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Osteocyte Ultrastructure in Renal Osteodystrophy

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Summary. The ultrastructure of the osteocyte has been studied in 80 needle biopsies from the iliac crest of uremic subjects with renal osteodystrophy.

Different types of osteocytes were present in the osseous trabeculae. Those recognizable in completely uncalcified osteoid tissue looked like normal osteocytes, even though the matrix was not mineralized. Those present in hypomineralized areas showed enlarged and irregular lacunae when examined under the light microscope; under the electron microscope these osteolytic-like changes were not evident and were found to have been produced by defective calcification of the perilacunar matrix. Osteocytes placed in matrix whose mineralization was normal were often surrounded by a border of crystals protruding side-to-side from the bone matrix into the lacunar space. Other osteocytes were placed in unusually wide lacunae. They showed evidence of osteolytic activity, chiefly consisting of irregularity of the lacunar wall, presence of flocculent, granular and filamentous material in the pericellular space, and calcification of mitochondria. Degenerating and degenerate osteocytes were also recognizable.

Key words: Osteocytic osteolysis – Chronic uremia – Osteomalacia – Secondary hyperparathyroidism.

Introduction

The regulation of calcium and phosphate homeostasis is largely controlled by the activity of the osseous cells, which are able to induce both deposition and mobilization of inorganic substance into and from the organic bone matrix. For a long time, these cellular activities have been considered characteristic of osteoblasts and osteoclasts respectively, although as long ago as 1910 Reck-

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linghausen had suggested that osteocytes too might promote the resorption of the pericellular matrix. It is now accepted that osteocytes are not quiescent cells buried in a calcified matrix. They are active metabolic cells, but the role they play in calcium and phosphate metabolism is still largely a matter of debate.

There is general agreement that osteocytes are active in the synthesis and calcification of the matrix by which they are surrounded, although their function in these specific processes decreases with time and cell maturation (Robinson et al., 1973). There are in addition many reports showing that osteocytes can remove the calcified matrix from the wall of their lacunae by a process which has been called "osteocytic osteolysis" (Bélanger, 1969, 1971). The evidence in favour of this process consists firstly of the histological finding that most of the osteocytic lacunae are enlarged and irregularly shaped in cases of increased bone resorption (Rasmussen and Bordier, 1974), secondly of the histochemical findings that in these cases only the enlarged osteocytes show phosphatase (Majno and Rouiller, 1951) and collagenolytic activity (Bélanger and Migicovsky, 1963), and thirdly of ultrastructural results showing that the enlarged lacunae not only have irregular borders (Baud, 1962), but also contain fragmented and flocculent material and granular inorganic substance probably produced by breakdown of the bone matrix (Jande, 1971; Jande and Bélanger, 1971, 1973; Remagen et al., 1968, 1969). However, there is still no general consensus that perilacunar bone can be removed through a process of osteocytic osteolysis, and in 1972 Cameron spoke of "obvious contradictions in the views" about this process.

The greatest difficulty in accepting the role of osteocytes in bone resorption seems to derive from the lack of a steady criterion for evaluating which morphological changes, if any, are to be considered specific to the process. The enlargement and irregularity of the lacunae, which are often the only or main morphological findings on which the histological diagnosis of osteocytic osteolysis is based, do not seem to constitute completely reliable evidence in favour of perilacunar bone resorption. In fact, the same changes in lacunae can be found in the osteocytes of rapidly forming bone tissues (Jowsey, 1968) and could be produced by defective mineralization of the periosteocytic matrix (Lindenfelser et al., 1973; Sissons, 1969; Steendijk et al., 1966). It is evident that these conditions could lead to incorrect evaluation of osteocytic activity, especially when osteomalacia and hyperparathyroidism occur together.

This association typically occurs in renal osteodystrophy (Stanbury, 1968). Histologic investigations have shown that there is a significant increase in numbers of osteocytes with enlarged and irregular lacunae in uremic subjects (Bonucci et al., 1975; Krempien et al., 1973; Lindenfelser et al., 1973; Ritz et al., 1973). However, this finding has been interpreted in contradictory ways; it has usually been attributed to osteocytic osteolysis, but it has also been attributed to defective mineralization of the walls of the osteocytic lacunae (Lindenfelser et al., 1973).

It is for this reason that we have examined bone biopsies from two groups of uremic patients suffering from renal osteodystrophy, the first treated with conservative therapy, and the other with haemodialysis.

Material and Methods

Needle biopsies from the iliac crest of two groups of uremic patients were examined. The first group consisted of 40 patients with chronic renal failure, their age ranging from 22 to 59 years. All the patients had been kept on a low-protein diet (24–35 g/day) for 1–6 years before the investigation. The calcium content of the diet averaged 900 mg/day and the phosphorus intake was between 300 and 500 mg/day. Plasma calcium ranged from 6.80 to 11.60 mg% (mean value 9.49) and plasma phosphate from 2.80 to 9.30 mg% (mean value 4.54), while the plasma $Ca \cdot PO_4$ product ranged from 28.10 to 79.80 (mean value 42.65).

The second group consisted of 40 patients with chronic renal failure, age ranging from 13 to 61 years, submitted three times every week to haemodialysis. They had not been treated with vitamin D and were given an unrestricted diet. None of the patients had been dialyzed for less than 2 months; most of them had been treated for 2–6 years. Their plasma calcium ranged from 6.6 to 10.9 mg% (mean value 8.72) and their plasma phosphate from 3.7 to 9.3 mg% (mean value 5.94); their plasma $Ca \cdot PO_4$ product ranged from 32 to 85 (mean value 51.47).

The biopsies were fixed in 4% paraformaldehyde buffered at pH 7.2 with phosphate or cacodylate buffers. They were reduced to small specimens which were post-fixed with 1% osmium tetroxide (buffered as above) and embedded in Araldite without decalcification.

Semithin sections were examined under the light microscope after staining with Azure II – Methylene blue, and with the von Kossa method for calcium phosphate. These sections were mainly used in selecting osteocytes. Selection was carried out on the basis of the degree of bone matrix calcification as revealed by von Kossa stained sections. In this way, osteocytes from three zones were chosen: 1) Osteocytes placed in completely uncalcified bone matrix, i.e., in osteomalacic osteoid tissue; 2) Osteocytes found in incompletely calcified bone matrix next to the osteoid tissue; 3) Osteocytes located deep within fully calcified bone matrix. Once a suitable zone had been selected, the blocks were trimmed, the tissue area was reduced, and ultrathin sections were collected for electron microscope observation. These sections were examined unstained and after staining with uranyl acetate and lead citrate. Other ultrathin sections were decalcified by flotation on 2% formic acid, and were stained with uranium and lead, and with phosphotungstic acid at pH 1.8 (Marinozzi, 1968).

