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Stemonitis flavogenita (Myxomycetes) Plasmodial Phase (Aphanoplasmodium)

5 Illustrations

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INSTITUT FÜR DEN WISSENSCHAFTLICHEN FILM

Film E 2000

Stemonitis flavogenita (Myxomycetes) Plasmodial Phase (Aphanoplasmodium)

E. F. HASKINS, Seattle (Wash.)

General Remarks[1]

Systematic position

The myxomycetes or true plasmodial slime molds possess attributes of both plants and animals. The animal-like assimilative phase consists of amoeboid, multinucleate masses of protoplasm termed plasmodia which can differentiate into organized plant-like fruiting bodies containing uninucleate, thick-walled spores. Most existing knowledge of the myxomycetes is based on investigation of phaneroplasmodial species (GRAY and ALEXOPOULOS [9]). The reports of ALEXOPOULOS [1], [2], [3], BENEDICT [5], [6], BISBY [7], INDIRA [11], [12], MCMANUS et al. [13], [14], [15], MIMS [17], and ROSS [18], [19] on *Stemonitis*, an aphanoplasmodial slime mold (ALEXOPOULOS [2]), suggest that this unique plasmodial type deserves further study. This film records the developmental sequences of the plasmodial phase of *Stemonitis flavogenita* Jahn (Order Stemonitales, Class Myxomycetes, Division Mycota) in an attempt to document a representative aphanoplasmodial slime mold.

Life cycle

Because of the diversity of stages in their life cycle, the myxomycetes are especially favorable organisms for morphological studies on development. The spore of *S. flavogenita* gives rise to one myxameba which can transform into a flagellate cell (swarm cell) when placed in a liquid environment or can encyst under unfavorable conditions. Populations of swarm cells predominate over myxamebae and amebal cysts (microcysts) in an actively growing culture. Subsequently, swarm cells or myxamebae

[1] Film data and summary of the film (English, German, French) see p. 16.

produce microscopic, multinucleate masses of delicately netted transparent protoplasm (aphanoplasmodia). Individual aphanoplasmodia can differentiate into clusters of stalked cylindrical fruiting bodies or can form strands of multiple cysts when unfavorable conditions arise (Fig. 1).

