Development of the Incubated Tyloses in *Quercus serrata* THUNB. *

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コナラにおける培養チロースの発達*

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Résumé

In small blocks numerous and ellipsoidal incubated tyloses developed simaltaneously. In higher temperature (25–35°C) incubated tyloses developed rapidly, while they deflated before lignified. In lower temperature (10–25°C) incubated tyloses developed slowly, but their walls were well lignified. Hence, experiments were done at 20°C in order to observe the whole process of incubated tylosis formation. During the enlargement stage, incubated tylosis wall appeared to consist of one layer and was joined with unlignified "protective layer". Incubated tylosis wall was 0.3–0.5 μm thick, which did not thicken afterwards and no pit-like structure was observed. Using TEM the tylosis wall was beginning to be lignified gradually soon after the enlargement. Golgi apparatus, ER, and mitochondria increased in number at the enlargement stage. They were observed until the last stage of development.

要　　旨

小さな培養ブロックを用いると，多数の培養チロースが一斉に発生し楕円体状に発達した。高温（25—35℃）で培養すると，培養チロースは急速に発達したが，木化する前にしぼんでしまった。低温（10—25℃）では発達に時間を要したが，壁の木化段階まで観察できた。そこで，20℃でチロースを培養し，チロースの発達の全段階を観察した。伸張中の培養チロース壁は一層からなっており，未木化の protective layer と連続していた。培養チロースの壁厚は 0.3—0.5μm で，伸張後の肥厚は認められなかった。また，壁孔様構造も観察されなかった。チロース壁の木化は，電顕観察によると，壁の伸張終了後間もない時点から段階的に始まっていた。ゴルジ体，ER，ミトコンドリアはチロースの伸張段階に数を増したが，これらは発達の最終段階まで観察された。

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INTRODUCTION

Tyloses have been of interest to researchers in wood science, and their structure and development process have been observed by many investigators. KÓRÁN and CÔTÉ[1] and FOSTER[2] studied the wall structure of "natural tyloses", which are formed in the natural circumstances.

"Incubated tyloses", which are induced in small wood blocks under artificial conditions, have also been investigated by JURÁŠEK,[3] KÓRÁN and CÔTÉ,[4] MEYER,[5] MURMANIS,[6] and FUJITA et al.[7] The so-called "protective layer" in *Quercus serrata* THUNB. was observed by SUGIOKA et al.[8]

In this paper, the following observations are described.

(1) As a preliminary examination, natural and incubated tyloses were observed with special emphasis on their shape and structure.

(2) The difference in tylosis development at different incubation temperature was observed, at proper time interval, in the pore zone of the current year during the dormant season.

(3) The development of tylosis wall and the difference of cell organelles in the whole process of incubated tylosis formation (from budding to lignification, at 20°C) were observed by electron microscopy.

MATERIALS AND METHODS

Several sample trees, 10–30 year-old *Quercus serrata* THUNB. growing in Kamigamo Experimental Forest Station of Kyoto University Forest, were cut down and only pore zones were examined.

(1) The preliminary observation on shape and structure of natural and incubated tyloses: The tyloses that had been formed before cutting are regarded as natural tyloses in this paper. For the observation of incubated tylosis formation, 3 cm-thick discs were taken from the stem immediately after it was cut, and some of them were cut to semicircular pieces (3 cm width) containing both bark and several outer growth rings. These discs and semicircular pieces were placed in a growth chamber as shown in Fig.1. The growt hchamber was kept in the laboratory maintained mainly at 20°C or 25°C.

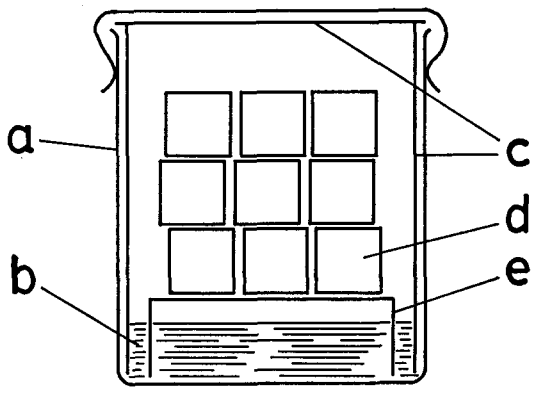

Fig. 1. Growth chamber : consisting of 1 liter beaker (a) which contains a small amount of distilled water(b) at the bottom. The inside of the beaker as well as the top cover is lined with moistened filter paper(c). Incubation blocks (d) are placed on a stand(e) to avoid direct contact of water.

