Effects of Parathyroid Hormone on Bone of Thyroparathyroidectomized Rats

An Ultrastructural and Enzymatic Study

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Osteoblasts and osteocytes in adult thyroparathyroidectomized (T_xPT_x) rats fed a low calcium vitamin D-free diet and given parathyroid (PTH) had ultrastructural evidence of increased activity compared to controls. Osteoblasts in PTH-treated rats had prominent rough endoplasmic reticulum and Golgi apparatuses and large mitochondria. The plasma membranes were extensively convoluted and associated with initial loci of mineralization in osteoid. Osteocytes contained increased rough endoplasmic reticulum, well-developed Golgi apparatuses and large mitochondria. Lacunar walls were roughened, but osteocytic osteolysis was not observed. Osteoclasts were encountered more frequently in PTH-treated rats, but their ultrastructural features were similar to those of controls. Osteoblasts and osteocytes in controls appeared to be inactive cells lining quiescent mineral surfaces. Parathyroid hormone treatment increased serum calcium levels and urinary hydroxyproline excretion, indicating enhanced resorption of bone mineral and matrix. Bone alkaline phosphatase and calcium–adenosine triphosphatase activities were elevated after PTH treatment and may be related to increased calcium transport by bone cells. These findings were interpreted to suggest that PTH mobilizes bone mineral by osteoclasis and increases metabolic activity of the osteocyte-osteoblast pump (Am J Pathol 75:529–542, 1974).

A MAJOR ROLE OF PARATHYROID HORMONE (PTH) in calcium homeostasis is to translocate calcium from bone to the extracellular fluid. The cellular and biochemical mechanisms of this function still are uncertain.[1] Parathyroid hormone is known to stimulate synthesis and release of lysosomal acid hydrolases and lactic and citric acids in bone cells.[2] Direct and indirect evidence suggests that PTH is able to increase the activity of bone alkaline phosphatase (APase).[3,4] Alkaline phosphatase [5,6] and calcium–adenosine triphosphatase (Ca–ATPase) [5,7] have been reported to be intimately associated with calcium absorption in the intestine. These enzymes have been localized ultrastructurally in bone cells [8,9] and have been implicated in calcium homeostasis.[9–11]

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Increased osteoclasis has been considered inadequate to explain the hypercalcemia of hyperparathyroidism and recent interest has focused upon the response of the osteocyte and osteoblast to PTH.[1] An insight into the role played by osteocytes and osteoblasts in calcium homeostasis can be achieved with the electron microscope.[12] However, results of ultrastructural studies on the effects of PTH on bone have been equivocal [13,14] and none have eliminated possible secondary influences of endogenous calcitonin secretion. Therefore, the objectives of this investigation were to evaluate the effects of PTH on bone of adult thyroparathyroidectomized (T_xPT_x) rats by light and electron microscopy and to correlate these findings with changes in serum calcium and phosphorus, urinary hydroxyproline excretion, and bone APase and Ca–ATPase activities.

Materials and Methods

Animals and Experimental Design

Male Sprague-Dawley rats weighing 300 to 350 g were caged individually and housed in rooms free of ultraviolet light. All rats were surgically thyroparathyroidectomized. Rats with serum calcium below 7 mg/100 ml 48 hours postoperatively were given either 25 USP units of bovine parathyroid extract (PTH) (kindly supplied by Eli Lilly and Company, Indianapolis, Ind) or placebo twice daily and pair fed a low calcium (0.05%), normal phosphorus (0.3%) diet with no added vitamin D for the experimental period of 7 days. All rats were supplemented daily with 10 μg sodium levothyroxine (Synthroid,® Travenol Laboratories, Inc, Morton Grove, Ill).

Urine was collected during the 24 hours prior to euthanasia for determination of total urinary hydroxyproline (HOP) by the method of Kivirikko *et al* [15] and creatinine (CR) according to Clark.[16] These data were expressed as HOP:CR ratios to correct for variability in urine concentration. At the end of the 7-day period all rats were exsanguinated from the abdominal aorta under light ether anesthesia. Terminal serum calcium was measured by atomic absorption spectrophotometry (Perkins-Elmer Model 303) and serum phosphorus according to Fiske and Subbarow.[17]

Morphologic studies were performed on 5 rats receiving PTH and 5 rats given placebo. For light microscopy, tibias were dissected free of soft tissue, divided longitudinally, fixed in buffered formalin, decalcified to effect in 10% ethylenediaminetetraacetic acid disodium salt (EDTA), dehydrated in alcohol, embedded in paraffin, sectiond at 6 μ and stained with hematoxylin and eosin.

Electron Microscopy

Bone sections from the tibial diaphysis and metaphysis (approximately 1 cu mm) were removed quickly, dissected free of soft tissue and fixed in 3% glutaraldehyde with 0.1 M sodium cacodylate buffer at pH 7.4. The tissue was postfixed in 1% osmium tetroxide with *s*-collidine buffer at pH 7.4, dehydrated through ascending concentrations of ethanol, transferred to propylene oxide and embedded in "hard" Epon (Shell Chemical Company, New York, NY). Thin sections were cut

with a diamond knife on an LKB ultramicrotome and floated on a water bath buffered at pH 7.4 to prevent demineralization. Sections were stained with uranyl acetate and lead citrate and examined with a Philips 200 electron microscope.

