Fine Structure of the Membranes and Organelles in Parenchyma Cells of Poplar Shoot Observed by Freeze Etching Technique

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Summary——The cross- and surface-fractured membranes and organelles such as plasmalemma, tonoplast, endoplasmic reticulum, Golgi bodies, mitochondria, plastids and nucleus were investigated by freeze etching technique.

Particles, about 110 A in diameter, were found on both convex and concave planes of almost all the membranes and organelles. A great number of the particles were found on the convex plane of plasmalemma, while they were much less numerous on the concave plane. On the convex plane of tonoplast, and the membranes of endoplasmic reticulum and Golgi bodies, however, much less numerous particles were found than those on the concave plane. In mitochondria, plastids and nucleus which are known to have a double membrane, particles on the convex plane of outer membrane were much less numerous than those on the convex plane of inner membrane. On the other hand, the particles on the concave plane of outer nuclear membrane were numerous than those on the concave plane of inner nuclear membrane.

Similar particles as observed on the membrane surfaces were found on the fractured plane of cytoplasm, on the cross-fractured starch grain, and on the cross-fractured nuclei and nucleoli.

Because of general occurrence of the particles on almost all the membranes and organelles, it is unreliable that plasmalemma particles may be involved in the synthesis and orientation of cellulose microfibrils of poplar parenchyma cells.

Introduction

Freeze etching technique was first applied by STEERE (1957)[1] to the biological specimens. Later, MOOR and MÜHLETHALER (1963)[2] established the technique as a reproducible laboratory one by showing the fine structure of yeast cells in which the cytoplasmic membrane contains hexagonal arrangements of the particles which apparently involved in the production of the glucan fibrils. Further freeze etching studies of lower plants such as *Cladophora*, *Chaetomorpha* and *Oocystis* strengthened that the regular arrays of plasmalemma particles were involved in the synthesis and orientation of cell wall fibrils[3~7].

BRANTON and MOOR (1964)[8] proposed that the freeze etching technique could be applied to the ultrastructural studies on the multicellular tissues of higher plants. NORTHCOTE and LEWIS (1968)[9] further studied the freeze-etched surfaces of membranes and organelles in the cell of pea root tips. Although the particles on the plasmalemma surface distributed randomly through these and other investigations[10~12], they were also

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considered to be involved in the synthesis of cellulose microfibrils. CHAFE and WARDROP (1970)[13] studied the particle distribution on the plasmalemma of differentiating xylem fibers of *Eucalyptus maculata*, of the cortical parenchyma of *Avena sativa* coleoptiles and of the collenchyma of *Apium graveolens* by the freeze etching technique. However, they found no correlation between the particle distribution and the microfibril orientation. Thus, the participation of plasmalemma particles on the involvement of orientation and synthesis of cellulose microtibrils in higher plants is not fully understood.

Previous papers on the freeze-etched study of higher plants[8~15], have indicated that the particles occur on almost all the membranes of organelles. MATILE and MOOR (1968)[14] first pointed out the different distribution of particles on convex and concave planes of the membranes of endoplasmic reticulum, Golgi bodies (and their vesicles) and vacuoles during the genesis of vacuoles of corn root tip cells. BRANTON (1969)[15] summarized the distribution and dimension of particles from the figures reported previously by several authors. However the distribution of particles is not fully investigated on all the membranes and organelles.

The present paper reports the distribution of the particles on the membranes and organelles of poplar parenchyma cells and discusses them in relation to the synthesis and orientation of microfibrils.

Material and Methods

Fresh branches were cut in resting time from poplar tree (*Populus nigra* var. *italica* Koehne) growing in the experimental field of Wood Research Institute. They were cultured in water in a dark room conditioned at 28°C. After about a week etiolated shoots grew out. Tissues near the tip were cut from actively growing young shoots. They were then subdivided into small pieces, which were fixed in 2.5 % glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) for 6~12 hr. After fixation the specimens were washed thoroughly and immersed into 50 % glycerol solution in 0.05 M phosphate buffer (pH 6.8), and then the specimens were put in a small holder and frozen rapidly in liquid nitrogen.

The freeze etching technique employed was that of NISHIURA (1972)[16], using a commercial Hitachi freeze etching apparatus. A small holder was transferred to the main apparatus which was kept at −196°C with liquid nitrogen. After the desired pressure and temperature relations in a vacuum chamber have been met, the internal structure was exposed by cutting or fracturing the frozen tissue. Thereafter, etching was conducted on standard conditions of specimen; temperature at −100°C, pressure under 1×10^{-5} Torr and sublimation time for 2 min.

Replication was carried out by Pt/Pd shadowing and carbon coating. Replicated

tissue was soaked in a solution composed of acetic acid and sodium chlorite for 1 hr at 60~70°C. The tissue was then soaked in 72% (w/w) sulphuric acid for 1 hr at 60~70°C, washed with water and soaked in a commercial bleaching solution for 1 hr at 60~70°C. The tissue was then washed with water again and taken up on a grid mesh, and examined with JEM T6S electron microscope at 60 kV.

Results

As the specimens used in the present investigation were all chemically fixed and immersed in a high concentration of glycerol (50%), they were presumed to be fairely modified from those of normal physiological conditions. In spite of the above shortcoming, it is still valuable to apply the freeze etching technique to observe the organelles of the cells of young poplar shoots because not only the surface structures of membranes and organelles but also their three-dimensional structures can be seen. Fig. 1 shows such a typical freeze etching image of poplar parenchyma cell. Freeze-etched replicas commonly show a fine granular appearance on membranes, organelles and on the fractured plane of the cytoplasm. The appearnce of all the organelles was similar to that observed by sectioning technique. When the specimens were freeze-etched, particles having average diameter of 110 A were found, and when they were fractured without further etching, particles having average diameter of 150 A, were found on the membranes and organelles (Figs. 2 and 3).

Plasmalemma

Plasmalemma surfaces were characterized first by the 110 A particles as in the case of the membrane faces of organelles. Besides, plasmalemma particles on the convex plane were much more numerous than on the concave plane (Fig. 1). These particles distributed randomly without any regular arrangement. Other characteristics of plasmalemma were the small pores and protuberances at the pimary pit fields (Fig. 4), lomasome like structures (Fig. 5) and fibrillar structures (Fig. 4).

In this article, the distribution of particles is discussed and the more detail structure of plasmalemma surface will be delt with another report.

Tonoplast and Vacuole

A cross-fractured vacuole can be identified by a single membrane which surrounds quite a smooth surface of cell sap without any particles (Fig. 6). Vacuoles included large and/or small spherical bodies (Figs. 7 and 8), some of which were characterized by the regular pattern of their surface; these structures looked like a golf ball (Fig. 8). Particles on the convex plane (Fig. 9) were much less numerous than those on the concave plane (Fig. 7).

