FULIGO CINEREA REPORTED FROM TAIWAN

Some observations on its plasmodia, sclerotia, spore germination and fructification[1]

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Abstract: When *Fuligo cinerea* (Schw.) Morg. of Taiwan arrived in my laboratory, it was a huge, yellow mass of plasmodium. In the next day it changed its color into brown or black and aethalia of various sizes were observed. Spore germination revealed a germination rate of 50%, which was probably determined by the age of spores. Swarm cells emerged by a slit in the wall usually one cell escaped from one spore. Biflagellated swarm cells produced synchronously were observed. Both yellow and colorless plasmodia were observed. Veins, fans and clamps were vivid and vigorous streaming occurred nearly in all the cultures. Myxamoebae of both large sized and smaller ones were observed. Sclerotia of angular cysts and microcysts often formed under unfavorable conditions. When the plasmodia became drier and smaller, fructification and sporulation followed. It takes about 18 days to complete the life cycle of *Fuligo cinerea.*

INTRODUCTION

Fuligo cinerea, collected on the ground from the forest floor at Lin-kuo (林口), Taipei Hsien, Taiwan was sent to me by Dr. C. H. Wang of the Agricultural Chemistry Department of this University on March 23, 1971. It was a huge mass of yellow plasmodium, measuring more than one foot in diameter and growing on the surface of a substrate which was a mixture of soil, grass, dead branches and other plant debris. The whole mass consisted of viscous, interwoven, ribbon-like structures (Pl. V, 6↗shows a portion of the ribbon in the lower right corner). The mass had an uneven, porous surface, was an unpleasant sight and had a bad smell. The next morning, its color changed from yellow to purplish-brown and its edges had tints of bright reddish-yellow which finally became dark brown or nearly black. In the meantime, the whole plasmodium became smaller, thicker and much drier in consistency and this was followed by sporulation and fructification the next day. The resulting aethalia were of various sizes, shapes and colors (Pl. V, 6). Germination of spores and cultivation of plasmodia have been experimented on synthetic media. Thin and rather smooth aethalia developed in the petri dishes (Pl. V. 4). Some notable events were observed during the course of this study, which are reported in the following account.

MATERIALS AND METHODS

The materials used for this study were obtained from the specimen collected at Lin-kuo, Taipei Hsien, as mentioned above. It was wrapped in a cellophane sheet

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and placed in a paper box about 35cm×35cm×15cm.

This myxomycete was classified as *Fuligo cinerea* (Schw.) Morg. based on the external characteristics of the aethalia (Gray and Aloxopculos, 1968, 193; Hatteri, 1935; Macbride, 1922).

The germination of spores in distilled water was attempted. After the occurrence of a few swarm cells, the whole suspension was poured over a weak agar plate, on which surface, plasmodia usually developed. About 2 dozens of such plates were prepared at a time and on nearly all of them developed transparent, colorless plasmodia. A complete study of the life cycle of this myxomycete, from spore to spore, is based on these plate cultures.

OBSERVATIONS AND RESULTS

1. Spore germination

Mature spores taken from newly formed aethalium were sown in distilled water for germination. Microscopic examination revealed that they were spherical in shape, about 4–7 μ in diam. (Pl. I, 1) having papillose walls (Pl. I, 3) and with a slight tint of violet- purple. Among these there were the capillitia with inflated, large nodes joined by long transparent filaments (Pl. I, 1). Upon germination, the spores generally open by the slit method (Pl. I, 2), while the pore method was also occasionally found. Germination did not occur until three or four days or up to a week after sowing, all depending on the age of the spore. Older spores required a longer time for germination, and this decided the percentage of the germination rate.