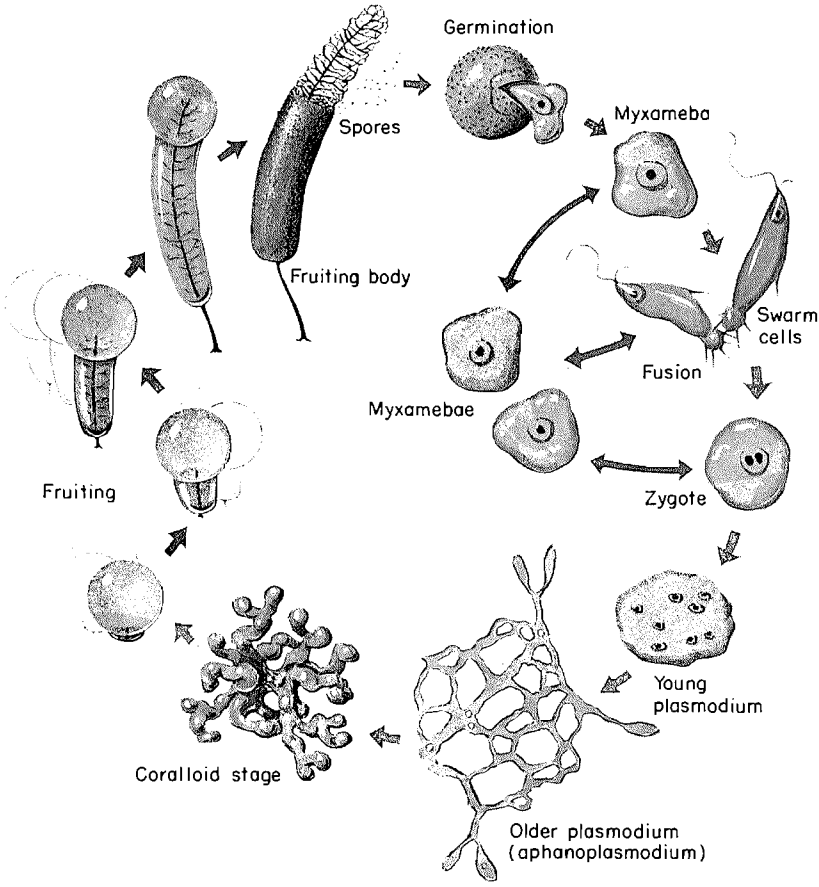

Fig. 1. Life history diagram of *S. flavogenita*

Plasmodial types

Until recently it was thought difficult to distinguish between plasmodia of myxomycetes. Today through the studies of various workers we recognize three general types of myxomycete plasmodia: the

phaneroplasmodium, the aphanoplasmodium, and the protoplasmodium (GRAY and ALEXOPOULOS [9]). The phaneroplasmodium, characteristically developed by members of the Order Physarales, has granular cytoplasm, displays a fleshy fan at its advancing front and has thick, tubular veins which possess a gellified ectoplasm and a rhythmic reversible streaming endoplasm. An aphanoplasmodium, typically formed by members of the Order Stemonitales, consists of a system of slender, flattened, non-granular veins which, with the exception of the presporulation phase, lack a gelled ectoplasm. The aphanoplasmodial veins display a rhythmic reversible flow which may vary from rapid to slow or even imperceptible. The protoplasmodium, exhibited by some species of the Order Liceales and apparently all species of the Order Echinostemliales, is granular, lacks a system of veins or channels, displays irregular protoplasmic streaming, and always remains diminutive.

The Aphanoplasmodium

Undoubtedly de BARY [4] was aware of the delicate nature of the plasmodial strands of *Stemonitis fusca*. He implied that its plasmodium was inconspicuous and not visible to the naked eye until it formed sporangia. ČELAKOVSKÝ [8] noted the transparency of the plasmodial veins of *S. fusca* (*S. dictyospora*) and made the observation that the veins were not differentiated into ecto- and endoplasmic zones. Apparently MILLER [16] was the first worker to illustrate what is now designated the aphanoplasmodium. The drawing of an unidentified species of *Stemonitis* which he presents shows few plasmodial veins which exceed 20 μm in width. More recently, THOM and RAPER [20] described the closed networks of delicate strands of colorless plasmodial protoplasm of *Stemonitis*. However, it was ALEXOPOULOS [1], [2] who first pointed out that the plasmodium of *Stemonitis* possesses a number of distinct characteristics which separate it readily from other plasmodial types. This led him to propose the term aphanoplasmodium for this plasmodial habit.

It remains to be proved that the plasmodial phase of the strain of *S. flavogenita* used for this film develops as a consequence of syngamy. Young plasmodia, four- to eight-nucleate, often have a linear orientation. Other young plasmodia, irregularly lobed, possess peripheral finger-like pseudopodia. It is unclear whether the former stage gives rise to the latter or if they represent two different development pathways. In both growth forms the peripheral pseudopodia spread out, branch, coalesce and thus give rise to the delicate, transparent aphanoplasmodial reticulum. When adjacent aphanoplasmodia contact one another they coalesce. This process usually leads to the production of a single extensive aphanoplasmodium in a culture dish. Under unfavorable conditions such as lack of food or accumulation of metabolic wastes an aphanoplasmodium may

encyst by withdrawing its veins into one or more chains of refractile cysts. Under favorable conditions these cysts are capable of germination.

Sporulation

The first morphological indication of the onset of sporulation is the development of the opaque plasmodial phase. The veins thicken, lose their transparency because of the accumulation of granular material, and become pale-white. Subsequently, the veins condense further to form the coralloid phase which consists of yellowish-white dendritic elements more or less erect in habit. This phase migrates actively over the substratum for one to several days before sporophores are formed. Sporangial initials develop from the coralloid phase as close packed pulvinate masses yellowish-white in color. As the initials elongate they become more cylindrical and the development of the internal stalks can be observed. The stalks grow in length and elevate the sporangia from the substratum. Progressively the maturing sporangia become yellowish-white, pale pinkish, and brown as columellar, capillitial, and spore formation occurs.

