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Osteoclast abnormalities in idiopathic osteopetrosis

Reference to the ultrastructural histochemistry study

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Summary. In order to investigate skeletal abnormalities in a case of idiopathic osteopetrosis, a bone biopsy was taken from the anterior iliac crest and prepared for ultrastructural and histochemical study. There was a drastic reduction in osteoclastic bone resorption. The ruffle border and sealing zone, which are the osteoclast cell surface markers of bone resorption, were absent. The cells were highly vacuolated, and the vacuoles contained large amounts of a residual organic material which reacted strongly with acid phosphatase. Acid phosphatase activity was never found outside the cell, and in particular, not at the bone-cell interface.

This suggests that the defect in bone resorption is caused by cell membrane abnormalities and the lack of ruffle border formation, rather than the inability of the lysosomal enzymes to digest the bone matrix.

Key words: Idiopathic osteopetrosis – Osteoclast – Acid phosphatase – Bone resorption

Introduction

Osteopetrosis (op) is a group of diseases of man and laboratory animals, characterized by abnormal bone density and brittleness, and obliteration of the medullary spaces (Jaffe 1972).

The congenital form of osteopetrosis has usually been described (Albers-Schönberg 1904) and it has been shown that the disease can be subdivided into 2 groups (Johnston et al. 1968; Brown and Dent 1971). The most common form (benign osteopetrosis) is generally inherited as an autosomal dominant trait. Affected subjects have a normal life expectancy and show no haematological abnormalities. In contrast malignant osteopetrosis is a rare disease which is inherited as an autosomal recessive trait and always has a fatal outcome.

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The pathogenesis of the disease remains unknown. However, available histological studies (Frost et al. 1969; Coccia et al. 1980; Shapiro 1982; Glorieux et al. 1981; Teitelbaum et al. 1981; Bonucci et al. 1975) and findings in experimental animals with a disease similar to human osteopetrosis (Marks 1973; Marshall 1982; Schofield et al. 1974) suggested that the skeletal lesions mainly result from a marked decrease in bone resorption.

Very few investigators have examined the cells and bone matrix from affected subjects under the electron microscope and other forms of osteopetrosis apart from congenital osteopetrosis have been described rarely. We therefore thought it useful to report the results of an ultrastructural and histochemical study of bone in a case of idiopathic osteopetrosis.

Case report

A 31 year old white female noted the onset of backache with diffuse pain in the thighs and legs, 3 months before first admission. At this date physical examination revealed absence of clinical abnormalities. X rays of the lumbar column and of the pelvis were normal. On laboratory examination the sedimentation rate, haemoglobin level, WBC count and differential were normal. Renal function was normal. Blood and urine calcium and phosphate levels were normal but blood alkaline phosphatase was high at 960 mU/ml (normal range 55–115). Analgesic treatment was prescribed. Persistance of the same symptoms was the cause of a second hospital admission 10 months later. Clinical examination was normal. Radiography demonstrated a diffuse and homogenous osteocondensation of the axial and limb skeleton.

Sedimentation rate, haemoglobin level, WBC count and differential, bone marrow biopsy, blood and urine immunoelectrophoresis and renal function were normal. Antinuclear antibodies were absent. Phosphocalcium metabolism studies showed a discrete diminution of blood calcium level (2.11 to 2.18 mmol/l, normal range: 2.28 to 2.52 mmol/l) marked diminution of urine calcium level (0.3 to 0.4 mmol/24 h, normal range 2.5 to 6.2 mmol/24 h), normal level of blood and urine phosphates, marked increase in urine hydroxyproline level (0.77 to 0.88 mmol/24 h, normal range 0.10 to 0.26 mmol/24 h), high level of blood alkaline phosphatases (207 to 232 UI/l, normal range (25 to 65 UI/l). Blood and urine fluoride level were normal. Parathyroid hormone was increased (99 to 187 pg/ml, normal range 20 to 45 pg/ml). Vitamin D metabolites were normal.

Bone histomorphometry on iliac crest bone biopsy showed marked increase in trabecular bone volume at 59% (normal range 15 to 22%).

Material and methods

A bone biopsy was taken from the anterior iliac crest. The specimen was fixed in 2% glutaraldehyde buffered at pH 7.4 with 0.1 M sodium cacodylate, for 3 h. Following fixation, the tissue was transferred to 0.1 M cacodylate buffer containing 7.5% sucrose and was left overnight at 4° C.

