Fine Structure and Formation of Cell Wall of Developing Cotton Fiber*

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Abstract——In the stage of elongation growth of cotton fiber, there is a plenty of amoeboid plastids, lipid droplets and rough-surfaced endoplasmic reticulum (RER) in the cytoplasm. On the other hand, in the stage of secondary wall thickening, cytoplasm is poor in amoeboid plastids and lipid droplets, but rich in smooth- and rough-surfaced ER.

The fact that microtubules alter their orientation from perpendicular to parallel to the cell axis in the course of transition from primary to secondary wall deposition suggests that microtubules are responsible for the orientation of microfibril.

Through secondary wall growth, vesicle-secreting ability of Golgi bodies seems to be very poor. In view of almost all cellulose that composes the cell wall components of cotton fiber, Golgi-derived vesicles could not excrete cellulose precursor to the wall.

It was found that smooth-surfaced ER (SER) is derived from RER during the secondary wall thickening. In addition, vesicles (larger than Golgi-derived vesicles) and cisternae of SER appear most frequently near the plasmalemma and seems to be incorporated into the cell wall. Consequently, a close correlation between SER and wall synthesis is presumed in the development of cotton fiber.

The occurrence of intensively electron dense substances in a vacuole and paramural bodies is also discussed in relation to the formation of cell wall.

Introduction

Cotton fiber has been investigated of their physical and chemical nature since it is of great importance as a raw materials. Detailed information on the anatomy of cotton fiber has already been obtained using light microscope[1~4].

The outermost layered cell (epidermal cell) of cotton seeds, which develop just after the flowering, begins to elongate and reaches its full length after 20 days. Cotton fiber, in its early stage, has only primary wall and undergoes elongation growth, while after about 20 days secondary wall begins to deposit, followed by continuous thickening until the bolls open.

In the stage of elongation growth, the fiber develops with both the tip growth such in pollen tubes and root hairs and side wall thickening such in tissue parenchyma[5].

The earlier submicroscopic informations on cotton fibers are summarized by ROLLINS[6]. Some of them are as follows: (1) the wax coated pectinaceous cuticle corresponds to the middle lamellae of the cells from stem tissues: (2) the primary wall, less than 0.5 micron thick, is a net like woven fabric of cellulose microfibrils which, on the inner surface, appear to be almost transverse and, on the outer surface, nearly parallel to fiber axis: (3) the S-1 layer is very thin, probably not more than 1/2 micron in thickness; the S-2 layer constitutes the bulk of the secondary wall which may be as much as 5 micron thick; the cellulose of the S-2 layer appears to occur in compact layers of closely aligned microfibrils which lay down almost parallel each other and at a slight angle to the fiber axis; the evidence for the occurrence of the S-3 layer is inconclusive.

However, little or no informations about electron microscopical and cytological study are

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available for the development of cotton fibers. In view of the uniqueness of the constituents of the cotton fiber in which the amount of cellulose is more than 95 %, and lignins are not contained[7], it is important to investigate the correlation between wall formation and organelles such as microtubules, Golgi bodies and ER.

Material and Methods

After flowering of cotton plant (*Gossypium hirsutum* L.) growing in the experimental field of Wood Research Institute, Kyoto University, some of the cotton bolls in different developmental stages were collected and differentiating seeds picked out of the bolls were immediately fixed in 2.5 % glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 6–12 hrs. The specimens were fully washed in the same buffer solution and post-fixed again in 1.0 % osmium tetroxide in 0.05 M phosphate buffer (pH 7.2) for 6–12 hrs. After thoroughly washing, they were dehydrated through a graded concentration of acetones and embedded in Epon 812.

Thin sections were cut using glass knives and observed under ordinary and polarized light for distinguishing the initiation of secondary thickening. Ultra-thin sections were cut using glass knives on a Porter-Blum type MT-1 ultramicrotome and were mounted on 150 or 180 mesh copper grids. Sections were stained on the grids with uranyl acetate and lead citrate. The grids were examined in a JEM T6S electron microscope using 60 kV.

Results

Elongation Growth

As generally accepted, cotton fiber develops from single layered epidermal cells of the seed surface (Fig. 1). In the early stage of elongation growth, young cotton fibers are connected by the intercellular layer just as general tissue cells and with the proceeding of growth they begin to separate each other, leaving the remnants of intercellular layer at the outermost part of primary wall (Fig. 2). Though almost all of epidermal cells grows at the same time, some of them delay to grow. Accordingly as can be seen in Fig. 1 (arrow), separately growing epidermal cells often adhere each other at the distance of two growth-delayed cells. A cotton fiber in this stage has a dense cytoplasm. Above all, the cytoplasm is rich in amoeboid plastids, lipid droplets and RER (Figs. 5 and 6). Coated vesicles can sometimes be seen, and microtubules arrange in almost constant interval from plasmalemma (Fig. 3). The orientation of microtubules is perpendicular to the fiber axis in longitudinal section (Fig. 7).

One of the most conspicuous features in epidermal cells is the occurrence of electron dense substances, which are found in the vacuole (Figs. 1 and 4).

Secondary Wall Thickening

Fig. 8 shows a section of a developing seed obtained from a cotton boll elapsed after ca. 20 days of flowering. Cotton fibers are found to be already deposited with the secondary wall as judged from their birefringence (Fig. 9). Nearly cross sectional view in this stage is shown in Fig. 10. Since the outermost layer of the walls which seems to correspond to the primary wall is more electron dense, it may be rich in osmiophilic substances such as unsaturated fatty acids and/or pectinaceous substances. In further advanced stages of wall thickening, the cell wall is characterized with darkly stained layer, which is osmiophilic in nature (Fig. 11). By the occurence of this layer which seems to consist of some discontinuous membranes just like unit membrane (Fig. 12), the walls appear to be periodical. Some SER seems to be continuous with the membranes (Fig. 13).

Amoeboid plastids and lipid droplets are scarcely seen, compared with the stage of elongation growth. The most frequently observable organelles during wall thickening are ER, including both

SER and RER. It is also found that the cisternae of SER occur by separating from that of RER (Figs. 14 and 15). In particular, many SER appear close to the plasmalemma (Fig. 16), and in grazing section the cisternae arrange in the direction which microtubules orient (Fig. 17).

Nearly cross sectional view of microtubules is shown in Fig. 18, and at the same time perpendicularly arranged microfibrils are seen as judged from electron stained or dotted appearance of the cell wall. In grazing and longitudinal sections, microtubules are also found to run parallel to microfibrils (Figs. 19 and 20).

Golgi bodies occur sparsely in the cytoplasm, although they sometimes assemble (Fig. 22), and have very few vesicles around themselves. Besides, Golgi-derived vesicles seen near the plasmalemma and in the cytoplasm are very few (Figs. 19 and 21 ; the evidence is supported from more than 300 electron-micrographs).

Elecrton dense substances are seen even in the thickening cells (Fig. 18, arrows) and decrease through the development of cotton fiber (compare the arrowed fibers with each other in Fig. 8).

Plasmalemmasome-like structures are found very often close to the plasmalemma (Figs. 16, 23 and 24). On the other hand, lomasome-like structure could be seen around the plasmalemma and within the vacuole (Figs. 25 and 26).

