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# The Ultrastructure of Bone Cells and Bone Matrix in Human Primary Hyperparathyroidism

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**Summary.** An electron microscope investigation has been carried out on needle biopsies of the iliac crest of 8 patients suffering from primary hyperparathyroidism.

A marked increase in bone resorption was the most conspicuous finding. It was due both to increased osteoclastic activity and to periosteocytic osteolysis. The osteoclasts had a more strongly developed brush border and contained more cytoplasmic vacuoles than those in controls. Many osteocytes were found within enlarged, irregular lacunae, and were surrounded by a space containing amorphous, granular and filamentous material. Their mitochondria were sometimes calcified. Osteoblasts were more active than in controls as shown by the developed rough ergastoplasmic cysternae and thick osteoid borders found near some of them. The osteoid tissue, however, was uncalcified; ultrastructurally, lack of the calcification front and incomplete matrix calcification were demonstrable. Mast cells, and osteoclast- and macrophage-like giant cells were often found in the fibrotic marrow spaces.

These results confirm that both the resorption and the formation of bone are stimulated in hyperparathyroidism. The calcification process is delayed and often remains incomplete.

**Key words:** Primary hyperparathyroidism – Bone cells – Bone matrix – Ultrastructure.

## Introduction

The skeletal changes brought about by primary hyperparathyroidism are well known. The main histological findings are active resorption of bone matrix

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by osteoclasts and osteocytes, fibro-cellular proliferation in marrow spaces, only partly successful attempts at bone regeneration, and the possible formation of "osteoclastomas" (brown tumours) (Jaffe, 1972).

To the best of our knowledge, the ultrastructure of bone cells and matrix has not been studied in cases of human primary hyperparathyroidism; the electron microscope investigations that have been carried out were on bone specimens of laboratory animals, after experimental administration of parathyroid extract and parathyroid hormone (Cameron et al., 1967; Weisbrode et al., 1974). Recently, a transmission electron microscope investigation of osteoclasts (Schulz et al., 1977) and scanning electron microscope investigations of osteoblasts and osteocytes in primary and secondary hyperparathyroidism have been reported (Krempien et al., 1977; Lindenfelser et al., 1973).

This report gives the results of our electron microscope studies on bone cells and bone matrix in biopsies of patients suffering from primary hyperparathyroidism.

## Material and Methods

Eight patients have been studied; their clinical data are summarized in Table 1. Twelve normal adult subjects had previously been studied (Maschio et al., 1974); their bone biopsies have also been used as controls in the present study.

The primary clinical symptoms which led the patients to seek advice were related to the kidneys, i.e., renal colic or painless haematuria. Of these patients, 6 had renal stones, 1 nephrocalcinosis, only 2 had bone pain.

Radiological evidence of primary hyperparathyroidism was found in 4 patients. Two of these patients had cystic bone lesions without subperiosteal resorption in their phalanges; two had cystic lesions and subperiosteal resorption.

Concentrations of serum calcium, phosphate, blood urea nitrogen (BUN), alkaline phosphatase and creatinine, and urinary phosphate and creatinine were evaluated in all patients by conventional methods using an autoanalyzer. Serum bicarbonates were determined by an Astrup instrument (Radiometer, Denmark). Ionized calcium was evaluated by a flow-through electrode system (Orion research, Inc., Cambridge, Mass., model SS-20). Immunoreactive PTH was determined in a heterologous system ($^{125}$I-bPTH-1-84), using guinea pig anti-bPTH (Wilson Co., USA) and bPTH as

**Table 1.** Summary of the clinical data

| Pat. | Sex | Age | Ca mg/DL | $Ca^{++}$ mg/DL | P mg/DL | BUN mg/DL | Cr.Cl. ml/min | $HCO_3$ mEq/L | $TmPO_4$ mgDL | Alk.Ph. KAU/ml | PTH ng/ml |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal range | | | 8.5–10.5 | 4.0–4.4 | 2.5–4.5 | 12–20 | 80–120 | 24–48 | 2.8–4.5 | 4.5–12 | 0.2–0.8 |
| G.A. | M | 61 | 10.5 | 5.60 | 2.6 | 31 | 25 | 23.0 | 1.76 | 8.40 | 1.20 |
| A.G. | F | 68 | 12.4 | 6.20 | 2.3 | 26 | 41 | 22.0 | 1.34 | 24.00 | 3.80 |
| G.G. | M | 33 | 11.3 | 5.28 | 2.2 | 16 | 90 | 24.5 | 1.51 | 10.50 | 0.55 |
| D.J. | F | 49 | 13.0 | – | 2.1 | 21 | 88 | 23.0 | 1.56 | 28.20 | 2.15 |
| B.G. | F | 51 | 12.0 | 4.46 | 2.4 | 18 | 82 | 23.0 | 1.56 | 12.30 | 2.50 |
| A.M. | F | 60 | 11.0 | 6.04 | 2.4 | 16 | 92 | 23.0 | 1.84 | 24.00 | 2.00 |
| N.M. | M | 68 | 14.0 | 9.52 | 1.6 | 20 | 82 | 24.4 | 1.00 | 205.00 | 1.50 |
| M.I. | F | 51 | 11.7 | 5.92 | 1.9 | 18 | 91 | 24.0 | 1.80 | 9.80 | 1.50 |
| B.E. | F | 58 | 13.5 | 6.14 | 2.1 | 21 | 80 | 24.0 | 1.73 | 16.90 | 1.80 |
| B.D. | F | 42 | 12.0 | 5.20 | 1.9 | 15 | 80 | 22.9 | 1.18 | 10.80 | 0.70 |