Enzyme Assay

Ten rats receiving PTH and 10 control rats given placebo were used for assay of bone APase and Ca–ATPase activity. Enzyme activities were determined on bone from each rat. Methods of bone collection have been described previously.[9] Alkaline phosphatase activity was assayed according to Hausamen *et al.*[18] Calcium–adenosine triphosphatase was determined by the technic of Nagode.[19] The substrate media contained 5 mM calcium, 5 mM magnesium and 2 mM Tris-ATP in 100 mM Tris-HCl buffer at pH 7.4. Phosphorus release was measured according to Baginski *et al.*[20] Enzyme activity was determined for each bone section which was placed in either 5 ml of Ca–ATPase substrate media or 6 ml of APase substrate media with constant agitation. The bone sections were dried, weighed and lyophilized. Lyophilized bone was pulverized in a mortar and pestle, and protein was extracted from the powder using 0.1% Triton X-100 in 0.01 M sodium acetate buffer at pH 4.0. Protein estimations were made according to Lowry *et al.*[21] Enzyme activity was determined for bone of each individual rat and expressed as nanomoles phosphorus per minute per milligram protein for APase and Ca–ATPase.

Results

Serum Electrolytes, Urinary HOP, Bone Enzyme Assay

PTH significantly elevated serum calcium and lowered serum phosphorus compared to controls (Table 1). Urinary HOP excretion expressed as HOP:CR ratio also was increased significantly by PTH treatment.

Table 1—Terminal Serum Calcium and Phosphorus and Urinary HOP Excretion in T_xPT_x Rats Receiving Either Placebo or PTH

	Placebo	PTH
Serum calcium (mg/100 ml ± SE)	4.73 ± .34	6.80 ± .23*
Serum phosphorus (mg/100 ml ± SE)	14.09 ± 82	8.79 ± 29*
Urinary HOP/CR	0.241 ± .04	0.389 ± .05†

* $P < .001$
† $P < .025$

Bone APase and Ca–ATPase activities were significantly higher in rats receiving PTH than in controls (Table 2).

Light Microscopy

In control rats, little metaphyseal trabecular bone was present. Projections of mineralized cartilage matrix and hypertrophied chondrocytes were capped with a blunt scalloped border of bone (Figure 1). Spicules of bone in the metaphysis were lined intermittantly by flattened osteo-

blasts (Figure 2). In rats receiving PTH primary and secondary spongiosa were abundant with cartilage cores and bone spicules covered with numerous plump osteoblasts and many osteoclasts (Figure 3).

Table 2—Bone Alkaline Phosphatase and Calcium–Adenosine Triphosphatase Activity in T_xPT_x Rats Receiving Either Placebo or PTH

	Placebo	PTH
Bone APase (nM P/min/mg protein ± SE)	308 ± 20	389 ± 20*
Bone Ca-ATPase (nM P/min/mg protein ± SE)	17.9 ± 1.5	25.2 ± 1.6†

* $P < .01$
† $P < .005$

Vascular channels in diaphyseal bone in control rats were narrow and smooth and lined with a layer of flattened osteoblasts. In PTH-treated rats, frequent vascular channels in diaphyseal cortical bone were irregularly widened, presumably by osteoclastic activity, and filled with hyperplastic osteoblasts (Figure 4).

Electron Microscopy

In control rats osteoblasts were fusiform with little organellar development and a smooth plasma membrane. They bordered a narrow osteoid suface. The mineralizing front appeared relatively regular and even (Figure 5). Most osteocytes from rats receiving placebo were interpreted to be inactive cells having few organelles and mild scalloping of their plasma membrane. They resided in lacunae that were well mineralized and smooth-walled (Figure 6).

Osteoblasts in rats receiving PTH appeared to be active cells with abundant rough endoplasmic reticulum, numerous mitochondria and prominent Golgi apparatuses. The plasma membranes on the osteoid surfaces were extensively convoluted and sent cytoplasmic projections deep into the moderately wide zone of osteoid. The mineralizing front was irregular with loci of mineral coalescing into sheets of bone (Figure 7). Mineralization appeared to be initiated in the cytoplasmic processes of osteoblasts (Figure 8).

Many osteocytes in rats receiving PTH had increased rough endoplasmic reticulum, mitochondria and Golgi apparatuses. The plasma membranes were corrugated, and the lacunar walls were roughened having hydroxyapatite crystals jutting out perpendicularly into the pericellular space (Figure 9). Unmineralized or demineralized zones of mature collagen of varying widths were present around some osteocytes (Figure 10).

Osteoclasts were present in increased numbers in rats treated with PTH but their ultrastructural features did not vary from controls (Figure 11).

