

FIBRILLAR DIFFERENTIATION IN MYXOMYCETE PLASMODIA

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ABSTRACT

Myxomycete plasmodia of four different types (not including *Physarum polycephalum*) were studied in thin sections viewed in the electron microscope. In the cytoplasm of the protoplasmodia of *Clastoderma debaryanum* and the phaneroplasmodia of *Fuligo septica* fixed *in situ*, fibrillar differentiations of three rather distinct kinds were observed. One of these is filamentous and closely resembles the filaments (or "microtubules") of the mitotic apparatus of other species. The larger phaneroplasmodia of two species belonging to the Physarales and the plasmodium of *Hemitrichia vesparium* showed fewer and less well defined fibrils, and no fibrils were seen in the aphanoplasmodium of *Stemonitis fusca*. Good stabilization of such fibrils in larger plasmodia may require fixation methods more rigidly controlled than those which succeed with microscopic protoplasmodia. The function of the observed fibrils cannot yet be determined. Their presence in cytoplasm fixed *in situ*, however, lends support to those theories of protoplasmic movement which are dependent on integral cross-bonding of one or a few molecular species.

The rhythmically reversing flow of the protoplasmic streaming in myxomycete plasmodia has been much studied, but never satisfactorily explained. Delimited only by the plasma membrane, the protoplasmic mass has no constant shape. In the species most studied, *Physarum polycephalum*, it is sometimes stretched out into a thin network of veins and at other times heaped together in an irregular bulbous mass. When migrating, the plasmodium has a constantly changing pattern in which the protoplasm flows in veins in the rear and as a flattened fan-like sheet in the advancing portion (Fig. 1).

Because movement is one of the most dramatic signs of the presence of life, interest in protoplasmic movements has a long history. The earliest studies were of ameboid movement, and all explanations invoked contraction of the ectoplasmic gel as the motive force (*e.g.*, 18, 34, 43). Ameboid movement has been explained in theories involving sol-gel in-

teractions, surface tension, elasticity of ectoplasm, solute drag force, syneresis, and others. Allen's "contraction-at-a-bend" theory (2, 3) and the posterior contraction theory of Goldacre (11, 12) based on the folding and unfolding of protein molecules are much discussed currently. A great variety of other theories include the tube-wall contraction theory (33), electrophoresis (7), gelation-syneresis (72), surface membrane formation (6), posterior-contraction hydraulic-pressure (47), sol-gel change (28), and jet propulsion (26). For movements in other types of cells, active shearing at sol-gel interfaces (24, 25) and pitch change in protein helices (22) have been proposed.

Is there one basic mechanism which is common to all types of protoplasmic movements? If so, does it depend on "contraction"? Many modern theories require contraction of some kind, and there are constant attempts to find a correlation between movements in cells and the presumably

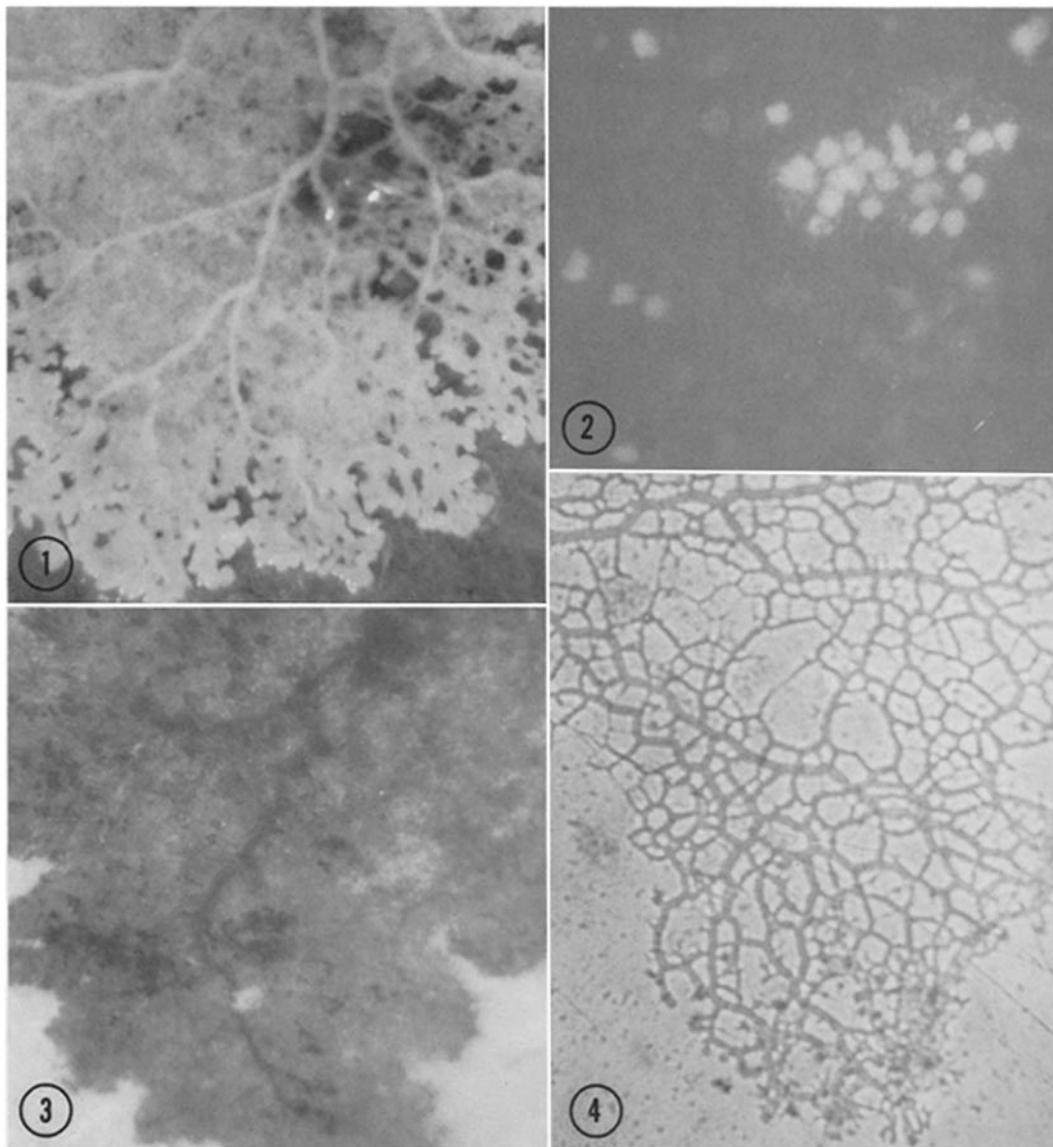


FIGURE 1 Phaneroplasmodium of the type found in *Physarum polycephalum* and *Didymium clavus*. $\times 49$.

