Virchows Arch. A Path. Anat. and Histol. 378, 287–295 (1978)

Virchows Archiv A

Giant Cell Tumor of Bone

Fine Structural Localization of Alkaline Phosphatase

Tomas Aparisi [1], Bengt Arborgh [2], and Jan L.E. Ericsson [3]

Department of Orthopedic Surgery [1] at Karolinska Hospital and Department of Pathology [2] at Huddinge Hospital, Karolinska Institute Medical School, Stockholm, and Central Laboratory of Clinical Pathology [3] at the University Hospital, University of Uppsala, Sweden

Summary. The fine structural localization of nonspecific alkaline phosphatase was elucidated in two giant cell tumors of bone using lead as capturing ion and β-glycerophosphate as substrate in the incubation solution. Lead phosphate precipitate – indicating presence of alkaline phosphatase – was demonstrated on the plasma membranes, and the membranes bordering vesicles and vacuoles of presumed endocytotic nature, in giant cells and type 1 stromal cells (fibroblast-like cells). The findings support the view that stromal cells type I and giant cells are histogenetically related.

Key words: Giant cell tumor – Ultrastructure – Alkaline phosphatase – Human bone neoplasias.

Introduction

The use of electron microscopy and histochemistry applied at the fine structural level has aided in the elucidation of the histogenesis, functions and interrelations of various types of cells in normal and neoplastic calcified tissues.

Giant cell tumors of bone constitute a group of neoplasms composed of cells of enigmatic derivation and function. Recent electron microscopic studies have unravelled the appearance of three types of cells in these tumors in great detail (Aparisi et al., 1977a, b). However, despite detailed ultrastructural analysis and characterization of these cells, a conclusion concerning their derivation and possible interrelationships has not been reached.

Alkaline phosphatase is an enzyme known to be associated with the plasma membrane and associated structures in bone-forming cells (preosteoblasts and osteoblasts). It cannot be demonstrated on the plasma membrane of macrophages-histiocytes or osteoclasts in non-neoplastic tissue (for example fracture callus) (Göthlin and Ericsson 1973). Giant cell tumors of bone are made up

Send offprint requests to: Dr. Tomas Aparisi, Department of Orthopedic Surgery, Karolinska Hospital, S-104 01 Stockholm, Sweden

0340-1227/78/0378/0287/$01.80

of fibroblast-like cells, macrophage-like cells and giant cells (bearing some resemblance to osteoclasts). On purely morphological grounds, fibroblasts and preosteoblasts are difficult or impossible to distinguish; thus the possibility exists that the fibroblast-like cells observed in giant cell tumors of bone are of preosteoblastic nature. If so, they can be expected to carry alkaline phosphatase in their plasma membrane.

Earlier studies on the localization of alkaline phosphatase in giant cell tumors of bone at the light and electron microscopic level have given uncertain results or have failed to demonstrate this enzyme in the tumor tissue (Gilmer, 1954; Kraievski et al., 1970; Mnaymneh and Ghandur-Mnaymneh, 1967; Ores et al., 1969; Pepler, 1958; Schajowicz, 1961; Thiery et al., 1975). Recent developments in ultrastructural histochemistry of phosphatases in calcifying tissues prompted us to reinvestigate the localization of alkaline phosphatase in giant cell tumors of bone. The demonstration of the enzyme in any type of cells would give clues to the derivation and functional differentiation of the cell or cells and might further aid in clarifying the interrelationships of the different cell types in the tumor tissue.

In this report, we present the results of a study on the fine structural localization of nonspecific alkaline phosphatase in two giant cell tumors of bone.