Methods

The strain of *S. flavogenita* (ATCC #24714, American Type Culture Collection, Rockville, Md. U.S.A.) used for this film was originally isolated from a banana peel by Professor O'Neil Ray Collins, Department of Botany, Berkeley, California. Spores were spread on the surface of one-half strength Difco corn meal agar poured in 100 mm diameter disposable plastic petri dishes and the surface was flooded with a dilute suspension of *Enterobacter aerogenes* (Chester) Hormaeche and Edwards in distilled water. Five days later four equidistant piles of sterile, pulverized oat flakes were added to each culture dish. The cultures were maintained on a laboratory shelf where they were subjected to indirect sunlight during the daily light-dark cycle. Care was taken to maintain a film of water on the agar surface. Under these conditions abundant swarm cells developed in several days, hyaline aphanoplasmodia were present in less than a week, opaque aphanoplasmodia were abundant in less than two weeks, and the coralloid phase regularly formed in a little more than two weeks. Sporulation occurred within several days after the formation of the coralloid phase. Fruiting body formation occurred in place in the culture dishes or following subculture to plates of 1.5% (w/v) Ionagar #2 (Oxoid). Plasmodia were photographed on a thin layer of agar pressed against a coverslip (HEUNERT [10]), or at an agar/air interface in a glass microchamber. Photographs were taken on 35 mm film (Eastman Double X, London and Ektachrome negative film) using an Askania-Z-camera.

Zeiss bright-field, Zernike phase-contrast, Nomarski interference-contrast, and Tessovar optics were used.
The technical assistance of Miss BRIGITTE MILTHALER IWF, is gratefully acknowledged.

Film Contents

Mitosis

Gross Structure

1 f/s to 8 f/min

24 f/s

1. Mitosis in a uninucleate aphanoplasmodium. A uninucleate plasmodium is distinguished from a myxameba by the occurrence of intranuclear mitosis and the absence of cytokinesis following nuclear division. At the beginning of the scene the uninucleate plasmodium is in metaphase. The nucleus rotates constantly and migrates actively around the cell. Anaphase and telophase occur and at the end of the division two rotating polynucleolate nuclei are seen. During nuclear division the plasmodium protrudes small pseudopodia, displays cyclosis, and exhibits active contractile vacuoles.
Frame width 89 μm, exposure frequency 1 f/s, phase-contrast, elapsed time 24 min.

2. Mitosis in a binucleate aphanoplasmodium. Nuclear rotation occurs as the two nuclei proceed through metaphase, anaphase, and telophase in synchrony. In anaphase the bands of minute chromosomes move toward the spindle poles and the spindle apparatus elongates noticeably. At the end of division four nuclei are observed in close proximity. During nuclear division the plasmodium displays prominent contractile and food vacuoles.
Frame width 94 μm, exposure frequency 1 f/s, phase-contrast, elapsed time 19 min.

3. Mitosis in a 4-nucleate aphanoplasmodium. The linear plasmodium shown in this sequence possesses prominent contractile and food vacuoles. Finger-shaped pseudopodia protude occasionally as the four rotating nuclei undergo synchronous mitosis thereby producing an 8-nucleate plasmodium.
Frame width 120 μm, exposure frequency 1 f/s, phase-contrast, elapsed time 17 min.

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[1] The headlines in italics correspond with subtitles in the film.

4. Mitosis in a multinucleate aphanoplasmodium. All of the nuclei in this irregularly lobed plasmodium are in metaphase. Some nuclei rotate clockwise, others rotate anticlockwise. Certain nuclei appear to reverse the direction of their rotation as the synchronous division proceeds. At the end of nuclear division active cyclosis occurs and several rudimentary veins are formed.

Frame width 195 μm, exposure frequency 30 f/min, phase-contrast, elapsed time 25 min.