FIGURE 2 Numerous small white protoplasmodia of *Clastoderma debaryanum*. $\times 49$.

FIGURE 3 The black plasmodium of *Hemitrichia vesparium*. $\times 49$.

FIGURE 4 The aphanoplasmodium of *Stemonitis fusca*. $\times 12$.

better understood movements in striated muscle (4, 6).

Many of the various theories which have been proposed to explain protoplasmic streaming in general have been applied to the shuttle type char-

acteristic of *P. polycephalum*: contractile gel (24, 25), Rashevsky's diffusion drag force (63, 64), and others (65); however, no convincing evidence for any of these has been advanced.

In 1952, Loewy (30) extracted from the proto-

plasm of the plasmodium of *P. polycephalum* a protein that behaved like muscle myosin B, and Ts'o *et al.* (68) later named it "myxomyosin." A contractile protein isolated from the same source by Nakajima (39) has slightly different physicochemical properties; he has called it "plasmodial myosin B."

Hatano and Takeuchi (15) found that the plasmodium contains ATP in amounts which change with the generation of motive force, and Kamiya (24, 25) and his group showed that the ATP used in producing the protoplasmic streaming is generated anaerobically in the ground plasm. Morphological evidence which could explain the mechanochemical coupling necessary for protoplasmic movement has, therefore, been sought, and the finding by Wohlfarth-Bottermann (70) of fibrils (which he believes are contractile) in the cytoplasm of the plasmodium of *P. polycephalum* has been of great interest.

The purpose of the present investigation was to examine species of myxomycetes other than *P. polycephalum* for evidence of such fibrils and to test on this type of material methods which have been found successful in stabilizing filaments of the mitotic apparatus in other species (*e.g.*, 13, 29, 50-56). Other observations of the protoplasm have also been made and are presented elsewhere (38).

MATERIALS AND METHODS

Plasmodia were grown on 1.5 per cent Difco corn meal agar. Four types of plasmodia were used: the gray-white phaneroplasmodium of *Didymium clavus* (Fig. 1); an unidentified yellow phaneroplasmodium; the yellow phaneroplasmodium of *Fuligo septica*; the transparent aphanoplasmodium of *Stemonitis fusca* (Fig. 4); the microscopic protoplasm of *Clastoderma debaryanum* (Fig. 2); and the intermediate type black plasmodium of *Hemitrichia vesparium* (Fig. 3).

In the first series, whole plasmodia of *C. debaryanum* and portions from the advancing fan and from the veins in the rear of the other types of plasmodia were cut on blocks from the agar surface, placed in Stender dishes, and covered immediately with 2.5 per cent glutaraldehyde buffered at pH 7.6 with veronal-acetate. After 40 minutes, the specimens were washed with buffer for 2 minutes and postfixated with similarly buffered 1 per cent osmium tetroxide for 1 hour. They were dehydrated through a graded series of ethanols (50 per cent, 75 per cent, 95 per cent, and absolute), embedded in dried 3:2 butyl-ethyl methacrylate containing 1 per cent benzoyl peroxide, and polymerized over night at 60°C. When necessary, trimming was done in 75 per cent ethanol. Sections 40 μ thick

were cut with freshly broken glass knives on a Porter-Blum microtome and stained for 1 hour with 1 per cent potassium permanganate. They were examined in an RCA EMU 3F microscope and photographed at 100 kv.

A number of other fixation methods were tried with mature and young plasmodia of *C. debaryanum*. One per cent osmium tetroxide was used alone or with added calcium, barium, or calcium and magnesium together, each additive as the chloride and at 0.002 M final dilution. These solutions were buffered at pH 7.6 with veronal-acetate. One per cent osmium tetroxide buffered at pH 7.0 with sodium phosphate was also used for some fixations. In the first series, a total of thirty-five fixations (of an average of 10 specimens each) was carried out and examined.

In a second series, plasmodia of *H. vesparium* and *F. septica* growing on paper toweling in Petri dishes were fixed whole in the Petri dish by flooding with 1 per cent OsO₄ buffered at pH 6.0 with veronal-acetate. The procedure used was otherwise the same as that described above, and parts were cut from the plasmodia for sectioning when they had been carried to 70 per cent ethanol.

A third series of plasmodia of *C. debaryanum* and small plasmodia of *H. vesparium* and *F. septica* growing on coverslips were fixed by drawing off as much fluid as possible without causing drying of the plasmodium and placing the coverslip, plasmodium down, over 2 per cent OsO₄ for 10 minutes (23).

OBSERVATIONS

Grossly, the plasmodia of *Clastoderma debaryanum*, *Stemonitis fusca*, and *Hemitrichia vesparium* differ markedly from the much studied yellow phaneroplasmodium of *Physarum polycephalum*, and the phaneroplasmodium of *Didymium clavus* (Fig. 1) differs at least in the pigment content. The protoplasm of *C. debaryanum* (Fig. 2) remains nearly spherical and microscopic until fruiting and shows only intermittent and irregular protoplasmic streaming. This plasmodium never stretches out into a network of veins (36). Streaming in other plasmodia is of the vigorous shuttle type considered typical for myxomycetes (1, 35-37). In *H. vesparium* (Fig. 3) there is rapid streaming which reverses its direction of flow at regular intervals, and in *D. clavus* and *F. septica* there are rather heavy veins in which the endoplasm flows within an encircling layer of gelled protoplasm. In the aphanoplasmodium of *S. fusca* (Fig. 4) many of the veins run parallel to each other, and the outer encircling layer of non-flowing protoplasm is very much reduced or absent. The protoplasm of *S. fusca* is so transparent as to be almost invisible,

macroscopically, in contrast to the jet black color of *H. vesparium* and the gray-white color of *D. clavus*. The plasmodium of *F. septica* is grossly rather similar to the yellow phaneroplasmodium of *P. polycephalum*.