Discussion

PTH administered to mature male T_XPT_X rats fed a low calcium diet resulted in enhanced osteoclasis, hyperplasia of osteoblasts, organellar hypertrophy in osteoblasts and osteocytes, and changes in the lacunar wall around osteocytes compatible with mineral resorption. These changes were associated with a significant rise in serum calcium and urinary HOP excretion, suggesting increased bone mineral release and greater bone matrix turnover,[22] respectively, and a fall in serum phosphorus, reflecting the phosphaturic action of PTH. Rats receiving PTH had greater amounts of metaphyseal trabecular bone lined by large osteoblasts and many osteoclasts compared to controls. The differences in amount of metaphyseal trabecular bone probably represent a combination of PTH-induced osteosclerosis[23] and a reduction of bone apposition in controls.[24] Although osteoclasis is reported not to be a constant response to PTH administration, the increased osteoblastic and osteoclastic activity in metaphyseal bone is in agreement with findings in intact growing rats giving PTH.[25]

Electron microscopically the PTH-stimulated osteoblasts differed dramatically from controls. Control osteoblasts were interpreted to be inactive cells on narrow osteoid seams abutting a smooth mineralizing surface. These ultrastructural findings in osteoblasts of control rats were considered to be compatible with the results of morphometric studies, indicating a decrease in organic matrix synthesis by osteoblasts and diminished bone apposition in T_XPT_X rats.[24] Osteoblasts in PTH-treated rats were interpreted to be metabolically active cells that were synthesizing collagen and participating in matrix mineralization. These observations are different from findings of degenerative changes in osteoblasts of intact growing rats given very high levels of PTH.[13] Although these changes may have been a manifestation of PTH-induced cell damage, they might represent a response to increased endogenous calcitonin secretion.

Osteocytes in control rats appeared, electron microscopically, as inactive cells with few organelles. Their lacunar walls were well mineralized and smooth. After PTH administration, osteocytes appeared to be more metabolically active and resembled younger newly embedded osteoblasts in organellar development. Lacunar walls were moderately roughened or occasionally rimmed with bands of dense mature collagen

devoid of mineral suggesting mineral mobilization. The increased development of Golgi apparatuses in osteocytes, irregular plasma membranes and roughened lacunar walls around osteocytes after PTH all are compatible with early stages of osteocytic osteolysis that have not progressed to matrix destruction.[26] These stimulatory effects of PTH on osteocytes differ from the degenerative and disruptive changes described by Cameron [13] and Jande [14] in intact animals. The PTH-induced elevated metabolic activity of osteocytes may represent increased production of organic acids capable of mineral disolution.[2] The failure of PTH alone unsupplemented with vitamin D to induce complete osteocytic osteolysis (defined as mineral and matrix destruction of bone) is compatible with the well-established interaction of PTH and vitamin D for a calcemic response [27,28] and is supported by our recent electron microscopic demonstration of osteocytic osteolysis in T_XPT_X rats given both PTH and vitamin D.[29]

Osteoclasts were more numerous in T_XPT_X rats given PTH but ultrastructurally similar in both groups. Exogenous calcitonin induces rapid flattening and loss of cytoplasmic coating of osteoclast brush borders.[30] Osteoclasts in T_XPT_X rats receiving placebo possessed all the electron microscopic features characteristic of these cells,[31] implying that the presence of the brush border, vesicles and lysosomal bodies are not hormone dependent.

Parathyroid hormone significantly increased the activity of bone APase and Ca–ATPase compared to placebo. Alkaline phosphatase [5,6] and Ca–ATPase [5,7] have been implicated in vitamin D-induced calcium translocation in the intestine. The role these enzymes play in bone is unknown, but recent studies have postulated that they function as hydrolases of inorganic pyrophosphates, which might act to prevent formation or resorption of hydroxyapatite [11] or as energy-dependent calcium transport systems.[10]

We speculate that the increased activity of bone APase and Ca–ATPase following PTH are biocheimcal reflections of enhanced metabolic activity of an osteocyte-osteoblast unit or network functioning as an energy-dependent calcium pump.[1,32] It is concluded that in T_XPT_X rats fed a low calcium vitamin D-free diet, PTH releases calcium from bone by osteoclasis and stimulation of the osteocyte–osteoblast pump.

