AN ELECTRON MICROSCOPE OBSERVATION ON CELLS FOUND IN BONE RESORPTION AREA INCIDENT TO EXPERIMENTAL TOOTH MOVEMENT

BY

Saburo Kurihara*¹

ABSTRACT

For the purpose of investigating many of the cells in the bone resorption area incident to experimental tooth movement and characterizing the role of these cells for the periodontal tissue resorption at the ultrastructural level, orthodontic force was applied to 24 male rats. Orthodontic elastics were inserted into the interproximal space of the upper first and second molars. The mesial side of the interradicular septum of the second molar was observed.

The electron microscopic findings were as follows:
1. Cell cohorts were found in the undermining bone resorption area. They were mainly composed of fibroblasts, endothelial cells, undifferentiated cells, macrophages and several kinds of osteoclasts.
2. Four different kinds of osteoclasts could be recognized from the morphological features: a) small osteoclasts situated apart from the bone surface, b) large osteoclasts rich in rough ER, c) classic large osteoclasts and d) degenerating osteoclasts.
3. Two different types of undifferentiated cells were observed in these area. One was a spindle-shaped bright cell rich in rough ER and the other was a round-shaped dark cell with numerous mitochondria and free ribosomes.
4. Macrophages showing phagocytosis were also found in the bone resorption area.

INTRODUCTION

Numerous light microscopic studies¹-⁸ on the histologic changes of the periodontal tissues during experimental tooth movement have been reported. However, the stage has been reached where it is very important to clarify the tissue response incident to the mechanical force on the basis of cellular changes. For this purpose, some electron microscopic studies have been carried out on account of its excellent resolution. Koumas and Matthews⁹ showed electron microscopic photographs of the periodontal membrane after experimental tooth movement in the guinea pigs. Rygh and Reitan¹⁰, and Rygh¹¹-¹³ discussed the changes in the collagen fibers and capillaries on the pressure side of the rats and of the orthodontic patients. Koga¹⁴ reported on the features of the periodontal membrane not only on the pressure side but also on the tension side. Hirashita¹⁵ described ultrastructural details of the osteoblasts and osteocytes on the tension side. Ichinokawa¹⁶ studied the osteoblastic and osteoclastic activity during experimental tooth movement after lead acetate vital staining.

However, little attention has been paid to the ultrastructural changes in the bone re-

*¹ 秋原三人: Department of Orthodontics (Chief: Prof. F. Miura), School of Dentistry, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
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sorption area during experimental tooth movement. Therefore, in the present study, it was attempted to investigate many of the cells in the bone resorption area incident to the experimental tooth movement and to characterize the role of the cells for the periodontal tissue resorption at the ultrastructural level. In particular studies were made on the osteoclasts, undifferentiated cells and macrophages in these areas.

**Materials and Methods**

A total of 32 Wistar strain male rats, weighing 180 to 210 g, were used for this study. These animals were divided into two groups, eight rats for the control group and 24 rats for the experimental group. Mechanical force was applied to the upper molars on both sides and the experiment was performed for one day, two days and three days, respectively. A segment of the orthodontic elastic band (1/4 light, 400–130, Unitek) was inserted into the interproximal space between the upper first and second molars (modified Waldo’s method).

After the animals were decapitated, the upper maxillary bones were dissected immediately, fixed in 10% neutral formalin, decalcified in a solution of 10% disodium EDTA for about three weeks and embedded in paraffin. They were cut into mesio-distal sagittal sections and stained with hematoxylin and eosin to study the tissue changes with light microscope.

For the electron microscopic study, the animals were fixed by perfusion (Hirashita) with a phosphate-buffered solution, at pH 7.4, of 2% glutaraldehyde for 30 minutes under ether anesthesia. The maxillary first and second molar region was dissected (Fig. 1) and immersed in the same fixatives (0–4°C) for two hours. After these blocks were washed in 0.1 M phosphate buffer, they were decalcified in a phosphated buffered solution (4°C), at pH 7.4, of 25% disodium EDTA, containing 0.2M sucrose, for about three weeks. During the subsequent washing in phosphate buffer, the specimens were trimmed and then post-fixed with 1% phosphate-buffered osmium tetroxide for two hours. They were dehydrated in a series of graded ethanol solutions and embedded in Epon 812. The ultrathin mesio-distal sagittal sections were cut with glass knives on an ultramicrotome (Porter-Blum MT-1). These sections were stained with uranyl acetate and lead citrate and were examined by an electron microscope (Hitachi HU-11A) operated at 75 KV. For orientation, about 1 μ sections stained with 0.1% toluidin blue were used.