Electron micrographs of *C. debaryanum* protoplasmodia and of *F. septica* phaneroplasmodia showed a range of cytoplasmic fibrillar structures which we have placed in three categories. (1) Fibrils of the type described by Wohlfarth-Bottermann (70, 71) in *P. polycephalum* (Figs. 5 and 7, *F*). These varied in width and with various fixation methods appeared sometimes rather coarsely granular and sometimes more homogeneous and dense. The ends of the fibrils could not be identified, and they sometimes divided dichotomously, as noted by Wohlfarth-Bottermann (70). Some were adjacent to elongate vacuoles (Fig. 7), but the association was not invariable. At times, the fibrils (Fig. 8, *F*) lay beneath and parallel to the plasma membrane (Fig. 8, *P*) but were never observed to insert upon it. (2) Fibrils similar in density to those just described and in similar locations but having a regularly repeating linear substructural unit (Fig. 6, *S*). These units are about 5 m μ in diameter, are closely packed, and are arranged in parallel about 5 m μ apart. (3) Fibrils composed of filaments (Figs. 9 and 10) similar to those of the mitotic apparatus in *Pelomyxa* (Fig. 11, *F*) (53) and other cells (*e.g.*, 13, 14, 16, 29, 60). Each filament appears as two dense parallel lines separated by a less dense region when observed in longitudinal section; the whole measures about 15 m μ across. The filaments often occur in bundles and are usually spaced 10 to 25 m μ apart. No limiting structure bounds or separates the fibrils from the surrounding cytoplasm. Their terminations are not obvious, and they are not all of the same length as seen in sections. Usually they are straight, but oc-

asionally they curve and sometimes form such figures as the U-loop seen in Fig. 13.

Table I shows the occurrence of these fibrils and filaments in the various plasmodia with the several fixation methods as described.

In *C. debaryanum* the fibrils were most clearly visualized when osmium tetroxide was used as the fixative at pH 7.0 (Figs. 5 and 8), and the filaments were optimally shown when both calcium and magnesium were added to the osmium tetroxide fixative (Figs. 9 and 10). Filaments were also well shown with osmium tetroxide vapor fixation (Fig. 13). Fibrillar differentiation was excellent in *F. septica* fixed in osmium tetroxide at pH 6.0 (Fig. 6); in *H. vesparium* (Fig. 12) and in *D. clavus* and the yellow phaneroplasmodium the differentiation was less pronounced, and no fibrils of any kind could be found in *S. fusca* fixed with osmium tetroxide alone at pH 7.6.

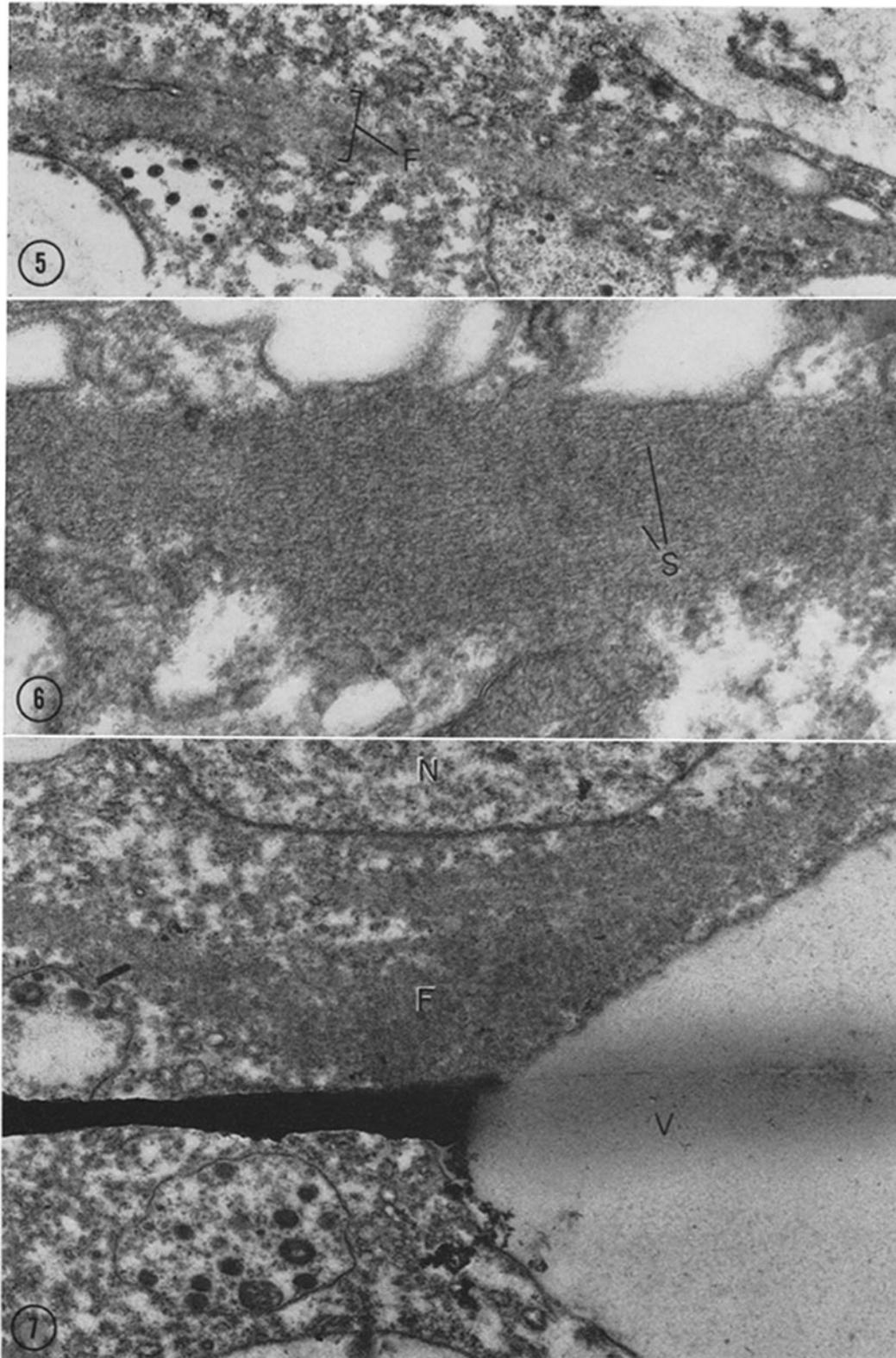
Fibrils that are of the type illustrated by Wohlfarth-Bottermann (70, 71) were found frequently in our specimens, but not always in the locations that he described as the usual ones. They were seen peripherally, were not observed to insert on the plasma membrane, and were sometimes, but not always, found close to elongated vacuoles. It is noteworthy that they were most numerous and well defined in the species in which protoplasmic streaming is most sluggish, *C. debaryanum*. However, this plasmodium is much smaller than that of the other species used. In fact, the entire plasmodium probably corresponds well to the droplets of protoplasm expressed from the veins of *P. polycephalum* which Wohlfarth-Bottermann used for many of his observations (70, 71).

