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Giant Cell Tumor of Bone

Fine Structural Localization of Acid Phosphatase

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Summary. The fine structural localization of acid phosphatase in the different cells in a benign giant cell tumor of bone has been studied. Stromal cells type 1 and 2 (fibroblast-like and macrophage-like, respectively) showed the presence of lead phosphate precipitate following incubation in a Gomori-type lead salt medium only in conventional lysosomes. In the multinucleated giant cells, the final product was deposited over lysosome-like organelles, and also over Golgi cisternae, vesicles, and vacuoles. Furthermore, evidence for presence of acid phosphatase was obtained in smooth-surfaced tubular, sausage-, horse-shoe-, and ring-shaped structures and over digestive vacuoles of autophagic or heterophagic origin. Finally, in these cells, many of the tubular and vacuolar elements located subjacent to areas of the plasma membrane with microvillous specializations (abortive brush borders?) were shown to carry acid phosphatase.

Key words: Giant cell tumor – Ultrastructure – Acid phosphatase – Lysosomes – Bone tumors.

Introduction

In a previous report, we have analyzed in some detail the fine structure of different cell types in a giant cell tumor of bone (Aparisi et al., 1977). The findings indicated (a) that there are two ultrastructurally distinct types of stromal cells; and (b) that although the giant cells shared certain properties with osteoclasts, clear differences do exist between the two types of giant cells. On the basis of the fine structural appearance alone, the function of and relationships between the various cell types in the tumor tissue could not be completely elucidated. In particular, the genesis of the giant cells remained obscure.

Electron microscopic studies of certain histochemical reaction patterns in bone cells (callus) have indicated that these cells differ with respect to the

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distribution and localization of alkaline phosphatase acid phosphatase and adenosine triphosphatase. Such methods (Göthlin and Ericsson, 1973a, b, c) applied to giant cell tumors would appear to be potentially useful in resolving problems concerning the genesis and interrelations of the different cells contained in such tumors.

In the literature, there are many studies on the histochemistry of giant cell tumor of bone at the light microscopical level (Schajowicz, 1961; Jeffre et al., 1965; Oliva et al., 1974). However, only few and fragmentary reports have been published on the ultrastructural localization of typical marker enzymes (Ores, 1969). It appears that so far the techniques for enzyme demonstration in giant cell tumors have not been sufficiently precise to allow insight into the histochemical properties of the tumor cells. Furthermore, the possibility that enzyme inhibition due to tissue processing influences the results on the ultrahistochemical level has not often been considered.

In this report, we describe the localization of acid phosphatase at the ultrastructural level in the same case of giant cell tumor of bone that was previously characterized with regard to the fine structure of its different cell (Aparisi et al., 1977). The results are discussed with special emphasis on the functional and histogenetic characteristics of the different tumor cells and the structure of the lysosomal vacuome, taking into account the fixative-dependent enzyme inhibition.