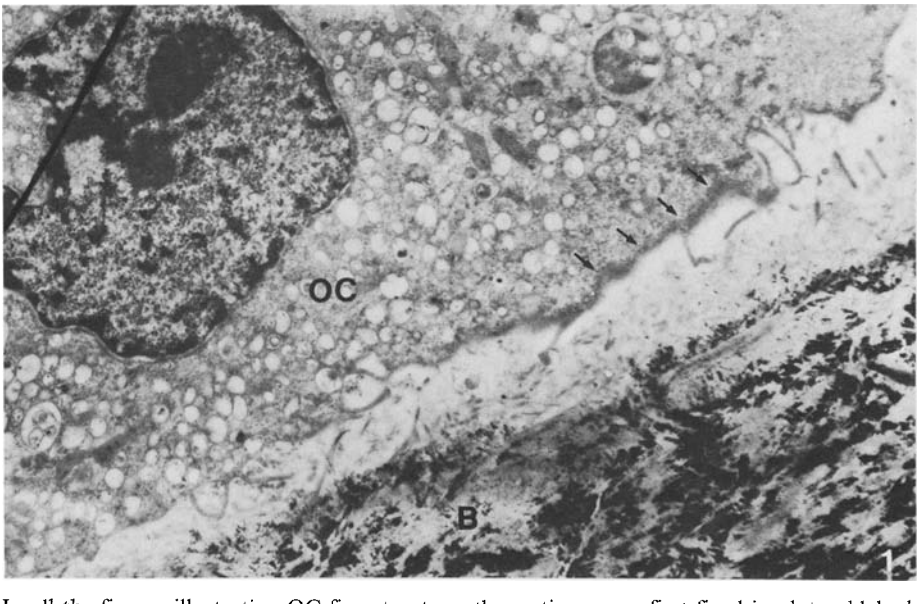
A portion of the biopsy was embedded for E.M. study wihout decalcification and the rest was decalcified in 10% EDTA, pH 7.4 during 5 days for enzyme histochemical study.

The tissue blocks without decalcification were post-fixed in 1% Palade's osmium for 1 h, dehydrated in graded alcohol and embedded in Epon 812. For enzyme histochemical study, the decalcified tissue blocks were chopped with the Oxford vibratome into 50 μ sections, rinsed in cacodylate buffer and incubated at 37° C in Gomori solution for 30 min in order to demonstrate acid phosphatase activity. The control consisted of sections incubated without the substrate. The sections were collected, post-fixed, dehydrated and embedded as previously described. Semi-thin sections, one micron thick, were prepared with a glass knife and stained with 1% toluidine blue. Fifteen osteoclasts (OCs) were selected by the following criteria: they had a typical appearance under the light microscope (multinucleated and located on the bone surface). The serial ultrathin sections of about 2 microns interval were cut with a diamond knife and double stained with uranyl acetate and lead citrate. These sections were examined and photographed with a Philips 300 E.M. operated at 60 kv.

Results

When examined under the electron microscope the osteoclasts from the patient were found to present morphological abnormalities.

The OC plasma membrane opposed to the bone surface showed neither the classical described sealing zone nor the ruffle border (RB), which are the characteristic cell surface markers (Fig. 1) of bone resorption. Facing the bone-cell interface, the plasma cell membrane is usually smooth and often in close contact with an electron dense material (Fig. 2). This material is composed of a fine granular material, a dense network of microfilaments



In all the figures illustrating OC fine structure, the sections were first fixed in glutaraldehyde and double stained with uranyl-acetate and lead citrate

Fig. 1. Undecalcified bone section. Part of an osteoclast (*OC*). A few short and undulating cytoplasmic digitations are visible along the plasma membrane facing the bone surface (*B*). Part of the cell membrane is coated by a electron dense material (*arrows*). × 7000

