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# Electron Histochemical Demonstration on the Localization of Activities of Alkaline and Acid Phosphatases in the Cartilage of Mice

by

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There have been several light histochemical studies performed on the alkaline (LORCH, '47[5] ; GREEP et al., '48[4] ; TAKAMATSU et al., '57[13] ; Loe, '59[6] ; MORI et al., '62[7]) as well as acid phosphatase activity (SCHAJOWICZ et al., '58[11] ; BURSTONE, '59[1] ; MORI et al., '62[7]) in the hard tissues. Several electron microscopic studies on the hard tissues have also been reported by SCOTT & PEASE ('56)[12], ROBINSON & CAMERON ('56)[9], and GODMAN & PORTER ('60)[2]. However, no work has been published so far on the electron histochemical demonstration on the localization of activities of alkaline and acid phosphatases in the cartilage.

In the present investigation attempts were made to elucidate the role of alkaline as well as acid phosphatase activity by the electron histochemical method in the cartilage cell of the femoral head of mice.

The femoral head of new born mice, 7 to 14 days old, were used in this study. Animals were killed by the decapitation without anaesthesia.

TAKAMATSU method (TAKAMATSU et al., '57)[13] for demonstration of alkaline phosphatase activity in the hard tissues was applied in cold formol-calcium-fixed frozen sections (OGAWA et al., '62)[8]. For the demonstration of acid phosphatase activity GOMORI method (GOMORI, '52)[3] was used in sections fixed in glutaraldehyde with cacodylate buffer (SABATINI et al., '63)[10]. Substrate-free incubating media were taken for controls. After incubation sections were postfixed in PALADE's osmium tetroxide and processed according to the routine procedure for the electron microscopic observation.

In the present study alkaline phosphatase activity was demonstrated in territorial peripheries and cytoplasm (probably endoplasmic reticulum) of the proliferating and hypertrophic cartilage cells (Figs. 1 & 2). On the other hand acid phosphatase activity was present in the rough-surfaced endoplasmic reticulum of the cells of calcifying cartilage (Figs. 3 & 4), but was not seen in that of the proliferating and hypertrophic cartilage. Occasionally lysosome-like structure containing acid phosphatase was observed (Fig. 3). In the epiphyseal zone of cartilage no enzymatic activity was demonstrated. No lead deposits were noticed in specimens incubated in substrate-free media.

In the present investigation it was shown that the proliferating and hypertrophic cartilage cells revealed alkaline phosphatase activity, but not acid phosphatase activity. Whereas calcifying cells revealed acid phosphatase activity, but not alkaline phosphatase

activity. These findings may lead to the concept that alkaline phosphatase activity seems to have an intimate functional relationship with the metabolism of precalcification stage during the cartilage cell differentiation, and that acid phosphatase activity seems to play a direct role in the process of calcification per se.

This study was carried out under the helpful suggestion by Dr. KAZUO OGAWA, Department of Anatomy, Kansai Medical School, Moriguchi Osaka, Japan.

## REFERENCES

1) Burstone, M. S : Acid phosphatase activity of calcifying bone and dentin matrices. J. Histochem. Cytochem., **7** · 147, 1959.
2) Godman, G. C. and Porter, K. R. : Chondrogenesis, studied with the electron microscope. J. Biophysic. Biochem. Cytol., **8** : 719, 1960.
3) Gomori, G. : Microscopic Histochemistry. Principles and Practice, Chicago, University of Chicago Press, 1952.
4) Greep, R. O., Fisher, C. J. and Morse, A. : Alkaline phosphatase in odontogenesis and osteogenesis and its histochemical demonstration after demineralization. J. Amer. dent. Ass., **36** : 427, 1948.
5) Lorch, I. J. : The localization of alkaline phosphatase in mammalian bones. Quart. J. micr. Sci., **88** : 367, 1947.
6) Löe, H. . Bone tissue formation. A morphological and histochemical study. Acta odont. Scand., **17** : 311, 1959.
7) Mori, M., Takada, K. and Okamoto, J. : Histochemical studies on the localization and activity of acid phosphatase in calcifying tissues. Histochemie, **2** : 427, 1962.
8) Ogawa, K., Shinonaga, Y. and Suzuki, T. : Electron histochemical demonstration of acid phosphatase activity in the rat cerebellum. J. Electronmicr., **11** : 111, 1962.
9) Robinson, R. A. and Cameron, D. A. . Electron microscopy of cartilage and bone matrix at the distal epiphyseal line of the femur in the newborn infant. J. Biophysic. Biochem. Cytol., **2** : 253, 1956.
10) Sabatini, D. D., Bensch, K. and Barrnett, R. J. : Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell Biology, **17** : 19, 1963.
11) Schajowicz, F. and Cabrini, R. L. : Histochemical localization of acid phosphatase in bone tissue. Science, **127** : 447, 1958.
12) Scott, B. L. and Pease, D. C. : Electron microscopy of the epiphyseal apparatus. Anat. Rec., **126** : 465, 1956.
13) Takamatsu, H. and Akahoshi, Y. . A new technique for the histochemical demonstration of alkaline phosphatase in hard tissues. Arch. Jap. Chir., **26** : 301, 1957.