(2) The observation on incubated

tylosis formation at various temperature : Incubated tyloses formed in the current year were observed twice during dormant season (from Nov. to Jan.). The incubation temperature was varied to six gradations, namely 10, 15, 20, 25, 30, and 35°C. The specimen blocks (semicircular pieces) were incubated at the same time in each temperature within the growth chamber. They were observed every six or eight hours for first three days, every twelve hours for subsequent two days, and once per every one or two days, and then every three or four days after that. For each time, one block was taken out from each growth chamber and checked for tylosis development. From the result of these experiments, it became clear that the incubation blocks were dried gradually. For the incubations hereafter, the growth chamber was sealed up in a polyethylene bag which contained a small amount of water at the bottom, and the incubation blocks themselves were also moistened suitably once in a while.

(3) More detailed observation on the development of incubated tyloses at 20°C : Specimen blocks (semicircular pieces) were incubated in the growth chamber at 20°C in June and December. The incubated tyloses formed at pore zone in the current year were observed.

For all observations described above, the materials were prepared as follows. The materials collected were observed with a binocular to check the developmental stages of tyloses. Some of them were fixed with 3% glutaraldehyde or preserved in 0.8M D-mannitol solution. For transmission electron microscopy (JEM-7), fixation and embedding were made by the ordinary method. Ultra-thin sections were stained with 4% uranyl acetate. All observations with TEM were on cross sections. For scanning electron microscopy (JSM-U3), preserved materials were rinsed well with distilled water and dehydrated. They were dried with a critical point dryer and coated with C-Au or Au only. For the observation on inner surface of tylosis wall, materials were treated with 1N KOH to dissolve the protoplasm after rinse.

RESULTS AND DISCUSSION

(1) The preliminary observation on the shape and structure of natural and incubated tyloses

Natural tyloses formed in *Quercus serrata* THUNB. were found in heartwood and intermediate wood, and moreover in sapwood sporadically. In the current year, only a few tyloses were found. Individual natural tylosis was generally large in size and more or less spherical (Fig. 3a). The wall was thick (about 2 μm), and a pit-like structure was observed on the inner surface of the wall. It was found not only where the tylosis touched the adjacent tylosis but also where tylosis touched the vessel wall (Fig. 3b).

Incubated tyloses, in 3 cm thick discs during the enlargement stages, were biggest in the current year and became smaller in the older annual rings. Moreover, they decreased in number to some extent in older vessels. On the other hand, incubated tyloses were not formed when water was present in the vessel lumina. In the small

blocks incubated tyloses were formed numerously. They were ellipsoidal shape and the walls were thin. This shape difference between natural and incubated tyloses seems to be resulted from whether numerous tyloses develop at once or develop one by one, as being pointed out by MEYER.[5]

(2) The observation on incubated tylosis formation at various temperature (at pore zone in the current year)

The results of two individual experiments (in dormant season) were in good agreement, which were summarized in Fig. 2. In this diagram each developmental stage of incubated tyloses is interpreted as follows.

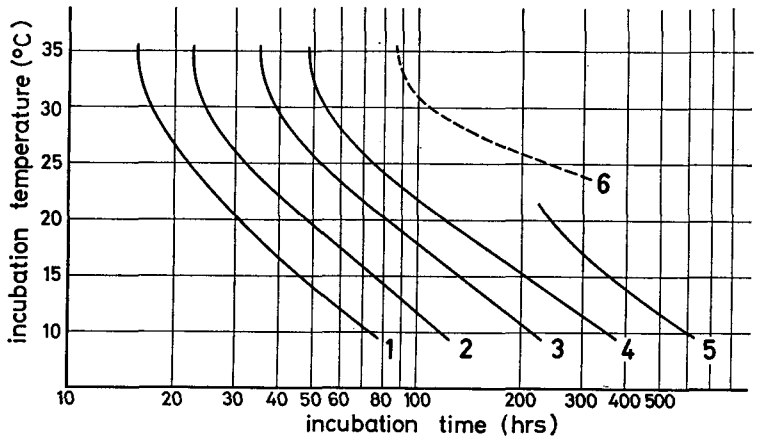

Fig. 2. Development of incubated tyloses at different temperature (at pore zone in the current year) 1–6 : developmental stage, see the legend in the text.

