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Abstract

A total of 19 cases with bone tumors, including six osteosarcomas. three giant cell tumors of bone, one malignant fibrous histiocytoma, four nonossifying fibromas, four chondromas and one chondrosarcoma, were examined as to enzyme histochemistry; the enzymes consisted of alkaline phosphatase (ALPase), acid phosphatase (ACPase), nonspecific esterase (NSE), adenosine triphosphatase (ATPase), 5'-nucleotidase (5'-Nucl) and beta-glucuronidase (beta-Gl). Osteosarcoma was strongly positive for ALPase followed by 5'-Nucl. Giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma showed enzyme histochemistry similar to each other: multinucleated giant cells and round cells in these tumors were strongly positive for ACPase, NSE, ATPase and 5'-Nucl simulating osteoclasts and histiocytes, whereas spindle cells were positive for ATPase and 5'-Nucl in their cytoplasm and weakly positive for ACPase. Chondroma and chondrosarcoma were focally positive for ACPase and NSE; the ACPase was sensitive to tartaric acid treatment. These observations showed that ALPase activity is very characteristic to osteosarcoma, and is useful for its diagnosis. From enzyme histochemistry, giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma can be regarded as a histiocyte-derived tumor of bone in contrast to osteosarcoma and cartilaginous tumors.

KEYWORDS: bone tumors, enzyme histochemistry, ultrastructure, tissue culture, histiocytes

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ENZYME HISTOCHEMICAL STUDY ON BONE TUMORS

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Abstract. A total of 19 cases with bone tumors, including six osteosarcomas. three giant cell tumors of bone, one malignant fibrous histiocytoma, four nonossifying fibromas, four chondromas and one chondrosarcoma, were examined as to enzyme histochemistry; the enzymes consisted of alkaline phosphatase (ALPase), acid phosphatase (ACPase), nonspecific esterase (NSE), adenosine triphosphatase (ATPase), 5'-nucleotidase (5'-Nucl) and β-glucuronidase (β-Gl). Osteosarcoma was strongly positive for ALPase followed by 5'-Nucl. Giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma showed enzyme histochemistry similar to each other: multinucleated giant cells and round cells in these tumors were strongly positive for ACPase, NSE, ATPase and 5'-Nucl simulating osteoclasts and histiocytes, whereas spindle cells were positive for ATPase and 5'-Nucl in their cytoplasm and weakly positive for ACPase. Chondroma and chondrosarcoma were focally positive for ACPase and NSE; the ACPase was sensitive to tartaric acid treatment. These observations showed that ALPase activity is very characteristic to osteosarcoma, and is useful for its diagnosis. From enzyme histochemistry, giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma can be regarded as a histiocyte-derived tumor of bone in contrast to osteosarcoma and cartilaginous tumors.

Key words : bone tumors, enzyme histochemistry, ultrastructure, tissue culture, histiocytes.

The diagnosis of tumor is, in general, based on the presence of a characteristic pattern simulating tissues or organs from which the tumor originated. Therefore, the diagnosis will be established with ease, whenever one can identify the well-differentiated tissues in a given tumor. On the other hand, tumors originating from the bone *per se* or from bone marrow usually present with variegated structures and often lack an identifiable, characteristic pattern; this causes a certain diagnostic difficulty in our daily clinical practice, in particular, in the field of surgical pathology.

Technical aspects of enzyme histochemical study have developed to give results much quicker than before enabling the technique to be used for precise diagnosis as a subsidiary method. A systematic study, however, on the enzyme histochemistry in the field of bone tumors has been reported in only few occa-

sions (1-4). Alkaline phosphatase has been investigated not only in osteosarcoma but in other bone tumors; for instance, Aparisi *et al.* (5, 6) identified alkaline phosphatase activity electron microscopically in giant cell tumor and chondrosarcoma and emphasized an enzymological similarity of osteoblasts with multinucleated giant cells in these tumors. On the other hand, the multinucleated giant cells seen in giant cell tumor and nonossifying fibroma and osteoclasts may share a common cytogenetic origin because of positive acid phosphatase and nonspecific esterase in both these types of giant cells (7-9), although a certain ultrastructural difference is present (1, 10).

We have made enzyme histochemical study on a total of 19 cases with bone tumors; among these, tumor tissue derived from a giant cell tumor was investigated immunologically through tissue culture. We report here the significance of enzyme histochemistry for diagnosis and discuss pathogenesis of bone tumors.

MATERIALS AND METHODS

Bone Tumors. The present study includes six cases of osteosarcoma, three giant cell tumor of bone, one malignant fibrous histiocytoma, four nonossifying fibroma, four chondroma and one chondrosarcoma (Tables 1 and 2).

