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THE ASPECT OF ULTRASTRUCTURAL CHANGES OF THE OSTEOBLASTS AND SURFACE AREAS OF ALVEOLAR BONE APPEARING IN EXPERIMENTAL TOOTH MOVEMENT

BY

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ABSTRACT

Molars of mature Wistar rats were moved experimentally by orthodontic elastic for four days. Then, the aspects of ultrastructural changes of the osteoblasts and structure of the alveolar bone surface which appeared in experimental tooth movement were studied.

The following results were obtained.

1) These osteoblasts are classified into three groups according to their position. 2) The most active response to the orthodontic force is exhibited by the second group of cells with the ability of rapid production of abundant acid polysaccharide; i) Abundant rough surfaced endoplasmic reticulum with markedly dilated cisternae. ii) Well developed Golgi apparatus and electron opaque granules with a limiting membrane are found in the cytoplasm. Large granules are frequently seen to be secreted out of the cell. iii) The mitochondria are of large size and round shape with well developed cristae. 3) The surface of the new alveolar bone is covered with a belt-shaped structure consisting of small dense spherical-shaped structures. 4) Osteoclasts are rarely seen, but the original function of the cells appears to be almost inactive.

INTRODUCTION

Sandstedt (1904)[1], Oppenheim (1944)[2], Reitan (1962)[3] and others[4,5,6] conducted studies with light microscopice and reported that the osteoblasts and the osteoclasts in experimental tooth movement play an important role in the remodeling changes of the alveolar bone. Studies on the fine structure of the bone were made with electron microscope and reported by a number of investigators, beginning with Wolpers (1904). However, these studies dealt with the bone matrix rather than with the cells involved in its formation. But, information about the fine structure of the osteoblasts in the metaphysis or tibia is reported in detail in the papers by Scott and Pease (1956)[7], Sheldon and Robinson (1957)[8], Durning (1958)[9] and Cameron, Paschall and Robinson (1964)[10]. Only Fitton Jackson (1957)[11] described about the osteoblasts in the tendon by comparing the embryo fowl and adult fowl. They studied the different kinds of bone tissues, so that their results were not always the same. Therefore, I have conducted studies on the ultrastructural changes of the osteoblasts and surface areas of the alveolar bone appearing in experimental tooth movement.

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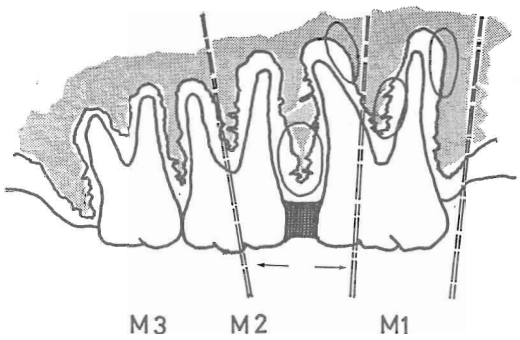

Fig. 1. Elastic insertion and observed areas
M1: First molar M2 Second molar
M3: Third molar
The slant line shows cutting by the dental disk. Arrow shows the migration of the teeth by the elastic force and observation carried out on the tension side (round marks).

Materials and Methods

The upper molars of the mature Wistar rats (50 rats, each weighing 220–240 g) were moved experimentally by orthodontic elastic (Waldo's method[12]) for four days.

Anaesthetized rats were perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 through their ascending aortae for 30 minutes.

The upper jaws were subsequently excised out (right molars as experimental groups, left molars as control groups) and they were cut into a few small blocks by dental disk as shown in Fig. 1. After these blocks were fixed in freshly prepared 2.5% glutaraldehyde (pH 7.2) for 90 minutes, they were rinsed with buffer solution (pH 7.2) and were then decalcified with a cold 2.5% EDTA-Na_2 solution for about three weeks.

After decalcification, they were rinsed with buffer solution and postfixed in 1.0% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2, 0°c–4°c) for 90 minutes. After they were dehydrated with a grated ethanol series (50%–100%), they were embedded in Epon 812[26]. A part of these specimens was used for metachromasia staining (0.1% toluidine blue, pH 5.6, for 15 mins.) in order to study the localization of sulfate-polysaccharide by light microscope. The best condition for toluidine blue staining was 0.1% toluidine blue, pH 5.6, for 15 minutes. This condition was obtained from the results which were prepared by the combination of 0.1%, 0.08% and 0.05% toluidine blue with the variation in pH from 5.8 to 5.6 and staining time of 5, 10, 15 and 20 minutes. For electron microscopy, the other specimens were ultrathin-sectioned in a thickness of about 70–90 mμ by glass knife, and stained with uranyl-acetate and lead citrate.

Results

Electron microscopic studies were carried out on the osteoblasts and the surface areas of the alveolar bone on the tension side (Fig. 1).

I) Light microscopy

The upper molars of the rats were moved by Waldo's method; the mean distance between the first molar and the second molar was about 0.6 mm.

On the tension side (the mesial and the distal surface of the alveolar bone between the first and second molars, the alveolar surface on the mesial side of the root apex of the first molar, the alveolar bone surface on the distal side of the root apex of the second molar), bone aposition was seen markedly[14]. The bone surface was strongly stained with 0.1% toluidine blue (pH 5.6) and the distance between the surface and the osteoblasts was about 2.0-3.0 μ. On the other hand, in the control groups, such a distance as shown in the experimental groups was not seen and the border was obscure.

On the tension side, logical differences

were not observed between the experimental groups and the control groups. These forms showed various forms: polyhedral-shaped, oval-shaped, etc. However, the nucleus of the osteoblasts in the experimental groups was stained deep blue and the cytoplasm was stained red-purple by toluidine blue staining, the latter showing a positive metachromasia staining reaction. Also, the metachromasia reaction of the osteocytes was not observed, being the same as that in the control groups.