Discussion

The results obtained from Fig. 1 clarify in the ultrastractural level that the cotton fibers develop by the elongation of epidermal cells. In the early stage of elongation growth, intercellular layer are found between two developing fibers just as the general tissue cell. Sometimes the cell walls of two immature fibers, apart from each other during their elongation, fuse to form the intercellular layer. In this case the remnants on the outermost part of the immature fibers may involve in the attachment of these cell walls. Consequently, the chemical nature of the remnants has more adhasive one. Generally, each cotton fiber detaches from the intercellular layer with the proceeding of cell elongation. Primary wall becomes strongly electron dense in the secondary thickening, which suggest that the chemical nature of its constituents becomes osmiophilic one.

Generally, primary wall of cotton fiber consists of 2 layers ; the microfibril orientation of the outer layer is random or almost parallel, whereas the inner one is transverse to the fiber axis[5,6]. The present investigation showed that the orientation of microtubule during cell elongation was perpendicular to the fiber axis which corresponded to that of microfibril in the inner layer of primary wall as already known. On the other hand, in the stage of secondary wall deposition, the orientation of microtubule is just parallel to the one of microfibril in any cross, grazing and longitudinal sections, and both runs almost parallel to the fiber axis.

MARX-FIGINI and SCHULZ[8] performed a kinetic experiment on cotton cellulose, which indicated complete uniformity of the degree of polymerization at every stage of secondary wall synthesis. So, they presented that the biosynthesis of secondary wall cellulose for cotton fiber must be a structure-controlled process, but not a time-controlled one and postulated that a template with the length of cellulose molecule (about 7μ) is situated inside the microtubule and the orientation of microfibril in particular directions, however, may be regulated by the protoplasmic streaming. They stated, on the contrary, another mechanism for the synthesis of primary wall which results in a lower average degree of polymerization and a non-uniform distribution.

However, in the transition from the stage of primary to secondary wall deposition, the orientation of both microfibril and microtubule changes in the same manner ; both are transverse to the cell axis in the former stage and parallel to the cell axis in the latter one. If microtubule acts as template of the synthesis of cellulose microfibril, the change of microtubule orientation could not be necessarily needed. Thus, the similar pattern of changes of microfibril and microtubule orientation during the transition from primary to secondary wall formation is well illustrated with the view that microtubule control the orientation, but not the biosynthesis of micro-

fibril. This view is consistent with the findings of MARX-FIGINI[9] that there is no involvement of microtubules in the biosynthesis of cellulose.

SER may be considered to play an important role on the formation of secondary wall of cotton fiber in view of the following results: (1) ER is the most frequently observable organelle in the cytoplasm; (2) SER is derived from RER, and can be seen near the plasmalemma as more or less inflated cisternae or vesicles: (3) in grazing sections, cisternae of SER arrange to the direction of microtubules close to the plasmalemma, and some of them seems to be incorporated into the cell wall.

Since cotton fiber consists of almost all cellulose, the role of SER might be more clearly seen in fine structural level than that in the tissue cells including hemicellulose and lignins, although the possibility that ER (mainly RER) involves in the formation of cell wall has been suggested by several investigators[10~14]. Vesicle-secreting ability of Golgi bodies is supposed to be very poor because Golgi bodies possess few vesicles around themselves, and Golgi-derived vesicles are scarcely seen in the cytoplasm and even near the plasmalemma. In view of earlier data that the walls of cotton fiber consist of 95 % or more cellulose[7], Golgi-derived vesicles may not participate in the transportation of cellulosic precursor. Recently, NOBUCHI and FUJITA[15] described the fine structure of differentiating tension wood in which the gelatinous layer is composed of almost cellulose[16]. Their figures on which Golgi-derived vesicles are scarcely seen may support the above finding.

One of the most remarkable features in a cotton fiber is the occurrence of intensively electron dense substances throughout its development. Since they occur densely in early stage of the development of cotton fiber and decrease gradually, the conversion of them into the cell wall substances is possible to occur.

MARCHANT and ROBARDS[17] suggested that all membraneous or vesicular structures assciated with the plasmalemma are classified as paramural body regardless of their origin. These paramural bodies were subdivided into two classes according to their derivation as lomasomes and plasmalemmasomes. In the present investigation, a variety of lomasomes- and plasmalemmasomes-like structure were found frequently during the wall thickening. However, lomasome-like structure was sometimes found within the vacuole. Since MATILE and MOOR[18] presented that the vacuoles are secondary lysosomes (digestive vacuoles) of higher plant, lomasome-like structure found in the vacuole is possible to an artifact formed during sample preparation.

Accordingly, the autoradiographic investigations should be further conducted to envisage whether paramural bodies are concerned with the synthesis of cell wall in higher plants.

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Explanation of Figures

The following abbreviations are used in the figures.

Ep : epidermal cell	EDS : intensively electron dense substance	Fi : cotton fiber
G : Golgi body	IL : inner brown layer	L : Lipid droplet
Lo : lomasome	M : mitochondrion	MT : microtubule
OL : outer brown layer	P : plastid	PaL : palisade layer
PL : plasmalemma	PLo : plasmalemmasome	

RER : rough-surfaced endoplasmic reticulum SER : smooth-surfaced endoplasmic reticulum

Fig. 1. The epidermal cell of cotton seed elongates to become cotton fiber. Separately growing epidermal cells often adhere each other (arrow) at the distance of two growth-delayed cells. ×6,600.

Fig. 2. Immature cotton fibers are just separating at the intercellular layer. Remnants of intercellular substances are shown with arrows. ×28,700.

Fig. 3. Longitudinal section of an elongating fiber. Microtubules run perpendicular to the fiber axis (arrow). ×22,400.

Fig. 4. Electron dense substances occur in the vacuoles of epidermal cells. ×1,000.

Figs. 5 and 6. In the stage of elongation growth, cytoplasm is rich in amoeboid plastids (Fig. 5, ×4,400), lipid droplets and RER. (Fig. 6, ×2,600).

Fig. 7. Microtubules run perpendicular to the cell axis (arrow) in the grazing-longitudinal section of an elongating fiber. ×31,000.

Figs. 8 and 9. Sections of a developing seed taken under ordinary (Fig. 8, ×110) and polarized light (Fig. 9, ×110). Arrows indicate different states of the occurrence of electron dense substance.

Fig. 10. Nearly cross sectional view in the stage of secondary wall deposition. Outermost layer of the wall is seen to be electron dense. ×3,800.

Fig. 11. Cell wall appears to be periodical by the occurrence of osmiophilic layers. ×3,800.

Fig. 12. Osmiophilic layers appear to consist of some discontinuous membranes like unit membrane. Arrow indicates the plasmalemma. ×50,000.

Fig. 13. SER is incorporated into the cell wall and continuous with osmiophilic layer (arrow). ×31,000.

Figs. 14 and 15. Cisternae and vesicles of SER which are deriving from RER (arrows). ×31,000 (Fig. 14). ×17,200 (Fig. 15).

Fig. 16. Occurrence of many SER close to the plasmalemma is the characteristic in the stage of wall thickening. ×21,600.

