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On the Plasmodium of Myxomycetes*

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INTRODUCTION

The Myxomycete plasmodium has always been a much sought-after object of study for biologists, since it offers a ready tool for studying the various features of living protoplasm. The major aspects studied are: cytology, by light microscopy and, more recently, by electron microscopy; protoplasmic streaming; the movement of the plasmodium; nutrition and culture. Interest in the morphology and observable features of the plasmodium has been aroused by the studies of Alexopoulos *(1960)*, which have been confirmed and extended considerably (McManus, *1961a, b, 1962, 1963, 1964:* McManus and Taylor, *1961;* Alexopoulos, *1964;* Benedict, *1964*). The introduction of Petri dish cultures (see McManus, *1961a*) makes such studies simple and easy.

Six species of Myxomycetes were successfully cultivated by the author on agar media from spore to spore. The plasmodia of these were studied in detail in culture, and the results are presented here. The species studied were:

Physarales	*Stemonitales*
Physarum cinereum (Batsch) Pers.	*Stemonitis herbatica* Peck
P. compressum Alb. & Schw.	*Trichiales*
P. gyrosum Rost.	*Arcyria cinerea* (Bull.) Pers.
P. vernum Somm.	

MATERIAL AND METHODS

For microscopic studies, plasmodia were grown in Petri dishes, under water. The Petri dish was sterilized, and a small block of agar bearing the plasmodium was cut out from a stock culture and transferred to the dish. About 5-10 ml of sterile water and a pinch of sterilized

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powdered oats were then added. The cultures were incubated at 25°C until the plasmodia had attained sufficient growth, after which they were maintained in moist chambers at room temperature. For examination, the cover was removed and the Petri dish placed directly on the microscope stage. Once thus opened, the culture was subject to contamination. While bacterial contamination was common, fungal and protozoan contaminants were rare. The cultures could be maintained in the vegetative state for several weeks by periodically (5-7 days) changing the water and adding fresh powdered oats, but eventually they either fruited or formed microcysts.

For a closer examination of the protoplasmic components, a portion of the plasmodium was mounted on a slide under a cover glass in a film of water and examined quickly under high magnification.

To make permanent preparations, the plasmodia were cultured on glass slides under water. In some cultures, a 2 per cent carrot decoction in water was used in the place of oats and water, as oat starch grains when ingested have a tendency to obscure plasmodial structure. After the plasmodia had attained sufficient growth, the slides were taken out, dried under cover to make the plasmodia adhere to glass, and fixed in Allen's modified Bouin's fluid: 100 ml of Bouin's fluid (saturated aqueous picric acid, 75 ml; formalin, 25 ml; glacial acetic acid, 5 ml) was heated to 37°C; 1 g of chromic acid added, stirred thoroughly, followed by 2 g of urea which was also thoroughly stirred in; the fluid was maintained at 37-39° C while placing the slides, then cooled gradually to room temperature and left overnight. After fixation, the slides were washed through several changes of 70 per cent alcohol, carried through mordant and stained with Heidenhain's iron-alum haematoxylin (Johansen, *1940*); destaining was carried out with picric acid, after which the slides were washed, dehydrated and mounted in balsam, without any counterstain.

THE PLASMODIUM

The plasmodium of the four physaraceous species, *Physarum compressum, P. gyrosum, P. cinereum,* and *P. vernum,* had several common features and could be fitted into a type distinct from those of *Stemonitis herbatica* or of *Arcyria cinerea.* The three types are therefore described separately.

The Physaraceous Type

Morphology: The plasmodia of *P. compressum, P. cinereum,* and *P. vernum* are whitish and nonpigmented in glass cultures, whereas that of *P. gyrosum* is bright yellow, with a deep pigment which diffuses into the surrounding medium, rendering it yellow within a few days of transfer.

Regardless of the species, the plasmodium has the following characteristic organization: a reticulum with fairly wide meshes having one or more fan-shaped advancing fronts *(Pl. 1, Fig. 1).* The fan usually marks the anterior end, and growth and movement are concentrated here. It is a continuous, perforated sheet of protoplasm, gradually merging into the reticulate posterior region through a fine-meshed network; the veins of this network increase progressively in thickness, and the meshes increase in width towards the posterior end. However, sometimes the veins may differ in thickness. The fan may at times become reticulate almost up to the edge *(Pl. 1, Fig. 1).* When the plasmodium is feeding, for example, on a larger oat particle, it attaches itself to the particle and radiates from it in one or more fans, the posterior end of each fan terminating in the oat particle.

While the plasmodia of the four species show a basic similarity in organization, they differ in their dimensions. Measurements of vein thickness were made from several plasmodia of each species at the posterior, middle and anterior regions, and these are shown in Table I. In *P. gyrosum* the plasmodium is large, with thick terete veins. The fans are very prominent, appearing thick and fleshy at the margins. The large veins appear opaque under the microscope *(Pl. 1, Fig. 2).* In strong contrast, *P. cinereum* and *P. vernum* usually present a delicate appearance with small plasmodia having small fans and slender veins, which may be terete or often flattened. On the whole, the plasmodium in these two species has a delicate appearance *(Pl. 1, Fig. 3).* The plasmodium of *P. compressum* is more or less intermediate between these two types in its dimensions *(Pl. 1, Fig. 4).*

Table I. Dimensions of veins in different plasmodia

Species	Diameter in microns				
	*Posterior region		*Middle region	*Anterior region	
	Usual range	Maximum	Usual range	Usual range	Maximum
Physarum gyrosum	125-250	400	50-100	15-50	–
P. compressum	100-200	350	50-70	25-40	–
P. cinereum	60-80	–	30-40	10-15	40
P. vernum	50-100	120	–	10-25	50
Stemonitis herbatica	30-40	–	15-20	5-10	–
Arcyria cinerea	40-50	130	25-35	8-15	–

* In *S. herbatica* these indicate simply the large, medium and small veins, as there is no anterior and posterior region.

The Protoplasm: The plasmodial protoplasm is found to consist of a firm outer layer which is very thin, colorless and transparent, homogeneous and entirely free from granules; this will be referred to as the ectoplasm. The bulk of the protoplasm within is highly granular, with various kinds of inclusions; this will be referred to as the endoplasm. The ectoplasm cannot be easily distinguished in the veins, but is clearly seen along the margins of the fans *(Pl. 1, Figs. 4, 5).*

In the veins, the granular endoplasm is usually further differentiated into an outer stationary gelated layer and an inner core of streaming protoplasmic sol. In *P. compressum* and *P. vernum* the gel layer is rather thick, comprising about half the diameter of the vein *(Text Fig. 3, E).* On the other hand, in *P. gyrosum* and *P. cinereum* much of the endoplasm is involved in streaming, the gel layer being comparatively thin in the former, and very thin or nearly lacking in the latter species. However, the sol and gel states are reversible. Surrounding the veins, there is usually a layer of slime in which egested starch grains become embedded when the plasmodium is actively feeding on oats.

In the region of the fan, there is no clear-cut differentiation of the endoplasm into gel and sol, most of it being involved in regular or irregular streaming movements along definite channels. Between these channels are sheets of static protoplasm. The endoplasmic layer in these sheets is very thin, and absent in some areas, which appear hyaline. The fan thus presents an uneven appearance, having numerous thin, hyaline areas which are traversed by thick, granular, vein-like channels. The margin of the fan is not even, but bears numerous irregular, blunt protrusions or pseudopods, and the ectoplasmic layer may be clearly seen in these regions. At times, vesicular swellings are seen at the margin. These are densely filled with granular protoplasm and appear opaque *(Pl. 1, Fig. 5).*

There is some indication that the protoplasm, at least in the gel state, has a vesicular organization. Spherical, transparent vesicles were often observed filling up the veins, but usually they were obscured by the granular nature of the protoplasm. The vesicles were seen most regularly in *P. gyrosum,* where they occurred closely aggregated and measured 20μ to 30μ in diameter, and the protoplasmic granules seemed to be arranged around the vesicles, presenting a reticulate appearance.

While the protoplasm appears very granular in *P. gyrosum* and *P. compressum (Pl. 1, Fig. 6),* in *P. cinereum* and *P. vernum* it is almost homogeneous and transparent because of the very small size of the

granules *(Pl. 1, Fig. 3).* The granules seem to be of more than one type, varying in size as well as in refractivity. They measure 1-2μ or more in thickness in *P. gyrosum* and about 1μ in *P. vernum;* whereas, in *P. cinereum,* some granules are 0.5-1.0μ thick while others are extremely fine, about 0.2-0.5μ.

In actively feeding plasmodia the protoplasm appears highly granular in all the species, being filled with the ingested starch grains of oat. Most of these occur free, without being enclosed in vacuoles, and are carried in the endoplasmic stream along with the protoplasmic granules.

Vacuoles are seen in plasmodia of all four species. They are particularly abundant in *P. gyrosum,* imparting a characteristic appearance to this plasmodium *(Pl. 1, Figs. 7, 8).* Normally they measure 10-20μ, up to 30μ in diameter, and are spherical, except when passing through narrow veins, when they tend to become elongated. The vacuoles are present in the endoplasmic gel, as well as in the sol, where they are carried about in the stream along with the granules though at a slower rate. Most of the vacuoles are found to contain some granular matter, or some amorphous stainable material. It is possible that they are not food vacuoles but enclose waste material, as food granules are seen to occur free in the protoplasm.

A few large, spherical, highly refractive bodies, up to 30μ in diameter are seen scattered in the protoplasm in all the species. These are presumably oil globules.

As far as could be seen from a study of *P. gyrosum* from fixed and stained material, the nuclei occur scattered throughout the plasmodium. In the veins they are more or less concentrated along the central axis; in the fans they are seen in larger numbers along the edge and in the thickened channels of protoplasmic streaming than in the thin or hyaline areas. However, there is no regularity in their distribution *(Pl. 1, Fig. 8).*

The nuclei seem to exhibit extreme plasticity, and are often seen to be variously elongated and irregularly shaped. When spherical, they are about 3-4μ in diameter.