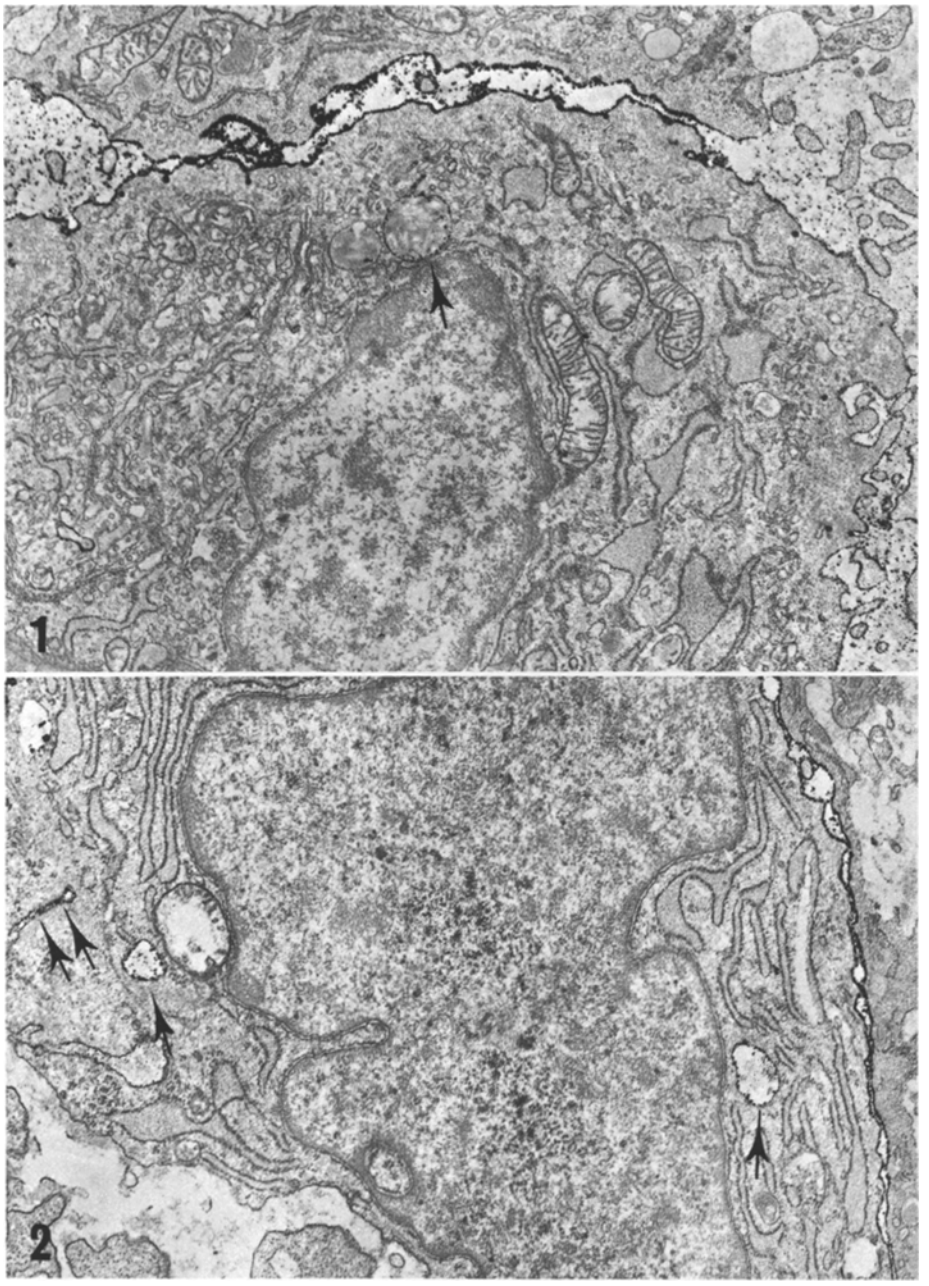
Materials and Methods

Small specimens of tumor tissue were fixed by immersion in glutaraldehyde solution for periods varying from 75 min to 20 h. The fixative was composed of 2% highly purified glutaraldehyde, 0.1 M cacodylate buffer and 0.1 M sucrose, pH 7.2. The material was subsequently washed and stored at +4° C in 0.1 M cacodylate buffer (pH 7.2) containing 0.1 M sucrose.