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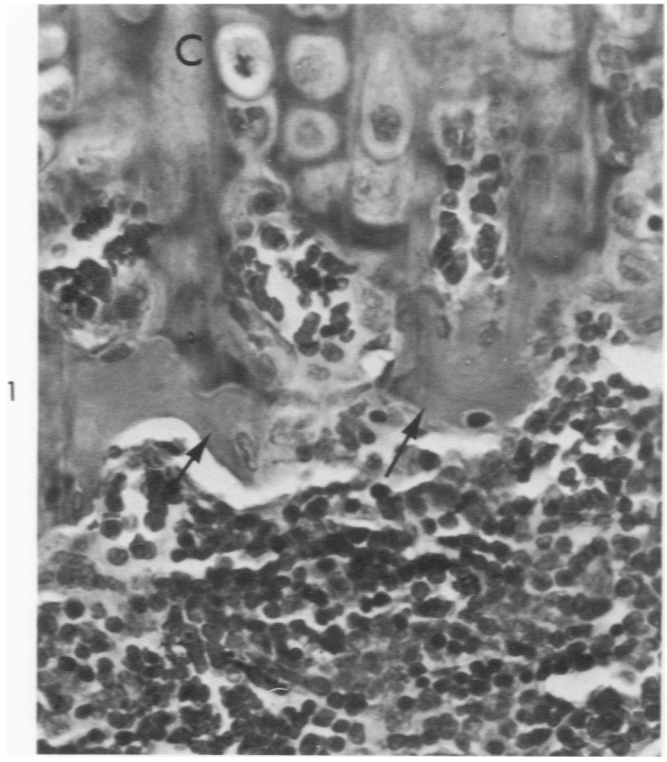

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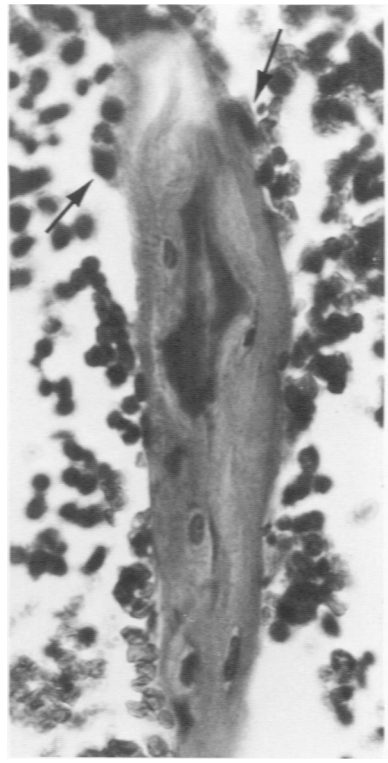

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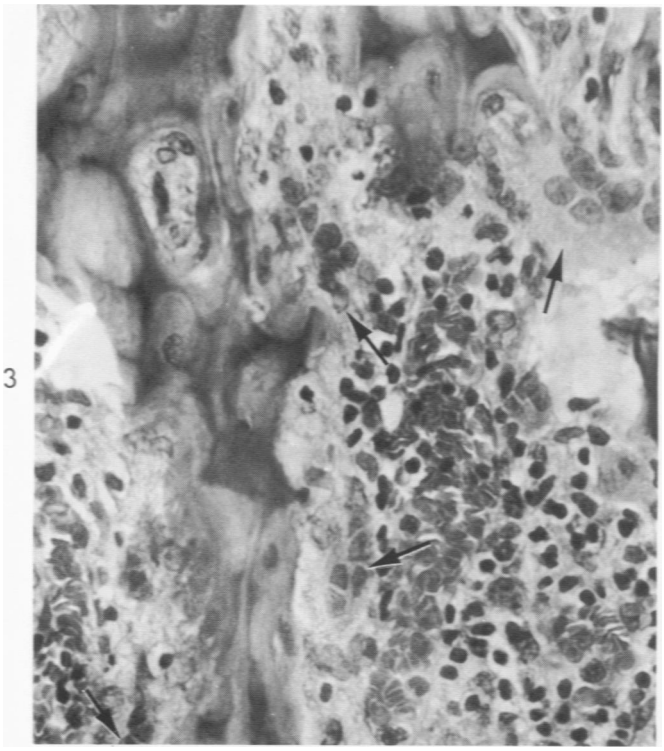

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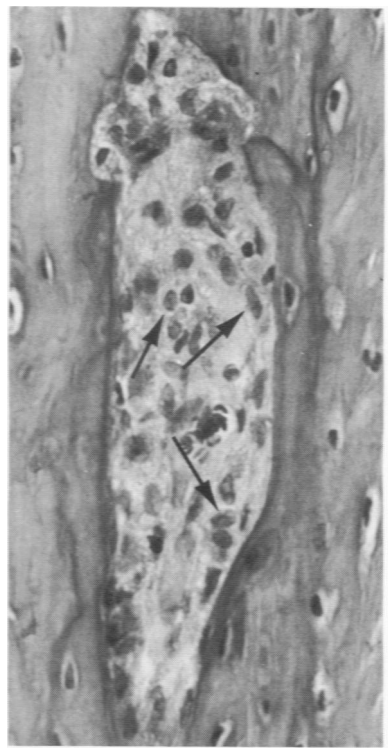