standard, as described elsewhere (Lo Cascio et al., 1977). The maximum tubular resorption rate of phosphate ($TmPO_4$) was evaluated utilizing the nomogram of Bijvoet and Morgan (1971).

The diagnosis of primary hyperparathyroidism was confirmed by histological examination of parathyroid glands; all the patients had parathyroid adenoma.

Needle biopsies were taken from the iliac crest. They were fixed in 4% paraformaldehyde buffered at pH 7.2 and then divided into small specimens which were washed in water, post-fixed with 1% osmium tetroxide buffered at pH 7.2, and embedded in Araldite.

Semithin sections (about 1$\mu$ thick) were examined under the optical microscope after staining with Azure II-Methylene blue, and using the von Kossa method. Ultrathin sections (about 700 Å thick) were examined unstained and after staining with uranyl acetate and lead citrate.

## Results

### *Optical Microscopy*

The histological picture of the bone biopsies varied to some extent from specimen to specimen, but common features were recognizable. The biopsy specimens were made up of an outer layer of compact bone and an inner one of trabecular bone. The border of the calcified matrix was irregular in both layers, because of the presence of many Howship's lacunae (Fig. 1). Most of these contained osteoclasts, many of which were also found in wide lacunae within compact bone (Fig. 2). There were more osteoclasts and more Howship's lacunae than in controls. The osteocytes very often showed enlarged, irregular lacunae and enlarged canaliculi, and were sometimes seen to be coalescing (Fig. 3). These osteocytes were not evenly distributed; more of them were found near areas of osteoclastic resorption than near resting areas. In all sites, however, they were much more frequent than in controls.

The borders of most of the osseous trabeculae were bounded by long, thick areas of osteoid tissue (Fig. 4). This was either completely uncalcified, or showed patchy areas of calcification, sometimes surrounding osteocyte-like cells. The amounts of osteoid tissue and its frequency varied considerably; in all cases, however, they were greater than in controls.

Two types of cells were visible along the border of the bone matrix and osteoid tissue. One type consisted of roundish cells easily recognizable as active osteoblasts because of their basophilic cytoplasm, large Golgi apparatus and polarized nucleus. There were more of these osteoblasts than in controls, where hardly any were found. The other type consisted of very thin, elongated cells shaped like fibroblasts. These cells were usually in contact with the calcified matrix.

The bone marrow spaces were sometimes occupied by a fibrous tissue consisting mainly of loosely arranged collagen fibrils and elongated, spindle-shaped fibroblasts. In some cases, osteoclast-like giant cells and mast cells were present too.

### *Electron Microscopy*

Many typical osteoclasts were present at sites of bone resorption (Fig. 5). They usually had a developed brush border whose channels contained many loose

