CHANGES IN THE FINE STRUCTURE OF BONE CELLS AFTER THE ADMINISTRATION OF PARATHYROID EXTRACT

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ABSTRACT

This paper describes the effect of parathyroid extract on the fine structure of bone cells in the proximal metaphysis of the tibia. The extract was given to rats intraperitoneally, in single or repeated doses of 1–14 units (USP) per gram body weight, over short or extended periods, and the tissue was examined between 6 and 26 hr after the commencement of injection. During this period, osteoblasts showed the greatest changes. Their mitochondria became swollen and contained dense granules; both rough- and smooth-surfaced vesicles were distended; ribosomes were separated from the membranes; and various dense bodies appeared in the cytoplasm. Osteocytes were similarly but less affected, and osteoclasts differed little from those in normal animals. Eventually after repeated injections, the fine structure of many of the cells was disrupted. These changes were accompanied by erosion of the bony trabeculae without any great increase in numbers of osteoclasts. It is possible that the alterations in fine structure were the result of damage to the cells by the parathyroid extract. However, since the extract did not seem to stimulate classical osteoclastic activity, the mitochondrial swelling and dense bodies could have been related to the removal of mineral and matrix, respectively.

INTRODUCTION

The parathyroid glands control the level of calcium ions in the serum by acting chiefly at three sites: the gut, the kidneys, and the skeleton (Rasmussen, 1961; Bartter, 1961; McLean and Urist, 1961). An extract of the glands injected intraperitoneally into experimental animals has been shown to produce a rise in serum calcium and extensive bone resorption (McLean, 1958). When large amounts are injected the osteoblasts seen in the normal animal are replaced by spindle-shaped cells and multinucleated osteoclasts (Heller et al., 1950). The extract presumably has a direct effect on the bone cell population because glands transplanted to the calvarium in vivo (Barnicot, 1948; Chang, 1951) or explanted with bone in vitro (Gaillard 1961) can produce local resorption. It has been proposed that the extract alters the metabolic processes (Firschein et al., 1958; Nichols, 1963) in the bone cells in such a way that the mineral and organic matrix can be dissolved and dissipated into the extracellular fluid, but there is no information about accompanying structural alterations in the cells.

Studies of the fine structure of bone cells have shown that there are characteristic differences between osteoblasts and osteoclasts (Scott and Pease, 1956; Dudley and Spiro, 1961; Gonzales and Karnovsky, 1961; Cameron, 1963; Hancox and Boothroyd, 1963; Robinson and Cameron, 1964). Osteoblasts have a structure organized for the

synthesis of protein for export to form bone matrix (Fitton Jackson and Randall, 1956), but the appearance of multinucleated osteoclasts suggests that they are equipped to do the opposite; that is, to break down what osteoblasts have built up. Their endoplasmic reticulum is not well developed, and loose inorganic crystals and fragmented collagen matrix under and between intricate folds of the brush border (Scott and Pease, 1956) are clear evidence of bone destruction. Because bone removal is increased by excess parathyroid extract, it is reasonable to expect that there might be an increase in the number of multinucleated osteoclasts, or of cells with only one nucleus having the same ability as osteoclasts and that this increase should be accompanied by a change in the fine structure of the previously existing cells.

Heller et al (1950), in their classical paper on the relationship between the parathyroid gland and bone, have described the transformations in the cells that can be seen with the light microscope as the result of the injection of large amounts of parathyroid extract (PTE.). We have used their work as a starting point, and our object was to study the changes in the fine structure of bone cells that could be seen with the electron microscope after PTE had been given to young rats.

MATERIALS AND METHODS

The rats were given PTE (Parathormone, Eli Lilly & Co., Indianapolis), inactivated PTE, or a substitute solution (Vaes and Nichols, 1962) by intraperitoneal injection. Table I gives the weight of each animal, the number of units injected, and the time at which each was killed. The animals were kept on a normal laboratory diet. Untreated animals provided further material for comparison.

The bone for study was taken from the proximal end of the tibia after each animal had been killed by dislocating the cervical vertebrae. The tissue was embedded in Araldite and the procedures for fixation, etc. were the same as those previously

TABLE I

Animal Weight, PTE Dose, and Time Interval

Rat No.	Weight	Dose	Time rat killed after 1st injection
	g		*hr*
		PTE	
26	100	100 units single injection	13
29	135	300 units single injection	13
30	125	100 + 100 units 1 hr apart	13½
45	40	40 units every 4 hr	26¼
46	40	80 units every 4 hr	26½
47	48	48 units every 4 hr	16
48	48	96 units every 4 hr	16
49	50	50 units every 4 hr	9½
50	53	106 units every 4 hr	9¼
52	100	500 + 500 units 1 hr apart	67
53	100	1000 units single injection	16¼
54	100	1000 units single injection	9¾
67	95	500 + 500 units ½ hr apart	12
68	113	500 + 500 units ½ hr apart	9
69	139	500 units single injection	6
94	31	100 units single injection	13
95	39	120 units single injection	10¼
		Inactivated PTE (Controls)	
251	40	200 units (equivalent) single injection	17
256	50	200 units (equivalent) single injection	8½
		Substitute solution	
255	160	600 + 600 units (equivalent) 1 hr apart	8½
257	100	500 + 500 units (equivalent) ½ hr apart	16¾

described in detail (Robinson and Cameron, 1964).

OBSERVATIONS

Light Microscopy

The histological picture depended on the size of the dose of PTE and on the time that had elapsed between the administration of the 1st dose and the killing of the animal. In the animals given the smallest dose or killed within 6 hr of a larger dose the changes were minor. Some of the osteoblasts were more elongated and spindle shaped than those in the normal animal, but there was no evidence of resorption of calcified tissue. After about 9 hr, an animal given a single injection of 3 units/g or 3 injections of 2 units/g at 4-hr intervals (Fig. 2) showed increased spindling and separation of the osteoblasts, and occasional dense bodies in their cytoplasm.

