ULTRASTRUCTURE OF SPORES AND PLASMODIUM OF *RETICULARIA LYCOPERDON*[1]

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ABSTRACT

Spores and plasmodium of *Reticularia lycoperdon* Bull. were sectioned by electron microscopy. Organelles have been studied and described. The mitochondria were found particularly clear in both spores and plasmodium. The spore walls were thick, with coarse protuberances and usually were surrounded by a delicate slimy layer. Nuclear divisions were occasionally observed before the spore ruptured. The emerging of the swarm cells, possibly 1-4 from each spore, came out through a crack (6) in the wall. Some of the swarm cells were transformed into myxamoebae shortly after emerging. The myxamoebae moved rapidly, each showed only one flagellum at the tapering end. Fibrils were seen when protoplasmic streaming was active. The spore to spore culture was completed in about 16 days, and this is the first time it has been reported in science.

INTRODUCTION

Along with the study on spore germination and plasmodium development of *Stemonitis splendens* Rostof. and *S. flavogenita* Jahn (Yang 1968), attempts were made to study the spore to spore culture of *Reticularia lycoperdon* Bulliard. Aethalia developed in petri dishes were smaller, much thinner and darker in color than those found in nature. The ultrastructure of spores and plasmodia of *Reticularia lycoperdon* collected in July 1967, were sectioned and studied by electron microscopy in the summer of the same year. The present study therefore, consists of respective results of the above investigations, i. e. the life cycle and the fine structure of the spores and plasmodia of *Reticularia lycoperdon.*

MATERIALS AND METHODS

Spores taken from the aethalia of *Reticularia lycoperdon,* which were collected by the author in a private garden in Taipei city, were used to start cultures in petri dishes containing 1.5% agar medium. Culture plates were incubated in dark at 25°C. Different stages in the life cycle i. e. swarm cells, myxamoebae, plasmodium and fructification, were examined under high magnification. Fructification and spores arising from the culture were compared with those collected from the field. Spores and plasmodia for electron microscopic study were fixed in 1% osmium tetraoxide,

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buffered in veronal acetate pH 7.6, embedded in *n*-butyl methacrylate EPON 812 vestopal or araldite and stained with 1% $KMnO_4$. Sections were cut at 1/20 μ thick and photographed. They were examined under the electron microscope.

OBSERVATIONS AND RESULTS

A. Spore to spore culture:

1. Germination of spores—The spores of the present specimens were brown in color, spherical and warty, and 8–12 μ in diameter. When they were sown in distilled water, they germinated readily. The emerging of the swarm cells was observed in about 7 minutes after sowing. They emerged by cracking the spore wall (Pl. I, Figs. 1–4). The rate of germination of this myxomycete was approximately 42%. Spores of older specimens showed a much lower rate of germination. Thus, spores of the same specimen, but sown 20 days later, required about 2 hours or more to start germination and those of 3 months later required about one month to show germination. This confirms the results of others that the viability of the spores of myxomycetes often corresponds with their age (6).
2. Swarm cells—The size of the swarm cells is not larger than that of the spores. When they were examined under the microscope, they were transparent, spherical, and minutely warted. As soon as they emerged from the spores they scattered freely on the surface of the medium. Suddenly a host of myxamoebae appeared in the culture. Such a sudden change from swarm cells to the myxamoebae stage has not been previously observed, either in *Stemouitis splendens* or *S. flavogenita.*
3. Myxamoebae—The myxamoebae of the present myxomycete were capable of moving rapidly. One flagellum was seen at the tapering end, while the opposite end was blunt and contained vacuoles and engulfed food particles. They are highly metabolic. This amoeboid stage was maintained for a much longer period of time than the swarm cell stage. Some of the myxamoebae united with swarm cells, some with other myxamoebae (Pl. I, Figs. 5, 6, 7).
4. Copulation—The myxamoebae, after moving around for some time gradually slowed down. Copulation between two myxamoebae was observed (Pl. I, Figs. 5–7). Some retracted their fleaglla before copulation, and some did not. Pl. I, Fig. 9 shows a flagellated myxamoeba and Fig. 8 a zygote with thick wall and a long flagellum still attached (indicated by an arrow).
5. Plasmodia—The plasmodia of *Reticularia Lycoperdon* is yellowish in color, thin and irregular in shape. It is formed by the union of swarm cells or of myxamoebae or unions between swarm cells and myxamoebae. Small plasmodia became coalesced into larger ones as shown in Pl. I, Figs. 3–4.