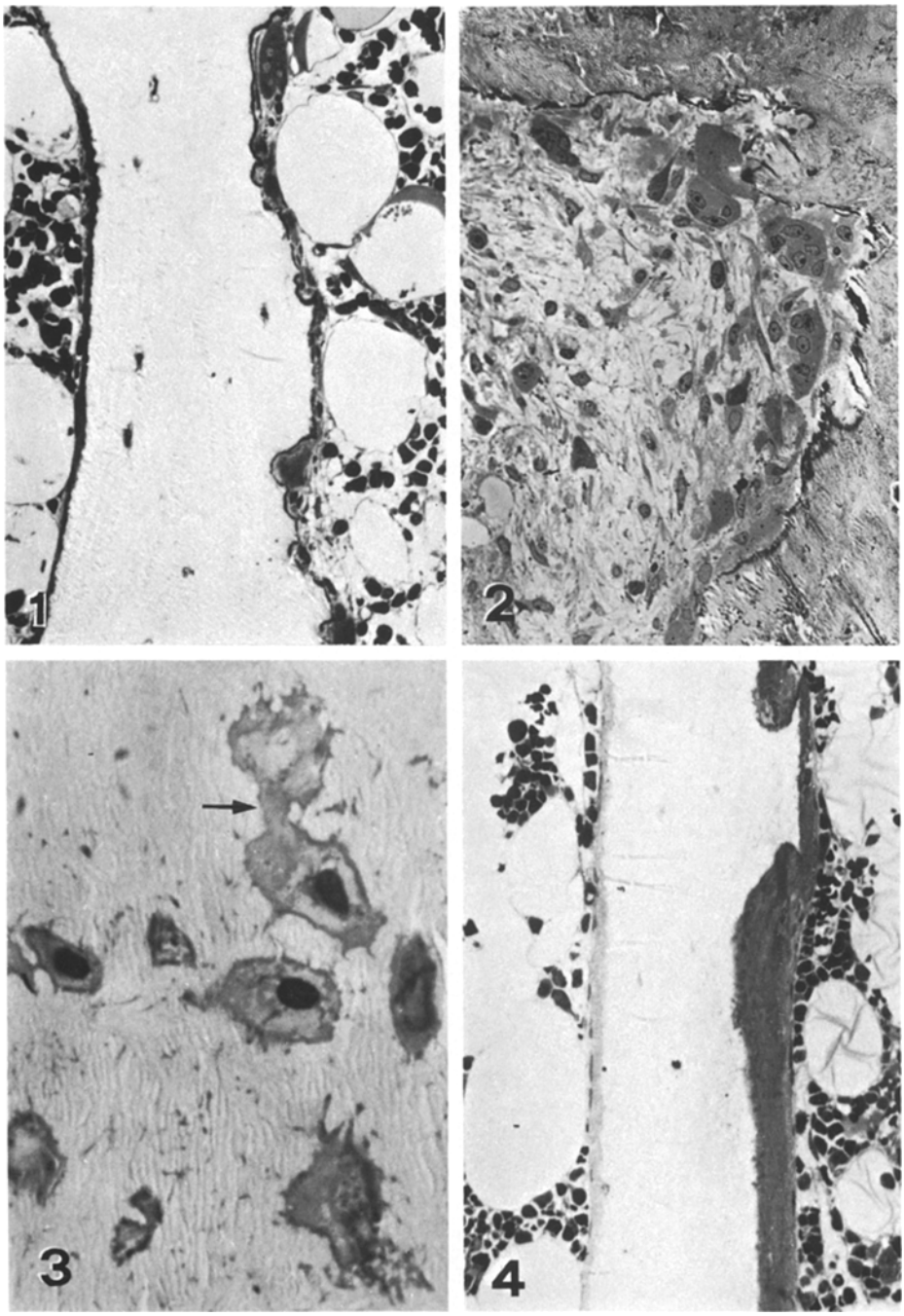



**Fig. 1.** Semithin section stained with Azure II-Methylene blue, showing a trabecula whose right border is irregular because of the presence of many Howship's lacunae, some of which contain osteoclasts. ×400

**Fig. 2.** Semithin section showing a wide area of osteoclastic resorption and marrow fibrosis. Azure II-Methylene blue, ×400

**Fig. 3.** Osteocytes with irregular and enlarged lacunae; two of them (arrow) seem to have coalesced. Azure II-Methylene blue, ×800

**Fig. 4.** Detail of a trabecula whose right border shows a thick layer of uncalcified osteoid tissue. This osteoid border is visible on left; it is in contact with small, spindle-shaped cells. Azure II-Methylene blue, ×400