Endoplasmic Reticulum

In a great number of ER their cross-fractured plane were exposed and, in lesser degree, their surface-fractured plane were exposed. The appearance of cross-fractured ER was quite similar to that observed by sectioning methods. The concave plane was densely sculptured with particles, while the convex plane holded fewer particles (Figs. 6 and 9). Some ER had pores (about 0.1～0.2 μ in diameter) on their membrane (Figs. 6 and 11).

Golgi body

Cross-fractured Golgi bodies were similar to those observed by sectioning method. A Golgi body consisted of 6～7 cisternae. However, in Fig. 13, it seems that a Golgi body consists of twelve cisternae, because of a Golgi cisterna revealing both convex and concave planes. Golgi bodies of poplar species did not show the fenestrated and anastomosed system as diagramatically illustrated by MOLLENHAUER and MORRE (1966)[14], but more simple one although many vesicles located around Golgi cisternae. The cisternae was found to have much more particles on the concave than on the convex plane (Figs. 12 and 13). Particles on the convex plane of Golgi vesicles were much less numerous than on the concave plane (Figs. 12, 13 and 14).

Mitochodria

Cristae of mitochondria were observed in their cross fracture (Figs. 9 and 10). Surface fractured plane of mitochondria, however, sometimes showed a very uneven face (Fig. 14). This may be because the membrane of mitochondria has two fractured face and the outer membrane is fractured away, leaving the convex plane of outer or inner membrane just like the islands. Particles on the convex plane of outer membrane were much less numerous than those on the convex plane of inner membrane. Particle distribution on the concave plane of both outer and inner membrane was not confirmed.

Plastid

Much less particles were found on the convex plane of outer membrane than on the convex plane of inner one (Fig. 15), while the distribution of particles was not discernible on the concave plane of the outer and inner membranes. Some plastids were found to have many protuberances on the concave plane of inner membrane (Figs. 16 and 17). Particles similar to those on the plastid membranes were also found on the cross fracture plane of starch grains (Fig. 18).

Nucleus

Fig. 19 shows the nucleus at the telophase of mitosis of poplar parenchyma cells. Nuclear membrane was recognized as a double membrane in cross-fractured sections (Figs. 1 and 19). The interior of the cross-fractured nucleus showed a granular appearance, and the localized distribution of these granules was found. Particle-assembled regions (Fig. 19, short arrows) might show the occurrence of chromosomes, and nucleoli

could easily be identified by their strongly granular appearance (Fig. 19, long arrows). Fig. 20 shows the large magnification of the inlet of Fig. 19, and Fig. 21 is an interpretative diagram of what can be seen in Fig. 20. It shows that during fracturing, both outer and inner nuclear membranes are split to expose their internal faces. Fig. 22 shows the surface-fractured nuclear membrane. On the convex plane of outer membrane (A), the pores were convoluted below the fractured plane and particles attached to it were very few. On the other hand, the convex plane of inner membrane (B) was slightly raised at the edges of the pores and carried much more particles. Fig. 1 shows a part of the surface of fractured nuclear membrane. The concave plane of outer membrane (A′) was slightly raised at the edges of the pores and holded many particles. On the other hand, the pores on the concave plane of inner membrane were convoluted below the fracture face and particles attached to it were few. Thus, it was assumed that as A and A′ were complementary, so B and B′ were in relation with the distribution of particles.

Discussion

Almost all the organelles such as ER, Golgi bodies, mitochondria, plastids and nucleus were similar to those observed by sectioning method. 150 A particles observed by freeze fracturing without further etching were somewhat larger than their native form possibly by the presence of a little water (or ice crystals) surrounding them. Thus, 110 A particles observed by freeze etching seemed to show their original dimension.

The distribution pattern of particles on the membranes and organelles is classified into three groups:

1. Plasmalemma carries numerous particles on the convex plane, although the particles on the concave plane are much less numerous.

2. The convex plane of tonoplast, ER and Golgi bodies (and Golgi vesicles) carries fewer particles, while the concave plane is densely sculptured with particles.

3. Cell organelles such as mitochondria, plastids and nucleus which consist of a double membrane have normally two fractured planes; the convex plane of outer membrane carries fewer particles than that of inner membrane, while particles on the concave plane of outer membrane are much numerous than those on the concave plane of inner membrane. The latter fact was confirmed in the case of nucleus, but not of mitochondria and plastids. If the concave plane of the membranes of mitochondria and plastids shows an alternative case, then the fourth group will be settled. Although randomly scattered particles on plasmalemma surface have been considered to be involved in the formation of cellulose microfibrils as described in "Introduction", the present results suggest that plasmalemma particles may not be concerned with the syn-

thesis and orientation of cellulose microfibrils. Because the fact that similar particles were found on the plasmalemma, tonoplast and the membranes of all the organelles is considered as the common feature of the fractured plane of the membranes and organells.

The exact behavior of the fractured planes along plasmalemma, tonoplast and the membranes of cell organelles is still not clearly understood. MOOR and MÜHLETHALER (1963)[2] described that freeze etching normally reveals the true surfaces of biological membranes, and this view was subsequently accepted by many authors[5,6,8~10,12,17,18]. BRANTON[15,19,20] presented an alternative interpretation of frozen etched membrane structures by showing that a small ridge is present at the base of many exposed membrane faces and that the ridge is continuous with and identical to one of the ridges assumed to represent a part of a unit membrane. NORTHCOTE and LEWIS (1968)[9] stated that the images produced from the pea root tissue indicated the best interpreted replicas of the external surfaces of the membranes or of the underlying surfaces after the membranes had been fractured away. On the other hand, HEREWARD and NORTHCOTE[21] examined the localization of freeze fracture planes of yeast membranes by the technique of freeze substitution using OsO_4 and stated that not only tonoplast but also plasmalemma split between two darkly staining lines, although the other cases were also found and interpreted. In the present investigation, it can be best interpreted that the membranes were split at the internal face from the evidence of the complementary distribution of particles both on the convex and concave plane of the plasmalemma, tonoplast and the membranes of organelles, and especially from the evidence of complementary appearance of nuclear pores both on convex and concave planes, and from the results similar to BRANTON[19] that a small ridge present at the base of exposed nuclear membrane faces.

Although the particles are thought to be composed of grobular proteins[22], their dense and general occurrence on the fractured plane of the cytoplasm, and on cross-fractured starch grains, nucleus and nucleoli may suggest their different nature for the particles for which cellulose synthesis has been ascribed.

The functional significance of newly found spherical bodies in the vacuole and the protuberances on the concave plane of plasid membrane is remained to be solved in the further studies.