Materials and Methods

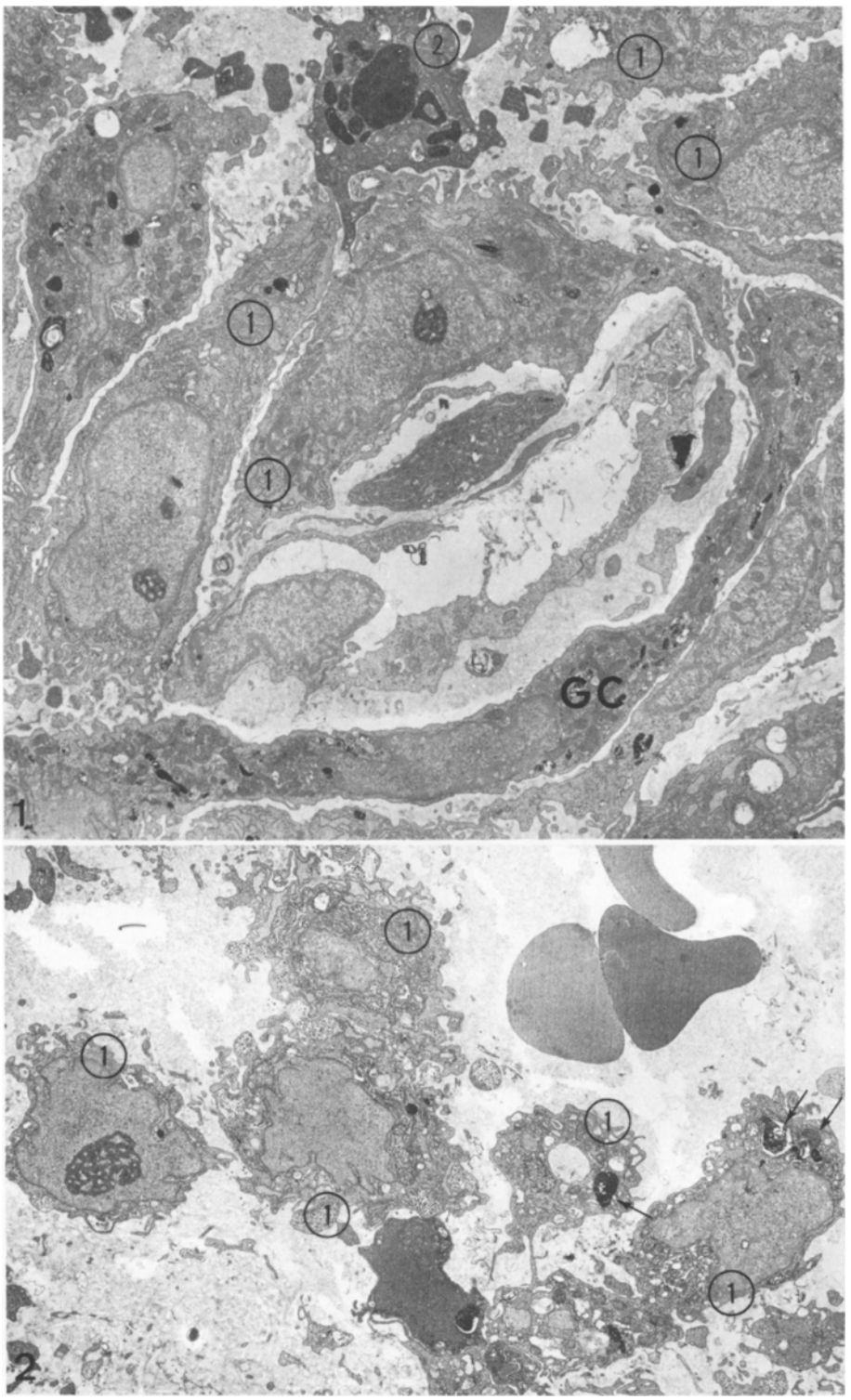
Histochemistry

Small pieces of tumor tissue were excised and immersed in glutaraldehyde solution for fixation (3% purified glutaraldehyde, 0.1 M cacodylate buffer, 0.1 M sucrose, pH 7.2) for 24 h; they were subsequently transferred to 0.1 M cacodylate buffer containing 0.1 M sucrose. 12 h before cutting, the material was immersed in a solution of 0.1 M cacodylate buffer and 0.1 M sucrose with 10% dimethylsulfoxide (Helminen and Ericsson, 1970; Göthlin and Ericsson, 1973a). Sections cut at 50 μ were then prepared on a freezing microtome (Leitz) and were incubated in a sodium-β-glycerophosphate medium for the demonstration of acid phosphatase using lead as capture ion. Control incubations were performed in a medium containing an enzyme inhibitor (sodium fluoride or sodium-L-tatrate), or in a medium lacking the substrate. Incubation times varied between 10 and 60 min.

Following incubation, the sections were rinsed several times in 0.1 M cacodylate buffer and were then postfixed in 2% OsO_4 buffered with *s*-collidine. Dehydration was performed in alcohol and the material was embedded in Epon. Thin sections, cut on an LKB-Ultrotome, were stained with lead citrate and were studied in a Jeol 100 C electron microscope.