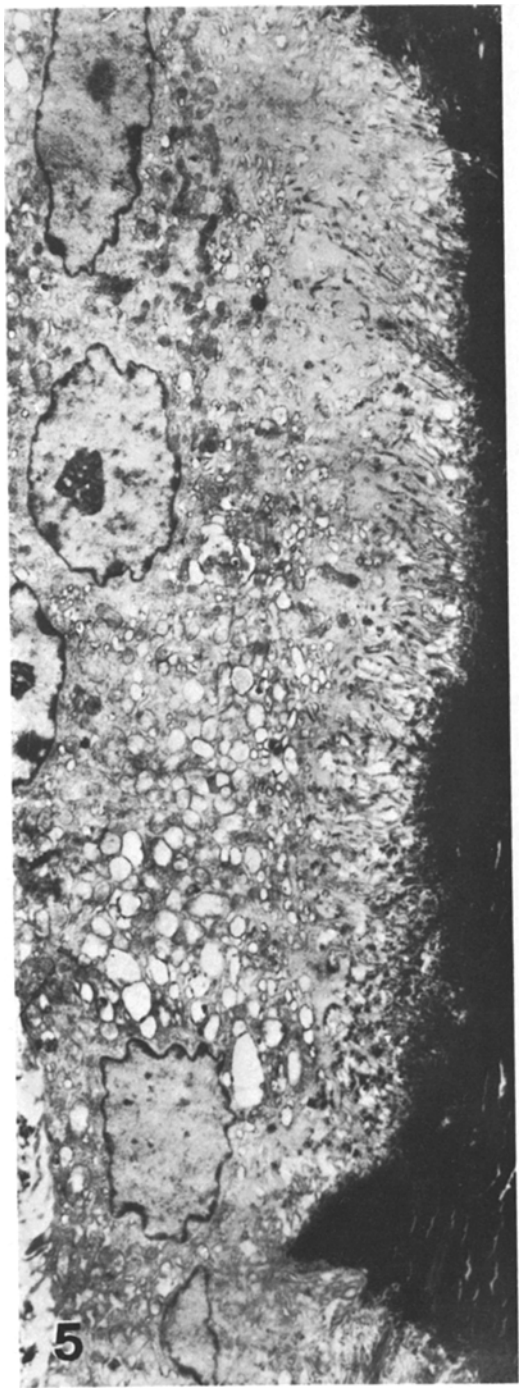



**Fig. 5.** Detail of an osteoclast with a developed brush border. Uranyl acetate and lead citrate, ×2500

crystals and fragments of still calcified collagen fibrils. Where the brush border touched the bone matrix, this was disarranged and consisted of isolated organic crystals and loosened collagen fibrils or fragments of fibrils. Apparently decalcified collagen fibrils were also occasionally present. Isolated crystals were found within cytoplasmic vacuoles. A few mitochondria contained small roundish aggregates of intrinsically electron-dense granules. These osteoclasts were like those found in controls, but they often had a more clearly developed brush border and a higher number of cytoplasmic vacuoles.

The osteocytes were frequently contained in large, irregularly shaped lacunae (Fig. 6). They were not in direct contact with the lacunar wall because of a space between their peripheral membrane and the bone matrix. This space contained granular, filamentous and amorphous material (Figs. 6, 7) and, sometimes, fragments of calcified collagen fibrils. The irregular border of the lacunae often showed crystals protruding side by side from the calcified matrix into the lacunar space. The osteocytes were themselves irregularly shaped because of the presence of many cytoplasmic processes protruding from them in every direction, and because their peripheral membrane was arched and wavy. Their nuclei were sometimes pyknotic. Their cytoplasm contained very few organelles and small aggregates of glycogen. The mitochondria were often swollen and some contained clusters of intrinsically electron-dense, needle-shaped crystals. A few lacunae contained degenerate osteocytes and/or fragments of them; in some cases the lacunae were completely empty.

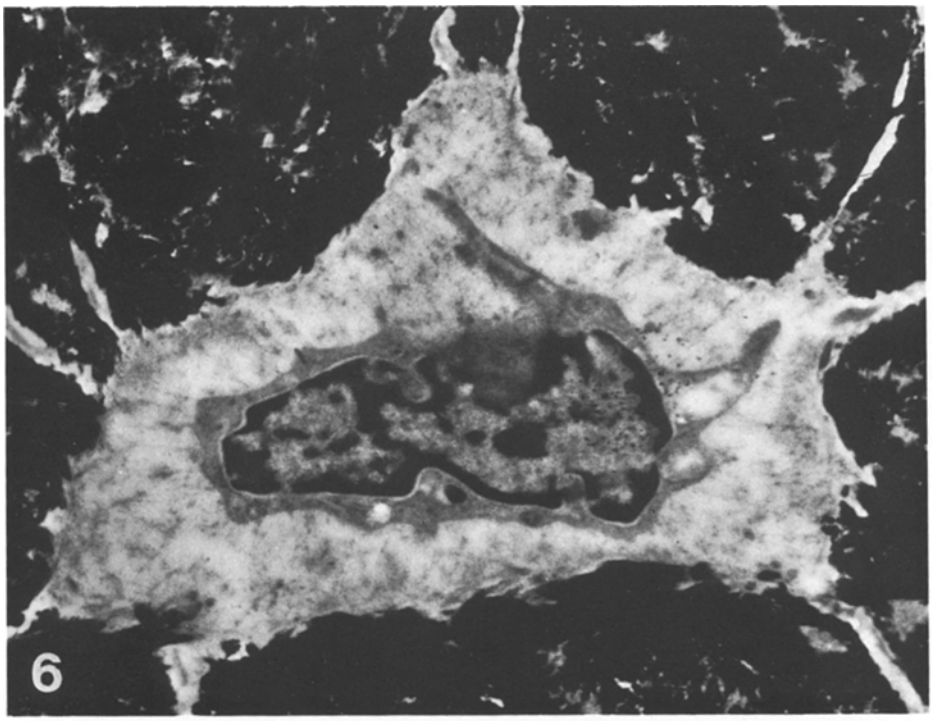
The osseous trabeculae were often surrounded by wide sheaths of osteoid tissue. This consisted of loosely arranged, irregularly oriented collagen fibrils (Fig. 8) which had a periodic banding of about 650 Å and were about 800 Å thick. This tissue was almost always uncalcified; in a few cases, it showed roundish or elongated clusters of needle- and filament-like crystals which were either irregularly scattered in the matrix, especially in periosteocytic matrix, or formed a very irregular calcification front.