When larger single or repeated small doses of PTE were used, a loss of trabeculae became apparent between 9 and 16 hr (Fig. 3). Most of the cells were spindle shaped, separated from one another, and more of them contained dense bodies, but still there was no obvious increase in number of osteoclasts. Occasionally osteoclasts contained cells in vacuoles in their cytoplasm, but there was no evidence of fusion of spindle cells or osteocytes to form multinucleated cells. Some single nucleated cells had pyknotic nuclei, and small areas of hemorrhage were common. The bone from the animals given a large dose at the beginning of the experimental period showed maximum resorption and cellular alteration by about 16 hr, but, with repeated small doses, the changes progressed. In the animal given 2 units/g at 4-hr intervals and killed at 26½ hr (Fig. 4) most of the primary spongiosa had disappeared, many of the bone cells were necrotic or contained dense bodies, and there was a large amount of hemorrhage. The osteoclasts were more numerous than earlier, but there was still a preponderance of spindle cells on and between the few remaining trabeculae. Dividing cells were present in small numbers at this time as well as earlier. In the animal given half this dose, the destruction was not so extensive and necrosis and hemorrhage were less obvious.

In the animals given inactivated PTE or the substitute solution, the bone was similar to that in the normal animal (Fig. 1).

Electron Microscopy

The changes seen with the light microscope were paralleled by changes in fine structure. Fig. 5 shows the appearance of many of the cells between 9 and 16 hr after administration of 3–10 units of PTE.

OSTEOBLASTS: These cells showed the greatest difference from the normal. The earliest changes (6 hr after 4 units/g) were the swelling of mitochondria and the appearance of numerous dense bodies before the cells became elongated. By 9 hr, the mitochondrial swelling was widespread (Figs. 5 and 6), although not every spindle cell was affected. The swollen mitochondria were also seen in dividing cells. The cristae did not pass from one wall to the other and were often widely separated, so that the organelles appeared vacuolated (Fig. 6). Many mitochondria contained small, dense bodies, each composed of a rosette of fine granules (Figs. 7 and 7 *a*).

The Golgi region was present in the spindle-shaped cells (Figs. 6, 6 *a*, and 11) although the smooth-surfaced cisternae became increasingly swollen as time passed (Fig. 8). Two changes appeared in the rough-surfaced endoplasmic reticulum. In some cells, much of the membrane system had disappeared and the ribosomes lay free in the cytoplasmic matrix (Fig. 9). In other cells, the cisternae were widely dilated (Figs. 1 and 8) and were eventually disrupted. Polysomes could often be seen in the cytoplasm (Figs. 7–9). Dense bodies of various types (Figs. 5, 6, 9–11) resembling lipid droplets, lysosomes, and myelin figures were present in many of the cells when most of the resorption was occurring.

The plasma membranes were like those in normal cells. They were relatively smooth with few projections and did not appear to fuse with those of neighboring cells (Figs. 6, 9, and 12). After 26 hr of being repeatedly dosed, many of these cells had washed-out nuclei with irregularly clumped chromatin; cell membranes were discontinuous and organelles were free between the cells.

OSTEOCYTES: The changes in these cells were less marked than in the osteoblasts. Mitochondria became swollen and dense bodies appeared in many, but not all cells (Figs. 12 and 13). There was not so much distension of the cytoplasmic membrane systems. Many of the cells did not fill their lacunae and were separated from the calcified matrix by a fine amorphous material that was free of collagen fibrils (Fig. 12). The plasma mem-

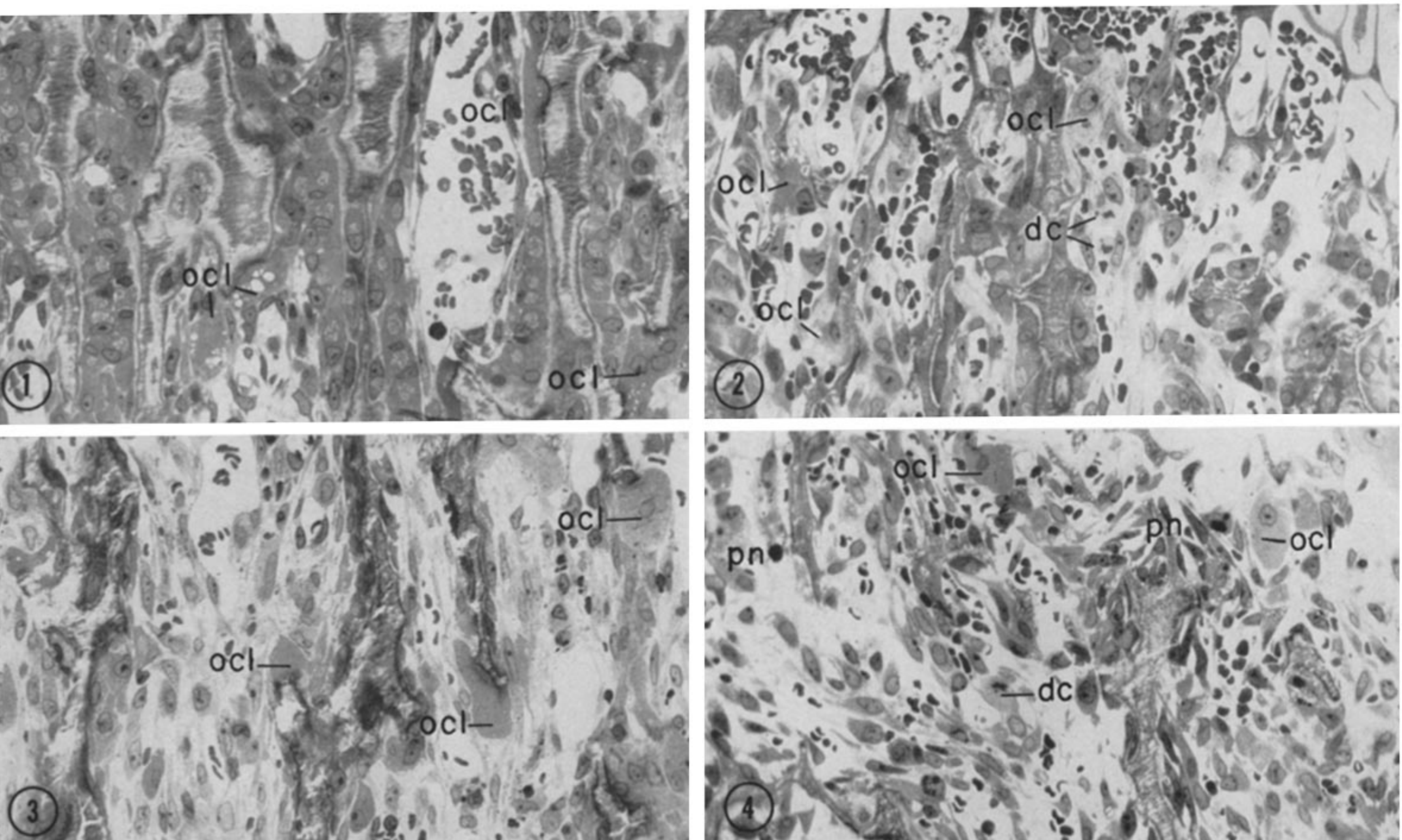


FIGURE 1 Normal primary spongiosa. Trabeculae are covered with a mass of polyhedral-shaped osteoblasts in most areas. Several osteoclasts (*ocl*) are present. Araldite embedding; toluidine blue staining. × 360.