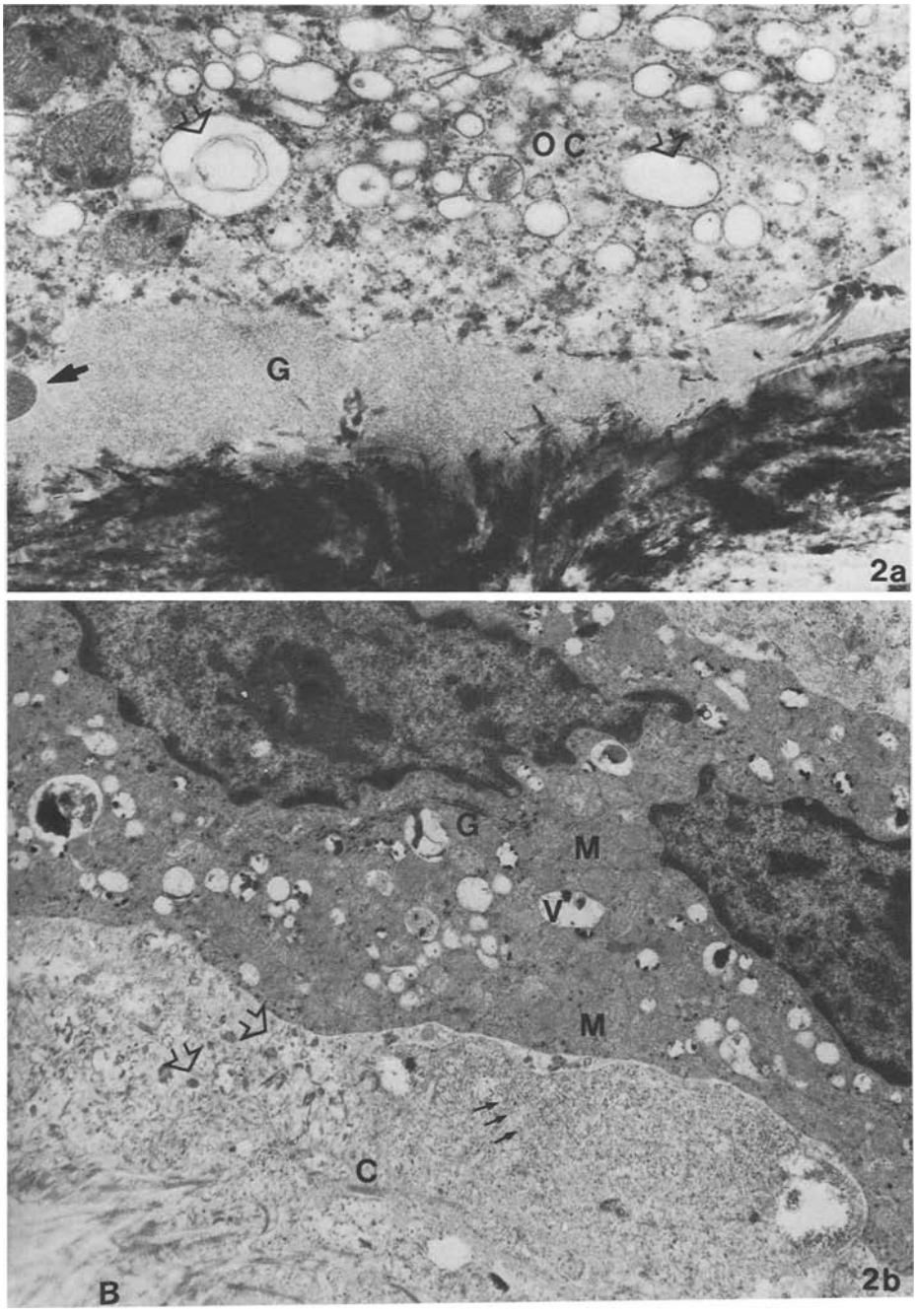


Fig. 2a. Undecalcified bone section. Part of an *OC*. The *OC* membrane is in contact with granular material (*G*) and organelles from damaged cells (*arrow*). The vacuoles are filled by large amounts of an amorphous material and ribosome residues (*open arrows*). ×24000. **b** Decalcificied bone section, stained for acid phosphatase. The granular material in contact with the *OC* is mainly composed of a dense network of filaments (*arrows*), organelles from damaged cells (*open arrows*) and a few collagen fibers (*C*). *B* (decalcified bone). ×20000. The OC is heavily stained for acid phosphatase: *G* (Golgi); *V:* vacuoles; *M*: mitochondria

