

Ultrastructure of *Sclerotinia sclerotiorum* (Libert.) de Bary

MITSUO ARIMURA and HIROSHI KIHARA

(Laboratory of Plant Pathology)

Much of the fungal morphology has been left beyond the resolution-limits of the light-microscope. Recently, however its accurate visualization has been brought into realization through the development of the techniques in the electron microscopy. Hitherto, no accurate informations have been obtained concerning the ultrastructure of sclerotium, which prompted the authors to make it clarified. In this paper, the experimental results of the observation of the ultrastructure of the mycelium and sclerotium of *Sclerotinia sclerotiorum* were reported.

Materials and Methods

The strain of *Sclerotinia sclerotiorum* (Libert.) de Bary used in this observation was isolated from a rape (*Brassica napus* L. var. *oleifera* DC.) in Kagoshima. The purified one was plated on a potato-dextrose-agar (PDA) (2.2 % mush potato, 3% dextrose, 1.7% agar), and incubated at 15°C for 2 weeks. After incubation, mycelium and sclerotium in maturity were used. They were cut into a proper size for fixation. Subsequently the cut ones were immersed in a fixative solution of 1% OsO₄ in a collidine buffer (PH 7.3) for 2.5 hours at 0°C. The cells were dehydrated through a graded series of acetone water mixtures (50 %, 70 %, 95 %). After being washed twice with 100 % acetone, the cells were transferred to propylene oxide. Then the cells were transferred to a 1:1 mixture of propylene oxide and Epon. Lastly the cells were embedded in a mixture of Epon 812, DDSA and MNA of Luft in gelatin capsules, and polymerized at 60°C for 2 days. In order to improve the contrasting of some sections the staining procedure was followed. Electron micrographs were taken with a Hitachi HS-7 electron microscope.

Observations and Discussion

I. Ultrastructures of Hyphal Cell on PDA

Between the hyphal cells on PDA and in sclerotium, there are few remarkable differences excepting those appertaining to the part of cell wall (C. W.). The comparative observation of the Figures (1-3) indicates that the older the hyphal cells grow, the thicker their cell wall and outer fibrous structure (F. S) become. As is to be explained later, a remarkable difference between a hyphal cell and a cell in sclerotium (Fig. 1-3) comes to be recognizable through the development of these parts. It seems that the mitochondria

are not located evenly in a cell, but maldistributed considerably (Fig. 4-6). As hyphal cells grow older, they begin to have vacuoles occupying wide space in a cell, and some unknown structures come to be observable in the vacuoles (Fig 5). Contrary to this, the young ones have almost no vacuoles in a cell, and they are small, in size if present.

II. Ultrastructures of Sclerotial Cell

The sclerotium of *S. sclerotiorum* is covered with black layers, resembling a dung of a rat in shape, 5-10 mm in size. Electron micrographs of sclerotium indicate that their outside part is in possession of distinct layers of very high electron density and that the electron-dense layers are observable from the outest to the 4-5th inner layers. The comparative observations of the other electron micrographs indicate that the outside sclerotial cells are larger than the inside sclerotial ones (Fig. 7-12). Furthermore, it seems that the shapes of the outside sclerotial cells are not so obviously uniformed as those of the inside sclerotial cells are. The cell wall and the outer fibrous structure of the outside sclerotial cells are not so thick as those of the inside sclerotial cells are. The sclerotial cells have a little thickened cell wall as in case of an inner layer and the fibres stand in a row at a nearly right angle to the inside cell wall, and the confused ones follow one after another to be contacted each other, occasionally appearing spiriferous, and the whole inner layers are surrounded by fibre layers at the outest side. The inner part of a sclerotia is crowded, being endowed with few spaces, because the sclerotial cells are surrounded by the thick fibrous layers (Fig. 9-11). The cells in sclerotium are distributed irregularly. It seems that this fibrous layer has been secreted from the inner parts of cells. It is of much interest that the sclerotial cells are formed with such thick fibrous structures. The measured values of the cell wall, the outer fibrous structure of the hyphal cells on PDA and the sclerotial cells are shown in the Table 1.

Table 1. The Measurement Values of C. W and F. S (Unit: μ)

cells str.	young hyphal cells	old hyphal cells	sclerotial cells
C. W	0.09	0.38	0.42
F. S	—	0.70	2.7-4.5

The sclerotial cell has few vacuoles or the vacuoles are usually very small, if present, in other words the sclerotial cell is considerably substantial. The mitochondria of the sclerotial cells have few crista. This fact suggests that the respiratory function of the sclerotial cell may be very low. Fig. 10 and 11 show two unknown structures, especially the structure in Fig. 11 is the one observable often.