Enzyme Histochemistry. Tissue obtained by biopsy was fixed with 10% formalin for routine hematoxylin-eosin staining. At the same time, for enzyme histochemistry, the tissue was fixed with 4% paraformaldehyde for 5 h at 4℃, replaced with Gum's sucrose solution, and kept at 4℃. Whenever time was available for study, but within one week after biopsy, the tissue thus prepared was quickly frozen using dry-ice with added hexane, sectioned to 4-5 μ thickness with a cryostat (Bright) at −20℃, and stained for the following enzymes: alkaline phosphatase, ALPase (according to the method described by Bengi *et al.*), acid phosphatase, ACPase (Barka and Anderson), nonspecific esterase, NSE (Leder), adenosine triphosphatase, ATPase (Müller-Hermelink), 5′-nucleotidase, 5′-Nucl (Müller-Hermelink), and β-glucuronidase, β-Gl (Hayashi).

Electron Microscopic Enzyme Histochemistry. Tissue was fixed for one h at 4℃ with 3% glutal aldehyde buffered with cacodyl oxide (pH 7.4), washed with the buffered solution for 30 min, sectioned to 40 μ thickeness, and stained using lead citrate for ALPase, and lead nitrate for ACPase, ATPase and 5′-Nucl. After fixing the tissue with osmium tetroxide for

TABLE 1. SUBCLASSIFICATION OF SIX CASES WITH OSTEOSAROMA

Age	Sex	Affected sites	Osteoid & bone	Cartilage	Fibroblastic component	Subclassified types
11	F	R femur	+++	+	−	Osteoblastic
13	M	R. humerus	++	−	+	Osteoblastic
13	M	R. femur	+	+++	−	Chondroblastic
18	F	L. tibia	+	++	+	Chondroblastic
19	M	R. tibia	+	−	+++	Fibroblastic
51	M	L. iliac bone	+	+++	−	Chondroblastic

TABLE 2. DETAILS OF OTHER BONE TUMORS THAN OSTEOSARCOMA

Age	Sex	Affected sites	Histologic diagnosis	Note
29	M	R. humerus	Giant cell tumor	Grade 1
35	M	R. radius	Giant cell tumor	Grade 3 with metastasis
65	F	R. tibia	Giant cell tumor	Grade 1
55	F	R. femur	Malignant fibrous histiocytoma	Recurred three times
15	F	R. femur	Nonossifying fibroma	
17	M	R. fibula	Nonossifying fibroma	
17	M	L. tibia	Nonossifying fibroma	
15	F	R. femur	Nonossifying fibroma	
3	F	L. 4th finger	Enchondroma	
23	F	L. femur	Chondromatosis (both femurs)	With osseous metaplasia
55	F	L. 4th finger	Enchondroma	
54	M	R. 1st toe	Enchondroma	
70	F	R. 5th finger	Chondrosarcoma	Secondary to chondroma

one h, the materials thus prepared were sliced for electron microscopy.

Immunology. Tumor tissue was finely minced, trypsinized and cultured with solution containing minimum essential medium and 10% fetal calf serum under 5% CO2. Cells thus cultured were tested for glass-adhering capacity, and for the presence of Fc and Cs receptors using sheep red-cell rosette formation.

Control Study. The following tissues were stained for enzyme histochemistry under identical conditions to those from bone tumors.

(a) After the fifth metatarsus of male rabbits weighing 5 kg was fractured manually (11), chondrocytes in the fractured site were observed for two weeks as to healing process.

(b) Two weeks after rabbits were injected subcutaneously and into the bone marrow with a solution containing powdered egg-shells, foreign-body granulomas thus produced were examined.

RESULTS

Osteosarcoma

As to subclassification of osteosarcoma, there were two cases of osteoblastic type, three chondroblastic and one fibroblastic (Table 1).

Histology. All these six cases formed more or less osteoid and bone, around which osteoblastic round cells with hyperchromatic, atypical nuclei proliferated together with pleomorphic giant cells. Chondroid matrix was present in four cases; three of these showed conspicuous, malignant cartilaginous component forming irregular-shaped islets. Fibroblast-like spindle cells were present in three cases; these cells were arranged in a coarse, fascicular pattern between myxoid matrix. One case of a boy aged 19 years showed sclerotic cortical bone and densely proliferating spindle cells, which simulated a fibrosarcoma; osteosarco-

ma was confirmed, however, by the presence of malignant osteoid.

Enzyme histochemistry. ALPase was the most characteristic enzyme for osteosarcoma, being positive in all the six cases (Table 3). Osteoblastic round cells accompanied by osteoid and bone formation were strongly positive, especially in their cytoplasmic membrane and in matrix adjacent to the cells (Fig. 1). Tumor giant cells were also strongly positive, fasciculated fibroblast-like spindle cells were moderately positive (Fig. 2), and almost all intralacunar chondroblastic cells forming cartilaginous islets were positive (Fig. 3).