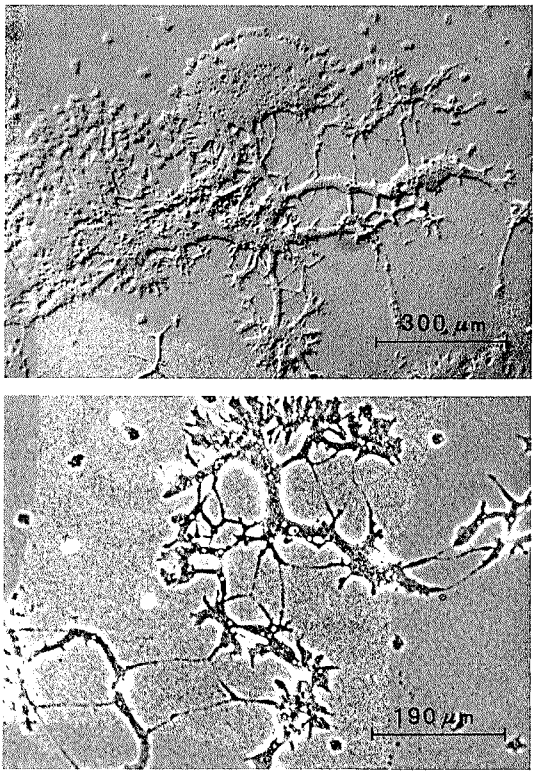


Fig. 2.
Hyaline aphanoplasmodia
a. Normarski interference-contrast (from Scene 5)
b. Zernike phase-contrast (from Scene 10)

5. Migrating aphanoplasmodium. The anterior of the migrating plasmodium consists of fan-shaped confluent pseudopodia. The posterior is composed of a network of veins which terminate in vesiculose or fan-shaped swellings. The veins appear to undergo rhythmic contractions (Fig. 2a).

Frame width 1.2 mm, exposure frequency 8 f/min, interference-contrast, elapsed time 40 min.

6. Overview of a mature aphanoplasmodium. Cyclosis appears imperceptible in the peripheral veins of this aphanoplasmodium. Scanning to a prominent central vein rapid, unidirectional streaming is observed.

Frame width 600 μm, exposure frequency 24 f/s, phase-contrast, elapsed time 14 sec.

7. Protoplasmic streaming in an aphanoplasmodium. Protoplasmic streaming is observed within a portion of an aphanoplasmodium. Large contractile vacuoles, cytoplasmic granules, and nuclei flow for a short distance in one direction and then reverse their movement. Thus this plasmodial network is divided into multiple, short length zones of rhythmic reversing streaming.

Frame width 195 μm, exposure frequency 30 f/min, phase-contrast, elapsed time 19 min.

8. Protoplasmic streaming in an aphanoplasmodium. In this scene unidirectional protoplasmic streaming is more prolonged than in the preceding sequence. As in the foregoing scene, the flattened aphanoplasmodial veins possess contractile vacuoles, cytoplasmic granules, and nuclei but lack distinct ecto- and endoplasmic zones.

Frame width 195 μm, exposure frequency 24 f/s, phase-contrast, elapsed time 18 sec.

Plasmodial Coalescence

1 f/s

9. Self coalescence. A portion of a plasmodial network is shown in which a vein bifurcates and then coalesces with two adjacent veins thus forming two new meshes.

Frame width 195 μm, exposure frequency 1 f/s, phase-contrast, elapsed time 9 min.

10. Aphanoplasmodial coalescence. A white arrow designates the region in which two young hyaline aphanoplasmodia coalesce at their advancing fronts. When coalescence is completed there is active mixing of protoplasmic material (Fig. 2b).

Frame width 765 μm, exposure frequency 30 f/min and 15 f/min, phase-contrast, elapsed time 20 min.

11. Aphanoplasmodial coalescence. A white arrow designates the area of coalescence between two plasmodia.

Frame width 120 μm, exposure frequency 1 f/s, phase-contrast, elapsed time 4 min.

12. Aphanoplasmodial coalescence. Coalescence is observed between a fan-shaped advancing front and a vesiculose pseudopodium.
Frame width 195 μm, exposure frequency 1 f/s, phase-contrast, elapsed time 4 min.