Fig. 1. Acid phosphatase; incubation time 20 min. Survey electron micrograph showing the various cellular elements of a giant cell tumor. A capillary partly surrounded by a giant cell (*GC*) and a mononuclear stromal cell (*1*) is present in the mid-portion of the picture. The giant cell contains distinct reaction product located in numerous vesicular and tubular structures and in some lysosome-like bodies. In the type 1 stromal cells (*1*), organelles (lysosomes) with positive reaction are sparse. Deposition of reaction product is on the other hand prominent in the type 2 stromal cell (*2*). ×4600

Fig. 2. Acid phosphatase; incubation time 20 min. Low magnification electron micrograph of tumor tissue composed of abundant collagen and type 1 stromal cells. Precipitate is located over lysosome-like bodies (*arrows*). ×4600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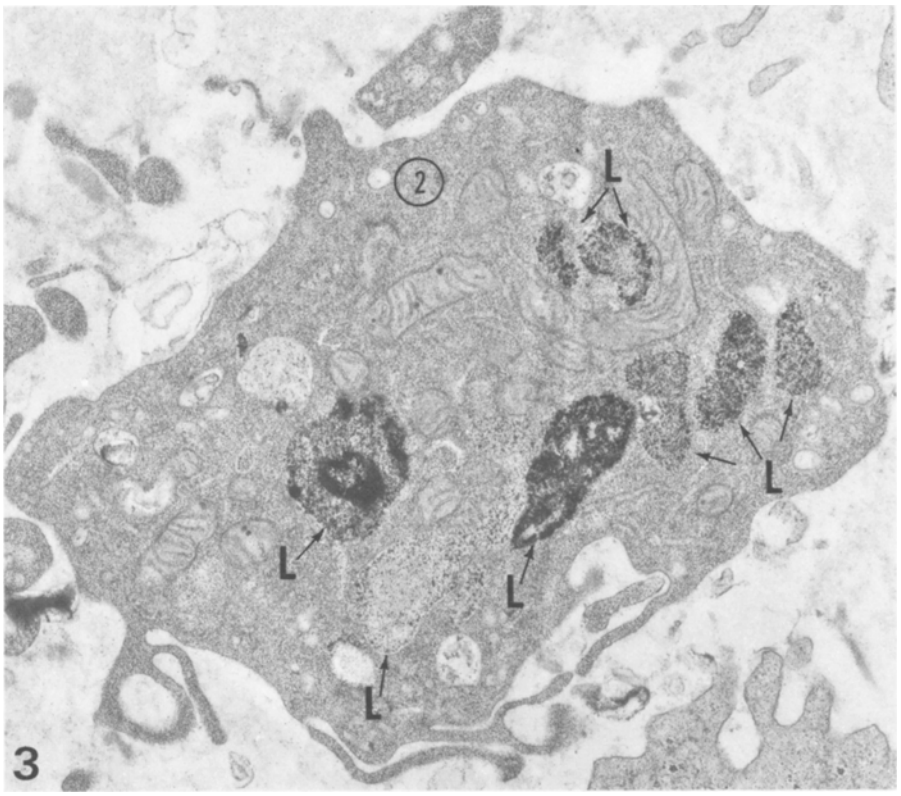

Fig. 3. Acid phosphatase; incubation time 20 min. Type 2 stromal cell (*2*) with numerous, irregularly shaped large lysosomes (*L*) covered by granular precipitate. ×19,800

Biochemistry

Fresh tumor tissue—from the same case that was used for the histochemical experiments—was homogenized in distilled water. The homogenate was then exposed to the fixative solution (final composition: 3% glutaraldehyde, 0.1 M cacodylate buffer, 0.1 M sucrose, pH 7.2) for 60 min and 24 h at 0° C and 37° C. The activity of acid phosphatase was then determined in fixed material and in unfixed control homogenate as described earlier (Berthet et al., 1952; Bowers et al., 1967).

Results

The fine structural appearance of the cells constituting the tumor tissue—giant cells and type 1 and 2 stromal cells—has been described previously (Aparisi et al., 1977).

Histochemistry

Type 1 Stromal Cells. Reaction product in these cells was confined to lysosome-like organelles of varying size and shape (Figs. 1 and 2).

Type 2 Stromal Cells. Final product was precipitated over the large and abundant bodies showing the typical appearance of lysosomes (Figs. 1 and 3).