Summary

1. Between the hyphal cells on PDA and the sclerotial cells, there are few remarkable differences excepting the parts of cell wall.
2. The sclerotial cells have very thick fibrous layers (detailed in Table 1).
3. The mitochondria of the sclerotial cell have few crista.

The excessive weakness of the respiratory function of the cells seems to have been suggested by this fact.

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References

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Explanation of Plates

- Fig. 1. Longitudinal section of the hyphal cells on PDA. $\times 14,000$.
- Fig. 2. Cross section of the young hyphal cell on PDA.
Cell wall without outer fibrous structure. $\times 22,000$.
- Fig. 3. Cross section of the old hyphal cell on PDA.
Cell wall and a little thickened outer fibrous structure. $\times 13,000$.
- Fig. 4. Longitudinal section of the hyphal cell on PDA.
The maldistribution of mitochondria. $\times 4,500$.
- Fig. 5. Same to Fig. 4. $\times 14,000$.
- Fig. 6. Enlarged plate of mitochondria area. $\times 13,000$.
- Fig. 7. Cross section of the outside of a sclerotium. $\times 3,400$.
- Fig. 8. Slightly inside section of a sclerotium.
Endoplasmic reticulum and mitochondria with few crista. $\times 10,000$.
- Fig. 9. Inner part of a sclerotium. $\times 3,400$.
- Fig. 10. Highly magnified plate of the inner part of a sclerotium.
Much thickened outer fibrous structure. $\times 12,000$.
- Fig. 11. Same to above.
Including an unknown structure. $\times 14,000$.
- Fig. 12. Same to above. $\times 12,000$.