Results

1. Completely Uncalcified Areas (Osteoid Tissue)

These areas were characterized by the presence of loosely arranged collagen fibrils which were either irregularly oriented or collected in lamellae. They were usually completely uncalcified. In some cases, however, scattered areas of calcification were visible, especially near the border between the calcified and uncalcified matrix. Cells of various shape were recognizable between the uncalcified collagen fibrils in the osteoid tissue (Fig. 1). Most were roundish or elongated and contained a rather irregularly shaped, often indented nucleus with dispersed chromatin and a clearly recognizable nucleolus. The cytoplasm contained an easily recognizable Golgi apparatus, a few rough ergastoplasmic cysternae, a few mitochondria, isolated lysosome-like bodies and ribosomes irregularly scattered through the cytoplasm and sometimes forming polyribosomes. Centrioles were also occasionally present.

Many cytoplasmic processes protruded from these cells and penetrated the surrounding uncalcified matrix in every direction. There was often very close contact between cytoplasmic processes in adjacent cells. Invagination of one

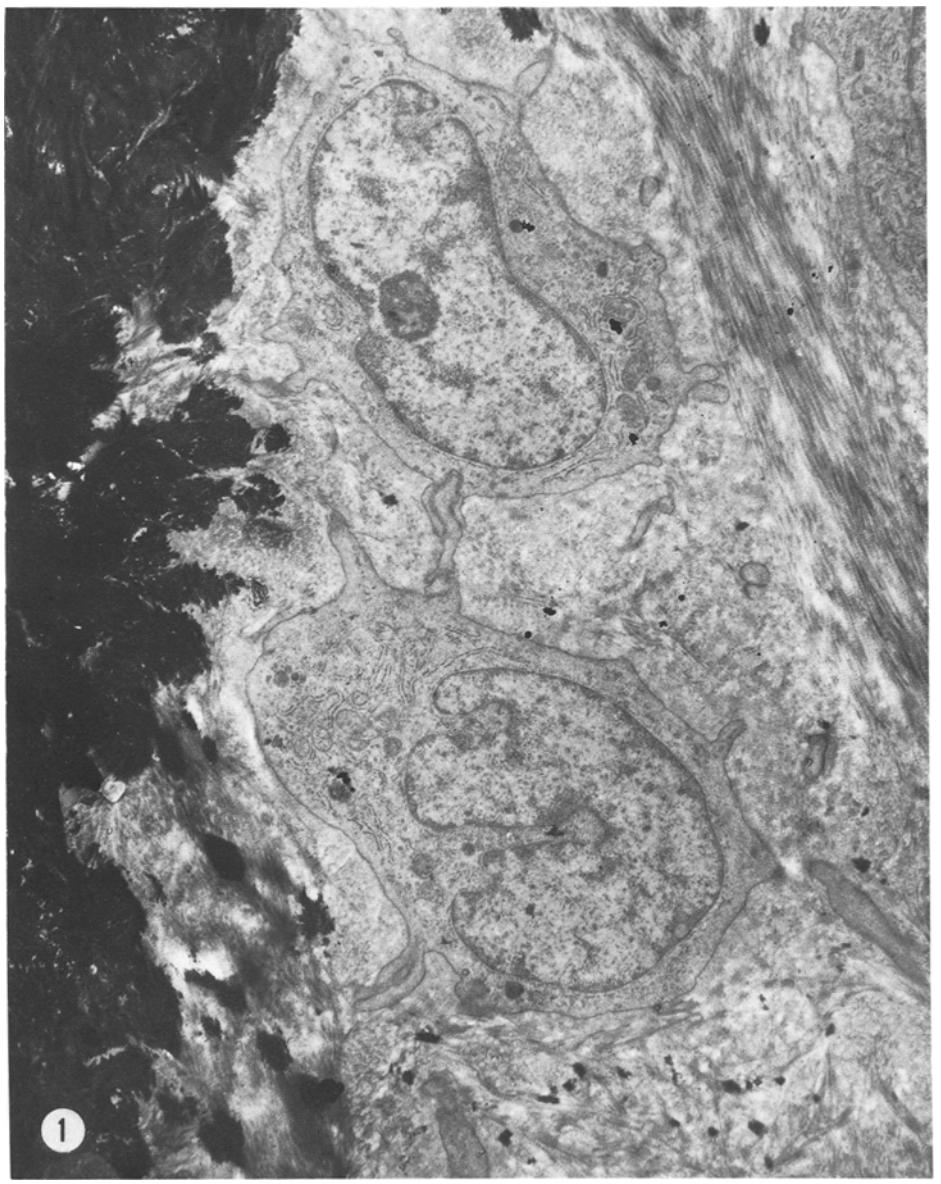
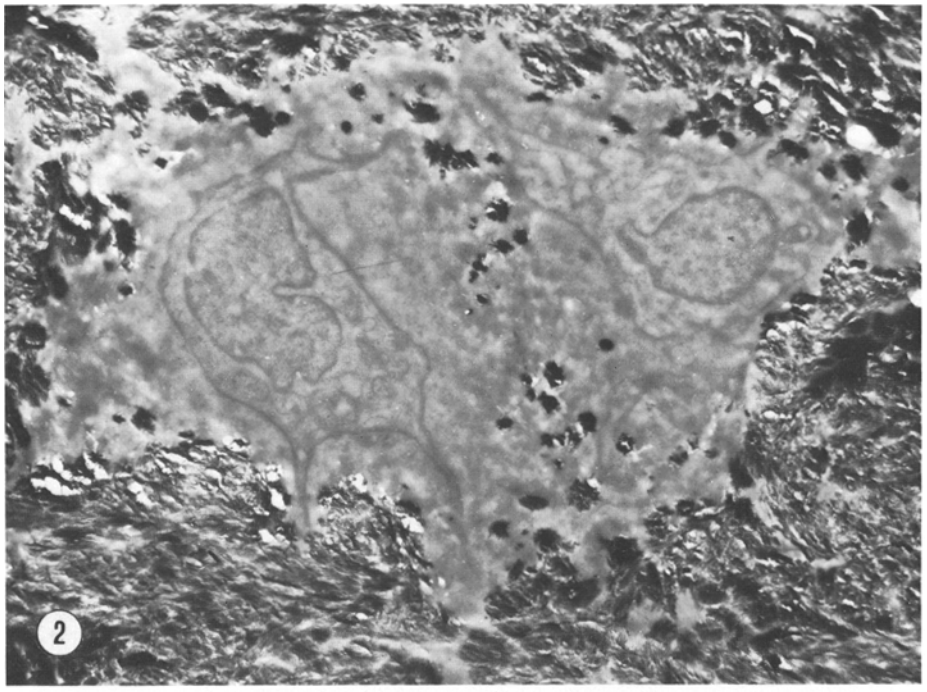


Fig. 1. Two osteocyte-like cells in osteoid tissue in the neighbourhood of the fully calcified matrix. Note interdigitation of cytoplasmic processes. Uranyl acetate and lead citrate, ×7000

Fig. 2. Two osteocytes in incompletely calcified bone matrix. Their lacunae are irregular and appear enlarged; moreover, they seem to be coalescing. These appearances are due to the incomplete calcification of the pericellular matrix. Unstained, ×6000