Prior to incubation for enzyme histochemistry, tissue was transferred to 0.1 M cacodylate buffer with 10% DMSO and was kept in this solution for 24 h (Helminen and Ericsson, 1970; Göthlin et al., 1973). Sections, approximately 50 μ thick, were then prepared on a Leitz freezing microtome and incubated in a Gomori type medium for the demonstration of alkaline phosphatase (Göthlin and Ericsson, 1973). Controls were incubated in a medium lacking substrate, and in a medium with EDTA as enzyme inhibitor (Aparisi et al., 1978a, b). After incubation, the sections were rinsed in the cacodylate-sucrose buffer and were post-fixed in *s*-collidine buffered osmium tetroxide (OsO_4). Preparation for electron microscopy was performed as described earlier (Aparisi et al., 1977a). The thin Epon sections were stained with uranyl acetate and lead citrate and were examined in a Jeol 100C electron microscope.

Case Histories

Case 1. A 29 year old nurse presented with pain in the right knee region without prior trauma. X-ray revealed a predominantly osteolytic lesion in the proximal part of the tibia. Operation in a local hospital was performed on the assumption that the lesion represented a bone cyst but histopathologic examination of the curetted material revealed giant cell tumor of bone, Jaffe type II. A recurrence occurred about 6 months later and she was then reoperated on in the orthopedic clinic at the Karolinska hospital. Thorough curettage and autologous bone transplantation were carried out. The tumor tissue was examined by light and electron microscopy and was processed for the fine structural demonstration of alkaline phosphatase, as described in "Materials and Methods". The diagnosis was again giant cell tumor of bone, Jaffe II. There is no recurrence one year after the last operation and the patient is free of symptoms.



Note: All the figures are from electron micrographs of tissues incubated for the demonstration of alkaline phosphatase, as described under "Materials and Methods"

Fig. 1. Portions of two fibroblast-like cells with deposits of reaction product located over the plasma membranes. In the right hand portion of the lower cell, the plasma membrane forms irregular, deep infoldings. A vacuole with a tail-like extension directed toward the cell surface and showing the presence of precipitate over its limiting membrane is indicated by an *arrow*. Incubation time, 90 min; × 11,500

Fig. 2. Fibroblast-like cells with focal deposits of final product on the plasma membranes, on the membranes delimiting 2 cytoplasmic vacuoles (*arrows*), and a deeply infolded channel (*double arrows*) connecting with the cell surface. Incubation time, 30 min; × 14,000

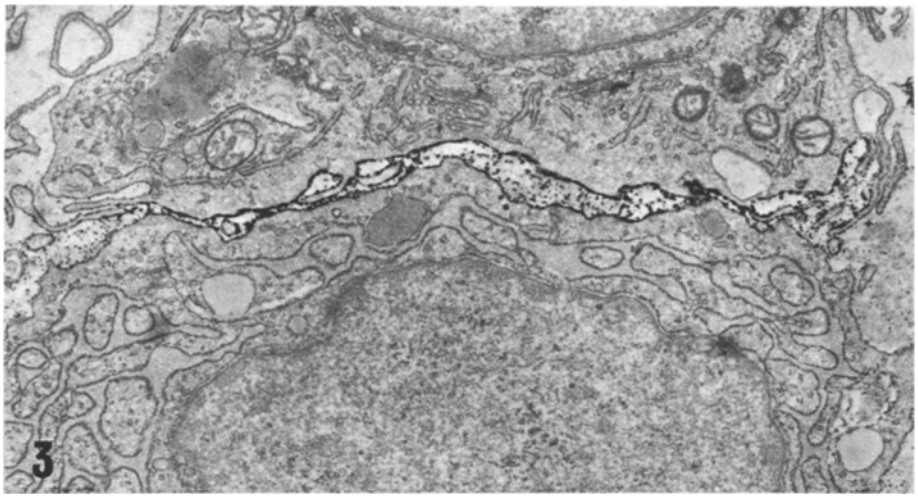


Fig. 3. Parts of two fibroblast-like cells with deposits confined to adjoining areas of the plasma membranes. Incubation time 90 min; ×12,500