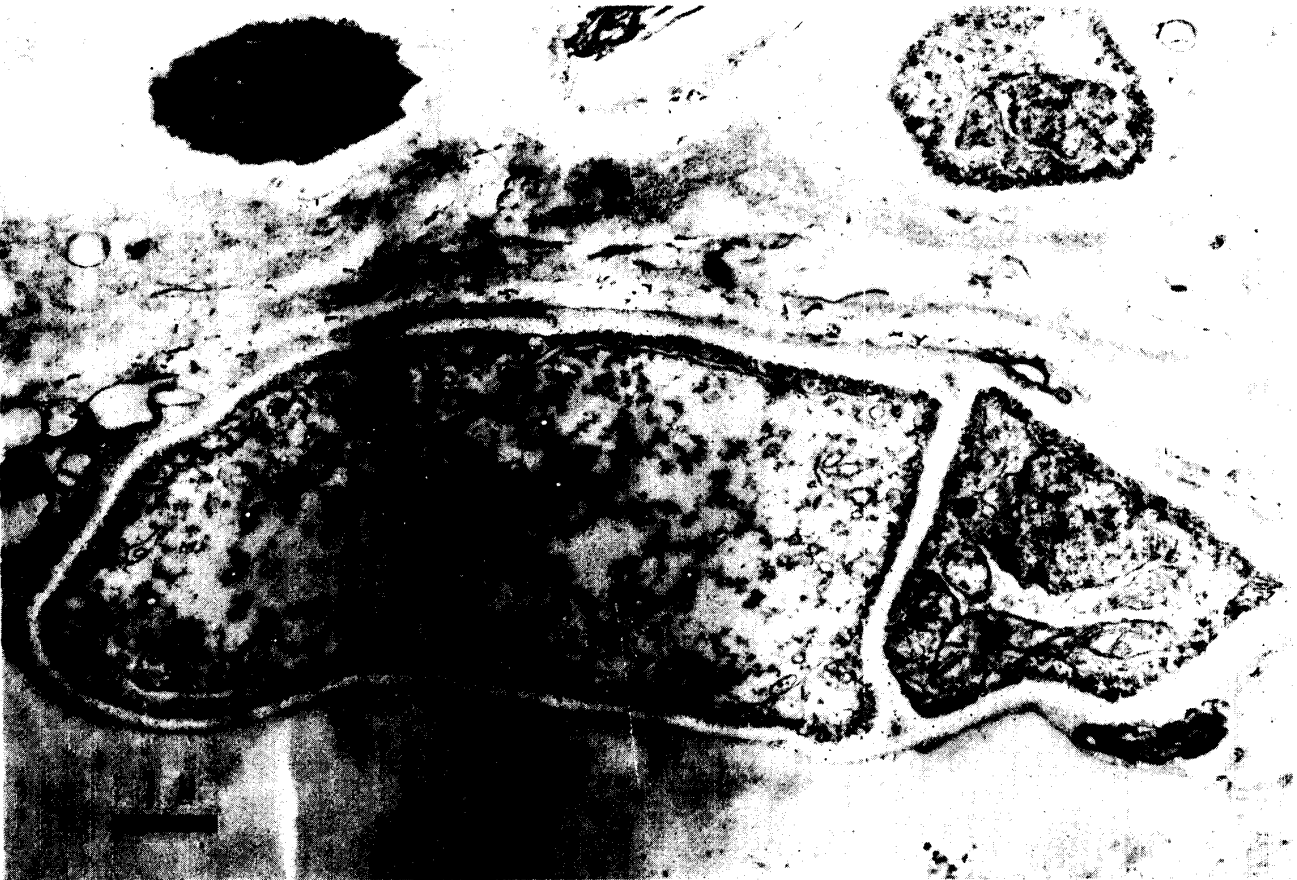


Fig. 1



Fig.2