Protoplasmic streaming: The shuttle type of endoplasmic streaming occurs regularly in the veins. In a few large veins, the movement may be continuous along the length of the plasmodium up to the region of the fan, with a definite direction. Streaming appears to be well-regulated in these veins, being usually fast and vigorous, and the continuity of flow in each direction is maintained for a long time, especially in regions of active feeding. On the other hand,

streaming is slow and irregular in the smaller veins. These veins take their contents into each other or into the larger veins, and show a total lack of coordination. The large veins continue into the fan, where they branch and anastomose. The boundaries of these veins or channels within the fan may change constantly, causing some movement in the adjoining regions also. Eventually these veins merge into the protoplasmic sheet of the fan, their streams spreading out into it, thus keeping the protoplasm of the fan in a constant state of movement. Occasionally, as in the somewhat transparent fan of *P. cinereum*, the channels may be continued up to the edge of the fan *(Pl. 1, Fig. 3)*.

The duration of streaming in the large veins is sometimes longer in one direction than in the other. Measurements of the duration of streaming in the forward and reverse directions indicate that the longer durations are neither consistent nor correlated with the direction of plasmodial movement *(Text Figs. 1, 2, A, B, C)*. However, there is some indication that a larger amount of protoplasm is brought into the fan than is taken away from it during these streaming movements. In growing or migrating plasmodia this additional material, which is brought in with great force, causes forward movement along the margin. But sometimes, when the plasmodium is not actively moving, it results in the formation of vesicular swellings at the margins, the endoplasm densely filling up these vesicles *(Pl. 1, Fig. 5)*.

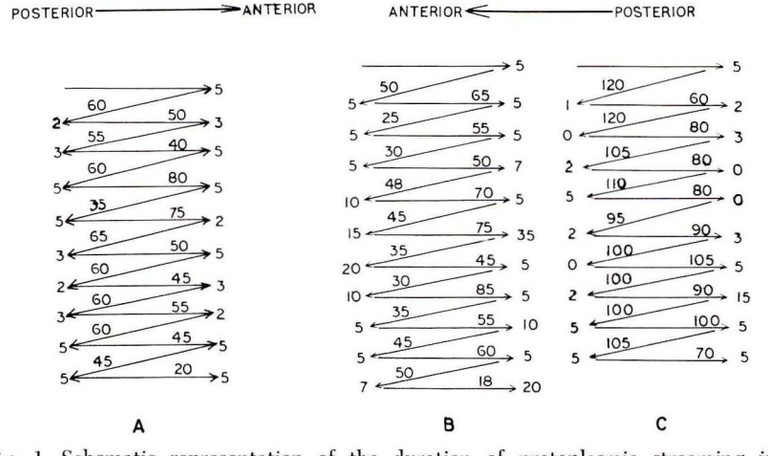


Fig. 1. Schematic representation of the duration of protoplasmic streaming in physaraceous plasmodia. The figures over the lines indicate the duration of flow in the direction of the arrow; the figures at the ends, the pause before changing the direction; all in seconds. A. *Physarum compressum;* B and C. *P. gyrosum*, readings from different plasmodia.

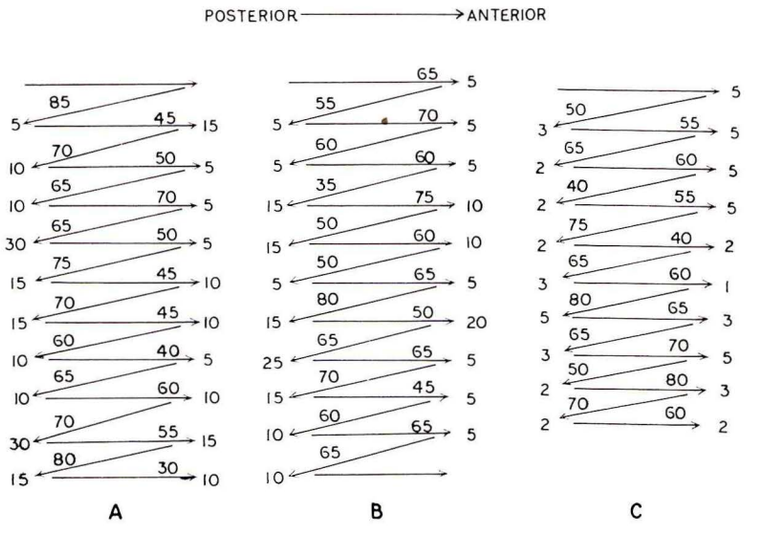


Fig. 2. Schematic representation of the duration of protoplasmic streaming in physaraceous plasmodia (Details as in Text, Fig. 1). A and B. *P. cinereum;* C. *P. vernum*.

Plasmodial movement and formation of the reticulum: The development of the characteristic network of the plasmodium is closely associated with its forward movement, which occurs as follows: along the anterior edge of the fan, the hyaline, transparent ectoplasm moves forward, to a very slight extent, in the form of numerous blunt, irregular pseudopodial protrusions. Subsequently these become continuous by coalescing along their sides. This process usually occurs when the endoplasm is receding, i.e., flowing away from the fan. During the next forward flow, the granular endoplasm is brought forward with such force that some of it pushes into the advanced, transparent region, filling it up. Much of the endoplasm then retracts with the receding current, leaving only a thin layer; and the ectoplasm advances once again, to be filled in similarly with the oncoming current. Thus, movement seems to depend to a large extent on the excess protoplasm brought by the forward current. This naturally presupposes that the quantity of protoplasm brought during forward streaming should be greater than that taken back with the receding current.

When a plasmodium radiates from a feeding center (in this case an oat particle), this extra material may be provided by the food

material taken in. In addition to this, the extra material may also be provided by a continuous withdrawal of small lateral veins, which connect the larger ones, progressively from the posterior region *(Pl. 2, Figs. 9, 10; Text Fig. 3, F-I)* in the following manner: a constriction suddenly appears in the middle of the vein and extends gradually on either side, resulting in the withdrawal of the granular endoplasm to the ends where they join the larger veins, and these ends become swollen. As the process advances, only a thin transparent ectoplasmic strand remains, connecting the two swollen ends. Next, this snaps in the middle and the broken ends retract in opposite directions. The bulbous swellings are gradually drawn into the larger veins as their protoplasm merges with the protoplasmic stream of the latter. This withdrawal of veins proceeds gradually from the posterior towards the anterior end. It is thus that the meshes of the reticulum are progressively larger towards the posterior end, where eventually only one or two long, thick veins will remain, all the interconnecting veins having been withdrawn in the course of forward movement.

In those plasmodia which have no feeding center, the extra protoplasmic material required for forward movement should come solely from the withdrawal of veins. As the plasmodium moves along, the ends of the few long posterior veins are dragged, and these retract and move forward in an amoeboid fashion, leaving a slimy track.

As movement continues, the protoplasmic sheet of the fan does not simply expand, but develops the reticulum progressively from the posterior end by a process of continuous perforation, which in principle is closely similar to the process of withdrawal of the veins *(Pl. 2, Figs. 11, 12, 13)*. Hyaline areas of various dimensions make their appearance in the protoplasmic sheet between the streaming channels *(Text Fig. 3, A)*. Each hyaline area seems to be formed by a pressing down of the upper ectoplasmic layer over the lower, resulting in the movement of the granular endoplasm away from this region. This is similar to the appearance of a constriction in the vein described earlier. However, the pressure point seems to be in the center of the area; along the margin of this area the endoplasmic granules are kept moving in and out by the force of adjacent streaming currents, resulting in constant changes in outline of the area. The hyaline area may gradually increase in size or remain constant for a considerable time, even as long as an hour in *P. compressum*. But enlargement eventually does occur, and as two adjacent hyaline areas enlarge, protoplasmic movement becomes channeled along a narrow stream in the intervening region.

Presently, a perforation appears in the center of the hyaline area—a

process analogous to the snapping of the thin ectoplasmic strand in a withdrawing vein *(Text Fig. 3, B)*. This indicates that the upper and lower ectoplasmic layers have united along the edge of the perforation. The latter enlarges rapidly, and with the continued enlargement of two adjacent perforations, the region separating them narrows down and organizes itself into a well-defined vein *(Text Fig. 3, C, D)*. As this process occurs throughout the sheet, a small-meshed reticulum results and the fan moves on, leaving this reticulum behind.

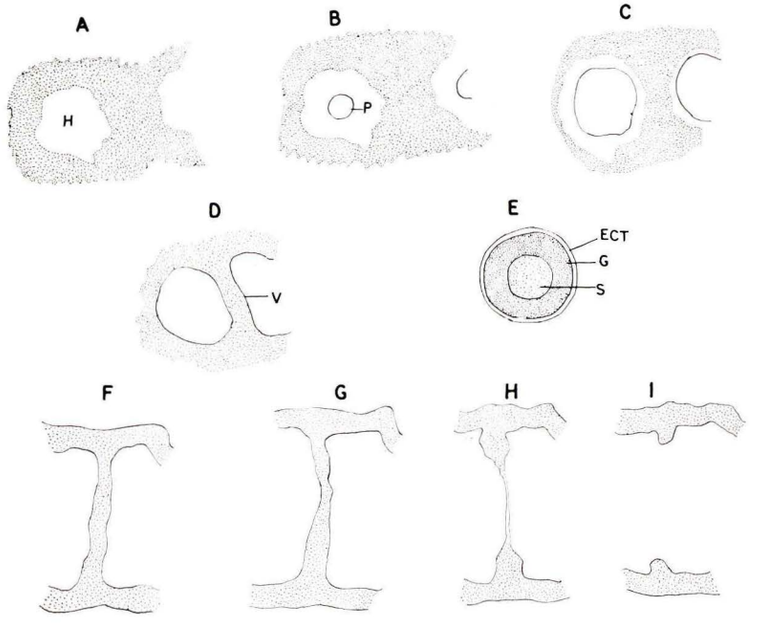


Fig. 3. A-D. Diagrammatic representation of the method of perforation in the fan of physaraceous plasmodia (H-hyaline area; P-perforation; V-vein. Further explanation in text). E. Diagrammatic cross-section of the vein in physaraceous plasmodia (ECT-ectoplasm; G-gel and S-sol of the endoplasm). F-I. Diagrammatic representation of the stages in the withdrawal of a vein in physaraceous plasmodia (Explanation in text).

When a plasmodium is changing the direction of its movement, the fan is gradually withdrawn and another fan develops in the new direction of movement by a process of flattening out. Withdrawal of the fan occurs by a continuous process of perforation unaccompanied by forward movement, resulting in the formation of veins up to the

edge of the fan. These veins are withdrawn in the usual manner *(Pl. 2, Fig. 14)*. Correlated with this withdrawal there is also a gradual reduction in the rate of protoplasmic flow in this region, since greater emphasis is placed on streaming in the new direction of movement. The new fan develops by a process of gradual flattening and expansion.

