論　　説

有機溶媒中に保存した花粉粒の微細構造

中　村　澄　夫

Fine structure of Lily pollen grains soaked in organic solvent

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It is a well known fact that pollen grains are able to be stored in dry, cold conditions or under vacuum without losing the viability for a long time. Most of these investigations are concerned with the possibility of giving pollen grains a longer existence. So far little ultrastuctural investigation on the pollen grains kept for a long term with these various treatments has been done.

Iwanami (1971) found that the lily pollen grains which had been soaked in various kinds of organic solvents such as acetone, ethyl ether and chloroform etc. for 24 hr retained their viability (It was confirmed by the pollen germination), and that generative nucleus of *Lilium auratum* pollen grains, which had been soaked for 80 days in acetone, had divided into 2 sperm nuclei.

In this paper, the relation between the fine structure and the germinative capacity of pollen grains which were soaked in ethyl ether for a long term (300 days) was investigated in detail.

Materials and methods

The pollen grains of *Lilium longiflorum* were collected at the anthesis stage and stored in plastic bottles with silica gel at —10°C. After 1 day, 20 mg of pollen grains were soaked in 5 ml of ethyl ether (at 5°C and —15°C) for 300 days in a refrigerator.

After 300 days, these soaking pollen grains were filtered by a filter paper and dried. The dried grains remained on the surface of the filter paper were collected and sown on a culture medium (sucrose 10%, agar 2% and bolic acid 100ppm) with the straight line method as reported by Iwanami (1959). The average length of the pollen tube was measured after 24 hr.

For ultrastructural investigation, these pollen grains were fixed in 3% glutaraldehyde and postfixed in 1% osmium tetraoxyde. The materials were dehydrated by ethanol-acetone series and were embedded in Epon 812-Araldite 6005 mixture (1 : 1 v/v). Thin sections were made with a glass knife on Reichert--

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OmU2 ultramicrotome and were doubly stained with uranyl acetate and lead following Sato (1968).

Observations were made with a JEM-100B electron microscope.

The following symbols are used.

C I : control I (fresh pollen).

C II : control II (pollen grains stored at 5°C for 300 days).

E I : Pollen grains stored in ethyl ether and at 5°C for 300 days.

E II : Pollen grains stored in ethyl ether and at —15°C for 300 days.

Results and discussion

The tube length of pollen grains of *L. longiflorum* which were soaked in ethyl ether for 300 days and cultured on the culture medium for 24 hr is shown in Table 1. As is evident from table 1, pollen grains stored in ethyl ether for 300 days showed shorter tube length than the fresh ones. The fine structure of each pollen grain will be shown later.

Table 1.

In the fresh pollen grains (CI), the three layers of the pollen wall are seen distinctly (Fig. 1). In CII, they are almost the same as CI (Fig. 2). On the contrary, the exine of the pollen grains soaked in ethyl ether (EI, EII) is thinner in comparison with CI (Fig. 3, 4).

In CI, many mitochondria of the so called orthodox conformation (Hackenbrock 1968) are observed. These mitochondria consist of an outer membrane and an inner one, which have cristate. Furthermore the mitochondrial granules are also seen (Fig. 5). In CII the membranes of mitochondria and the mitochondrial granules are seen, but their membrane structures are transformed. Especially the structural transformation of cristae are conspicuous (Fig. 6). In EI the distinct membrane structure and the mitochondrial granules are not seen, and the survival of them is only observed (Fig. 7). On the other hand, in EII the membrane of mitochondria and the mitochondrial granules are seen. However the transformation of these membrane structures is remarkable (Fig. 8).

The stratiform rough ER near the pollen wall in CI is often observed (Fig. 9, 10). In CII the ER is seen also near the pollen wall, but the ribosomes are not seen on the surface (Fig. 11). On the contrary, in EI and EII, the ER is not seen at all.