Fig. 3. Two osteocytes in incompletely calcified matrix. Note that uncalcified collagen fibrils are in close relationship with the peripheral membrane of these cells, and that the lacunae have an irregular shape. Uranyl acetate and lead citrate, ×6000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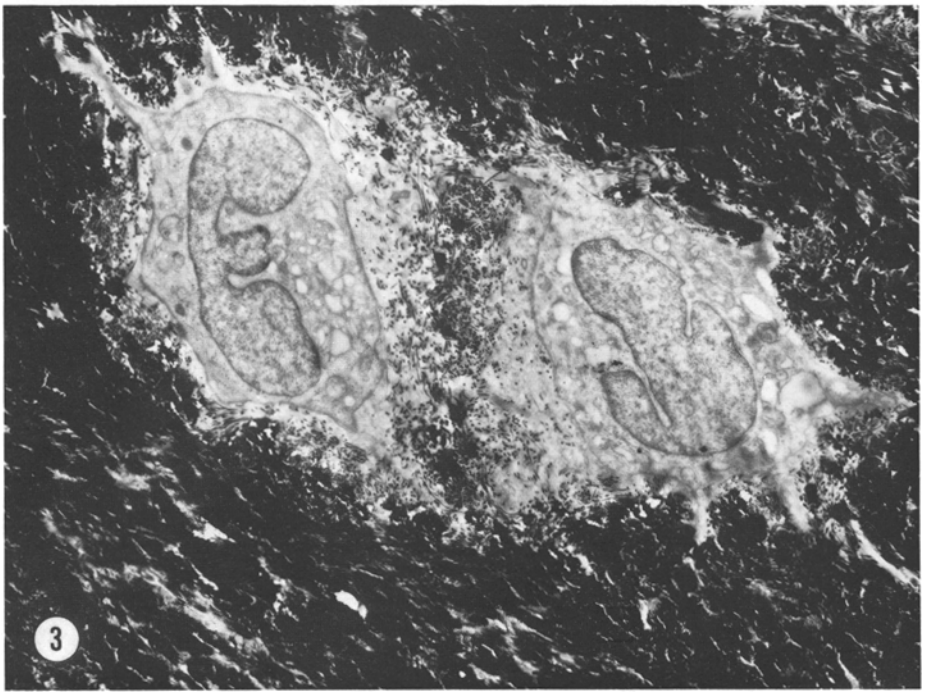
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cell process into another was not uncommon (Fig. 1). The peripheral cell membrane was always closely connected with the collagen fibrils in the surrounding osteoid matrix (Fig. 1).

2. *Incompletely Calcified Matrix*

Incompletely calcified matrix was easily recognizable under both the light and electron microscope. In semithin sections treated according to the von Kossa method, these areas, unlike the fully calcified ones, did not appear homogeneously black. Under the electron microscope, the distribution of the inorganic substance appeared irregular. Wide zones of the matrix were left completely uncalcified; others contained irregularly distributed, roundish clusters of crystals; and others showed granular, intrinsically electron-dense substance closely connected with the periodic banding of the collagen fibrils, while hardly any crystals were present.

These incompletely calcified areas contained cells of various shapes; most of them tended to be roundish. Under the light microscope they all seemed to be located within enlarged, irregularly shaped lacunae. Under the electron microscope (Figs. 2, 3), they did not appear to be closely connected with the calcified matrix, since uncalcified collagen fibrils separated the cell membrane and the mineralized matrix (Fig. 3).

The cytoplasm of these cells was abundant, especially in cells near the uncalcified osteoid tissue, and a wide Golgi area was almost constantly present. Rough cysternae of the ergastoplasmic reticulum were present only in the largest cells; in the smallest Golgi vesicles, a few mitochondria, lysosome-like bodies and occasional centrioles were recognizable. Cytoplasmic processes irregularly protruded from these cells into the adjacent incompletely calcified matrix.

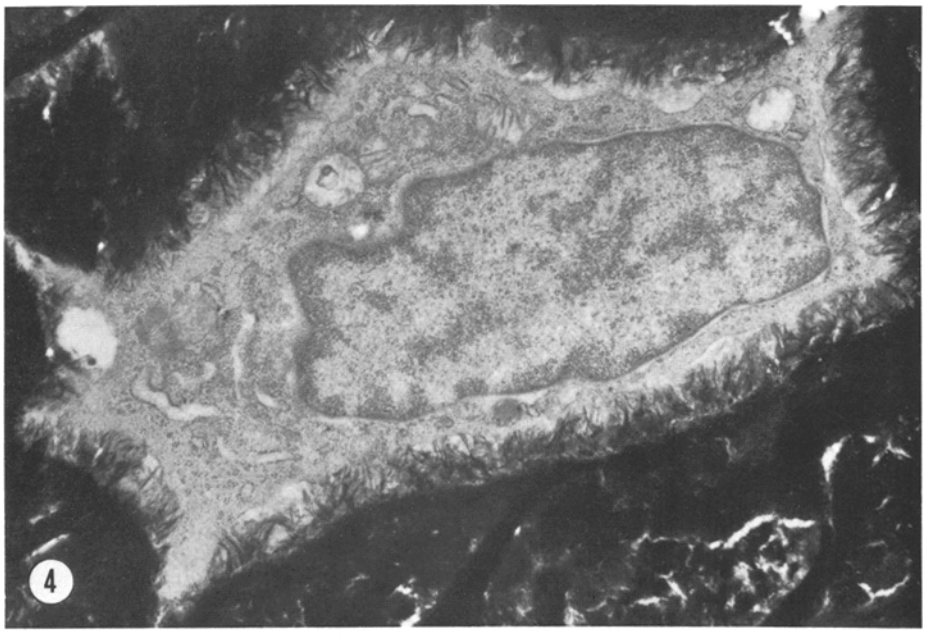
The nuclei were almost always irregularly shaped and indentations in their membrane were often observed (Figs. 2, 3). The chromatin was dispersed and the nucleoli were not as easy to see as in the cells found in the osteoid tissue.

3. *Calcified Matrix*

The fully calcified matrix appeared homogeneously black under the light microscope in von Kossa-stained sections. Similarly, the intrinsic electron density of the inorganic substance completely masked the collagen fibrils of the organic matrix.

Under both the light and the electron microscopes *different types of osteocytes* were recognizable in fully calcified areas. Their shape varied from roundish to elongated and fusiform. The width of their lacunae also varied. However, most could be roughly collected into two groups, those with small and those with wide and irregular lacunae; those with wide lacunae predominated and a few lacunae of intermediate width were also present.