1: Smaller incubated tylosis buds were formed in some vessels.

2: Incubated tylosis buds were formed in every vessel (Fig. 4a).

3: Most of incubated tyloses were elongated to the length of a quarter of vessel diameter.

4: Most of incubated tyloses were elongated to the length of a half of vessel diameter and began to block the vessel lumina (Fig. 4b).

5: Lignification of incubated tylosis walls could be confirmed barely with phloroglucin-hydrochloric acid.

In high incubation temperature incubated tyloses became deflated after some elongation. Stage 6 showed the time when this phenomenon was observed first in some vessels.

As is evident from Fig. 2, incubated tyloses were formed at every incubation temperature (10–35°C), of which development was dependent significantly on the temperature. In higher temperature (above 25°C) they developed rapidly and the effect of incubation temperature was less remarkable. However, they became deflated before lignified. In lower temperature (under 25°C) the effect of temperature was remarkable. The lower the incubation temperature was, the more hours were needed to develop. On the other hand, numerous incubated tyloses developed simultaneously at higher temperature, while their extent of development more or less varied at lower temperature. From these experiments, it was revealed that the whole process of incubated tylosis formation (from the budding to the lignification) could be observed at lower temperature.

Deflatting of incubated tyloses is mainly due to the drying of the incubation wood blocks. From a more cautious subsequent experiment not to dry incubation blocks, it became clear that incubated tyloses could be developed to the stage of lignification (though not perfectly) even at 25°C or 35°C. In this incubation method, some incubated tylosis buds were observed at 40°C, but they did not develop any further. No tylosis formation was observed at 50°C. These temperature values agree with the result by JURÁSEK.[3] The lack of tylosis development at such a high temperature seems to be resulted from the decrease of various physiological activities in a parenchyma cell. On the other hand, tylosis formation was also observed in the case of incubation in a refrigerator (at about 5°C) in *Quercus serrata* THUNB. The lowest allowable formation temperature probably depends on the difference in species.

Incubated tylosis development is more or less different depending on the season (growth or dormant) in which they are incubated. For example, the tyloses that were incubated in June needed about 24 hours at 25°C, 34 hours at 20°C, and 70 hours at 10°C to bud to the developmental stage 2. The time needed for incubation is always shorter than that in dormant season. However, no remarkable difference was found in the subsequent developments. The development of incubated tyloses seemed to be less variable in dormant season.

(3) More detailed observation on the development of incubated tyloses at 20°C in the current year

(a) The development of incubated tylosis wall

In *Quercus serrata* THUNB. tylosis wall was formed from the "protective layer" as well. In June, the tylosis wall was connected with unlignified "protective layer" that had formed in the neighbouring ray parenchyma cell (Fig. 5a). In December, the tylosis wall was joined similarly with "protective layer" but only with the unlignified inner layer. The outer layer touching to the secondary wall of the ray parenchyma cell had been lignified in this season (Fig. 5b). These "protective layers" with which the tylosis walls were joined stained darker with GA and OsO_4 than the outer "protective layers" and the secondary walls of other cells. During the enlargement stage of the incubated tylosis, the wall appeared to consist of one layer in terms of the stainability. This does not agree with the results by MURMANIS[6] and FOSTER.[2] They claimed two-layered walls. The difference is, perhaps, due to the harvesting method or observing method. Besides, it may be due to the amorphous substance with which tylosis bud is covered in early stages of development.

The following is the results on tyloses incubated in December. After the elongation stages, the incubated tylosis walls had rather uniform thickness (0.3–0.5 μm thick, Fig. 6a and b). The measurements were made on cross sections and at the sites where tyloses touched to the inner surfaces of the vessels. Judged from scanning electron microscopical observations, the wall was also thin and no pit-like structures were observed. Therefore, it appears that incubated tylosis wall did not thicken after enlargement as far as this incubation method is concerned. This phenomenon is probably due to the difference between incubating conditions and natural ones. Lignification of incubated tylosis

wall was observed with phloroglucin-hydrochloric acid staining after about 10 days of incubation. As for the stainability with $KMnO_4$, however, lignification appeared to begin partially before 10 days of the growth. Outer part of 6-day-old tylosis walls were stained heavily only in the area where some tyloses were gathered but not touched one another (Fig. 7). The tylosis walls of 14-day old had been lignified considerably, for even in the inner part of the wall it was stained in the same manner as a vessel wall. The lignification process on the incubated tylosis walls appears to be similar to that on other cell walls. On the other hand, hemispherical materials were often observed on the outer surface after about 6 days of growth.