Fig. 17. Cisternae of SER orient parallel to microtubules and seem to be incorporated into the cell wall. ×31,000.

Fig. 18. Nearly cross sectional view of a thickening cotton fiber. Microtubules in cross sectional view are shown. The wall is characterized by the dotted appearance. ×31,000.

Fig. 19. Grazing and longitudinal section. Microtubules run parallel to microfibrils. Any Golgi-derived vesicles are not seen around the plasmalemma. ×31,000.

Fig. 20. Longitudinal section. Microtubules run parallel to microfibrils, and both orient to fiber axis (arrow). ×31,000.

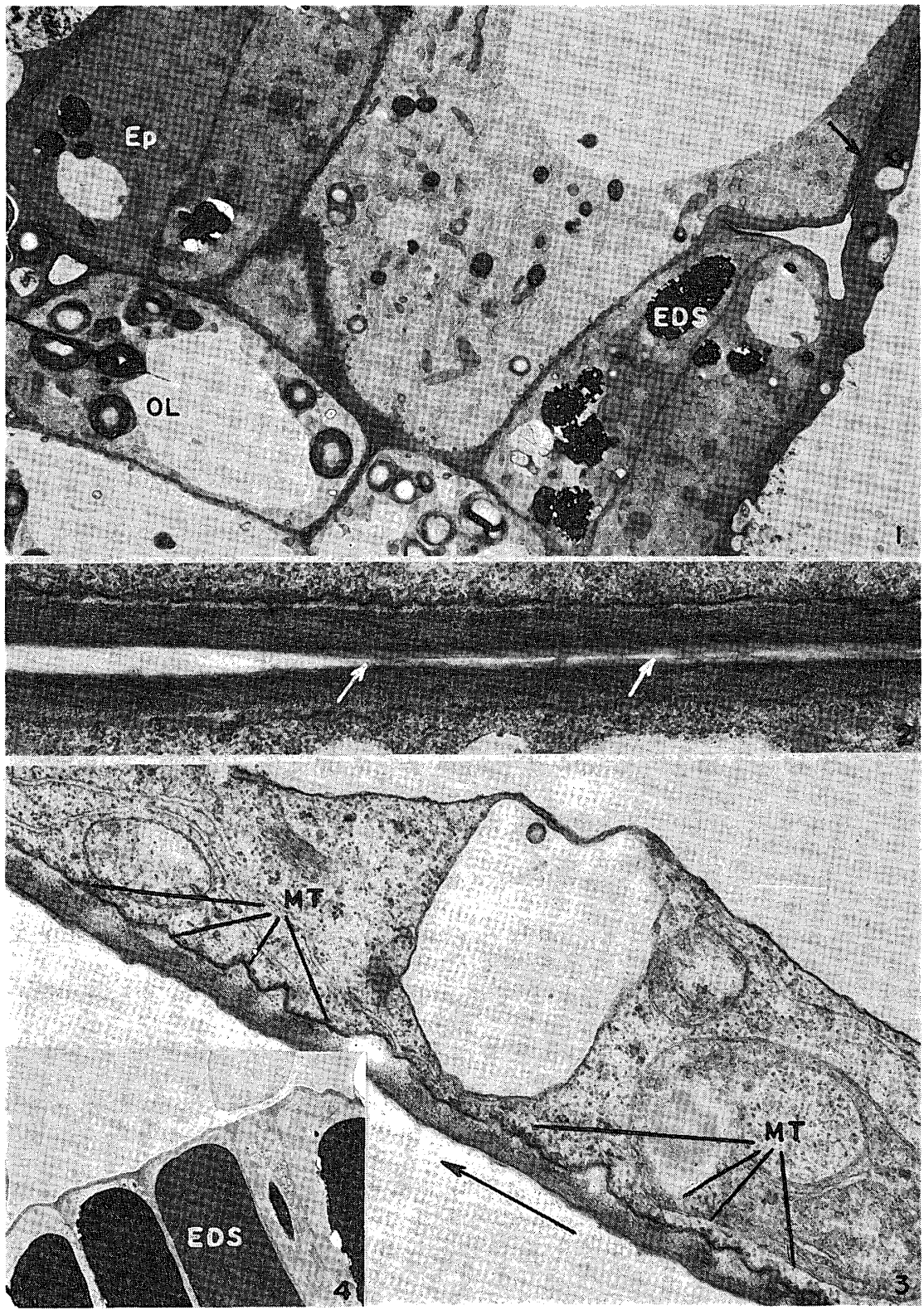
Fig. 21. Few Golgi vesicles are found not only arround plasmalemma but also in the cytoplasm. ×20,500.

Fig. 22. In some cases, assembled Golgi bodies are seen as if they are functionally connected one another. ×20,500.

Figs. 23 and 24. Occurrence of plasmalemmasome-like structures. ×25,300 (Fig. 23). ×24,500 (Fig. 24).

Fig. 25. Occurrence of lomasome-like structure. ×25,300.