2. Swarm cells

The protoplasts first emerging from the spores were mostly non-moving, transparent, spherical bodies (Pl. I, 8). As they escaped through a slit in the spore wall, it was not difficult to see that there was only one protoplast emerging from one spore. At the time of germination, when much water is absorbed, the size of the emerging protoplast is as large as the spore itself; the largest are about 7–12μ in diameter. They move about rather passively, as observed under the microscope. There were also some swarm cells with flagella found in these same cultures. The long flagella were usually twice as long or longer than the body of the cell and always were attached to the pointed end while the opposite end was blunt. The flagellated swarm cells may be interpreted as being transformed from the spherical protoplasts which first emerged from the spores. But when and how they were transformed was not determined in this study. In spite of the fact that usually one swarm cell emerges from one spore yet in a few cases it seemed that more than one protoplast came out from the cracked spores. When they emerged, it was always, all of a sudden, and then they immediately dispersed into the surrounding media and were lost to view.

Another type of swarm cells which was observed were those found in some of the cultures which developed into yellow plasmodia. They were biflagellated with rapid motility. When a few drops of distilled water were added to an old culture their activity was greatly increased. These swarm cells were especially slender and active. But they only maintained their activity for a few hours and then became quiet.

3. Plasmodia

Both colorless and light yellow plasmodia were produced by the present myxomycete. The former produced spherical, non-moving and also flagellated swarm

cells while the latter produces elongated, rapidly moving, biflagellate swarm cells. The former was thin and spreading on the agar plates with certain limits, while the latter was more watery, and grew indefinitely. The former consisted of a mixture of bacteria and other substances, such as food particles, while the latter consisted of a more or less pure culture of bacteria (mostly Bacilli). The former developed veins, fans, clumps and finally reached the fructification stage (Pl. V, 1–4), while the latter exhibited no veins, and did not reach the fructification stage, except that in one plate some minute veins and 2 small black spots (as large as a pin point) were observed.

Two of the plate cultures (labeled 3/27a and 3/27b) formed colorless plasmodia, these showed the best results—including the formation of veins, exhibiting vigorous streaming, fans and clumps and finally reached sporulation and fructification. From these cultures the present observations on the life cycle of *Fuligo cinerea* were made.

4. Myxamoebae

Two types of myxamoebae were observed in *Fuligo cinerea*. One was the large, highly vacuolated type found in the yellow plasmodium (Pl. II, 1–4). They often occurred in great numbers synchronously. They became thick walled and underwent a dormant stage (Pl. II, 5) when the environment was not favorable, but returned to the myxamoebae condition when conditions were favorable. Aggregation of these myxamoebae was not observed. The other type was smaller and spherical or irregular (Pl. I, 9); and they were scattered in the colorless plasmodium. Aggregation of these produced large plasmodia (Pl. III, 3, 4, 6). (It is believed that from these arose the veins and later fructifications.)

5. Sclerotia

Three kinds of sclerotia were found in *Fuligo cinerea*. The first type observed was the ribbon like spherules formed before fructification (Pl. V, 6 arrow). The second type were the microcysts occasionally found in certain areas of the colorless plasmodium or on the surface of the fructification, and the third type was the triangular or closely packed angular cysts found abundantly in the yellow plasmodium. They sometimes became dormant and later germinated to form large, highly vacuolated myxamoebae (Pl. II, 1–4). In this myxomycete the formation of closely packed angular cysts, was obviously due to the vast number of bacteria contained in the culture which may have induced its rapid growth, and caused the color of the plasmodia (Gray and Alexopoulos, 1968, 153–4).

6. Fructification

When plates with profuse veins were moistened and nutrient material (e. g. flakes of oat meal) was added, they immediately revived and gave rise to more veins, and the streaming of protoplasm likewise returned. The factors influencing the formation of fructification have been studied by many investigators. In *Fuligo cinerea* it was observed that lack of moisture and of nutrient material were perhaps the major factors that induced the development of fructification. The light factor had no effect on fructification and this was shown by the fact that a culture plate (No. 3/25) left in dark showed developing aethalia about the same time as others where onesided light was present.