No fundamental differences were recognizable between roundish and fusiform osteocytes, whereas there were easily recognizable ultrastructural differ-



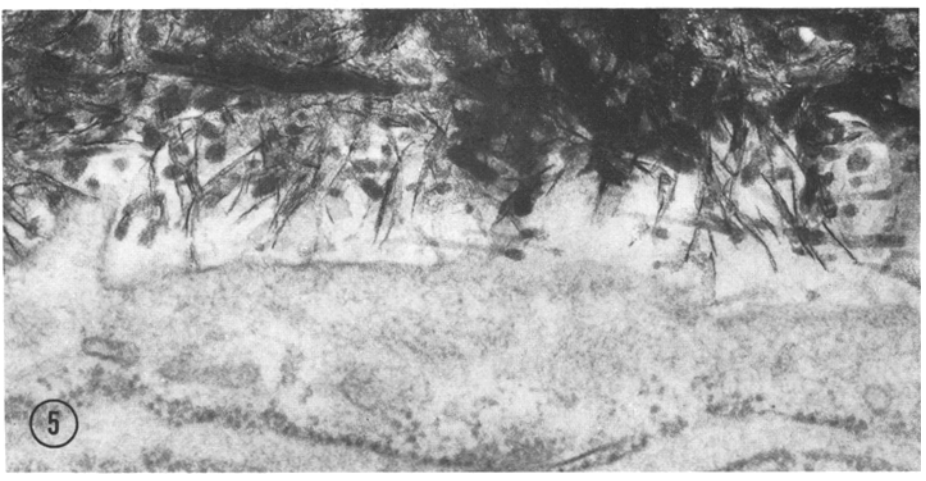


Fig. 4. A small osteocyte in fully calcified matrix. Its peripheral membrane is in close contact with the lacunar brush border, i.e., with crystals protruding from the calcified matrix into the lacunar space. Uranyl acetate and lead citrate, ×11,000

Fig. 5. Detail of the peripheral membrane of a small osteocyte and of the lacunar brush border. Uncalcified collagen fibrils are evident between the crystals, probably because the lacunar brush border is still incompletely formed. Uranyl acetate and lead citrate, ×18,000

ences between the osteocytes found in small and wide lacunae. Small lacunae were completely filled by osteocytes (Fig. 4). These showed nuclei similar to those found in the cells described in the preceding sections, and little cytoplasm, with a few rough ergastoplasmic cysternae and cytoplasmic organelles.

The most interesting feature of these osteocytes was found at the border of their lacunae. This border was smooth in some cases, but in most it showed a lot of intrinsically electron-dense, filament- and needle-like crystalline structure protruding side-to-side from the calcified matrix into the lacunar space like the hairs of a brush (Fig. 4). These crystals will be called the "lacunar brush border" from now on; they were found whether the sections were obtained from phosphate- or cacodylate-buffered specimens, and had the same appearance in both cases. In some osteocytes, the lacunar brush border consisted of relatively few crystals lying between uncalcified collagen fibrils (Fig. 5), but there were usually plenty of crystals and their tips apparently touched the peripheral membrane of the osteocytes, completely masking the collagen fibrils (Fig. 4).

The lacunar brush border was either continuous all around the lacuna (Fig. 4) or discontinuous (Fig. 6). In this case, it was present in some zones of the lacunar border and absent in others; where present, its crystals appeared somewhat disarranged and were found in spaces apparently formed by the retraction of the peripheral membrane of the osteocytes (Fig. 7). In some cases, there were so many of these spaces that the cortical cytoplasm of the osteocytes appeared scalloped and vacuolized. This pattern was chiefly observed in osteocytes found in rather irregularly shaped lacunae of intermediate width.

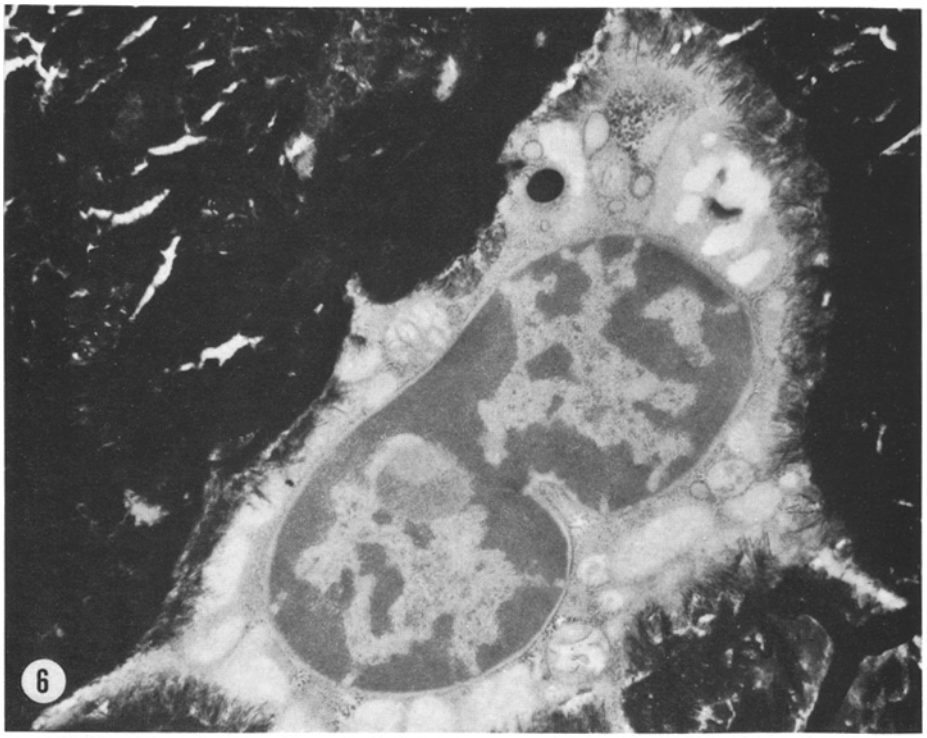
The lacunar brush border was indirectly recognizable in sections decalcified by flotation on formic acid and stained with uranyl acetate and lead citrate or with phosphotungstic acid. In these sections, filament- and needle-like structures were recognizable. They had no intrinsic electron density in unstained sections; after staining, they formed a brush border like that found in undecalcified sections (Fig. 8). These structures were not constituents of the collagen fibrils of the perilacunar matrix, although they were in contact with, and some of them protruded from the tips of the fibrils themselves (Figs. 8, 12).