Fig. 26. Lomasome-like structure occurs in the vacuole. ×25,300.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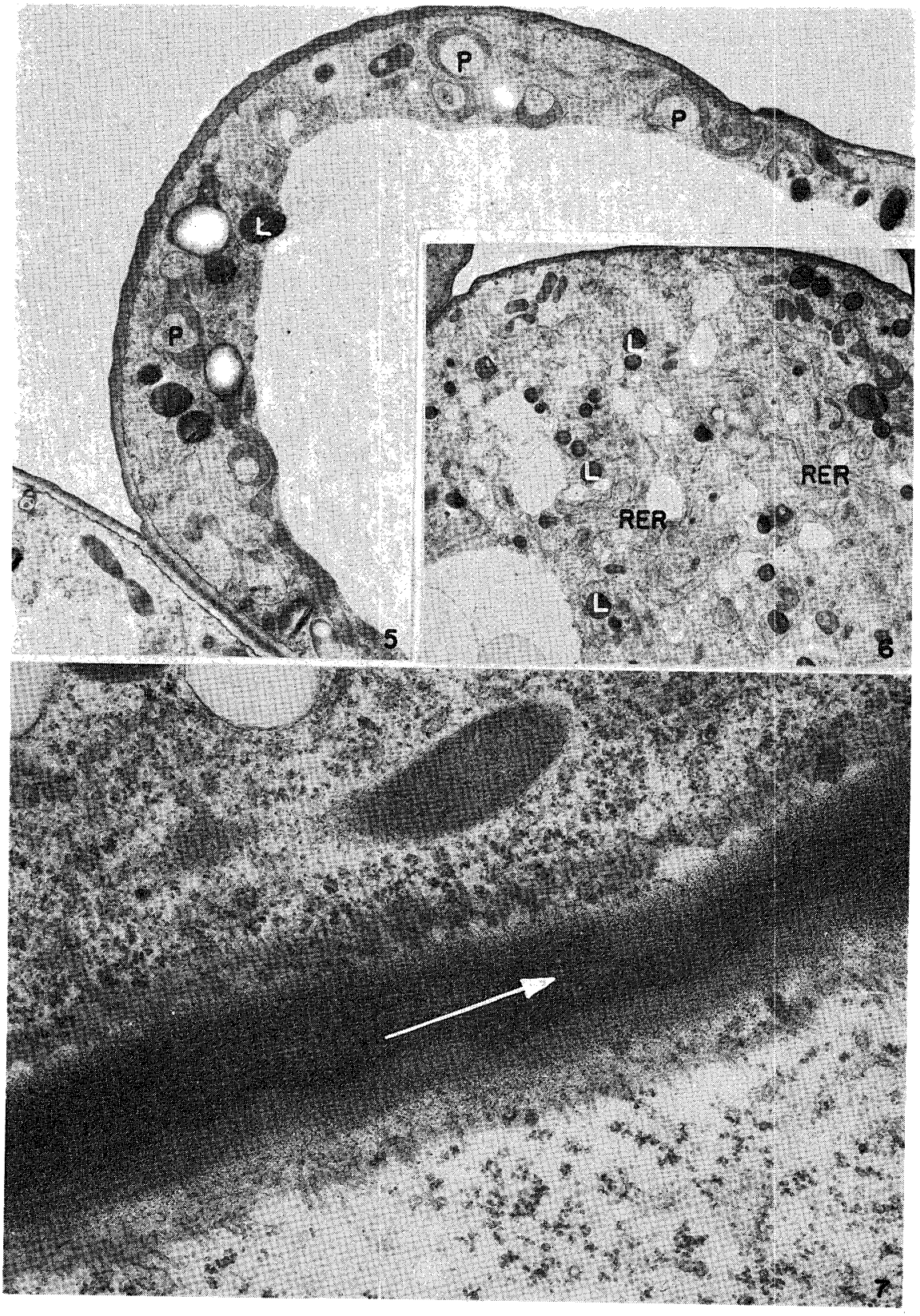
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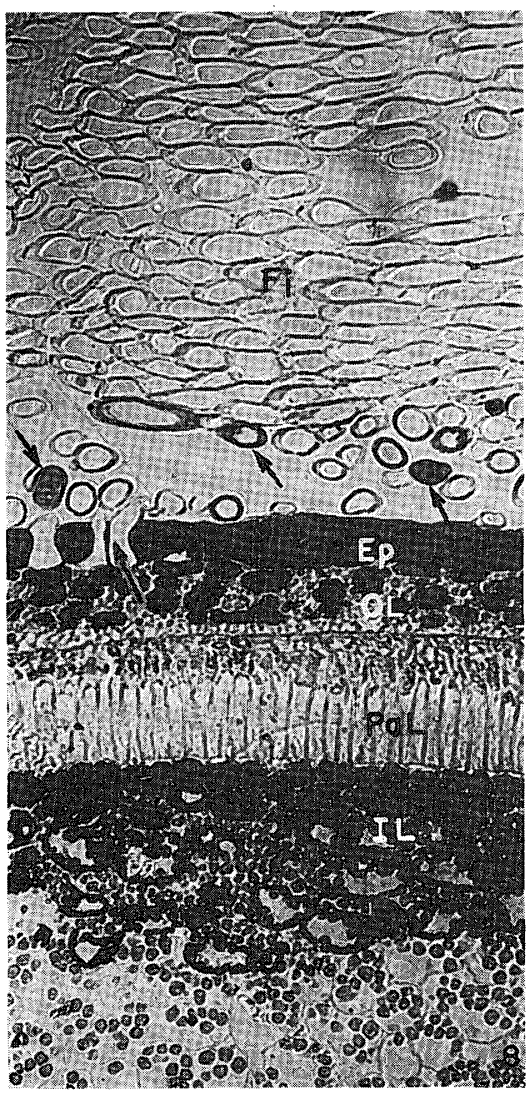
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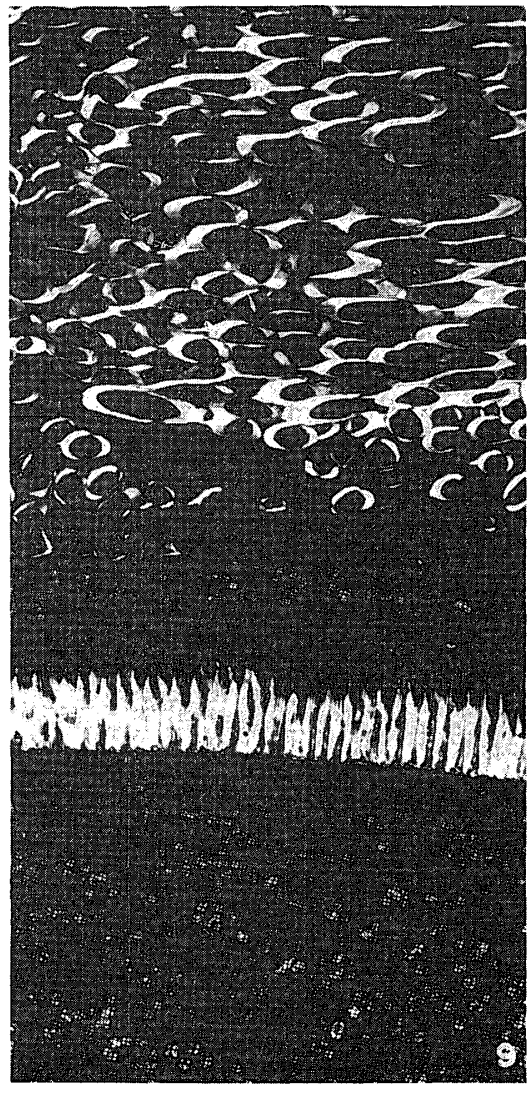
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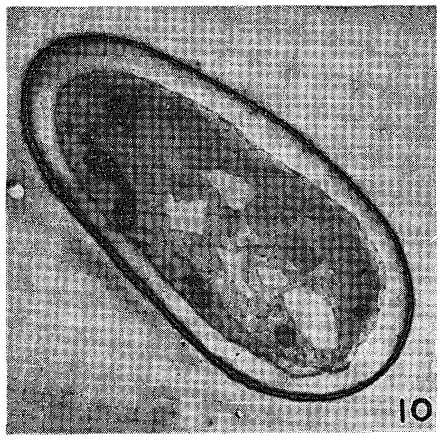