In CI, there are a lot of lipid bodies in the cytoplasm (Fig. 12). Most of them are surrounded by the double membranes (Fig. 19 arrows). It seems that these double membranes are the same as endoplasmic reticulum (Fig. 21 arrows). In CII many lipid bodies are also seen, but there are fewer than those in CI. On the other hand, in EI there are few lipid bodies in the cytoplasm. The double membranes surrounding lipid bodies are not observed either. Meanwhile, in EII there are a lot of lipid bodies in the cytoplasm without the double membranes surrounding them. And the aggregations of lipid bodies are observed (Fig. 4). A point to which special attention should be paid is that many ribosome-like dots surrounding lipid bodies are found in EII (Fig. 20), which the double membranes seem to disappear. In the pollen grains soaked in ethyl ether (EI, EII), a structural denaturation in lipid bodies is seen (Fig. 14 and 15). From these observations, it is assumed that the organic solvent (ethyl ether) causes some damages on

the metabolism of lipid. In EI and EII, some black spots on lipid bodies are found, but their origin is not clear.

In CI, there are a few starch grains (Fig. 12) and amyloplasts (Fig. 17, 18). Some proamyloplasts are also observed (Fig. 16, 17). The states of starch grains in CII are almost the same as those in CI (Fig. 13). In EI, few starch grains and no amyloplasts are seen (Fig. 14). In EII, there are a few starch grains and no amyloplasts (Fig. 15).

Very few golgi bodies are observed in CI (Fig. 21). They are undeveloped golgi bodies which have a few cisternae, while in CII, EI and EII golgi bodies are not seen.

It seems that the preservative state of the ordinary structure of mitochondria is associated with the viavility of each pollen grain. For example, the fine structure of protoplasm in CII is roughly similar to EII except for pollen wall, mitochondria and endoplasmic reticulum. The fine structure of the whole cell in CII, if anything, seems to better than it in EII. However, the pollen tube length of EII which preserves the ordinary structure of mitochondria comparatively well is longer than of CII (Table 1).

As seen in EI and EII, the main differences of the fine structure in pollen grains are observed by the differnt soaking temperatures in the same organic solvent. In the cold condition (—15°C), the ordinary structures of organella are preserved pretty well in comparison with the pollen grains stored in 5°C. This results also coincides with the pollen tube elongation (Table 1). Therefore, it can be concluded that, in the same organic solvent, the viability of pollen grains soaked in the cold condition (—15°C) is kept better than that in 5°C.

From these observations descrived above, it may be concluded that the organella of pollen grains undergoes a considerable damage, especially to pollen wall, mitochondria, ER and lipid bodies, and their germinative capacity decreases, when pollen grains are soaked in organic solvent for a long time (300 days), though pollen grains retain their viability well and their ordinary structures are preserved pretty well in soaking for a few months (Fig. 22).

Acknowledgement

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Referances

Hackenbrock, C. R. 1968. Ultrastructural bases for metabolically linked mechanical activity in mitochondria. J. Cell Biol., *37*:345-369.

Iwanami, Y. 1959. Physiological studies of pollen. J. Yokohama City Univ., *116*:1-137.

———. 1971. The soaking of the pollen grains into organic solvents. Jap. J. Palynol., *8*:39-43.

——— and N. Nakamura. 1972. Storage in an organic solvent as a means for preserving viability of pollen grains. Stain Technol., *47*:137-139.

———. 1975. Studies on pollen grain and seed in organic solvents. Jap. J. Palynol., *15*:3-15.

Sassen, M. M. A. 1964. Fine structure of *Petunia* pollen grain and pollen tube. Acta Botan. Neerl., *13*:-175-181.

Sato, T. 1968. J. Electron Microscopy, *17*:158.