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References

1) R. L. STEERE, J., Biophys. Biochem. Cytol., **3**, 45 (1957).
2) H. MOOR and K. MÜHLETHALER, J. Cell Biol., **17**, 609 (1963).
3) R. D. PRESTON, In "Formation of Wood in Forest Trees", 169, M. H. Zimmermann, ed., London and New York, Acad. Press, 1964.
4) J. R. BARNETT and R. D. PRESTON, Ann. Bot., **34**, 1011 (1970).
5) D. G. ROBINSON and R. D. PRESTON, J. Cell Sci., **9**, 581 (1971).
6) D. G. ROBINSON and R. D. PRESTON, Planta (Berl.), **104**, 234 (1972).
7) D. G. ROBINSON, R. K. WHITE and R. D. Preston, Planta (Berl.), **107**, 131 (1972).
8) D. BRANTON and H. MOOR, J. Ultrast. Res., **11**, 401 (1964).
9) D. H. NORTHCOTE and D. R. LEWIS, J. Cell Sci., **3**, 199 (1968).
10) K. MÜHLETHALER, In "Cellular Ultrastructure of Woody Plants", 51, W. A. Cote, Jr., ed., Syracuse Univ. Press, 1965.
11) L. A. STAEHELIN, Z. ZELLFORSCH,. **74**, 325 (1966).
12) K. MÜHLETHALER, Ann. Rev. Plant Physiol., **18**, 1 (1967).
13) S. C. CHAFE and A. B. WARDROP, Planta (Berl.), **92**, 13 (1970).
14) P. MATILE and H. MOOR, Planta (Berl.), **80**, 159 (1968).
15) D. BRANTON, Ann. Rev. Plant Physiol., **20**, 209 (1969).
16) M. NISHIURA, Kagaku (In Japanese), **42**, 431 (1972).
17) L. A. STAEHELIN, Proc. Roy. Soc. B, **171**, 249 (1968).
18) H. MOOR, Intern. Rev. Cytol., **25**, 391 (1969).
19) D. BRANTON, Proc. Natl. Acad. Sci. U. S., **55**, 1048 (1966).
20) D. BRANTON and R. B. PARK, J. Ultrast. Res., **19**, 283 (1967).
21) F. V. HEREWARD and D. H. NORTHCOTE, J. Cell Sci., **10**, 555 (1972).
22) S. J. SINGER and G. L. NICOLSON, Science, **175**, 720 (1972).

Abbreviations used

CW	Cell wall	ER	Endoplasmic reticulum
G	Golgi body	L	Lipid droplet
Lo	Lomasome	M	Mitochondrion
Mi	Inner membrane of mitochondrion	Mo	Outer membrane of mitochondrion
N	Nuclei	Ni	Inner nuclear membrane
No	Outer nuclear membrane	Pi	Inner membrane of plastid
Po	Outer membrane of plastid	PL	Plasmalemma
PPF	Primary pit field	R	Small ridge at the base of exposed membrane
S	Starch grain	Sb	Spherical body like a golf ball
T	Tonoplast	V	Vacuole
⌒	Convex plane	⌣	Concave plane
⊖⇒	Direction of shadowing		

Fig. 1. Typical freeze etched image. Cytoplasm is occupied by a large nuclei. The concave planes of outer and inner nuclear membranes are shown. The convex and concave planes of plasmalemma are shown in the bottom. 110 A particles are seen not only on the plasmalemma, nuclear membrane and on the cross-fractured nuclei but also in the cytoplasm. ×26,500.

Fig. 2. Freeze etched plasmalemma. Particles, average diameter of 110 A, are shown on its convex plane. ×122,000.

Fig. 3. Freeze fractured plasmalemma. Particles, average diameter of 150 A, are shown on its

convex plane. ×122,000.

Fig. 4. Freeze fractured. Fibrillar structures and primary pit fields are seen on the convex plane of plasmalemma. ×8,000.

Fig. 5. Freeze etched. Lomasome like structures are seen on the convex plane of plasmalemma. ×25,000.

Fig. 6. Freeze etched. ER is sometimes characterized by 0.1～0.2 μ pores. Convex plane of ER holds lesser particles. No particles can be seen on the cross-fractured vacuole. ×26,500.

Fig. 7. Freeze fractured. Particles on the concave plane of plasmalemma are much less numerous than those on the concave plane of tonoplast. Cytoplasm is localized in the peripheral region. Cross fractured vacuole shows quite smooth plane. Spherical bodies are found in the vacuole (arrows). ×9,500.

Fig. 8. Freeze etched. Large or small spherical bodies like a golf ball are found in a vacuole. ×8,000.

Fig. 9. Freeze fractured. Many cross and surface fractured mitochondria are shown. Cristae of mitochondria are discernible. Convex plane of tonoplast holds lesser particles. ×26,500.

Fig. 10. Freeze fractured. Convex plane of ER carries much less particles than concave plane. ×28,000.

Fig. 11. Freeze etched. Three-dimensional conformation of ER is clearly seen. Many pores are found on its membrane. ×23,000.

Fig. 12. Freeze fractured. Many Golgi vesicles occur near the cisternae. Concave plane of Golgi cisternae holds numerous particles. ×28,000.

Fig. 13. Freeze fractured. Convex plane of Golgi cisternae carries lesser particles. ×48,000.

Fig. 14. Freeze etched. Surface-fractured mitochondria show very uneven face. ×22,000.

Fig. 15. Freeze fractured. The convex plane of a plastid is shown. The convex plane of its outer membrane carries much less numerous particles than that of its inner membrane. ×28,000.

Fig. 16 and 17. Freeze etched (Fig. 16). Freeze fractured (Fig. 17). Several protuberances are seen on the concave plane of inner membrane of a plastid. ×26,500 (Fig. 16). ×28,000 (Fig. 17).

Fig. 18. Freeze etched. Cross-fractured plane of two starch grains carries numerous particles. ×22,500.

Fig. 19. Freeze etched. The nuclei at the telophase of mitosis are shown. Cell plate is formed between two nuclei. Nuclear membranes show a double membrane structure. In cross-fractured nucleus, somewhat aggregated regions of particles correspond to the site of chromosome, while densely aggregated regions of them correspond to the site of nucleolus. ×21,500.

Fig. 20. The large magnitication of the inlet of Fig. 19. ×55,000.

Fig. 21. Diagrams of imaginary sections of nuclear membrane perpendicular to the plane of the page through the fractured tissue along the dashed arrows.

Fig. 22. Freeze etched. The convex plane of outer nuclear membrane carries much less numerous particles than that of inner nuclear membrane. Nuclear pores show concave feature on outer nuclear membrane, while they show convex feature on inner nuclear membrane. ×26,500.