The osteocytes which were contained in wide and irregularly shaped lacunae could be divided into two types: the first consisted of osteocytes whose cytoplasmic organelles and nuclear structure were preserved and still clearly recognizable, and the second consisted of degenerating osteocytes. The osteocytes of the first type appeared small compared with their lacunae. This was chiefly due to an irregular enlargement of the lacunae which led to the formation of a space of variable width between the bone matrix and the cell (Fig. 9). The

Fig. 6. Osteocyte showing initial discontinuity of the lacunar brush border. The upper and lower parts of the cytoplasm appear vacuolized. Uranyl acetate and lead citrate, ×15,000

Fig. 7. Detail of a disarranged lacunar brush border; note crystals apparently detached from the calcified matrix and cytoplasm retractions. Uranyl acetate and lead citrate, ×26,000

Fig. 8. Detail of the lacunar brush border in a section decalcified with formic acid and stained with phosphotungstic acid; crystal-like organic filaments protrude from the perilacunar matrix (above) into the lacunar space (below). ×100,000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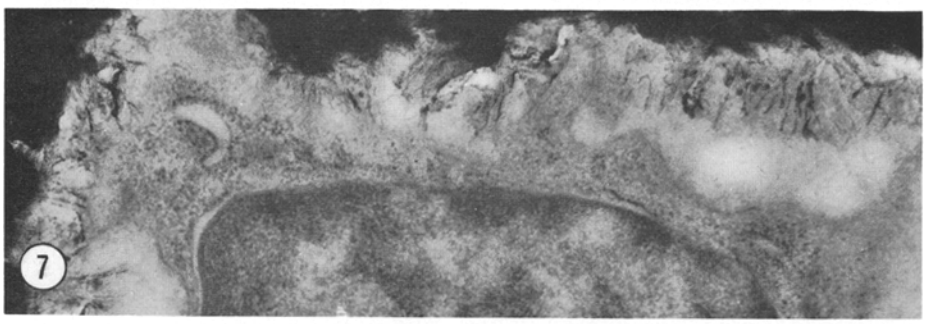
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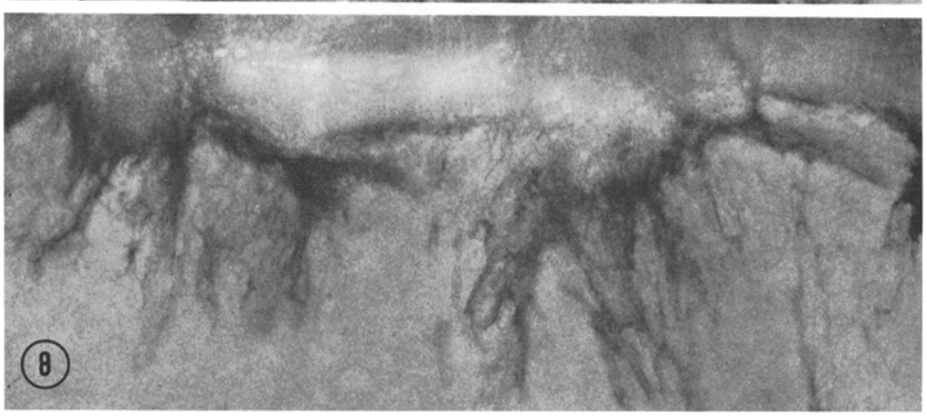
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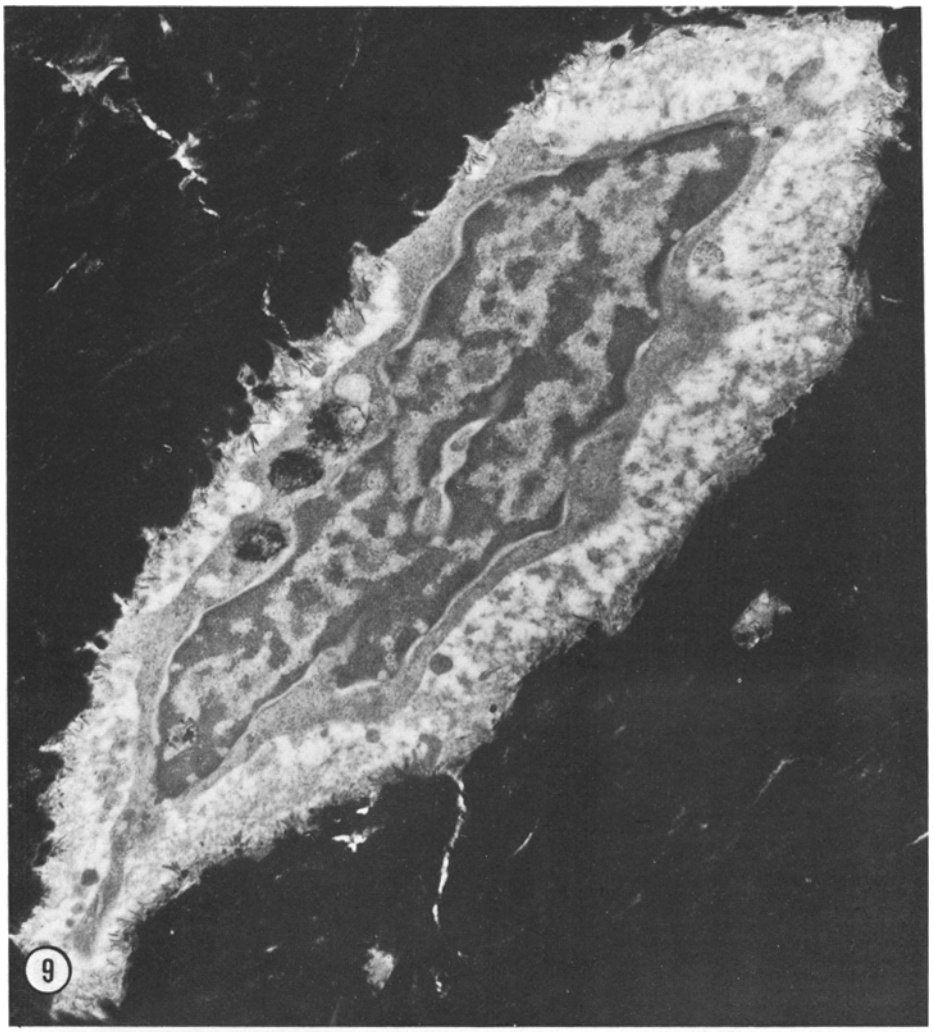
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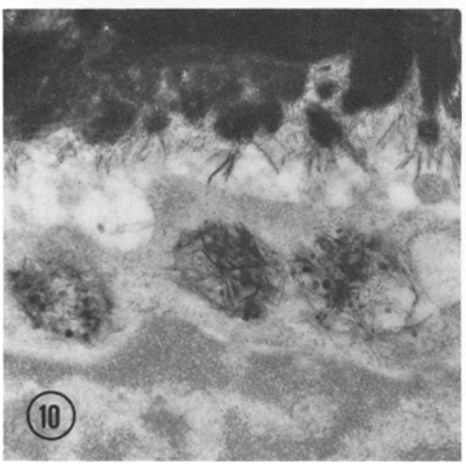
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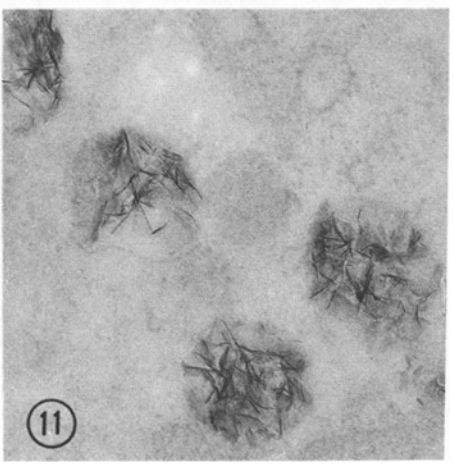
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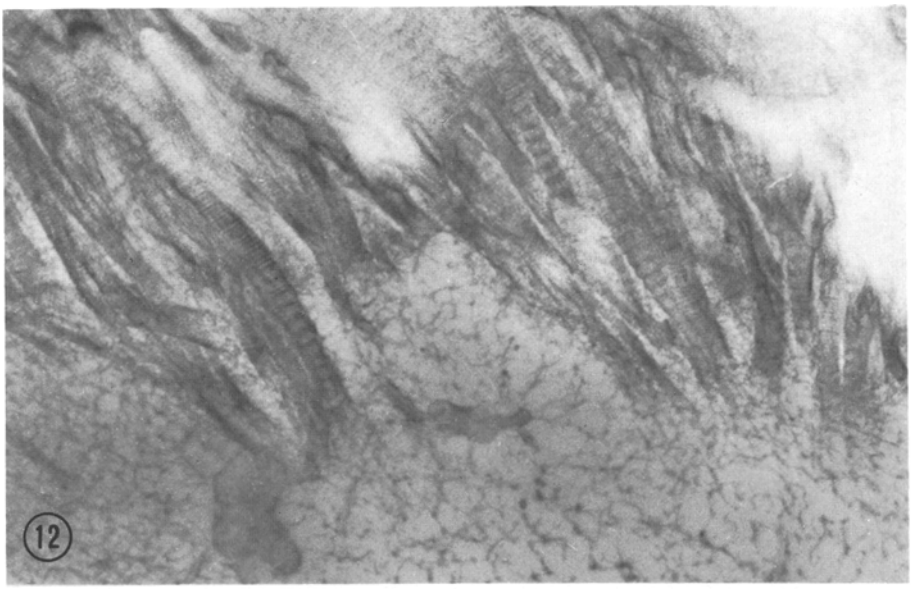