(b) The difference in the structure or number of cell organelles prior to or in the process of tylosis formation (in December)

After 16 hours of incubation, Golgi apparatus had still a few vesicles and showed dormant structure (Fig. 8). After 32 hours of incubation (at the developmental stage 1, as defined above), Golgi apparatus began to increase in number and to be accompanied with many large vesicles. After 50 hours of incubation, numerous Golgi apparatus were scattered all over the bud (Fig. 9). Golgi vesicles were large and numerous. These changes of Golgi apparatus were observed not only in the tylosis buds but also in the ray parenchyma cells forming tyloses. Golgi apparatus kept the same structure until the tyloses began to blockade the vessels. The nucleus and amyloplasts were sometimes removed from the ray parenchyma cell to the tylosis. After 10 days of incubation, most of Golgi apparatus had been transformed into the structure with a few vesicles (Fig. 10).

In the stage of incubated tylosis enlargement, the change of cell organelles and starch grains almost agreed with the results by FUJITA et al.[7] In these experiments, however, dark (osmiophilic) precipitates were scarcely formed in this stage. This is probably due to the longevity of cells thanks to the improvement of incubation method. Golgi apparatus, ER, and mitochondria that had increased in number could be observed until the later stages, even after 26 days of incubation, within most of tyloses and ray parenchyma cells. However, it is still difficult to give a clear-cut answer why Golgi apparatus had transformed into dormant structure (with a few small vesicles) in the lignification stage. More minute investigation would be necessary for the structure change and the functions of cell organelles.

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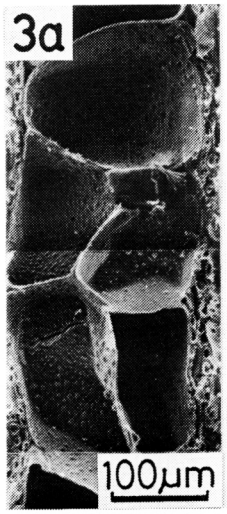
EXPLANATION OF PHOTOGRAPHS

Fig. 3. Natural tylosis a: tangential view of a vessel lumen b: inner surface with pit-like structure
Fig. 4. Incubated tyloses in the current year at 35°C a: developmental stage 2 (after 24 hours), b: developmental stage 4 (after 48 hours)
Fig. 5. Incubated tylosis bud in the current year fixed with GA and OsO_4 a: incubated at 20°C for 48 hours in June, b: incubated at 20°C for 50 hours in December.
Fig. 6. Incubated tylosis wall fixed with GA and $KMnO_4$ on cross section a: incubated at 20°C for 5 days (5-day-old tylosis), the last stage of enlargement; b: 26-day-old tylosis, the last stage of lignification
Fig. 7. 6-day-old incubated tylosis walls fixed with GA and $KMnO_4$
Fig. 8. A ray parenchyma cell contacted with a vessel after 16 hours of incubation, fixed with GA and $KMnO_4$
Fig. 9. A part of a tylosis bud after 50 hours of incubation, fixed with GA and $KMnO_4$
Fig. 10. 10-day-old incubated tyloses fixed with GA and $KMnO_4$