FIGURE 2 Same region as in Fig. 1. $10\frac{1}{4}$ hr after 3 units/g PTE single injection. A number of the cells are elongated. *ocl*, osteoclast; *dc*, dividing cell. Araldite embedding; toluidine blue staining. × 360.

FIGURE 3 Same region as in Fig. 1. 9 hr after 10 units/g PTE single injection. Most of the cells are spindle shaped and separated from one another. *Ocl*, osteoclast. Araldite embedding; toluidine blue staining. × 360.

FIGURE 4 Same region as in Fig. 1. $26\frac{1}{4}$ hr after 2 units/g PTE at 4-hr intervals. More advanced changes with little remaining bone. *dc*, dividing cell; *pn*, pyknotic nucleus; *ocl*, osteoclast. Araldite embedding; Toluidine blue staining. × 360.

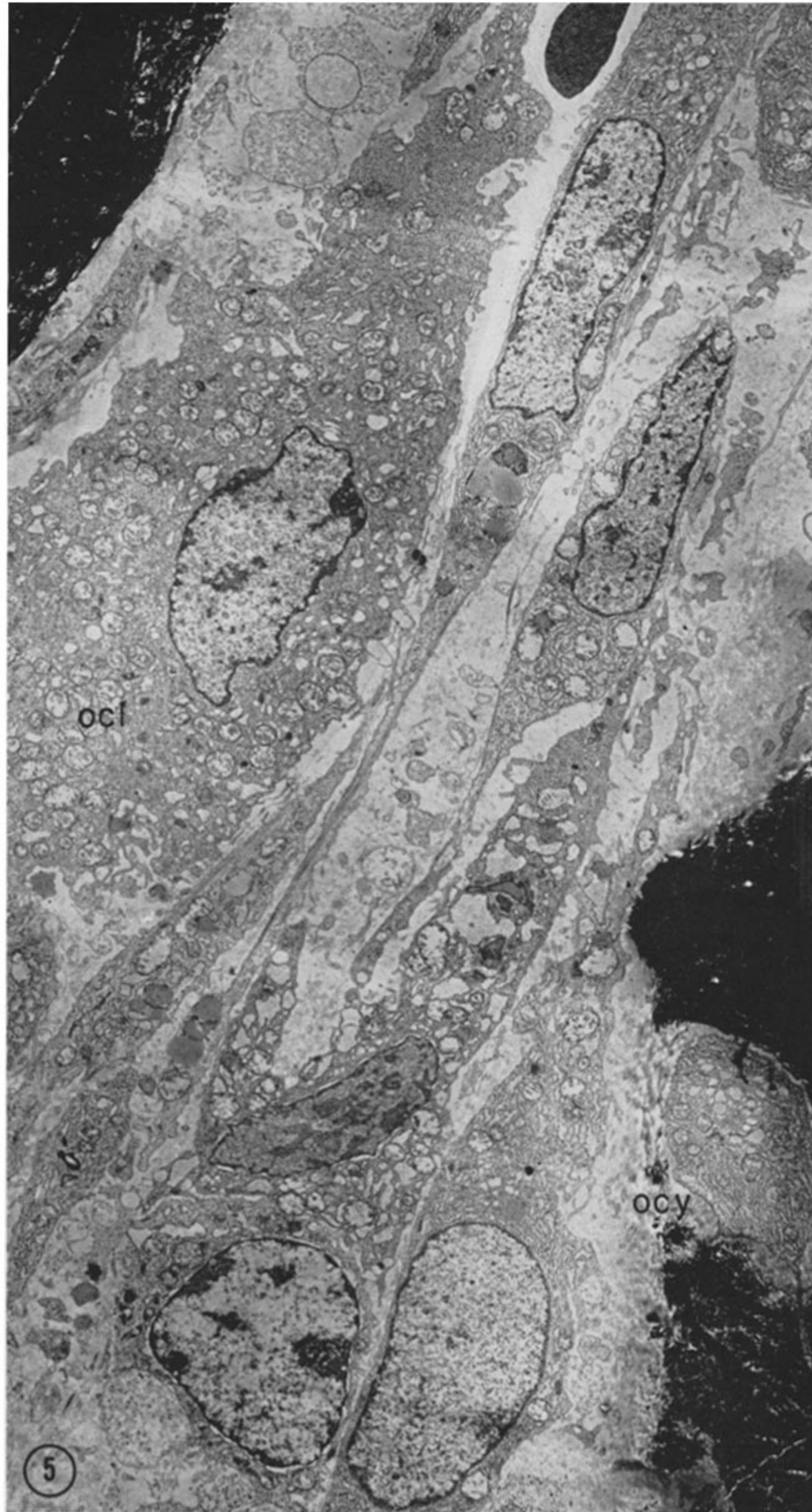
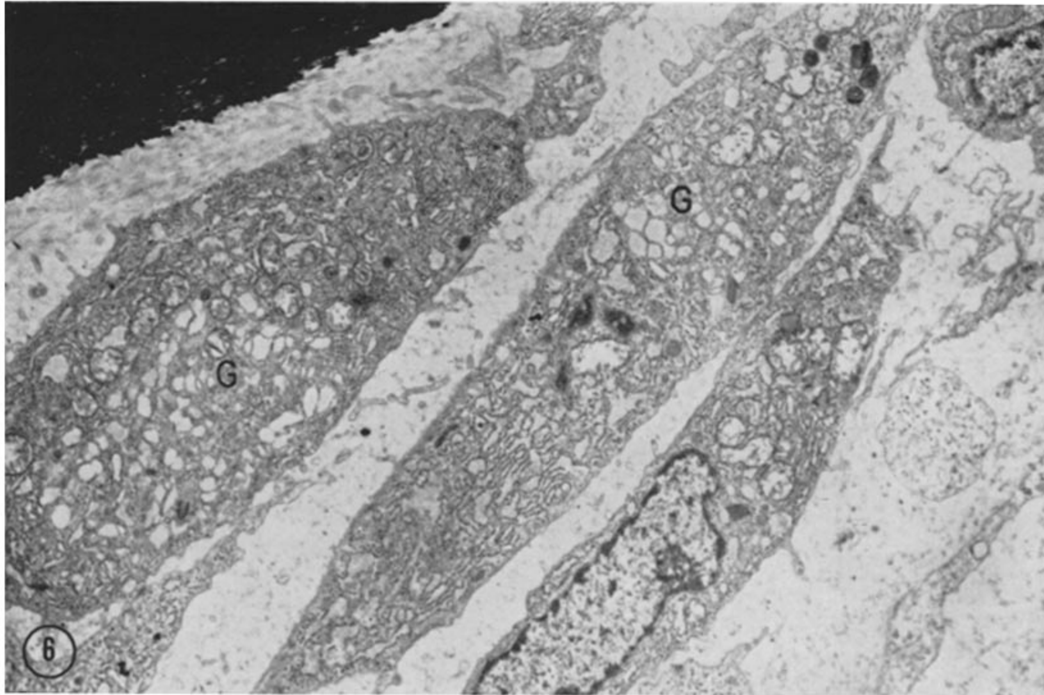