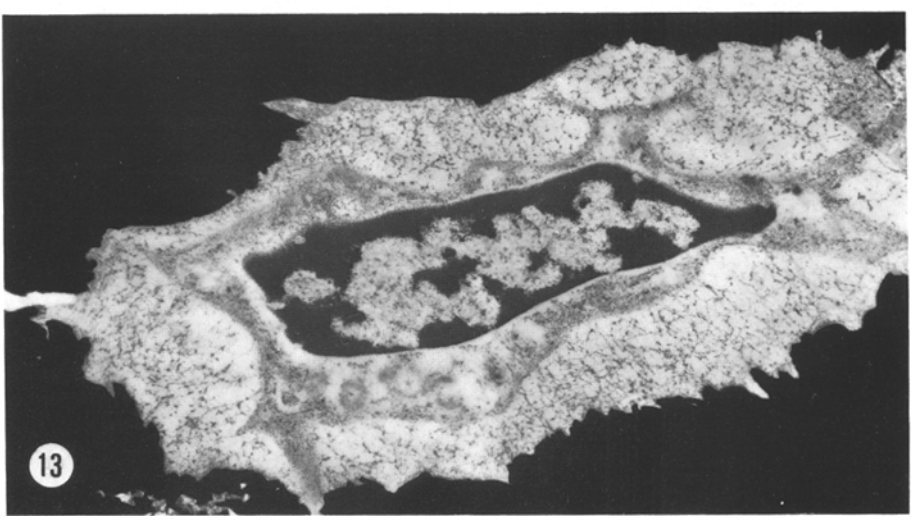

Fig. 9. Osteocyte engaged in osteolysis, as shown by enlargement and irregularity of its lacuna, almost complete disappearance of the lacunar brush border, presence of granular and filamentous material in the lacunar space, and of inorganic material within mitochondria (see also Fig. 10). Uranyl acetate and lead citrate, ×10,000

Fig. 10. Detail of Figure 9 showing calcified mitochondria, disarranged lacunar brush border, and electron-dense bodies probably representing cross-sections of calcified collagen fibrils. Uranyl acetate and lead citrate, ×45,000

Fig. 11. Clusters of inorganic crystals, probably intramitochondrial, in the cytoplasm of a resorptive osteocyte. Unstained, ×55,000

Fig. 12. Detail of the lacunar space of a resorptive osteocyte in a section decalcified and stained with phosphotungstic acid; the lacunar space contains filaments which are in direct contact with the tip of apparently dissociating collagen fibrils. ×50,000

Fig. 13. Degenerate osteocyte contained in an enlarged and irregular lacuna. Note condensed chromatin, lack of cytoplasmic organelles, and filaments in the lacunar space. Uranyl acetate and lead citrate, ×11,000