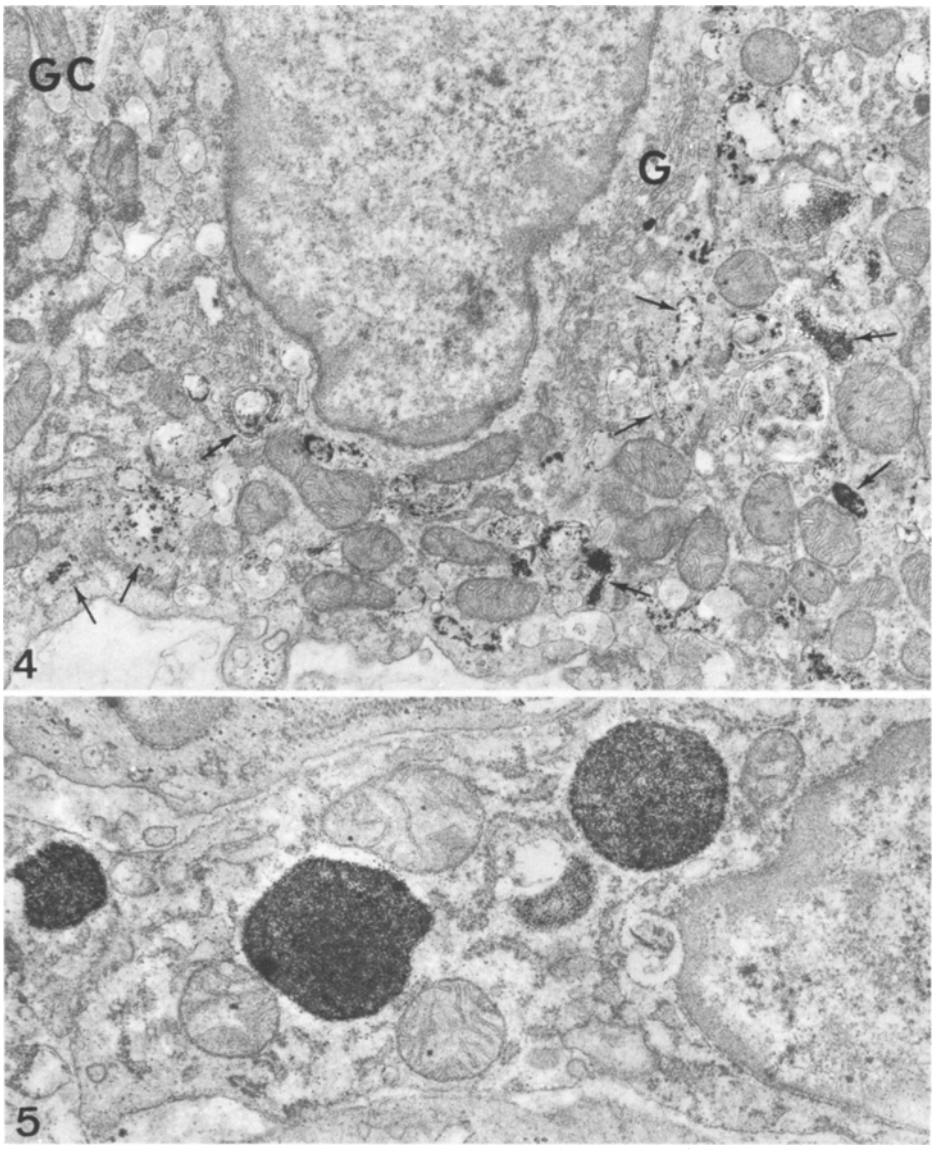

Fig. 4. Acid phosphatase; incubation time 20 min Portion of a multinucleated giant cell (*GC*) with presence of distinct reaction product over numerous tubular, vesicular and vacuolar structures in the cytoplasm (*arrows*). Small amounts of precipitate are also deposited over parts of the Golgi apparatus (*G*). ×17,500

Fig. 5. Acid phosphatase; incubation time 20 min. Detail of a giant cell containing several conspicuous lysosome-like bodies covered by reaction product. ×24,900

Giant Cells. Reaction product was precipitated over single membrane limited bodies of the conventional lysosome type distributed randomly throughout the cytoplasm of these cells (Figs. 4–6). Some of the lysosomes showed tubule-shaped extrusions which were also covered by reaction product (Fig. 6). In many cells, there was also a distinct lead phosphate precipitate over Golgi