Aggregation of zygotes of swarm cells or myxamoebae presently occurred. The thickness of the plasmodium varies. In the thick region, it is yellowish; while in the thinner areas it is whitish and transparent, and here protoplasmic streaming was occasionally observed. A fine network of veins was faintly seen but the veins did not give rise to short, coarse branches as in *Stemonitis splendens* (23).

6. Fructification—Plasmodium became aggregated into larger masses (Pl. II, Fig. 3) when its watery content gradually evaporated into a thin film. After being exposed to fluorescent light (20 foot candles, at 30 cm. distance) some dark patches appeared (Pl. II, Fig. 4) on cultures growing on weak agar medium, but the next morning all these patches disappeared; instead, some spherical, dark aethalia (8–12 mm in diam.) appeared in their place. Some cultures growing on Sabouraud's medium, which was casually picked up from laboratory, also gave rise to fruiting stages but they did not follow the regular sequence of development, that is, they did not have the vein-formation stage nor showed vigorous protoplasmic streaming. Besides, they did not result in the aethalium stage, and their spores were exposed on the surface of the blackish patches which occurred on the edges of petri dishes. The spores that developed in weak agar plates were enclosed in a thin membrane of the spherical aethalium (Pl. II, Fig. 5). Morphologically, the spores produced from both the weak agar and Sabouraud's media did not show much of any difference from those collected from the field, except being smaller in size. Those grown in the laboratory measured 7.5 μ while those from the field measured 8–12 μ. The life cycle of *Reticularia lycoperdon*, from spore to spore was completed in about 16 days.

B. Ultrastructure of spore and plasmodium

1. Fixation and embedding—The spores and plasmodium of *Reticularia lycoperdon* were fixed in 1% osmium tetraoxide, buffered in acetate pH 7.6, and embedded in *n*-butyl methacrylate EPON 812 vestopal or araldite and stained with 1% potassium permangnate.
2. Sectioning and photography—Specimens prepared as above were examined under electron microscope model HU–11A (Jap.). Sections were cut at 1/20 μ thick by Sorvall MT–2 Porter-Blum Ultra-microtome and photographed.
3. The fine structure of the spore under the electron microscope revealed that the outer layer was deeply stained with conspicuous protuberances, while the inner layer, which was nonstainable, was thicker than the former. There was a slimy layer surrounding the outer layer (Pl. IIIs). The nuclear envelope and nucleolus were clear; other organelles found in the cytoplasm included: mitochondria, dictyosome, polyribosomes in net arrangement and endoplasmic reticulum.

4. Division of protoplast was observed on several occasions showing different stages as Pl. V, Figs. 1a, 2b. This may indicate that each spore of this specimen will produce more than one swarm cell, possibly four, at least, in some of the spores.
5. The fine structure of plasmodium showed various kinds of organelles such as mitochondria, vacuoles, droplets of lipid density, pigment granules and one zygotic union was seen. The thick region of the plasmodium was darker in color, beside the organelles mentioned above, it contained other particles and numerous lipid droplets (Pl. IV, Figs. 1–2). The thin areas were transparent and contained less particles. Fibrils were found in three sections (Pl. VI, Fig. 1; Pl. VII, Figs. 1–2) where protoplasmic streaming was observed. Spores in different germinating stages including the cracked empty spore cases were seen mingled in the plasmodium.

CONCLUSIONS

1. *Reticularia lycoperdon*, the myxomycete used in the present study was found to be the first record for this species on Taiwan.
2. Spore to spore culture was completed in about 16 days. Repeated experiments gave the same results.
3. The germination of spores revealed that the rate of germination and the time required for germination corresponded with the age of spores. The younger the spores, the higher the rate of germination and less time was required before germination began. The older the spores, the lower the rate of germination and the longer the time required for germination. This agrees with what Elliott reported in his investigation on germination of myxomycete spores in general. But he also found abundant germination obtained from his experiment with material of 17 years old (7). This may indicate that age of the spores of *R. lycoperdon* Bull. is not an important factor in its spore germination.
4. Cultures developed on Sabouraud's medium did not show the complete sequence of development.
5. Sections of the spore of *Reticularia lycoperdon* examined under electron microscope revealed a two-layered cell wall with slimy material surrounding the outside; a large nucleus with nuclear envelope and nucleolus, large mitochonodria, polyribosomes in net arrangement and endoplasmic reticulum in the cytoplasm.
6. A study of the fine structure of plasmodium disclosed that in the thick regions there were more pigment granules, vacuoles, and mitochonodria, while in the transparent protoplasm less granular material and only slight streaming and fibrils were observed.