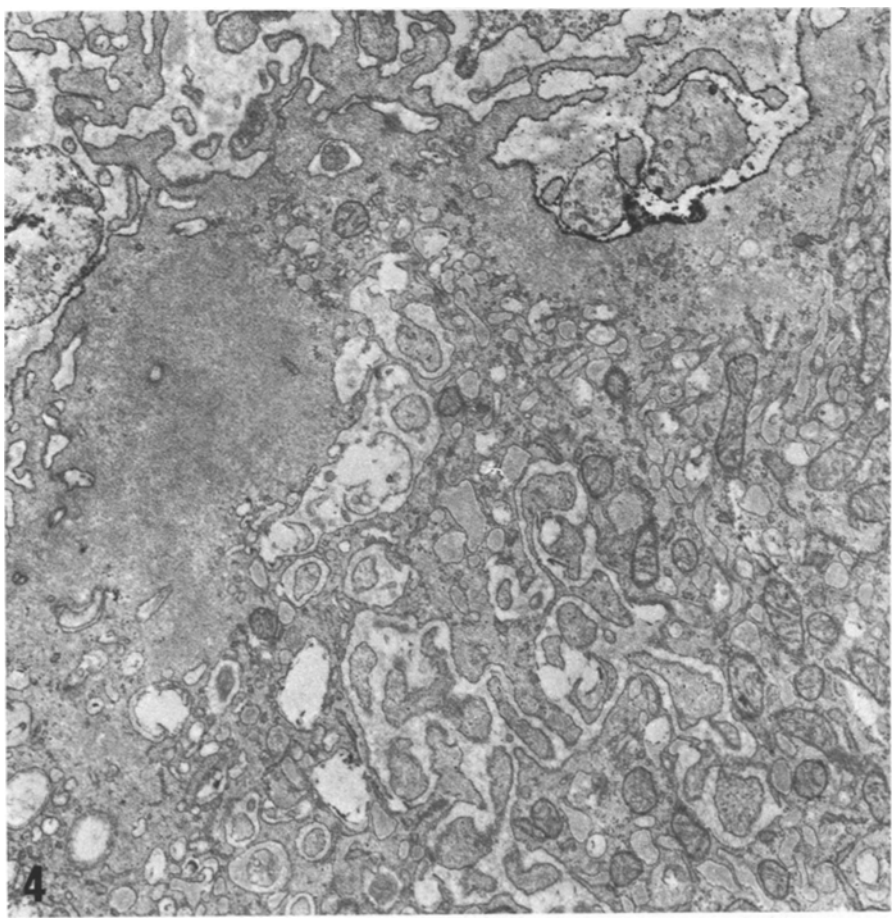


Fig. 4. Portion of multinucleated giant cell with prominent ectoplasmic layer subjacent to the plasma membrane (thickest in the left hand portion of the picture). Focal deposits of precipitate are present on the plasma membrane. Incubation time, 90 min; ×14,000

Case 2. A 32 year old lawyer with a history of one recurrence of giant cell tumor of bone following operation on the left distal metaphysis of the radius. Three months later there was a further recurrence and the patient was then transferred to the orthopedic clinic at Karolinska hospital. The lesion was carefully curetted and autologous bone transplantation was performed. The material for the electron microscopic studies reported here was obtained on this last occasion and was treated as in previous case. The histopathologic diagnosis on all three occasions was giant cell tumor of bone, Jaffe type II. The patient is well and no signs of recurrence have been noted 5 months after the last operation.

Results

The fine structure of giant cell tumor of bone has been described previously (Aparisi et al., 1977a). This study has demonstrated that the tumors are composed of three morphologically distinct types of cells: giant cells; stromal cells type 1 (fibroblast-like); and stromal cells type 2 (macrophage-like). The appearance of the cells constituting the two tumors studied in the present report did not deviate significantly from that of the cells described and studied in other giant cell tumors (Aparisi et al., 1977a). There was nothing to suggest that the tumors were of the malignant variety (Aparisi et al., 1978c).