The filaments (or "microtubules") which were seen in our material and which resemble filaments of the mitotic apparatus (13, 29, 50-56) had no obvious or constant relationship to other organelles or inclusions. The adjacent cytoplasm showed no

FIGURE 5 An electron micrograph of a fibril of type I (*F*) in cytoplasm of *C. debaryanum* fixed in osmium tetroxide at pH 7.0. $\times 22,000$.

FIGURE 6 A fibril from *Fuligo septica* fixed in osmium tetroxide at pH 6.0. This fibril has subunits (*S*) rather regularly arranged and is termed type II. $\times 37,000$.

FIGURE 7 Fibril from *C. debaryanum* fixed in osmium tetroxide at pH 7.6 with barium chloride added. Fibrils (*F*) were sometimes but not always found close to vacuoles (*V*). A portion of a nucleus (*N*) is included $\times 26,500$.



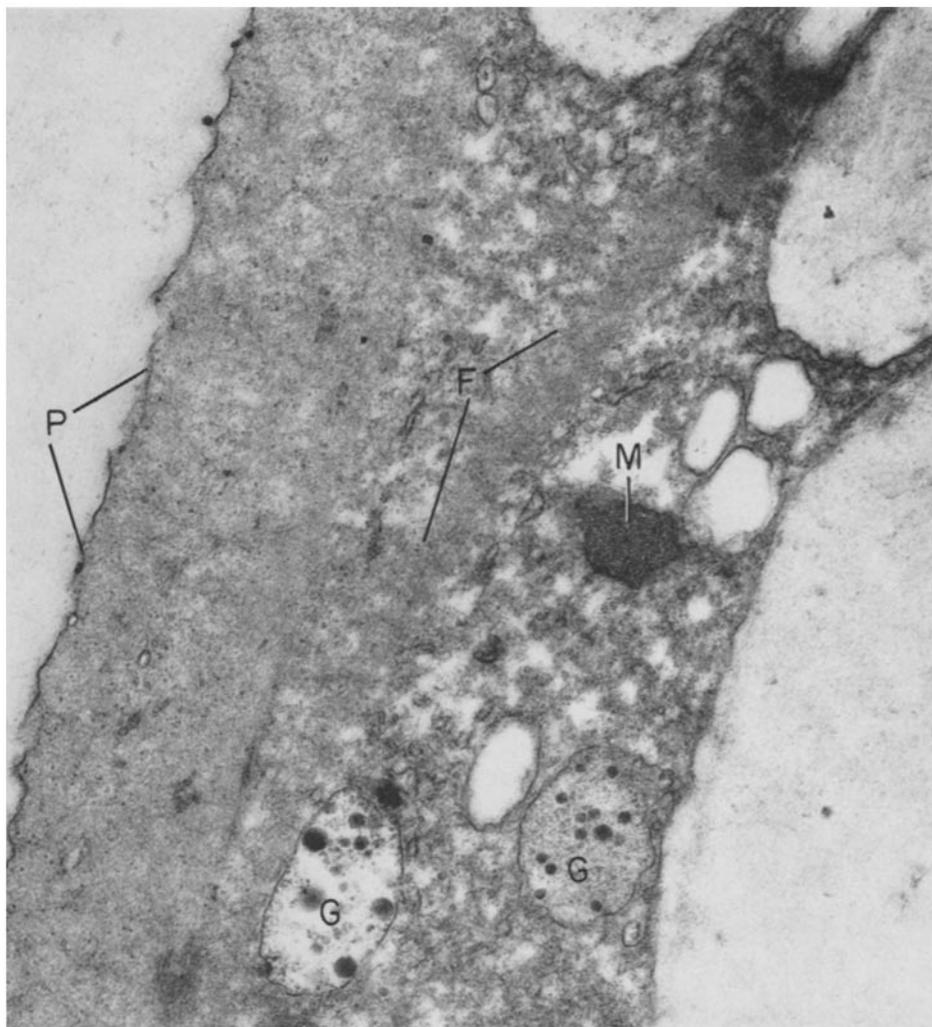


FIGURE 8 In *C. debaryanum*, fibrils (*F*) are sometimes observed lying parallel to the plasma membrane (*P*). An unidentified granular vacuole (*G*) is frequently seen in this species; a mitochondrion (*M*) is also included. Fixation in osmium tetroxide at pH 7.0. $\times 30,000$.

evidence of tension nor any obvious difference in orientation of structures lying close to or at the ends of the filaments. No recently divided nuclei were seen near them, and no nuclei in the process of mitosis were ever observed.