II) Electron microscopy

a) Osteoblasts

The osteoblasts in the control groups (Fig. 10) are closely adjacent to the surface of the alveolar bone. The cell organellae are well developed in the cytoplasm, such as the rough surfaced endoplasmic reticulum, Golgi apparatus and mitochondria. It seems that they keep the cell function normal. In contrast to the osteoblasts in the control, the osteoblasts found on the tension side in the experimental groups migrate and scatter within a short distance from the surface of the alveolar bone. These osteoblasts are classified into three groups according to the position they occupy. The first group consists of young osteoblasts at some distance from the surface of the alveolar bone. Each osteoblast (Figs. 2 and 3) has a large nucleus surrounded by a clear marginal chromatin and a small amount of cytoplasm. The second group consists of mature osteoblasts scattered on the surface of the alveolar bone. Each osteoblast has a relatively small nucleus, with abundant and well developed organellae in the cytoplasm.

This finding indicates that these osteoblasts (the second group) show a marked activity of the cell function as follows: *i*) Abundant rough surfaced endoplasmic reticulum with markedly dilated cisternae was observed. The cisternae contain small fibrils (Fig. 7). ii) The Golgi apparatus was observed and contains amorphous materials of high electron density (Fig. 7). iii) Many electron opaque granules are found in the cytoplasm, which may start from the well developed Golgi region. iv) Often, we are able to observe the transportation of a part of the Golgi complex and smooth endoplasmic reticulum (large arrow) and a part of the smooth endoplasmic reticulum and rough endoplasmic reticulum (thin arrow) (Fig. 6). v) Secretory granules in the cytoplasm are enveloped by a limiting membrane, showing a very high electron opacity. The size is 0.2 μ–0.5 μ in diameter (mean 0.3 μ) and shows a round or oval form. Also, large granules occupy the margin of the cytoplasm (Figs. 2 and 7). Also, large granules are frequently seen secreting out from the cell (Fig. 7). vi) The mitochondria were observed with a large size and round shape with well developed cristate (Figs. 2 and 3). The third group consists of mature osteoblasts close to the surface of the alveolar bone or partly embedded in the newly formed bone matrix. The volume of the cytoplasm is decreased as compared with that of the second group. It resembles that of the osteocytes.

b) Areas of alveolar bone surface

Surrounding the osteoblasts near the bone surface in the experimental groups, a number of new capillaries was seen behind the cell (Fig. 9A).

The growing endothelial cells forming new capillaries were attached to each other by the desmosomes (Fig. 9A: arrow). However, the growing endothelial cells were often very thin and the wall of the newly formed capillary clearly shows many fenestration structures (Fig. 9B). The surface of the new alveolar bone was covered by

a belt-shaped structure consisting of small dense spherical-shaped structures (Figs. 3 and 4: arrow). It looks as if it identifies the finding that was seen during the forming of the young dentin by light microscope. Also, it was observed that the cytoplasmic process spreads to the bone matrix through small dense spherical-shaped structures and it forms bone canalicules. The interface between the bone surface and the osteoblasts in the second group was filled with numerous collagen fibrils running in various directions. Bundles of collagen fibers were frequently enclosed by the osteoblasts (Fig. 5). On the other hand, in the control group, the belt-shaped structure consisting of small clear dense spherical-shaped structures was not observed on the bone surface. Next, the osteoclasts were observed at the rate of an osteoclast to several osteoblasts in the experimental group (Fig. 12). But, osteoclasts were observed seldom. And even if they were observed, the organellae (the microvilli, smooth vesicles and lysosomes as seen in Fig. 8) in the cytoplasm of the osteoclast were not observed (Fig. 5).

Discussion

I) Methods

It is very difficult to prepare the small hard tissue segments (alveolar bone and teeth), because it is necessary to cut the section of the hard tissue after the decalcification procedures, and it is not so easy to maintain the fine cellular structures during the decalcification procedures.

However, Warshawsky and Moore[15] stated "Provided the initial fixation by perfusion is good, the decalcification method by EDTA-Na_2 gives satisfactory preservation of the fine structures of the cells and matrices, in addition to maintaining the relationship of hard and soft tissues"[16]. The author also obtained satisfactory results by using their method. In order to study the biochemical acid polysaccharide on the alveolar surface (tension side) in the experimental gruops, metachromasia staining of the segments embedded by Epon was done.

II) Osteoblasts

Each of the osteoblasts in the control groups (no influence of orthodontic force) is closely adjacent to the surface of the alveolar bone at the rate of an osteoclast to a few osteoblasts. The osteoblasts in the control groups (Fig. 10) are characterized by a large round nucleus and relatively abundant organellae in the cytoplasm. These organellae are well developed in the rough endoplasmic reticlum, Golgi apparatus and mitochondria[7,10,17]. Therefore, the osteoblasts in the control groups may carry on the natural function, being closely adjacent to the surface of the alveolar bone. On the other hand, the osteoblasts in the experimental groups migrate and scatter within a short distance from the surface of the alveolar bone. This aspect seems to be the response of the osteoblasts, which migrate and scatter to the orthodontic stimulus (force) on the tension side; this is the first characteristic of the osteoblasts as the response to the orthodontic stimulus. Osteoblasts which migrate are classified into three groups according to their position.

The first group consists of young osteoblasts distant from the surface of the alveolar bone. The second group consists of mature osteoblasts close to the surface of the alveolar bone or partly embedded in the newly formed bone matrix. The classification into these three groups, therefore, is the second characteristic on the tension side. So, with regard to the osteoblasts in

the first group, they have a large nucleus surrounded by a clear marginal chromatin, a few organellae in the cytoplasm and processes of the cytoplasm.

Probably, they may be relatively young. Also, it seems that they are waiting to proceed to the second group (Fig. 3).

The osteoblasts in the second group, in comparison with those in the first group, have a relatively small nucleus and abundant and well developed organellae (rough endoplasmic reticulum, Golgi apparatus, mitochondria and secretory granules) in the cytoplasm. These findings may suggest that the active production of acid polysaccharide and the secretion of collagen may be necessary as the elements to form a new bone matrix. Therefore, the second of the three groups shows the most active response to the orthodontic stimulus or force (on the tension side).