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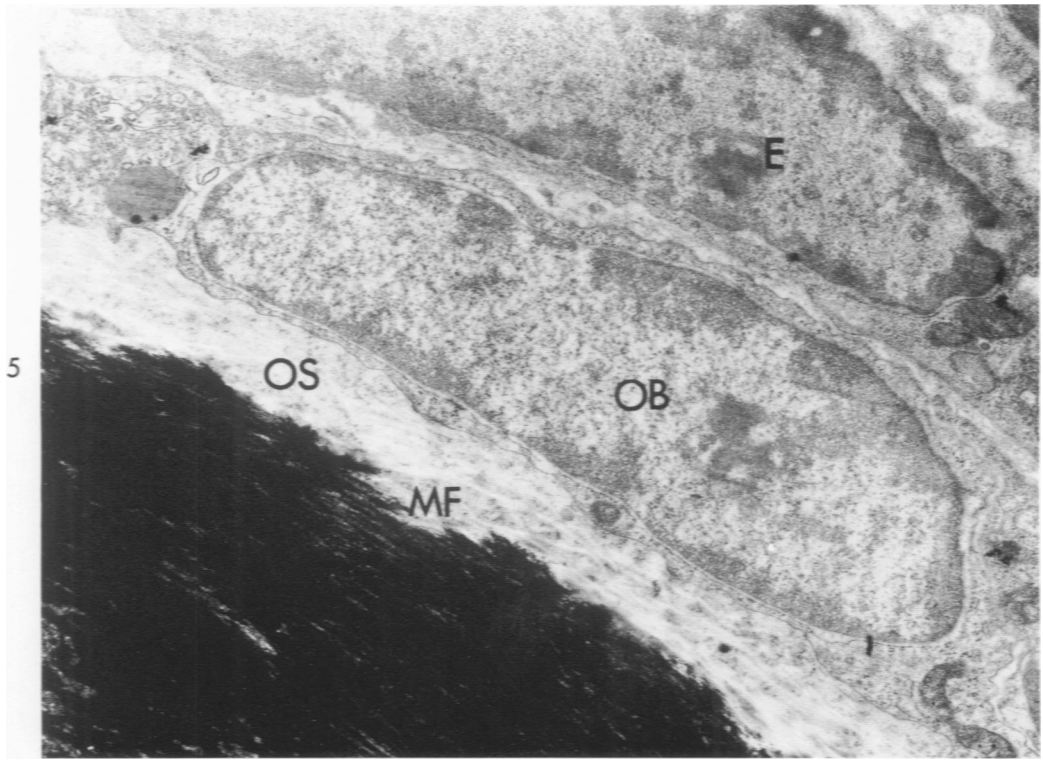
Fig 1—Hypertrophied chondrocytes (*C*) and shortened primary spongiosa (*arrows*) with little osteoblastic activity in T_xPT_x rat receiving placebo (H&E, × 315). **Fig 2**—Metaphyseal trabecular bone in T_xPT_x rat given placebo. Few osteoblasts with little cytoplasm (*arrows*) line the bone surface (H&E, × 500). **Fig 3**—Primary spongiosae in T_xPT_x rat given PTH lined by osteoblasts and several osteoclasts (*arrows*). Section is from region just below epiphyseal plate (H&E, × 315). **Fig 4**—Widened vascular channel in cortical bone filled with hyperplastic osteoblasts (*arrows*) in T_xPT_x rat given PTH (H&E, × 315).



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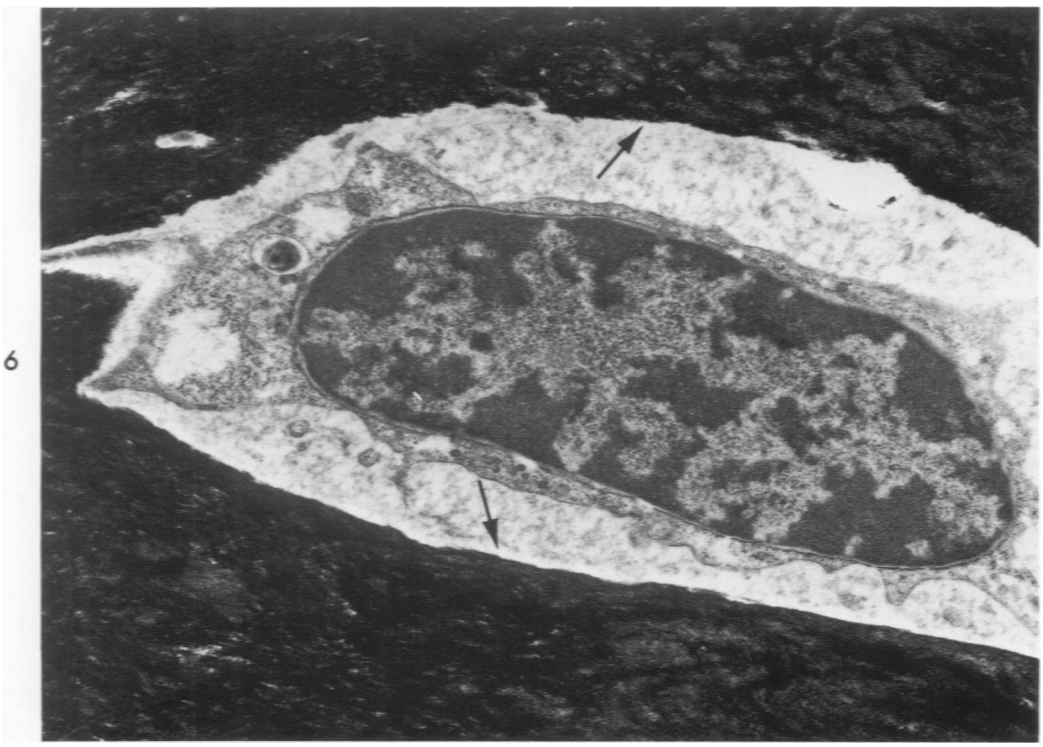