FIGURE 5 9 hr after 9 units/g PTE over 30 min. Most of the cells are elongated and separated from one another. The mitochondria and some cisternae are swollen and dense bodies are present. The bone matrix is completely obscured by mineral at the upper left, but shows patchy calcification at the lower right. Osteoclast, *ocl*; osteocyte, *ocy*. × 5000.



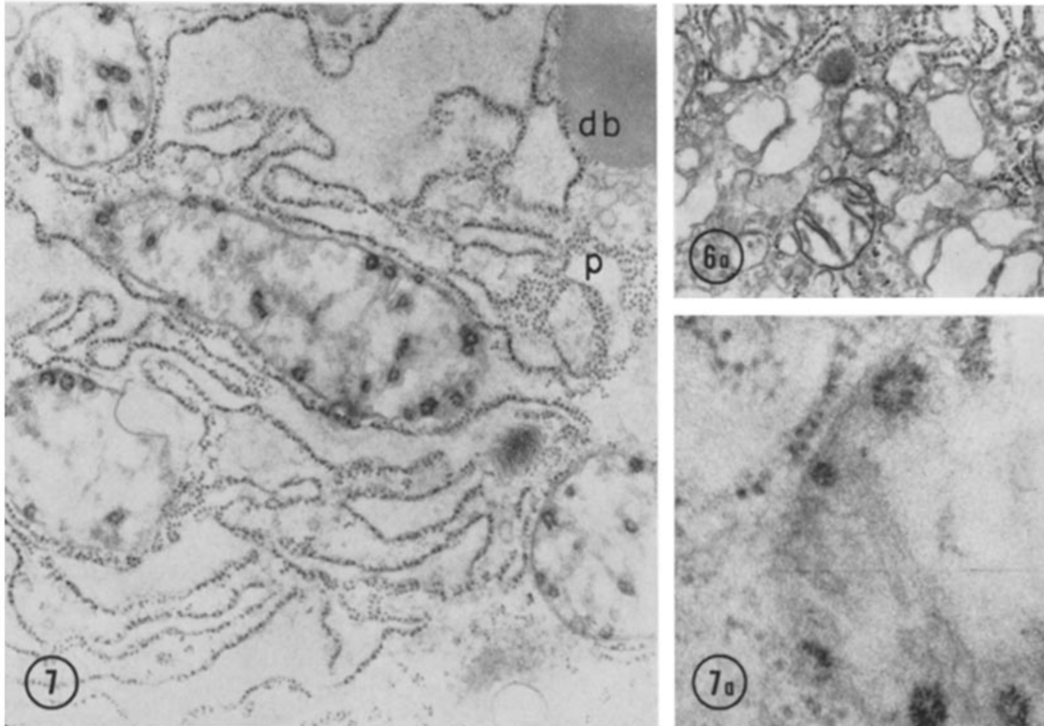


FIGURE 6 9 hr after 9 units/g PTE over 30 min. Spindle cells with Golgi zones (*G*), swollen mitochondria, and dense bodies. Uncalcified fibrils can be seen at the bone matrix surface. × 6000.

FIGURE 6 *a* Golgi and rough-surfaced vesicles and vacuolated mitochondria. × 18,000.

FIGURE 7 16¼ hr after one injection of 10 units/g PTE. Granules in swollen mitochondria. Polysomes, *p*; dense body, *db*. × 30,000.

FIGURE 7 *a* Mitochondrial granules composed of rosettes of small particles. × 80,000.