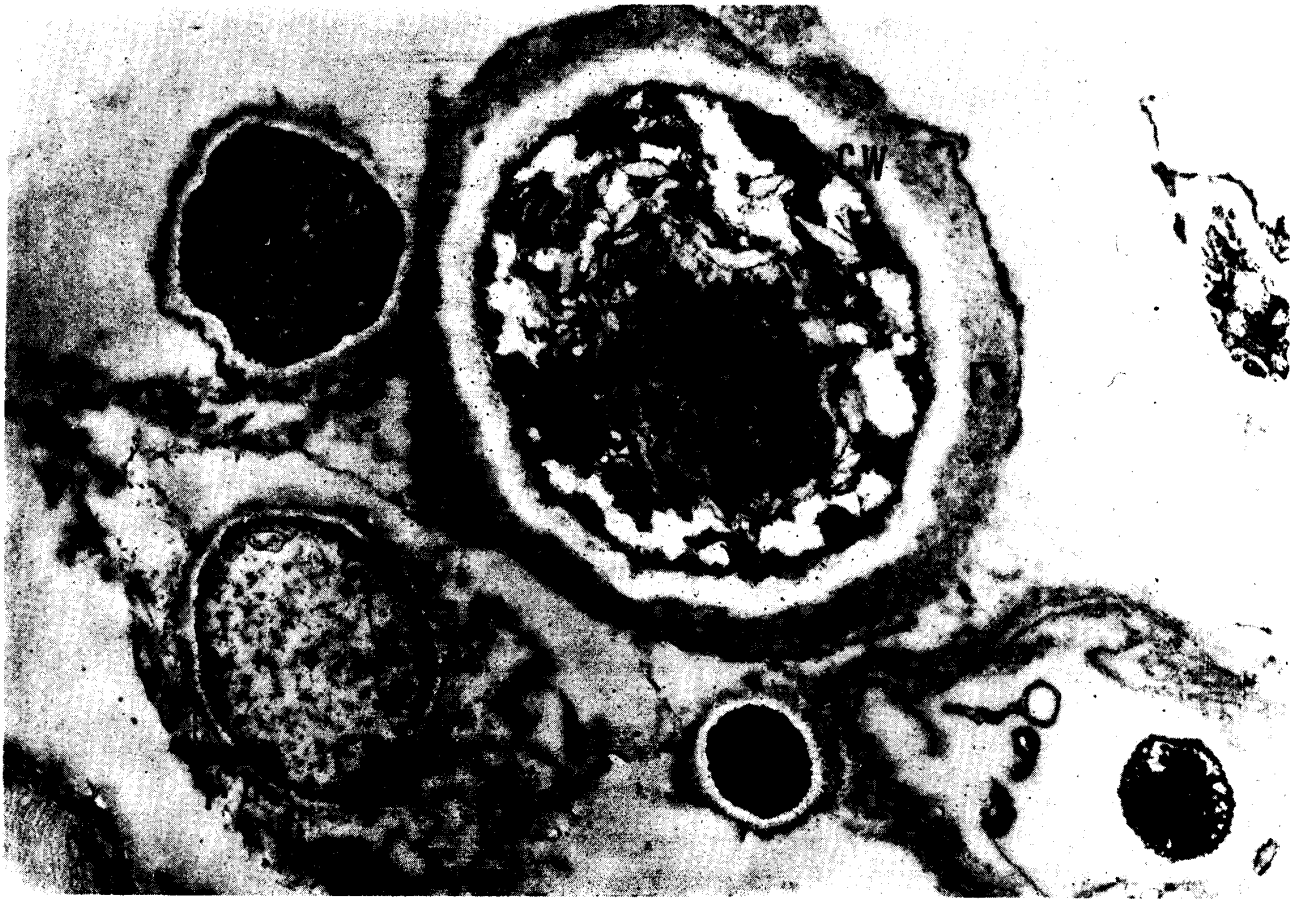


Fig. 3

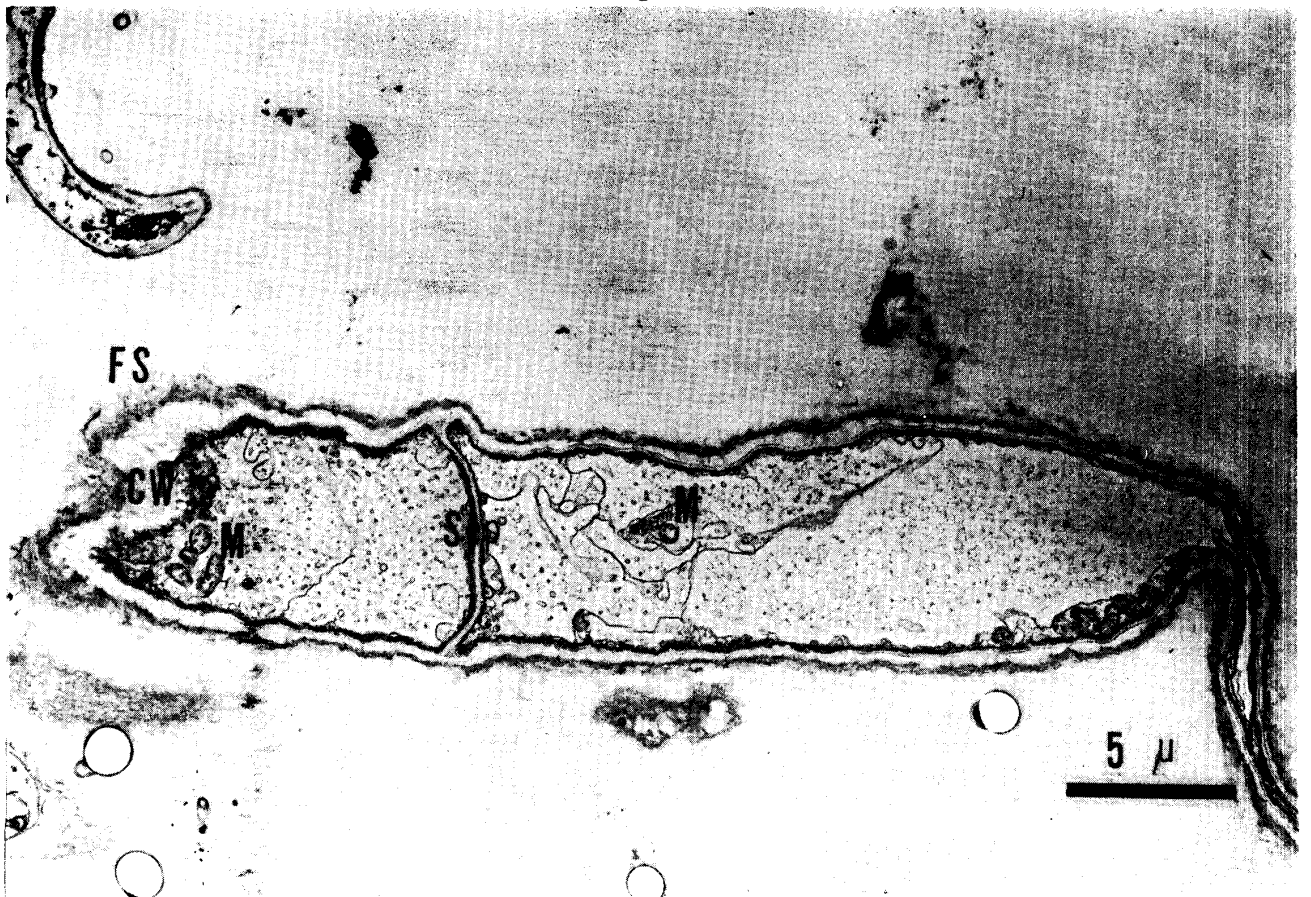