A second method of reticulation is occasionally observed, i.e., by branching and anastomosis. The fan gives rise to one or two stout processes which elongate, branch, and unite with already existing veins. This process, however, is not common to these plasmodia and was observed only once, in *P. compressum*, in a withdrawing fan of reduced size.

It may be mentioned that reticulum formation is a very slow process in *P. compressum* and *P. gyrosum*. Considerable time, up to an hour or more, may sometimes elapse between the appearance of a hyaline area and the perforation in its center; even subsequent processes are very slow. In *P. cinereum* and *P. vernum*, the events follow each other at a fairly rapid rate.

Response to external factors: The plasmodium is sensitive to several factors. Staling of cultures, depletion of food, and even slight movements, as when the culture dish is carried about from one place to another, bring about certain morphological changes. Most of these changes usually occur when a culture is left without changing the water or renewing the food supply. The fans are withdrawn, the veins become very thick, forming an irregular, wide-meshed network. Sometimes the veins may become knotted, irregularly shaped or undulate *(Pl. 2, Fig. 15)*. They may lose their adhering properties and float in the water. The veins are also seen to develop wing-like lateral extensions in some cases *(Pl. 2, Fig. 16)*. These wings may give way to even-sized lateral branches by a method of continuous perforation similar to the formation of the plasmodial network from the fan *(Text Fig. 4, A-C)*. Occasionally, numerous short, stout branches are seen projecting from all over the vein, laterally as well as on the dorsal side. These veins may at times be swollen at the tips and present a coralloid appearance. Long filiform pseudopods, resembling those of *Stemonitis herbatica*, were seen radiating from a vein-ending on one occasion in *P. vernum*. Occasionally, the plasmodium breaks up into cysts *in situ*, the latter following the contour of the plasmodium. This was seen in *P. cinereum*.

In some cases, all the veins are withdrawn and the plasmodium is transformed into a continuous, irregular perforated sheet in which

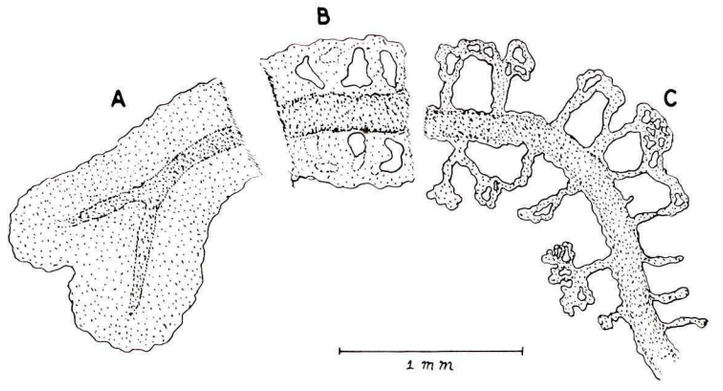
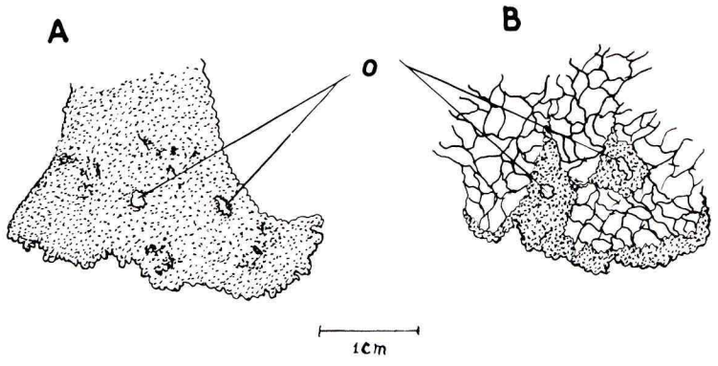


Fig. 4. Morphological irregularities in old physaraceous plasmodia. A. Vein of *P. gyrosum*, with wing-like lateral extensions. B and C. Formation of lateral veins from the "wings" at the posterior end, by progressive perforation.

streaming occurs along certain channels and the remaining region is gelated. This was seen in *P. compressum* and *P. gyrosum*. In the latter species the sheet is thick with a very uneven surface *(Text Fig. 5, A; Pl. 2, Fig. 17)*. If a small particle of oat is placed on such a sheet, however, an immediate transformation is seen. The sheet becomes progressively perforated, starting from around the oat particle, and within an hour's time it has organized itself into a typical reticulum surrounding the latter *(Text Fig. 5, B)*. A very peculiar formation is at times observed in *P. gyrosum*. The plasmodium forms a fine, small-meshed reticulum. Into each mesh project one or more small veins, ending in miniature fans containing mostly gelated protoplasm. The structure is reminiscent of the aerolae in the venation of dicotyledonous leaves *(Text Fig. 5, C)*.

The plasmodium is very sensitive to drying. A few minutes of sudden exposure to dryness may kill the plasmodium. On one occasion, when a plasmodium of *P. compressum* growing on a glass slide was being examined microscopically, the region directly in the microscope field began to dry up. Immediately, the entire protoplasm was withdrawn into the adjacent regions which were still moist. This was achieved not by a movement of the plasmodium as such, but by a unilateral streaming which showed the fastest rate ever observed. Almost the entire protoplasm had solated and was flowing away, leaving behind an insignificantly thin outer layer.



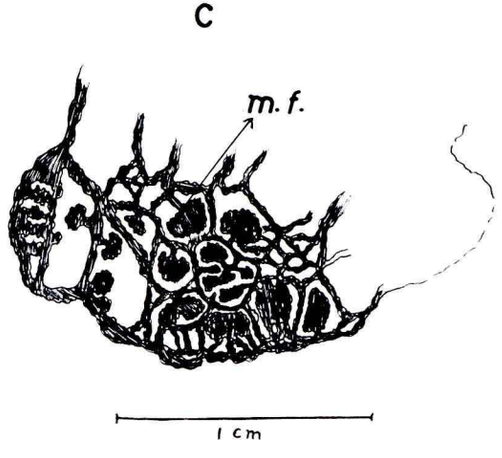


Fig. 5. Morphological irregularities in old physaraceous plasmodia *(P. gyrosum)*. A. Thick, continuous sheet, on which two particles of oat (o) are placed. B. Development of a reticulum from this sheet subsequent (and consequent) to the placing of oats. C. Fan with numerous meshes and miniature fans. (All semidiagrammatic. Explanation in text).

Propagation: In glass cultures, plasmodia of some of the species produce myxoamoebae and swarm cells. The function of these is perhaps the propagation of the plasmodium. This phenomenon was seen in *P. compressum* and *P. cinereum*.

The plasmodium of *Stemonitis herbatica* is whitish and non- pigmented. In glass dishes, it is not readily visible to the unaided eye, except as a few long, whitish strands (veins) which may be branched and anastomosed. Microscopic examination, however, reveals the presence of an exquisitely fine network of delicate veins interconnecting these larger veins. The organization of the plasmodium is thus in the form of an extensive network of broad meshes formed by fairly large veins, which are inter-connected by a fine-meshed network of slender veins *(Pl. 3, Figs. 18, 19)*. In its exquisiteness, the reticulum is comparable to a delicate lacework. Unlike the physaraceous plasmodium, there is no antero-posterior organization. The entire plasmodium grows and spreads in a more or less radial manner *(Pl. 3, Fig. 21)*. There are no fans, and the margins of the plasmodium are rather irregular, with numerous free vein-endings, the tips of which may be swollen into vesicles *(Pl. 3, Fig. 22)*. Long, finger-like processes, which may be called pseudopods, arise in bunches here and there on the network and are concentrated at the ends of veins *(Pl. 3, Figs. 20, 22)*. The entire plasmodial network is more or less uniformly small-meshed *(Pl. 3, Fig. 23)*. Continuous sheets of protoplasm are very rarely found, as in occasional fan-like developments *(Pl. 3, Fig. 24)*. Also, when the plasmodium is feeding on oats, the region immediately surrounding the oat particle appears as a thin, highly perforated sheet.

The veins are extremely slender (Table I), and are remarkably different from those of the physaraceous plasmodia: 1) a single vein is usually of uniform thickness throughout its length; 2) it usually traverses straight lines in its course, branches at sharp angles, and hence the meshes of a network are often remarkably angular *(Pl. 3, Fig. 23)*. It may be noticed from Table I that in contrast to the physaraceous plasmodia, no great difference exists in the size of the veins, and the plasmodium thus has a very regular appearance.

The Protoplasm: The protoplasm is divisible into a thin layer of nongranular ectoplasm which is hyaline and transparent, and a granular endoplasm. However, the latter is very fine-granuled and hence appears transparent *(Pl. 3, Fig. 25)*. Moreover, differentiation of the endoplasm into an outer gel layer and inner sol is rarely seen, almost the entire endoplasm being in the sol state. The ectoplasm is very plastic, and appears to be much less viscous than in physaraceous plasmodia; this is manifest in the slender pseudopods which are produced in great abundance and constantly change their size and shape.

The granules in the endoplasm are abundant, but characterized by minute size (C. 0.5μ) and low refractivity. In plasmodia feeding on oats, the endoplasm is filled with numerous starch grains. Numerous egested starch grains are also seen embedded in the layer of slime covering the veins. This slime layer is much thinner than is commonly seen in physaraceous plasmodia.

The protoplasm is characterized by a high degree of vacuolation. In plasmodia actively feeding on oats, most of the vacuoles enclose some granular matter and, infrequently, large starch grains. They are spherical, up to 25 or 30μ in diameter, and often occupy the entire width of the veins. When the plasmodium is not feeding on oats, small vacuoles measuring 3-5μ are seen.

The nuclei, as seen from fixed and stained preparations, are scattered rather irregularly all over the plasmodium and are often concentrated in the expanded axils *(Pl. 3, Fig. 26)*. They are seen in large numbers in regions of active feeding around oat grains. Very slender veins usually do not contain nuclei, but occasionally a nucleus may be seen lodged in a very thin vein, becoming elongated due to lateral compression. Sometimes they are seen as spherical bodies, 3-4μ in diameter. But, as in the case of *P. gyrosum*, they seem to be very plastic and usually appear irregularly elongated. In the veins they are elongated parallel with the long axis.

