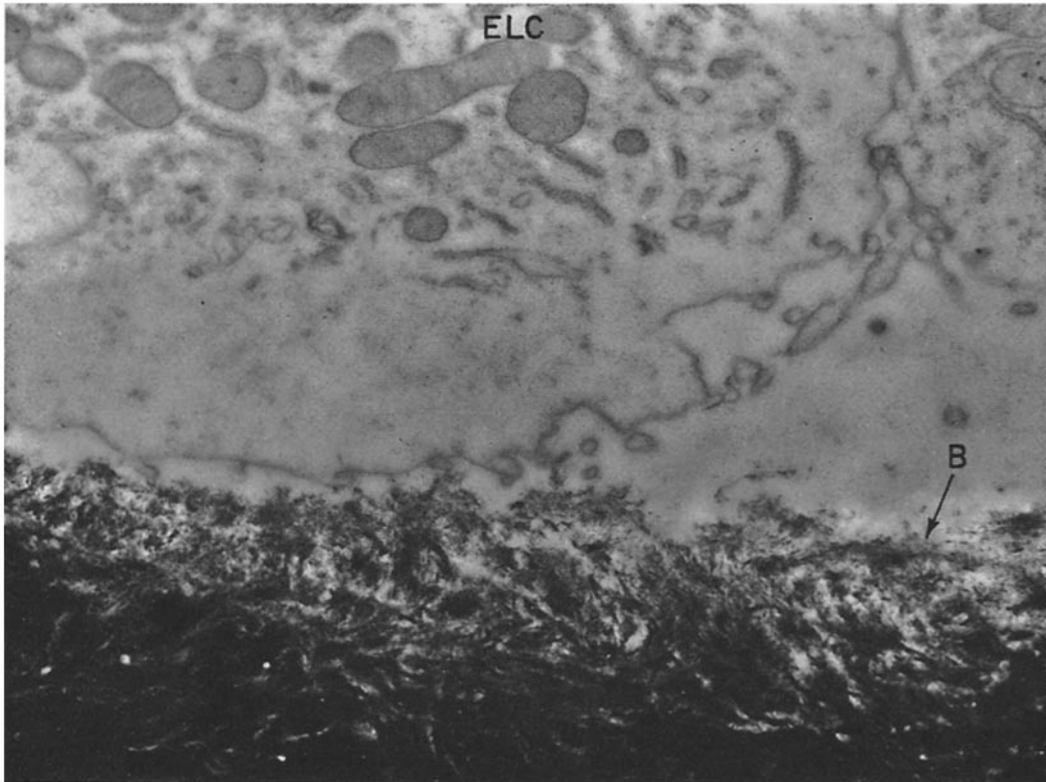


FIGURE 21

The endosteal lining cell (*ELC*) has few processes and is not associated with either osteoid formation or resorption of the bone (*B*). × 17,000.

of the mineral in the electron beam with selective reprecipitation in respect to collagen, since no such deposits occurred on cellular constituents in the same field. In areas of active bone formation, one almost invariably finds either totally unmineralized collagen fibers in the osteoid or massively mineralized collagen in the bone with no transition between the two states. This observation suggests that once nucleation has occurred, the rate of *in vivo* crystal growth is extremely rapid.

Addendum: After completion of this manuscript, a study entitled "Electron microscopy of osteoclasts in healing fractures of rat bone" by Drs. F. Gonzales and M. J. Karnovsky appeared in *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 299. We are in substantial agreement with their conclusions.

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