The uncalcified osteoid tissue was in contact with fully calcified bone matrix (uniformly electron-dense under the electron microscope), or with incompletely calcified bone matrix. This displayed characteristic areas—some roundish and some elongated (Fig. 9)—containing inorganic crystals and granular inorganic material closely related to the periodic banding of the collagen fibrils. These calcified areas were separated by interposed uncalcified collagen fibrils.

As observed under the optical microscope, many osteoblasts were present along the edge of the trabeculae and in contact with the osteoid border. Many of them had a roundish shape: under the electron microscope, these cells showed a wide cytoplasm containing many rough ergastoplasmic cysternae with many ribosomes and a developed Golgi apparatus. A few of them contained scanty cytoplasmic organelles and had swollen mitochondria.

Thin, elongated cells were found close to the bone matrix were bone formation was not occurring. The morphology of these cells was close to that of fibroblasts. They had few cytoplasmic organelles, undeveloped Golgi areas, and contained granules of glycogen.

In some places, the bone matrix was not in contact with osteoblasts, osteoclasts or elongated cells, but with seemingly undifferentiated cells. These were



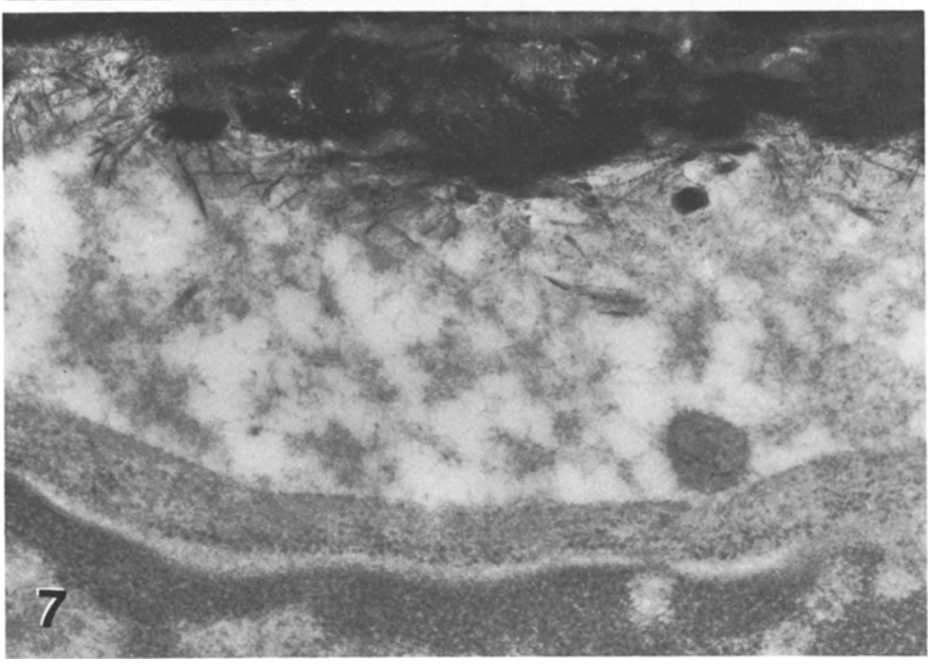




**Fig. 6.** Osteocyte contained in enlarged and irregular lacuna in fully calcified matrix; a pericellular space containing granular and filamentous material is visible. Uranyl acetate and lead citrate, ×18,000

**Fig. 7.** Detail of enlarged osteocytic lacuna; osteocyte partly visible below, calcified matrix above. Note flocculent and granular material in the pericellular space and crystals irregularly protruding from the bone matrix. Uranyl acetate and lead citrate, ×47,000