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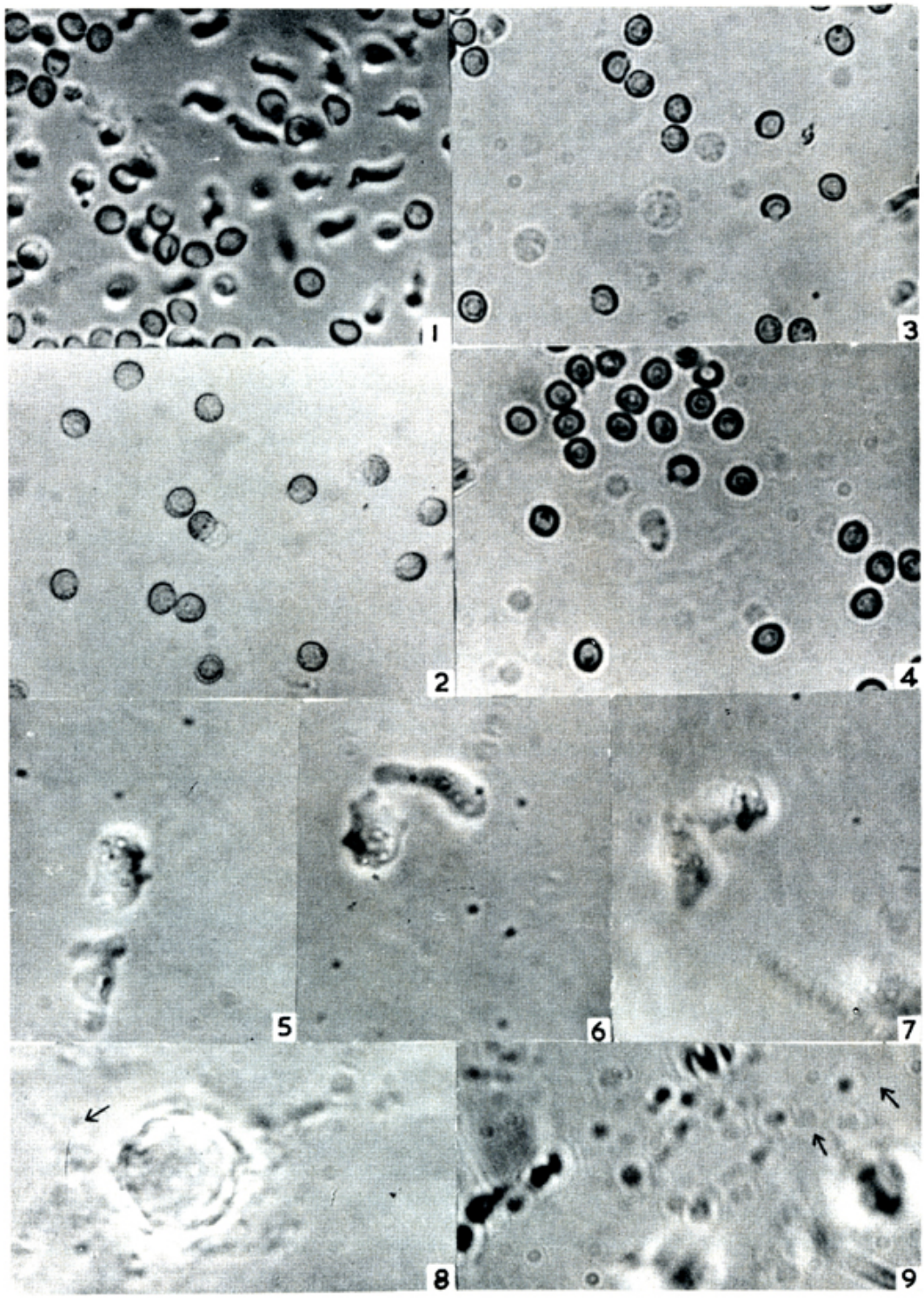
Plate I