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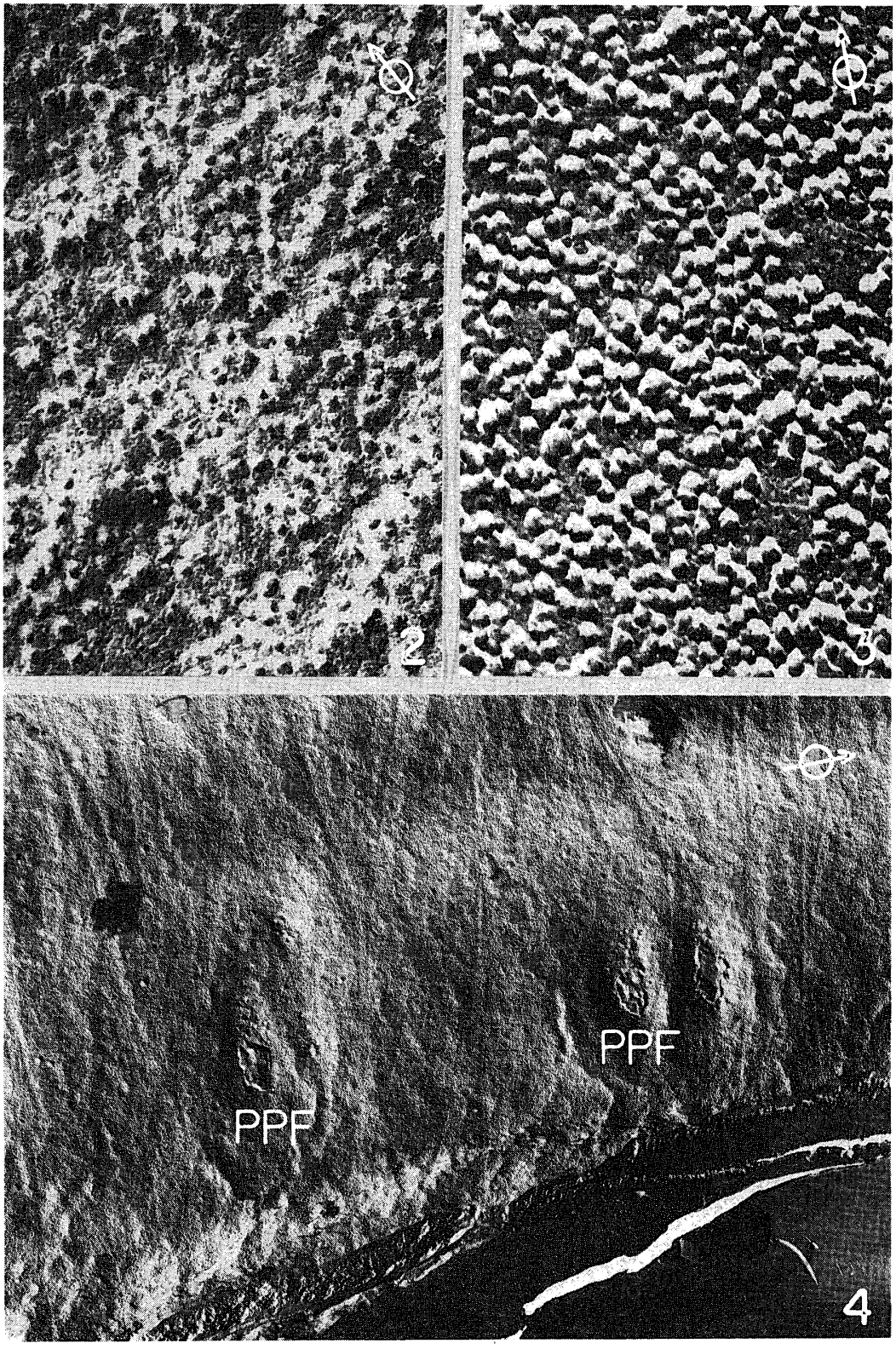
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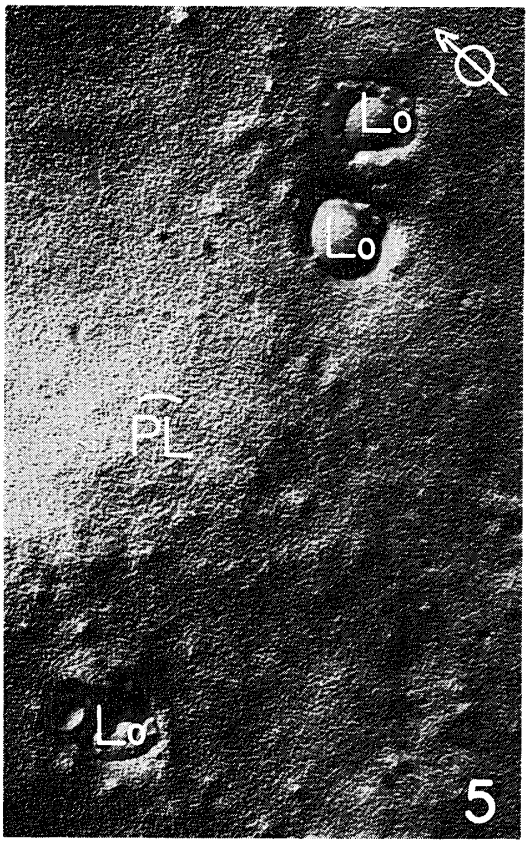
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