The aethalia developed in the laboratory were black, thin and smooth as compared with those produced by the specimen collected in the nature which were cushion-shaped and of various sizes and colors, and with rough surfaces (Pl. V, 3, 4, 6).

DISCUSSION AND CONCLUSION

1. The time required for the germination of spores of *Fuligo cinerea* is about 4 days to a week. The rate of germination is probably governed by the age of spores. Spores collected from newly formed aethalium showed a higher percentage of germination, i.e. 50%, while those from aethalia of 8-months old or older showed only a 13.8% of germination.

2. The swarm cells which first escaped from the spores were spherical and non-flagellated, but later flagellated ones were also observed. The latter, were perhaps transformed from the non-flagellated ones.

3. The aethalia produced on synthetic media were thin, and not at all like the cushion-shaped mass that fruited on natural substratum. Probably the formation of these under-developed aethalia was due to improper nutrient material in the controlled environment.

4. The life cycle of *Fuligo cinerea* (Schw.) Morg. was much interrupted by the formation of spherules. It has been mentioned in the foregoing paragraphs that fructification only developed from the colorless plasmodium and rarely from yellow plasmodium. The only case observed of the latter was the presence of very fine veins and minute aethalia of almost microscopic size in one of the plates.

5. The life cycle of this myxomycete is normally completed in 16–18 days.

6. Just why two types of plasmodia and two kinds of swarm cells were observed is not clear. Perhaps the original specimen contained a mixture of two different myxomycetes or contamination may have occurred in our laboratory, or perhaps some pigment present in the bacteria may have caused the yellow color of the plasmodium. But in any case, the yellowish plasmodia did not give rise to aethalia of *Fuligo cinerea*.

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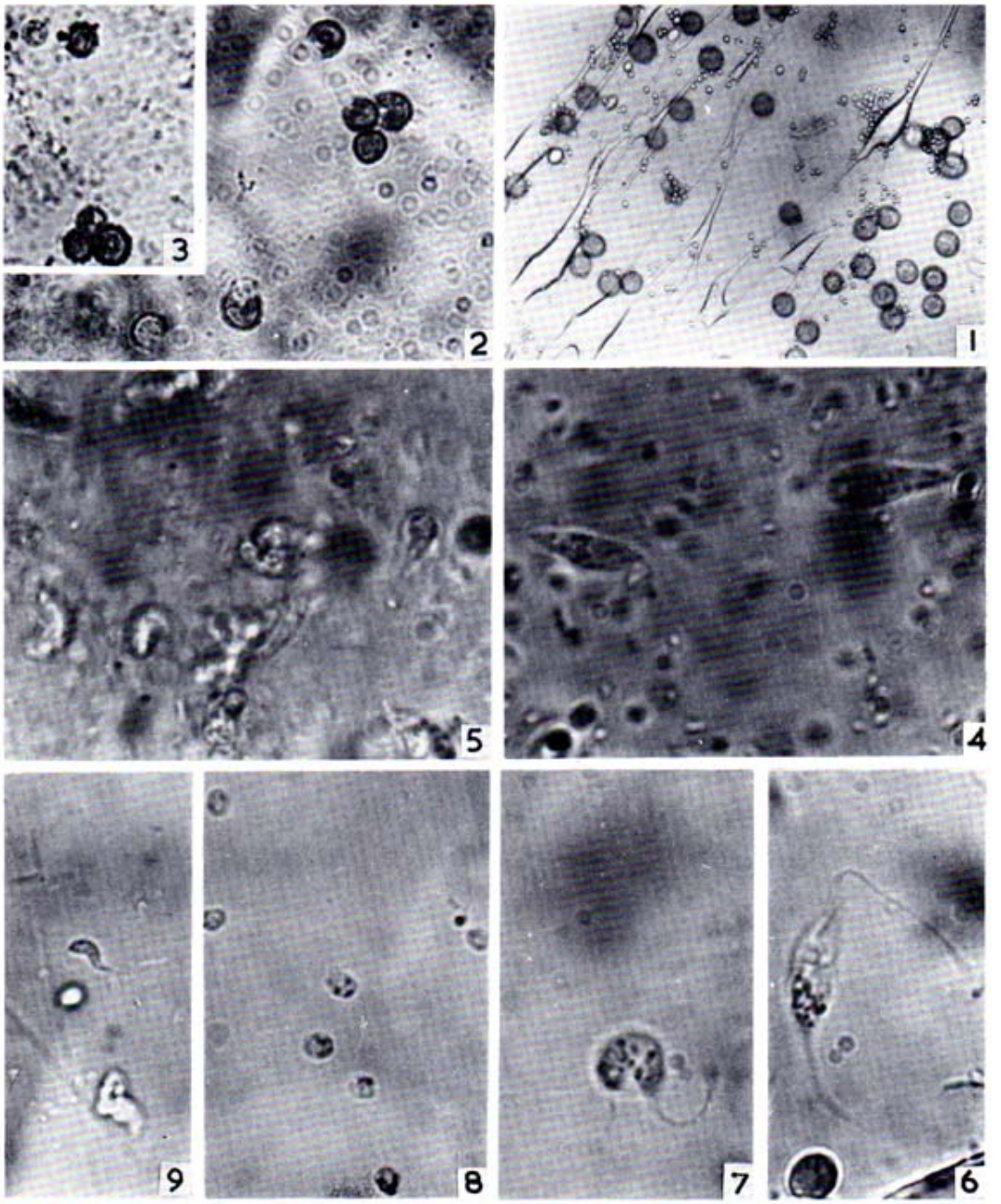