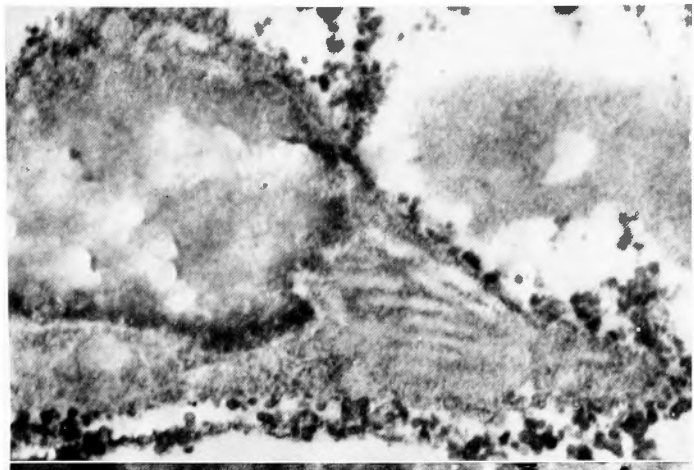


**Fig. 1** Alkaline phosphatase activity is noted in territorial peripheries of the proliferating cartilage. No lead deposition is observed in the nucleus of the cell.

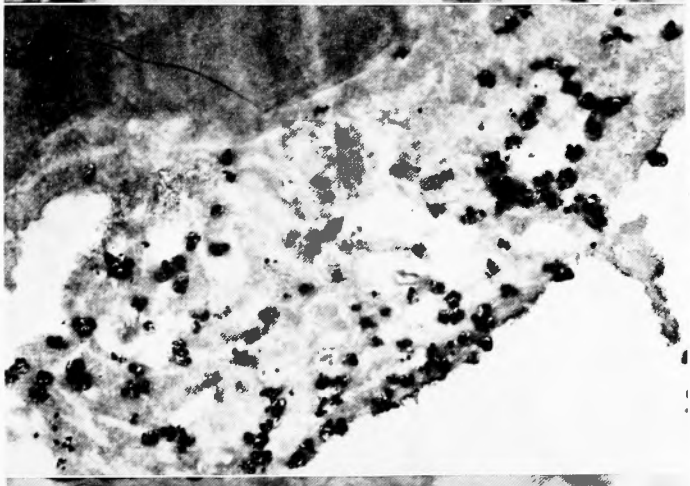
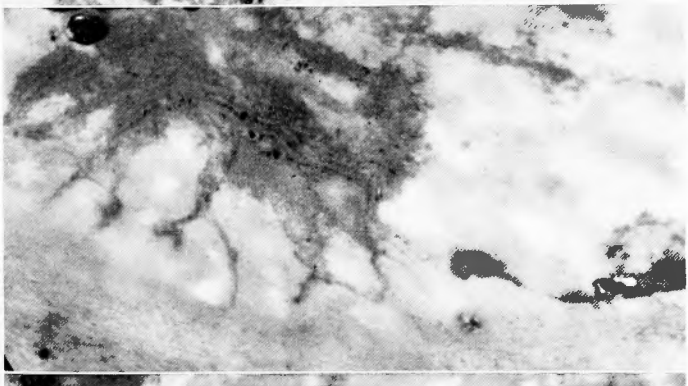
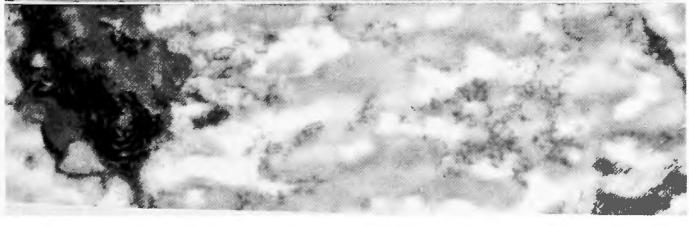


**Fig. 2** Note alkaline phosphatase activity in periferies and cytoplasm of the hypertrophic cartilage.





**Figs. 3 and 4** Rough surfaced endoplasmic reticulum of the calcifying cartilage is positive for the acid phosphatase activity. Lysosome-like structure is noted.

和文抄録

# マウスの軟骨におけるアルカリ性および酸性フォスファターゼ活性の電子顕微鏡のレベルでの組織化学的研究

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田　中　清　介

硬組織のアルカリ性および酸性フォスファターゼ活性の組織化学的研究および電子顕微鏡学的研究は，それぞれいくつかの発表が既になされている．しかしながら，電子顕微鏡のレベルでの組織化学的研究は硬組織では未だ報告されていない．

本研究ではマウスの大腿骨頭の軟骨細胞におけるアルカリ性および酸性フォスファターゼの活性を電子顕微鏡のレベルで組織化学的に同定することを試みた．

アルカリ性フォスファターゼ活性の同定には冷 formol-calcium で固定し，更に凍結切片にした後，高松法を用いて行なつた．酸性フォスファターゼ活性は cacodylate 緩衝液を加えた glutaraldehyde で固定し，更に凍結切片とした後，Gomori 法を用いて同定した．基質液に浸漬した後 Palade の osmium tetroxide で固定し，epon 包埋を行なつた．

本研究ではアルカリ性フォスファターゼ活性は増殖細胞層および肥大細胞層の軟骨細胞内周辺と細胞質の endoplasmic reticulum と思われる部分に認められた．一方，酸性フォスファターゼ活性は石灰化層の軟骨細胞の rough-surfaced endoplasmic reticulum に認められ，時には lysosome 様構造をしたものが認められた．軟骨細胞の骨端層には酵素活性を認めなかつた．

軟骨細胞の増殖層と肥大層ではアルカリ性フォスファターゼ活性が陽性であるが，酸性フォスファターゼ活性はなく，石灰化層では酸性フォスファターゼ活性を認めて，アルカリ性フォスファターゼの活性を認めない．この所見からアルカリ性フォスファターゼは軟骨細胞の分化，即ち増殖層から肥大層へ，更に石灰化層へと分化するための代謝に何らかの役割を演じ，酸性フォスファターゼは石灰化に重要な役割を演ずるものと思われる．