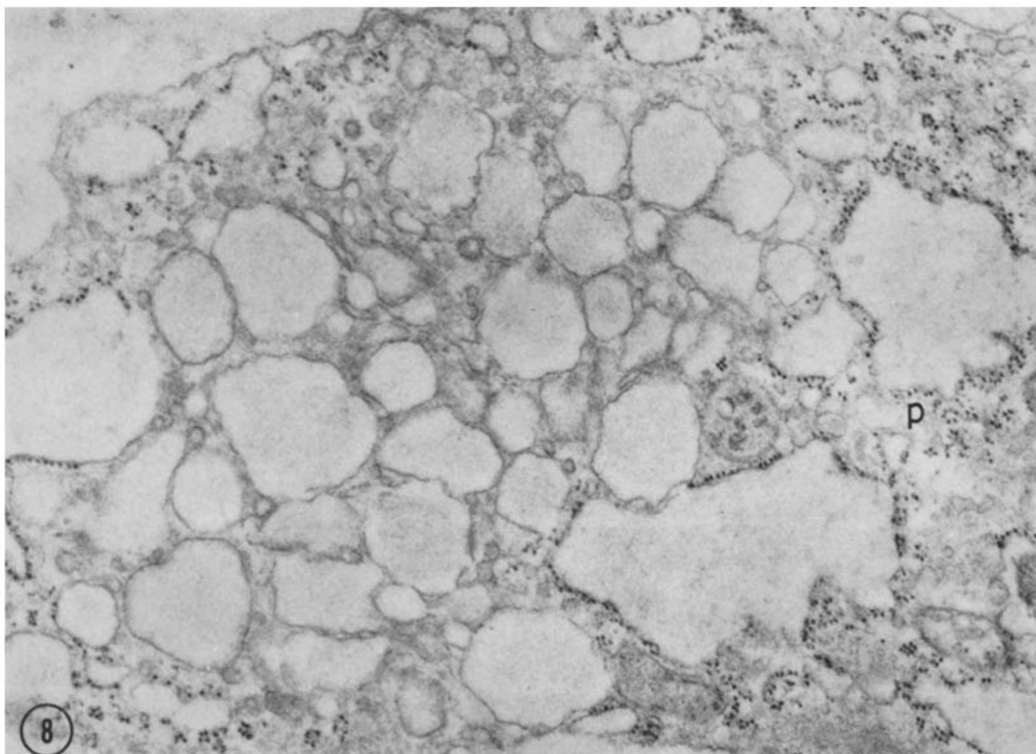


FIGURE 8 9 hr after 9 units/g PTE over 30 minutes. Distended Golgi and rough-surfaced vesicles. Polysomes, *p*. × 25,000.

brane in such cells was relatively smooth between the processes that passed into the canaliculi.

OSTEOCLASTS: These were little different from those in normal animals. They were sometimes spindle shaped (Fig. 5), but the main change was the swelling of the mitochondria (Figs. 5 and 14), although this was not always so pronounced as in the spindle cells. They had considerably fewer dense bodies than the osteoblasts. The brush border (Fig. 14) was present and there was no evidence of fusion with other cells (Fig. 13). The osteoclasts seemed less affected than the other cells after they were dosed repeatedly for 26 hr; their brush borders remained relatively intact and in relation to disrupted bone surfaces.

BONE MATRIX SURFACE: This looked very much like that in normal bone. Under the osteoblasts, there was either a layer of collagen with or without patches of mineral or else the surface was completely calcified (Figs. 5 and 6). Under the brush border of the osteoclasts the matrix had the usual disrupted appearance (Figs. 13 and 14).

CONTROLS: In the control animals, the cells appeared normal. They formed a compact layer over the calcified tissue. The cells had few dense bodies, and only occasional cells contained swollen mitochondria.

DISCUSSION

After the intraperitoneal injection parathyroid extract into rats, two main changes occurred in the tibial metaphysis. Alterations developed in the fine structure of osteoblasts and osteocytes, beginning with the swelling of mitochondria and the formation of numerous dense bodies, and progressing to eventual death of many cells after 26 hr of repeated injections. During the same period, the bony trabeculae were eroded and many eventually disappeared without any great increase in multinucleated osteoclasts such as is described by Heller et al. (1950). Several questions follow from these results. It would be interesting to know (*a*) whether the changes in fine structure were an expression of the physiological effect of the extract

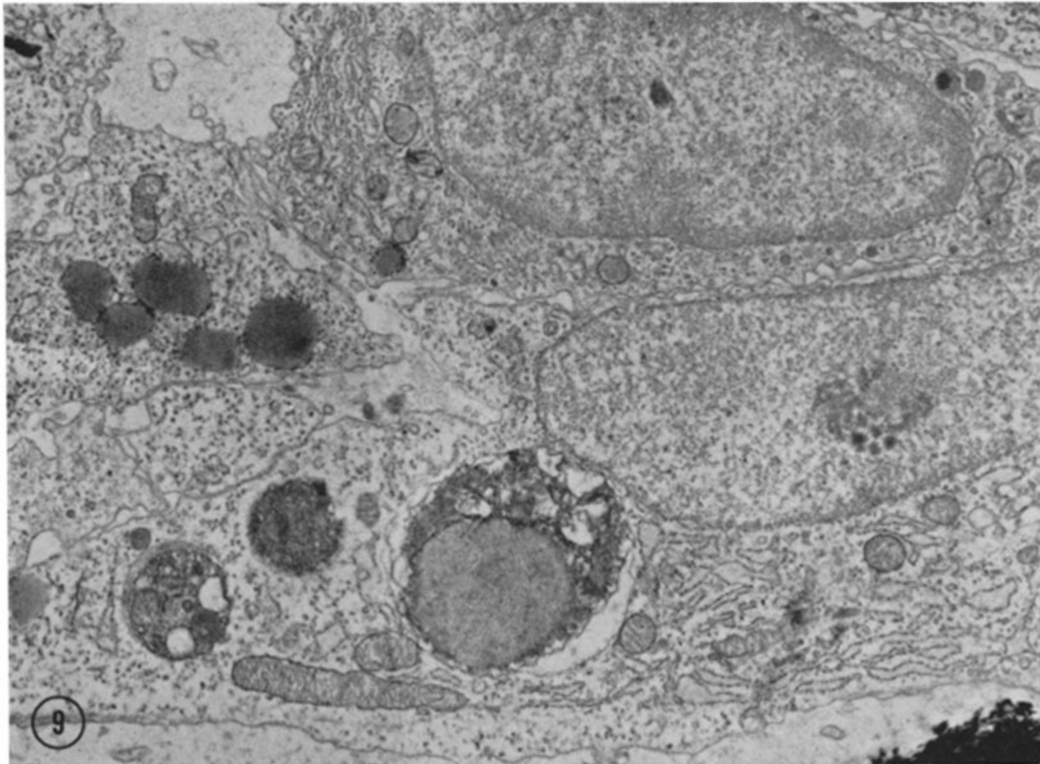


FIGURE 9 13 hr after one injection of 3 units/g PTE. Various dense bodies are present. The endoplasmic reticulum is rudimentary and many ribosomes lie free in the cytoplasm. × 10,000.

or of damage produced by toxic doses; (*b*) how the calcified tissue disappeared without an obvious increase in numbers of multinucleated osteoclasts; and (*c*) whether the changes in fine structure could be related to the removal of mineral and organic matrix.