ACPase was weakly positive in osteoblastic round cells, partly positive in intralacunar chondroblastic cells, and negative in fibroblast-like spindle cells. The ACPase reaction was suppressed by 25 mM tartaric acid. ACPase was

TABLE 3. ENZYME HISTOCHEMICAL FINDINGS OF NINETEEN CASES WITH BONE TUMORS

Tumor components	Enzymes[a]					
	ALPase	ACPase	NSE	ATPase	5′-Nucl	β-Gl
Osteosarcoma						
Fibroblast-like spindle cells	++	−	+	−	+	−
Osteoblastic round cells	+++	+*	−	+	+++	+
Chondroblastic cells	++	+*~−	+	−	+	+
Osteoblasts	+++	+*	−	+	++	+
Osteocytes	++	+*		−	+	+
Osteoclasts	−	+++	+++	+++	+++	−
Giant cell tumor						
Round cells	−~(+)**	++	+++	++	++	+
Giant cells	−~(+)**	+++	+++	+++	+++	−
Spindle cells**	++	+	+	++	+	+
Malignant fibrous histiocytoma						
Fibroblast-like spindle cells	−	+	+	+	+	+
Histiocyte-like round cells	−	++	+++	++	+	+
Giant cells	−	+++	+++	+++	+++	−
Nonossifying fibroma						
Spindle cells	−	+	−	+	+	+
Foam cells	−	+	−	+	−	−
Giant cells	−	+++	+++	+++	++	−
Chondroma	−~(+)***	+*	+	−	−~(+)***	+
Chondrosarcoma	−	+*	+	−	−	+

* Sensitive to 25 mM tartaric-acid treatment. ** Positive in a malignant giant cell tumor. *** Positive in the osseous metaplastic lesion.

a ALPase: alkaline phosphatase; ACPase: acid phosphatase; NSE: nonspecific esterase; ATPase: adenosine triphosphatase; 5′-Nucl: 5′-nucleotidase; β-Gl: β-glucuronidase.

also positive in apparent histiocytes scattered in the interstitium. NSE was weakly positive in intralacunar chondroblastic cells, and weakly positive or negative in osteoblastic round and fibroblast-like spindle cells. ATPase was almost negative, except for cytoplasmic membrane of osteoblastic round cells. 5'-Nucl was strongly positive in cytoplasmic membrane of osteoblastic round cells (Fig. 4) as well as in osteoid, whereas moderately to weakly positive in intralacunar chondroblastic and fibroblast-like spindle cells. β-Gl was weakly positive only in the perinuclear area of the above tumor cells.

Electron microscopic enzyme histochemistry. An 11-year-old female with an osteoblastic type was used for this experiment. Tumor cells showed relatively ample cytoplasm with numerous large mitochondria, dilated rough endoplasmic reticula containing low-density substance, well-developed Golgi's apparatus, and microvesicles scattered along the peripheral part of cytoplasm. Intercellularly, there were some collagen fibers and amorphous substance.

As to enzyme histochemistry, ALPase was strongly positive in cytoplasmic membrane and in microvesicles found along the cytoplasmic membrane (Fig. 9). ATPase and 5'-Nucl (Fig. 10) were confined to a part of cytoplasmic membrane, whereas negative in the cytoplasm *per se.*

Reactivity of ALPase against inhibitors. The first case, an 11-year-old female, was also used in the present study (Table 4). ALPase was markedly inhibited

TABLE 4. ALKALINE PHOSPHATASE REACTIVITY AGAINST INHIBITORS*

Non-treated	Heating**	EDTA	L-triptophan	L-phenylalanine	L-leucine
+++	−~+	+	−	+++	++

* An 11-year-old female with osteosarcoma was used in this study. Concentration of all the four inhibitors was 5 mM. ** Either at 56℃ for 30 min or at 60℃ for 20 min.

by heating either at 56℃ for 30 min or at 60℃ for 20 min, and also by adding 5 mM EDTA and 5 mM L-tryptophan. On the other hand, 5 mM L-phenylalanine and 5 mM L-leucine almost failed to suppress the enzyme activity.

Giant Cell Tumor of Bone

Two cases were benign, *i.e.*, grade I. The third case, a 35-year-old male, had recurred three times within five months, metastasized to the dorsal subcutis of the right hand, and histologically simulated a fibrosarcoma (Table 2).