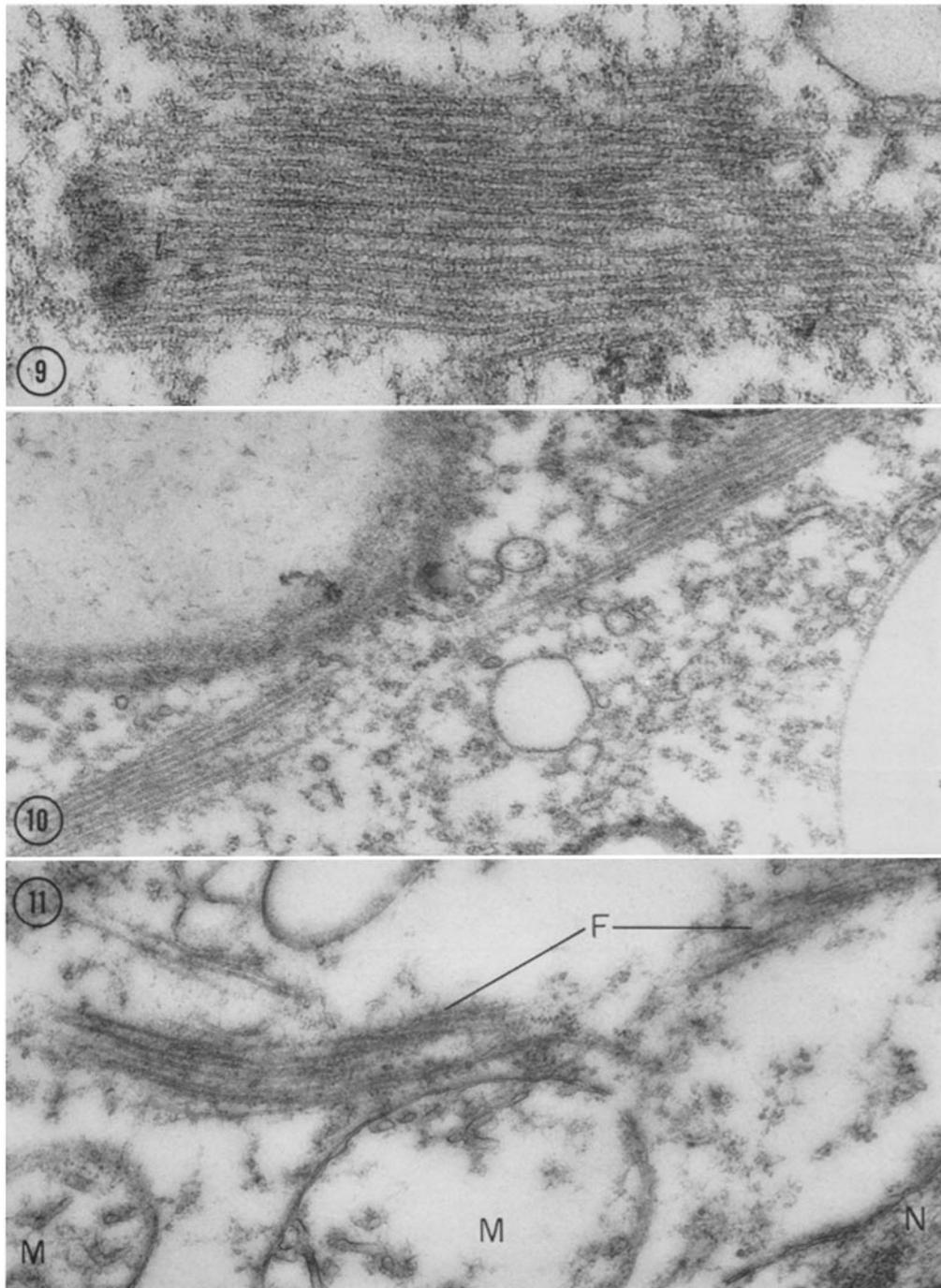
DISCUSSION

Contractile Component and Fibrils in Myxomycete Plasmodia

The myxomyosin extracted from the plasmodium of *P. polycephalum* by Loewy (30) has con-

tractile and other properties similar to those of actomyosin, and plasmodial myosin B, isolated by Nakajima (39), has slightly different physicochemical properties and consists of units 6 to 8 $m\mu$ in diameter.

In suspensions prepared from the plasmodium of *P. polycephalum*, Sponsler and Bath (61, 62) found long fibrils 50 to 60 $m\mu$ in diameter which they suggested might be related to myxomyosin. In a later study, Dugas and Bath (10) mention the question of the presence of fibrils, but in discussing their electron microscopic observations on sec-



FIGURES 9 AND 10 Filament bundles from *C. debaryanum* fixed in osmium tetroxide with added calcium and magnesium chlorides. Under these conditions, well defined filaments are observed and are separated from each other by distances about equal to their diameter. Fig. 9, $\times 47,000$; Fig. 10, $\times 36,000$.

FIGURE 11 Filaments (*F*) in the interzone of late anaphase in mitosis in the giant amoeba, *Pelomyxa carolinensis*. The morphology and arrangement of filaments is quite similar to that shown in Figs. 9 and 10 above. A portion of the nucleus (*N*) and two mitochondria (*M*) are included. Fixation in osmium tetroxide at pH 7.6 with calcium chloride. $\times 31,000$.

tions of the plasmodium of *P. polycephalum* they do not refer to fibrils.

Others who have studied protein extracts of the plasmodium of this same species have reported network arrangements or rigid rods (68), but Stewart and Stewart (63) could find no such structures in sections of plasmodia fixed from the living state. They suggest that, since fibril formation often occurs as a *post mortem* change, these fibrils might not be present *in vivo*.

responsible for streaming. He suggests that the fibrils are not the only structures responsible for contraction and assumes that the ectoplasmic gel layer of the veins can and does contract. He says that these contractions produce the motive force for streaming and that both the gel layer and the fibrils are composed of the same substance as the ground cytoplasm.