Plate I

1. Spores and thread-like capillitium with swollen endings. ×400 2. Germinating spores showing emerging protoplast by slits ×600 3. Spores with papillose walls ×600 4. Flagellated swarm cells. ×1500 5. A synchronous culture of flagellated swarm cells. ×1000 6, 7. An unusual kind of swarm cells with one flagellum at each end, one long and the other one short, or possibly the conjugation of two swarm cells ×1500 8. Spherical non-moving swarm cells shortly after their escape from the spores. ×600 9. A flagellated swarm cell and a myxamoeba observed 4 days after sowing of spores in distilled water. ×600

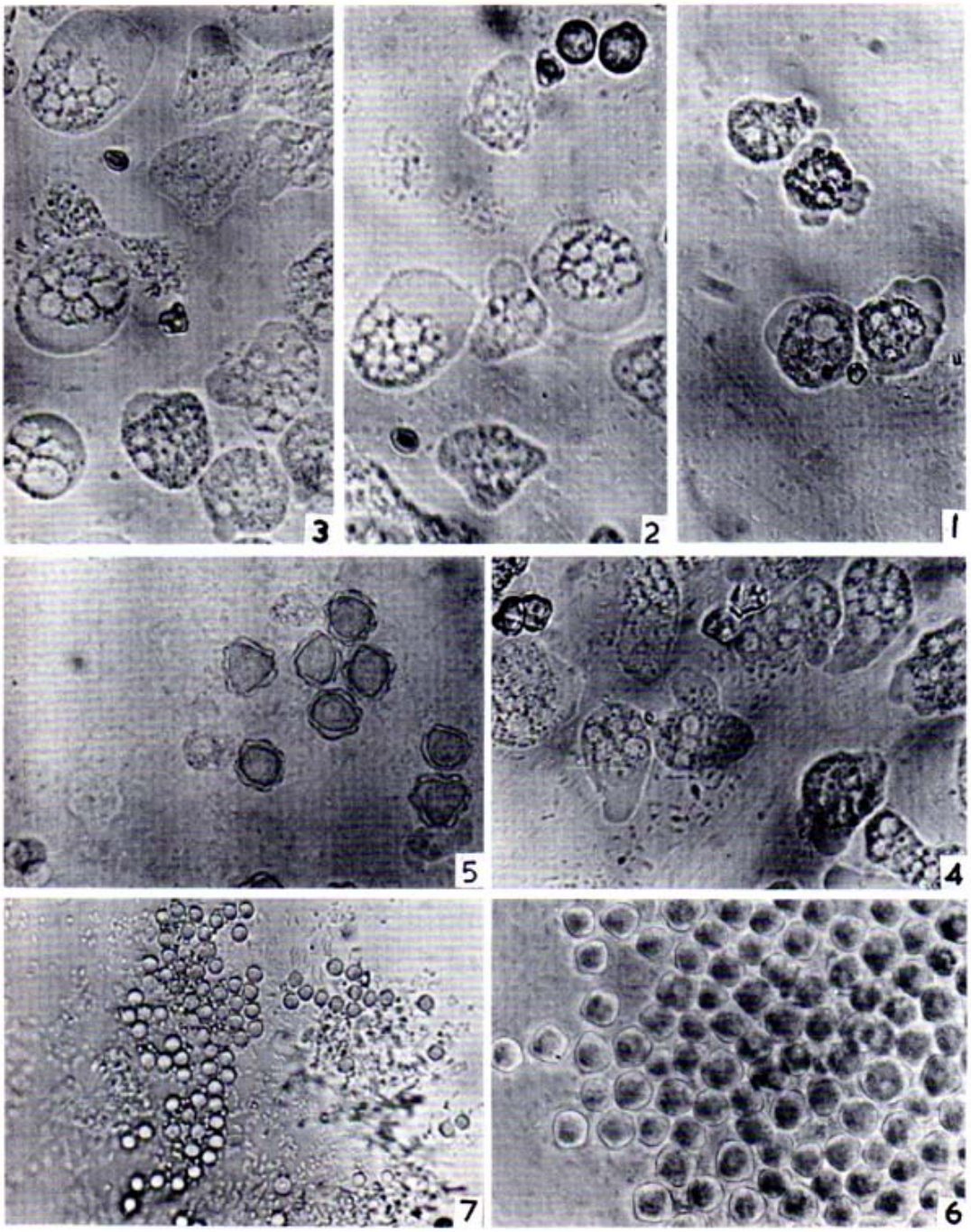

Plate II

1-4. A large number of myxamoebae mildly active developed from angular cysts. The plasmodium contained abundant bacteria. ×600