Fig. 4

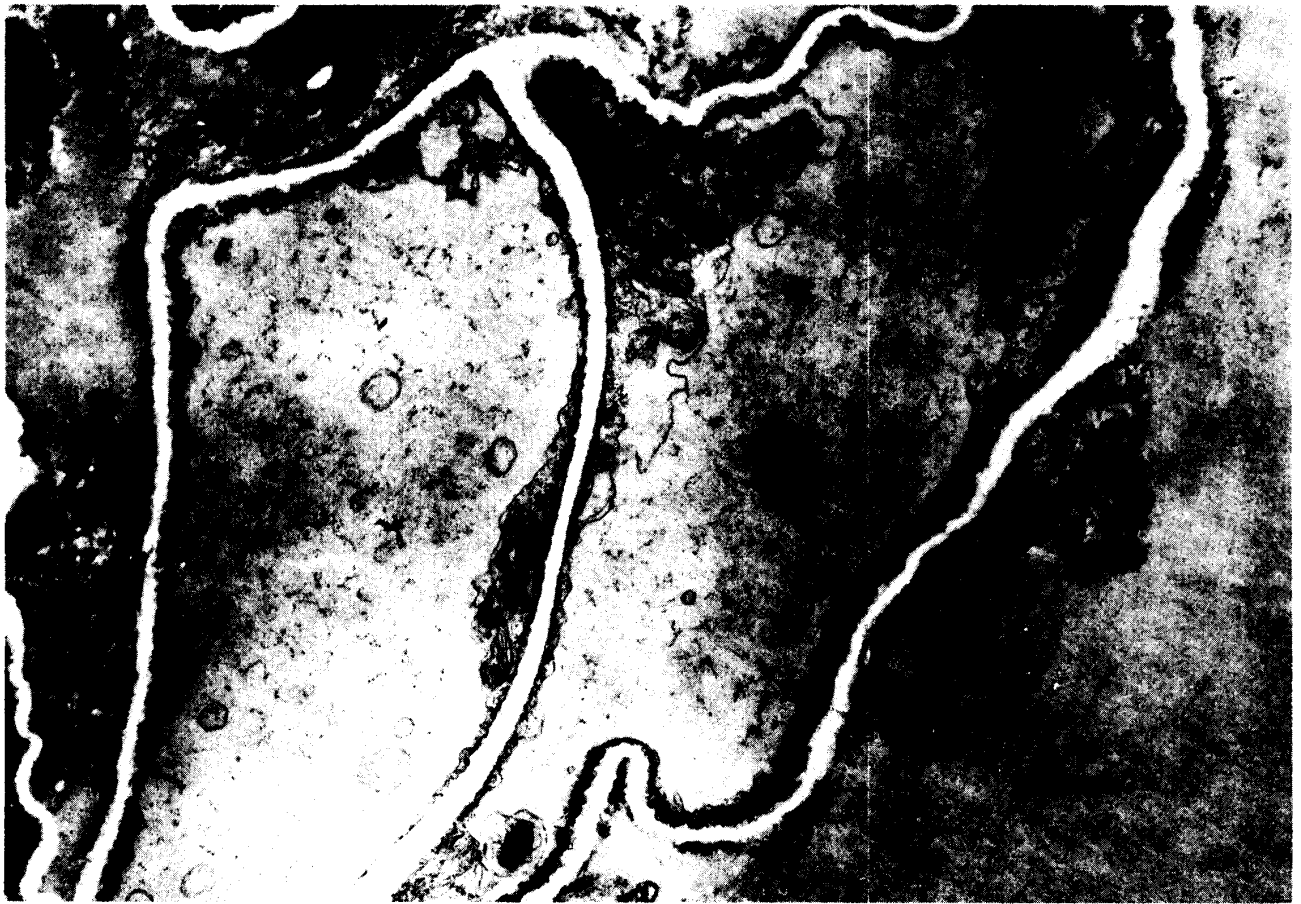


Fig. 5