Nakajima (40) has found birefringent fibrillar structures in living plasmodia in which there was

TABLE I

Fibrils and Filaments Seen in Myxomycete Plasmodia Following Fixation by Various Methods

All fixations were at pH 7.6 unless indicated otherwise.

Species	Fixative	Fibrils		Filaments
		Type 1	Type 2	
<i>C. debaryanum</i>	OsO ₄	+	A	—
<i>C. debaryanum</i>	OsO ₄ pH 7.0	+	+	—
<i>C. debaryanum</i>	Glutaraldehyde + OsO ₄	A	—	—
<i>C. debaryanum</i>	OsO ₄ + Ca	—	—	—
<i>C. debaryanum</i>	OsO ₄ + Ba	+	+	+
<i>C. debaryanum</i>	OsO ₄ + Ca + Mg	—	A	A
<i>C. debaryanum</i>	OsO ₄ Vapor	+	+	+
<i>C. debaryanum</i> (young plasmodia)	OsO ₄ + Ca	+	A	+
<i>C. debaryanum</i> (young plas.)	OsO ₄ + Ba	—	—	+
<i>C. debaryanum</i> (young plas.)	OsO ₄ + Ca + Mg	+	—	+
<i>H. vesparium</i>	Glutaraldehyde + OsO ₄	+	—	+
<i>H. vesparium</i>	OsO ₄ Vapor	+	+	—
<i>D. clavus</i>	Glutaraldehyde + OsO ₄	+	—	+
<i>F. septica</i>	OsO ₄ Vapor	+	+	—
<i>F. septica</i>	OsO ₄ pH 6.0	+	+	+
<i>S. fusca</i>	OsO ₄	+	—	—
Yellow phaneroplasmodium	OsO ₄ + Ca + Mg	+	—	A

+ = typical, — = none seen, A = found, but somewhat atypical.

Wohlfarth-Bottermann (70) in 1962 published electron micrographs of sections showing fibrillar differentiation in protoplasm expressed as droplets from the veins of the plasmodium of *P. polycephalum* and fixed in 1 per cent osmium tetroxide plus 1 per cent potassium dichromate at pH 6.8–7.2 for 30 minutes. At time zero after release, fixed droplets showed no fibrils, whereas droplets fixed at intervals later showed increasing numbers of fibrils. Isolated veins also showed fibrils in the areas from which the protoplasm was flowing. He believes that this reconciles morphological findings with the physiological studies of Loewy (30). Kamiya (24, 25), and Ts'o *et al.* (68) and that the fibrils contract to produce a local pressure which is

active streaming, but these were also found in some plasmodia in which streaming could not be seen.

Our study demonstrates the presence of fibrils in plasmodia fixed *in situ* when suitable fixation conditions are used. The three different types of fibrillar differentiation seen in our material may represent the same type of structure differently affected by various fixations or in different physiological states at the time of fixation. Fibrils which might be designated as intermediate between the three types described (called atypical in Table I) were also found. Thus, a considerable morphological range of material that appears to be a cross-linked gel is present.

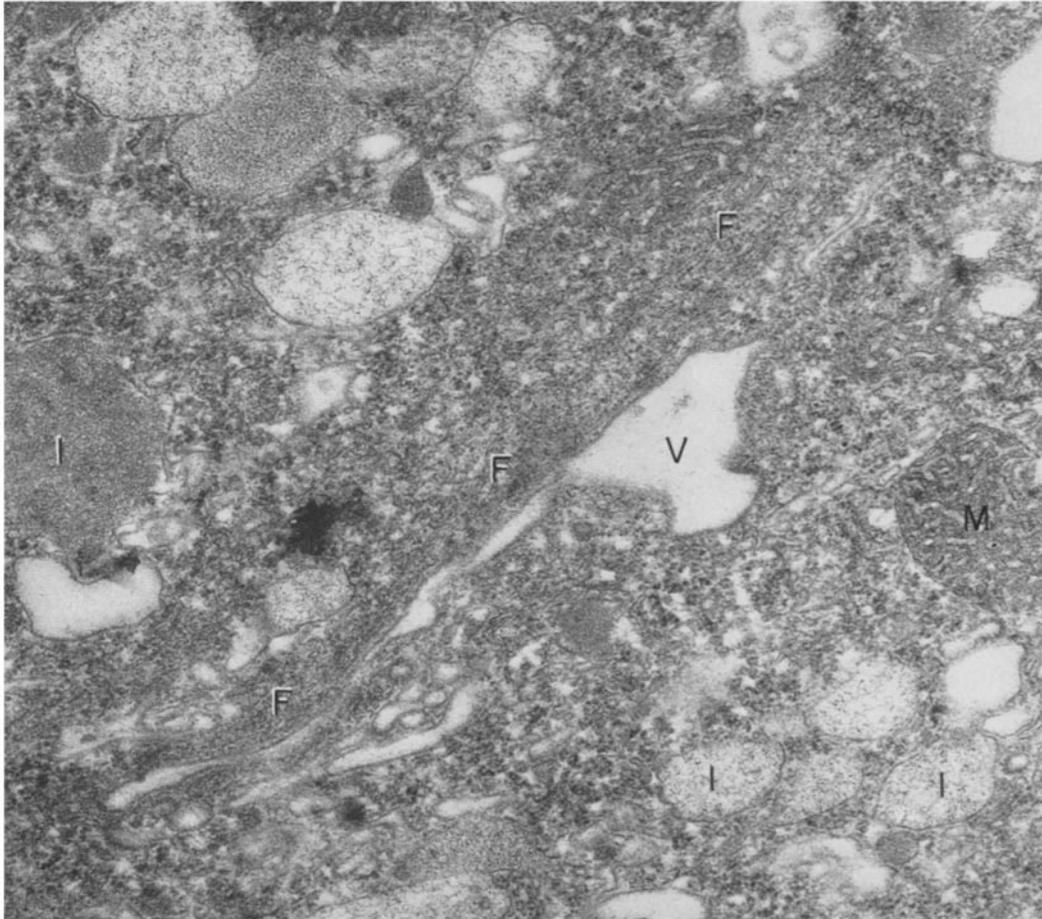


FIGURE 12 Fibril (F) beside an elongated vacuole (V) in *Hemitrichia vesparium*. A mitochondrion (M) and several inclusions (I) are also shown. Fixation in osmium tetroxide at pH 7.0. $\times 42,000$.