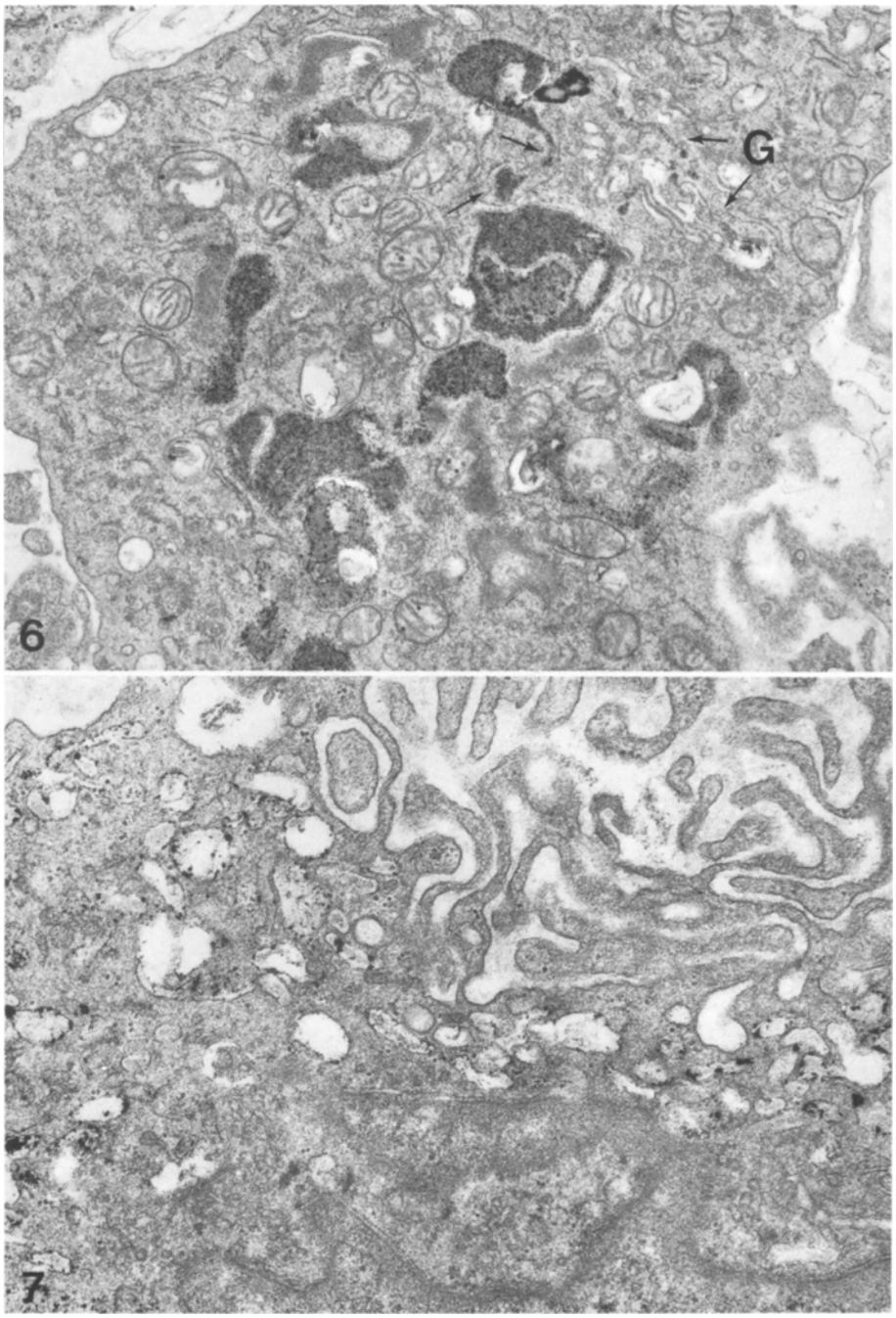

Fig. 6. Acid phosphatase, incubation time 20 min. Multinucleated giant cell with abundant irregularly shaped lysosome-like bodies showing deposition of finely granular reaction product. Observe that precipitate is also present in a Golgi area (*G*) and over a tubular structure connected with a lysosome (*arrows*). ×15,850

Fig. 7. Acid phosphatase; incubation time 20 min. Picture illustrating peripheral portion of a giant cell with numerous microvillous-like plasma membrane projections. Reaction product is present in tubular and vesicular structures adjacent to the cell surface. ×25,000