The top of the mesial area of the interradicular septum of the second molar was selected for light microscopic observation (Fig. 1), and the area of undermining bone

![Fig. 1. The maxillary first and second molar regions (within the square frame) were removed after perfusion. Arrow shows the area the electron microscopic observation was focused at. M1: first molar; M2: second molar; M3: third molar.](image)

![Fig. 2. Light microscopic photograph of the interradicular septum of the second molar (H-E stain). a: control group; b: after one day; c: after two days; d: after three days; Alv: alveolar bone; D: dentine; Nec: necrosis of periodontal membrane; arrow: osteoclasts. ×240.](image)
OBSERVATION ON CELLS FOUND IN BONE RESORPTION AREA

(a) Alv

(b) Alv

(c) Alv, Nec

(d) Alv, Nec
resorption in the experimental groups and the area around Howship lacunae incident to the physiological tooth movement\textsuperscript{5,18,19} in the control group were offered for electron microscopic study.

**FINDINGS**

**LIGHT MICROSCOPIC OBSERVATION**

In the control group, a few small multinucleated osteoclasts were found. Capillaries were also observed in the large lacunae. Slightly large cells with round or ovoid nuclei were found near these capillaries (Fig. 2 a). On the other hand, in the experimental group, cohorts of cells were observed in the large lacunae adjacent to the necrotic area after one day. The cohorts of cells consisted of osteoclasts, fibroblasts, endothelial cells and cells with large round or ovoid nuclei (Fig. 2 b). After two days, many more of the same kinds of cells, especially osteoclasts, were found in the same area where undermining bone resorption was present (Fig. 2 c). Some osteoclasts were large, regularly shaped and stained pale while the others were small, irregularly shaped and densely stained. Capillaries were always observed in the bone resorption area. These cellular and tissue changes were found dominantly after the third day of the experiment (Fig. 2 d).

**ELECTRON MICROSCOPIC OBSERVATION OF THE CONTROL GROUP**

A few multinucleated osteoclasts with ruffled border were seen on the bone surface of the lacunae. These osteoclasts were varied in size and the smaller ones were dominant. Fibroblasts as well as a small amount of collagen fibers were observed, being apart from the bone surface. A small number of cells like macrophages was also found adjacent to the capillaries.

A few relatively small (about 10×20 µ) multinucleated osteoclasts with two to four nuclei (Fig. 3) were observed independently on the bone surface. These cells showed the typical features of osteoclasts: the ruffled border with innumerable cytoplasmic folds or processes and the clear zone with plain cytoplasmic membrane\textsuperscript{20}. No prominent organelles were observed in the cytoplasm of the ruffled border and the clear zone except for the fine granular or fibrillar materials. The nuclei of the individual osteoclasts, even in the same cells, varied in shape, some were ovoid and the others were deeply fissured like the lobated nuclei. A large number of mitochondria was observed in the cytoplasm except for the area around the ruffled border. Their shapes were mostly globular, but a
few rod-shaped ones were also noted. A small number of short tubular rough endoplasmic reticulum (rough ER) and free ribosomes was found near the nuclei. Poorly developed Golgi apparatus and many vacuoles of different sizes were observed in the cytoplasm. Large vacuoles were seen adjacent to the ruffled border. A few dense bodies and residual bodies also existed.

A small number of *mononuclear cells like macrophages* was also observed close to the capillaries in the vicinity of the bone lacunae (Fig. 4). These cells had a rounded shape (about 7 μ in diameter) and many cytoplasmic processes were seen around the cell surface. The nuclei of these cells possessed deep fissures. One or two nucleoli were observed in the nuclei and the chromatin was almost aggregated near the nuclear membrane. The cytoplasm of these cells was not developed sufficiently. A few mitochondria and dense bodies were noted in the cells. Small pinocytotic vesicles were also observed on the surface of the cytoplasmic membrane.