Excellent visualization of fibrils and filaments is achieved in the small plasmodia of *C. debaryanum*, but fewer fibrils are seen in large plasmodia. This difference may reflect the greater effectiveness of penetration of the fixative and hence a better stabilizing effect on subtle, transient structures. Fibrils are thus more easily shown in the microscopic protoplasmodia than in the larger types, even though they may be present in all. More favorable conditions of fixation, when found, may reveal still more fibrils.

Fibrils in Protoplasm of Other Species

Jarosch (21), Kamiya (25), and Kuroda (27) have found moving fibrils in protoplasm squeezed out of cells of *Nitella* or *Chara* and observed by light

microscopy. Kamiya (25) says that in a drop of cytoplasm these fine fibrils are being continually gathered and dispersed and that by ultramicroscopy they can be observed to consist of still finer fibrils that seem to be transient. Movements of these fibrils, according to Kamiya, appear to be associated with a mechanism of countershifting with the immediately adjacent milieu. He remarks that if such fibrils were arranged in parallel on the interior of the cortical protoplasm, a motive force of shifting could be generated along the interface, causing the endoplasm to flow.

In cultures of plant callus cells, Mahlberg (31) observed that at 10 days some cells had developed an extensive system of parallel membranes. These formed an anastomosing network which underwent

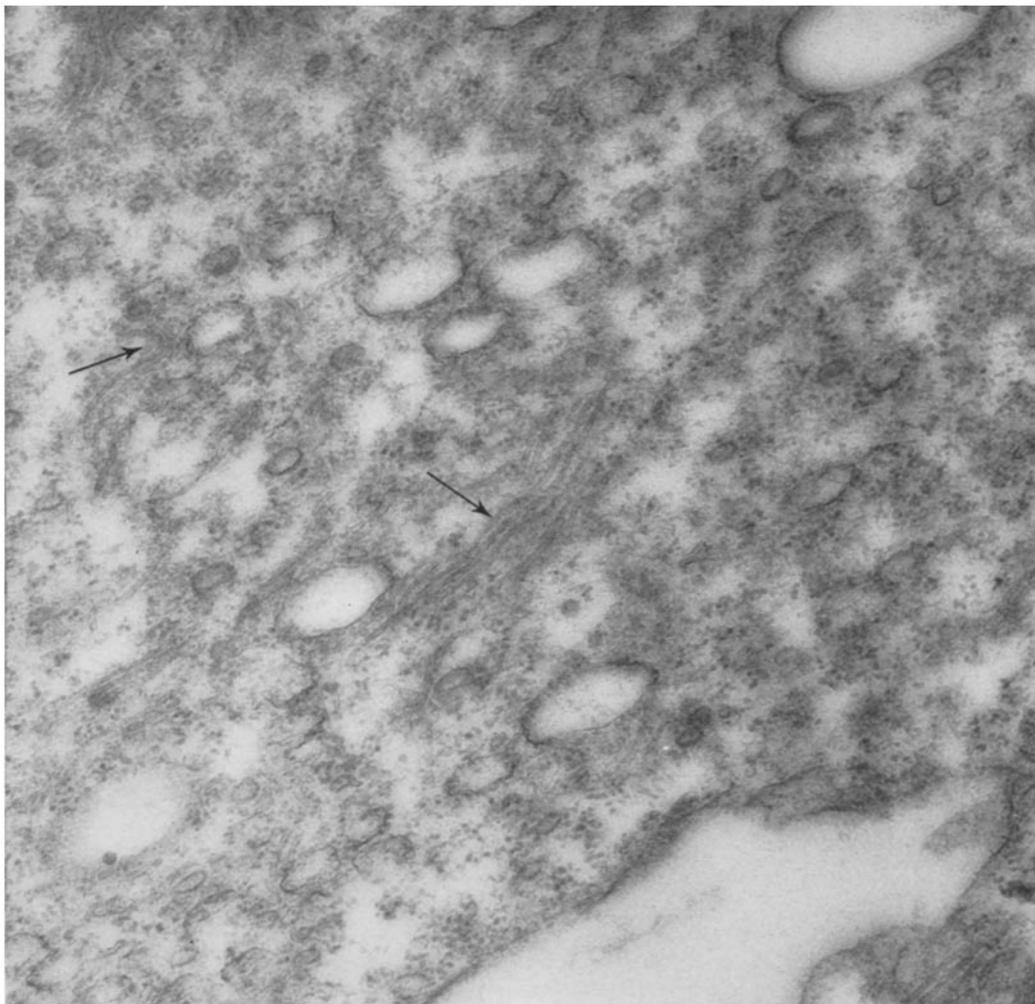


FIGURE 13 Filament (type III) groupings of less typical appearance in *C. debaryanum* show curves (arrows) and slightly non-parallel orientation. Fixation was in osmium tetroxide vapor, which allows a gradient of the fixation without addition of salts foreign to the culture. $\times 61,000$.

contortions during movement in the cells. The membranes developed dilatations and contracted to become tube-like and sometimes pieces were pulled off and became attached to other membranes. Mahlberg believes that these may be the same type of structures as the fibrils observed by Jarosch (21) which, however, do not appear until some time after cytoplasm has been expressed from the cell and are transitory.