Protoplasmic Streaming: Streaming is markedly different from that in the physaraceous plasmodium in certain aspects. The rate of streaming is comparatively slow. In the small veins, it is sometimes so slow as to be almost imperceptible. The pause before reversing the direction of flow is very long, as against the brief pauses noticed in the physaraceous plasmodium; the long pauses occur rather consistently at one end, the pause at the other end being brief *(Text Fig. 6, A, B)*. Streaming is more regular and rhythmic in the larger veins, into which the smaller ones take their contents. Some degree of coordination exists with reference to the rate and direction of streaming in these large veins, but the small interconnecting veins display a total lack of coordination. The larger veins seem to be the main channels of circulation.

Formation of the Reticulum: The reticulum develops as the plasmodium grows and spreads out radially. Apart from this, there is usually no forward movement; in this respect the plasmodium differs markedly from the physaraceous plasmodium. Correlated with the peculiar organization of the plasmodium, the pattern of development of the network follows a distinct course. The vein-endings all around

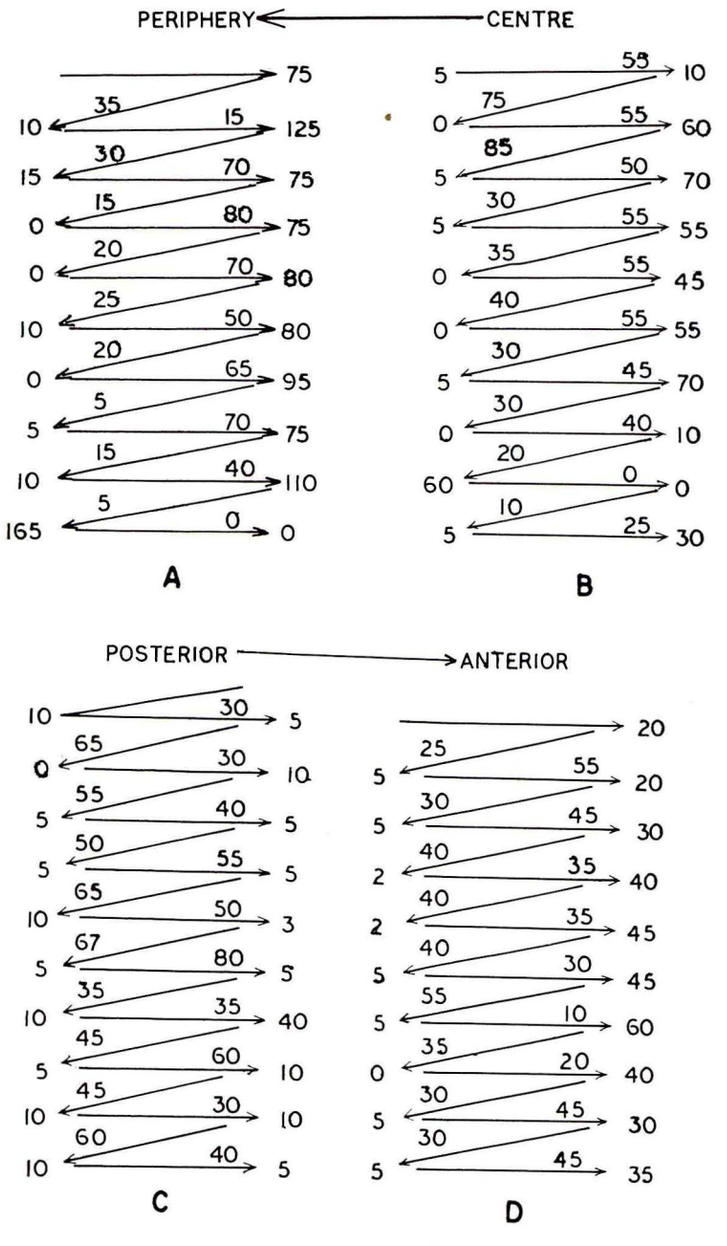


Fig. 6. Schematic representation of the duration of protoplasmic streaming in plasmodia (Details as in Text, Fig. 1). A and B. *Stemonitis herbatica.* C and D. *Arcyria cinerea.*

the edges of the reticulum put forth finger-like protuberances, adequately termed "filopods" by McManus *(1961a)*, which are transparent at first, consisting of the ectoplasm alone. Later the granular endoplasm flows into the base of these filopods. Protoplasmic streaming often causes them to enlarge, branch, and change their shapes constantly. Some of these are very slender, and they swing about in the water in a characteristic fashion. During such swinging movements a filopod may establish contact with a similar one from an adjacent vein. The ectoplasms coalesce, and during the next outward flow of protoplasm in any one of the branches, the endoplasm flows into the coalesced region and merges with the endoplasm of the other branch. With continued flow of protoplasm the newly formed vein enlarges to the proper size. Sometimes, the filopods may not swing about but grow directly towards each other and fuse. The veins continue to elongate and put forth more filopods. Thus the reticulum develops as the plasmodium grows and, as in the Physarales, the process is closely associated with protoplasmic streaming, although the pattern is different.

Perforation sometimes occurs in expanded regions *(Pl. 3, Fig. 24)* but this is very rare. The perforations in such cases appear to be preceded by vacuoles and not hyaline areas.

Response to External Factors: In cultures, the plasmodium is not very sensitive to changes in temperature. However, it is extremely sensitive to drying. If a portion of the reticulum growing on a glass slide under water is allowed to dry by lifting the slide, the protoplasm appears to coagulate almost immediately, resulting in the death of that region. When the slide is placed back in water, the veins float and do not recover. If the exposure is of very brief duration, there may be a prolonged cessation of streaming, which is eventually resumed.

In older plasmodia, or under certain conditions, as when the plasmodium is fed with an excess of oats, or when cultures remain for several days without the water being changed, several morphological irregularities are found. The characteristic organization of the plasmodium is lost and the network becomes irregular. Long strands are seen, and sometimes these bear numerous short laterals, giving the appearance of a thorny creeper. Sometimes the strands are large, being flanked on either side by a fine network *(Pl. 3, Fig. 25)*. Dense reticula, which look more like highly perforated protoplasmic sheets, surround the oat particles. These sheets are interconnected by large, branched veins with irregular outlines. These veins lose their evenness of outline and become irregularly flattened. Occasionally, fan-

like structures of continuous protoplasm develop at the edges. These are very thin and flat, and highly irregular in outline. They lack the characteristic organization of the physaraceous fans and are very temporary structures. Sometimes all the protoplasm is withdrawn into a few long veins which have an irregular and knotted appearance, with large swellings at irregular intervals. Eventually, the plasmodium breaks up into spherical cysts of various sizes *(Pl. 4, Fig. 27)*. If the cysts are formed without prior condensation into large veins, these follow the pattern of the plasmodium *(Pl. 4, Fig. 28)*. If the water is replaced, the cysts undergo no change, but the addition of a pinch of powdered oats or a few drops of carrot decoction often brings about a revival, the cysts reuniting to form a normal plasmodium.

Propagation: In old cultures, certain peculiar structures are occasionally seen. These are in the form of very large swellings scattered at long intervals on the plasmodium, bearing numerous long, flexible, transparent, finger-like processes all over *(Pl. 4, Fig. 29)*. Eventually, these structures become detached and float in water. Their ultimate fate is not known, but it is possible that they are propagules which may develop into new plasmodia if transferred to fresh medium.

As in some of the physaraceous plasmodia, this plasmodium also produces myxamoebae and swarmers in great abundance in a manner to be described later. These produce new plasmodia if transferred to a fresh medium in cultures.

The Intermediate Type

The single trichiaceous species studied, i.e., *Arcyria cinerea,* was found to have a plasmodium which exhibited certain features characteristic of the physaraceous plasmodium and certain others of the stemonitoid plasmodium, as well as certain peculiarities of its own.

Morphology: An antero-posterior orientation, such as in the physaraceous plasmodia, becomes manifest at an early stage. The young plasmodium has an anterior fan and a posterior vein and exhibits rhythmic streaming movements when barely a few days old *(Text Fig. 7, A-D)*. In the mature plasmodium, the organization shows a pattern which combines the characteristics of the physaraceous as well as stemonitoid plasmodia.

The mature plasmodium is nonpigmented, not extensive, slender-veined, very flat and transparent, bordering on invisibility *(Pl. 4, Fig. 30)*. In cultures it can be detected only on close scrutiny. In these cultures the plasmodium spreads out in a more or less radial fashion, in the form of a reticulum. In a few regions the reticulum has defi-

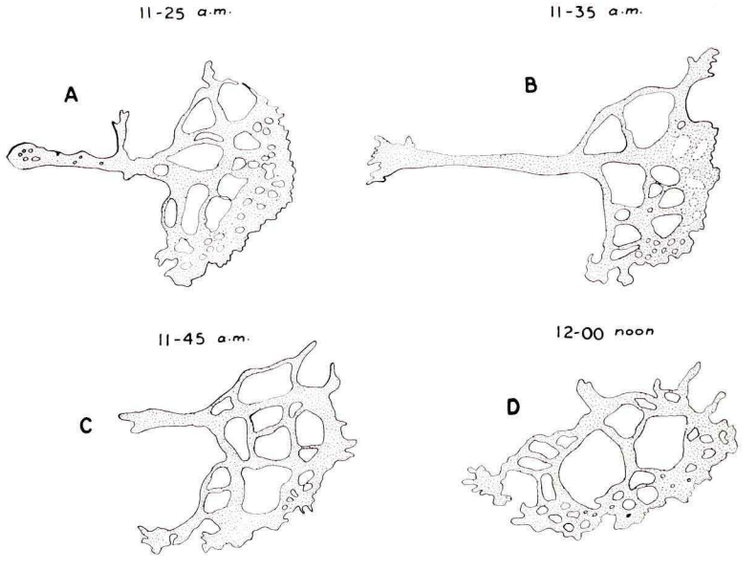


Fig. 7. Movement and reticulation in a 3-day old plasmodium of *Arcyria cinerea*. A and B. The posterior end of the vein is held firmly on the substratum while the plasmodium elongates forward. C and D. The anterior end flattens out, becoming reticulate, while the posterior vein draws up forward.

nite, fan-like edges *(Pl. 4, Fig. 31)*. Growth is not centered in the fans alone, but may occur freely all along the edges.

The plasmodial reticulum consists of a few prominent veins forming broad meshes, which in turn are interconnected by fine, slender veins of fairly uniform thickness (Table I) forming an intricate network of small meshes, as in the case of *S. herbatica (Pl.4, Fig. 32)*. Occasionally, in vigorous and actively feeding plasmodia, the large veins may be as thick as 130μ. Numerous finger-like processes are at times seen scattered over the veins, as in the stemonitoid plasmodium.