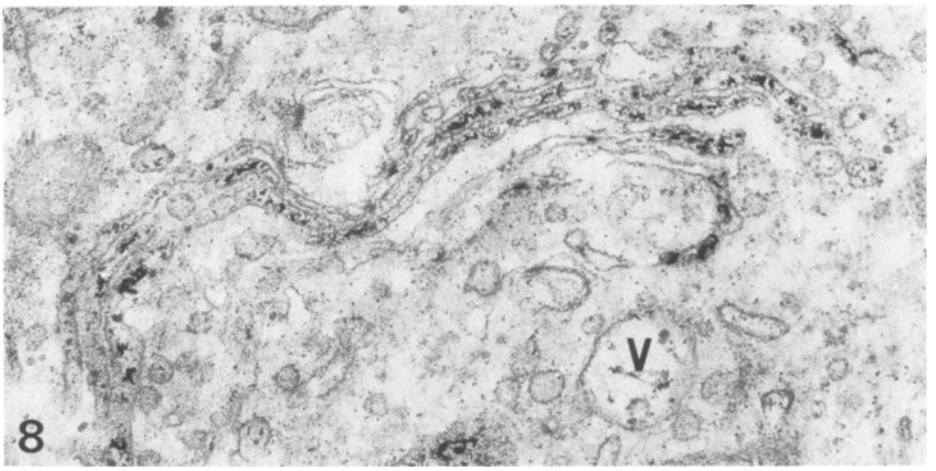

Fig. 8. Acid phosphatase; incubation time 40 min. Detail of a Golgi area in a giant cell. Reaction product is evident in the smooth surfaced Golgi cisternae and in a small vacuole (*V*). ×52,800

Table 1. Inhibitory effect of glutaraldehyde on the activity of acid phosphatase[a]

Fixation[b] Time and temperature	Inhibition of enzyme activity
10′ 37° C + 50′ 0° C	81%
60′ 0° C	65%
24 h 0° C	86%

[a] Substrate: Sodium-β-glycerophosphate, pH 5.0

[b] 3% glutaraldehyde in 0.1 M sodium-cacodylate buffer and 0.1 M sucrose, pH 7.2

cisternae and Golgi-associated vesicles and vacuoles (Figs. 6 and 8). It was not possible to decide from the pictures whether or not these vesicles and vacuoles were "coated". In addition, precipitate was localized over most of the tubular, sausage-, horseshoe- and ring-shaped elements, some of which showed a close spatial relationship to the Golgi regions (Figs. 1, 4 and 6). Reaction product was also present in digestive vacuoles. Finally, in regions of cells showing surface specializations with formation of microvilli-like structures and club-shaped extrusions, final product, indicating the presence of acid phosphatase, was found to be present over vacuoles and tubular structures localized superficially in the cytoplasm in relationship to the surface specialization (Fig. 7). Precipitate was not observed over identifiable rough surfaced endoplasmic reticulum or the plasmalemma.

Controls. No precipitate occurred in tissues incubated in a medium devoid of the substrate or containing sodium fluoride or sodium L-tatrate.

Biochemistry. Fixation in glutaraldehyde was parallelled by an inhibition in the activity of acid phosphatase. When fixation was performed at 0° C, the degree of inhibition was 65% after 60 min and 86% after 24 h (Table 1). If

the initial phase (10 min) in the procedure of fixation was performed at 37° C, the inhibition of activity was 81% after 24 h.

Discussion

When interpreting the results of enzyme histochemistry it is important to take into consideration the inhibitory effects of the tissue preparation procedure. As already pointed out earlier for tissues other than bone (Arborgh et al., 1971; Essner, 1973), fixation in glutaraldehyde is followed by a considerable decrease in the activities of many enzymes. Acid phosphatase is evidently sensitive to the effects of aldehyde. Consequently, absence of acid phosphatase reaction product in a tissue after histochemical incubations does not always mean a real lack of enzyme activity but merely reflects relative quantitative differences in activity. Another explanation for a varying histochemical result could be that iso-enzymes have different sensitivity to glutaraldehyde (Essner, 1973).