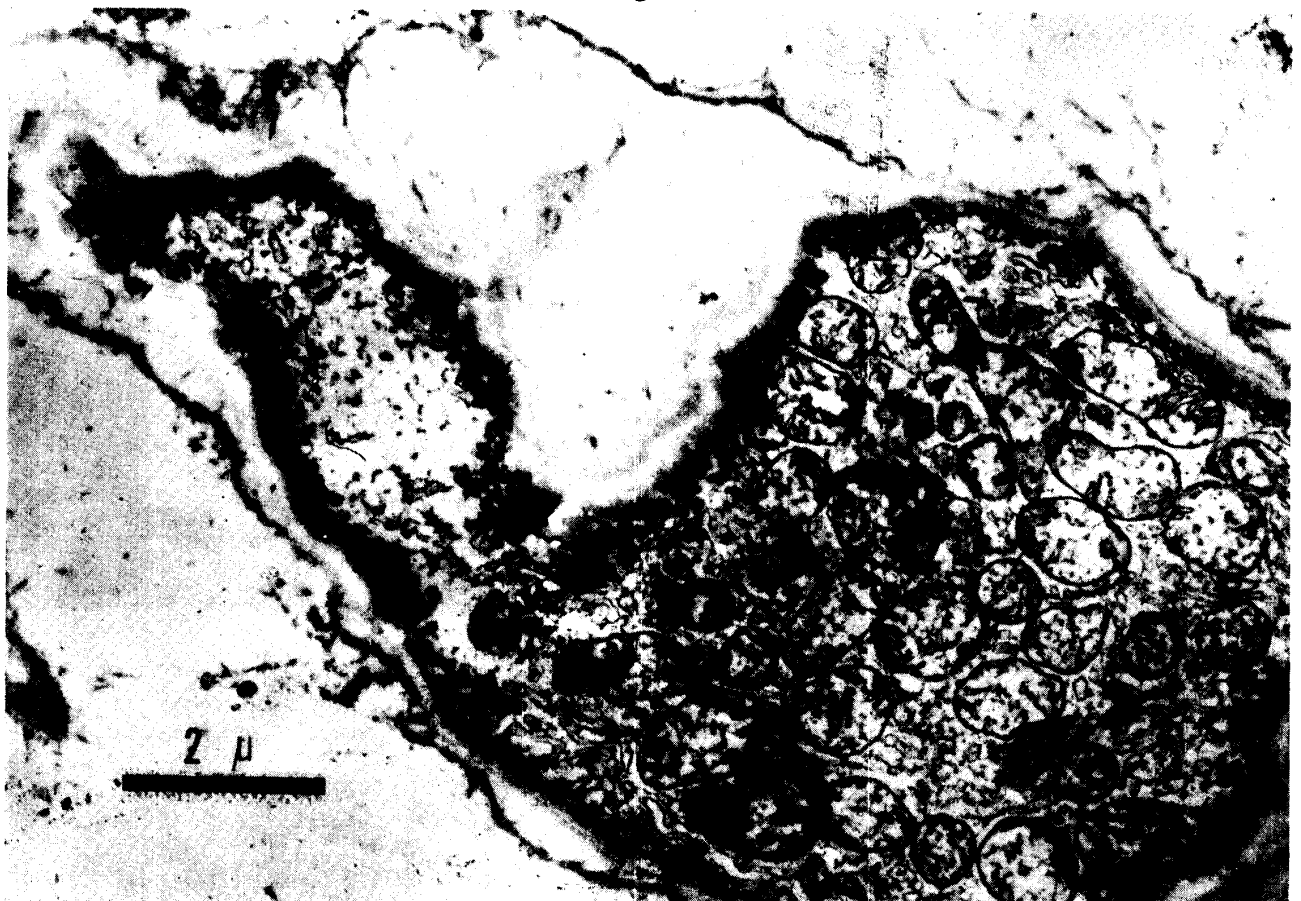


Fig. 6

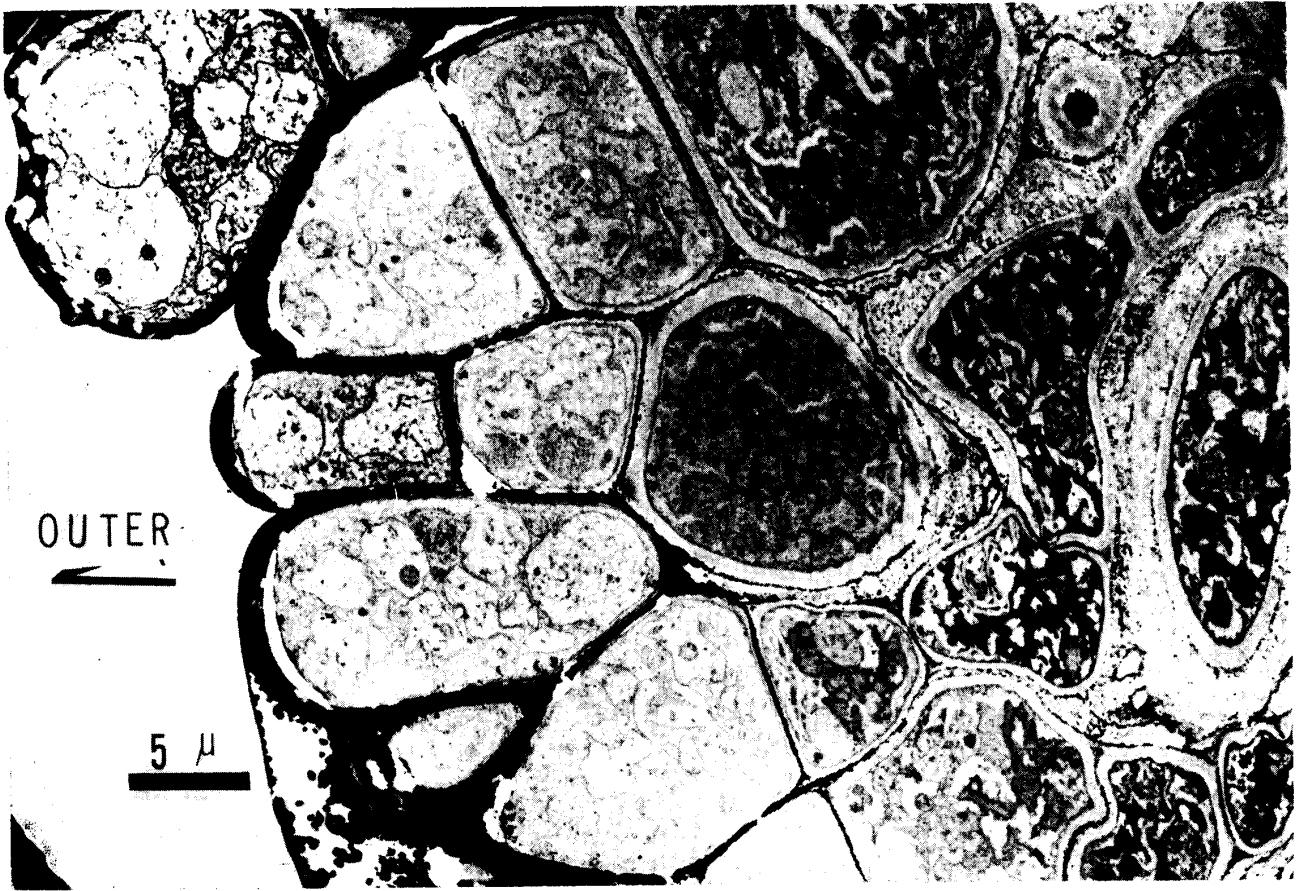