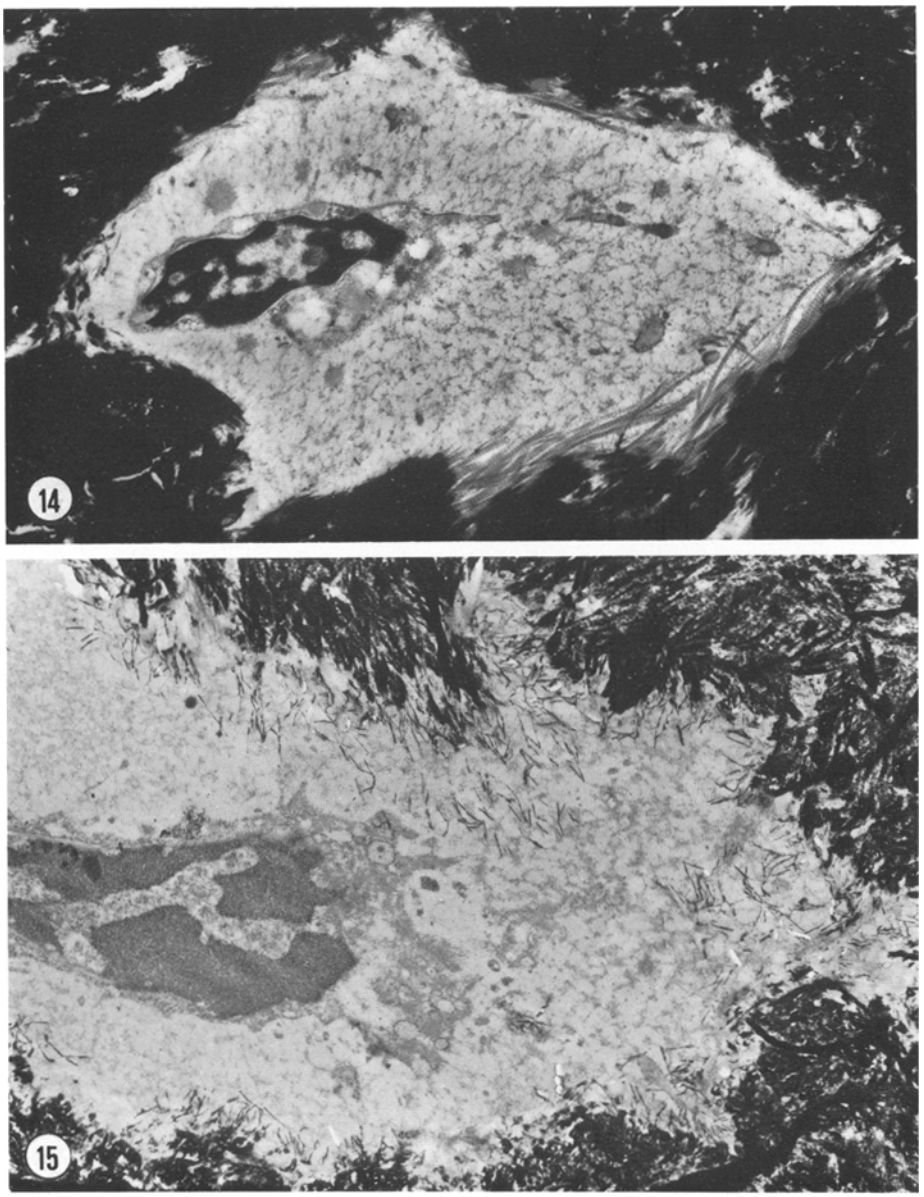

Fig. 14. Degenerate osteocyte placed in enlarged and irregular lacuna, whose border is partially decalcified as shown by unmasked collagen fibrils (lower right); the chromatin is condensed and the cytoplasm vacuolized and fragmented. Uranyl acetate and lead citrate, ×6000

Fig. 15. Degenerate osteocyte contained in a very irregular and enlarged lacuna; many crystals are scattered through the flocculent and filamentous material contained in the lacunar space. Uranyl acetate and lead citrate, ×10,000

nuclei of these osteocytes were very similar to those of the osteocytes described in preceding sections, although their chromatin was less dispersed and was occasionally condensed. There was very little cytoplasm, and it contained only a few mitochondria, vesicles of the Golgi apparatus and lysosome-like bodies. The mitochondria of these cells very often contained intrinsically electron-dense crystalline structures similar to those of the calcified bone matrix (Figs. 9–11). The space separating the osteocytes from the walls of their lacunae contained cytoplasmic processes and granular, filamentous and flocculent material (Fig. 9) stainable with phosphotungstic acid (Fig. 12).

The lacunar brush border was less evident in these lacunae than in those of the osteocytes described in the preceding section (compare Figs. 4 and 9). In some cases it seemed unchanged, but it usually appeared disarranged, containing irregularly distributed crystals which were apparently detached from the calcified matrix (Fig. 10). Moreover, roundish electron-dense bodies, about 700 Å across, were found between the crystals (Figs. 9, 10). Although the electron density of the inorganic substance masked the underlying structures, it seemed that these roundish bodies were cross-sectioned collagen fibrils probably about to become detached from the perilacunar matrix. This situation could be recognized in decalcified stained sections, where the pericellular space of the enlarged lacunae contained cross-sections of isolated collagen fibrils apparently detached from the bone matrix. The same space contained needle- and filament-like structures and flocculent and filamentous material (Fig. 12) similar to those found in undecalcified sections. In some cases, it was evident that the structures of the decalcified brush border and the filamentous material contained in the pericellular space were in direct contact with collagen fibrils which seemed about to become disaggregated (Fig. 12).

The osteocytes of the second type, i.e., those with a degenerating appearance, were chiefly characterized by an irregular nucleus with condensed and clumping chromatin and by a very small amount of cytoplasm which was often vacuolated and contained hardly any normal organelles (Figs. 13, 14). Even so, mitochondria more or less totally occupied by inorganic crystals were sometimes recognizable. Moreover, fragments of cytoplasm were found in the lacunar space, where an irregular network of filaments and clusters of flocculent material were recognizable too (Figs. 13, 14). The lacunar border was very irregular in these cases; no lacunar brush border was found, and uncalcified collagen fibrils sometimes took their place (Fig. 14). In some cases, the lacunar spaces in these osteocytes contained irregularly oriented, apparently dissociated crystals randomly dispersed between and over filamentous and flocculent material (Fig. 15).

Discussion

The present results show that in renal osteodystrophy, and especially in cases treated by haemodialysis, osteocytes have a variable morphology which seems to reflect their metabolic activity and to depend, at least partly, on the degree of calcification of the pericellular matrix.

As previously reported (Bonucci et al., 1975, 1976; Maschio et al., 1974, 1975), almost all the patients showed severe osteomalacic changes which were chiefly characterized by the presence of wide sheaths of osteoid tissue along the border of the osseous trabeculae. Many cells were recognizable in the osteoid tissue. Some had a relatively large cytoplasm with abundant cytoplasmic organelles and rough ergastoplasmic cysternae, so that they looked like osteoblasts; and some had little cytoplasm and very few organelles, and looked like osteocytes. The name "osteocyte" is usually restricted to cells surrounded by calcified matrix, whereas the cells found by us in osteoid tissue were surrounded by uncalcified collagen fibrils, so that it may seem an oversimplification to apply this term to them. On the other hand, their ultrastructure shows that they are true osteocytes, so that the conclusion must be drawn that the transformation of osteoblasts into osteocytes is not disturbed by the defective calcification which occurs in renal osteodystrophy, and that this transformation is independent of the calcification of the matrix which surrounds the cells.

The largest of these osteoid osteocytes resembled osteoblasts and their ultrastructural characteristics indicated that they were still capable of synthesis. They were similar to the "formative" osteocytes found in normally calcified bone matrix (Jande, 1971; Jande and Bélanger, 1971, 1973; Luk et al., 1974); these are considered to be young, bone-forming cells which gradually change into mature osteocytes. It may be that in osteoid tissue the large cells also synthesize pericellular matrix, so reducing the width of the space in which they are located. They may become mature osteoid osteocytes by a gradual fall in the numbers of cytoplasmic organelles they contain.

Formative and mature osteocytes were present even in incompletely calcified bone matrix. Under the light microscope, most of these osteocytes showed enlarged and irregular lacunae so that they could have been wrongly considered to be osteocytes engaged in osteolysis. However, electron microscopy showed that the apparent enlargement and irregularity of the lacunae in these osteocytes were due to defective calcification of the perilacunar matrix and not to periosteocytic osteolysis. This was shown by the finding that there was no evidence of a pericellular space, since the collagen fibrils were very close to the osteocyte peripheral membrane, while roundish areas of initial calcification were scattered between the fibrils, as often happens in normally calcifying osteoid tissue.

These findings show that even if the danger of incorrect evaluation can be limited or avoided by statistical analysis carried out on a large population of osteocytes (Krempien et al., 1973; Meunier et al., 1971), the degree of calcification of the periosteocytic matrix should always be taken into consideration when bone sections are to be examined for osteocytic osteolysis. In this connection, the presence of enlarged and irregular lacunae in hypomineralized areas must not be regarded as evidence of osteolysis.