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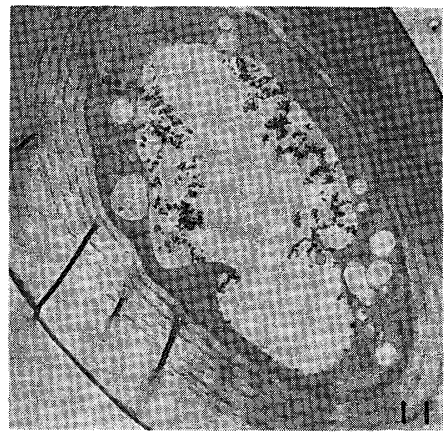
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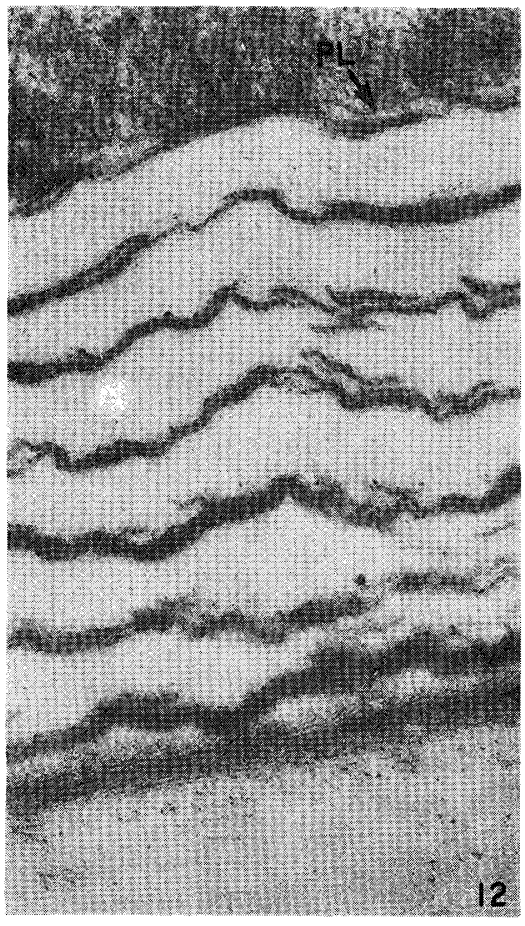
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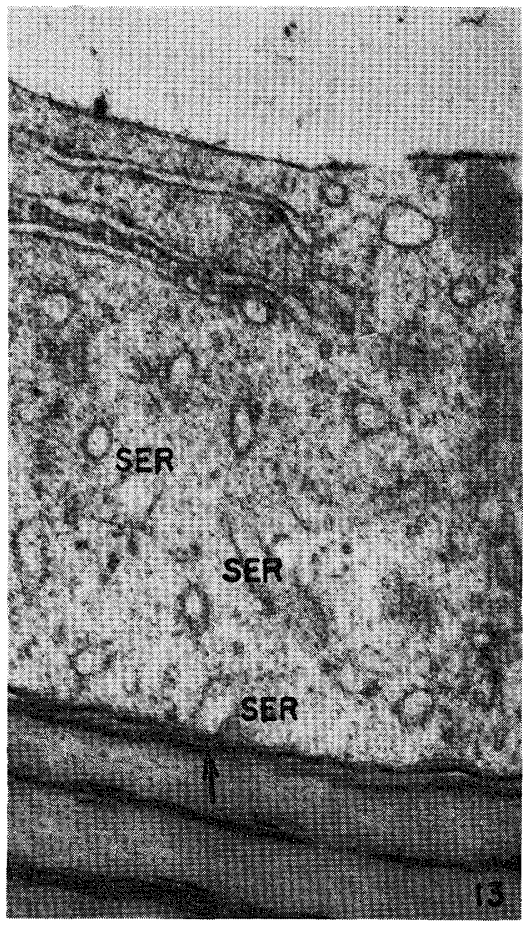
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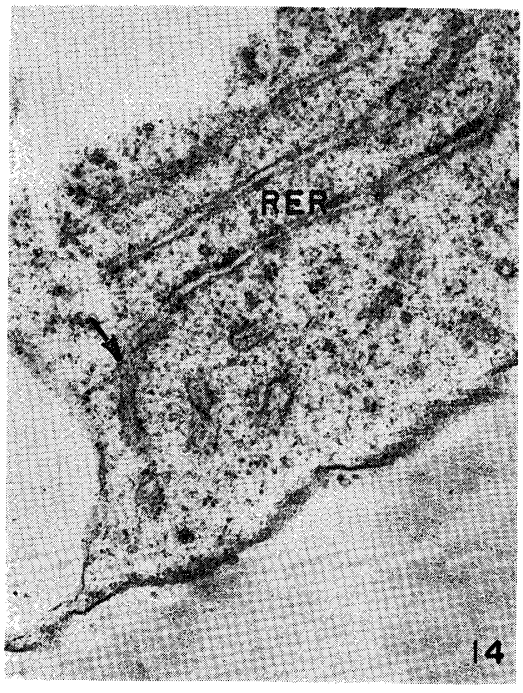