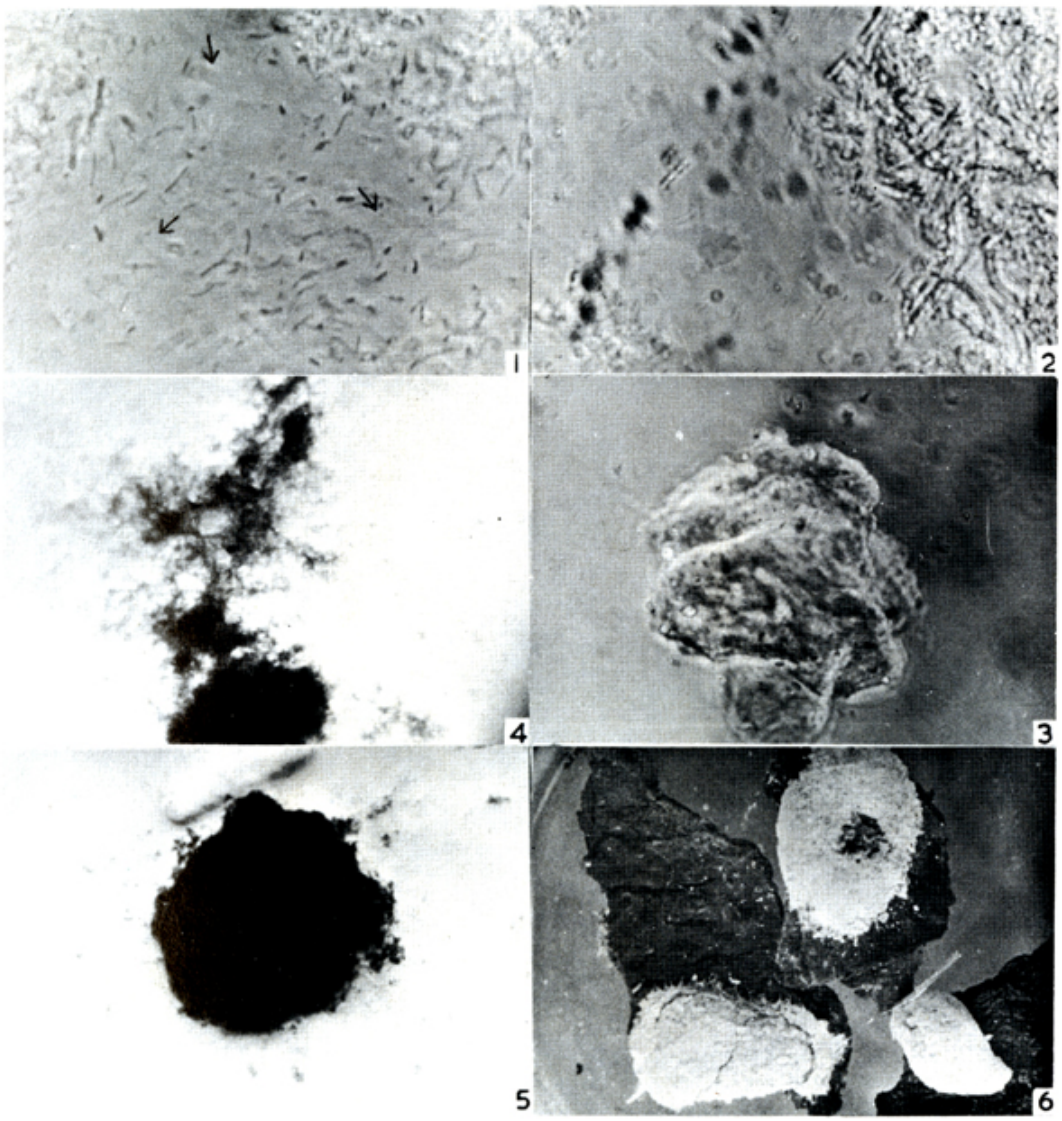


Reticularia lycoperdon—spore to spore culture

1-4. Germination of spores in 7 minutes ×600
1. Immediate emerging of swarm cells and myxamoebae from spores collected from dark grey aethalium, (in 7 minutes)
2. Showing 2 swarm cells half-emerging, and surface of the spores warted.
3. Swarm cells begin to gather forming plasmodia of various sizes, (1 hour after sowing of spores)
4. Ditto.