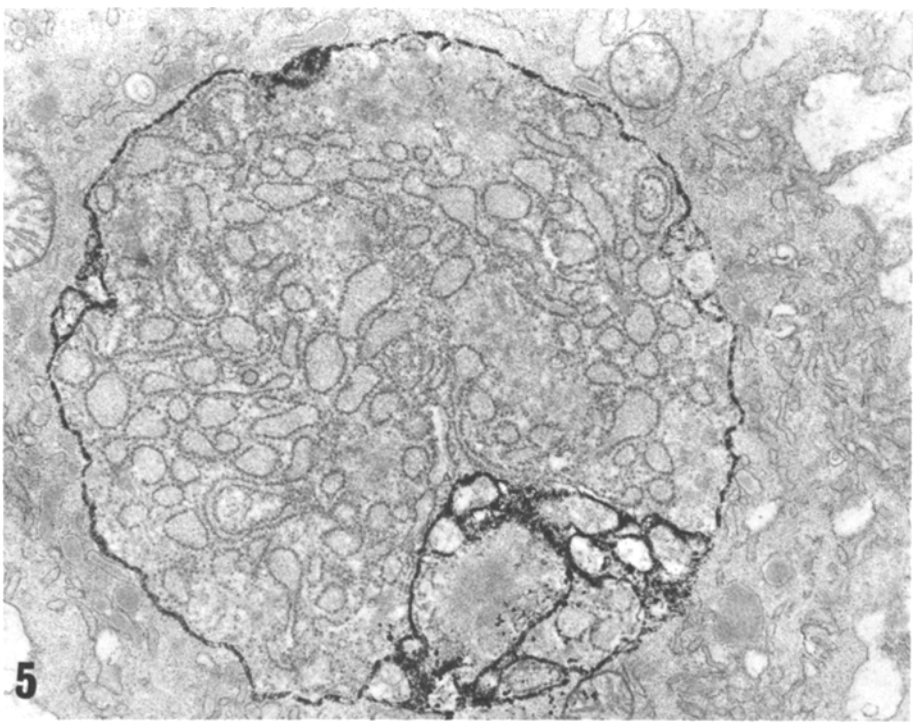


Fig. 5. Central area of giant cell with huge phagocytic vacuole containing well preserved cell (or portion of cell) rich in endoplasmic reticulum (most likely a fibroblast-like cell). Note prominent deposition of final product on and between bordering membranes. Incubation time, 30 min; ×21,000