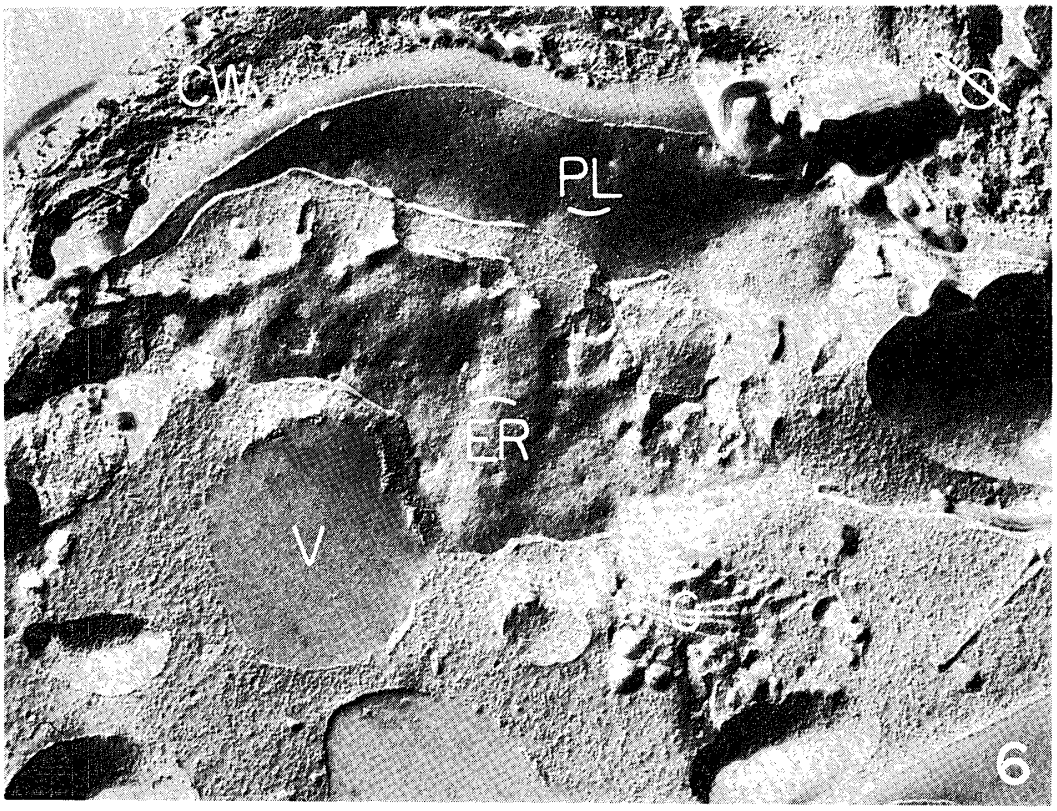
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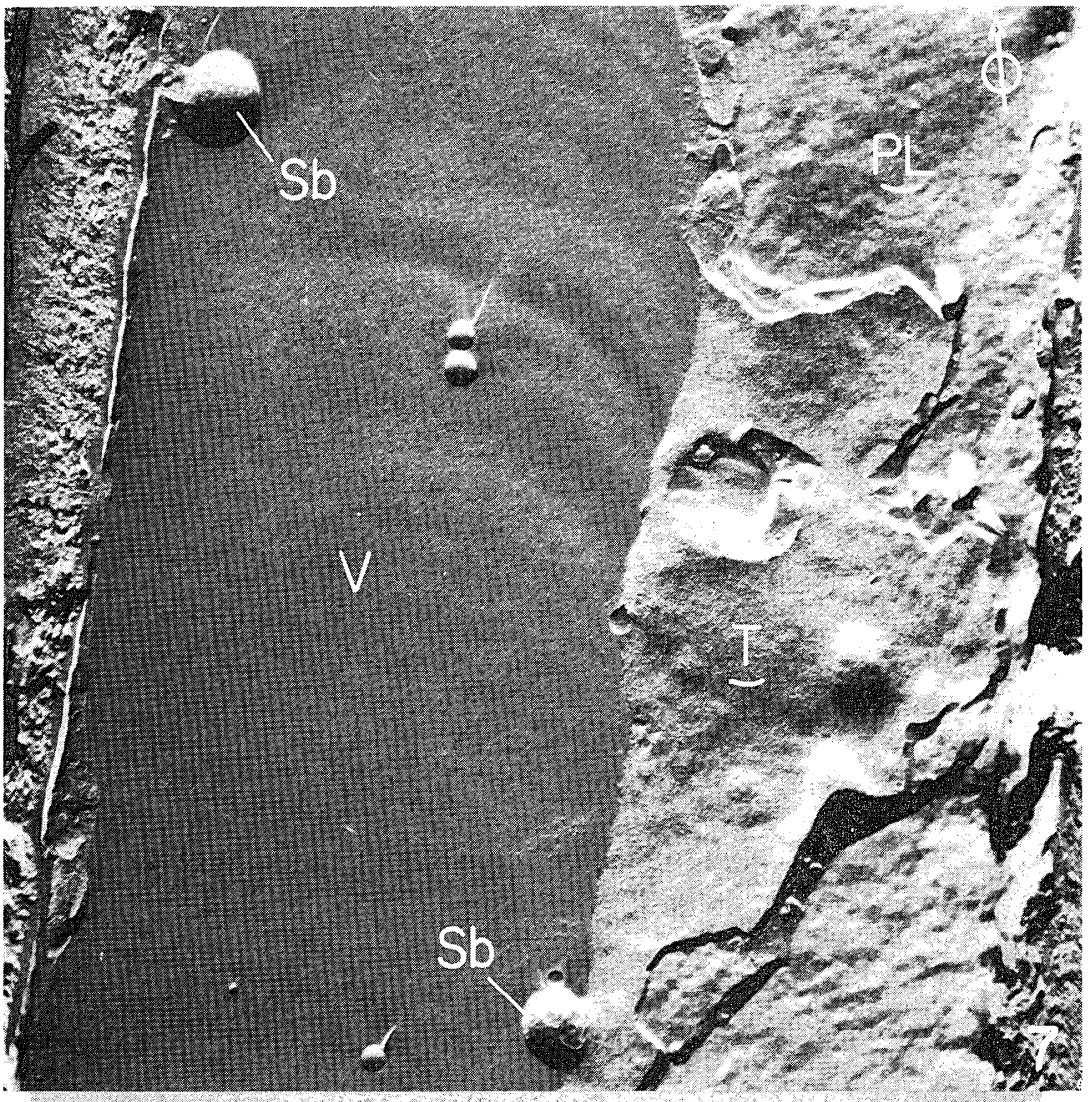
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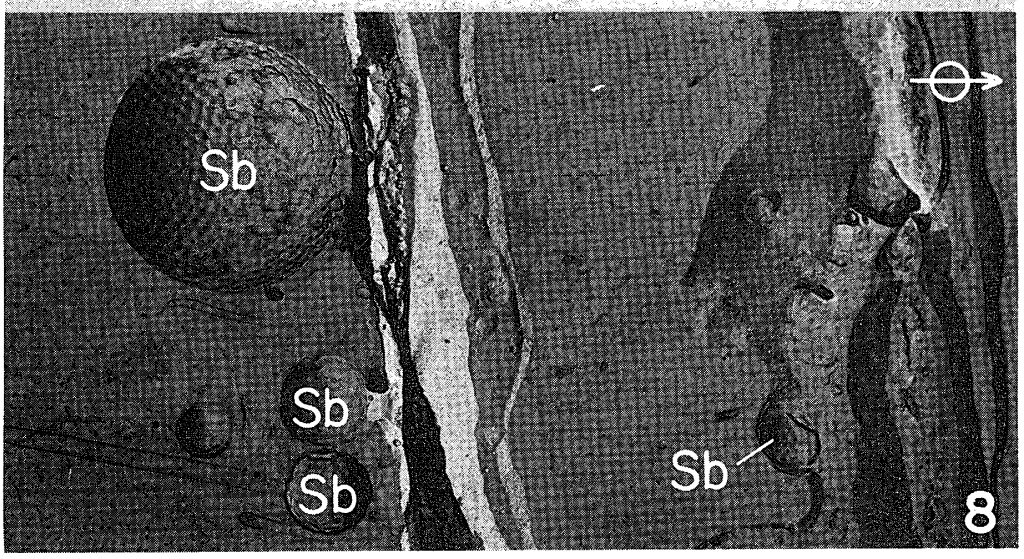