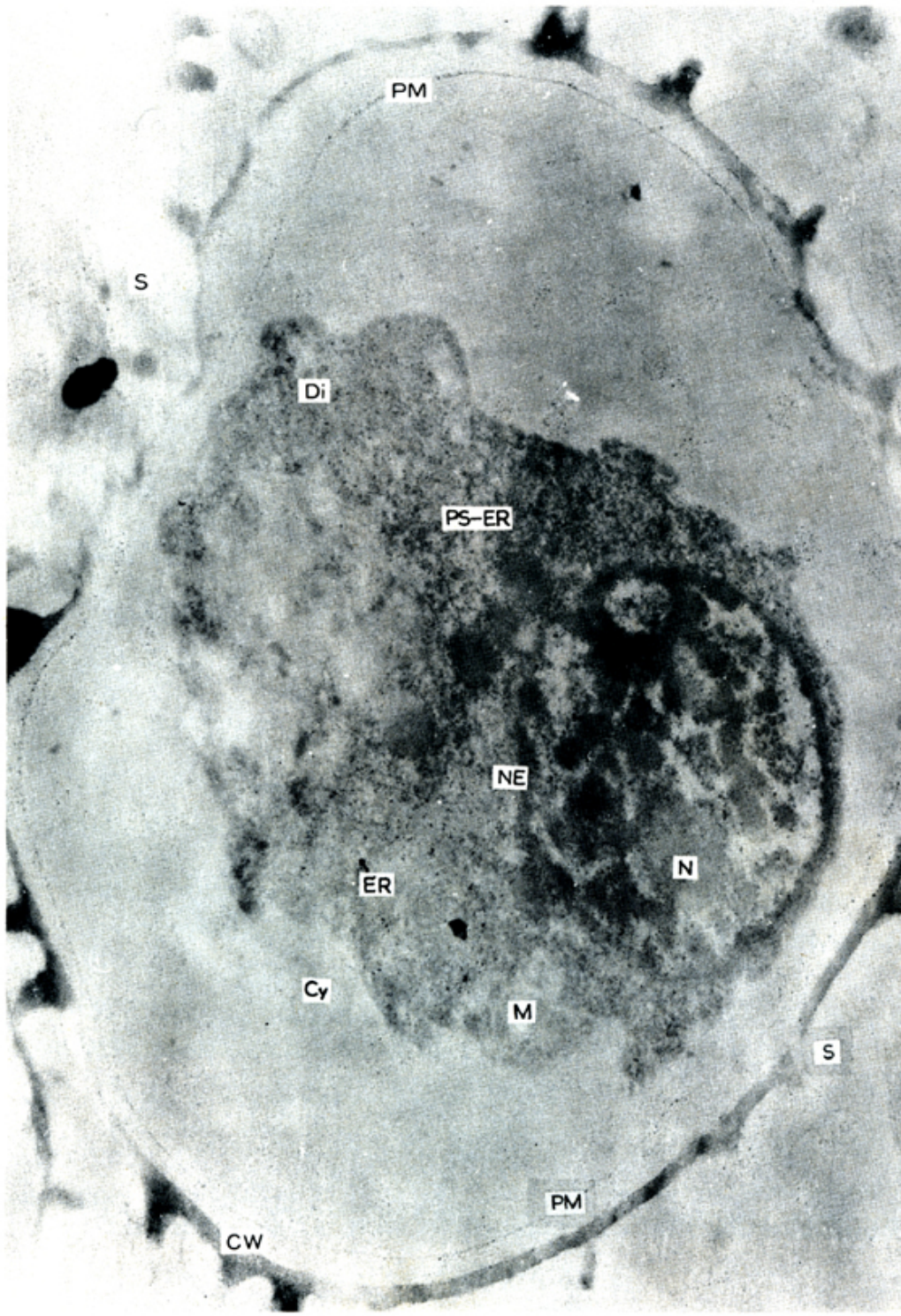
5-7. Amoeboid stage, showing copulating ×1,500
8. A thick-walled zygote with flagella still attached, appeared in 16 days ×1,500
9. Flagellated swarmers ×1,500

Plate II



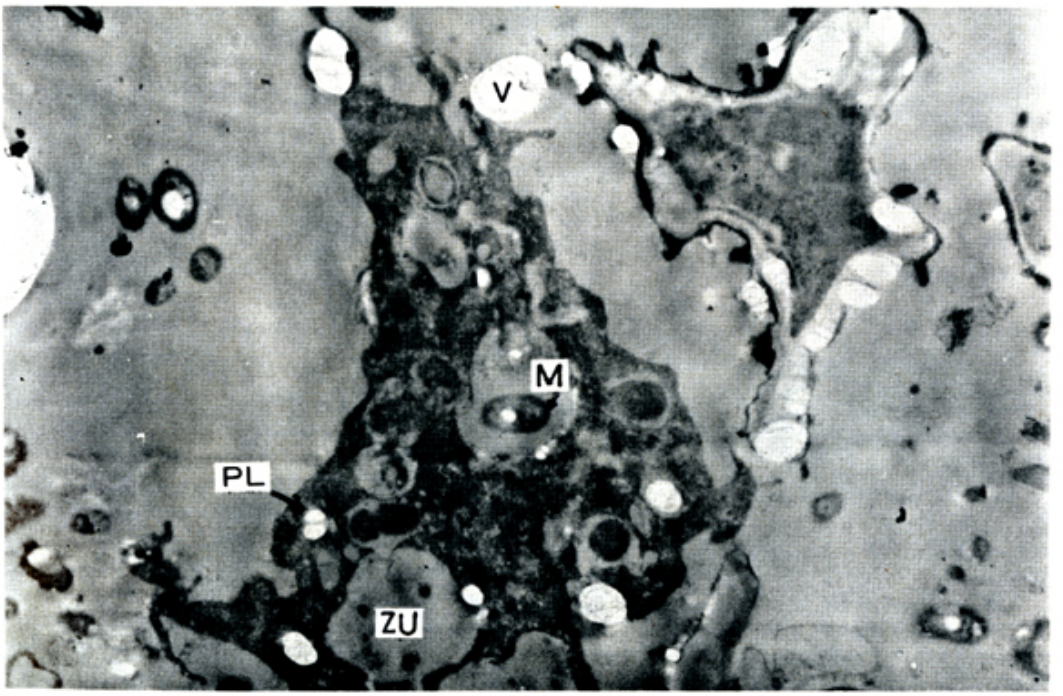
1-2. Thick areas of plasmodia yellow, streaming of protoplasm occurred in thin areas. ×600
3. Plasmodia aggregated in masses ×28
4. Beginning of fruiting ×28
5. Completion of an aethalium (dark brown in color) ×28
6. A light yellow aethalium collected September 1966 from a dead trunk of *Pinus palustris* ×1.5