(*a*) *Nature of the Changes in Fine Structure*

In assessing the nature of the changes, the fact that the doses of PTE were large must be taken into account. However, most attempts to understand the normal relationship between the parathyroids and bone have been based on large doses. Heller et al. (1950) certainly emphasized the fact that they were using toxic amounts of PTE, sufficient to cause degenerative changes in some of the bone cells. Many of the changes in fine structure in the present study could have been indications of degeneration. In the liver, swelling of mitochondria and distension of the endoplasmic reticulum have been regarded as signs of damage and analogous to the cloudy swelling seen with the light microscope (Ashworth et al., 1963). Some of the dense bodies seen in our study resembled lipid; while a small number of them may be found in normal bone cells (Bonucci, 1965), the number in our material may have been abnormal. Autolysis is reported to be accompanied by the appearance of various dense bodies (Ashford and Porter, 1962). The increase in the number of such dense bodies in the spindle cells in our material may have been manifestations of damage. On the other hand, there was evidence that the cells were continuing to function. Dividing cells, some containing swollen mitochondria, could be seen even after 26 hr of repeated doses. During the 9- to 16-hr period, when the resorption reached a peak after a single dose, many of the cells with the swollen mitochondria and dense bodies also had well developed Golgi areas, endoplasmic reticulum, and numerous polysomes.

It is difficult to say whether these changes in

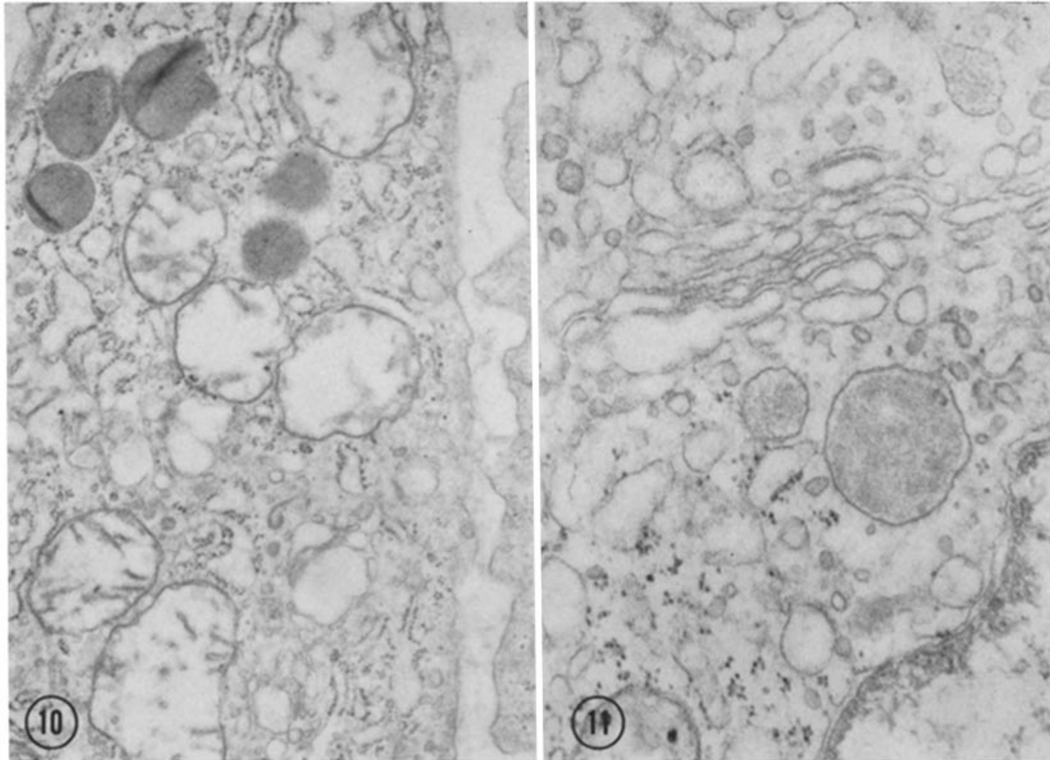


FIGURE 10 9 hr after 9 units/g PTE over 30 min. Dense bodies and swollen mitochondria in a spindle cell. × 45,000.

FIGURE 11 9 hr after 9 units/g PTE over 30 min. Dense bodies and Golgi vesicles in a spindle cell. × 20,000.

fine structure were an exaggeration of a physiological process. Swollen mitochondria are present in some cells in normal bone, and this may be an indication that the cells are responding to endogenous parathyroid hormone. When a small dose of PTE was given, the widespread swelling of mitochondria was the most notable change and occurred before resorption could be detected. On the other hand, with the larger doses some of the cells were spared, suggesting that these cells were in a particular functional state and so did not respond to the PTE. However, since the repeated doses produced considerable necrosis eventually, it is possible that the earlier changes were the result of damage to the cells.

(b) *Resorption without an Obvious Increase in Numbers of Multinucleated Osteoclasts*

Several possibilities come to mind to explain this result. One is that those osteoclasts present moved more rapidly than normally (Gaillard, 1961). Most of them were found in the usual places (e.g. tibial buttress and diaphyseal ends of the metaphyseal trabeculae), and the close relationship between disintegrating bone and brush border was maintained. On the trabecular surfaces, the osteoclasts were separated from each other by spindle cells which would seem to have provided a hindrance to their movement.

The extract may have stimulated a more rapid turnover of osteoclasts with an increased rate of bone destruction. This could not be determined on morphological grounds, and there is no evidence from H^3-thymidine studies that PTE changes the rapidity with which the cycle "osteoprogenitor" cell - osteoclast - "osteoprogenitor" cell takes place (Young, 1964).