In previous studies which have shown fibrils in amoebae (72) and in myxomycete plasmodia (61, 62, 70, 71), the protoplasm used was at least some-

what removed from its usual living state. In the present study, the fibrils seen in plasmodia of *C. debaryanum*, *F. septica*, and *H. vesparium* are in protoplasm that was undisturbed before fixation and was fixed under specified and varied conditions.

In a variety of cell types, fibrillar components, including those often referred to as "microtubules" because of their electron-transparent cores, are being reported with increasing frequency in various cytoplasmic locations. They have been observed apparently closely associated with developing primary (29) and secondary (16) cell walls in

plant cells, in intimate association with developing nematocysts in *Hydra* (60), in dendrite and axon terminals, and in cells of the nervous system of the rat (17, 42), the goldfish (41, 49), and the opossum (57), in germ cells of various species of animals and plants (8, 32, 45), in avian lung epithelium (69), and in frog epidermis (44). Roth (51) suggested the generalized occurrence of filaments in protozoa, and Pitelka (46) has recently reviewed this subject. Shalla (59) found, in tomato leaflet cells infected with tobacco mosaic virus, filaments which were four times the size of the mature virus; he interpreted them as a stage in the development of the virus. In his micrographs, these filaments appear morphologically quite similar to others mentioned here. In extracts from homogenized amoebae treated with ATP, Wolpert (72) found filaments 12 m μ thick and 0.5 μ long. If he further purified his extract and treated it with ATP, he found filaments 8 to 9 m μ thick.

It is a major contribution of this study to show that the filaments of the type III fibrils are closely related to those of the mitotic apparatus as regards both their morphology and their reaction to various fixation conditions. It should be noted, however, that no filaments were observed in some of the myxomycete plasmodia after some of the fixations that have successfully preserved filaments in the mitotic apparatus of certain species (13, 48, 53). In metazoan organs, demonstration of filaments in the mitotic apparatus is particularly difficult; preservation by adjustment of pH and divalent cations is unsuccessful, and only glutaraldehyde-osmium tetroxide methods have been useful thus far (9, 29, 55). The large volumes and the permeability characteristics of individual cells may inhibit a sufficiently rapid establishment of the desired intracellular fixation conditions. Thus, in older plasmodia of *C. debaryanum* are more impermeable than younger ones, an explanation could be given for some of the results, noted in Table I, for fixation conditions that did not preserve filaments.

As the number of studies utilizing appropriate methods of preservation increases, more fibrillar and filamentous organization is being demonstrated in movement systems. This study demonstrates the importance of rigid definition of fixation conditions in observations of streaming protoplasm and parallels similar demonstrations regarding the mitotic apparatus (13, 14, 23, 53, 54).

Possible Function of the Three Types of Fibrils Found in This Study

Wohlfarth-Bottermann (70, 71) believes that the fibrils found in plasmodia, which he calls "plasma filaments" and which resemble our fibrils of types I and II, are contractile. In myxomycete plasmodia he has found more fibrils in excised parts *from* which protoplasm is flowing than in parts *to* which it is flowing. Similarly, Allen *et al.* (5) found that more heat is generated at the point *from* which flow occurs than elsewhere in the plasmodium. In droplets which had flowed out after a vein was pricked, Wohlfarth-Bottermann (71) reported that fibrils form in an orientation that would justify the assumption that their contraction could force the protoplasm back into the vein. He believes that the fibrils are highly differentiated parts of the cytoplasmic matrix, as is the ectoplasmic network, and that both these structures contract to produce the reversible streaming in *Physarum polycephalum* plasmodia. Most of the fibrils shown in his micrographs of *P. polycephalum* are similar to those of type I found here in other species, and some of his micrographs show fibrils resembling our type II. He does not show filaments (our type III).

Palay (41, 42) observed filaments in axoplasm that are similar to our type III and suggests that they are differentiations of the endoplasmic reticulum. Similarly, Ruthmann (58) has speculated that the continuous filaments of the meiotic spindle in the crayfish may be endoplasmic reticulum, and Harris (14) reported that the asters in sea urchin embryos contain large amounts of endoplasmic reticulum. Slautterback (60) has suggested that two size categories of fibrils exist: (1) those with an outside diameter of 12 to 20 m μ , and (2) those with an outside diameter of 27 m μ . He speculates that the smaller ones function in synthetic or metabolic activities, and the larger ones, as elastic bodies. Involvement of filaments in coordination of ciliary beat has been strongly suggested by the merotomy studies of Taylor (67) on the protozoan, *Euplotes*; this subject was reviewed by Roth (50).

In our material, there is little evidence that any of the three types of fibrils seen insert on structures which could usefully be moved by them. A possible exception is the occasional presence of type I fibrils in contact with the membrane of a vacuole. The type III filaments or "microtubules" were often found in bundles and were usually straight, although occasional curvatures are seen.

The similarities demonstrated by this study between filaments in streaming cytoplasm and those in the mitotic apparatus draw attention to concepts presented from studies of mitosis. Inoué has studied the birefringence of fibers in mitosis (19, 20) and suggests that "spindle fibers are undoubtedly almost fluid in nature and are not stably cross-linked gels as the term 'fibers' may imply" (19). He further suggests that "as material is removed from the chromosomal fibers, . . . the fibers shorten in length to reach a new orientation equilibrium" (20). Electron microscope studies (52) suggest that the functional unit in the mitotic apparatus is a filament too small to be resolved in the above studies and that, since it probably does not change morphologically during mitosis, it could adequately produce contraction only by deletion of material or elongation by addition of material. The two types of studies together point toward mitotic movement being produced largely by formation or destruction of filaments that are com-

posed of subunits cross-linked in a precise and labile manner.

Functions currently attributed to cytoplasmic fibrillar structures include their contribution to structural rigidity, contractile movements, coordination of movements, and the piping of fluids. Explanations must be sought at the molecular level of function, and it is here that our level of understanding is lowest. The function of fibrillar material to which the present study lends support is circulation of materials within the cytoplasm by molecular mechanisms akin to those functioning in the mitotic apparatus.

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