Plate III



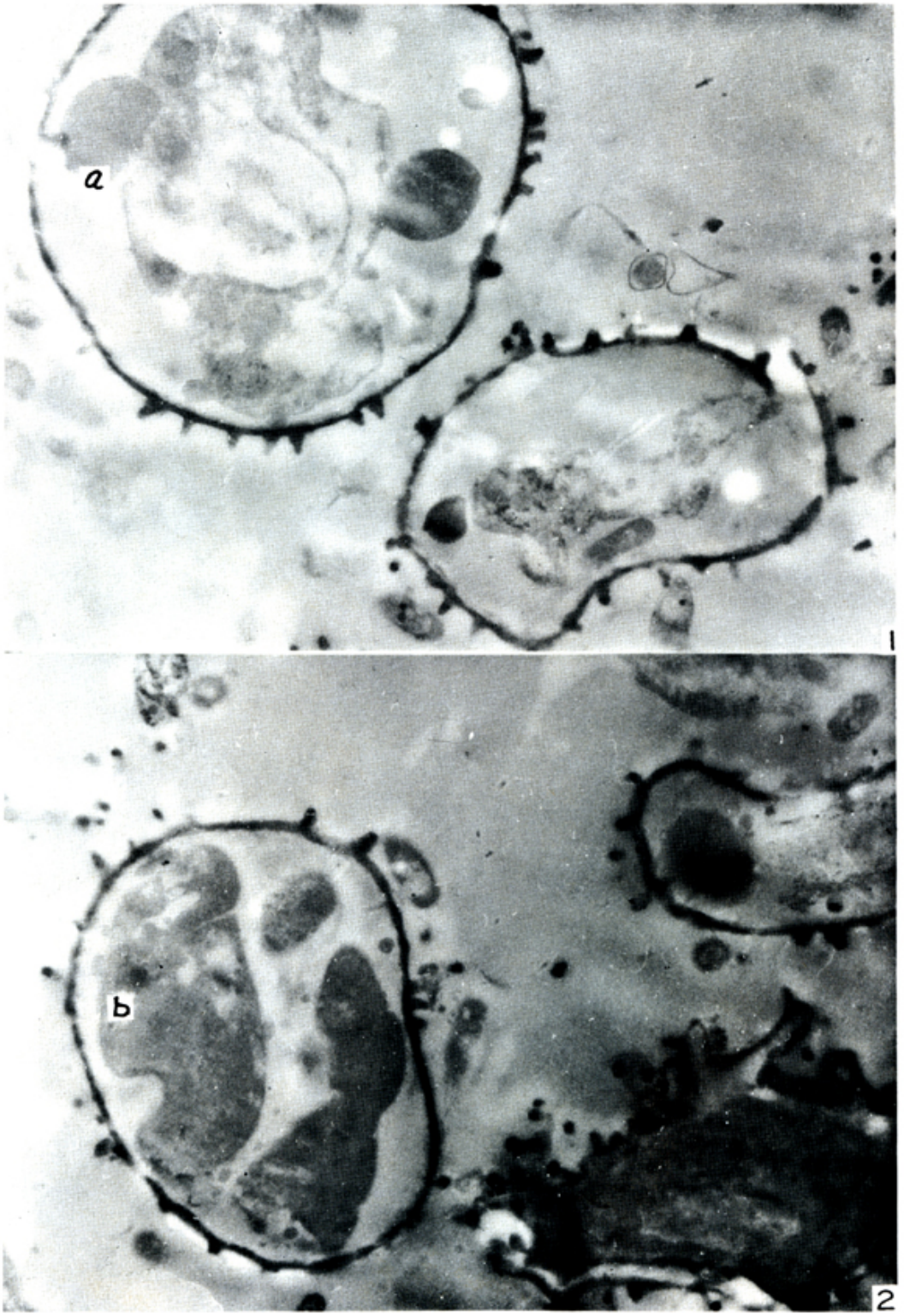
Organelles of a spore under electron microscope ×40,000
CW, cell wall with warts; PM, plasma membrane; N, nucleolus; NE, nuclear envelope; Di, dictyosome; ER, endoplasmic reticulum; Cy, cytoplasm; S, slime outside CW; M, mitochondrion; PS-ER, polyribosomes in net arrangement.