**ELECTRON MICROSCOPIC OBSERVATION OF THE EXPERIMENTAL GROUP**

After the first day of the experiment, many kinds of cells were observed near the bone surface. A small number of multinucleated osteoclasts was found in the lacunae and its size was larger than that of the control group. Many undifferentiated cells with a high nucleo-cytoplasmic ratio were seen in the same area. Many mononuclear cells like macrophages were also noted near the capillaries. However, granular leukocytes were never found in these areas. After two days, multinucleated osteoclasts, fibroblasts, undifferentiated cells and mononuclear cells like macrophages were observed much more dominantly in the bone resorption area compared with those of the control and one-day experimental group. Large osteoclasts were prominent in these areas. After three days, degenerated osteoclasts were found in the bone resorption area adjacent to the necrotic tissues.

After one day, nearly all of the *osteoclasts*, which had well developed cytoplasmic, had three or four nuclei (Fig. 5) and a few mononuclear cells showed a prominent ruffled border and wide clear zone (Fig. 6). The Golgi apparatus was well developed around the nuclei. Large vacuoles were frequently observed in the cytoplasm near the ruffled border and rarely on the other side of the cytoplasm. After two days, two different types of large multinucleated osteoclasts were differentiated according to the degree of cytoplasmic organization, one of them rich in rough ER and the other one containing numerous vacuoles. In addition, small osteo-
clasts (10 to 15 μ in diameter) were also observed, being situated apart from the bone surface (Fig. 7). These cells possessed neither a brush border nor a clear zone, but the cytoplasmic organization of these cells was similar to those of the large osteoclasts rich in rough ER.

The narrow ruffled border and the wide clear zone, in the large osteoclasts rich in rough ER, were observed to be in contact with the bone tissue, while a few cytoplasmic processes like the pseudopodia were noted on the other surface of the osteoclasts (Fig. 8). A small number of vacuoles was located near the ruffled border. These cells were richer in rough ER compared with those of the classic osteoclasts. Also, the free ribosomes were seen dispersed in the cytoplasm. The Golgi apparatus was moderately developed subjacent to the nuclei. Small dense bodies and few residual bodies were also noted.

The well developed ruffled border and the narrow clear zone were observed in the other types of large osteoclasts with numerous vacuoles (Fig. 9). The surface of these cells was almost smooth except for a few small cytoplasmic processes. Many vacuoles of variable size and numerous mitochondria with distinguishable cristae were observed in the cytoplasm near the bone surface, while a small number of short tubular rough ER was seen near the nuclei or at the other side, situated apart from the bone surface. A small amount of free ribosomes was seen dispersed. Many residual bodies and a few dense bodies were observed.

Some degenerating osteoclasts were observed to be in contact with the bone tissue, which contained nuclei showing pyknosis and karyolysis, indistinguishable cytoplasmic membrane, swollen mitochondria and a large number of vacuoles, as shown in Fig. 10. Other osteoclasts were seen situated far from the bone surface, the cytoplasmic features...
of which were more degenerated (Fig. 11).

A large number of immature cells with a high nucleo-cytoplasmic ratio was observed in the bone resorption area (Fig. 12). These undifferentiated cells were divisible into two types based on their shape and ultrastructural organization, the spindle-shaped bright cells with oval nuclei and the round-shaped dark cells with undulated nuclei. However, maturation in different stages could be seen.

The spindle-shaped bright cells were oval or spindle-shaped in profile (from $5 \times 10 \mu$ to $8 \times 16 \mu$) with large and oval nuclei. A few cytoplasmic processes were seen around the cell surface without any basement membrane. The rough ER of these cells was mostly of the short tubular type and some of them were of the parallel lamellae type. A few rod-shaped mitochondria, a small number of dense bodies, many small vesicles, microtubules and microfilaments were observed in the cytoplasm. The Golgi apparatus was clearly differentiated in the relatively matured cells. It was difficult to distinguish some of the relatively matured cells from the osteoblasts or fibroblasts. In addition, these cells were frequently observed adjacent to the multinucleated osteoclasts (Fig. 14). In a few instances, the degenerated cytoplasm of the other cells was surrounded by the cytoplasmic processes of these cells (Fig. 13).