ABBREVIATIONS

T: tylosis Tw: tylosis wall G: Golgi apparatus R: ray parenchyma cell Rs: secondary wall of ray parenchyma cell PL: "protective layer" Vw: vessel wall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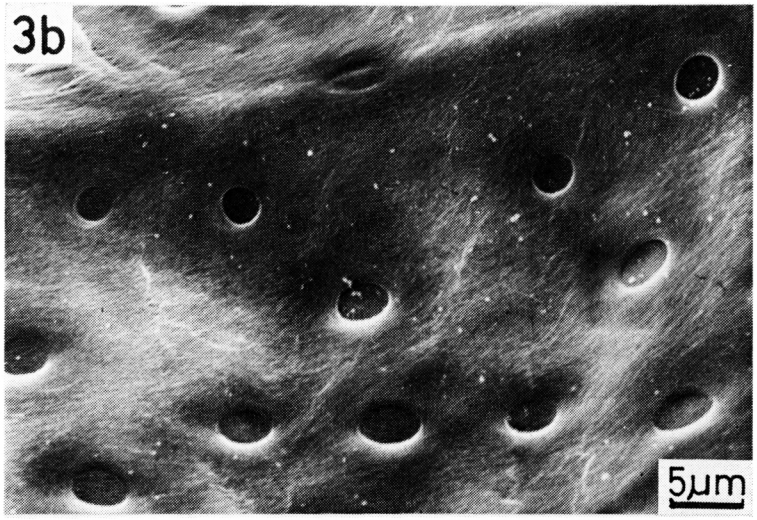
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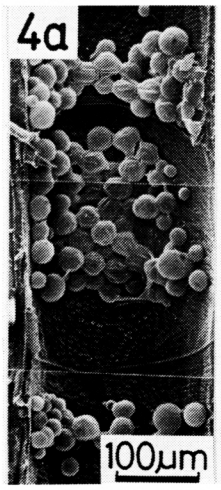
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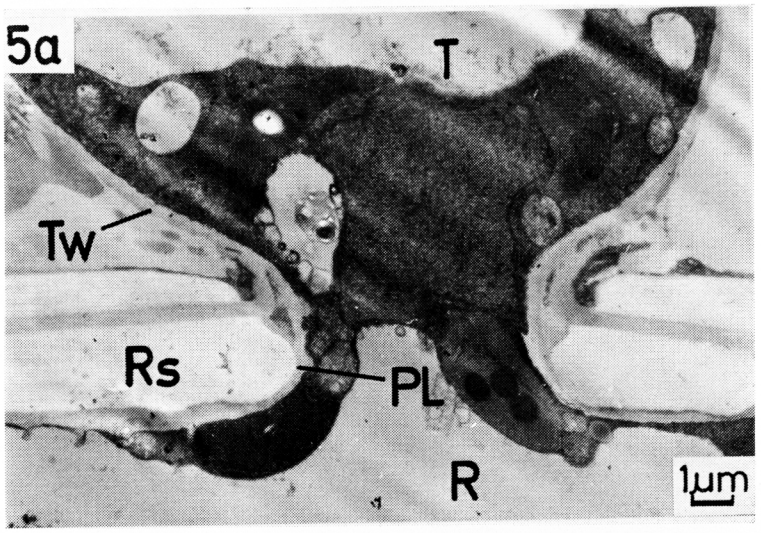
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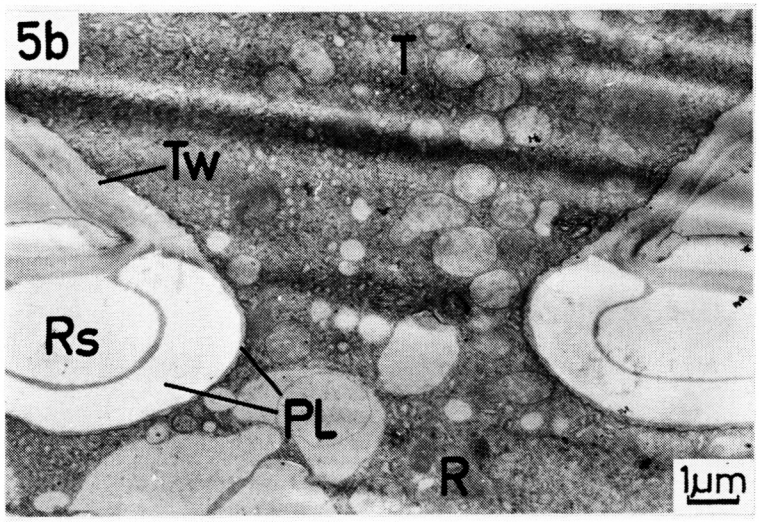