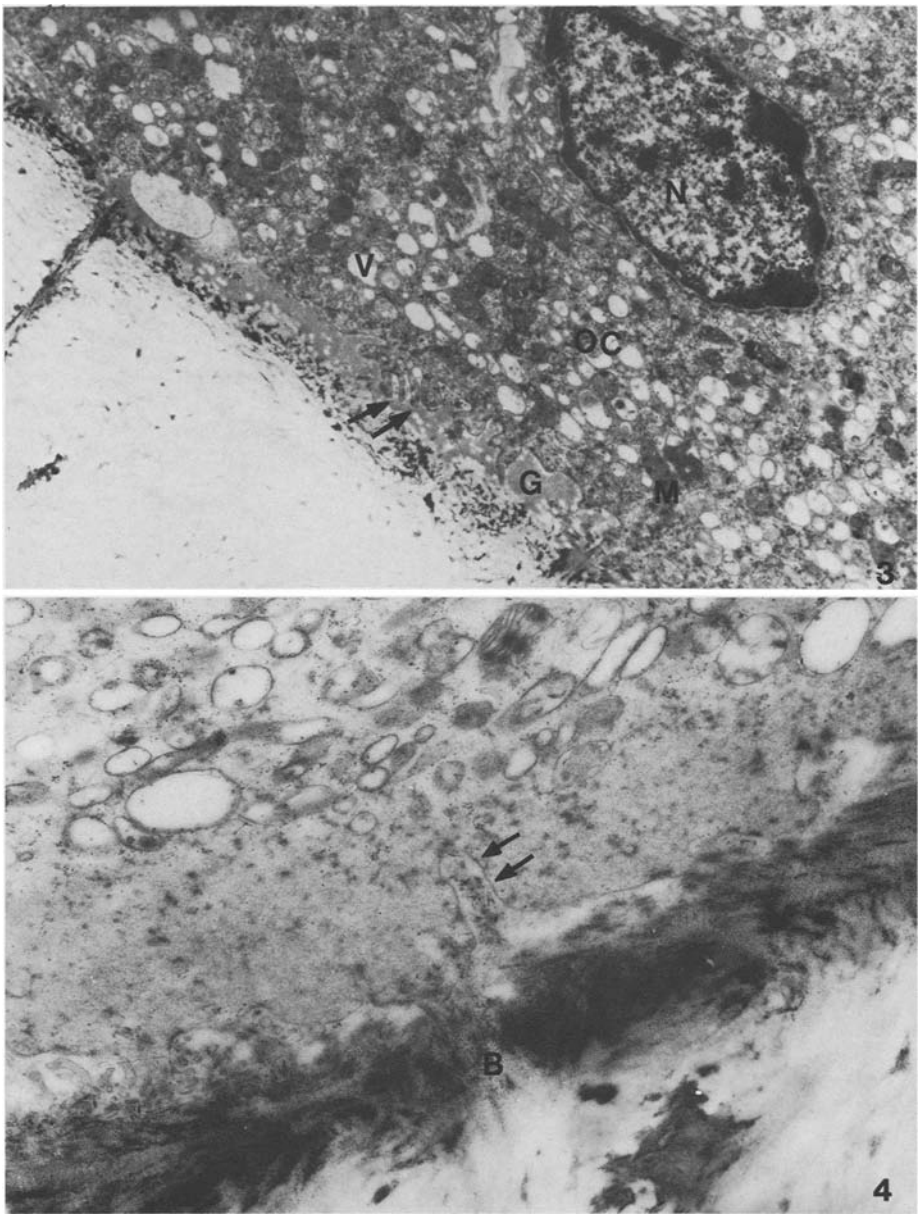


Fig. 3. Undecalcified bone section. OC cell membrane showed a ruffle border-like structure (*arrows*) opposed to the bone surface. ×8000. *N*: nucleus; *M*: mitochondria; *V*: vacuole; *G*: granular material in contact with OC

Fig. 4. Undecalcified bone section. OC showed some pinocytosis activity (*arrows*). ×15000; *B*: mineralized bone surface