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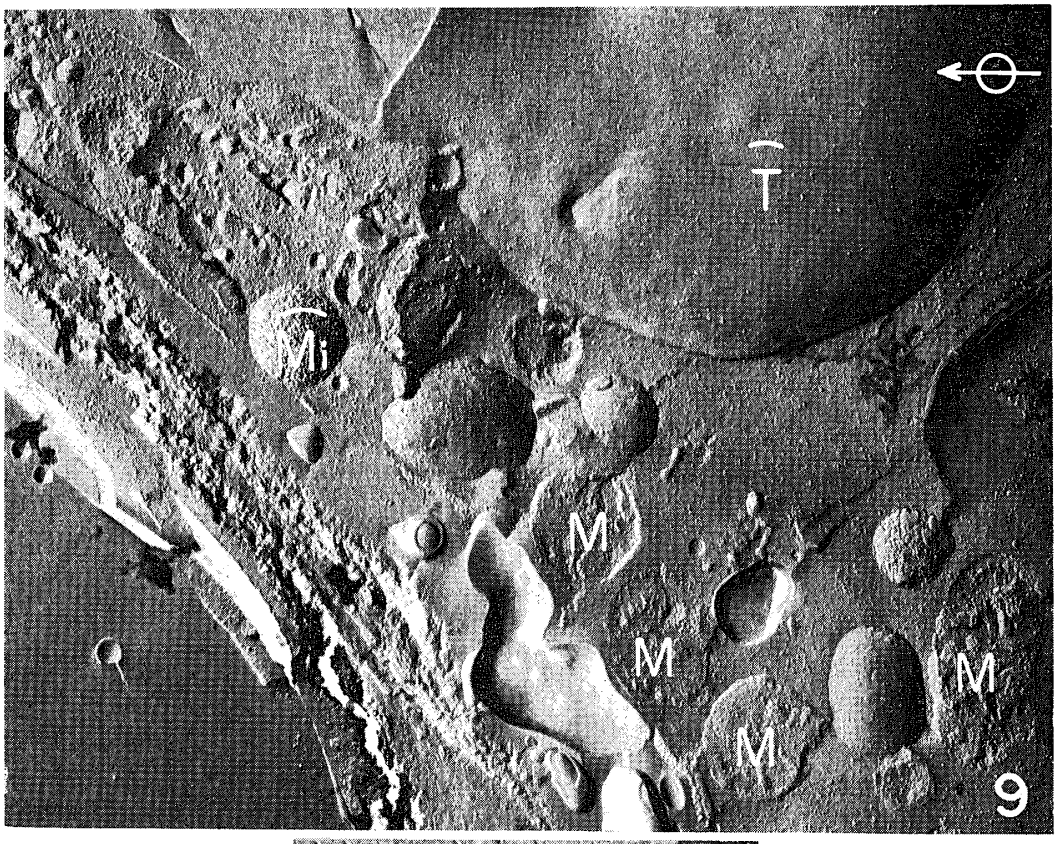
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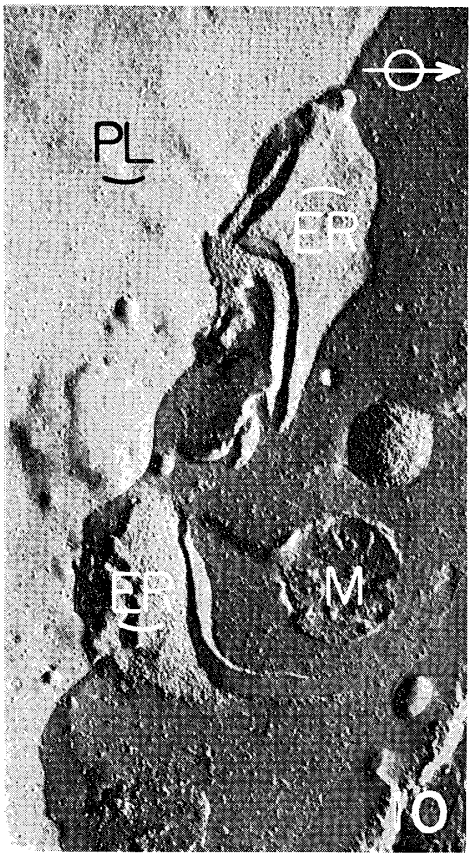
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