Histology. Two benign tumors were histologically typical, *i.e.*, showing a large number of multinucleated giant cells of osteoclastic type, mononuclear round cells, only a few mitoses, and vascular-rich interstitium. A malignant counterpart of this tumor presented with giant cells which became smaller in size and decreased in number as compared to the benign tumors; spindle cells which proliferate and infiltrate into the surrounding tissue by forming fascicles; and increased mitoses being 2-3 per one high power field.

Light and electron microscopic enzyme histochemistry. The results will appear elsewhere in more detail (12), and are described briefly (Table 3). Multinucleated giant cells and mononuclear round cells of all three cases were diffusely and strongly positive for ACPase, NSE, ATPase and 5′-Nucl in cytoplasm. On the other hand, spindle cells of a malignant case were weakly positive for ACPase, NSE, ATPase and 5′-Nucl in cytoplasm. ALPase was negative in the tumor cells of benign cases, but was strongly positive in spindle cells and weakly positive in round cells and multinucleated giant cells of a malignant case. The ALPase was heat-stable, and identified as the type 4 isozyme according to reactivity against several inhibitors and also to an electrophoretic pattern of the medium used for tissue culture study on tumor cells.

Cell surface marker. Cultured cells consisted of 72.1% spindle, 26.5% round, and 1.4% giant cells, the spindle cells being predominant. These cells adhered tightly on glass surface, and in spite of adding trypsin were separated from the surface only with considerable difficulty. Fc and C3 (Fig. 11) receptors were 18.2% and 42.3%, respectively; the receptors were mostly found in round cells, and in some spindle cells, whereas giant cells were too few to draw any conclusions from the present study.

Malignant Fibrous Histiocytoma

A tumor, originated from the distal portion of the right femur of a 55-year-old female, had recurred three times within six years.

Histology. The first resected specimen consisted of fibroblast-like spindle cells, being accompanied by collagen fibers and forming a clear-cut storiform pattern (Fig. 12). A small number of atypical multinucleated giant cells and histiocyte-like round cells, foam cells in clusters or scattered, and lymphocytes and plasma cells in diffuse infiltration or focal aggregation were also present. The second specimen looked similar to the first, and the third material showed more atypical giant cells and histiocyte-like round cells than in the previous two occasions. The fourth one became further predominated by atypical giant cells, although the storiform pattern was still recognized (Fig. 13).

Enzyme histochemistry. This was performed on the fourth specimen. ALPase was negative except for the vascular wall. ACPase, NSE, ATPase and 5′-Nucl were strongly positive in cytoplasm of atypical giant cells (Fig. 5, a and b). ACPase and NSE were strongly positive in cytoplasm of some histiocyte-like round cells, but weak in other types of cells; the latter mimicked fibroblast-like spindle cells. ATPase and 5′-Nucl were weakly positive in cytoplasm of histiocyte-like round cells. β-Gl was positive in the perinuclear area of histiocyte-like round cells.

Nonossifying Fibroma

This consisted of two specimens from a male and female aged 15 and 17 years old, respectively (Table 2).

Histology. Cortical bone and bone trabecullae were absorbed and replaced by proliferating spindle cells and collagen fibers. Multinucleated giant cells were scattered, a small number of foam cells formed clusters, and hemosiderin was deposited more or less in all four cases.

Enzyme histochemistry. ALPase was negative in tumor cells. ACPase, NSE, ATPase and, to a lesser extent, 5'-Nucl were strongly positive in cytoplasm of giant cells (Fig. 6, a and b). These enzymes were weakly positive to almost negative in spindle cells. ACPase and ATPase were weakly positive in cytoplasm of foam cells, and β-Gl was positive only in spindie cells.

Chondroma

This consisted of three cases of enchondroma, and one chondromatosis affecting both femurs of a 23-year-old female.

Histology. All four cases showed well-matured cartilaginous tissue consisting of chondrocytes which were embedded in matrix. The chondrocytes varied in morphology from large atypical cells arranging irregularly to normal-appearing cells. A chondromatosis focally had osseous metaplasia of cartilage.

Enzyme histochemistry. ACPase and NSE were positive in the chondrocytes of tumorous lesion; the ACPase was localized near the nucleus and was sensitive to 25 mM tartaric acid. ATPase was negative, ALPase and 5'-Nucl were also negative although positive in the osseous metaplastic area, and β-Gl was weakly positive in cytoplasm of chondrocytes.

Chondrosarcoma

The case was a 70-year-old female, whose right fifth metacarpus developed chondrosarcoma secondary to chondroma.

Histology. The tumor grew in multilobulations which were separated from each other by fibroblasts and collagen fibers. Malignant chondrocytes appeared large and pleomorphic, and were arranged irregularly.

Enzyme histochemistry. ACPase and NSE were positive near the nucleus of tumor cells; the ACPase was sensitive to 25 mM tartaric acid (Fig. 7, a and b). ALPase, ATPase and 5'-Nucl were all negative, and β-Gl weakly positive.