The round-shaped dark cells (5 to 10 $\mu$ in diameter) had round and irregularly undulated nuclei. The cytoplasmic processes like the pseudopodia were seen around the
Fig. 12. Undifferentiated cells. Spindle-shaped bright cell (A) and round-shaped dark cell (B). After two days. m: mitochondria; d: dense body; G: Golgi apparatus; arrow: rough ER; B: bone. ×15000.
cell surface. A few of the short tubular rough ER were found around the nuclei. Numerous free ribosomes were scattered and round-shaped or oval-shaped mitochondria were observed dominantly in the cytoplasm. Dense bodies varying in size were also noted in the cytoplasm. Moderately developed Golgi apparatus was generally seen adjacent to the nuclei. The cytoplasmic organization in the well developed cells was similar to that of the small multinucleated osteoclasts situated apart from the bone surface. After the second day of the experiment, two or more round-shaped dark cells in contact with each other were seen in the bone resorption area and their cytoplasmic membranes were indistinguishable in some intercellular contact areas, where interdigitation of mutual cytoplasmic processes was also found occasionally (Fig. 15 and 16).

The mononuclear cells like macrophages increased in number as compared with those of the control group. Most of them were seen near the capillaries in the bone resorption area. A large number of dense bodies was involved in these cells (Fig. 17). After the second day of the experiment,
Fig. 17. Cell with many dense bodies like a macrophage near the capillary (Cap). After two days. \( \times 10400 \).

the debris of the degenerated cells or periodontal soft tissues (Fig. 18) was observed in some of these cells.

**DISCUSSION**

1. **DIFFERENT TYPES OF CELLS OBSERVED IN THE BONE RESORPTION AREA.**

   In this study, undermining bone resorption occurred after the first or second day of tooth movement and cohorts of many cells were seen in the bone resorption area. These findings coincide with the light microscopic studies by Macapanpan\(^5\), and Zaki and Huysen\(^7\). By this electron microscopic observation, these cell cohorts were mainly composed of fibroblasts, endothelial cells, undifferentiated cells, macrophages and several

Fig. 18. Macrophage, in which the other cell debris are observed (arrow). Co: collagen fiber. \( \times 7750 \).

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Fig. 15 and 16. Round-shaped dark cells in contact with each other. Some parts of their cytoplasmic membrane (large arrow) were distinguishable and others (small arrow) were indistinguishable. After two days. \( \times 10400 \) (Fig. 15), \( \times 12000 \) (Fig. 16).
kinds of osteoclasts.

No infiltration of granular leukocytes was observed in the cohorts, which is in accordance with the findings of Zaki and Huysen. The tissue reaction in the bone resorption area induced by the experimental tooth movement seems to be slightly different from the exudative inflammation by trauma or invasion of bacteria.

In addition, endothelial cells of the capillaries were always observed in the bone resorption area. Nakamura and Zaki and Huysen found a close relationship between the distribution of the capillaries and bone resorption. However, the role of the capillaries in this area is still unknown.

The cells, except for fibroblasts and endothelial cells, in the bone resorption area can be mostly divided into three groups:

1) **Osteoclasts**: A large number of osteoclasts with different kinds of ultrastructural organization were observed after the second or third experimental day. Hancox described light microscopically that multinucleated osteoclasts are divided into three groups, the normal and healthy, early degenerative and senescent osteoclasts.

Few electron microscopic observations have been reported concerning the different types of osteoclasts. Dudley and Spiro found both the inactive osteoclasts and degenerative osteoclasts with swollen mitochondria. Rohrr and Bremer found two different types of osteoclasts, active and resting osteoclasts, in the rats administered parathyroid hormone.

In the present study, four different kinds of multinucleated osteoclasts can be recognized from the morphological features: a) small osteoclasts situated apart from the bone surface (Fig. 7), b) large osteoclasts rich in rough ER (Fig. 8), c) classic large osteoclasts (Fig. 5 and 9) and d) degenerating osteoclasts (Fig. 10 and 11).