The osteoblasts in the third group show a decrease in the cytoplasmic organellae and they are partly embedded in the newly formed bone matrix (Fig. 4). These cells may resemble those of the osteocytes. From these findings, it seems that the osteoblasts in the second group play the most important role of bone formation in response to the orthodontic stimulus. So, to discuss electron microscopically about this cell is the main object of this study.

The present observations are concerned with some features of the fine structures of the osteoblast cytoplasm in different stages (osteoblasts influenced by orthodontic stimulus and osteoblasts in the control group-no orthodontic stimulus) of function. These osteoblasts demonstrate abundant and well developed rough surfaced endoplasmic reticulum with some dilation of the cisternae[7, 18–22]. These results suggest that the cells are probably associated with the active secretion of proteins as seen in the exocrine cells of the pancreas and the plasma cells. Sheldon and Robinson[8,22] noted the dilated sacs[23] of the rough surfaced endoplasmic reticulum and similar sacs can be seen in the papers by Scott and Pease[7] and Fitton Jackson[24,25]. Sheldon and Robinson suggested that the increased distance between the membrane may be associated with the particular phase of secretion and the secretion may contribute to the formation of the bone matrix as a precursor[24–27]. In this study, there are recognized small filamentous materials or small granular materials in the dilated sacs of the rough surfaced endoplasmic reticulum. These collagen precursors may probably be the tropocollagen.

Also, it may indicate the presence of an aminopolysaccharide appearing partly as PAS-positive granules. From the findings of communication between the rough surfaced endoplasmic reticulum and the smooth surfaced endoplasmic reticulum or between the smooth surfaced endoplasmic and the Golgi apparatus which are described in this paper, it seems to be the transfer of the materials from the rough surfaced endoplasmic reticulum to the Golgi apparatus. Therefore, the materials within the vacuoles of the well developed Golgi apparatus, as seen in Fig. 6, may be concentrated within the vacuoles of the Golgi apparatus. Palade[28], in regard to the exocrine cells of the pancreas, has demonstrated that proteins are synthesized in the rough surfaced endoplasmic reticulum and the newly synthesized proteins of the pancreas are concentrated in the structures of the Golgi apparatus, especially in the large vacuoles filled with dense materials and develop into secretory granules, occasionally impinging on the surface membrane of the cell. The process is clearly demonstrated by electron[29] and

light[30] microscopic radioautography.

In the present study, many dense granules (mean diameter, 0.3 μ) enveloped by a limiting membrane were observed in the cytoplasm of the second groups of osteoblasts (Figs. 2 and 3). The large granules occasionally impinge on the surface membrane of the cell as if about to go through it (Fig. 7).

These secretory granules have not been proved biochemically yet, but from the results concerning the form, the density and the limiting membrane, they may be considered to be of the same structure as described by Fitton Jackson[24], Fitton Jackson and Randall[26], which were demonstrated in the cytoplasm of the fibroblasts and osteoblasts of the avian tendon.

On the other hand, Fitton Jackson showed that the granules were never recognized in vitro and in vivo within the fibroblasts of the adult fowl tendon. As we used adult rats in the present study, it may be reasonable to surmise that the secretory granules are never found in the cytoplasm of the osteoblasts in the adult rats of the control groups. On the other hand, occasionally, the granules impinging on the surface membrane of the cell, as if about to pass through it, become to be of a lower electron density content, which are not different from the surrounding extracellular substance. It is not clear how it contributes to the bone deposition, however, it is suggested that they are in close relationship with the formation of the collagen fibrils. Mitochondria in the second group seemed to have prominent cristae in comparison with those of the control group (Fig. 10), and the outer mitochondrial membrane and the adjacent endoplasmic reticulum were often in close apposition[18]. Probably, this may be related to the supply of energy necessary for the cell function.

III) Areas of alveolar bone surface

A belt-shaped structure observed on the bone surface is consistent with the parts showing a strong positive response by metachromasia staining when seen by light microscope. This suggests the ability of rapid production of abundant acid polysaccharide. Electron microscopically, the belt-shaped structure consists of small dense spherical-shaped structures, and it seems to be closely related to the process of ossification.

With regard to ossification, Scott and Pease[7] described that there is a fibrous preosseous zone between the osteoblasts and the calcified matrix, and the calcification of the preosseous matrix is a progressive accretion and aggregation of the inorganic crystals. They say that, at first, masses of crystals are randomly disposed and finally the individual crystals become associated with the cross-banded collagen fibers[31,32]. Small dense spherical-shaped structures seen in this study seem to be calcified materials followed by rapid acid polysaccharide.

In fact, there were often cross-banded collagen fibers in the belt-shaped structure covered by small dense spherical-shaped structures.

Therefore, the belt-shaped structures that are an aggregation of small dense spherical-shaped structures seem to be the first bone matrix formed rapidly as a response to the orthodontic stimulus. On the other hand, various directional collagen fibrils filled the fibrous preosseous zone on the tension side in the experimental groups and also there were often observed collagen fibers invading into the bone matrix or being surrounded by osteoblasts. In consequence, it seems that, by the ortho-

dontic stimulus, formation of collagen is vigorously stimulated in the preosseous zone as well as the small dense spherical-shaped structures or newly formed capillaries. Also, in these areas, osteoclasts were rarely observed. Generally, osteoclasts exist at the rate of one osteoclast to a few osteoblasts on the physiological bone surface, but a few osteoclasts in the experimental groups (on the tension side) show that these areas are vigorous fields of bone formation. Also, the osteoclasts in the experimental groups (on the tension side) seem as if the cell stopped its natural function[33], because the cell no had smooth vesicles, lysosomes and microvilli. Generally, the osteoclasts, as seen Fig. 11 and Fig. 12, are the giant cells with numerous nuclei, numerous mitochondria, smooth surfaced endoplasmic reticulum, lysosomes, smooth vesicles and well developed microvilli (ruffled border[7] or brush border[34])[35] on the cell surface adjacent to the bone matrix.

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Explanation of Figures

Fig. 2. The surface of alveolar bone and osteoblasts observed on the tension side in the experimental groups.

The surface of the alveolar bone is covered by a belt-shaped structure consisting of small dense spherical-shaped structures (arrow). There are fibrous preosseous zones (fb) between the osteoblasts and bone matrix.