5. Thick walled angular cysts in dormancy. ×400

6. Sclerotia formed of closely packed angular cysts. ×600

7. Spherical microcysts found in the colorless plasmodium and also in some of the newly formed aethalia. ×600

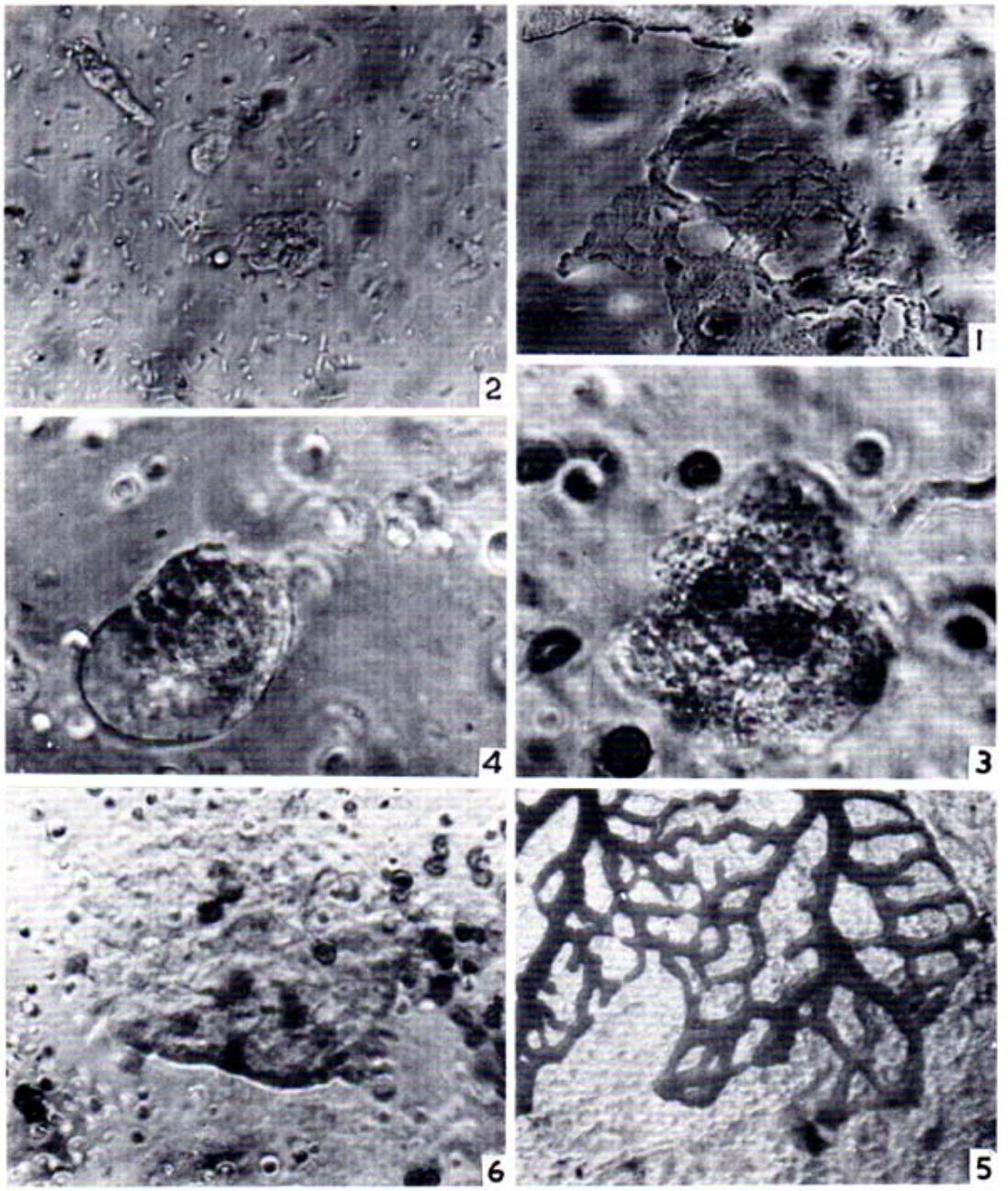

Plate III

1, 2. After actively moving for several hours the swarm cells became elongated myxamoebae (Fig. 2) and these formed an aggregation which became a much vacuolated plasmodium (Fig. 1) ×400

3, 4, 6. Coalescence of spherical swarm cells into colorless plasmodium.