〈抄録〉

ある種の有機溶媒中に数カ月間保存した花粉粒が、生命を維持し続け、かつ、いくつかの有機溶媒中ではコントロールよりも発芽能（花粉管の伸長）が高いことが報告されている（岩波 1972）が、本報では、エチルエーテル内に 300 日間保存したテッポウユリの花粉粒の発芽能とその花粉粒内のオルガネラの変化との関係を比較検討してみた。

その結果、核には大きな変化は見られなかったが、花粉壁、ミトコンドリア、粗面小胞体などの膜系、および、油滴、デンプン粒などの貯蔵物質に変化が認められた。すなわち、エチルエーテル中に保存した花粉粒内のミトコンドリアにはミトコンドリア顆粒は認められるが、クリステは不明瞭で変形が著しい。さらに、コントロールでは多数見られる粗面小胞体は全く見られなかった。また、デンプン粒もほとんど見られなかった。エチルエーテル中に 5 ℃で保存した場合には、油滴の数が極度に減少しているが、−15℃で保存した場合には、量的にはコントロールとほとんど変化がなかった。また、コントロールの油滴の囲りに見られる 2 重膜構造がなくなり、その周囲にリボゾーム様の dots が認められた。さらに、いくつかの油滴中に構造変性が見られた。

岩波（1971）の報告によると、エチルエーテル中におけるヤマユリの花粉の貯蔵日数と発芽能との関係は、貯蔵日数 60 日（2 カ月）までは、コントロールよりも発芽能が高いが、60 日を過ぎると急激に低下し、90 日（3 カ月）以降ではコントロールよりも低くなり、130 日（4.3 カ月）では、発芽能がコントロールの 1/5 に低下する、ということであるが、今回調べた貯蔵日数 300 日（10 カ月）のテッポウユリの花粉の結果（発芽能……表 1）は、この岩波の結果から予想される値と大体一致している。また、微細構造から見た貯蔵日数 300 日のテッポウユリ花粉粒のオルガネラの保存状態も発芽能の結果と平行している。

このように、2 ～ 3 カ月位までは有機溶媒中でよく保存される花粉粒も、約 1 年という長期間では、オルガネラに大きな変化を受け、発芽能の低下を示すことが考えられる。

Table 1. The tube length of pollen grains in *L. longiflorum* which were soaked in ethyl ether for 300 days and cultured on the culture medium for 24 hr.

Treatment	Symbol	Pollen tube length in milimeters
control I (fresh pollen)	C I	2.8
control II (300 days, 5℃)	C II	0.4
ethyl ether (300 days, 5℃)	E I	0
ethyl ether (300 days, −15℃)	E II	0.8

Key to abbreviations

A　: amyloplast
E　: exine
E R : endoplasmic reticulum
G　: golgi apparatus
I　: intine
L　: lipid body
M　: mitochondrion
P A : proamyloplast
P W : pollen wall
R　: ribosome
S　: starch grain
V N : vegetative nucleus

PLATE 1

Figs. 1-4. Each pollen grain showing the pollen wall and the other organelles.

Fig. 1. The pollen wall (PW) in CI containing the exine (E) and the intine (I) is seen distinctly. ×16,000.

Fig. 2. The state of the pollen wall in CII is almost the same as those in CI. ×16,000.

Fig. 3. The exine in EI is very thin in comparison with CI. ×16,000.

Fig. 4. The exine in EII is thinner in comparison with CI. The structural denaturation in lipid body is seen (arrow). ×16,000.

PLATE 2

Figs. 5-8. Enlargement of part of pollen grain with different treatment containing mitochondria (M).

Fig. 5. The mitochondria in CI. The orthodox conformation of mitochondria is clearly seen. ×40,000.

Fig. 6. The mitochondria in CII. The membrane structures of mitochondria are seen, but they are fairly transformed. ×40,000.

Fig. 7. The mitochondria in EI. The distinct membrane and the mitochondrial granules are not seen. The survivals of mitochondria only are observed. ×40,000.

Fig. 8. The mitochondria in EII. The membrane structure and the mitochondrial granules are seen. The transformation of the membrane structures is also remarkable. ×40,000.

PLATE 3

Fig. 9. The stratiform rough ER which is observed near the pollen wall in CI. ×13,000.

Fig. 10. Enlargement of stratiform rough ER. The ribosomes are seen on the surface. ×40,000.

Fig. 11. The ER near the pollen wall in CII. The ribosomes are not seen on the surface as seen in Fig. 15. ×20,000.