Two-week-old Fracture Healing of Rabbit's Metatarsus

The procedure of fracture has been described previously.

Histology. Cartilaginous islets were noticed beneath the periosteum and were surrounded by fibroblasts which grew in layers. At both ends of the islets, there was osseous metaplasia of the cartilage with osteoblasts and calcium deposition in matrix; in the metaplastic lesion, chondrocytes were enlarged and identical to hypertrophic chondrocytes.

Enzyme histochemistry. Hypertrophic chondrocytes were strongly positive for 5'-Nucl, and weakly positive for ATPase especially in cytoplasmic membrane. The cytoplasmic membrane of the hypertrophic chondrocytes accompanied by calcium deposition was also positive for ALPase. Small chondrocytes were posi-

tive for NSE; the small chondrocytes present outside osseous metaplastic lesion were negative for all the enzymes. ACPase was also negative in both types of the chondrocytes. Osteoblasts were strongly positive for ALPase, and especially in their cytoplasmic membrane, were positive for ATPase and 5'-Nucl. ACPase positivity was also confined to the cytoplasmic membrane and sensitive to 25 mM tartaric acid. Subperiosteal spindle cells, *i.e.*, the cells not situated in cartilaginous islets *per se*, were positive for ALPase and NSE, and negative for ACPase, ATPase and 5'-Nucl. β-Gl was all posisive including small chondrocytes, osteoblasts and spindle cells.

Rabbit Foreign-body Granuloma

Enzyme histochemistry concerning histiocytes and foreign-body type giant cells present in the granuloma will be described.

ACPase, NSE, ATPase and 5'-Nucl were diffusely and strongly positive in cytoplasm of both types of the cells (Fig. 8, a, b, c and d); the ATPase and 5'-Nucl were, in addition, found focally in cytoplasmic membrane. ALPase was negative in these cells.

DISCUSSION

To our knowledge, there has been little systematic enzyme histochemical study of bone tumors. We will discuss several enzymes investigated in the following orders.

Alkaline Phosphatase (ALPase). Jeffree and Price (2) compared ALPase activity of osteosarcoma, giant cell tumor of bone, chondroma, chondrosarcoma, fibrosarcoma, nonossifying fibroma and fibrous dysplasia, and demonstrated characteristic reactivity of ALPase in the osteosarcoma. Sanerkin (4) also emphasized the significance of ALPase in the diagnosis of osteosarcoma. On the other hand, ALPase was reported to be positive in giant cell tumor and chondrosarcoma (5, 6).

All six cases of osteosarcoma, regardless of their subclassification, were strongly positive for ALPase, especially in osteoblastic round cells forming osteoid and bone. On the other hand, giant cell tumor, malignant fibrous histiocytoma, nonossifying fibroma, chondroma and chondrosarcoma were all negative; a malignant giant cell tumor was positive for ALPase, which, however, belonged to the type 4 isozyme and proved to be a nonosseous type (12). Nonossifying fibroma was positive only around absorbed bone trabecullae; this may be due to enzyme diffusion from the trabecullae. Chondroma and chondrosarcoma were all negative except for in the osseous metaplastic lesion, and were different in ALPase activity from malignant cartilaginous component present in osteosarcoma. Jefree *et al.* (2) tried to account for the difference in reactivity between osteosarcoma and cartilaginous tumors on the basis of the presence of osteoblasts in the malignant cartilaginous component of osteosarcoma; this ex-

planation needs further clarification. By electron microscopic enzyme histochemistry, ALPase was reported to be positive in osteosarcoma and hypertrophic chondrocytes (1). The hypertrophic chondrocytes seen in healed fracture of our experiment were positive for ALPase only at places where calcium was deposited in the matrix.

Therefore, diffuse ALPase activity in a given tumor tissue highly suggests an osteosarcoma because of its identification with the osteosarcoma.

Acid Phosphatase (ACPase) and *Nonspecific Esterase (NSE).* These two enzymes are lysosome-derived, hydrolytic enzymes, and negative in osteosarcoma, chondroma and chondrosarcoma (2). Göthlin *et al.* (13) and Doty *et al.* (1) found positive ACPase in osteoblasts and chondroblasts of healed fracture. Cytoplasm of osteoclasts is diffusely and strongly positive for both ACPase and NSE (9). Giant cell tumors, in particular, multinucleated giant cells are also strongly positive for both enzymes, as seen in osteoclasts (7, 8, 12, 14). A similar finding to that in giant cell tumor was reported for multinucleated giant cells of nonossifying fibroma (2). In our series consisting of giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma, the multinucleated giant cells were, like osteoclasts, strongly positive for both enzymes, mononuclear cells were moderately to weakly positive, and ACPase was resistant to treatment by tartaric acid. This observation was different from osteosarcoma, chondroma and chondrosarcoma, in which ACPase was confined to cytoplasm and sensitive to tartaric acid. The multinucleated giant cells seen in the above three bone tumors react to both enzymes similar to histiocytes and histiocytic giant cells, and together with the findings obtained from ATPase and 5′-Nucl, can be grouped in the same category as the histiocytes and histiocytic giant cells.