Encystment

Cyst germination

1 f/min to 30 f/min

13. Overview of aphanoplasmodial encystment. The encysting aphanoplasmodium withdraws its central veins into peripheral, nodular strands. These nodules condense further, separate from one another and form cysts.
Frame width 760 μm, exposure frequency 1 f/min, interference-contrast, elapsed time 10 h 36 min.

14. Aphanoplasmodial encystment. A plasmodial vein aggregates into nodules which subsequently separate from one another and form cysts. Active peripheral contractile vacuoles are observed within the cysts.
Frame width 160 μm, exposure frequency 2 f/min, interference-contrast, elapsed time 6 h. 9 min.

15. Aphanoplasmodial excystment. The protoplast of the cyst appears to emerge through a pore during the process of germination. The netted plasmodial form is soon re-established.
Frame width 195 μm, exposure frequency 30 f/min, interference-contrast, elapsed time 37 min.

Coralloid Stage

Formation of Sporophores

15 f/h to 15 f/min

16. Development of the mature aphanoplasmodial network. As the scene begins six young, irregularly lobed plasmodia are observed. Numerous myxamebae migrate over the agar substratum. The plasmodia protrude stellate pseudopodia which branch, coalesce and form plasmodial nets. Progressively, adjacent plasmodia contact and coalesce. This ultimately leads to the formation of a single plasmodium. The rhythmic contractions of the plasmodial veins reflect the tempo of the reversible protoplasmic streaming.
Frame width 1.6 mm, exposure frequency 4 f/min, interference-contrast, elapsed time 8 h. 36 min.

17. Formation of the coralloid stage. Yellowish-white dendritic veins extrude upward from the central portion of the opaque white plasmodial

network. The formation of this more or less erect habit marks the end of assimilation and the beginning of the sporulation phase (Figs. 3a—e).

Frame width 19.3 mm, exposure frequency 4 f/min, bright-field, elapsed time 4 h. 53 min.

18. Migration of the coralloid phase. The dendritic veins of the coralloid phase spread across the agar substratum by extrusion of protoplasm into peripheral fan-shaped knobs (Fig. 3f).

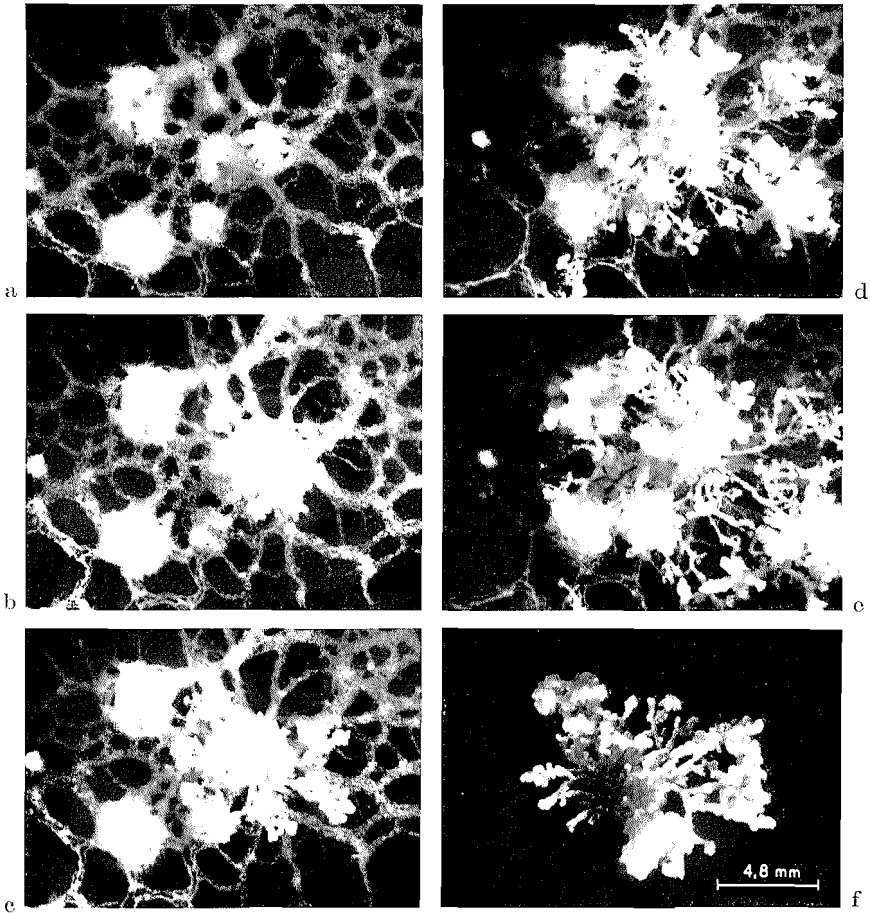
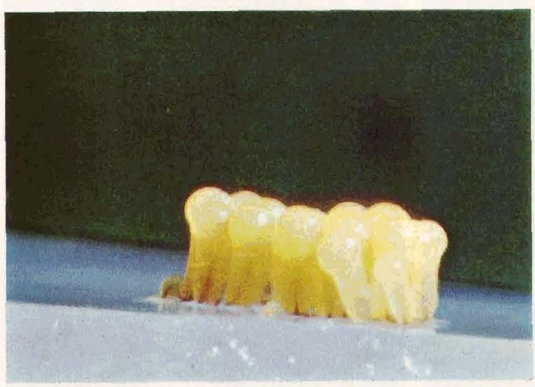