PLATE 4

Figs. 12-15. Lipid bodies (L) and starch grains (S) in each pollen grains.

Fig. 12. In CI, there are a lot of lipid bodies and a few starch grains in the cytoplasm. ×8,000.

Fig. 13. In CII, many lipid bodies are seen. The state of starch grains is almost the same as CI. ×5,000.

Fig. 14. In EI, there are a few lipid bodies and starch grains in the cytoplasm. The same denatured figure of lipid bodies as Fig. 18. is seen (arrow). ×8,000.

Fig. 15. In EII, a lot of lipid bodies are seen. The starch grains are fewer than those in CI. The structures which seem to be denaturation of lipid bodies are observed (arrows). ×8,000.

PLATE 5

Fig. 16. The proamyloplast (PA) and amyloplast (A) in CI. ×16,000.

Fig. 17. Detail of the proamyloplast in CI. The proamyloplast in the left is surrounded by a double membrane which seems to be ER (arrow). ×40,000.

Fig. 18. Detail of the early amyloplast (A) in CI. Two starch grains and membrane structure (arrow 1) are seen in amyloplast. There are some mitochondria, endoplasmic reticulum and free ribosomes near the amyloplast. The nuclear pores are seen in the nuclear envelope of the vegetative nucleus (arrow 2). ×45,000.

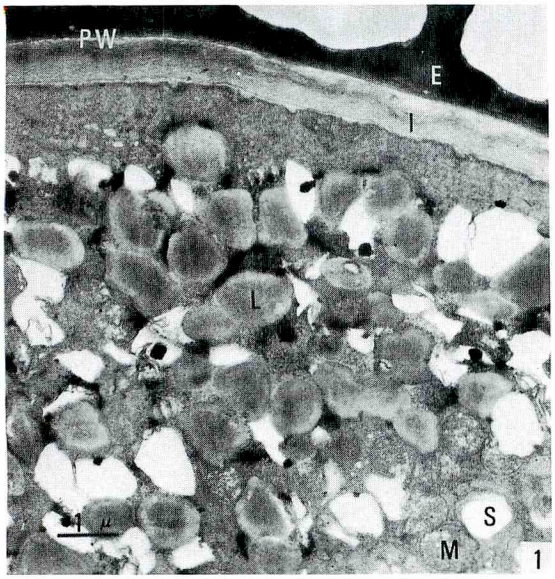
PLATE 6

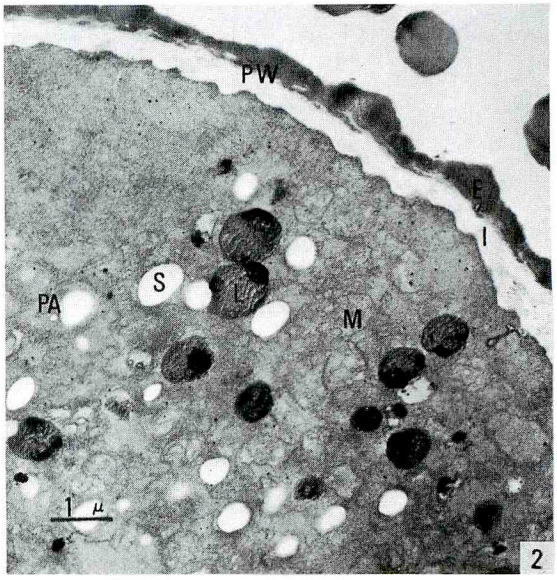
Fig. 19-21. Detail of the lipid bodies (L) in CI and EII. The lipid bodies in CI are surrounded by a double membrane (Fig. 19. arrows ×40,000). From Fig. 21 (arrow), it is clear that the double membrane is the same as the rough ER. In EII, many ribosome-like dots surrounding lipid bodies are seen (Fig. 20. arrows ×8,000).