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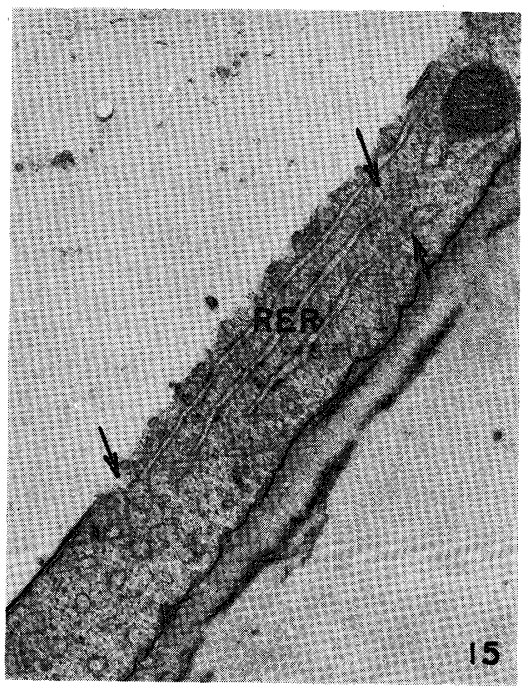
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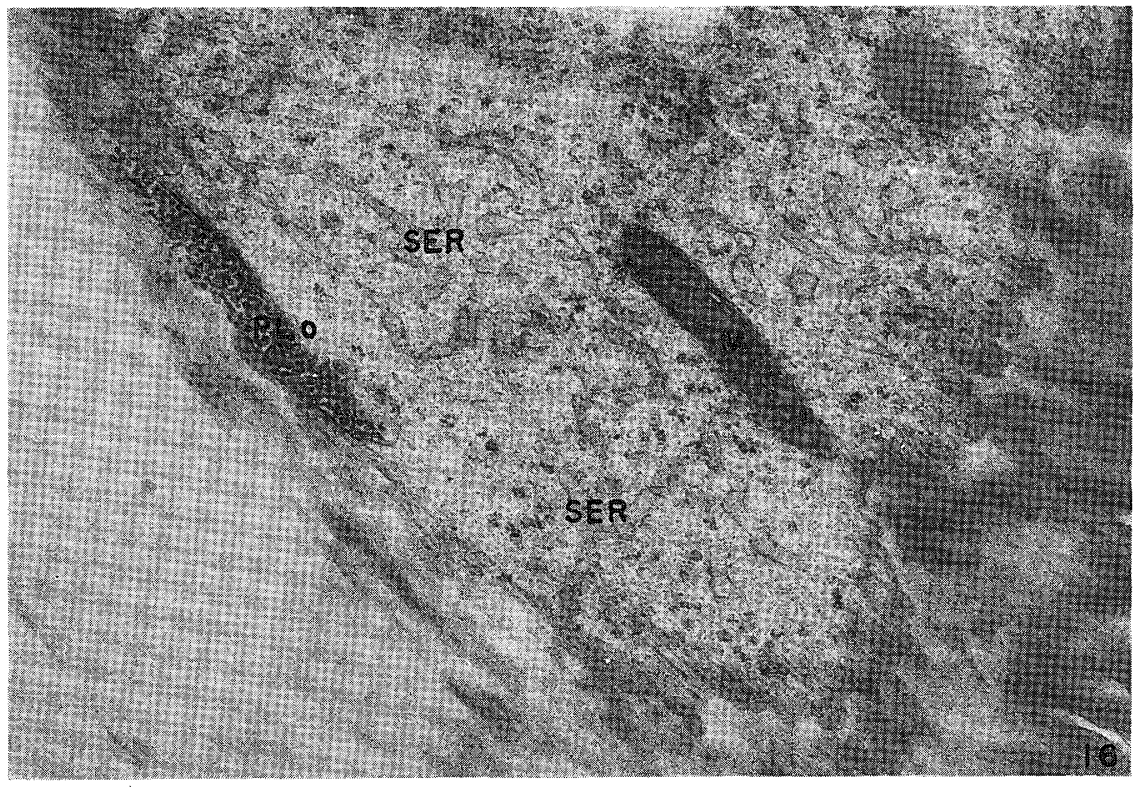
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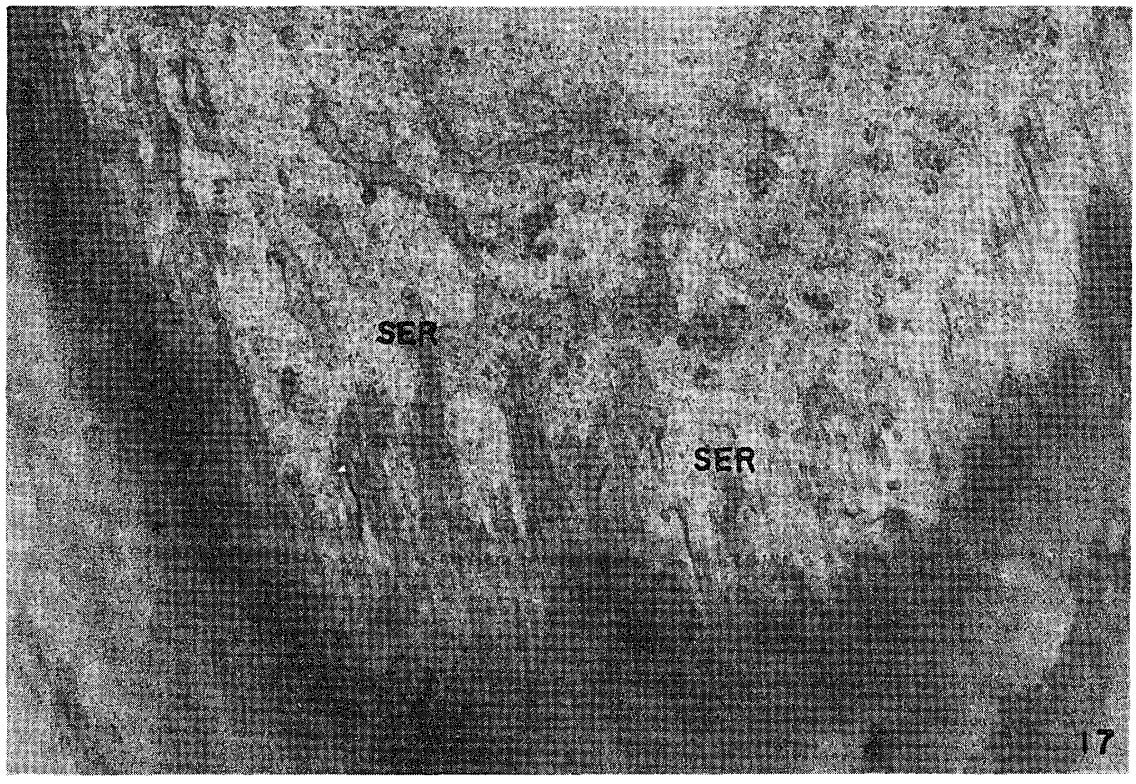
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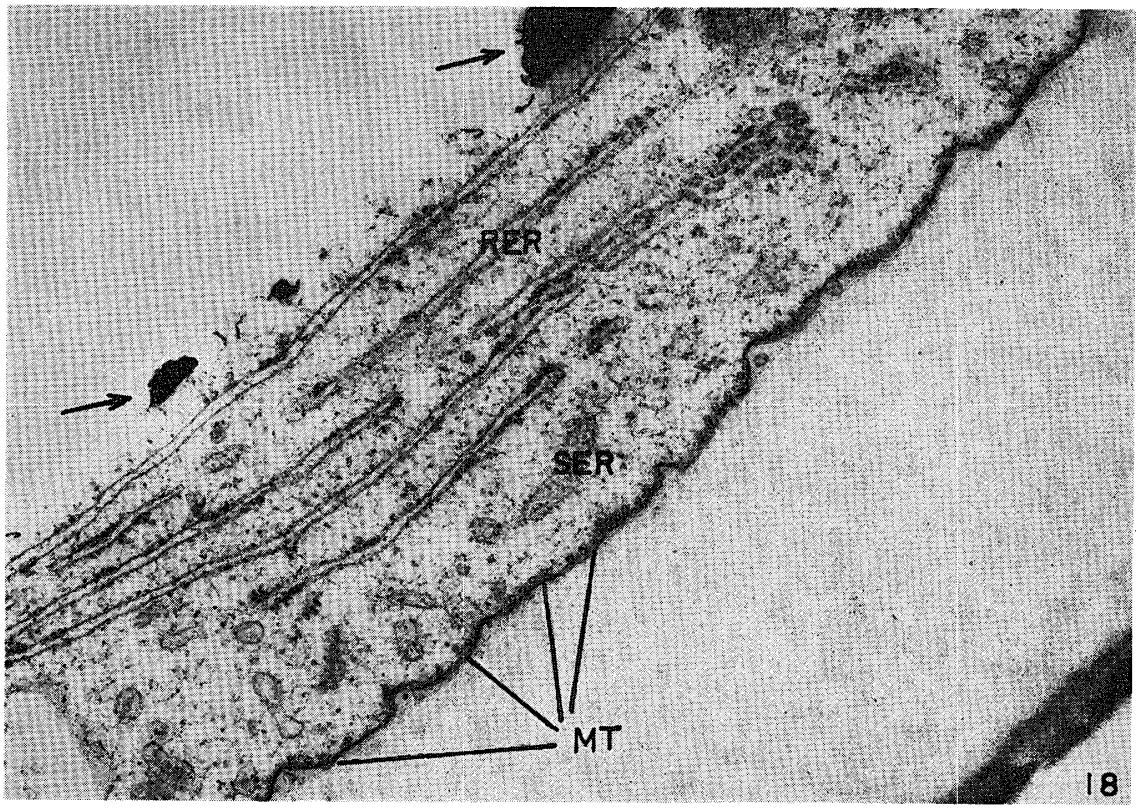