5. A portion of fan-like veins enlarged as in plate V, Figs. 1, 2.

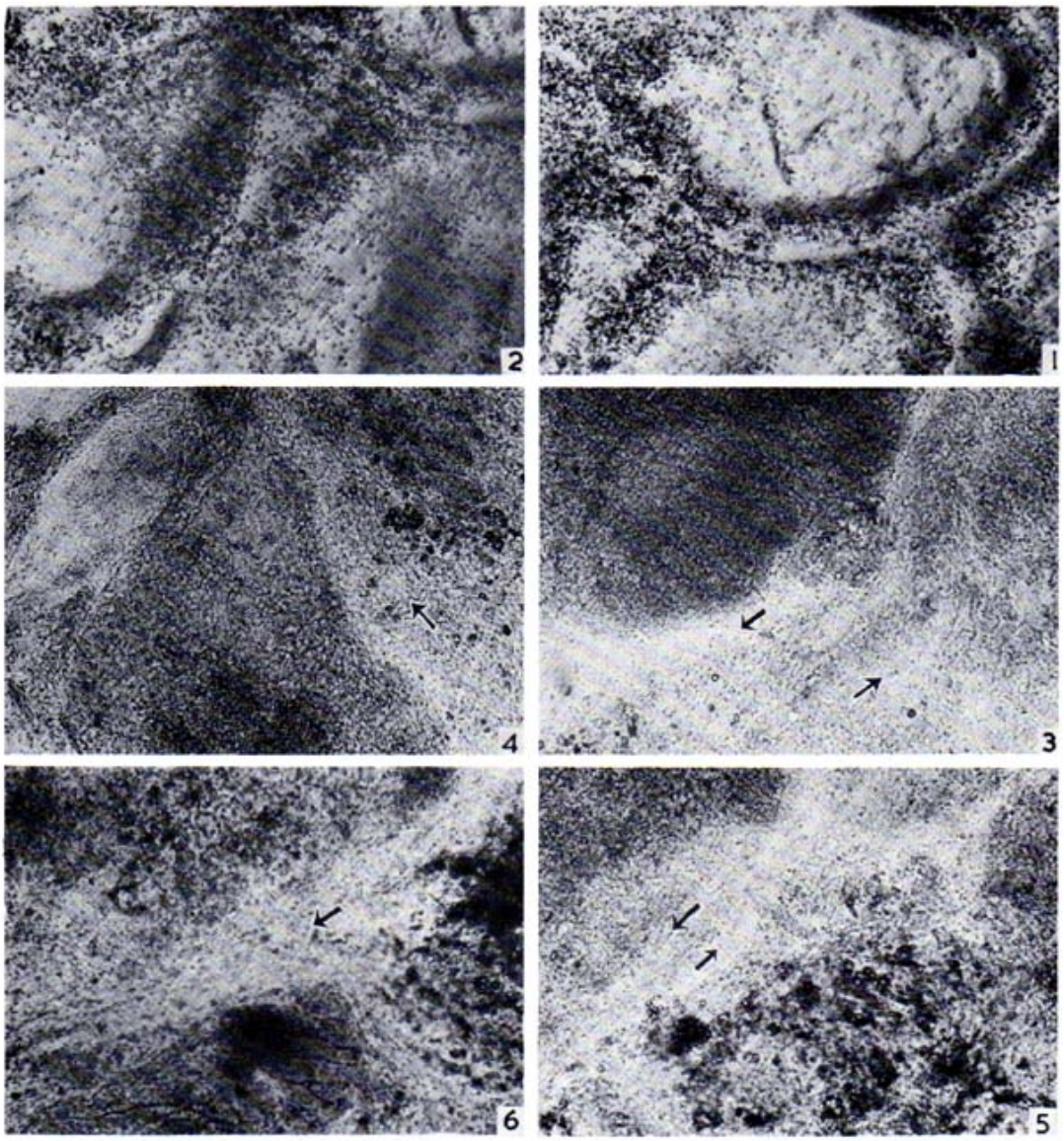

Plate IV

1-6. Prior to the formation of veins, streaming was observed in various areas of the colorless plasmodium. The light areas showed streaming which was actively going on, while the darker areas showed dense protoplasm which included spore shells, bacteria and other things in the plasmodium. ×60