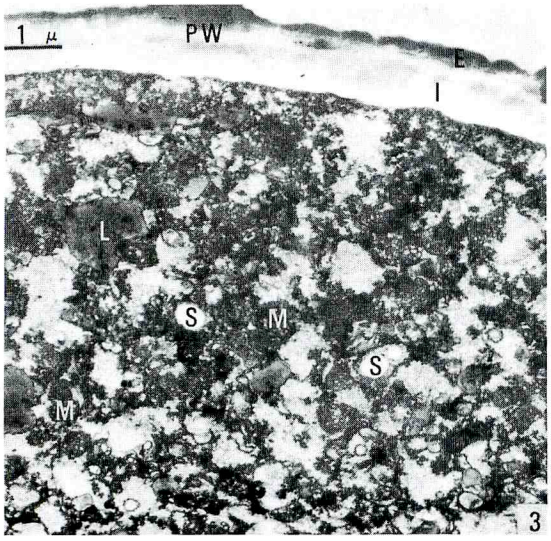
Fig. 21. The golgi apparatus (G) which has only a few cisternae. ×4,000.

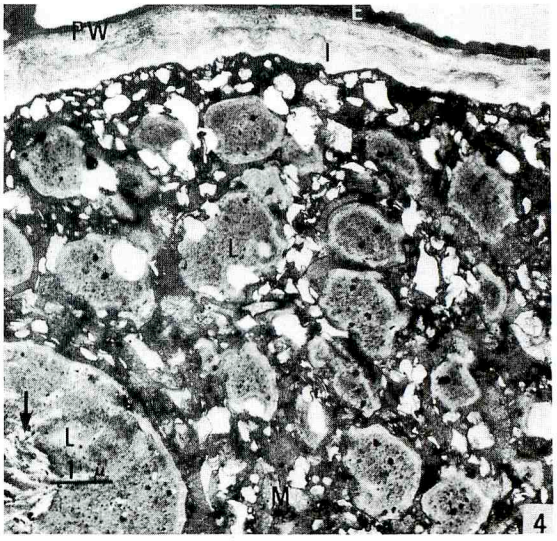
Fig. 22. The state of the pollen wall and the cytoplasm in the pollen grain which were soaked in ethl ether for 2 months (60 days).

PLATE 1









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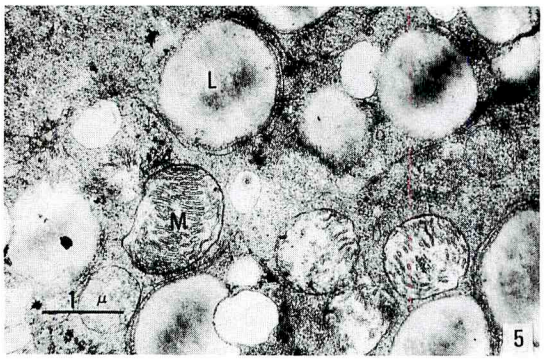