The plasmodial fan is usually small, very thin, and flat. The veins continue into the fan in such a way that usually, there is a well-organized, reticulate channel of streaming protoplasm with clear-cut boundaries in the fan, which may extend to the very edge of the fan. The protoplasm between these channels appears gelated and static, and it is so flat and transparent that its perforated nature is obscured *(Pl. 4, Fig. 34)*. This channelization of streaming gives a

distinct appearance to the fan in this species, which differs markedly from the physaraceous fan where protoplasmic streaming is somewhat indefinite and irregular, especially toward the edge.

The Protoplasm: As in other plasmodia, there is a thin, transparent ectoplasmic layer covering the plasmodium. The endoplasm is mostly in the sol state as in *Stemonitis*. The most striking feature of the endoplasm is a remarkable degree of homogeneity and transparency *(Pl. 4, Fig. 33)*, stemming from the fact that the granules are almost immeasurably fine, even-sized, and of low refractivity. A few large granules, 2-3μ thick, are sometimes seen scattered among these. When the plasmodium is feeding on oats the protoplasm is filled with starch grains.

Numerous vacuoles fill the protoplasm in actively feeding plasmodia, occurring to a small degree in the gelated regions of the fan but mostly moving with the endoplasmic current. Most of these enclose granular material. In the gelated region of the fan, some vacuoles are occasionally seen to open to the exterior *in situ* at the upper as well as lower surfaces, thus creating a perforation in the gel. The walls of the vacuole then become continuous with the ectoplasm and the contents of the vacuoles are liberated into the space thus created. The perforation may enlarge gradually.

When the plasmodium is not feeding on oats the number of vacuoles is comparatively small. A few of them are large, 10-20μ in diameter, enclosing granules but the rest are small, 5-10μ and devoid of granules. A few large, spherical, oil-globule-like bodies are also occasionally seen scattered in the endoplasm.

Protoplasmic Streaming: Streaming is usually vigorous and rapid in the larger veins. Readings of the time taken for streaming in the forward and reverse directions indicate that the pause before changing the direction is sometimes consistently longer at one end than at the other, and at other times inconsistent and irregular *(Text Fig. 6, C, D)*. The movements are to some extent co-ordinated in adjacent large veins, but slow and irregular, with prolonged pauses, in the small interconnecting veins.

Formation of the Plasmodial Reticulum: Because of its flat nature and transparent protoplasm, this plasmodium is well suited for the observation of forward movement and reticulum formation. These processes are basically similar to those of physaraceous plasmodia, but differ from them in certain respects. The course of the main veins which are to develop from the fan is predetermined by the clear channelization of streaming movement along certain tracts

which are slightly raised above the surface. In the remaining flat region the small interconnecting veins develop by the usual perforation method. The protoplasm in this region being very transparent, the perforations can be distinguished only with difficulty *(Pl. 4, Fig. 34)*. Moreover, the perforations seem to be preceded not by hyaline areas but by vacuoles, whose opening to the exterior creates perforations as previously described. As the plasmodium moves forward, the smaller veins are continuously withdrawn in the manner described for *P. compressum*. The whole process, however, proceeds at a much more rapid rate than in physaraceous plasmodia.

Response to External Factors: Like most plasmodia, the plasmodium of *A. cinerea* is sensitive to heat and drying. Staling and depletion of food in water cultures result in morphological irregularities. The characteristic organization of the reticulum is lost, the veins are few, thick, white, and knotted *(Pl. 4, Fig. 35)*. They lose their adhering properties and float in water. Eventually, cysts may form *in situ* in a moniliform pattern, following the contour of the veins *(Pl. 4, Fig. 36)*. The cysts may be spherical or elongated, uniseriate or multiseriate.

Propagation: Like *S. herbatica*, the plasmodium in *A. cinerea* also produces swarmers in abundance. These swarmers are very different from those of the other species in that the proportion of length to width is considerably larger, and instead of being comma-shaped, the swarmers are worm-like and slightly twisted. Their movement is comparatively slow, though characteristically rotatory.

Swarmer Formation From the Plasmodium

The constant presence in cultures, especially of *Physarum compressum*, *Stemonitis herbatica*, and *Arcyria cinerea*, of myxamoebae and free-swimming swarmers in great abundance, is a very interesting feature. The water and powdered oats used for culturing were presterilized, and sterile conditions were maintained during inoculation and up to the time of observation. Hence there was no chance of contamination. Moreover, that these organisms were swarmers or myxamoebae of the species concerned and not protozoan contaminants, could be ascertained by comparison with swarmers obtained from germination of spores of the respective species. Repeated microscopic observation established beyond doubt that these were produced from the plasmodia themselves, in the manner described below. The process was observed very often in *S. herbatica* and *A. cinerea*.

In *S. herbatica* the plasmodium has no definite margins. The edges consist of numerous free vein-endings. These bear numerous

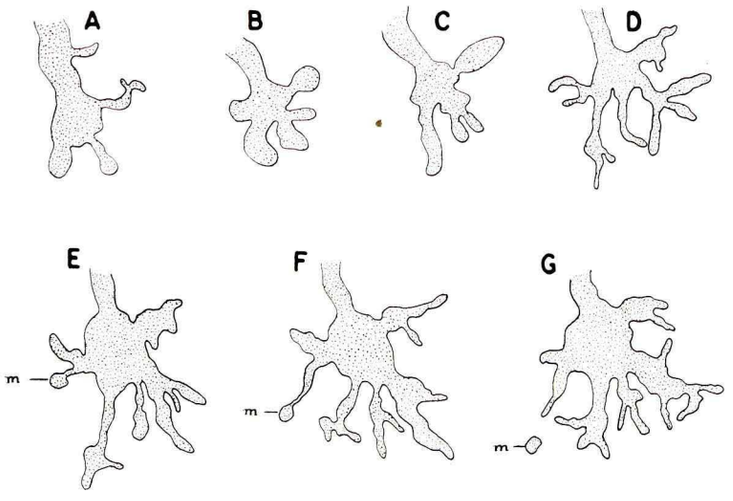


Fig. 8. Swarmer formation from plasmodial vein-ending of *Stemonitis herbatica*. A-D. Formation of filopods at the vein ending. E-G. Constriction and cutting off of myxamoeba(m) from a filopod.

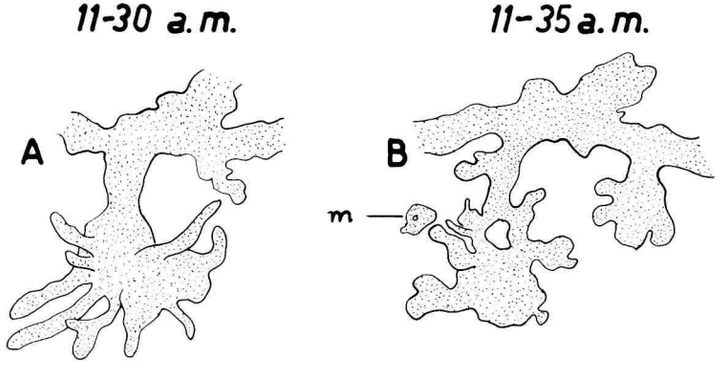


Fig. 9. Swarmer formation from plasmodial vein ending of *Stemonitis herbatica;* m, myxamoeba.

finger-like pseudopods which constantly change their sizes and shapes. Usually they arise in clusters, radiating in all directions. Besides being concentrated at the edges, such pseudopods may also occur scattered on the veins within the reticulum. In *Arcyria cinerea* the plasmodium has fans with continuous margins, but filiform pseudopods like those of *Stemonitis* usually protrude from these margins, as well as from the veins at random. It is from these pseudopods that the myxamoebae are usually cut off in both the species. They may arise from any of the pseudopods. Pseudopods which are about to cut off swarmers are in no way differentiated from those that are not. They undergo various changes of shape, but eventually the apex swells slightly and a constriction appears below it. The constriction deepens, and an amoeboid mass is pinched off. This crawls out for a short distance and comes to rest. In *Arcyria* it assumes the elongated form of a swarmer, develops flagella, and swims away within a few minutes. In *Stemonitis* it appears to remain as an amoeba for an indefinite period, and its conversion into a free-swimming swarmer has not been observed, but is assumed to take place, since swarmers are found in great abundance *(Text Figs. 8, 9, 10).*

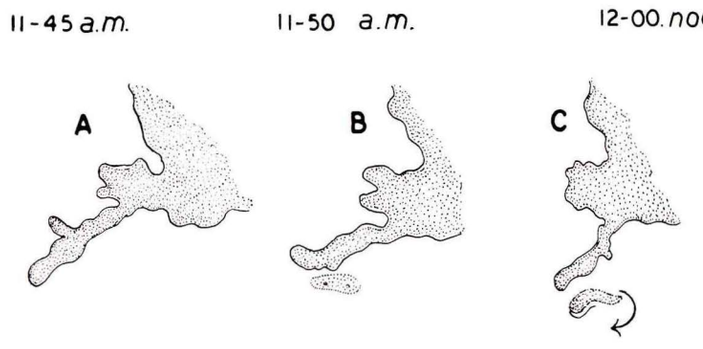


Fig. 10. Swarmer formation from plasmodial fan of *Arcyria cinerea.* A. Pseudopod from fan. B. Myxamoeba cut off from pseudopod. C. Swarmer, flagellated and rotating.

Sometimes, a small projection may be put forward from a vein and cut off as a whole into a myxamoeba, without forming an elongated pseudopod. Occasionally, the myxamoeba is cut off from a withdrawing veinlet in *Arcyria,* in a manner described earlier (Indira, *1964*).

In *Physarum compressum,* the actual formation of myxamoebae or

swarmers has not been observed, but it is probable that it takes place in a manner more or less similar to that in the other two species. Swarmers are found in cultures of *P. cinereum* also, but here again the process has not been observed.

The swarmers produced by the plasmodia seem to be potentially capable of forming new plasmodia. In *Stemonitis herbatica* about one ml of the fluid containing these swarmers was transferred from a Petri dish culture to a watch glass by means of a pipette. An equal amount of 2 per cent carrot decoction was added to this, and the culture incubated in a moist chamber. Within forty-eight hours a fairly extensive plasmodium had developed in the watch glass. Such a process is likely to occur in the other species also, in which case it may be assumed that these swarm-cells serve as a means of propagation of the plasmodium. It may also be added here that in *S. herbatica,* ingestion of haploid swarmers by young plasmodia has often been observed in cultures freshly derived from spores. But the swarmers produced from the mature plasmodium were never seen to be ingested, indicating perhaps that they had the same ploidy as the plasmodia. The terms primary swarmer and secondary swarmer could be used to denote swarmers produced respectively from spores and from plasmodia.