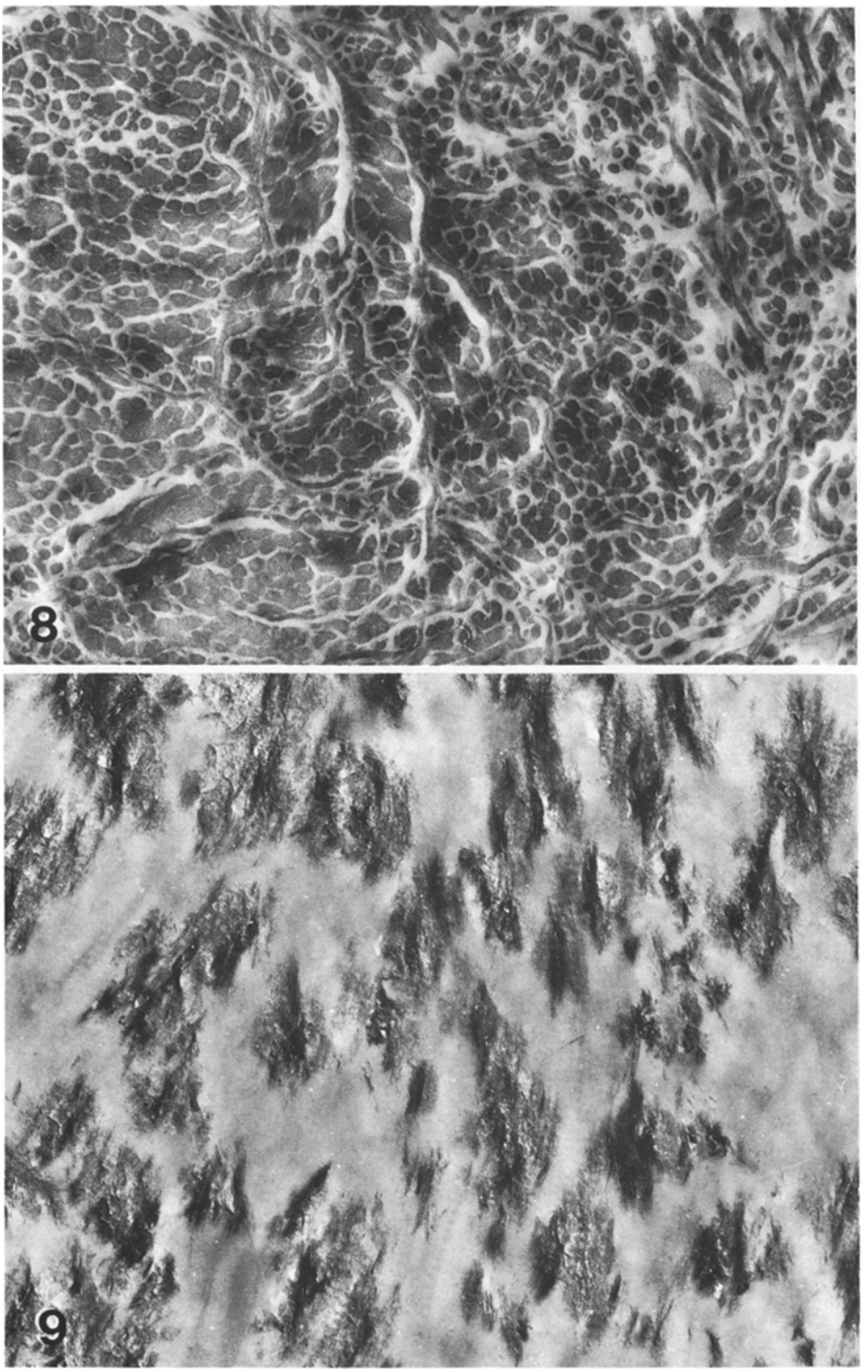
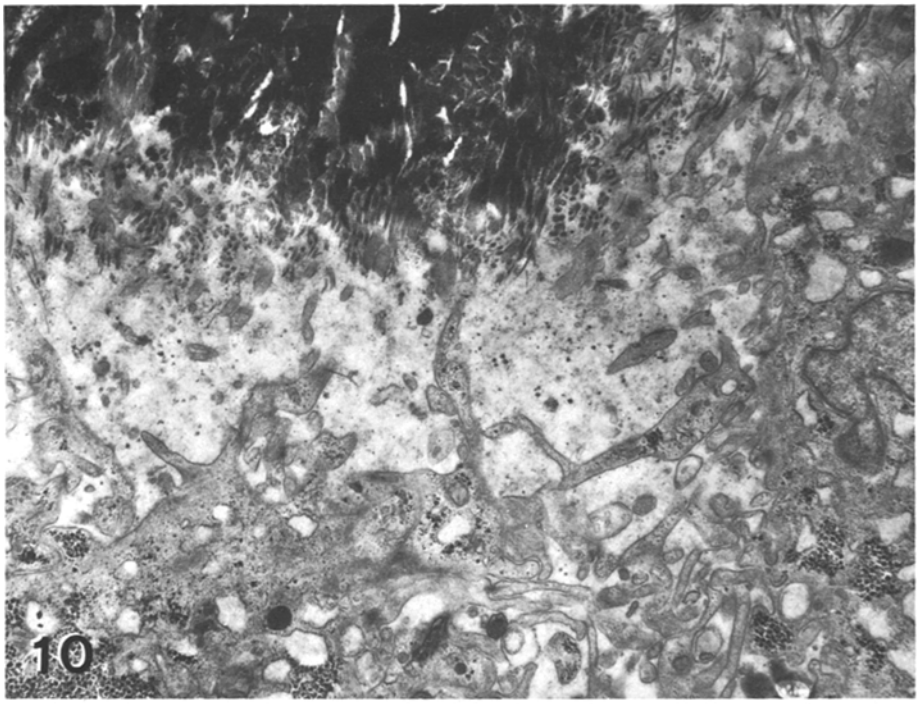