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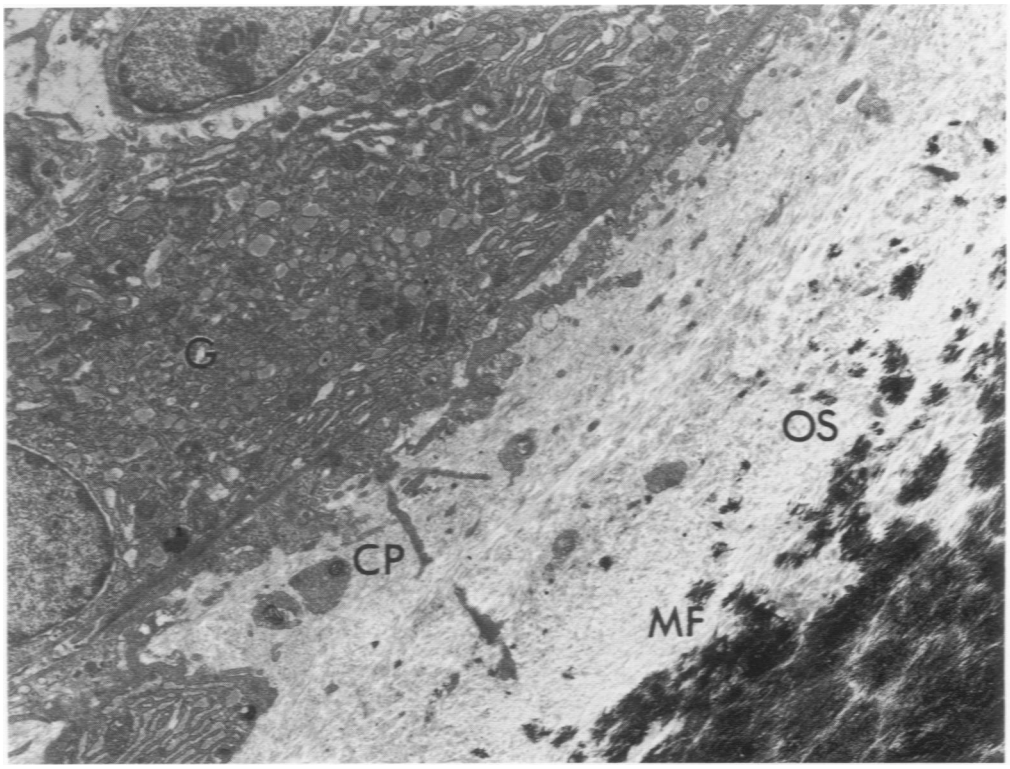
Fig 5—Osteoblast (*OB*) with few cytoplasmic organelles bordering a narrow zone of osteoid (*OS*) and a relatively even mineralizing front (*MF*). Portion of an endothelial cell (*E*) is seen at upper right (× 9900). **Fig 6**—Osteocyte with few organelles in a smooth walled and well-mineralized lacuna (*arrows*) in T_xPT_x rat given placebo (× 5900).



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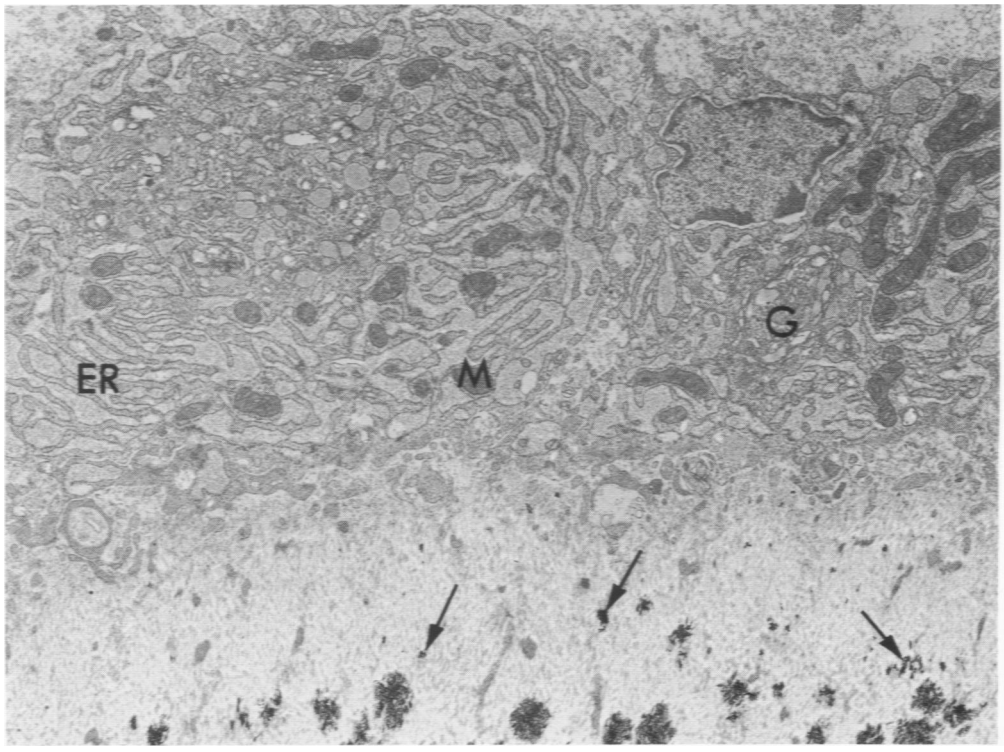