Many osteoblasts are migrated and scattered within a short distance from the surface of the alveolar bone. These osteoblasts are classfied into three groups according to their position. I: the first group. II: the second group. ×9000

Fig. 3. Osteoblasts in the Second Group

The most striking characteristics of the cytoplasm of the osteoblasts are as follows: i) Abundant rough endoplasmic reticulum with markedly dilated cisternae were observed. ii) Many electron-opaque granules are found in the cytoplasm, which may start from the well developed Golgi region. iii) The mitochondria were of large size and round shape with well developed cristae. ×10500

Fig. 4. Third Group of Osteoblast (III)

The volume of the cytoplasm is decreased. ×12000

Fig. 5. Second Group of Osteoblasts

The osteoclast is almost inactive. ×31500

Fig. 6. Second Group of Osteoblasts

The transportation of a part of the Golgi complex and smooth surfaced endoplasmic reticulum (large arrow) and a part of the smooth surfaced endoplasmic reticulum and rough surfaced endoplasmic reticulum can be observed (thin arrow). ×27000

Fig. 7. Second Group of Osteoblasts

Many electron dense granules are found in the cytoplasm of the osteoblast. Large granules are seen frequently secreting out from the cell (arrow). ×15750

Fig. 8. Osteoblasts and Osteoclasts

Osteoclasts are rarely seen, but the original function of the cells appears to be almost inactive. This indicates that this area represents an area of rapid and intense bone formation. ×13500

Fig. 9. Osteoblasts and Newly Formed Capillaries

A: The growing endothelial cells forming new capillaries are attached to each other by the desmosomes (arrow). ×9300

B: Growing endothelial cells are often very thin and the wall of the newly formed capillary clearly shows many fenestration structures (arrow). ×24800

Fig. 10. Osteoblasts in the Control Group

Each osteoblast is closely adjacent to the surface of the alveolar bone. And there are not seen any small dense spherical-shaped structures on the surface of the alveolar bone as seen in the experimental groups. ×9000

Fig. 11. Osteoblasts and Osteoclasts in the Control group

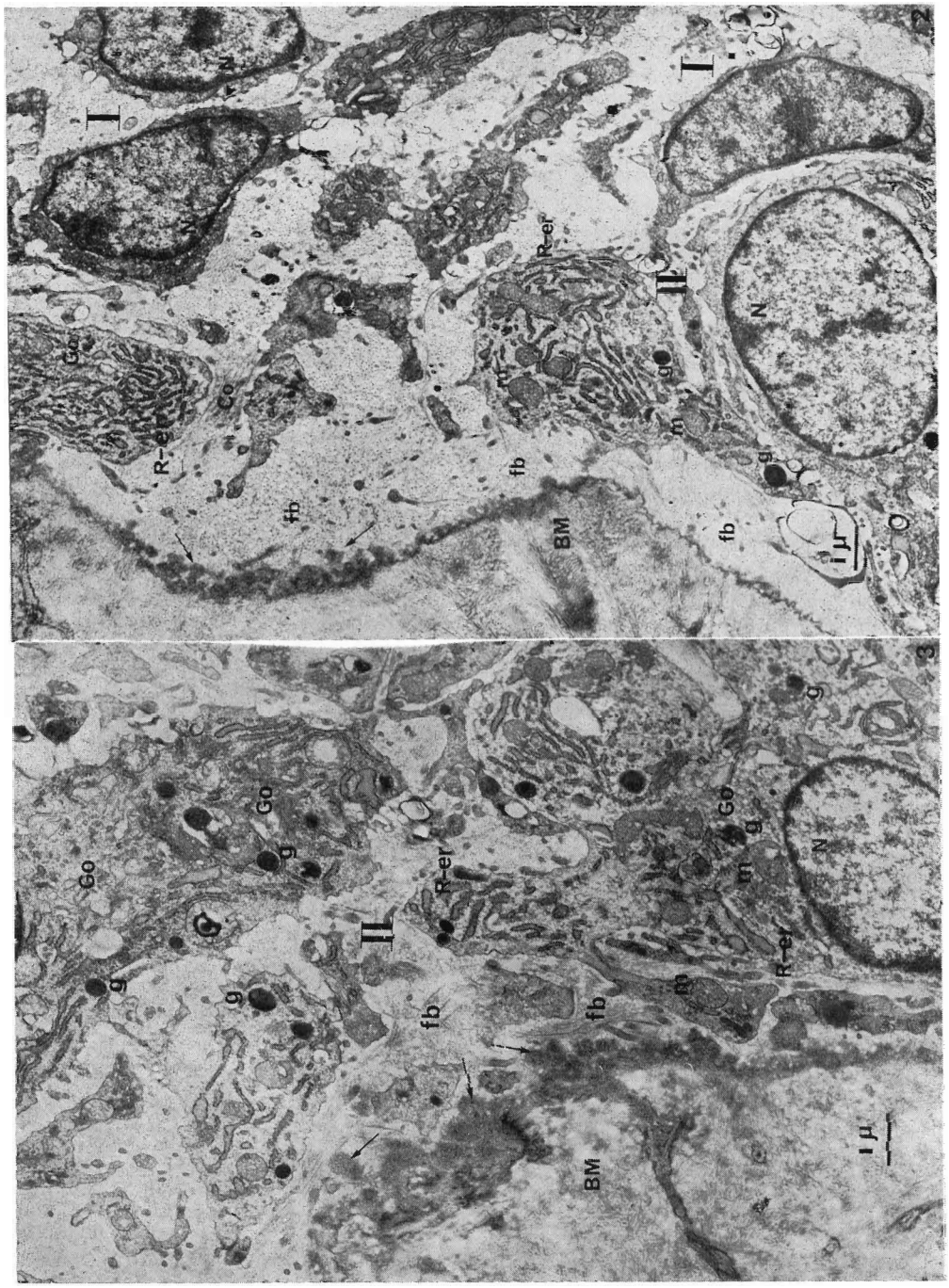
There is a difference between the osteoclast in the experimental group and the osteoclast in the control group. ×15750