Plate IV



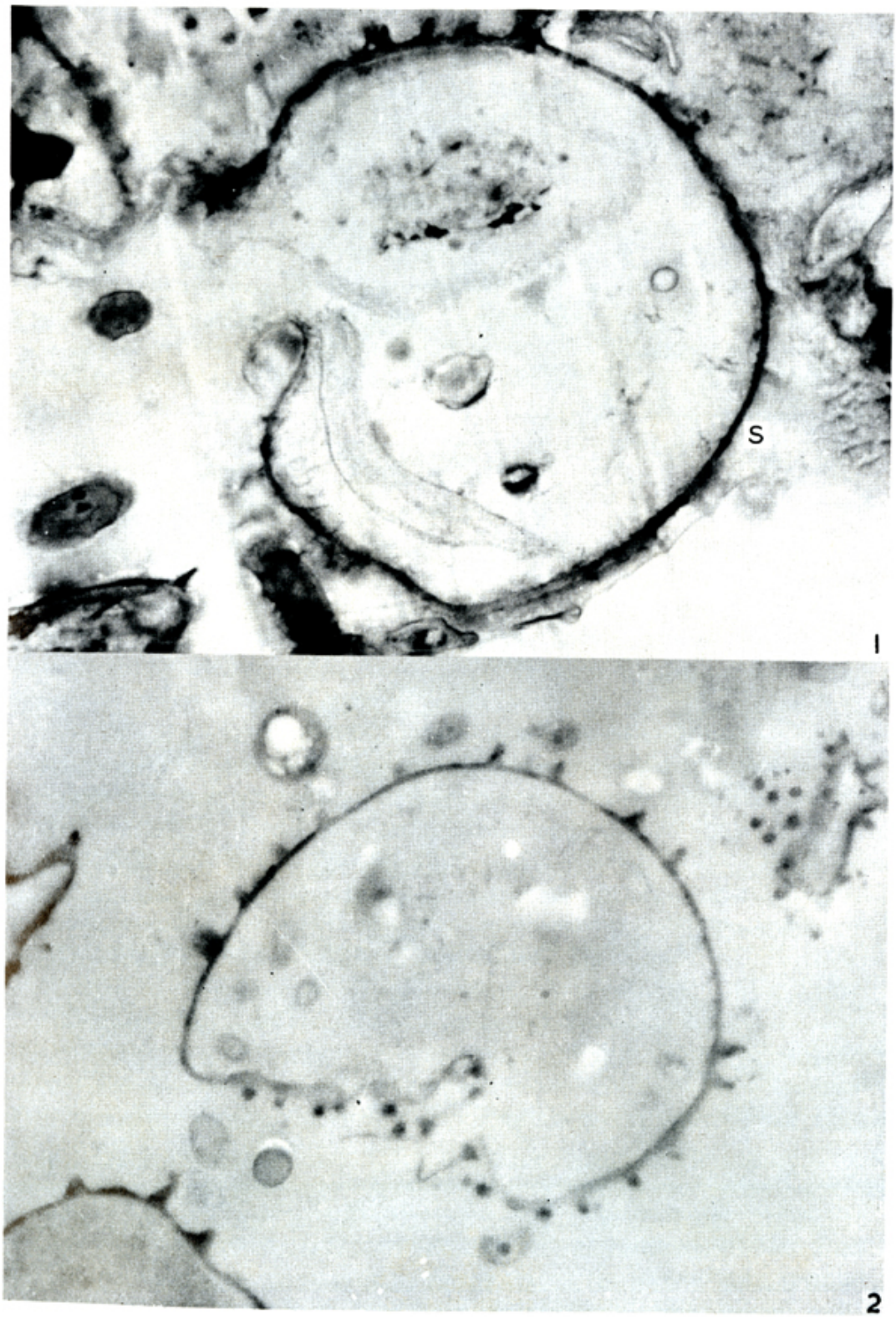
Portion of plasmodium showing; PL, platelet of lipid density; ZU, zygote union; M, mitochondrion; V, vacuole; etc. ×12,500