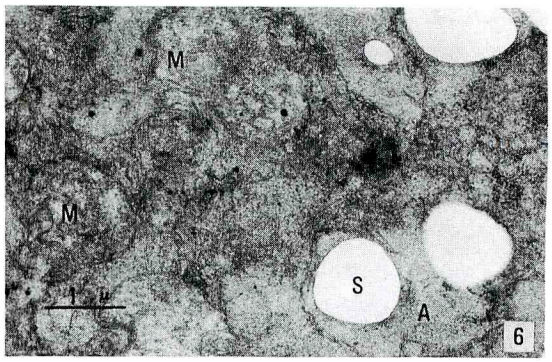

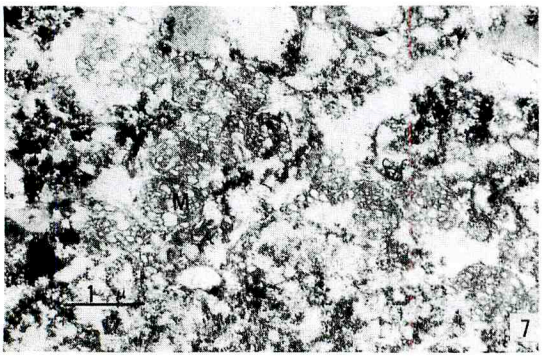

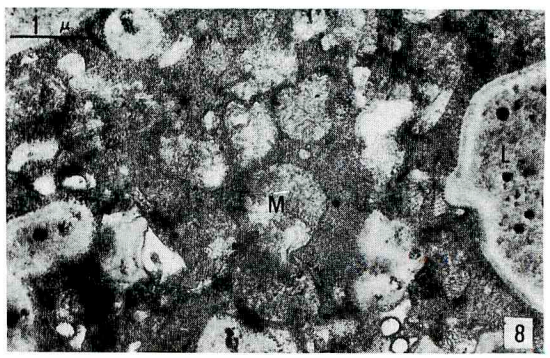

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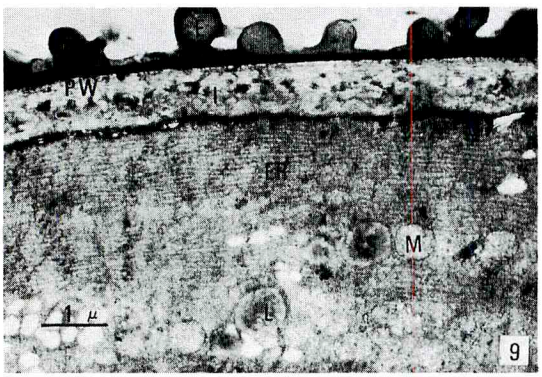

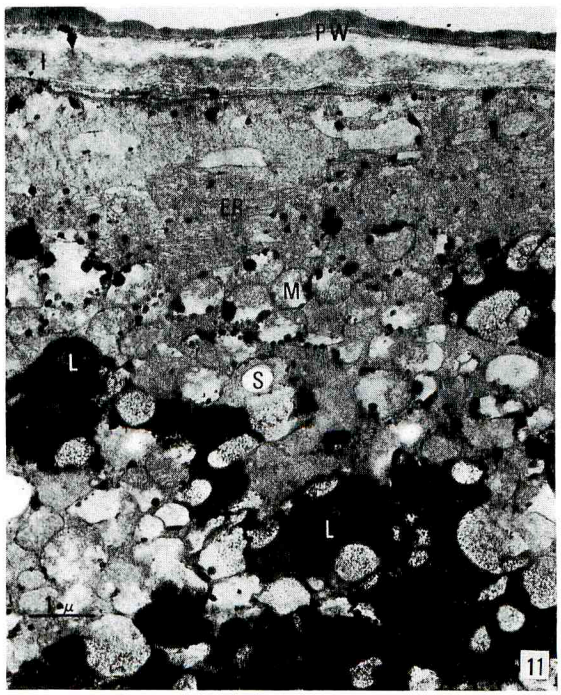

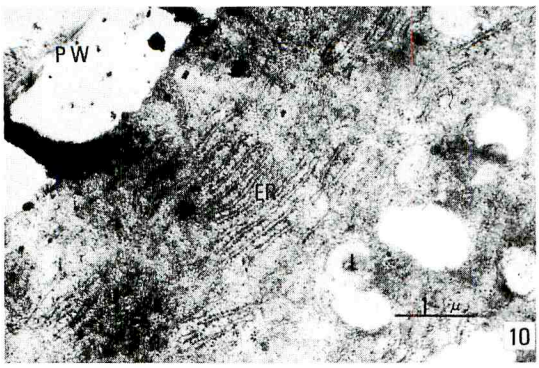

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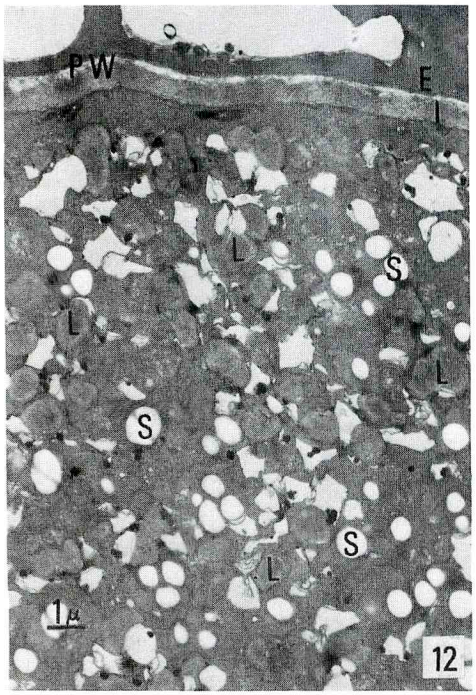