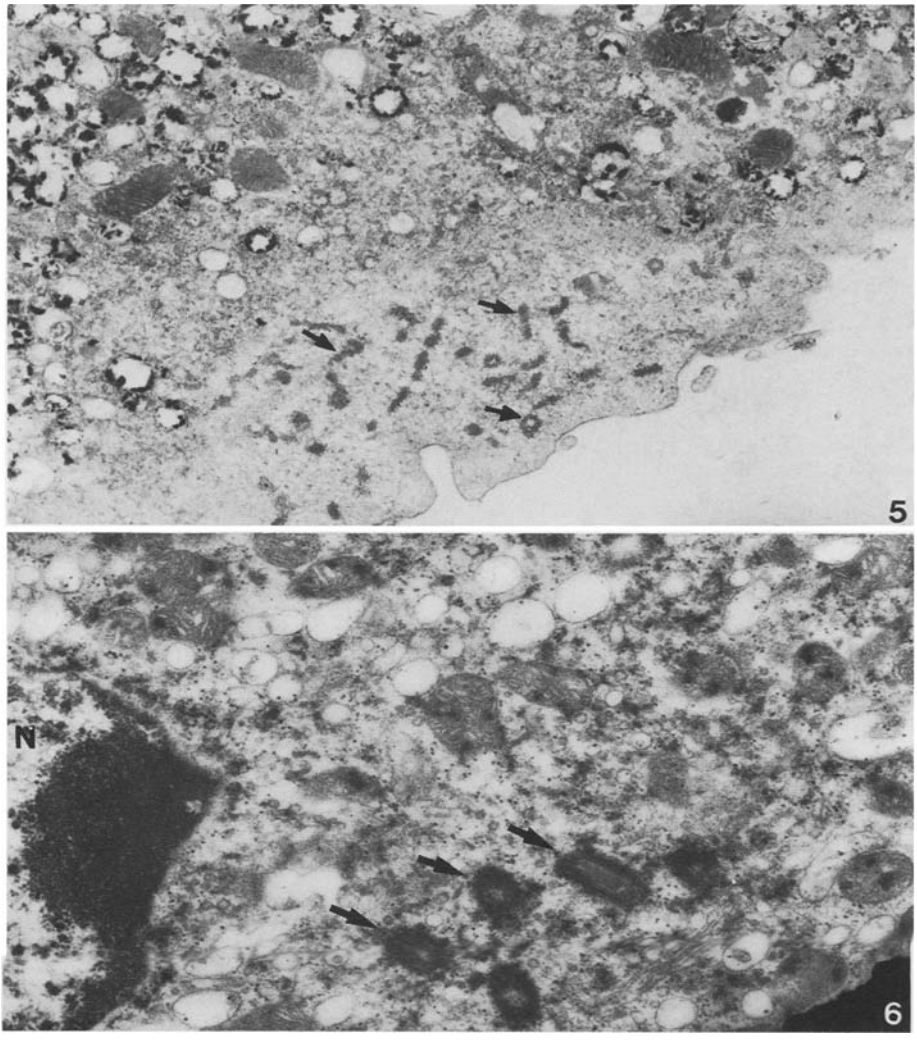


Fig. 5. Decalcified bone section, stained for acid phosphatase, the proximal portion of the OC facing the mineralized bone surface has a morphology similar to that of the classical sealing zone. This portion is not free from organelles however (*arrows*). ×15000

Fig. 6. Undecalcified bone section. Notice the presence of centrioles within the OC (*arrows*); *N*: nucleus. ×19000

and a few collagen fibers (Fig. 2a, b). However is some areas, thin and undulated cytoplasmic digitations were found opposed to the mineralized bone surface, some with a RB-like structure; however there was no sign of bone mineral mobilization (Fig. 1–3). In spite of the absence of the RB, the OCs appeared to influence the removal of bone matrix by a pinocytic process (Fig. 4). Only a small volume of bone is probably removed by this