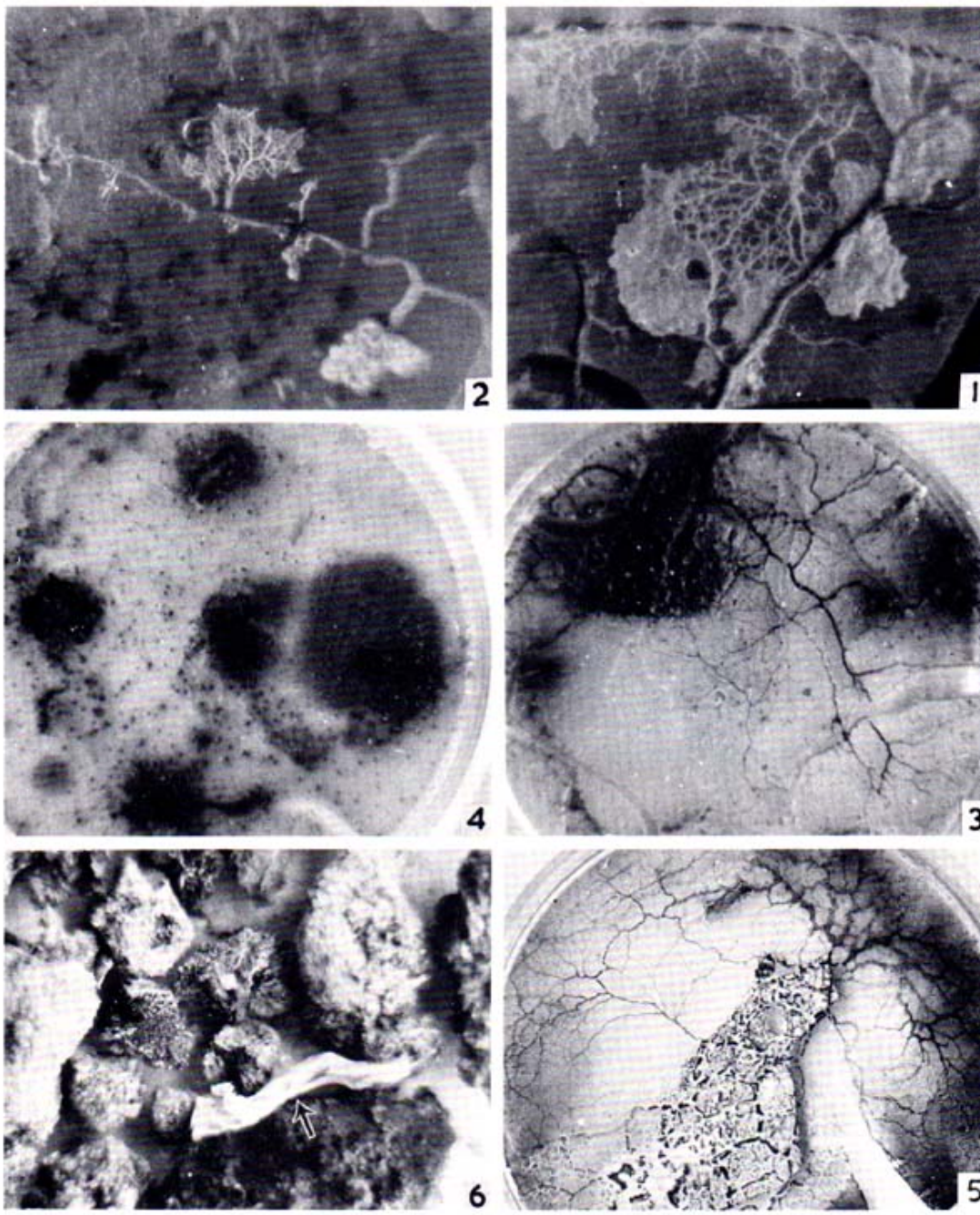

Plate V

1, 2. Fan-like veinlets arose from primary veins when plasmodium resumed growth in moist conditions. ×1.5
3. Vein formation and vigorous streaming leading to the formation of aethalia ×4/5
4. Formation of thin aethalia (dark areas) in petri plates.
5. Showing some veins originally on a piece of filter paper (center) creeping up onto the agar medium when planted in the agar plate. ×4/5
6. Portion of the specimen collected from Lin-kuo showing a ribbon like spherule aethalia in various shapes, sizes, thicknesses and shades of color. ×1