The small osteocytes observed in the small lacunae within the fully mineralized matrix showed ultrastructural characteristics which suggested that they had very low metabolic activity. In particular, they showed a few cytoplasmic organelles and rough ergastoplasmic cysternae. Another interesting finding was the presence on the border of their lacunae of crystalline structures protruding side-to-side like the bristles of a brush, from the calcified matrix in the direction

of the osteocytic peripheral membrane. These structures make up a continuous sheath which has been called "lacunar brush border" here.

The lacunar brush border does not seem to be peculiar to the osteocytes of uremic, osteodystrophic subjects, since it has already been found in the osteocytes of medullary bone (Bonucci and Gherardi, 1975) and in those of osteoporotic rats (Salomon and Volpin, 1972). Moreover, protruding crystals like those found in the lacunar brush border are also visible along the margin of calcified cartilaginous trabeculae (Bonucci, 1967; Cameron, 1963; Robinson and Cameron, 1956; Scherft, 1968, 1972). Such crystals have been considered evidence that calcification is proceeding slowly or has stopped altogether (Bonucci and Gherardi, 1975; Scherft, 1972). The presence of the same type of protruding crystals around apparently inactive or hardly active osteocytes seems to strengthen this concept.

The crystals which make up the lacunar brush border are not bound to collagen fibrils. In fact, after decalcification and staining no collagen fibrils were recognizable where the lacunar brush border had been found. Only filament- and crystal-like structures were present, sometimes in contact with, and sometimes protruding from the tip of the collagen fibrils of the surrounding bone matrix, but distinct from them. These structures looked exactly like those previously described as "crystal ghosts" (Bonucci, 1967) and found in decalcified and stained sections of bone, cartilage, and pathologically calcified tissues (Appleton, 1971; Bonucci, 1967, 1971, 1975; Bonucci et al., 1973; Bonucci and Gherardi, 1975; Smith, 1970). They have been considered non-collagenous organic structures, probably proteoglycans, which might offer a framework for the deposition of the inorganic substance (Bonucci, 1975).

Another important consideration is pertinent to the nature and function of the lacunar brush border. Its crystals are often loosely arranged and closely connected with the peripheral membrane of the osteocytes. In consequence of these factors, the crystals of the lacunar brush border may constitute a disposable, easily utilizable reservoir of mineral ions. Their resorption could be the first step in the process of osteocytic osteolysis, which could therefore proceed initially without appreciable enlargement of the osteocytic lacunae, so that nothing would be seen under the light microscope. The two processes of formation and resorption of the lacunar brush border may explain how significant amounts of calcium ions can be either removed from circulating fluids or introduced into them; this would occur under the control and by means of osteocytes, without significant changes in the shape of their lacunae.

Studies of osteocytes ultrastructure carried out on bone specimens decalcified before embedding have frequently shown an osmiophilic lamina along the border of the osteocytic lacunae (Donath and Delling, 1971; Jande, 1971; Jande and Bélanger, 1971; Luk et al., 1974; Scherft, 1972; Tonna, 1972, 1973; Wassermann and Yaeger, 1965; Whitson, 1972). The present results have been obtained from undecalcified bone specimens and from bone sections decalcified after embedding, in which the osmiophilic lamina was not recognizable. This is due to the masking effect of the crystals when undecalcified sections are examined (Wassermann and Yaeger, 1965), but the reason for the failure to detect the osmiophilic lamina is not at all clear when sections are decalcified after embed-

ding. It is, however, interesting to note that the position of the osmiophilic lamina with respect to the border of the lacunae is practically the same as that of the lacunar brush border. In this connection, Wassermann and Yaeger (1965) have suggested that the osmiophilic lamina could be a special area near the surface of the mineralized matrix whose crystals (called by these authors "coastal crystals") might be of special interest, since their location at the edge of the mineralized matrix and their unordered arrangement could make them readily available to osteocyte activity. This concept is in complete agreement with our finding of a lacunar brush border—which is probably identifiable with "coastal crystals"—and with the tentative explanation we have given of its function and metabolic role.

That the lacunar brush border can be reabsorbed by osteocytes is suggested by the fact that in the present series of biopsies it was often irregular and discontinuous. Moreover, the irregularity of the lacunar brush border was always associated with the presence of granular, flocculent and filamentous material in the lacunar space. The mechanism of resorption is still unclear, however. Certainly, the periosteocytic resorption process can overstep the lacunar brush border and occur in the perilacunar matrix. As a result, and as previously reported in other normal and pathological conditions of the skeleton (Baud, 1962, 1966; Jande, 1971; Jande and Bélanger, 1971, 1973; Luk et al., 1974; Remagen et al., 1968, 1969; Tonna, 1972, 1973; Weisbrode et al., 1974; Whitson, 1972), the volume of the lacunae increases, their borders become irregular, and a space containing granular, filamentous and flocculent material is formed between the osteocyte and the lacunar wall. Electron probe analysis (Remagen et al., 1969) and pyroantimonate precipitation technique (Doty and Schofield, 1972) have shown that this material is associated with calcium ions, but its nature is not exactly known. Jande (1971) considers that this material is a breakdown product of the organic bone matrix and that it consists of acid proteoglycans because it reacts with colloidal iron and colloidal thorium.

The osteocytes which contain enlarged, irregular cysternae, and show flocculent and filamentous material in the pericellular space have previously been called bone-resorbing or resorptive osteocytes, because they are considered to be engaged in osteolysis (Jande, 1971; Jande and Bélanger, 1971, 1973; Luk et al., 1974). A great many of these were found by us, probably as a result of the high degree of hyperparathyroidism present in our patients.

These resorptive osteocytes very often showed intramitochondrial accumulation of inorganic crystals. This could be due to excessively high intracellular concentration of calcium ions probably produced by osteocytic osteolysis. It is well known that mitochondria can control and regulate the movement and concentration of intracellular calcium (Lehninger, 1970; Rasmussen, 1966) and that if exposed to excessively high calcium concentration they can accumulate so much amorphous and crystalline inorganic material that they become completely masked by the mineral substance (Bonucci et al., 1973). It is possible that when the normal osteocyte calcium-loading limit is overstepped, intramitochondrial calcium overloading can occur and this, in turn, can promote or facilitate osteocytic degenerative processes.

Degenerating osteocytes were regularly present in our material, although

they did not seem to be as frequent as they have been reported to be in other material (Jande and Bélanger, 1973; Luk et al., 1974; Tonna, 1973). It is possible that their scarcity in renal osteodystrophy is related to the increased degree of renewal of the bone tissue which occurs in this pathological condition, mainly as a result of the increased activity of osteoclasts. It is, however, worth remembering that the first (indirect) reference to osteocytic osteolysis was that made by Recklinghausen (1910) in discussing cases of osteomalacia and rickets.

The bone matrix surrounding degenerate osteocytes was not only partly reabsorbed but also decalcified in some instances, as shown by the presence of collagen fibrils unmasked by the removal of inorganic crystals. It is possible that the degeneration and death of osteocytes cause an increase in the level of acidity within lacunae high enough to solubilize the mineral substance.

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