Fig. 12. Cytoplasm of the Osteoclast in the Control Group

This giant cell has numerous nuclei, numerous mitochondria, smooth surfaced endoplasmic reticulum, lysosomes, smooth vesicles and well developed microvilli on the cell surface adjacent to the bone matrix. ×19500

(Key to abbreviations used in figures)

BM:	Bone Matrix	N:	Nucleus
Bm:	Basement Lamina	O:	Osteoid
Co:	Collagen fibrils	OB:	Osteoblast
fb:	fibrils	OCL:	Osteoclast
Go:	Golgi apparatus	OC:	Osteocyte
g:	secretory granules	r:	ribosome
Ly:	lysosome	R-er:	rough endoplasmic reticulum
m:	mitochondria	S-er:	smooth endoplasmic reticulum
mv:	microvilli	SV:	smooth vasicle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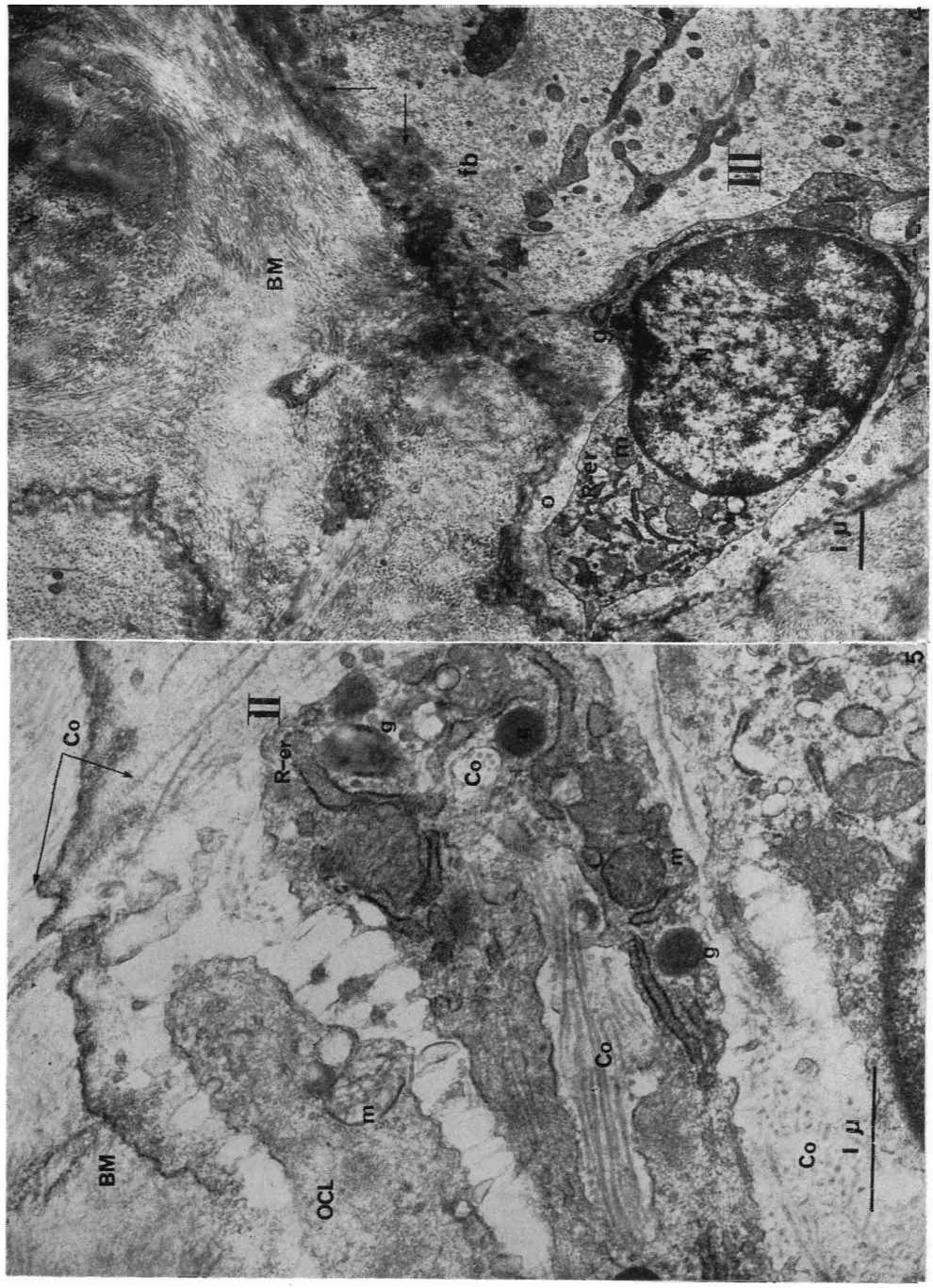
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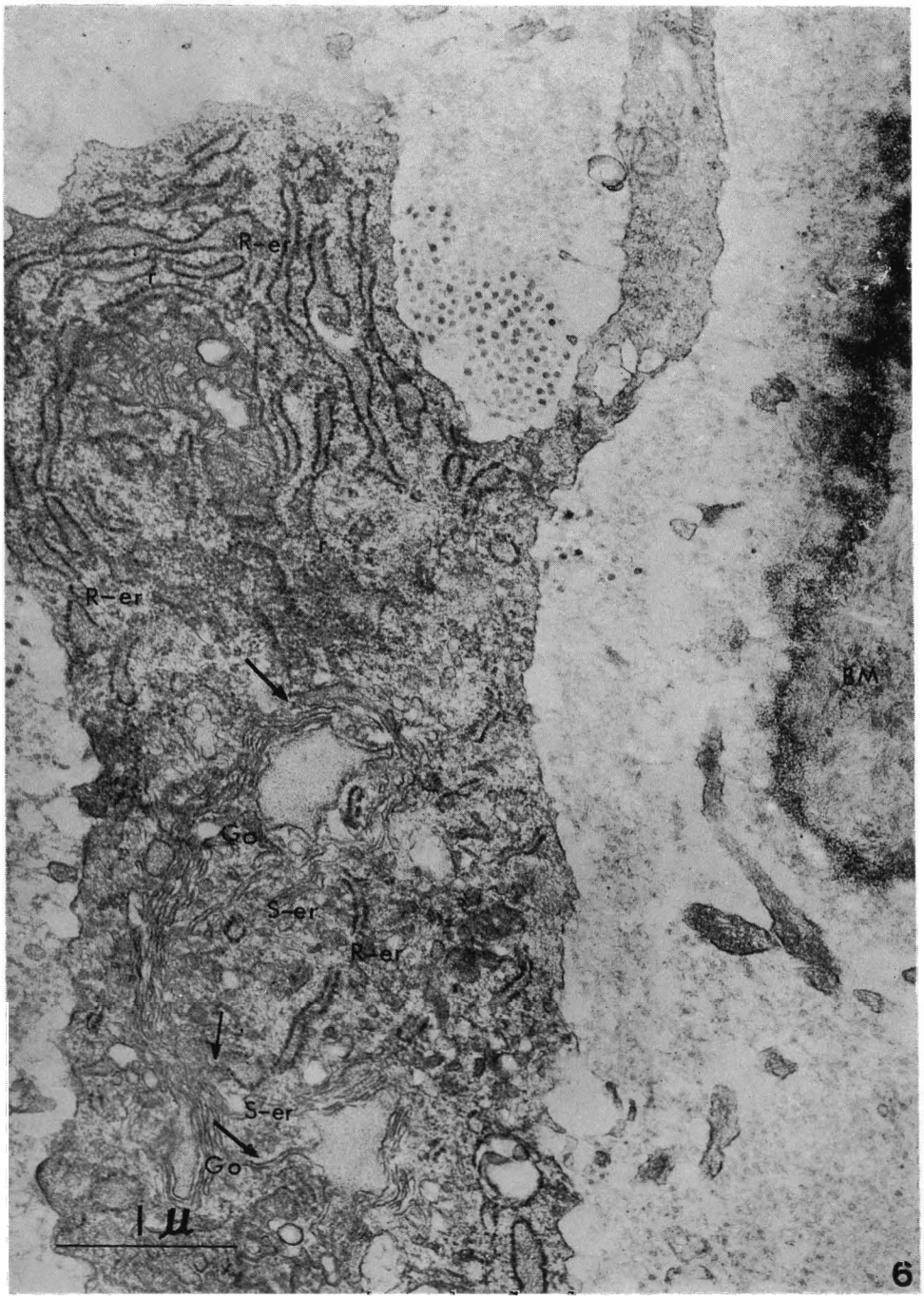
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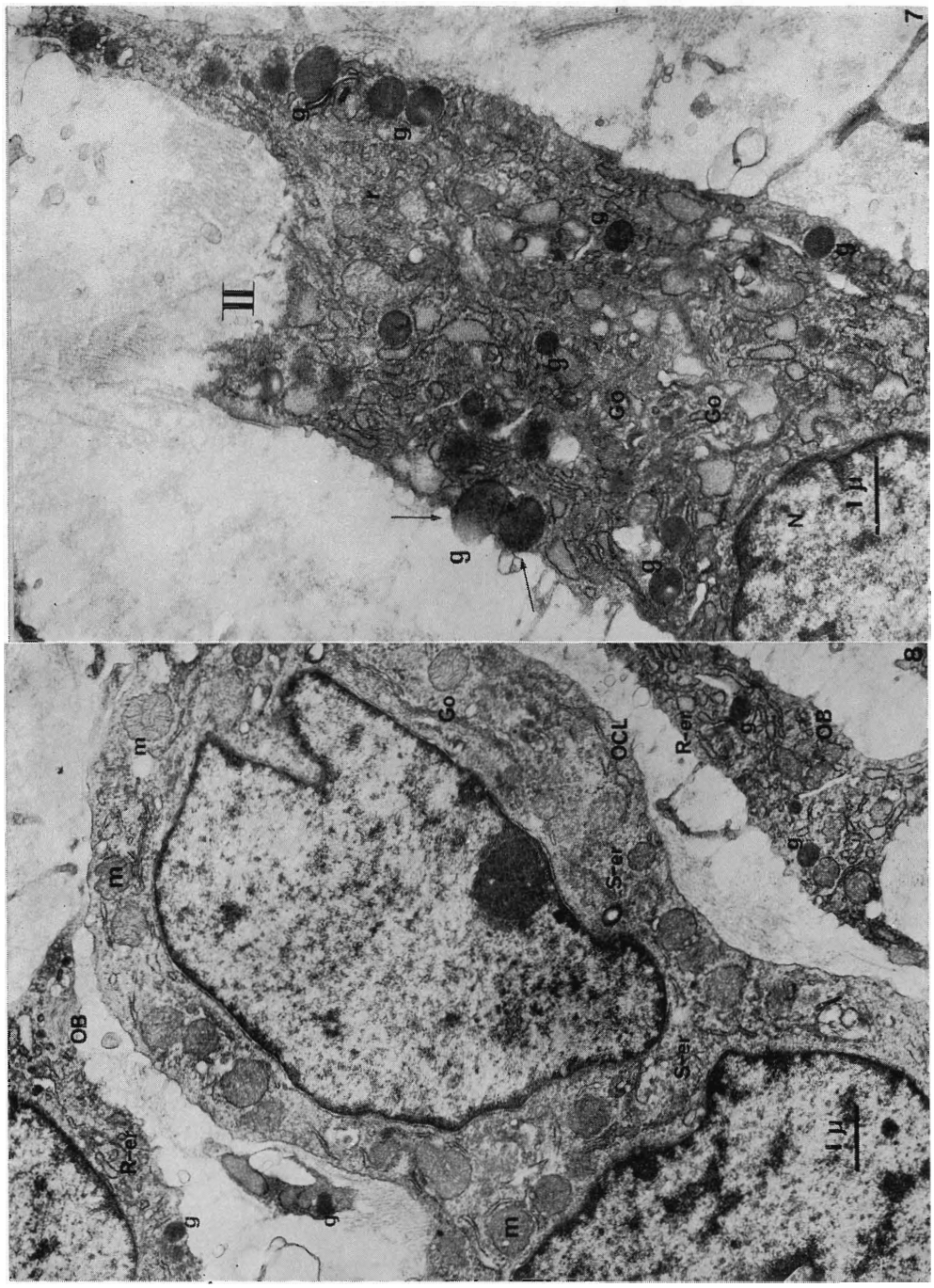