Another possibility is that bone cells with single nuclei took on the characteristics of osteoclasts. In some of them, the endoplasmic reticulum was not

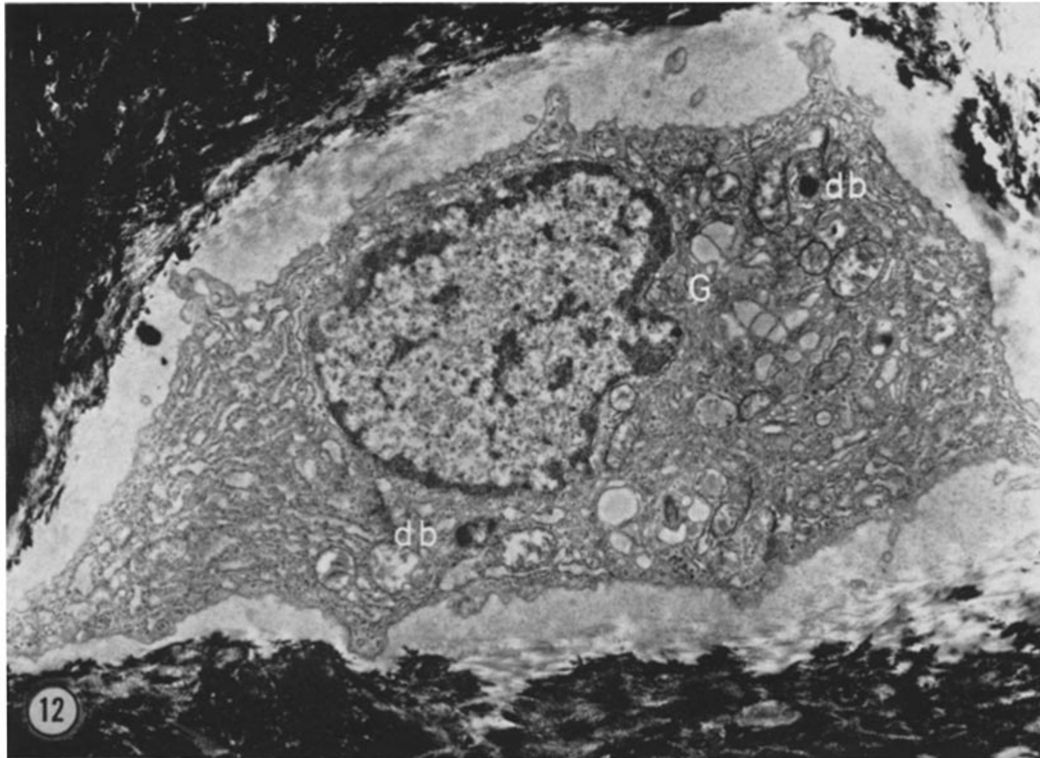


FIGURE 12 9 hr after 9 units/g PTE over 30 min. Osteocyte in its lacuna. The cell is separated from the lacunar wall by a space. The endoplasmic reticulum is well developed and the Golgi zone (*G*) is distinct. The mitochondria are swollen and some dense bodies (*db*) are present. × 10,000.

well developed (or had become "atrophic") and there were many free ribosomes in the cytoplasm, as in classical osteoclasts. However, none developed a brush border and none was adjacent to the areas of disrupted bone that can be seen under multinucleated cells.

It seems to us that none of these explanations is satisfactory. It is apparent that PTE did not stimulate classical osteoclasis in the early stages, and this is in agreement with the observation of Heller et al (1950), although those authors were impressed by the later appearance of osteoclasts (at 12 hr). If this observation is correct, it will be necessary to look for some other way in which the extract exerts its effects. In the normal animal, it is possible that exchange of calcium and inorganic phosphate between bone and tissue fluid is not mediated by osteoclasts (Macgregor, 1964). but by the other bone cells for which the cellular mechanisms and their morphological basis have not as yet been recognized. When the parathyroid glands are stimulated by peritoneal lavage, calcium removal is not dependent on the size of the osteoclast population (Talmage et al., 1965). PTE may also have its main effect on cells other than osteoclasts and cause the net movement of mineral to be in one direction, that is, out of bone. This effect must occur quite early, for Cameron and Copp (1963) have shown that the level of calcium in the serum may rise 6 hr after injection of small amounts of PTE into rats. The changes that we have seen by that time seem minor but later they become exaggerated and could be responsible for, or at least connected with, the increasing loss of both mineral and matrix from the bone.

(*c*) *Relation of Changes in Fine Structure to Removal of the Calcified Tissue*

So far as the mineral removal is concerned, a number of ions have been suggested as being involved, and fashions have changed from citrate