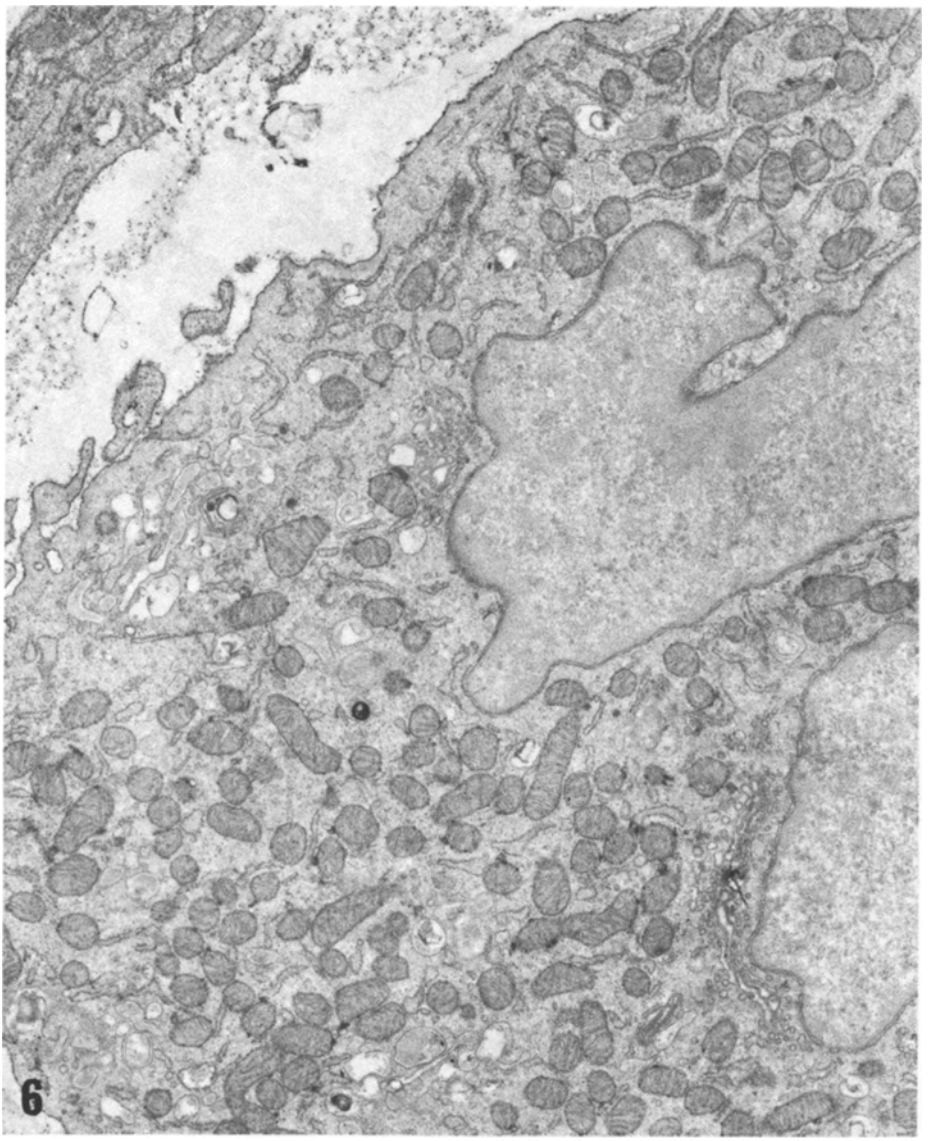


Fig. 6. Peripheral and intermediate area of giant cell with portion of two nuclei. Note faint sprinkling of final product of the plasma membrane. Incubation time, 30 min; ×15,000

Localization of Alkaline Phosphatase

The electron dense precipitates marking the localization of the enzyme activity were deposited over essentially the same types of elements in the two tumors; these are therefore described together.

(a) Stromal Cells Type 1. The majority of these cells showed faintly recognizable deposits, in sites described below, or lacked deposits of reaction product completely. In the remainder of the cells, conspicuous lead phosphate precipitates

were noted over portions of the plasma membrane in a focal, spotty fashion, or around the entire circumference of the cells (Figs. 1–3). The density of the precipitates varied considerably in different regions of one and the same plasma membrane. In many instances the precipitates tended to be thicker in areas where the cells were lying close together, and thinner on the "free" surfaces. Reaction product was also present over the membranes bordering vesicles and vacuoles subjacent to the plasma membrane (Figs. 1 and 2). It was also found to be associated with the membranes of infolded channels that emanated from the cell surface (Fig. 2) and occasionally with tail-like expansions of subplasmalemmal vacuoles (Fig. 1). Other structures in the cells lacked signs of enzyme activity.

(b) Stromal Cells Type 2. No deposits of final product could be demonstrated.

(c) Multinucleated Giant Cells. Many of these cells showed the presence of faint deposits of reaction product over their plasma membranes (Fig. 6) while in some cells there was a complete lack of precipitate. In occasional cells marked focal deposits were noted, often in areas with a cytoplasmic material resembling the "ectoplasmic layer" (Göthlin and Ericson, 1976) (Fig. 4). A giant cell with a large phagocytic vacuole containing a portion of a cell rich in endoplasmic reticulum was found to have strong deposits of final product associated with the bordering membrane of the vacuole (Fig. 5). Other components of the multinucleated giant cells than those mentioned above were devoid of precipitates.

(d) Other Cell Types. Precipitates of reaction product were observed on the membranes bordering erythrocytes and on the plasma membranes of endothelial cells in the small tumor vessels.

(e) Controls. No precipitates were found in tissues incubated in a medium lacking the substrate or in a medium containing EDTA as enzyme inhibitor.