2) **Undifferentiated cells**: Many undifferentiated cells with a high nucleo-cytoplasmic ratio were observed in the bone resorption area in the experimental group. Scott reported two types of osteogenic cells in the tibial epiphysis of the embryonic rats, spindle-cell type (A cell) and rounded-cell type (B cell). She concluded that the specialization of the osteogenic cells changing into osteoclasts and osteocytes may involve separate pathways of cytodifferentiation.

Two different types of undifferentiated cells were also observed in the present study. One is the spindle-shaped bright cell rich in rough ER (Fig. 12) and another is the round-shaped dark cell with numerous mitochondria and free ribosomes (Fig. 12).

3) **Macrophages**: Few description of the macrophages in the periodontal membrane incident to the experimental tooth movement has been reported by light or electron microscopic observation. This may be due to the difficulty in distinguishing the macrophages from the fibroblasts or from the other cells in the periodontal tissue. A small number of mononuclear cells like macrophages was observed adjacent to the capillaries in the control group and showed features similar in size and vacuoles to those of the immature monocytes or histiocytes.

A moderate number of mononuclear cells like macrophages with many dense bodies and well developed Golgi apparatus was observed close to the capillaries in the experimental group (Fig. 17). Some macrophages showed phagocytosis in the bone resorption area (Fig. 18). These findings are similar to the electron microscopic observation of Cohn et al. and Yamori. Cohn et al. reported that there was a prominent formation of large electron-opaque granules as well as an increase in the size of the Golgi apparatus during the induction of macrophage maturation. Yamori described about
the ingesting type of phagocytes appearing three days after the tuberculosis infection.

2. FUNCTION OF THE CELLS OBSERVED IN THE BONE RESORPTION AREA.

1) Osteoclasts: Bonucci\(^{(80)}\) thought that the osteoclasts were cells which might perform synthesis, exocytosis, endocytosis and digestion (Fig. 19). However, it is inferred in the present study that the degree of each function might be different at various periods of the life cycle because of the different morphological features in each osteoclast.

   a) Small osteoclasts—Since these cells are found situated apart from the bone surface without numerous vacuoles and ruffled border, they might not have the function of resorbing the bone tissue. However, the cytoplasmic organization of the mitochondria, rough ER and free ribosomes have similarities close to those of the large osteoclasts rich in rough ER. The term "multinucleated pre-osteoclast", therefore, is possible from these observations. In addition, these cells could be regarded as wandering cells because they have pseudopodia around the cell surface like macrophages.

   b) Large osteoclasts rich in rough ER—The cytoplasmic organization of these cells was analogous to those of the small osteoclasts. However, no ruffled border or well developed cytoplasm was present in the small osteoclasts, nor were there many nuclei. Fewer vacuoles were contained within the cytoplasm near the ruffled border of the large osteoclasts rich in rough ER compared to the classic osteoclasts. These cells, like the pancreatic exocrine cells\(^{(31)}\) might be synthesizing some material containing protein (hydrolytic enzyme?) from the point of view of being rich in rough ER or free ribosomes. There is an interesting correlation between this and that of Owen and Shetlar\(^{(32)}\), who demonstrated the uptake and secretion of \(^3\)H-glucosamin by the osteoclasts. The term

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Fig. 19. Schema of the presumed steps of the process of osteoclastic bone resorption. Quoted from Bonucci.
"pre-mature osteoclast", therefore, could be applied to these osteoclasts.

As to the rough ER of the osteoclasts, Scott and Pease\(^{33}\) stated that there is no conspicuous endoplasmic reticulum, but scattered masses have been observed in some osteoclasts of the kittens. Dudley and Spiro\(^{35}\) pointed out the presence of one or more smaller aggregates of endoplasmic reticulum in the patients or in the chicks. Gonzales and Karnovsky\(^{34}\) reported that the distal parts of the osteoclasts in the rats are richer in endoplasmic reticulum than the proximal parts. Kallio et al.\(^{20}\) observed a moderate amount of loosely organized granular endoplasmic reticulum in the mice.

These variable observations of the rough ER of the osteoclasts might be attributed to the different types of osteoclasts in each maturing stage as well as in each specimen. The same explanation would apply to the numerous free ribosomes and developed Golgi apparatus seen in the "pre-mature osteoclast".