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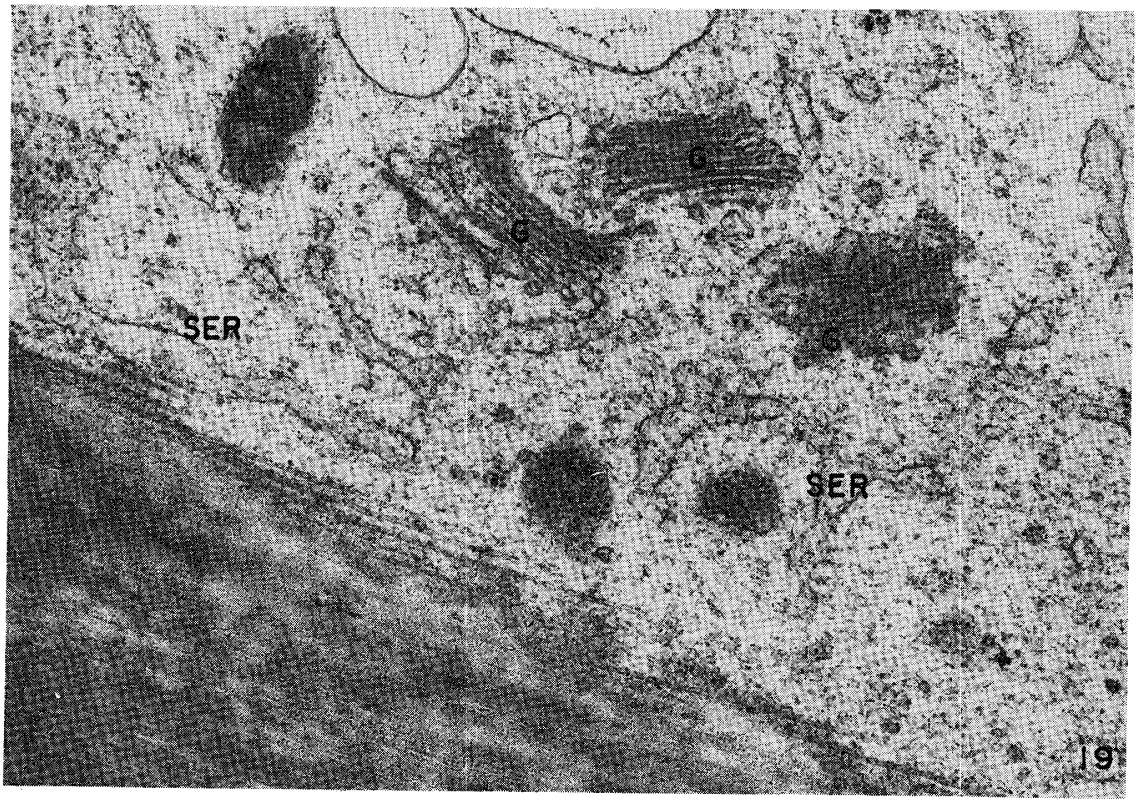
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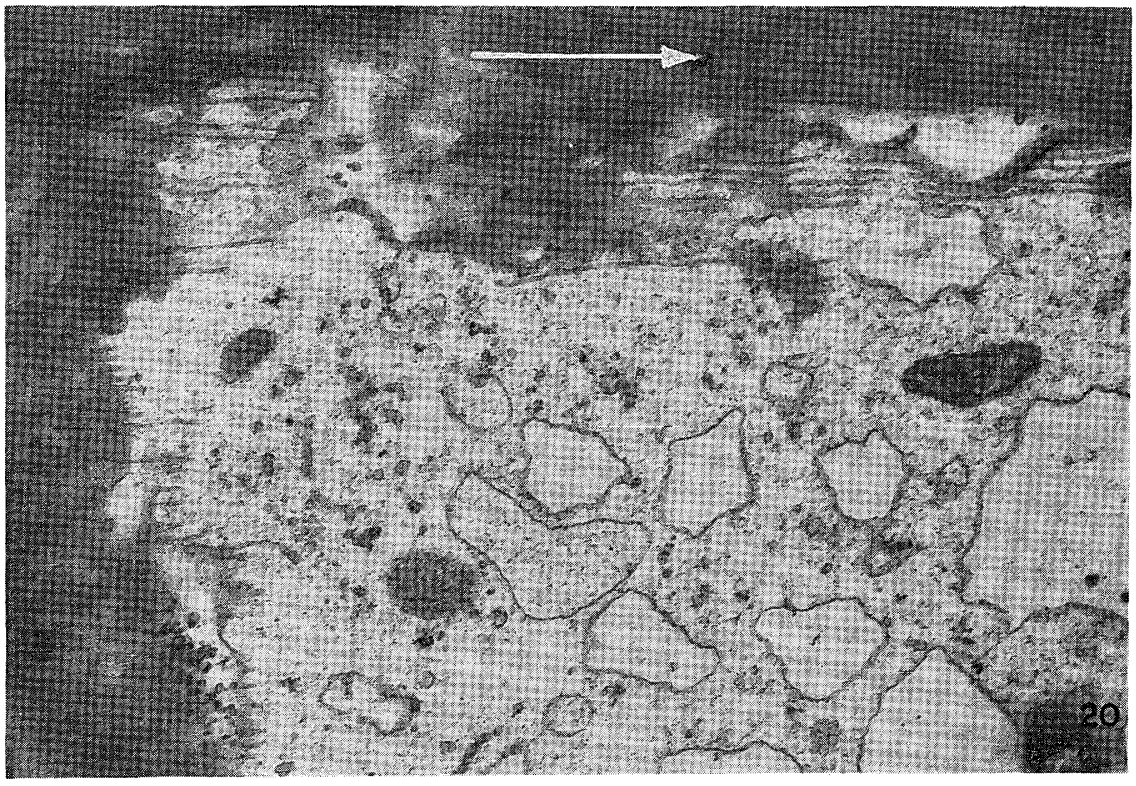
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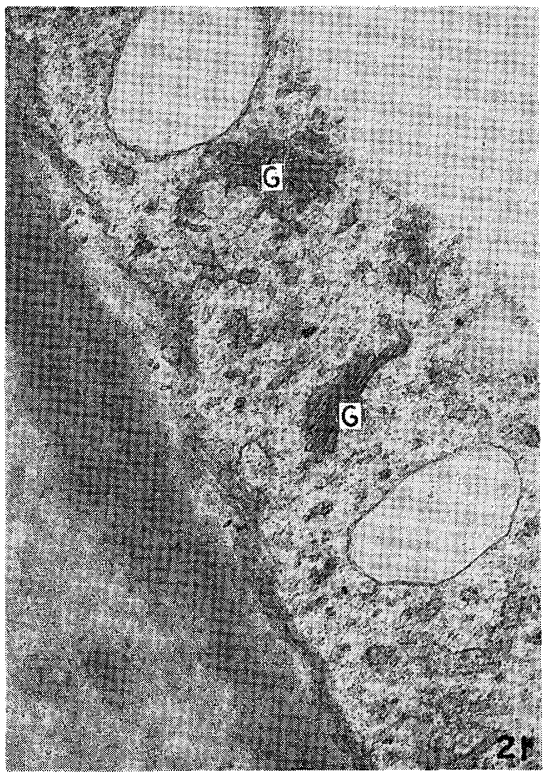
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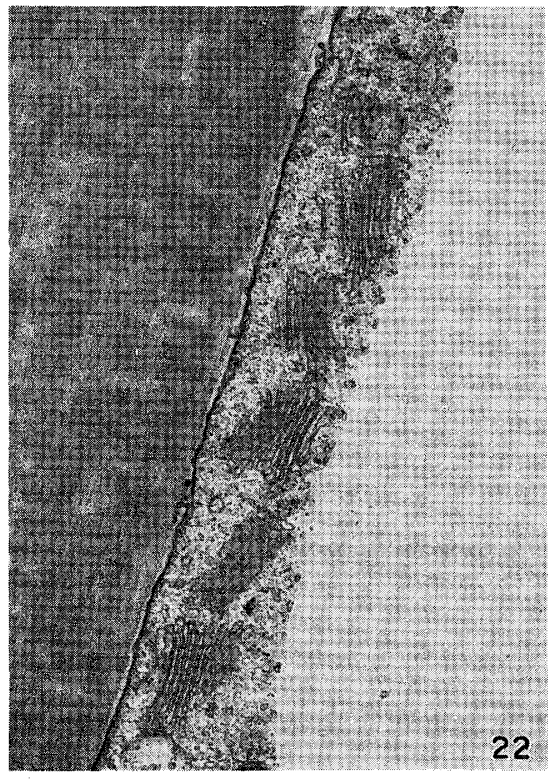