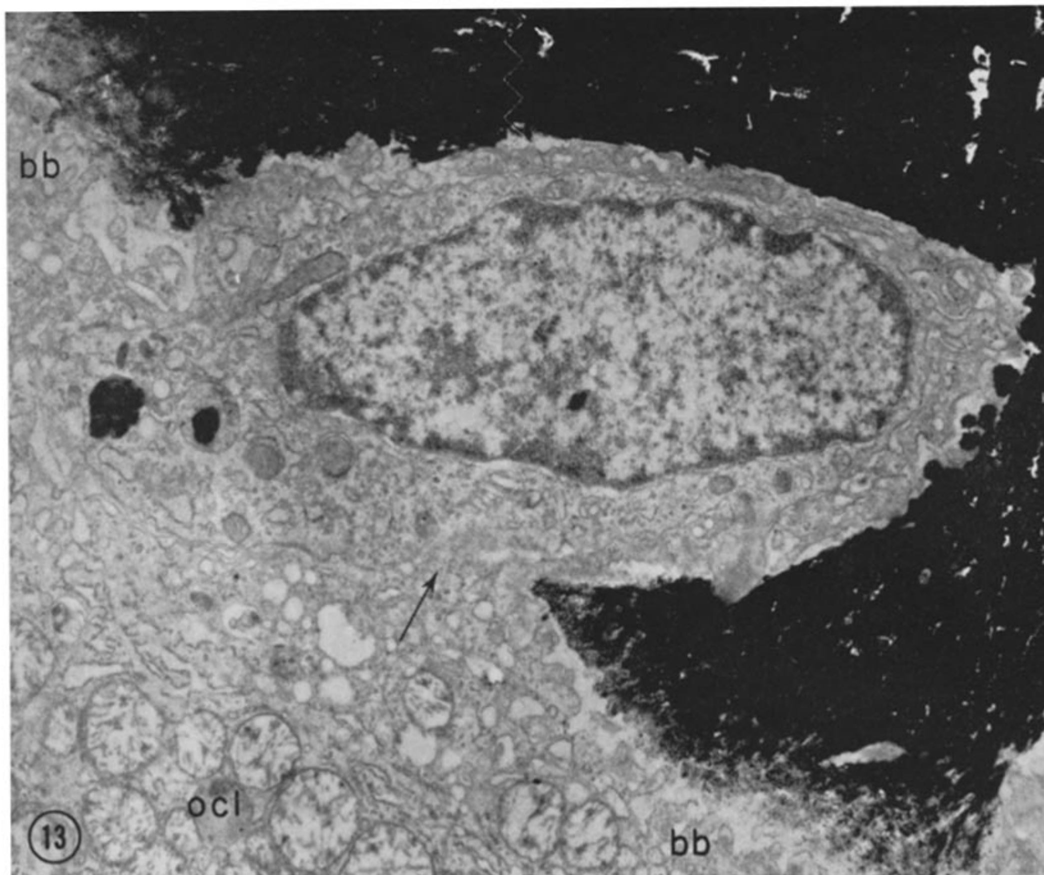


FIGURE 13 9 hr of 2 units/g PTE at 4-hr intervals. Osteoclast (*ocl*) with brush border (*bb*) and a neighboring osteocyte. The plasma membranes between them are blurred in one area (arrow), but this is probably because they have been sectioned obliquely rather than because the cells have fused. Mitochondria in the osteoclast only are swollen. The bone adjacent to the brush border has a frayed appearance, but there is no suggestion of this at the margins of the lacuna in which the osteocyte lies. × 10,000.

(Neuman et al., 1960; Walker, 1961) to lactate (Borle et al., 1960) and carbonate (Forscher and Cohn, 1963). All the acids invoked are related to respiration in cells, and changes in mitochondria may, therefore, be of some significance.

The swelling of the mitochondria may be secondary to the increase in calcium and phosphate ions in the tissue fluid. It is known that these ions will cause mitochondrial swelling in vitro (Wojtczak and Lehninger, 1961), and this may be the first indication that the PTE has caused mineral to be removed from the hard tissue. The granules in the mitochondria of the spindle cells may also be a measure of mineral movement (Caulfield and Schrag, 1964; Greenawalt et al., 1964) On the other hand, DeLuca and Sallis (1965) have shown that mitochondria provide a system that will respond specifically to parathyroid hormone and may control intracellular ion concentrations. The structural alterations in mitochondria may be a reflection of this control.

Removal of organic matrix would depend in part on the presence of proteolytic activity. Bélanger and Migicovsky (1963) showed that PTE increased the size of osteocyte lacunae in vivo and enhanced the ability of osteocytes to digest a gelatin substrate in vitro. We found osteocytes separated from the lacunar walls by a space that

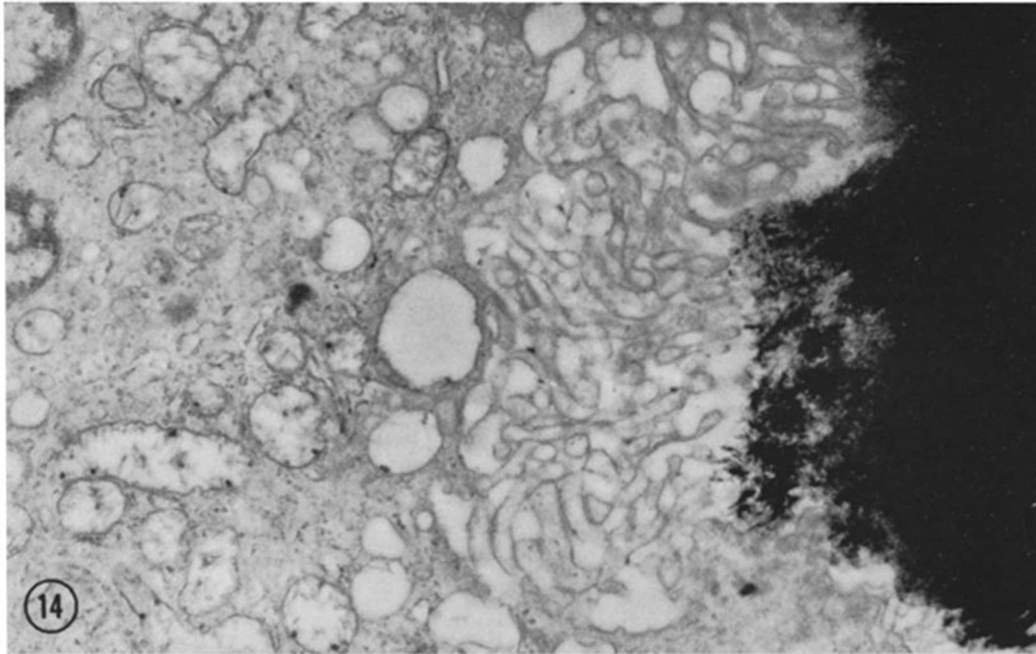


FIGURE 14 16 hr of 1 unit/g PTE at 4-hr intervals. Osteoclast with brush border and adjacent disrupted bone. The mitochondria are swollen and contain granules. × 12,000.

was larger than normal (Fig. 12). This space may have been evidence of "osteolysis," but the possibility of cell shrinkage cannot be excluded. Collagen breakdown in vitro is increased by PTE (Stern et al., 1965; Walker et al., 1964, Kaufman et al., 1965). Woods and Nichols (1965) have demonstrated collagenase in normal bone cells in the mitochondrial fraction and suggested that it might be contained in lysosomes. Many of the dense bodies in spindle cells (Fig. 10 and 11) may have been counterparts of the lysosomes described by others (de Duve, 1959; Novikoff, 1963), although we did not test them for the presence of acid phosphatase. Lysosomal enzymes may be responsible for the normal process of resorption (de Duve, 1963; Vaes, 1965) or that which occurs in hypervitaminosis A (Fell, 1964), so it is possible that some of the dense bodies that appeared after PTE were significant in the removal of the trabeculae.

In conclusion, it can be said that, while the work of Heller et al., (1950) has provided useful information in terms of conventional histology, the experimental technique in our hands has not yielded unequivocal evidence about the relationship between bone resorption and changes in fine structure of bone cells produced by PTE. We have shown (as they also did) that bone can disappear without an obvious increase in osteoclasts and suggested how the mitochondrial changes and appearance of dense bodies may have been related to the erosion of the bony trabeculae. However, the changes could be interpreted as having been the result of damage from large doses of the extract. A more refined approach will be necessary to show the way in which the parathyroid glands control bone cells.

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