Discussion

Earlier light microscopic studies on the localization of non-specific alkaline phosphatase in multinucleated giant cell tumors of bone have revealed faint enzyme activity in either the giant (Kraievski et al., 1970; Thiery et al., 1975) or the stromal cells (Pepler, 1958; Thiery et al., 1975) or have failed to demonstrate the existence of alkaline phosphatase activity in these cells (Schajowicz, 1961; Mnaymneh and Ghandur-Mnaymneh, 1967; Gilmer, 1954; Jeffree and Price, 1965). Marked activity was noted in vessels, however (Schajowicz, 1961). Ores et al. (1969) were unable to demonstrate occurrence of reaction product indicating alkaline phosphatase activity in either stromal or giant cells in an electron microscopic investigation of one case of giant cell tumor of bone. Mnaymneh and Ghandur-Mnaymneh (1967) concluded that the results of histochemical studies of the localization of alkaline phosphatase were "equivocal and contradictory".

The present study has demonstrated the existence, in or on the plasma membranes of giant cells and stromal cells type 1, of an enzyme causing the

hydrolysis of β-glycerophosphate at an alkaline pH. The evidence indicates that this enzyme is the same as was studied in previous reports and represents the non-specific alkaline phosphatase (E.C. 3.1.3.1.). The comparatively faint deposits of reaction product may explain why the activity was not revealed in some of the earlier light microscopic studies. Considerable inactivation by the fixative has probably contributed to the difficulties in earlier attempts of identification of the enzyme, since biochemical quantitation of alkaline phosphatase in giant cell tumors of bone have shown considerable inactivation following fixation in purified glutaraldehyde (Aparisi et al., 1976). It should be remembered, however, that in the biochemical assays the enzyme measured is associated not only with the cells constituting the tumor tissue but also with vessels. The extent of the inactivation of enzyme activity in giant and stromal cells thus remains to be determined.

Occurrence of reaction product on the membranes of subplasmalemmal vesicles and vacuoles of presumed endocytotic origin indicates that the enzyme activity is retained, following the budding-off procedure. In this connection it is interesting to note that Borgers et al. (1978) demonstrated the presence of alkaline phosphatase on the plasma membrane and the membrane of endocytic vacuoles of polymorphonuclear leucocytes in man. In what appears to be a phagocytic vacuole illustrated in Figure 6 it is not clear whether the alkaline phosphatase is located on the membrane of the vacuole or the bordering membrane of the phagocytized portion of cytoplasm.

Endocytic vacuoles and vesicles probably sooner or later fuse with lysosomes. Absence of reaction product on the membrane bordering the lysosomes in the giant and type 1 stromal cells may be taken to suggest that the alkaline phosphatase activity is lost or inhibited in the course of the fusion process.

Earlier electron microscopic studies have revealed comparatively strong alkaline phosphatase activity in bone forming cells (preosteoblasts and osteoblasts) (Göthlin and Ericsson, 1973). By light microscopy, marked activity was noted in osteoblastic osteosarcomas (Jeffree and Price, 1965). Occurrence of what appears to be enzyme activity in the type 1 stromal cells – which are fibroblast-like – suggests that these cells have a kinship with bone forming cells. Absence of reaction product on the plasma membranes of some of these cells may either signify inability to synthesize the enzyme, latency, or inactivation by fixation. Since no evidence of enzyme activity was obtained for the type 2 stromal cells, the results indicate that these cells differ histogenetically from the type 1 cells.

Alkaline phosphatase activity has never been demonstrated in osteoclasts or on the plasma membrane of such cells. Furthermore, differences exist in the localization of acid phosphatase between osteoclasts and giant cells in bone tumors (Aparisi et al., 1977b). It is therefore possible that the two cell types mentioned above are unrelated to one another, the giant cells in the presently studied tumors being histogenetically linked to the equally alkaline phosphatase-synthesizing stromal cells type 1 and the osteoclasts to macrophage-like cells (Göthlin and Ericson, 1976). The latter have never been shown to carry alkaline phosphatase on their plasma membranes, and resemble to some extent stromal cells type 2 in giant cell tumors of bone.

The possible relationships between the three types of cells in giant cell tumors

of bone have been much debated. Although not conclusive, the present results support the view that stromal cells type 1 and the giant cells are histogenetically related. It has been claimed that the giant cells are created by way of fusion of mononuclear cells. If so, the findings in this study favor the notion that the mononuclear cells represent stromal cells type 1.