CONCLUSIONS AND DISCUSSION

From the detailed study of limited but representative species in culture, a few broad conclusions may be drawn.

While the four species of *Physarum* show certain similarities in their plasmodia, each has certain distinctive characteristics of its own: the plasmodia of *P. gyrosum* and *P. compressum* are large, thick-veined, rather opaque, and highly granular, whereas those of *P. cinereum* and *P. vernum* are rather small with slender veins and fairly homogeneous and transparent protoplasm. Between the first two species, *P. gyrosum* is distinct from the other by its yellow pigment and the relative thinness of the gel layer in the veins. *P. cinereum* is distinguished from *P. vernum* by a higher degree of transparency and extreme thinness of the gel layer.

Though the individual species may thus be distinguished, collectively the physaroid plasmodia may be described as representing a single type, which fits into the "phaneroplasmodium" of Alexopoulos. This type, as observed in the present investigation, possesses the following characteristics:

1. A well-defined antero-posterior orientation which seems to be established at an early stage. The anterior end consists of a contin-

uous, fan-shaped, protoplasmic sheet and the posterior portion of a network of veins which usually increase progressively in thickness toward the rear. The fan seems to be the active center for growth and forward movement. The plasmodium may be unipolar, i.e., having a single fan, or multipolar, having several fans.

2. Generally, the veins are rather thick and readily visible, although sometimes, as in the case of *Physarum cinereum*, they may be slender and almost flat. The large veins may vary in thickness from 50-250μ in different species and the small ones, from 10-50μ.

3. The meshes of the plasmodial reticulum are fairly wide, becoming wider towards the posterior end as movement continues. Small interconnecting veins are not usually found between the large meshes. Sometimes they may be present towards the anterior end, but are soon withdrawn into the larger veins.

4. Within a single plasmodium, there is considerable variation in the thickness of veins, width of the meshes, etc. The outlines of the veins are not very even, and even in a short vein there may be variations in thickness at different regions.

5. The reticulum may be described as a closed one, as there are usually no free vein-endings. Pseudopodia usually arise only from the fan, and they are usually short and blunt, aptly termed lobopods (McManus, *1961a*).

6. The protoplasm usually presents a highly granular appearance due to the large size and high refractivity of the component granules, but in some species the granules may be sufficiently small and non-refractive to render the protoplasm transparent and homogeneous.

7. The granular endoplasm is differentiated into an outer static gel and an inner streaming sol. The gel layer may be fairly thick, comprising about half the diameter of the veins, or it may be thin or almost lacking.

8. The streaming movement is usually fast and vigorous under normal conditions, and the pauses at either end, before changing the direction, are brief.

9. Plasmodial migration is a common feature; the development of the plasmodial reticulum is correlated with growth and movement. The reticulum develops from a continuous sheet by a process of perforation.

The plasmodium of *Stemonitis herbatica* with its absence of fans, its slender veins, and even-meshed reticulum fits into the "aphanoplasmodium" type of Alexopoulos. It exhibits certain characters that contrast with those of the phaneroplasmodium:

1. There is a total absence of antero-posterior orientation. The

hypha-like, linear organization is established at a very early stage. There are no well-defined fans; growth occurs in a radial manner along the free vein-endings.

2. The veins are extremely slender; the large ones may measure 30-40μ and the small ones 5-10μ. Generally they are flattened, giving a two-dimensional appearance.

3. The reticulum is fine and delicate, and usually consists of large veins interconnected by a small-meshed network of slender veins.

4. Within a small area, the veins as well as the meshes of the network show a fair degree of uniformity in size. The veins are smooth with remarkably parallel sides, usually oriented in straight lines, and branched at sharp angles, giving a highly angular appearance to the network.

5. The reticulum is open, with numerous free vein-endings all around. Pseudopods occur scattered over the reticulum and in particular abundance at the vein-endings; they are often in clusters, and are very long, slender, and digitate, as suggested by the term filopod (McManus, *1961a*).

6. The protoplasm is very fine-granuled and hence appears homogeneous and transparent.

7. There is usually no differentiation of the endoplasm into gel and sol.

8. Streaming movements are comparatively slow, especially in the smaller veins. The pauses before changing direction are usually long.

9. Plasmodial migration appears to be altogether absent, except at the time of fruiting. The plasmodial reticulum expands at the margins by a process of branching and anastomosis of the free vein-endings.

The characteristics of the plasmodium of *Arcyria cinerea* are intermediate between the phanero- and the aphanoplasmodium.

1. The general orientation and organization of the plasmodium are typical of the phaneroplasmodium. A well-defined advancing fan is present but growth is at times radial, as in the aphanoplasmodium.

2. The veins are very slender and rather flattened; the larger veins measure 40-50μ in diameter, the smaller ones 10-15μ.

3. The meshes of the network are rather small. The characteristic structure of the aphanoplasmodium, with small meshes connecting larger ones, is seen very often.

4. The width of the meshes and of the veins is variable within a small range and irregular.

5. The reticulum may be closed in some regions, with a fanlike edge, and open with free vein-endings in some other regions. The

pseudopods advancing from the fan may be large and blunt, but those seen at vein-endings are long and slender.

6. The protoplasm is extraordinarily transparent and homogeneous, the protoplasmic granules being usually finer than those of *S. herbatica.*

7. The endoplasm in the veins is not clearly differentiated into gel and sol; it is usually in the sol state.

8. Protoplasmic streaming is usually vigorous and rapid. The pauses before changing direction are usually short, but sometimes long as in *S. herbatica.*

9. Movement and reticulum formation follow the basic pattern of the phaneroplasmodium, with some difference in detail.

Comparing the different types of plasmodia in the light of the criteria described by Alexopoulos *(1960)* and McManus and Taylor *(1961),* a few statements can be made.

Contrary to the statement of McManus and Taylor *(1961),* and in conformity with that of Alexopoulos *(1960),* the present studies indicate that the most prominent difference between the phaneroplasmodium and aphanoplasmodium is in growth habit. In the phaneroplasmodium, polarity is established at an early stage, one end flattening out and behaving as the anterior end. In the aphanoplasmodium there is no polarity. Initially there is a tendency for linear elongation which later expresses itself in a radial manner, i.e., in all directions.

The second striking difference is the "closed" network in the phaneroplasmodium and the "open" network in the aphanoplasmodium. This feature does not seem to have been sufficiently recognized in previous accounts.

A network of large meshes interconnected by small meshes has been found typical of aphanoplasmodia in contrast with phaneroplasmodia, where interconnecting veins are withdrawn at an early stage. Long veins with anastomosing laterals, which are described as typical of the aphanoplasmodium (Alexopoulos, *1959;* McManus, *1961a*) were found only in old cultures as a morphological variation. The thick veins and three-dimensional aspect of the phaneroplasmodium, which has been contrasted with the slender veins and two-dimensional aspect of the aphanoplasmodium, is not a universal feature, as may be seen from the descriptions. However, the unevenness of the veins in phaneroplasmodia can be contrasted with the even, hypha-like aspect of those in aphanoplasmodia, as mentioned by Alexopoulos *(1960).*

The transparent nature of the protoplasm, described for the stemonitoid plasmodium from very early times (Celakovsky, *1892;* Miller, *1899;* Thom and Raper, *1930;* Alexopoulos, *1959, 1960, 1963;* McManus, *1961a;* McManus and Taylor, *1961*), and stressed as being markedly different from physaraceous plasmodia, is characteristic of a few of the physaraceous species as well, e.g., *P. cinereum.* It should be stressed that there is no "granular" and "nongranular" protoplasm. The protoplasm is granular in all species, and the difference is one of coarse *versus* fine, and refractive *versus* nonrefractive granules, the latter types usually imparting transparency to the protoplasm.

Another aspect that requires some clarification is the organization of the protoplasm into "ectoplasm" and "endoplasm." Much has been made of the almost complete absence of a gelated ectoplasm in the aphanoplasmodia, etc. In the present paper, the term "ectoplasm" is used to indicate the very thin, transparent outermost layer of protoplasm which is devoid of granules. This is present in all plasmodia and does not vary much in thickness from species to species. It seems to vary in plasticity, however, being generally more plastic in aphanothan in phaneroplasmodia. It is perhaps because of this difference that the phaneroplasmodium produces blunt "lobopods" and the aphanoplasmodium fine, slender "filopods." Within the ectoplasm there is granular protoplasm, which is referred to here as the endoplasm. This endoplasm is further differentiated into a gelated cortex and a streaming sol. In some species, however, this cortical layer is very thin or absent, e.g., *S. herbatica, P. cinereum* and *A. cinerea.* This feature is not specific to any plasmodial type. Further, the gel and sol states are reversible and this reversibility is helpful to the plasmodium in its various activities.

Streaming movement is more irregular in rate and duration in the aphano- than in the phaneroplasmodium, and it is also characterized by very long pauses. McManus and Taylor *(1961)* found no such differences, but Alexopoulos *(1959)* has reported such slow streaming in *Stemonitis flavogenita.*

The method of formation of the plasmodial network is strikingly different between the phanero- and aphanoplasmodia. Such a difference had been suggested by Alexopoulos *(1960).* The perforation method of phaneroplasmodia observed here has been beautifully described by Camp *(1937),* but the method of branching and anastomosis is perhaps described here for the first time.

Another difference between the phanero- and the aphanoplasmodium, which has not been sufficiently emphasized, is a tendency for active migration in the former and its absence in the latter. Mi-

gration in phaneroplasmodia is a very commonly described phenomenon, though Guttes, *et al. (1961)* and Daniel *(1964)* state that there is no migration under optimum nutritional conditions. In the present study, however, migration was seen to be a constant feature in phaneroplasmodia under all conditions.

A physiological difference between the phanero- and aphanoplasmodia is pointed out by Alexopoulos *(1960)*, who states that plasmodia of *Stemonitis* and *Arcyria* can grow on agar only if a layer of water is present over the surface and hence require periodical addition of water. In the present studies, although the aphanoplasmodium did seem to be more delicate than the other, both *S. herbatica* and *A. cinerea* showed good growth and fruiting without any water being added over the agar surface.

Examining all these points, one feels that our concepts of plasmodial types need to be broadened in some respects, and modified in some others.