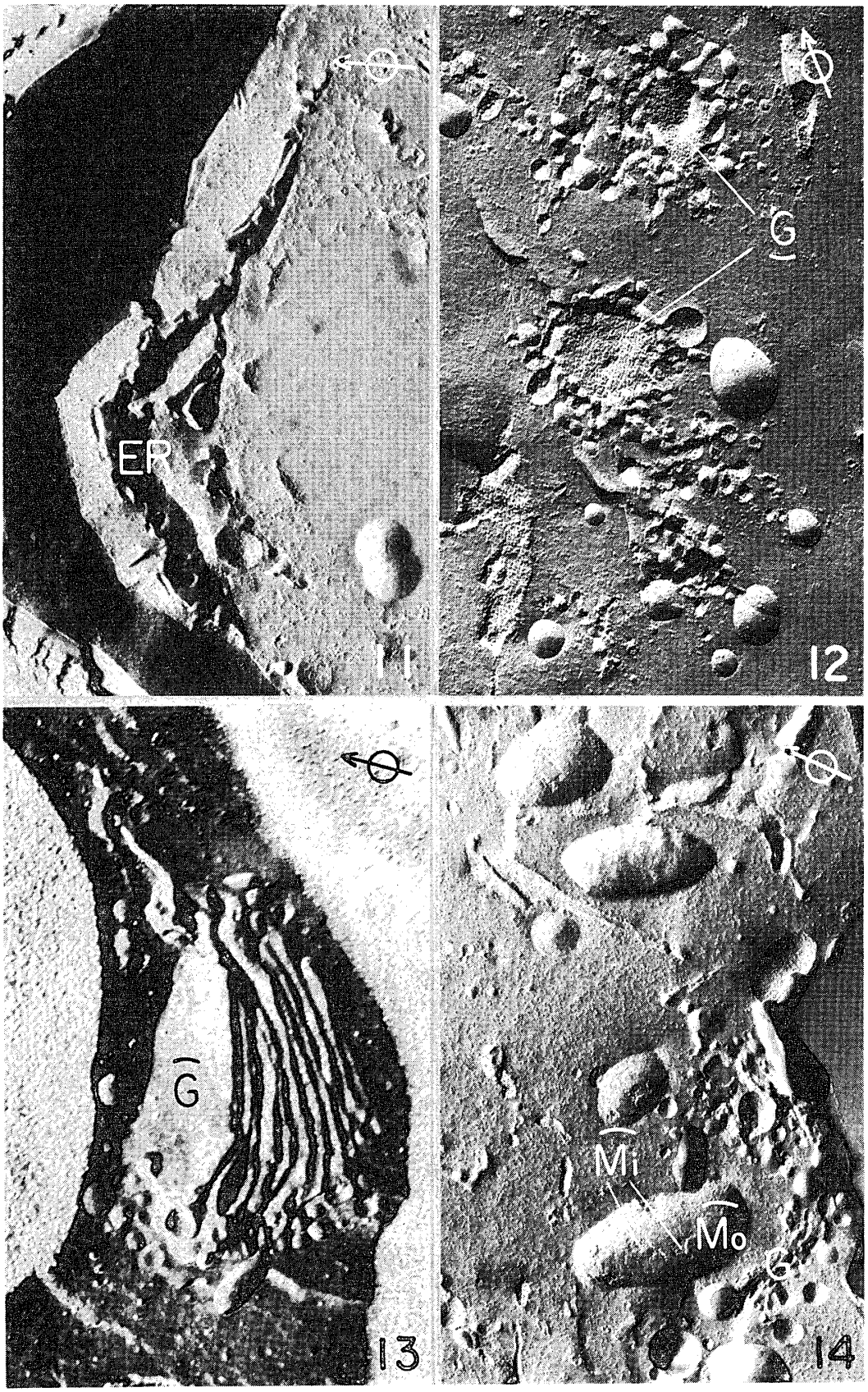
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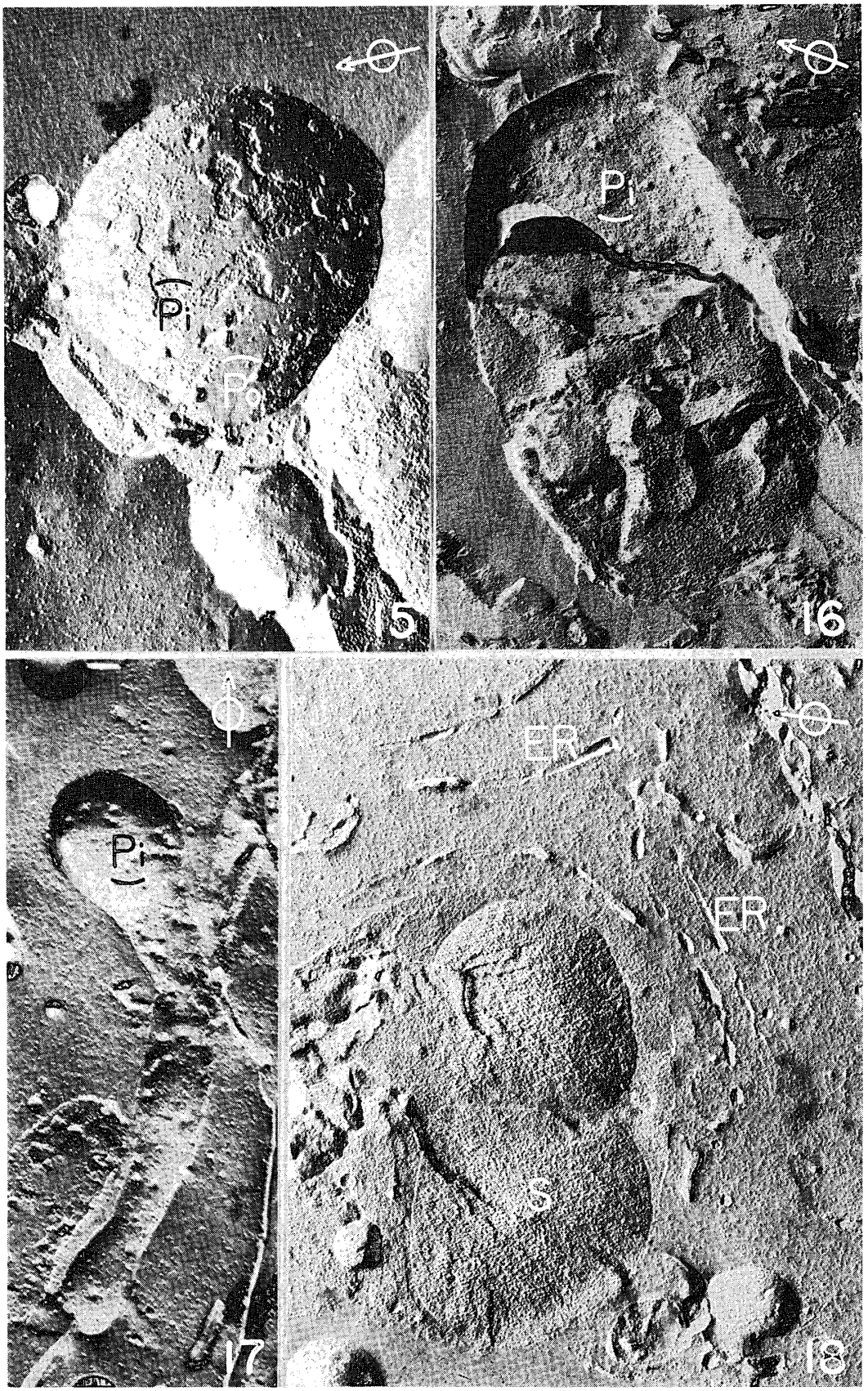
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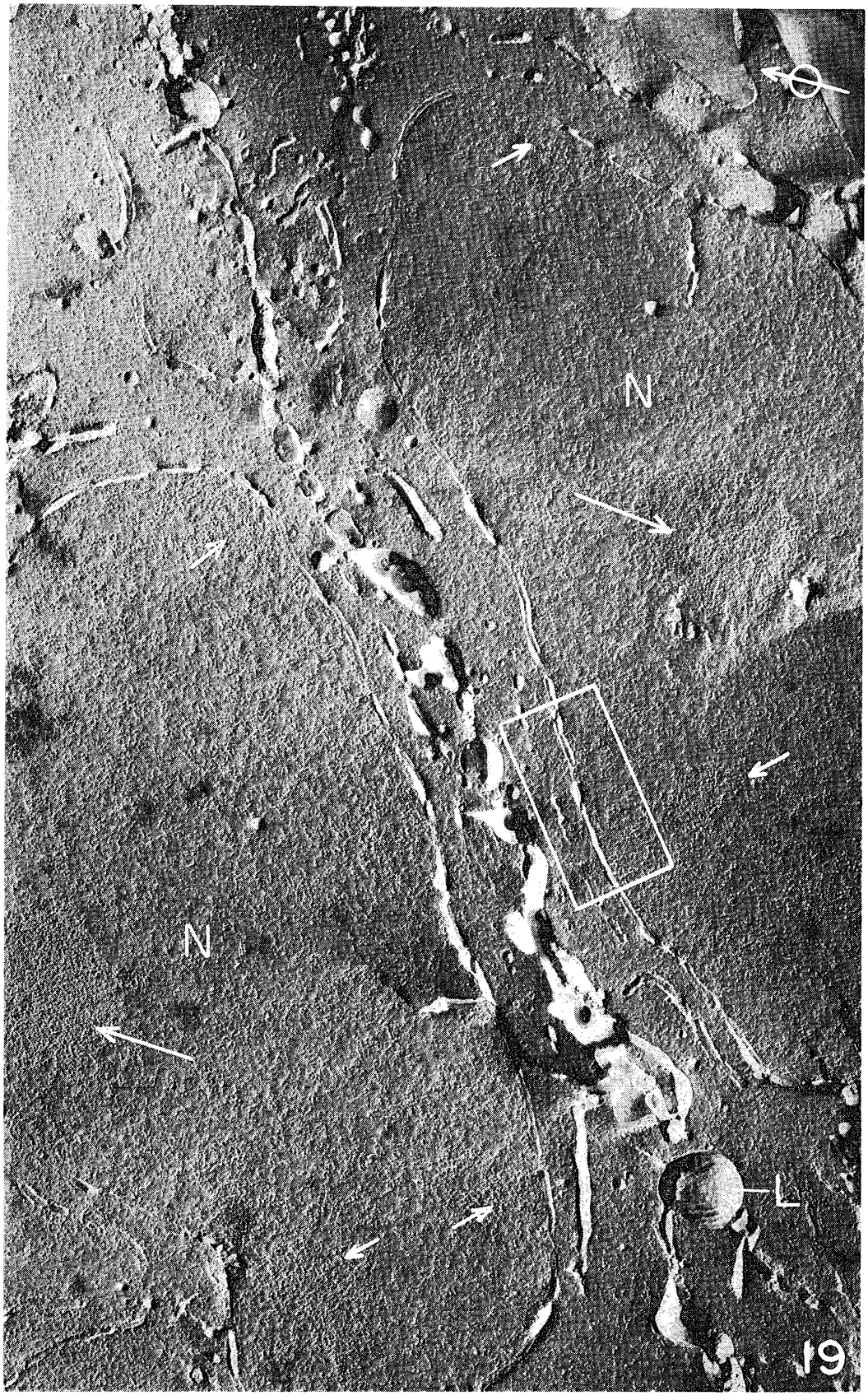