Acknowledgements. The skilful technical assistance of Miss Silwa Mengarelli and Mr. Magnus Norman is gratefully acknowledged. We thank Mrs. Brigitte Giertz for typing the manuscript.

This investigation has been aided by a grant from the Swedish Cancer Society (grant 77:64, project number 617-B77-06XA).

References

Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Giant cell tumor of bone. An ultrastructural study. Proceedings of the Annual Meeting of the Swedish Medical Association Hygiaea **85**, 386 (1976)

Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Giant cell tumor of bone. Detailed fine structural analysis of different cell compontns. Virchows Arch. A Path. Anat. and Histol. **376**, 273–298 (1977a)

Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Giant cell tumor of bone. Fine structural localization of acid phosphatase. Virchows Arch. A Path. Anat. and Histol. **376**, 299–308 (1977b)

Aparisi, T., Arborgh, B., Ericsson, J.L.E., Göthlin, G., Nilsonne, U.: Contribution to the knowledge of the fine structure of chondrosarcoma. With a note on the localization of alkaline phosphatase and ATPase. Acta Path. Microbiol. Scand. A, Pathology (in press) (1978a)

Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Studies on the fine structure of osteoblastoma. With notes on the localization of non specific acid and alkaline phosphatase. Cancer (in press) (1978b)

Aparisi, T., Arborgh, B., Ericsson, J.L.E.: Malignant giant cell tumor of bone. Fine structure and localization of acid phosphatase. (Submitted for publication) (1978c)

Borgers, M., Thoné, F., De Crée, J., De Cock, W.: Alkaline phosphatase activity in human polymorphonuclear leukocytes. Histochem. J. **10**, 31–43 (1978)

Gilmer, W.S.: Histochemical studies in bone disease. I. Distribution of acid and alkaline phosphatase in bone tumors as a diagnostid aid. J. Bone Joint Surg. **36 A**, 182–183 (1954)

Göthlin, G., Ericsson, J.L.E.: Fine structural localization of alkaline phosphatase in the fracture callus of the rat. Histochemie **36**, 225–236 (1973)

Göthlin, G., Arborgh, B., Ericsson, J.L.E., Helminen, H.: Histochemical and biochemical studies on the localization and activity of lysosomal enzymes in fracture callus. Histochemie **35**, 97–110 (1973)

Göthlin, G., Ericsson, J.L.E.: The osteoclast. Revue of ultrastructure, origin, and structure-function relationship. Clin. Orthop. **120**, 201–231 (1976)

Helminen, H., Ericsson, J.L.E.: On the mechanisms of lysosomal enzyme secretion. Electron microscopic and histochemical studies on epithelial cells of the rat's ventral prostate lobe. J. Ultrastruct. Res. **33**, 528–549 (1970)

Jeffree, G.M., Price, C.H.G.: Bone tumors and their enzymes. J. Bone Joint Surg. **47 B**, 120–136 (1965)

Kraievski, N.A., Raikhlin, N.T., Soloviov, J.L.: Histochemical characteristics of giant cell bone tumours. Fol. Histochem. Cytochem. **8**, 3–10 (1970)

Mnaymneh, W., Ghandur-Mnaymneh, L.: Giant cell tumor of bone. Progr. Clin. Cancer **3**, 245–280 (1967)

Ores, R., Rosen, P., Ortiz, J.: Localization of acid phosphatase activity in a giant cell tumor of bone. Arch. Pathol. **88**, 54–57 (1969)

Pepler, W.J.: The histochemistry of giant cell tumours (osteoclastoma and giant cell epulis). J. Path. Bact. **76**, 505–510 (1958)

Schajowicz, F.: Giant cell tumor of bone. An histochemical study. J. Bone Joint Surg. **43 A**, 1–29 (1961)

Thiery, J.-P., Mazabraud, A., Voisin, M.-C.: Etude cytologique de la tumeur à céllules géantes. Rev. Chir. Orthoped. **61**, 377–386 (1975)

Received February 13, 1978