In addition, the "pre-mature osteoclast" may be the stage which is just before vigorous bone resorption, judging from the narrow ruffled border and wide clear zone. This assumption seems to correspond to the suggestion by Malkani et al.\(^{35}\) that the clear zone of the osteoclasts transforms progressively into the ruffled border.

c) Classic large osteoclasts—Ultrasound structural organization of these cells in the present study corresponds to that of the osteoclasts reported by many authors\(^{20,22,23,25,23}\). These features of numerous mitochondria, large number of vacuoles and wide ruffled border represent rapid bone resorption; thus, the term "matured osteoclast" should be proposed for these cells. The most characteristic feature of the "matured osteoclast" is the numerous mitochondria in the cytoplasm. Lehninger\(^{36}\) described that the mitochondria has the role of saving energy in the cell by synthesizing ATP. Hamilton and Holdworth\(^{37}\) pointed out the role of the mitochondria as transportation of Ca ions across the cell. Matthews et al.\(^{38}\) presumed that the mitochondria might act as a calcium reservoir. It could be said from the present findings that a large number of mitochondria plays the most important role in bone resorption.

d) Degenerating osteoclasts—These were found adjacent to the bone surface after the third day of tooth movement (Fig. 10). They were very similar to the degenerating osteoclasts which were recently pointed out in the cases of Paget’s disease by Rabel et al.\(^{39}\). This is contrary to Rasmussen’s hypothesis\(^{40}\) that the osteoclasts might change into the osteoblasts. Rebel et al. concluded that the osteoclasts eventually degenerate completely, at least in the case of Paget’s disease.

Some of the degenerating osteoclasts in the present study are degenerate much more than those in Paget’s disease and seem to become necrotic (Fig. 11). It is uncertain whether this degeneration implies the changes after the osteoclasts have completed their function of bone resorption or the changes caused by mechanical stress.

2) Undifferentiated cells

a) Spindle-shaped bright cells—These undifferentiated cells were similar to Scott’s A cell\(^{26}\) in cytoplasmic organization. She divided the A cell into two subtypes, perivascular and endosteal types, and named the A cell as the "pre-osteoblast". Cameron\(^{41}\) observed analogous undifferentiated cells in the fracture callus, which clearly have some of the morphological features of the osteoblasts. In the giant cell tumor of the bone, Hanaoka et al.\(^{32}\) found stromal cells which were oval or spindle-shaped with less electron dense cytoplasm. These cells resemble ultrastructurally the spindle-shaped bright cells in
the present observation. In addition, the spindle-shaped bright cells possess an ultra-
structural similarity to the fibroblasts$^{45}$ and histiocytes or reticulum cells$^{48}$.

Although it is considered that the spindle-
shaped bright cells, which are relatively
similar to the mature cells, might be the
pre-osteoblasts or pre-fibroblasts because of
abundant rough ER and Golgi apparatus,
these cells might have the possible role of
ingesting or digesting the destroyed matrix
and cell debris like the histiocytes or reti-
culum cells.

Furthermore, Jee and Nolan$^{44}$ observed
light microscopically spindle-shaped cells
containing charcoal particles in the femur of
the rabbits and concluded that they might
fuse into osteoclasts. Hanaoka$^{49}$ showed an
electron microscopic photograph of the fusion
of this undifferentiated cell into an osteoclast.

Therefore, four possible descendants of
these cells were considered by this ultra-
structural observation: i) osteoblasts, ii)
fibroblasts, iii) phagocytes like histiocytes or
reticulum cells and iv) part of the osteoclasts.

b) Round-shaped dark cells—These cells
have a strong resemblance to Scott’s B cell$^{28}$
because of the distribution of the mito-
chondria and free ribosomes. She described
that these cells are easily distinguishable by
their marked density and reserved the term
“pre-osteoclast” for the B cell. Cameron$^{25}$
also described about the undifferentiated
cells with numerous free ribosomes. Recent-
ly, Saotome$^{46}$ inferred that mononuclear
cells with numerous free ribosomes and
many mitochondria are pre-osteoclasts.