Plate V



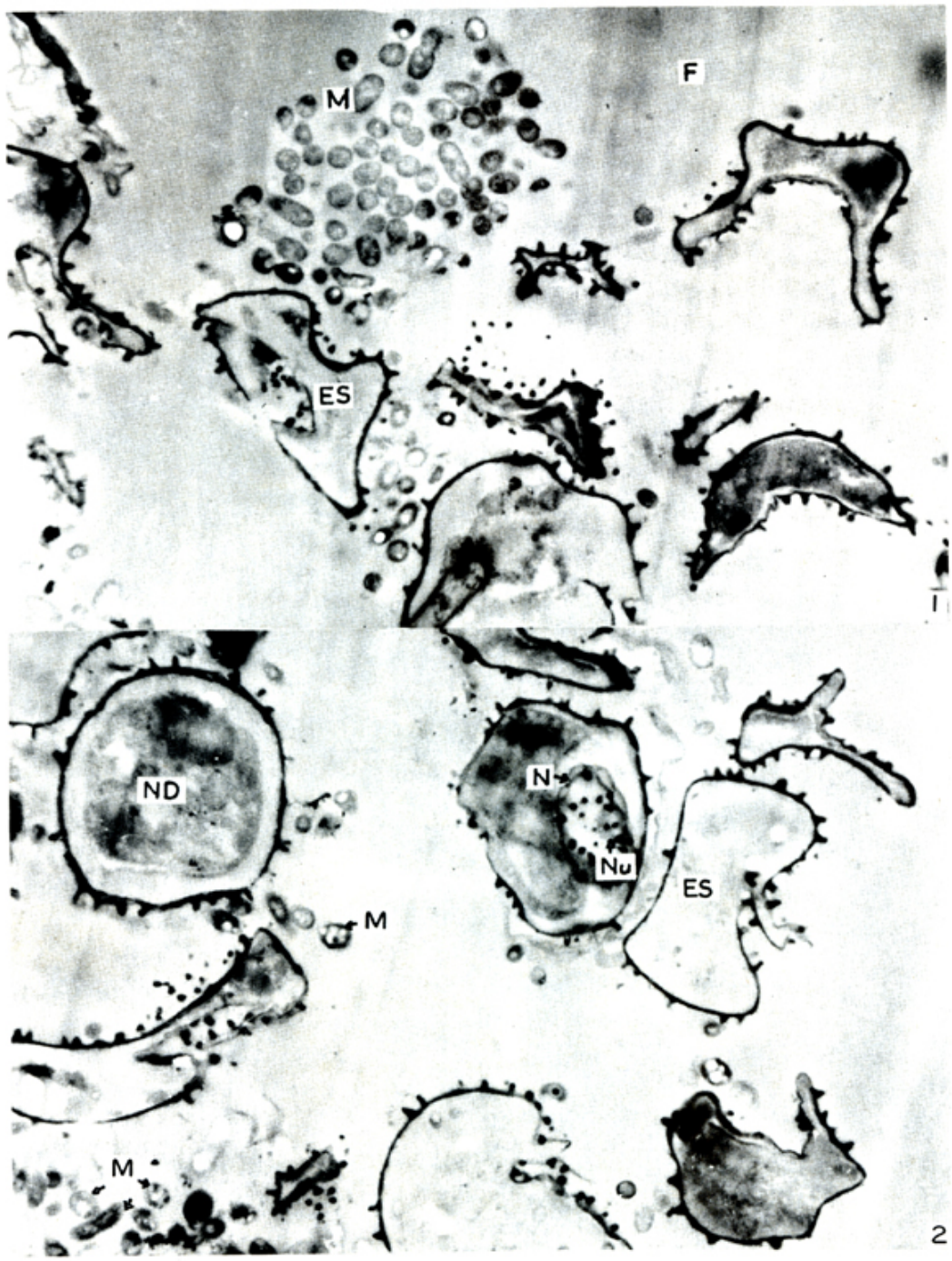
Spores showing:
1a. Division of protoplasts within the spore wall. ×12,500
2b. Ditto, showing another stage. ×12,500

Plate VI



1. Section of spore, showing some protoplasts still within the spore and a delicate slimy layer, S surrounding the spore wall. ×12 500
2. An empty spore showing the crack where the swarm cells emerging. ×12,500

Plate VII



1. Veins showing; F, fibrils; M, mitochondria; ES, empty spore; etc. ×7,500
2. Ditto, containing spores of various stages, mitochondria etc. dispersed in the plasmodium. M, mitochondria; ND, nuclear division N, nucleus; Nu, nucleolus. ×7,500