**Fig. 8.** Collagen fibrils of uncalcified osteoid tissue. Uranyl acetate and lead citrate, ×30,000

**Fig. 9.** Incompletely calcified bone matrix; the mineral substance is collected in roundish and elongated areas separated by interposed uncalcified matrix. Unstained, ×25,000



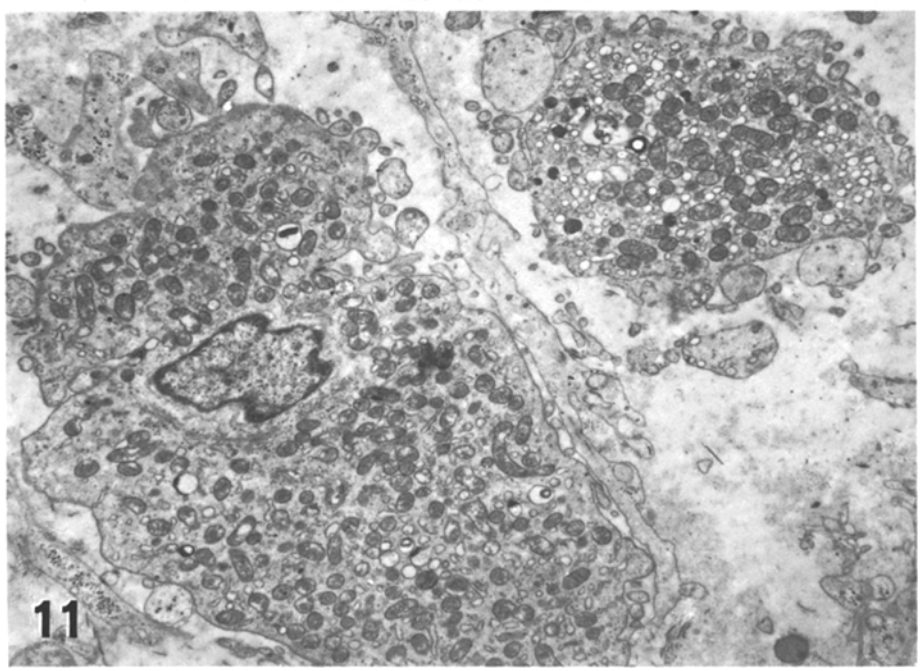




**Fig. 10.** Detail of the border of a bone trabecula, showing calcified matrix (above) and many cytoplasmic processes pertaining to cells whose cytoplasm contains granules of glycogen, vacuoles, and few organelles. These cells and the calcified matrix are separated by an area containing amorphous and filamentous material and a few uncalcified collagen fibrils. Uranyl acetate and lead citrate, $\times 10{,}000$

**Fig. 11.** Detail of a fibrotic bone-marrow space with two osteoclast-like giant cells. Uranyl acetate and lead citrate, $\times 10{,}000$

mononuclear cells, smaller than typical osteoblasts, and their cytoplasm contained very few organelles. In other cases, the cells placed near the bone matrix had an irregular shape and showed many cytoplasmic processes running irregularly in all directions. The cytoplasm of these cells contained lysosome-like bodies, granules of glycogen and very few rough ergastoplasmic cysternae (Fig. 10).

Osteocyte-like cells were occasionally found in the uncalcified osteoid tissue. They were also observed in incompletely calcified areas, where they were apparently contained in large, irregularly shaped lacunae bordered by uncalcified collagen fibrils which were in direct contact with the osteocyte peripheral membrane.

The bone marrow spaces did not always contain haemopoietic cells. In some cases, these were replaced by fat cells. In other cases, a fibrous tissue was present, consisting of elongated and roundish fibroblasts and a few collagen fibrils. There were also typical mast cells and giant cells; the giant cells were polynucleated and had an osteoclast-like structure, except that they had no brush border (Fig. 11).

## Discussion

The most evident finding in these biopsies was the histological and ultrastructural evidence of increased rate of bone resorption produced by osteoclastic activity and osteocytic osteolysis.