The cytogenesis of osteoclasts has been said (i) to be derived from osteoblasts (15), (ii) to share a common precursor with osteoblasts (16), and (iii) to be derived from monocytes or histiocytes (17-19). Touw *et al.* (20) have reported the similarity of LDH localization of osteoclasts to Ca-apatite-treated macrophages. On the other hand, the osteoclasts, like macrophages, possess glass-adhering and phagocytic activities, whereas they lack Fc and C3 receptors; this is also the case in multinucleated giant cells in giant cell tumor (21-23). Multinucleated giant cells in the primary culture of a malignant giant cell tumor in the present study were too few to confirm the presence of both receptors, although mononuclear cells had 18.2 and 42.3% of Fc and C3, respectively. The reasons why we do not see both receptors in the multinucleated giant cells are: (i) disappearance of the receptors while being cultured, (ii) disappearance of the receptors secondary to cell fusion (21), or (iii) masking of the receptors (22); these hypotheses need further investigation.

Adenosine Triphosphatase (ATPase). Osteoblasts are rich in ATPase in cytoplasmic membrane which is dependent on Mg^{++}, whereas osteoclasts, monocytes and histiocytes contain ATPase in lysosomes which are independent of Mg^{++}

(24, 25). As far as ATPase in bone tumors is concerned, only that of giant cell tumor and chondrosarcoma has been reported (3, 6). In the present experiment, osteosarcoma and chondrosarcoma were only focally positive in their cytoplasmic membrane.

In contrast to that, cytoplasm of multinucleated giant cells in giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma was strongly positive for ATPase, which was situated electron microscopically in lysosomes and microvesicles. In addition, mononuclear cells of the above three bone tumors were positive for ATPase in both cytoplasm and cytoplasmic membrane, while spindle cells of malignant giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma were positive only in cytoplasmic membrane and simulated fibroblasts in its reactivity. Since histiocytic giant cells in foreign-body granuloma produced in rabbits are positive for ATPase in cytoplasm and histiocytes are transformed to fibroblast-like cells (26, 27), together with the findings obtained from ACPase and NSE, the above three bone tumors, namely, giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma, can be enzyme histochemically regarded as a histiocyte-derived tumor of bone.

5'-Nucleotidase (5'-Nucl). There have been only a few reports concerning 5'-Nucl; Kraievski *et al.* (3) and Troyer (25) described its activity in osteoclasts and chondrocytes. In our series of osteosarcoma, cytoplasmic membrane of the tumor cells in the osteoblastic type was most strongly positive for 5'-Nucl followed by chondroblastic and fibroblastic types. Giant cell tumor, malignant fibrous histiocytoma and nonossifying fibroma were positive, as seen in ATPase, in lysosomes and microvesicles, and were different in reactivity from that of osteosarcoma. Chondroma and chondrosarcoma were negative for 5'-Nucl. Therefore, the 5'-Nucl, together with ALPase, can be a reliable marker for the diagnosis of osteosarcoma because of its strong activity on cytoplasmic membrane.

β-Glucuronidase (β-Gl). Activity of β-Gl has been described only for osteosarcoma and giant cell tumor (2). According to our study, β-Gl was not very helpful for diagnosis of bone tumors, because it lacked difference in intensity of enzyme activity and also specificity of enzyme localization.

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FIGURE LEGENDS

Fig. 1. Alkaline phosphatase activity of an osteosarcoma (a 13-year-old female), showing strongly positive activity in osteoblastic tumor cells with osteoid formation, × 320.

Fig. 2. Alkaline phosphatase activity of an osteosarcoma (a 19-year-old male), showing activity in spindle cells with a fasciculated arrangement, × 320.

Fig. 3. Alkaline phosphatase activity of an osteosarcoma (a 13-year-old male), showing activity in tumor cells accompanied by chondroid matrix, × 320.

Fig. 4. 5'-Nucleotidase activity of an osteosarcoma (a 13-year-old female), showing activity in osteoblastic tumor cells accompanied by osteoid formation, × 320.

Fig. 5. A malignant fibrous histiocytoma (a 55-year-old female). (a) Acid phosphatase activity, showing activity diffusely in cytoplasm of multinucleated giant cells and focally in mononuclear cells, × 250. (b) Nonspecific esterase with almost the same activity as acid phosphatase, × 250.