Fig. 3. Prior to sporulation strands of the aphanoplasmodium turn opaque, thicken and become coralloid

(Figs. 3a—e from Scene 17 of the film; Fig. 3f from Scene 18)

a

b



c

d

Fig. 4. Stages in the development of fruiting bodies (sporophores)

(Fig. 4a from Scene 21,
Figs. 4b—d from Scene 22)

Frame width 19,3 mm, exposure frequency 8 f/min, bright-field, elapsed time 55 min.

19. Migration of the coralloid phase. The dense strands of the coralloid phase spread across the agar substratum.
Frame width 19.3 mm, exposure frequency 15 f/min, bright-field, elapsed time 30 min.

20. Migration of the coralloid phase. In this sequence the protoplasmic contents of the posterior region of the plasmodium are extruded into anterior pulvinate nodules. The collapsed veins are observed clearly in the posterior region of the plasmodium.
Frame width 12 mm, exposure frequency 15 f/min, bright-field, elapsed time 1 h. 11 min.

21. Formation of sporangial primordia. Lateral view. The yellowish-white, erect, forked veins of the coralloid phase sink slowly to the substratum. They remodel and form pulvinate sporangial initials (Fig. 4a).
Frame width 7.8 mm, exposure frequency 15 f/min, bright-field, elapsed time 24 min.

22. Formation of sporophores. Lateral view. A cluster of yellowish-white pulvinate sporangial primordia elongate progressively and become more cylindrical in shape. The development of internal stalks can be observed within the sessile sporangia. As the sequence proceeds the stalks grow in length and elevate the sporangia from the substratum. The maturing sporangia undergo color changes from yellowish-white, to pale-pinkish, to brown, as columellar, capillitial, and spore formation occurs (Fig. 4b—d).
Frame width 6.1 mm, exposure frequency 4 and 2 f/min, bright-field, elapsed time 7 h. 34 min.

Capillitial Formation

Spore Formation

2 f/min and 4 f/min

23. Capillitial formation. Concluding aspects of capillitial formation is shown in a sporangium. The portion of the capillitium attached to the columella darkens progressively and elements of the peripheral capillitium form.
Frame width 490 μm, exposure frequency 2 f/min, oblique bright-field, elapsed time 3 h. 52 min.

24. Spore formation. A peripheral portion of the sporangium undergoes cleavage producing brown, firm walled spores. Nuclear details of cleavage are not observable by this optical method.

Frame width 195 μm, exposure frequency 4 f/min, bright-field, elapsed time 3 h. 11 min.