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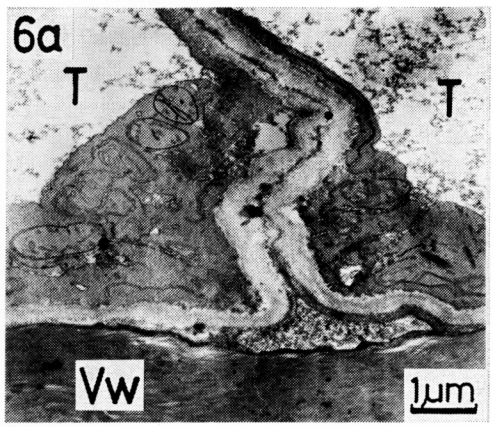
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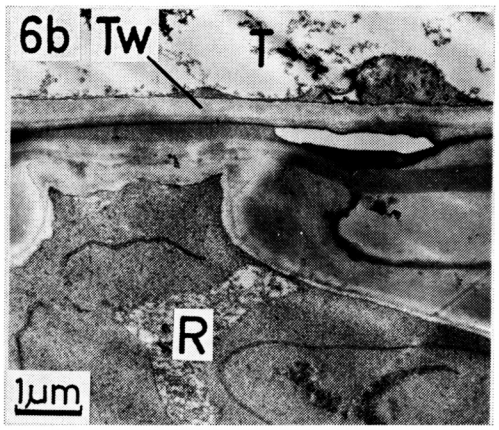
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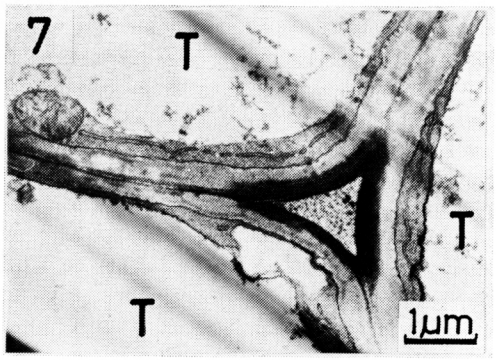
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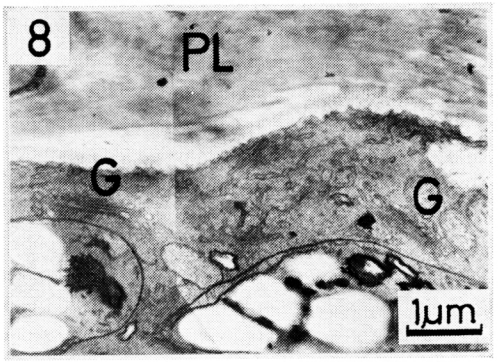
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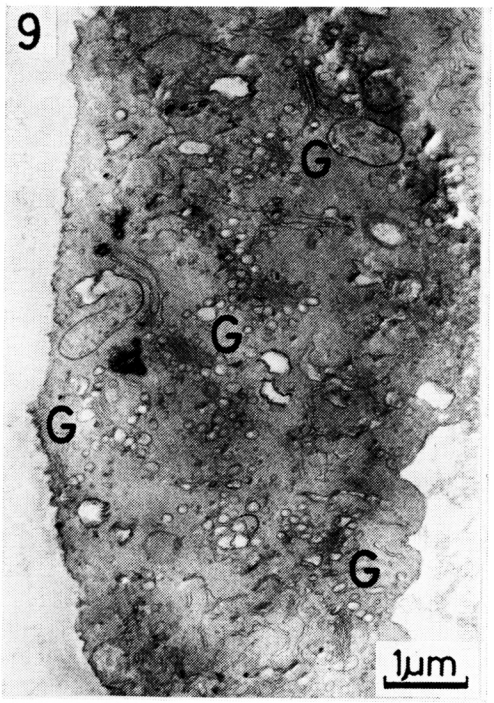
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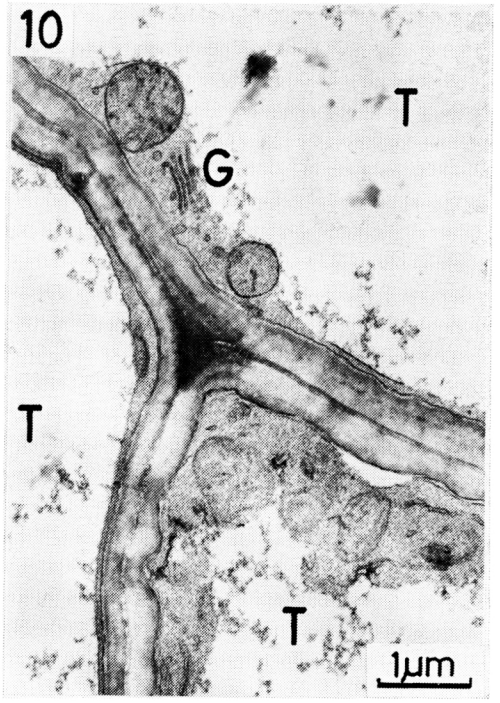
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