In the present study, considering that the
cytoplasmic organization of the round-shaped
dark cells was analogous to that of the small
osteoclasts without the ruffled border (Fig. 7)
and large osteoclasts rich in rough ER
(Fig. 8), it is reasonable to suggest that these
dark cells might be called “mononuclear
pre-osteoclasts”.

Some of these “mononuclear pre-osteoc-
lasts” were observed gathered each other
the bone surface in the bone resorption area.
Some parts of the cytoplasmic membrane
seemed to be indistinct (Fig. 15 and 16).
These findings show the possibility of the
fusion of these cells.

There is a general agreement that the pre-
cursor cells fuse to form osteoclasts.$^{22,46,48}$
The fusion was also observed ultrastructurally
in the other tissues. Okada$^{49}$ described about
the giant polymuclear cell formation caused by
the HVJ virus from Ehrlich’s ascites
tumor cells. Matsumoto$^{50}$ also described in his
observation on the tubercle formation in the
rabbits that the Langhans giant cells are
formed by the cellular fusion of the epithe-
lioid cells. Sutton and Weiss$^{51}$ reported that
in the tissue culture of the monocyte the
plasma membranes break down in places and
the epithelioid cells fuse to form giant cells.
The features of the fusion of the epithelioid
cells shown by them have a great similarity
to those found in the present study (Fig. 15).

From the above discussion, the cellular
transformation of the osteoclasts in the bone
resorption area incident to the experimental
tooth movement could be pictured as shown
in Fig. 20 and 21. First, the “mononuclear
pre-osteoclasts” might fuse into “multinucle-
ated preosteoclasts”, then they might differ-
entiate to “pre-mature osteoclast”. These
“pre-mature osteoclasts” might develop into
“matured osteoclast”. On rare occasions,
some “mononuclear pre-osteoclasts” might
develop into “matured osteoclasts”, having a
resorptive activity without any fusing me-
chanism. After all, the “matured osteoclast
transforms into the degenerating osteoclast.
3) Macrophages

The ultrastructural changes in the macro-
phages by this observation are similar to
those described by Cohn et al.$^{52}$. They sug-
Fig. 20. Group of osteoclasts: 1. round-shaped dark cell, 2. two round-shaped dark cells, 3. small osteoclast situated apart from the bone surface, 4. pre-mature osteoclast, 5. matured osteoclast and 6–7. degenerating osteoclast. ×2500.

Fig. 21. Possible schema of the changing process of osteoclast incident to experimental tooth movement.
gested that hydrolytic enzymes are initially synthesized in the endoplasmic reticulum, transferred to the Golgi apparatus and packaged into small Golgi vesicles which represent the primary lysosome. Golgi vesicles subsequently fuse with the pinosomes and then discharge their hydrolases and form digestive granules or secondary lysosomes.

Therefore, it is suggested that macrophages which increase in number in the bone resorption area incident to the experimental tooth movement might perform the important role of ingesting the other necrotic cells or soft tissues. As a result of their function, the periodontal membrane might be easily regenerated, thus replacing the necrotic tissue with the granulation tissue, forming new collagen fibers.

**Conclusion**

The present study on the electron microscopic observation of the cells found in the bone resorption area incident to the experimental tooth movement in the rats is concluded as follows:

1. The cells were mainly composed of fibroblasts, endothelial cells, undifferentiated cells, macrophages and several kinds of osteoclasts.

2. Four different kinds of osteoclasts could be recognized from their morphological features: a) small osteoclasts situated apart from the bone surface ("multinucleated pre-osteoclast"), b) large osteoclasts rich in rough ER ("pre-mature osteoclast"), c) classic large osteoclasts ("matured osteoclast") and d) degenerating osteoclasts.

3. Two different types of undifferentiated cells were observed in the bone resorption area: a) spindle-shaped bright cells rich in rough ER (possibly the originating cells for the osteoblasts, fibroblasts, phagocytes or reticulum cells, and a portion of osteoclasts) and b) round-shaped dark cells with numerous mitochondria and free ribosomes ("mononuclear pre-osteoclast").

4. Macrophages showing phagocytosis were also found in these areas and it is suggested that the ingestion of necrotic tissues is an important role in the regeneration of the periodontal tissue.

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