25. Spore formation. A peripheral portion of a sporangium undergoes two waves of cleavage. The ameboid protospores produced by these divisions display active cyclosis. The maturing spores develop firm brown

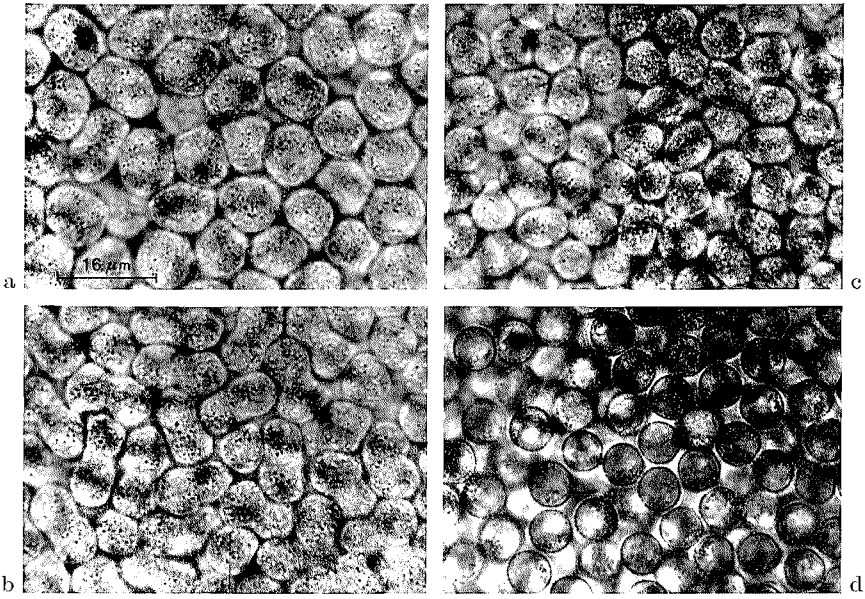


Fig. 5a—d. Stages in the second wave of spore cleavage
(From Scene 25 of the film)

walls decorated with faint granulations (Figs. 5a—d). Nuclear details of cleavage are not observable by this optical technique.

Frame width 64 μm, exposure frequency 4 f/min, bright-field, elapsed time 3 h. 48 min.

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Film Data

The film was published in 1974 for use in research and university education. Silent, 16 mm, color, 152 m, 14 min (running speed 24 f/s).
It was taken in 1972 at the Institut für den Wissenschaftlichen Film, Göttingen. Published by the College of Arts and Sciences, Department of Botany, University of Washington, Seattle, Dr. E. F. HASKINS and the Institut für den Wissenschaftlichen Film, Göttingen, Dr. H.-K. GALLE; photography: H. H. HEUNERT.
With financial support by N. S.F. grant GB-31243.
Professor O'NEIL RAY COLLINS, Department of Botany, University of California, Berkeley is thanked for providing the strain of *S. flavogenita* used in this film.
The help of Mrs. HELEN C. LYMAN is acknowledged in the preparation of Fig. 1.

Summary of the Film

Mitosis in uninucleate, binucleate, four-nucleate, and multinucleate plasmodia is illustrated. The growth, streaming patterns, and coalescence of plasmodia are presented. Encystment and excystment are included. Formation of the coralloid phase and sporulation are demonstrated. Capillitial and spore formation are illustrated.

Inhalt des Films

Der Film zeigt Mitose in einkernigen, zweikernigen, vierkernigen und mehrkernigen Plasmodien. Es werden sowohl Wachstum, Plasmaströme und Verschmelzung als auch Enzystierung und Exzystierung von Plasmodien dargestellt. Nach der Bildung des Koralloidstadiums folgt die Sporulation, bei der im einzelnen die Bildung des Capillitiums und Sporendifferenzierung aufgezeigt wird.

Résumé du Film

Le film montre la mitose d'un plasmodium à un noyau et de plasmodia à 2, à 4 et à plusieurs noyaux. Développement, courants de plasma et coalescence ainsi que enkystement et dékystement de plasmodia sont illustrés. Suivant à la formation de la phase coralloide la sporulation y compris la formation du capillitium et la ségrégation des spores est représentée.