Fig. 7



Fig. 8



Fig. 9

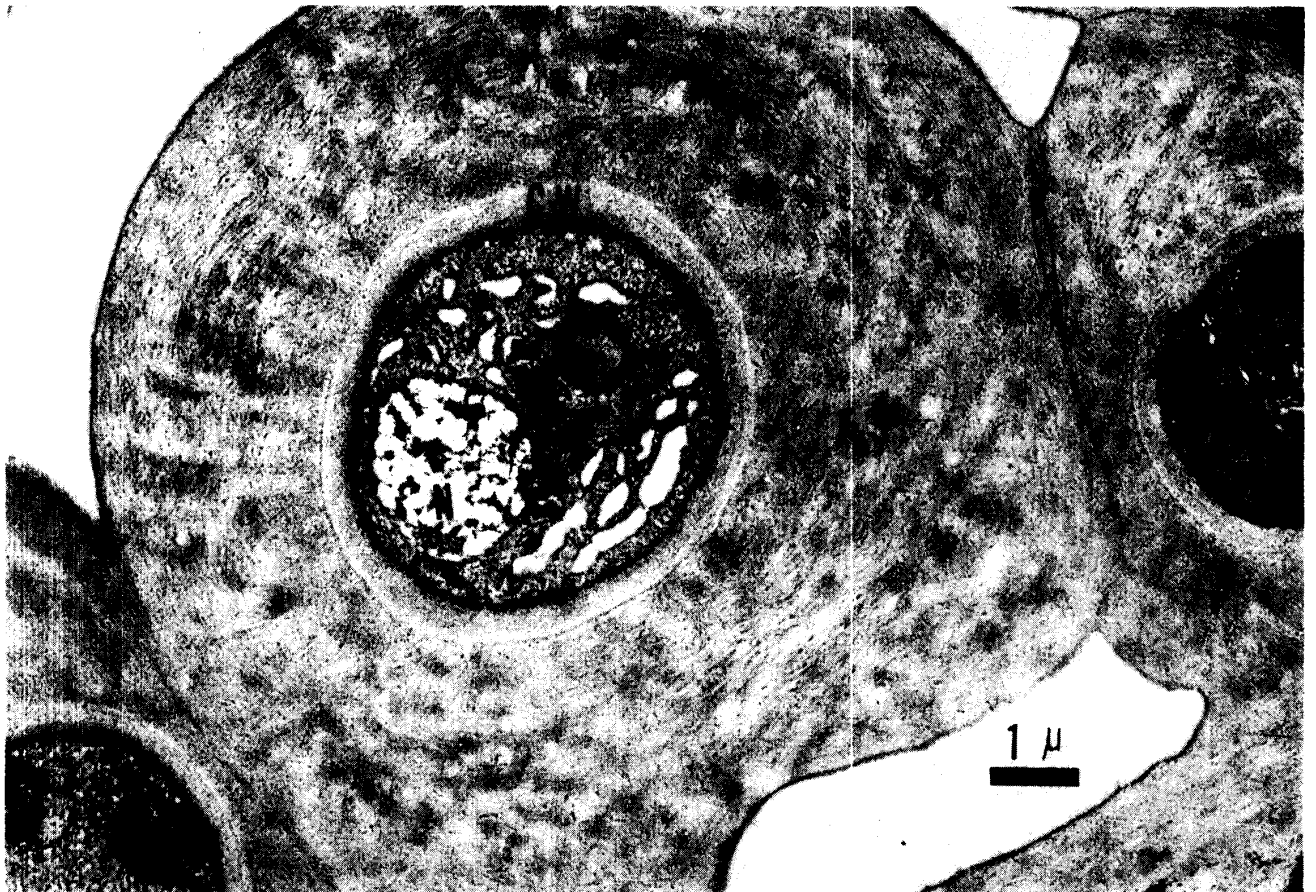