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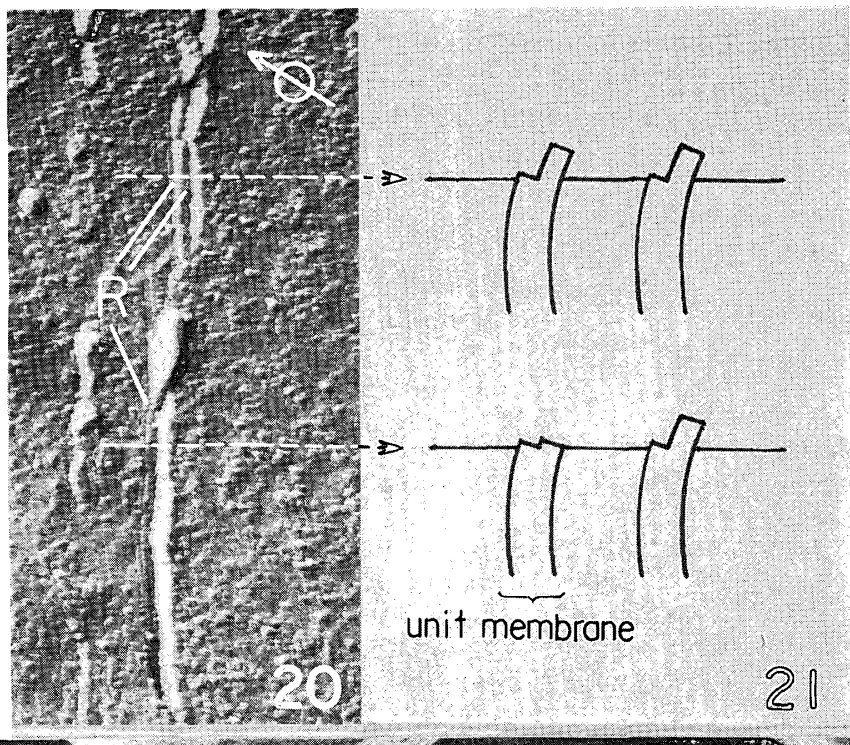
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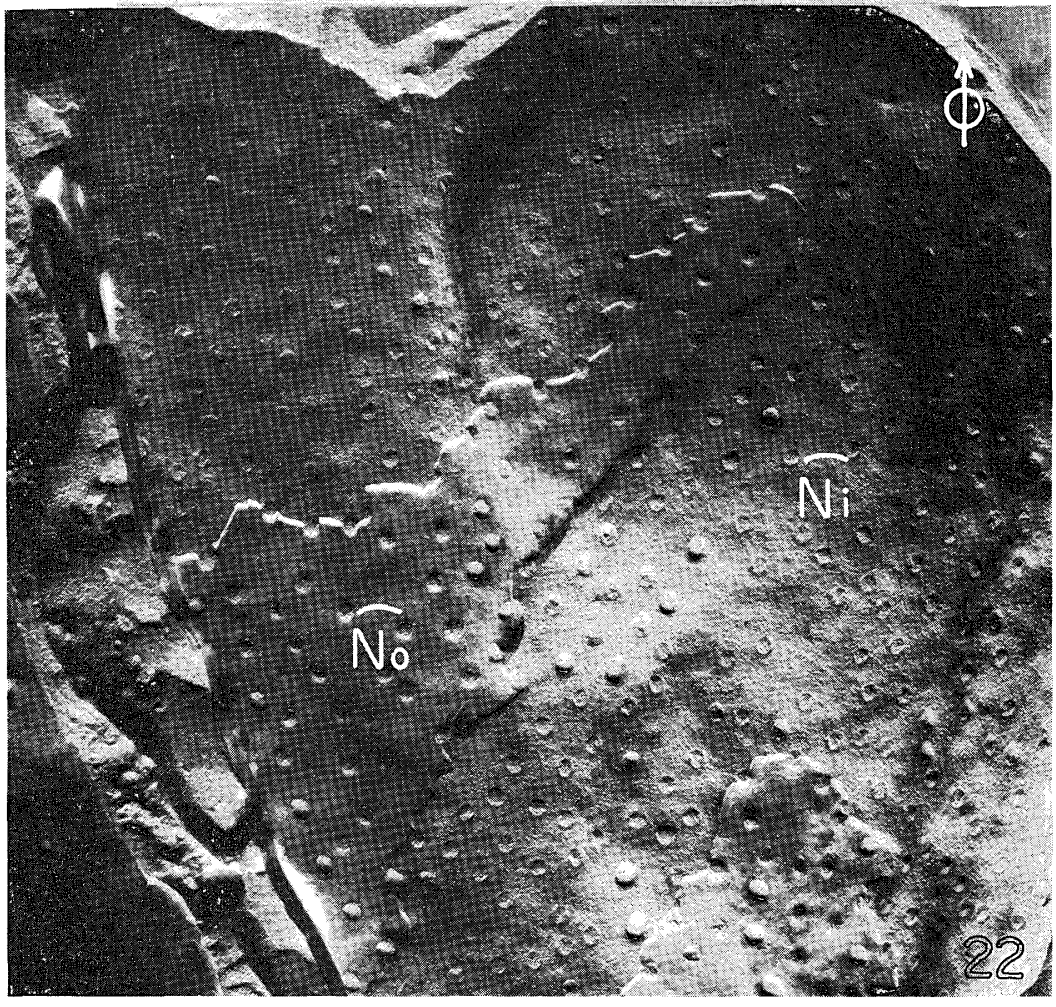
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