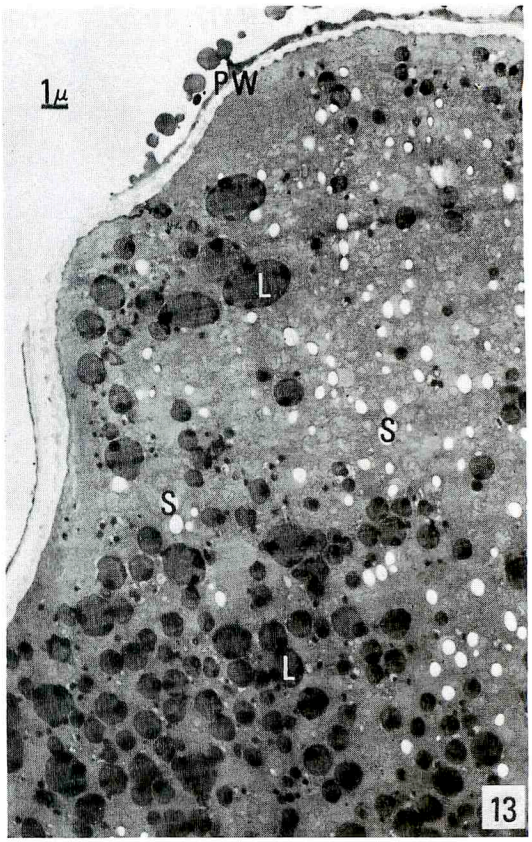

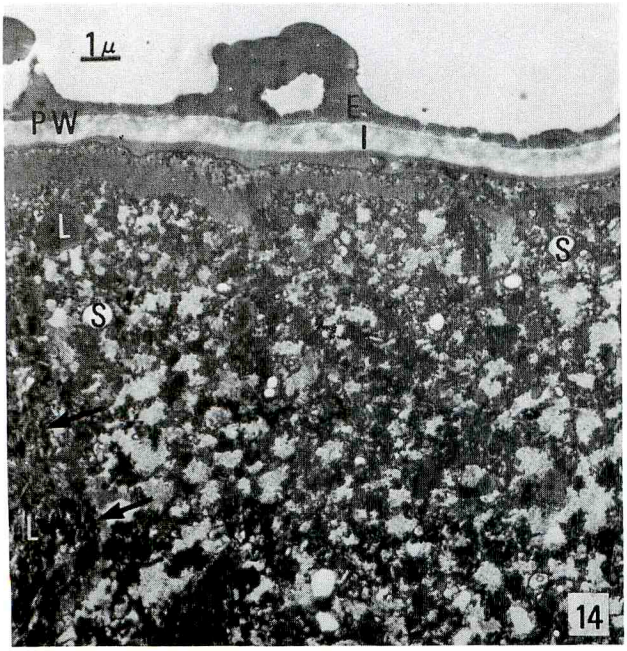

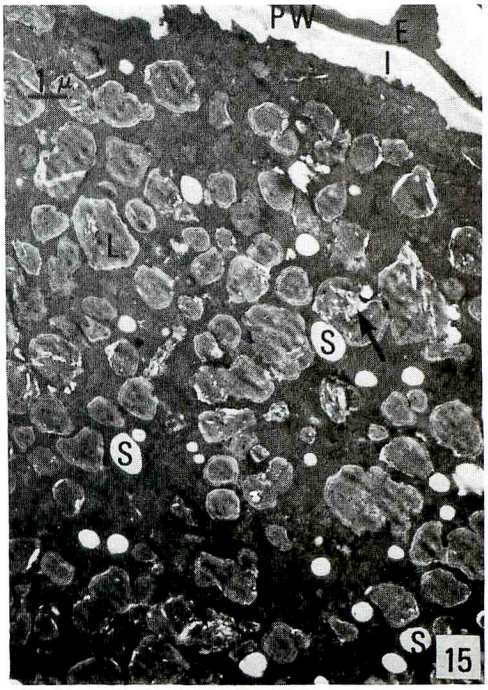

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