The findings presented in this report show that giant cell tumor of bone also contains acid phosphatase activity which is greatly inhibited by glutaraldehyde. The degree of inhibition increases with time. However, in the case studied we were unable to take advantage of short fixation time since 60 min of immersion fixation was not sufficient for good tissue preservation at the ultrastructural level. A rise in the temperature during the initial phase of fixation apparently increased the degree of inhibition of enzyme activity. This points to the importance of keeping the fixative properly chilled during the fixation.

In a previous study on the fine structural localization of acid phosphatase in giant cell tumor of bone, Ores et al. (1969) concluded that enzyme activity was only present in the giant cells (and thus appeared to be absent from the stromal cells). Such a statement must, however, be questioned on the basis of our biochemical findings and the preceding discussion. In the present report, distinct localization of reaction product was observed in the stromal cells indicating the presence of acid phosphatase in organelles with the characteristic morphology of lysosomes. Hence, the observations demonstrate that type 1 and type 2 stromal cells resemble other cell types in carrying acid phosphatase-containing lysosomes.

With regard to the giant cells, acid phosphatase appeared to be present in conventional lysosomes as well as the Golgi apparatus. However, conventional lysosomes are rather few and sparse in these cells, and the bulk of activity demonstrated was associated with elements different in fine structure from conventional lysosomes and Golgi cisternae and vesicles. These unique structures consisted of the tubular and sausage-shaped elements, vacuolar structures with tail-like expansions, ring- and horseshoe-shaped structures and some large vacuoles; furthermore, acid phosphatase appeared to be present in the subplasmalemmal vacuolar and tubular structures associated with specializations of the plasma membranes. With regard to the latter, it is interesting to note that in osteoclasts acid phosphatase is found in tubular structures subjacent to the brush border (Göthlin and Ericsson, 1973a). Although the tumor giant cells did not show a well developed brush border, the areas with microvillous-like protrusions may represent an attempt of the cells to mimic such a border

and the tubular structures may be the equivalent of similar organelles in the osteoclasts.

The nature of the vacuolar structures with tail-expansions, sausage-shaped tubular elements and ring- and horseshoe-shaped structures distributed randomly throughout the cytoplasm is uncertain. It could well be that the elongated elements are parts of the lysosomal vacuole in spite of their appearance. An irregularly shaped, partly flattened lysosome system with a three-dimensional arrangement would probably present as "unconventional" structures in a two-dimensional picture. However, the contents of most of these elements are somewhat unlike those which are usually present in lysosomes. Another possibility is that all or part of the tubular system may represent areas of GERL, as especially the sausage-shaped tubular elements appeared to be closely related to the Golgi regions. Ring-shaped elements might possibly correspond to newly formed autophagic vacuoles where the "isolation membranes" represented flattened – perhaps primary – lysosomes which had wrapped around and sequestrated a portion of cytoplasm. Similar images have been obtained in studies of autophagy in human glia cells (Hamberg et al., 1977). Any direct contact between the tubular structures and the rough endoplasmic reticulum has not been demonstrated in the sections studied.

Presence of the reaction product over vacuoles in different locations is compatible with the view that the giant cells perform endocytosis. Digestive vacuoles carrying acid phosphatase could either be autophagic or heterophagic in origin.

The derivation of the giant cells, and their relationship – if any – to the stromal cells has been much discussed. The observations in the present study indicate that, with regard to the fine structural localization of acid phosphatase, there are remarkable differences between the giant cells on the one hand and the stromal cells on the other. Thus, in the giant cells, the bulk of the enzyme activity appears to be bound to organelles which are not present – or at least not to such an extent – in any of the two types of stromal cells described. The findings do not exclude any of these cells as possible precursors of the giant cells, however. If any of them are, the presumed fusion of the cells and formation of the multinucleated giant cells must be associated with a profound change in the lysosomal vacuome. It appears likely that the unorthodox lysosomes so abundantly present in multinucleated giant cells have not been engaged in digestive events and thus may correspond to primary lysosomes. The findings would thus suggest that multinucleated tumor giant cells are very active in synthesizing lysosomal enzyme. In osteoclasts, such enzyme is – at least in part – released to the extracellular medium. It remains to be seen whether or not the lysosomal enzyme in tumor giant cells is also secreted to the extracellular medium.

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