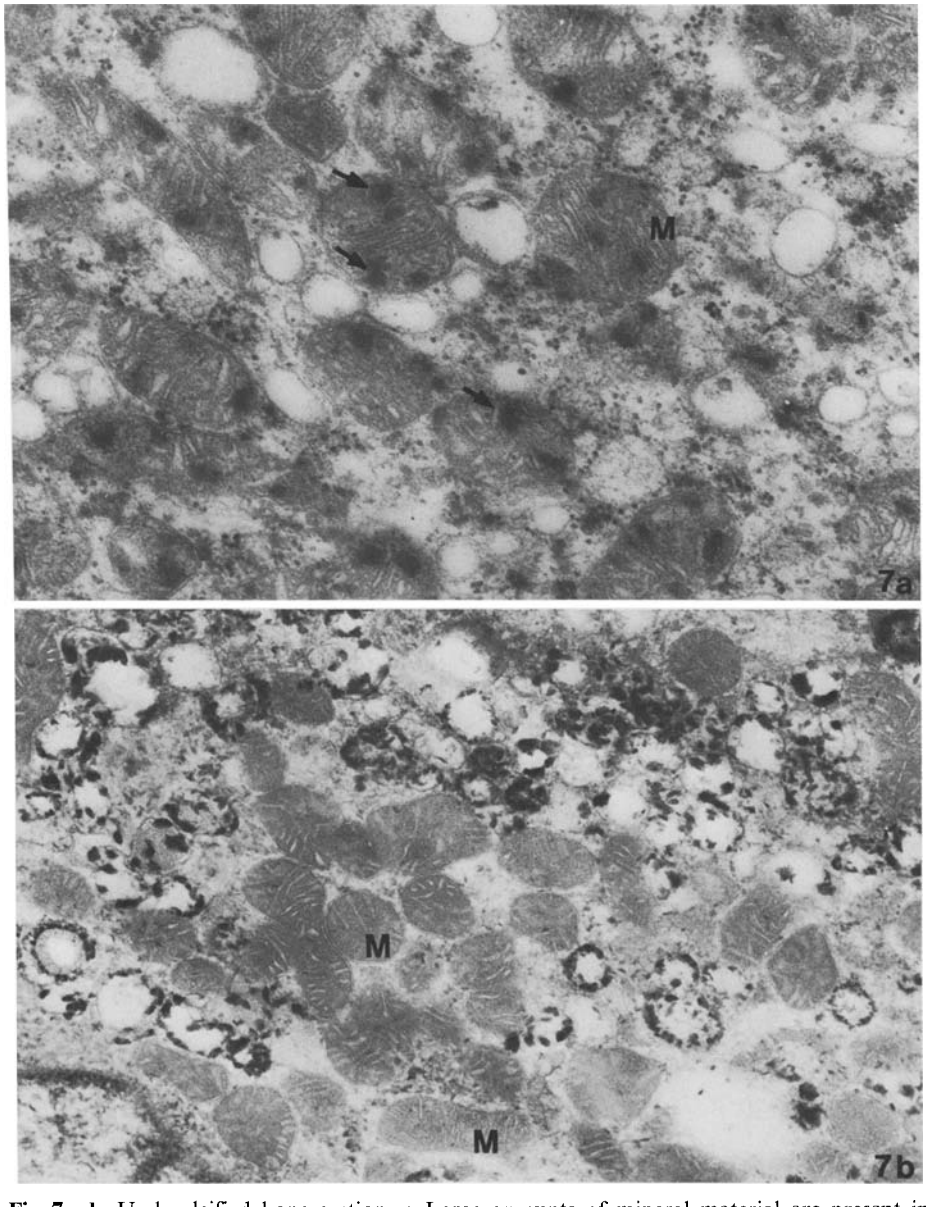


Fig. 7a, b. Undecalcified bone section. **a** Large amounts of mineral material are present in the mitochondria (*arrows*) but not in the decalcified section **b** stained for acid phosphatase. *M* mitochondria; **a**: ×30000; **b**: ×24000

method. The portions of the cell cytoplasm located on the proximal side of the cell membrane opposed to the bone surface have a similar morphology to the normal sealing zone. This part of the cell was not free from organelles and contained variable sizes of electron dense bodies which did not react to acid phosphatase (Fig. 5).