Fig. 6. A case of nonossifying fibroma (a 15-year-old female). (a) Acid phosphatase, × 250. (b) Nonspecific esterase, × 250. Both enzymes are shown diffusely positive in multinucleated giant cells, and focally positive in spindle cells.

Fig. 7. A case of chondrosarcoma (a 70-year-old female). (a) Acid phosphatase, showing the activity focally and confined to cytoplasm, × 320. (b) Acid phosphatase sensitive to tartaric-acid treatment, × 320.

Fig. 8. Rabbit's subcutaneous histiocytes, × 320; (a) acid phosphatase, (c) nonspecific esterase, and (d) adenosine triphosphatase. (b) Bone marrow histiocytes for acid phosphatase, × 320.

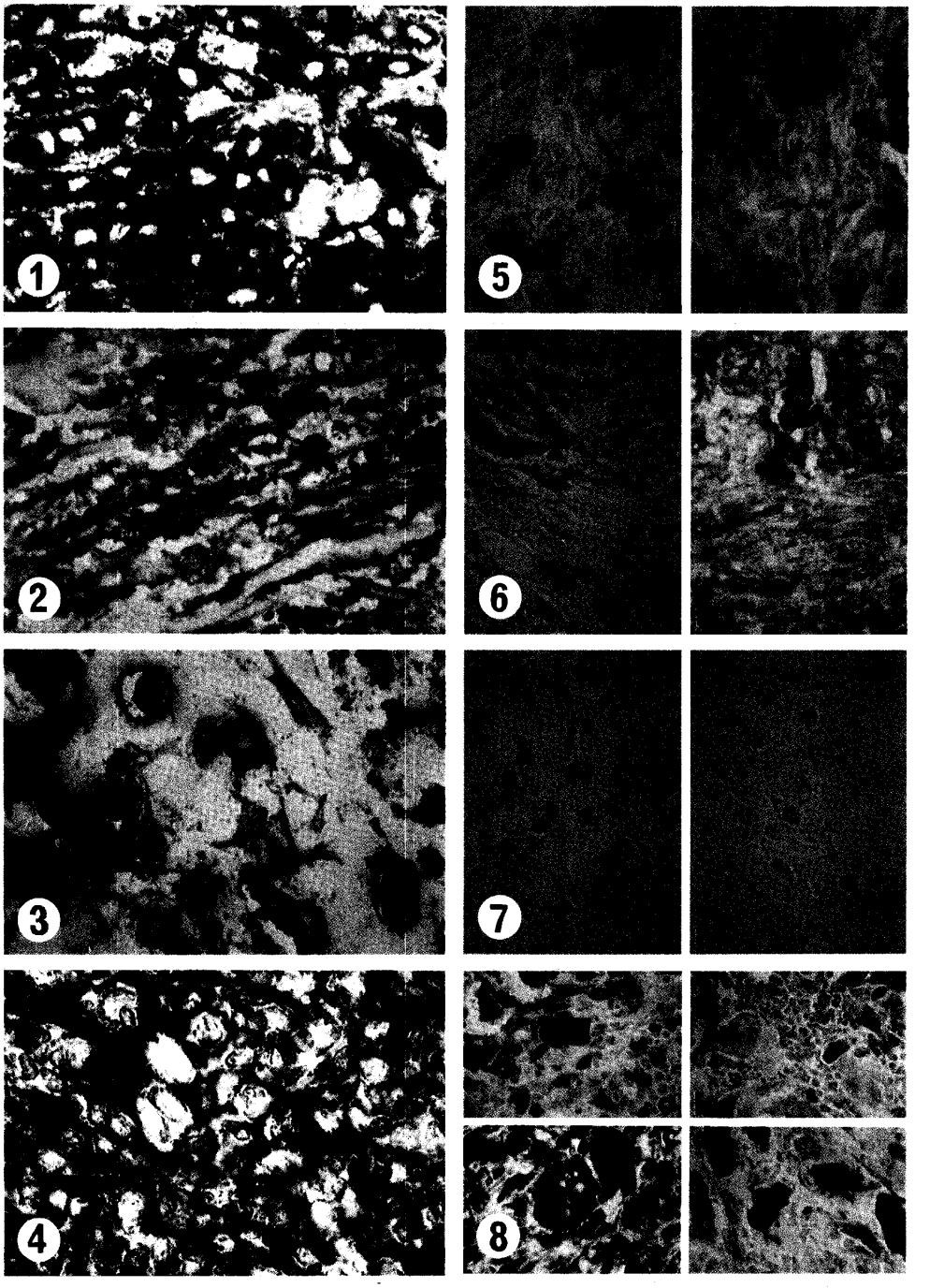
Fig. 9. Alkaline phosphatase activity of an osteosarcoma, osteoblastic type (an 11-year-old female), showing the reactivity found in cytoplasmic membrane and microvesicles along the cytoplasmic membrane of tumor cells (an arrow), and also the reactivity scattered in interstitium (an arrow), × 9,000.