The morphological variations produced in plasmodia under unfavorable conditions have rarely been described. Sobels and Cohen *(1953)* describe, as signs of injury, changes similar to those described here. In view of such variations, it is desirable that plasmodia in cultures be studied under optimum conditions to get a correct picture. In fact, the winglike extensions produced in old cultures of physaraceous plasmodia are very similar to the "ruffles" described by McManus *(1962)* as characteristic of trichiaceous plasmodia.

The observation of a vesicular organization of the protoplasm in *P. gyrosum* is interesting, but needs verification. Vacuoles of several types are seen. In young plasmodia they seem to contain mostly ingested swarm-cells. In older plasmodia they contain perhaps waste materials, as ingested starch grains of oat occur freely in the endoplasm. It is doubtful if these grains are ingested by phagocytosis. Some other process seems to be involved here. In *Stemonitis*, for example, the region of the plasmodium immediately surrounding the oat particle is a continuous protoplasmic sheet of low viscosity. It is likely that the starch grains are loosened here by some process, and then drawn into the protoplasm during streaming movements, digested so far as possible and later egested.

Contrary to the general view, direct measurements indicate that the streaming movements are not always consistently longer in one direction than in the other. When they are, the longer duration is not necessarily in the direction of movement. But on the whole, the shuttle type of streaming is a well-organized phenomenon, playing a major role in the intake, circulation, and digestion of food, growth,

movement, and the development of the plasmodial reticulum.

The formation of swarmers from plasmodia is indeed a phenomenon of great interest, reported for the first time by the author (Indira, *1964*). Its occurrence in most of the plasmodia studied here indicates that it may be a widespread, if not universal, phenomenon among myxomycetes. It is interesting to recall here the observation by Camp that small portions of the plasmodium may become severed from the main body by the appearance of constrictions leading to eventual breakage. These portions may pursue an independent course as small plasmodia, but sometimes they are so small that they do not develop into plasmodia (Camp, *1937*, p. 314). It would have been interesting to place these bits under water and observe them over a long period to see if they would develop into swarmers.

The phenomenon of swarmer-formation may represent an important stage in the life cycle of Myxomycetes which is not yet thoroughly understood. It may also have far-reaching evolutionary significance. Are these swarmers haploid or diploid? Their noningestion by the plasmodia perhaps indicates that they have the same ploidy (presumably diploid). Again, do they unite in pairs? Various conjectures are possible, but no definite statement can be made until the cytological changes accompanying this process are clarified. And this may not be easily accomplished, as the formation of a swarmer from any region of the plasmodium cannot be predicted with precision. Until these processes are more clearly understood, it is safe to assume that this may be a means of propagation of the plasmodium, as the formation of new plasmodia from these swarmers has been observed in one instance.

SUMMARY

The plasmodia of six myxomycetous species, of which four belong to the Physarales, one to the Stemonitales and one to the Trichiales, were studied in culture.

The physaraceous plasmodia fit into the "phaneroplasmodium" type and the stemonitoid plasmodium into the "aphanoplasmodium" type, while the trichiaceous plasmodium is intermediate between the two.

The phaneroplasmodium is characterized by a closed network with a definite antero-posterior organization and polar growth, irregular veins, short, blunt pseudopods, and active migration over the substratum, during which the reticulum develops from a fan-like sheet by a process of perforation. In contrast, the aphanoplasmodium has an open network with radial growth, smooth, hypha-like veins and slender, digitate pseudopods; there is no migration, and the network

develops by branching and anastomosis of pseudopods from veins.

Unfavorable circumstances may cause morphological changes in all the plasmodia.

While feeding on oats the endoplasm is filled with starch grains, and a process other than phagocytosis is indicated for their intake.

The duration of streaming movements is not always consistently longer in any one direction.

Most of the species produce swarmers or myxamoebae from plasmodia in culture. In one species, these were seen to develop into new plasmodia.

ACKNOWLEDGMENTS

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Addendum

Since the completion of this work in 1966, Ross and Cummings *(1967)* have produced photographic evidence for the formation of amoeboid bodies from plasmodia, citing the author's earlier work (Indira, *1964*). However, the phenomenon described by the above authors differs in certain respects:

The present author has observed the formation of swarmers and myxamoebae a) only in mature, well-organized plasmodia, b) in Petri-dish cultures under water, and c) very regularly in all the plasmodia of the species indicated, whereas, Ross and Cummings have observed it in a) very young (30 hours old) plasmodia, b) in microcultures (the cultural conditions are not described in the paper), and c) as a rare phenomenon occurring in one or two out of hundreds of microcultures. It is possible that the phenomenon described by them might be different. It may have resulted, as the authors themselves suggest, from the incompatibility of haploid nuclei following the coalescence of haploid swarmers with a theoretically diploid plasmodium, the haploid nuclei being cast off in the amoeboid bodies. Nevertheless, the suggestion of these authors that the uninucleate-haploid-amoeboid state and the multinucleate-diploid-plasmodial state are not completely rigid and separate phases of the life cycle, deserves careful consideration by all interested in studying this group.

Explanation of Plates

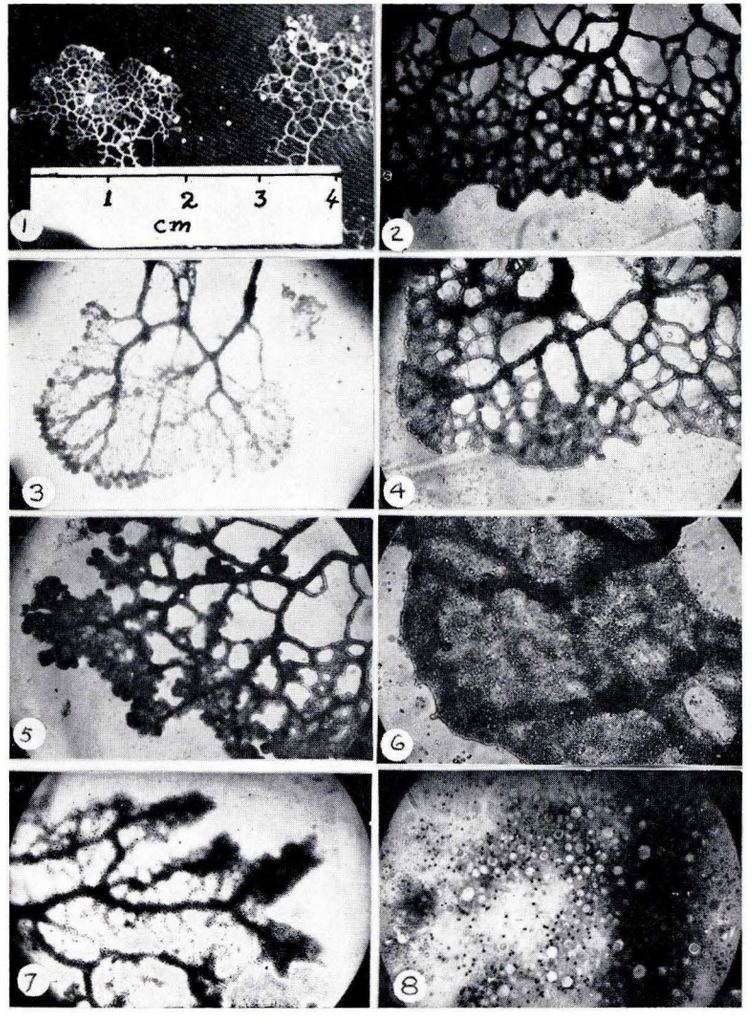

Plate 1

Plate 1. Figs. 1-8. Physaraceous plasmodia. 1. Plasmodial fans of *Physarum cinereum*, with oat particles (seen as white globules). 2. Plasmodial fan of *P. gyrosum*, with opaque veins, ca×18. 3. Flattened, transparent fan of *P. cinereum*, showing channelizing of veins up to the margin, ca×18. 4. Plasmodial fan of *P. cinereum*. Note demarcation of ectoplasm at the margin, ca×18. 5. Plasmodial fan of *P. compressum*, ca×18. 6. Plasmodial fan of *P. compressum*, showing granular structure, ca×55. 7. Fixed and stained plasmodial fan of *P. gyrosum*. Note highly vacuolate structure, ca×18. 8. Portion of Fig. 7 magnified, showing nuclei and vacuoles containing stained matter, ca×55.

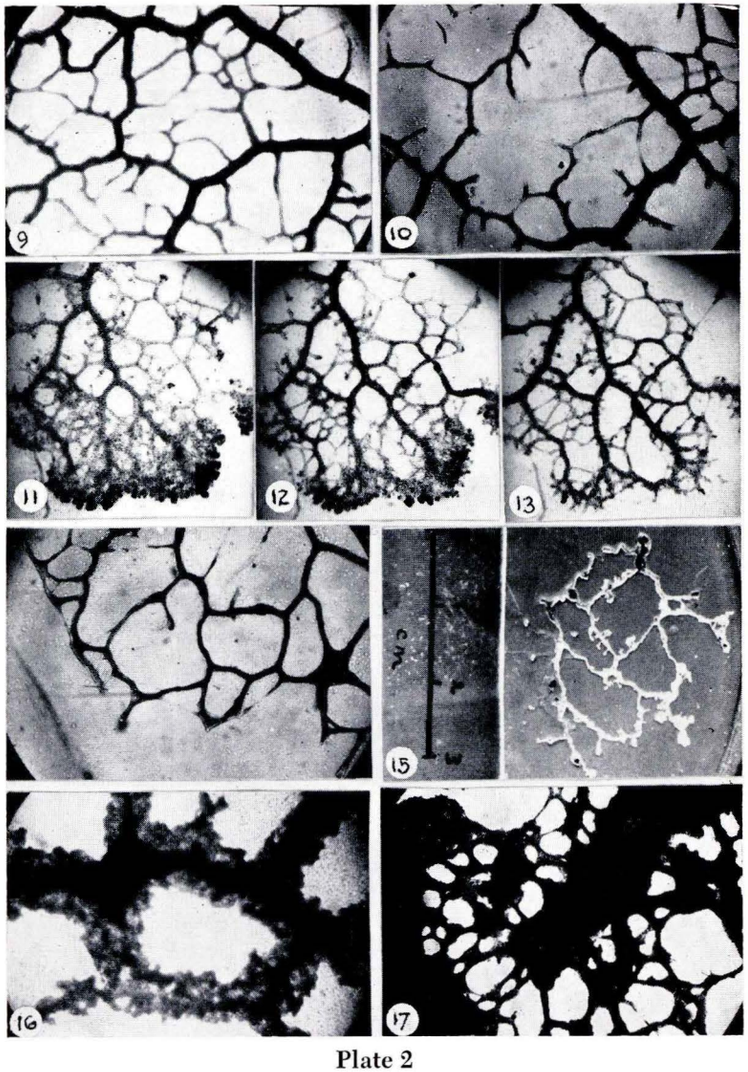

Plate 2

Plate 2. Figs. 9-17. Physaraceous plasmodia. 9, 10. Posterior region of the plasmodium of *P. gyrosum*, showing large veins, and small interconnecting veins in different stages of withdrawal, ca×18. 11-13. Stages in progressive reticulation of the fan in *P. compressum*. Photographs taken at 5-10 minute intervals, ca×18. 14. Withdrawing fan of *P. gyrosum*. Note that reticulation has progressed up to the edges, and the veins are in the process of withdrawal, ca×18. 15. Old plasmodium of *P. cinereum* showing dense, thick veins with short laterals. 16. Portion of an old plasmodium of *P. cinereum* with wing-like lateral extensions from veins, ca×18. 17. Portion of an old plasmodium of *P. gyrosum*, with a perforated sheet-like structure, ca×18.