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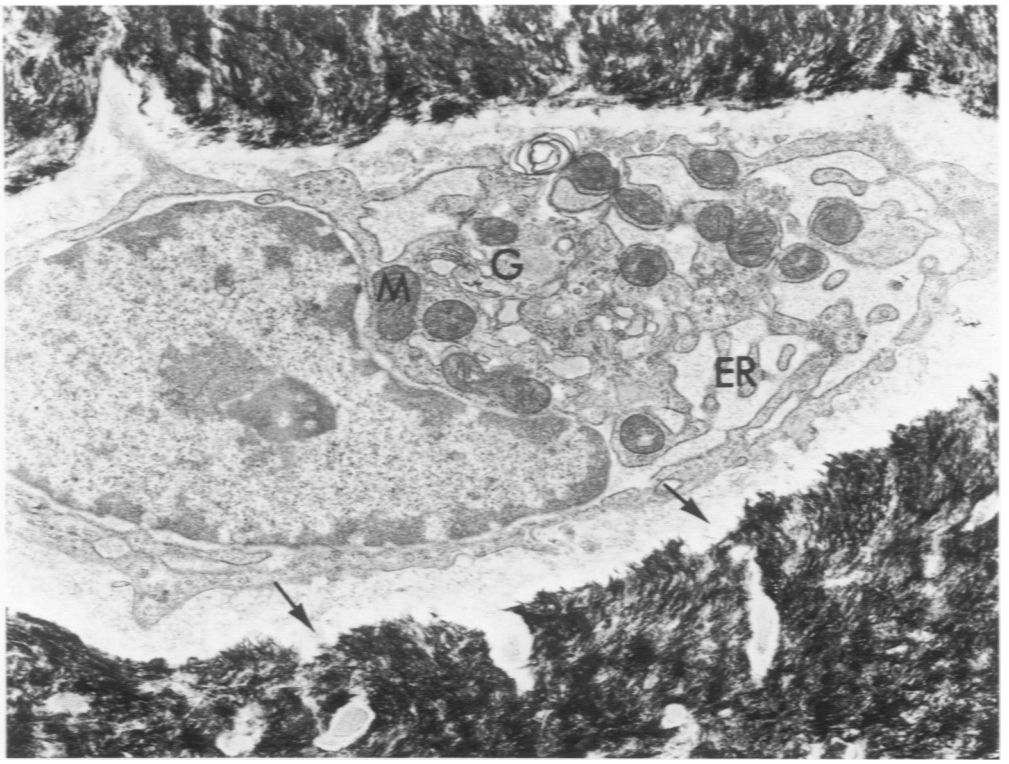
Fig 7—Osteoblasts from T_xPT_x rat given PTH with numerous Golgi vesicles (*G*), rough endoplasmic reticulum and mitochondria. Cytoplasmic projections (*CP*) extend into the osteoid (*OS*). The mineralizing front (*MF*) is irregular (× 3500). **Fig 8**—Initial loci of mineralization (*arrows*) are seen in cytoplasmic projections of osteoblasts from T_xPT_x rats treated with PTH. Osteoblasts contain abundant endoplasmic reticulum (*ER*), large mitochondria (*M*) and prominent Golgi apparatuses (*G*) (× 3600).