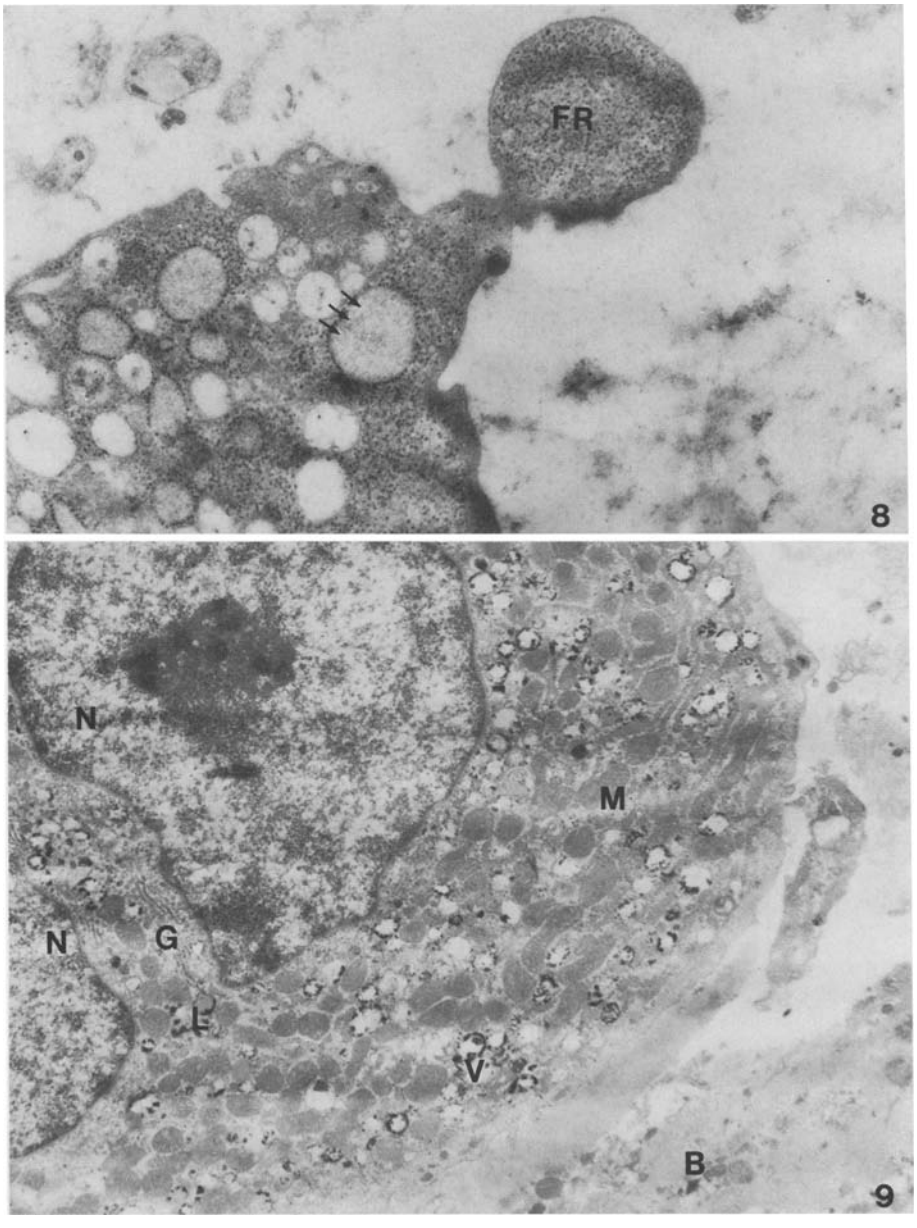


Fig. 8. Undecalcified bone section. Cross section of the RER. Notice the presence of organic material within the RER (*arrows*) ×19000; *FR*: free ribosomes

Fig. 9. Decalcified bone section, stained for acid phosphatase. Large amounts of acid phosphatase are present in the cell. ×7000. *L*: lysosome; *N*: nucleus; *G*: perinuclear Golgi apparatus; *M*: mitochondria; *V*: vacuoles; *B*: decalcified bone

The cytoplasm was characterised by the presence of an abnormal amount of vacuoles, which were often filled with flocculent or irregularly dense amorphous material as well as autophagosomes containing ribosome residues (Fig. 1–2a–3).

The centriole was also present within the cell (Fig. 6). Mitochondria were densely packed and large amounts of rounded mineral material with a diameter of 64 nm had accumulated within the mitochondria. Since this material was not found in the mitochondria of the decalcified sections, it is probably composed of calcium phosphate (Fig. 7a, b). Unlike normal OCs which are rich in polyribosomes, the cytoplasm of OCs from this patient contained free ribosomes which were scattered in a disordered way throughout the cell. The rough endoplasmic reticulum was extremely dilated and filled with an organic material (Fig. 3–8). In general, the surface profile of the OC plasma membrane was smooth and in contact with a mixture of an organic dense granular material and organelles from damaged cells (Fig. 2b–9). In most OCs, the nucleus had a normal structure although in some cells the heterochromatin occupied almost the whole of the nuclear surface.

Acid phosphatase was present within the cell; large amounts were accumulated in the lysosomes and in vacuoles of variable size; the reaction of this enzyme was never observed outside the cell, in particular at the bone-cell interface (Fig. 2b–9).