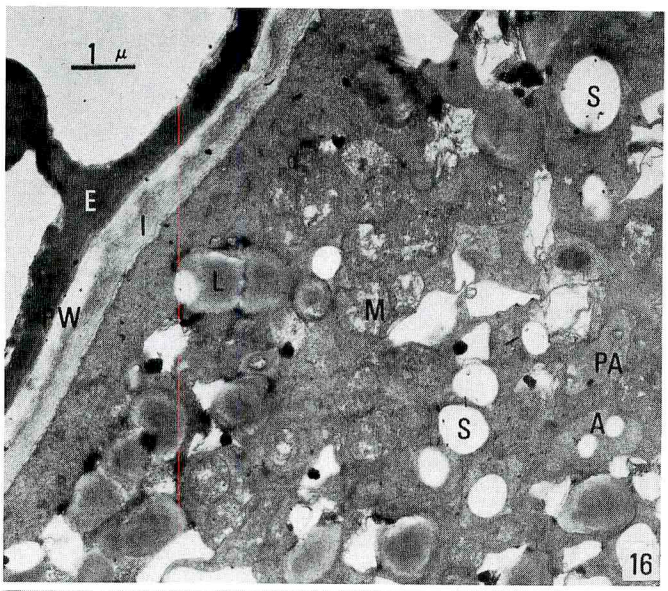

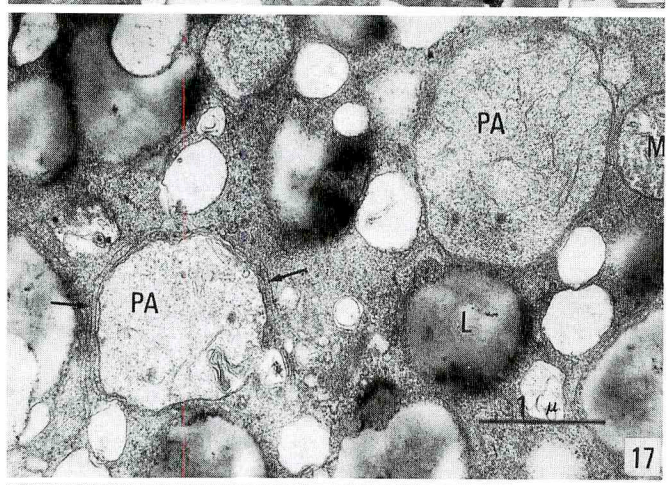

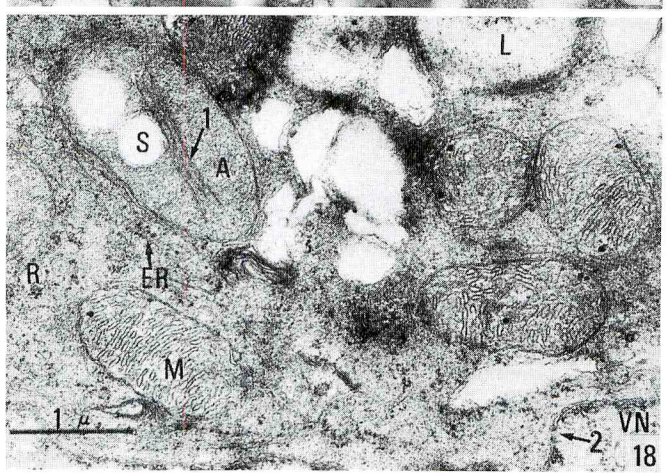

PLATE 6