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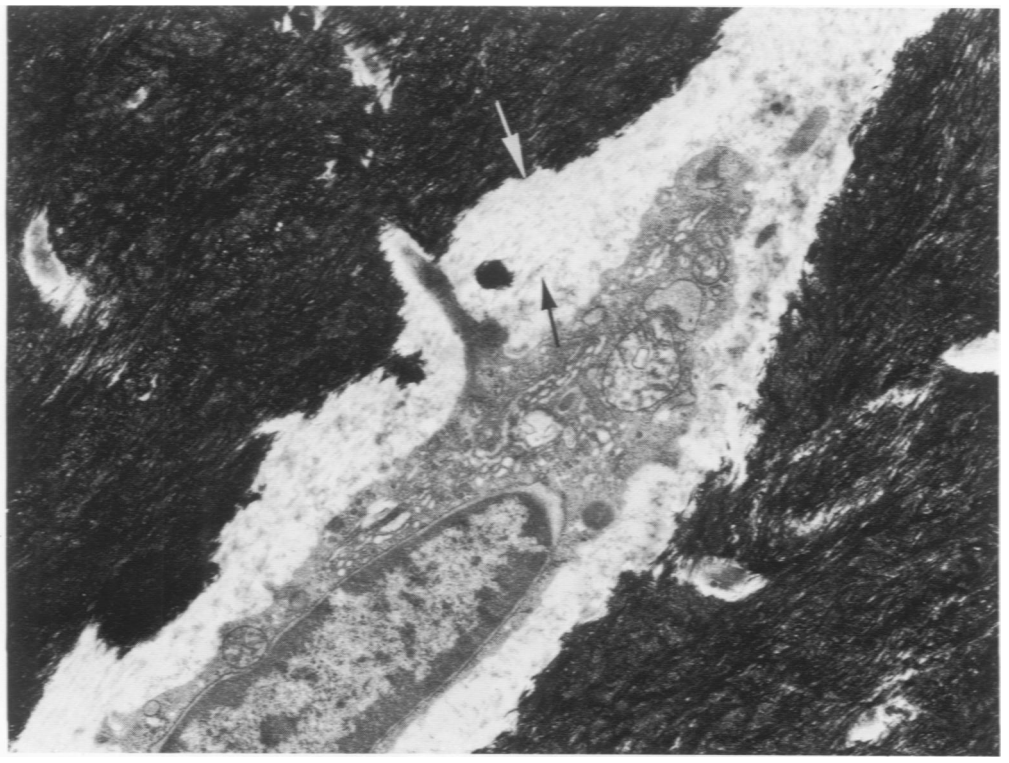

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Fig 9—Osteocyte from T_xPT_x rat receiving PTH. Rough endoplasmic reticulum (*ER*), mitochondria (*M*) and Golgi apparatus (*G*) are prominent. The lacunar wall is irregular and rough (*arrows*) (× 9900). **Fig 10**—Osteocyte with well-developed Golgi apparatus from T_xPT_x rat given PTH. The lacunar wall has zones of demineralized mature collagen (*arrows*) (× 9900).

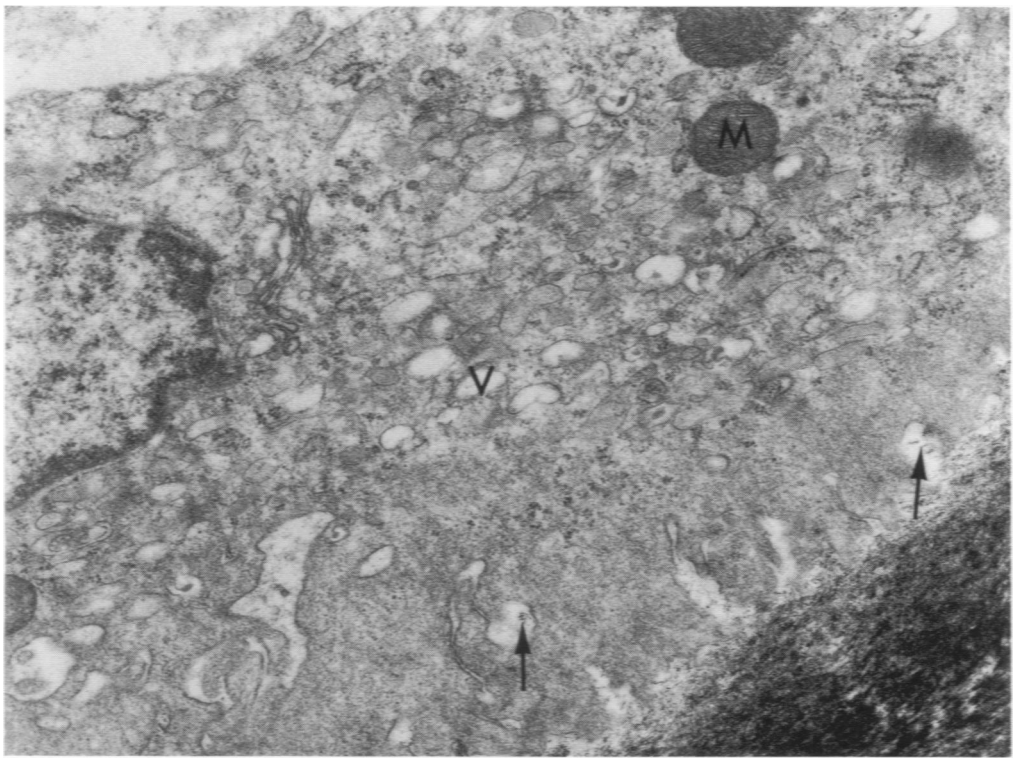

Fig 11—Tangential section of osteoclast brush border in T_xPT_x rat given placebo illustrating mineral spicules (*arrows*) between the cytoplasmic processes of the brush border. Numerous mitochondria (*M*) and vesicles (*V*) are present in the osteoclast (× 12,500).

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