Discussion

Examination with the electron microscopic showed that there is a marked difference between the morphology of normal OC and that of the OCs studied in this report from a patient with idiopathic osteopetrosis. Normal OCs have the characteristic ruffle border and sealing zone, which are the cell surface specifications of these cells and represent the hallmark of bone resorption (Holtrop and King 1977; Gothlin and Ericsson 1976; Chambers et al. 1984).

Immediately adjacent to the ruffle border, normal OCs contain large numbers of dense bodies and vacuoles which have been shown to be histochemically reactive for various lysosomal enzymes; and the reactions of these enzymes have been demonstrated on the bone matrix in contact with the RB (Lucht 1971; Dorey and Bick 1977; Doty and Schofield 1972).

In this clinical form of idiopathic osteopetrosis, OCs showed a drastic reduced osteoclastic bone resorption. The ultrastructure of OC was abnormal, the ruffle border and sealing zone which are the OC cell surface markers of bone resorption, were absent.

Even in the absence of the normal RB, some OCs appeared to remove the bone matrix by a pinocytic process. This finding suggests that OCs still retain some of their phagocytic activity, though only a small or insignificant volume of the bone matrix is probably removed. These findings are

quite similar to those observed recently in the ia rat (Marks 1973; Schofield et al. 1974) as well as those reported in human congenital osteopetrosis (Bonucci et al. 1975; Shapiro et al. 1980–1982; Glorieux et al. 1981; Teitelbaum et al. 1981). These authors concluded that the defect in bone resorption is due to a disorder of osteoclastic function. It is still not clear however if the defective OC function responsible for the reduced bone resorption is caused by the inability of the lysosomal enzymes to carry out their digestive function or by membrane abnormalities which render the OC unable to recognize the resorbing bone surface and to differentiate into the RB.

Glorieux et al. 1981, showed that the infusion of PTH in a male patient suffering from malignant congenital osteopetrosis caused a decrease in bone volume and the resumption of osteoclastic bone resorption. In the patient studied in our report, serum calcium and phosphorus were within the normal range, whereas serum PTH levels were extremely high. We therefore presume that in this clinical form of osteopetrosis, OCs do not respond to parathyroid hormone.

The ultrastructure of OCs from this patient was similar to that of OCs from diphosphonate-treated animals (Plasmans et al. 1980; Schenk et al. 1973). As mentioned, the administration of these agents inhibited bone resorption and caused a striking decrease in OC activity, characterized by a profound change in the ultrastructure, and an accumulation of large amounts of mineral deposits in the mitochondria. These findings suggest that, like diphosphonate-treated OCs, the OCs in this patient have a significantly reduced activity.

The relationship between OC-RB, cytoplasmic vacuoles, lysosomes and the release of lysosomal enzymes has been extensively discussed in the literature (Holtrop and King 1977; Gothlin and Ericsson 1976; Lucht 1971; Dorey and Bick 1977; Doty and Schofield 1972). The absence of infolding and a clearly defined OC-RB in this study shows probably that the bone matrix is not being resorbed or if occurs it will be not significant.

Moreover, large amounts of acid phosphatase are present in the lysosomes and in vacuoles of variables sizes, though this enzyme is not found outside the cell and in particular at the cell-bone interface; this suggests that the defect in bone resorption is not caused by digestive incompetence due to lysosomal enzyme abnormalities, but rather to OC membrane abnormalities which probably prevent RB formation. These finding are in agreement with Lucht (1971); Gothlin and Ericsson (1976); Doty and Schofield (1972) who suggested that the RB and its infolding are a prerequisite for the release of lysosomal enzymes.

In this form of osteopetrosis, the mineralized bone surface opposing the OC membrane is constantly coated with a dense organic material and OC profile is extremely smooth when in contact with this material. Thus, the question is whether this material inhibits osteoclastic resorption or may prevent the OC from recognizing the bone surface to be resorbed. Further investigation is necessary to know whether this layer plays a role in the control of osteoclastic bone resorption.

Conclusion

In this clinical form of idiopathic osteopetrosis, OCs were shown to have an abnormal ultrastructure. Our results suggest that the defect in bone resorption is a consequence of cell membrane abnormalities and a defect in ruffle border formation rather than the inability of the lysosomal enzymes to carry out their normal digestive function.

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