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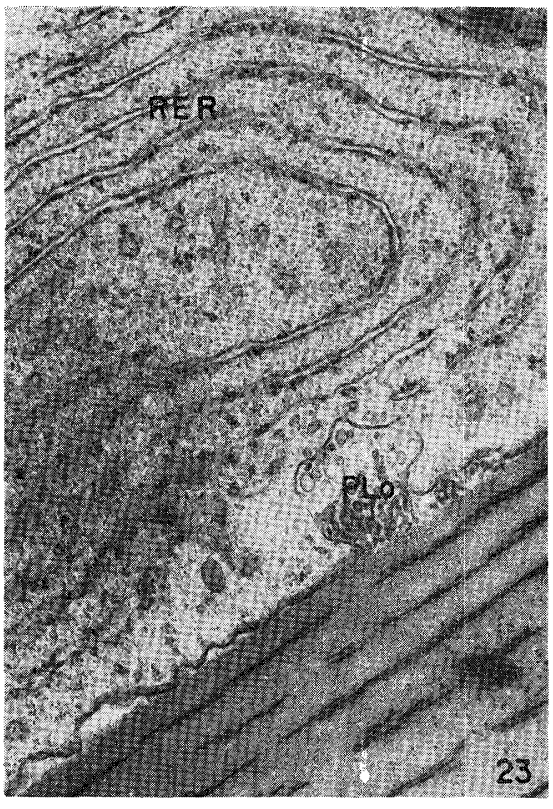
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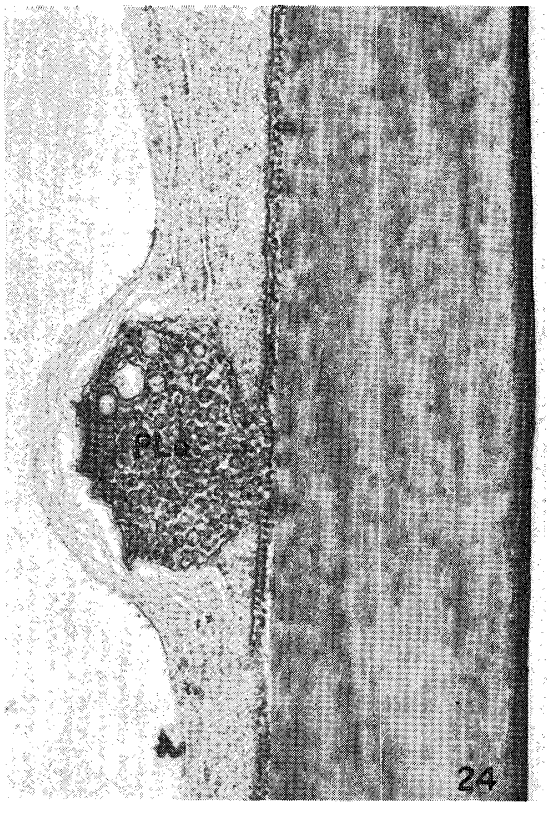
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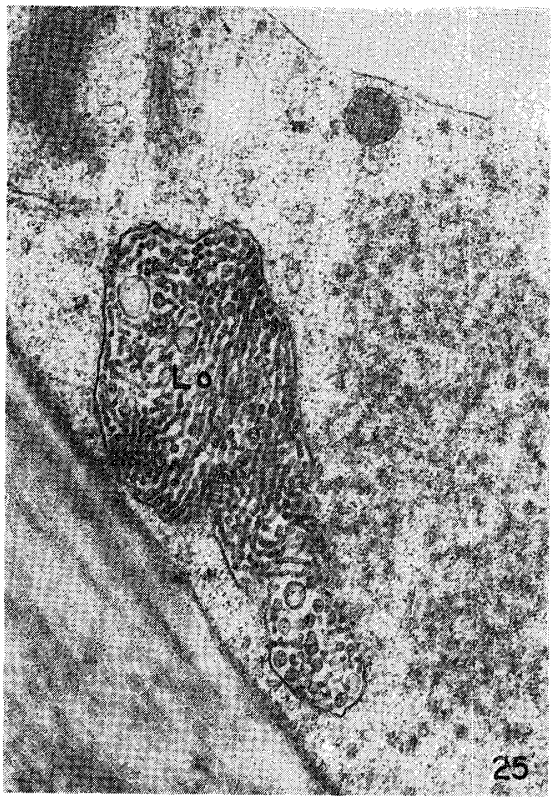
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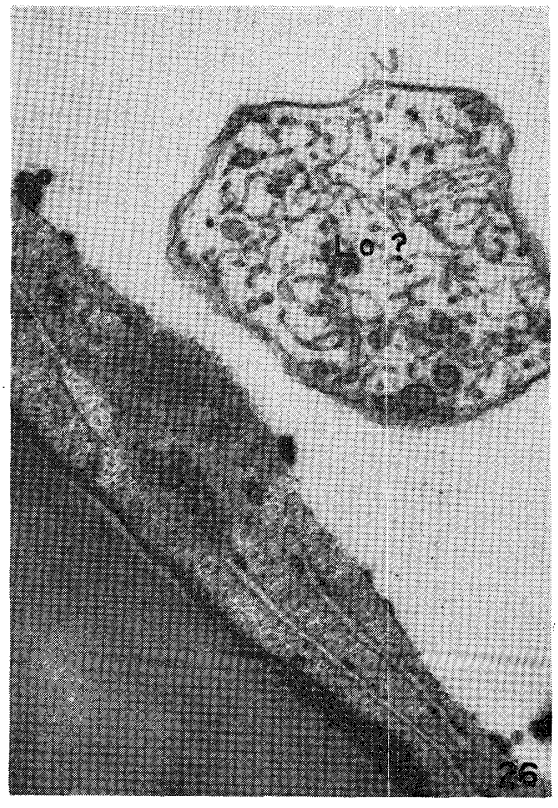
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