The osteoclasts were not markedly different from those found in controls and from those previously described in normal bone (Cameron, 1972). Despite this, and despite the impracticability of quantitative measurements, given the extreme variability of ultrastructural parameters and their close dependance on the plane of the section and on cellular orientation (Holtrop and King, 1977), a careful comparison between the osteoclasts of control subjects and those of patients with primary hyperparathyroidism showed that there was often a more highly developed brush border and a greater number of cytoplasmic vacuoles in the latter than in the former. Since these findings are considered to be ultrastructural evidence of increased resorption activity (Holtrop and King, 1977), and since parathormone increases the ruffled border of the osteoclasts both in vivo and in vitro (Holtrop et al., 1974), it is probable that in our cases osteoclast function was enhanced, not only because more osteoclasts were present than usual (see also Schenk et al., 1973) but also because each single cell had a more intense resorptive activity than those of the controls.

As previously reported (Meunier et al., 1971), the osteocytes were often surrounded by large, irregularly shaped lacunae. Electron microscopy showed that a space containing what looked like ramnants of fragmented bone matrix lay between the peripheral membrane of these osteocytes and the wall of their lacunae. All of these findings may be considered evidence of osteocytic osteolysis (Bélanger, 1971; Bonucci and Gherardi, 1977; Remagen et al., 1969) and their frequency in the present series of patients is not surprising if one considers that osteocytic osteolysis increases as parathormone secretion rises (Bélanger,

1971). It may therefore be concluded that bone resorption in primary hyperparathyroidism is not only due to osteoclast overactivity but to periosteocytic osteolysis as well.

Besides bone resorption, bone apposition was another evident feature, as shown chiefly by the presence of thick osteoid borders along the bone trabeculae. The stimulating effect of parathormone on osteoblast secretion and its role in the regulation of the anabolic-catabolic balance in bone has recently been discussed by Parsons (1976), who underlines that the stimulation of osteoblasts by parathormone has often been underestimated with respect to that of osteoclasts.

In parathormone-treated, thyroparathyroidectomized rats, osteoblasts have large mitochondria and prominent rough endoplasmic reticulum and Golgi apparatus (Weisbrode et al., 1974); that is, they have the ultrastructural characteristics of actively synthesizing cells. Most of the osteoblasts in the present series of cases had these characteristics. They were not sufficient to differentiate the parathormone-stimulated osteoblasts from active osteoblasts found in areas of ossification in controls, but only few osteoblasts had these characteristics in normal subjects over 20 years of age, whereas they often occurred in our patients although all of them were over 30.

These findings stand as further confirmation that osteoblastic overactivity really occurs in human primary hyperparathyroidism, although spindle-shaped, apparently inactive cells are found near the calcified matrix in even the severest cases of hyperparathyroidism.

Besides roundish and spindle-shaped osteoblasts, other cells were recognizable near the bone matrix. Some were small and showed very few cytoplasmic organelles; these were probably immature osteogenic cells. Others were irregularly shaped; their many cytoplasmic processes and their ultrastructural morphology suggested that they might be macrophages, but no evidence of phagocytosis was found.

It is known that the rate of bone formation and the thickness of the osteoid border often increase in primary hyperparathyroidism (Boyce and Jowsey, 1966; Delling, 1974; Miravet et al., 1967; Olah, 1973) and this has been confirmed by the present investigation. There has been frequent discussion as to whether the increase in amounts of osteoid tissue is simply due to the demonstrable increase in osteoblastic activity or to a normal rate of osteoid production combined with a delay in mineralization. Even if the rate at which osteoid tissue becomes calcified may sometimes be the same as in normal subjects (Olah, 1973), and if quantitative measurements have shown that the mean width of osteoid seams may be normal, suggesting that mineralization is undisturbed (Schenk et al., 1973), severe falls in osteoid mineralization have often been reported in primary hyperparathyroidism and considered to be dependent on an abnormal vitamin D metabolism (Bordier et al., 1973; Miravet et al., 1967; Stanbury, 1972). The present results not only confirm the presence of excessive amounts of osteoid tissue in areas of bone formation, but also show that the calcification process is delayed, as demonstrated by the frequent lack of the calcification front and the frequently incomplete mineralization of the bone matrix.

Osteoclast-like cells were present in marrow spaces affected by fibrosis. Here also, mast cells were often found. It has been shown by Urist and MacLean (1957) that mast cells increase in number in the endosteum of rats fed on a diet producing moderate rickets, osteoporosis and osteitis fibrosa. On the other hand, it has been shown that bone resorption is greatly enhanced in vitro if heparin is added to ineffective doses of parathormone (Goldhaber, 1965). As mast cells secrete heparin, and their number rises in primary hyperparathyroidism, it could be suggested that a direct (Jowsey et al., 1970) or indirect factor may be at work in stimulating bone resorption. If so, their function, like that of the associated osteoclast-like giant cells, has not been defined.

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