Fig. 10

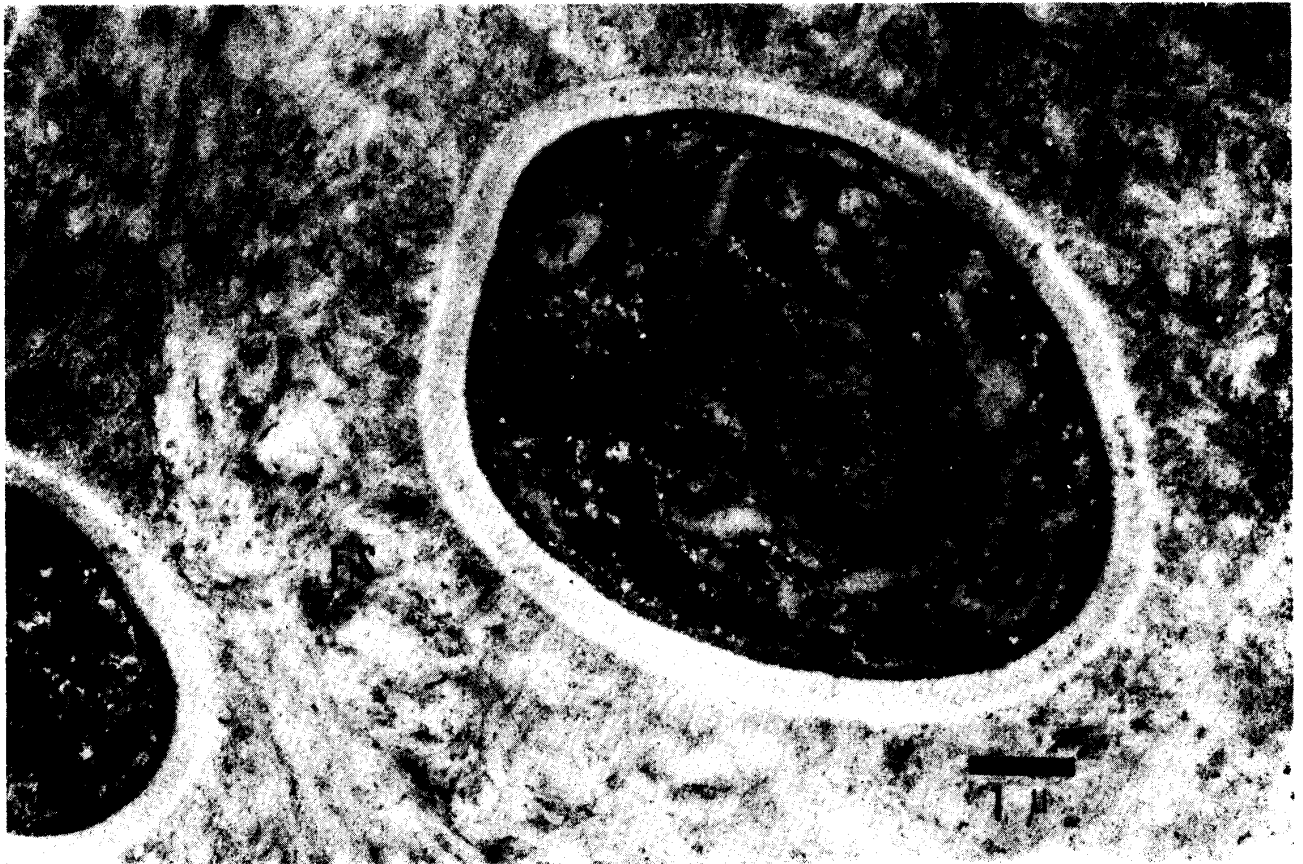


Fig. 11



Fig. 12