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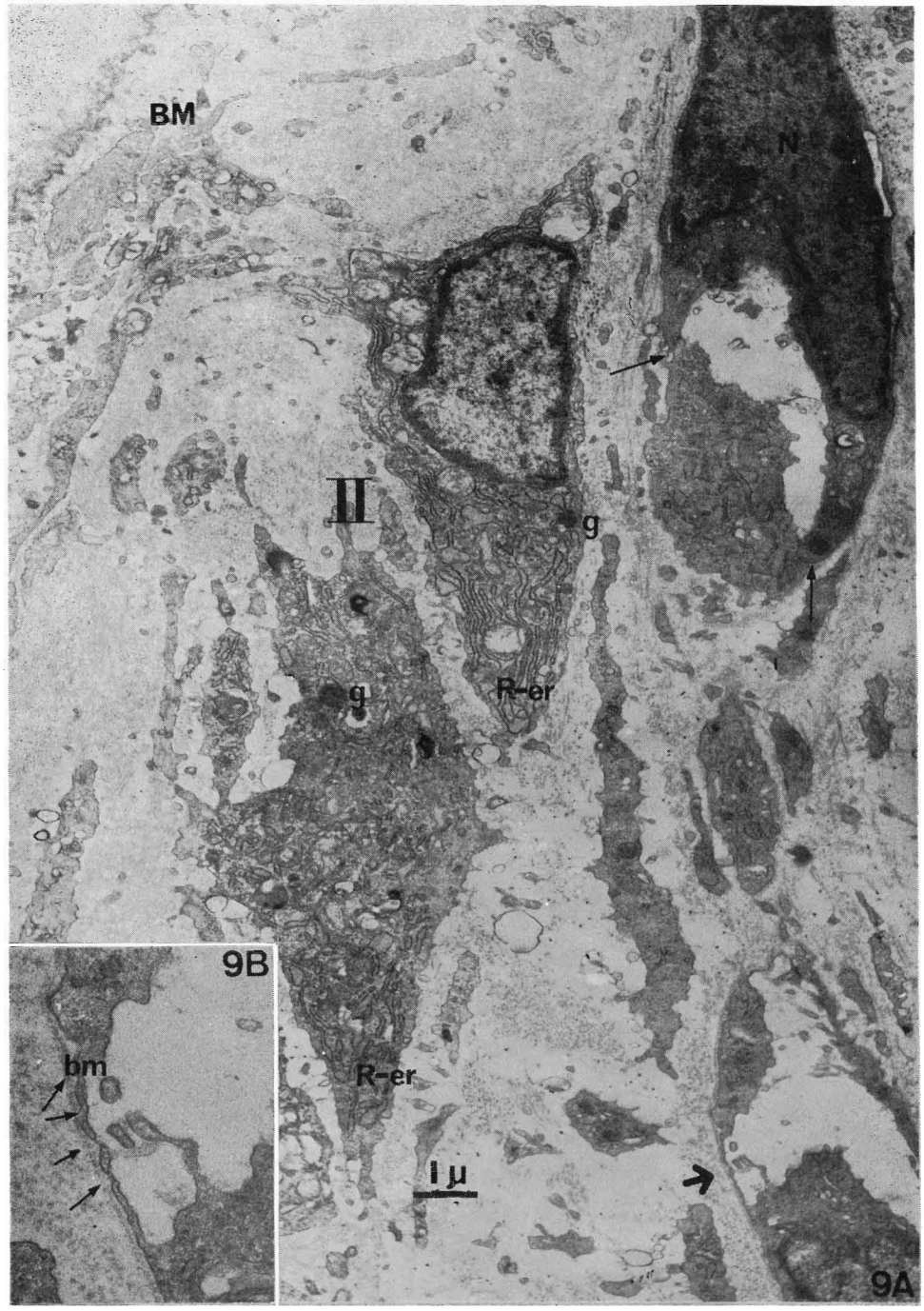
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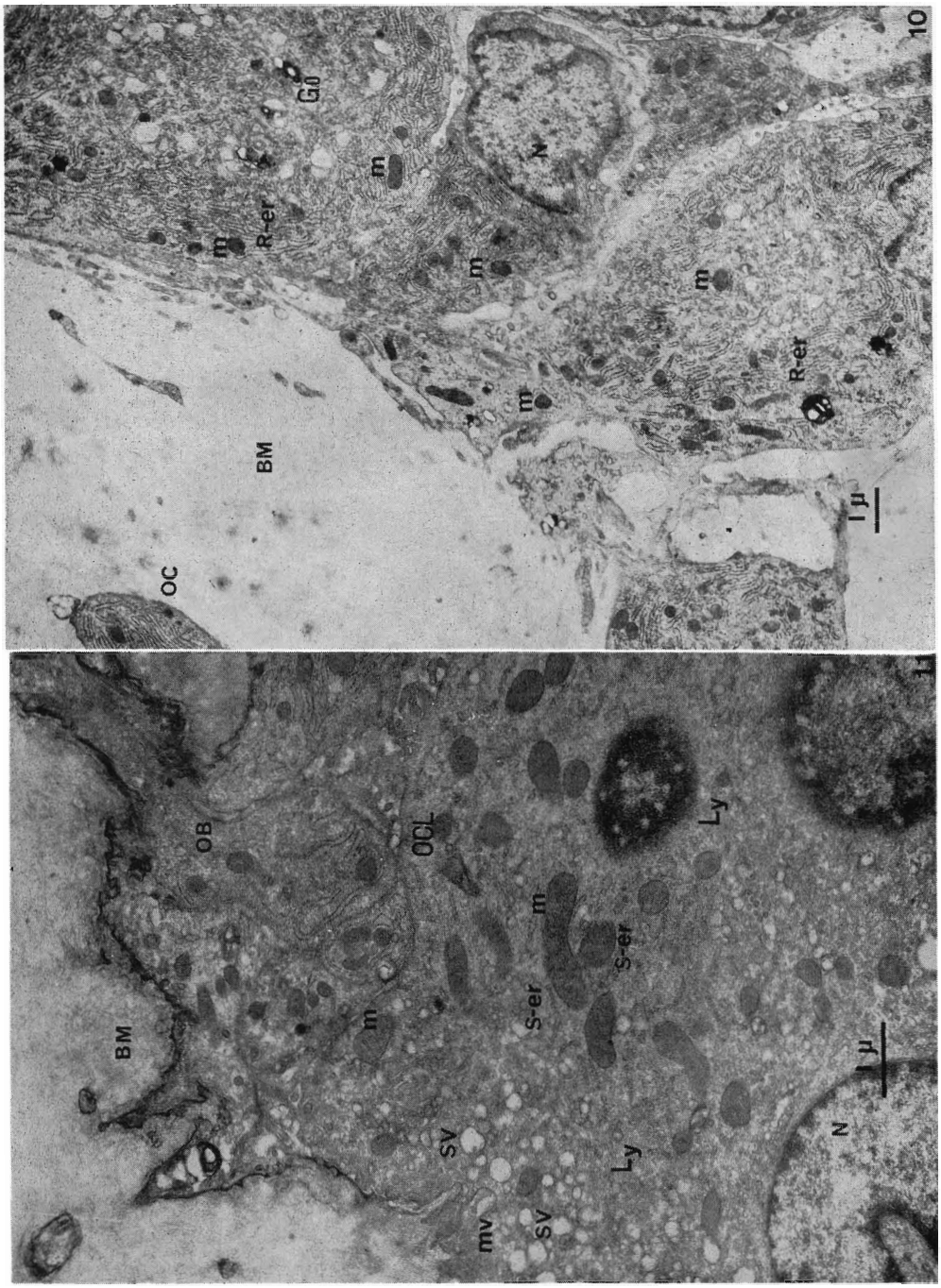
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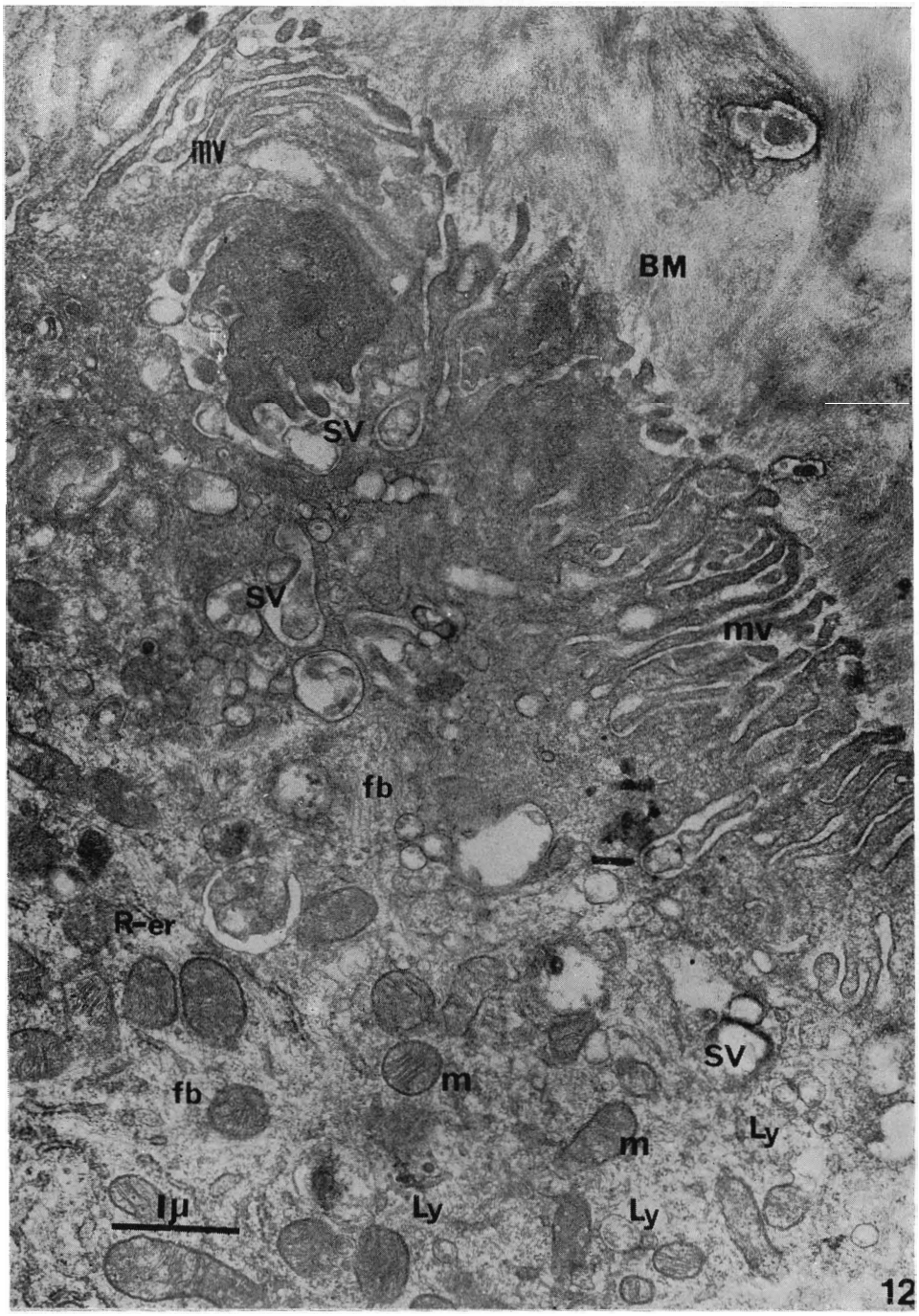
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