Fig. 10. 5'-Nucleotidase activity of the same case as Fig. 9, showing the reactivity confined to a part of cytoplasmic membrane of tumor cells (arrows), × 12,000.

Fig. 11. C3 receptor demonstrated in cultured tumor cells derived from a malignant giant cell tumor of bone (a 35-year-old male).

Fig. 12. The first specimen from a malignant fibrous histiocytoma (a 55-year-old female), showing proliferation of spindle cells accompanied by collagen fibers, and a storiform pattern, hematoxylin-eosin, × 160.

Fig. 13. The fourth specimen from the same patient as Fig. 12, showing increased atypical multinucleated giant cells and an abortive storiform pattern as compared to Fig. 12, hematoxylin-eosin, × 160.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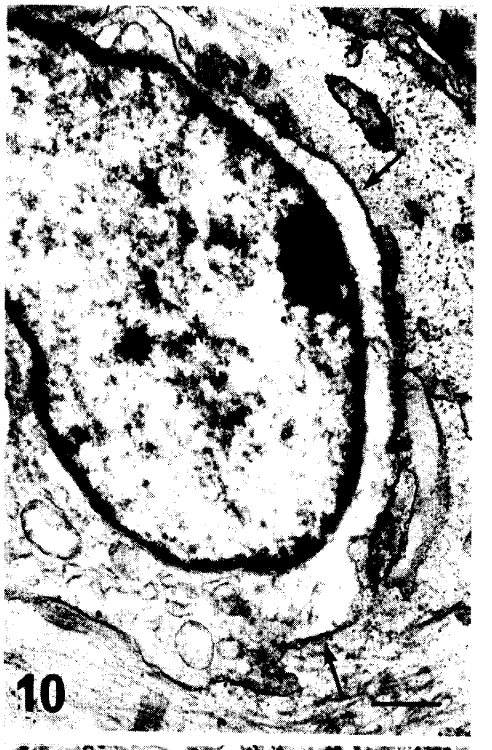
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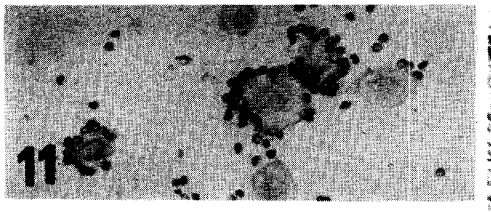
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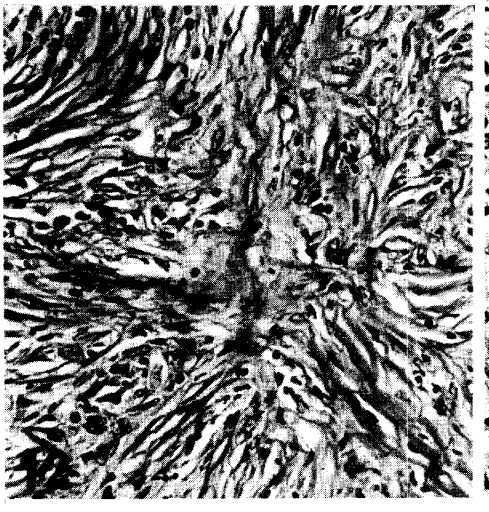

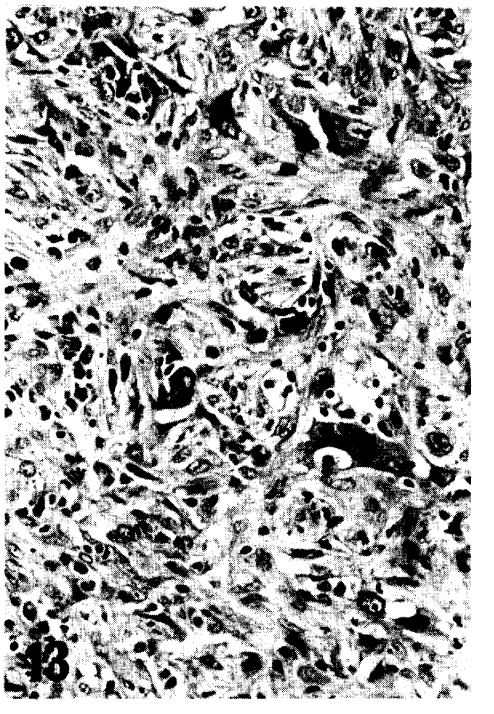
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