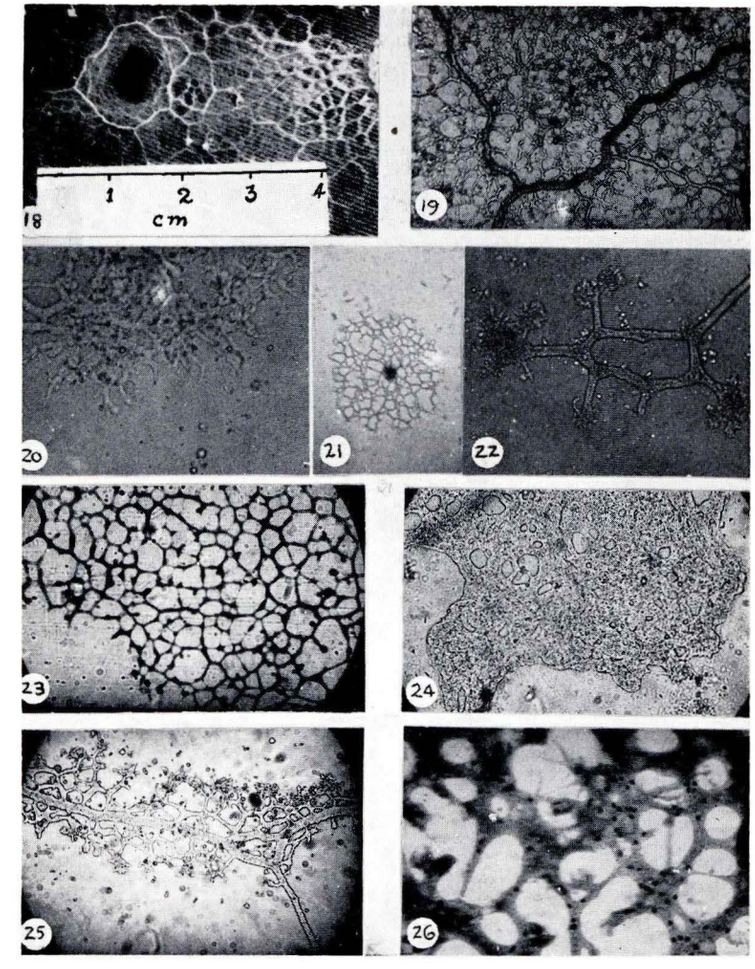

Plate 3

Plate 3. Figs. 18-26. Stemonitoid plasmodium: *Stemonitis herbatica*. 18. Plasmodium showing wide meshes interconnected by a fine network. 19. Portion of Fig. 18 enlarged to show detail, ca×50. 20. Marginal region of the plasmodium showing slender, filiform pseudopods, ca×35. 21. Small plasmodium, with oat-grain in the center, showing radial orientation, ca×18. 22. End of a vein showing free ends with vesiculose swellings and filopods, ca×83. 23. Fixed and stained plasmodium showing even-meshed, regular structure, ca×55. 24. Fan-like extension, with perforations, ca×125. 25. Large vein from an old plasmodium, flanked by irregular laterals. Note transparent nature of the protoplasm, ca×35. 26. Fixed and stained plasmodium showing distribution of nuclei, ca×290.

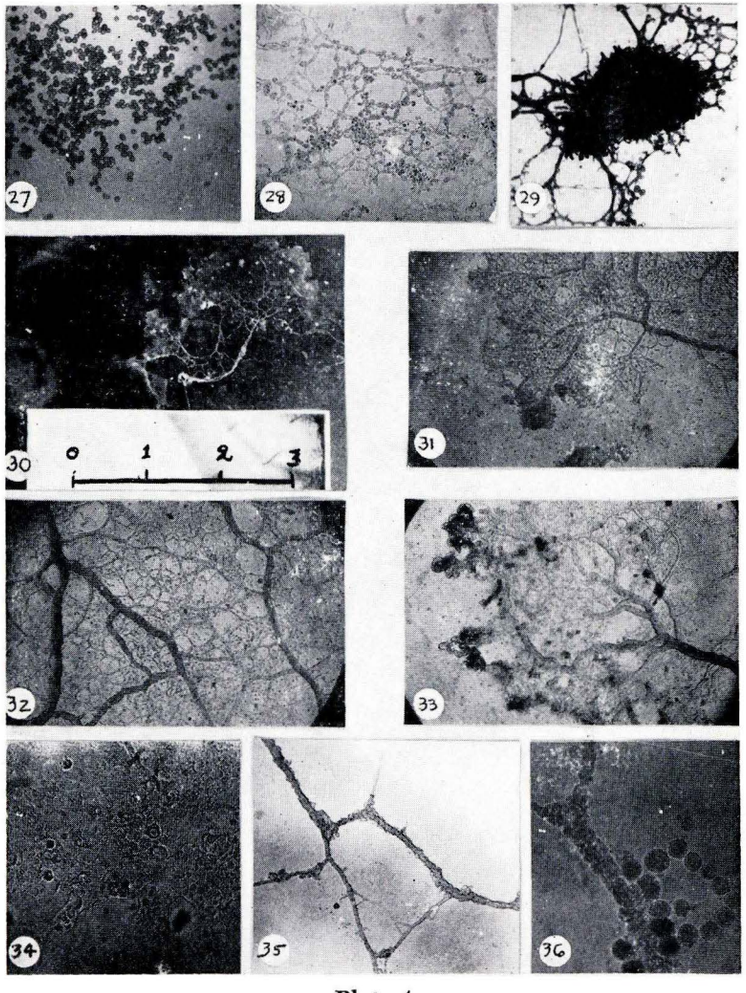

Plate 4

Plate 4. Figs. 27-29. *Stemonitis herbatica.* 27. Scattered cysts formed from an old plasmodium, ca×66. 28. Plasmodium about to encyst, showing beaded structure, ca×53. 29. Propagule in old plasmodium (stained) ca×53. Figs. 30-36. *Arcyria cinerea.* 30. Delicate plasmodium. 31. Fan showing fine venation, ca×18. 32. Interior region of plasmodium showing *Stemonitis*-like structure, ca×18. 33. Fan with vesiculose margins. Note transparent nature, ca×18. 34. Fan, magnified, to show transparent, perforated structure, ca×53. 35. Thick, knotted veins from old plasmodium, ca×80. 36. Encysting plasmodium, ca×80.

REFERENCES CITED

Alexopoulos, C. J. *1959.* The laboratory cultivation of *Stemonitis.* Am. J. Bot. *46:* 140-142.

_____. *1960.* Gross morphology of the plasmodium and its possible significance in the relationships among the myxomycetes. Mycologia *52:* 1-20.

_____. *1963.* The Myxomycetes II. Bot. Rev. *29:* 1-78.

Benedict, W. G. *1964.* Plasmodial activity in *Stemonitis fusca* Roth. Can. J. Bot. *43:* 1639-1643.

Camp, W. G. *1937.* The structure and activities of myxomycete plasmodia. Bull. Torrey Club *64:* 307-335.

Celakovsky, L. *1892.* Ueber die Aufnahme lebender und todter verdanlicher Korper in die Plasmodia der Myxomyceten. Flora, *76:* 182-244.

Daniel, J. W. *1964.* The nutritional requirements of a myxomycete as a function of its developmental cycle in pure culture. Proc. Int. Bot. Cong.

Guttes, E., S. Guttes and H. P. Rusch *1961.* Morphological observations on growth and differentiation of *Physarum polycephalum* grown in pure culture. Devel. Biol. *3:* 588-614.

Indira, P. U. *1964.* Swarmer formation from plasmodia of myxomycetes. Trans. Brit. Mycol. Soc. *47:* 531-533.

Johansen, D. A. *1940.* Plant microtechnique. McGraw-Hill Inc., New York and London.

McManus, Sr. M. A. *1961a.* Culture of *Stemonitis fusca* on glass. Am. J. Bot. *48:* 582-588.

_____. *1961b.* Laboratory cultivation of *Clastoderma debaryanum.* Am. J. Bot. *48:* 884-888.

_____. *1962.* Some observations on plasmodia of the Trichiales. Mycologia *54:* 78-90.

_____. *1963.* The plasmodium of the myxomycete *Cribraria violacea.* Proc. Iowa Acad. Sci. *70:* 97-100.

_____. *1964.* The plasmodia of the myxomycetes *Licea biforis* and *Cribraria violacea.* Mycologia *56:* 237-239.

_____. and M. K. Taylor *1961.* Some observations on the plasmodia of Myxomycetes. Proc. Iowa Acad. Sci. *68:* 72-78.

Miller, C. O. *1899.* The aseptic cultivation of Mycetozoa. Q. J. Microsc. Sci. *41:* 43-71.

Ross, I. K., & R. J. Cummings, 1967. Formation of amoeboid cells from the plasmodium of a myxomycete. *Mycologia 59:* 725-732.

Sobels, J. C. and A. L. Cohen *1953.* The isolation and culture of opsimorphic organisms. II. Notes on isolation, purification and maintenance of myxomycete plasmodia. Ann. N.Y. Acad. Sci. *56:* 944-948.

Thom, C. and K. B. Raper *1930.* Myxamoebae in soil and decomposing crop residues. J. Wash. Acad. Sci. *20:* 362-370.

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