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# Studies on Microplasmodia of *Physarum polycephalum*

## I. Classification and Locomotion Behavior

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**Summary.** Depending on the conditions of the axenic shuttle culture, microplasmodia of the acellular slime mold *Physarum polycephalum* can be subdivided into three classes regarding fine structural organization and protoplasmic streaming activity: (a) spherical and rod-shaped types, (b) ameboid types, and (c) symmetrical types.

In ameboid microplasmodia, the motive force for the irregular protoplasmic streaming activity is generated by alternative contraction and relaxation of a membrane-associated layer, morphologically consisting exclusively of thin filaments (probably actin). The protoplasm flows along a hydraulic pressure gradient produced by the filament layer within limited regions of the cell periphery. In dumbbell-shaped microplasmodia the motive force for the regular protoplasmic shuttle streaming between the two spherical heads is generated both by volume changes of the peripheral cell region (caused by the contractile activity of the membrane-associated filament layer), and by volume changes of the internal cell membrane invagination system (caused by fibrils attached to the basal region of the invaginations). The development from the unordered protoplasmic streaming pattern and less complicated fine structural organization in ameboid microplasmodia to the highly organized protoplasmic shuttle streaming and the more complicated morphology in dumbbell-shaped microplasmodia can be explained by intermediate stages. Whereas the motive force for the transport of smaller amounts of protoplasm can be generated by the exclusive action of a cortical filament layer, the existence of a filament cortex, the display of cytoplasmic fibrils, and the development of plasma

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membrane invaginations appear to be a necessary precondition for the transport of large amounts of protoplasm.

**Key words:** Microfilaments – Protoplasmic streaming – Microcinematography – Microplasmodia – *Physarum polycephalum.*

A special growing type of the acellular slime mold *Physarum polycephalum* is formed under conditions of axenic shuttle culture, in which the plasmodia are reduced to small protoplasmic fragments (50–400 µm) called "microplasmodia" (Daniel and Rush 1961). Because of their small size, microplasmodia proved to be suitable objects for in vivo experiments using high resolution light microscopy.

The present paper deals with the analysis of motile and contractile activities in two types of microplasmodia; an attempt was made to correlate these findings with the ultrastructural organization of the contractile apparatus. The observation of these two plasmodial types permitted investigation of (1) the formation of the plasmalemmal invagination system (Wohlfarth-Bottermann 1974), and (2) the differentiation of cytoplasmic actomyosin fibrils (Wohlfahrth-Bottermann 1962).

In recent investigations on endoplasmic drops, the transition between plasmodial stages that lack invaginations to those possessing them has been analysed (Götz von Olenhusen et al. 1979; Achenbach et al. 1979). The results of these papers show that the invaginations originate from fusion processes of membrane-vesicles among each other and with the plasmalemma. At the same time cytoplasmic actomyosin fibrils appear, which are in close contact with the invaginations, and are responsible for the motive force of the shuttle streaming of the protoplasm.

An invagination system and cytoplasmic fibrils are both observed in microplasmodia and are of essential importance also for the protoplasmic streaming of this growing type of *Physarum polycephalum*. However, the functional correlation between the two systems seems to differ in microplasmodia from the situation in macroplasmodia in some aspects. Furthermore, there exist microplasmodia lacking invaginations and distinct cytoplasmic actomyosin fibrils; these forms move like amebae (Wehland et al. 1978; Stockem et al. 1978; Wehland et al. 1979; Gawlitta et al. 1980). The transition between microplasmodia without invaginations and microsplasmodia with invaginations and cytoplasmic fibrils will be discussed in the present paper.

## Materials and Methods

*a) Culture*

Microplasmodia of the acellular slime mold *Physarum polycephalum* were grown in axenic stock cultures according to the method of Daniel and Rush (1961). The culture flasks were shuttled with a frequency of 1.5 Hz, i.e., 90 vibrations per minute. Different frequencies did not prove to be applicable. The age of the microplasmodia taken for the experiments ranged from 1 to 2 days.

*b) Light Microscopy*

The time of the single experiments did not exceed 1 h after taking the microplasmodia from the culture. Observations and microphotography were carried out with a Leitz-Ortholux and a Zeiss microscope using bright-field, phase-contrast and differential-interference contrast (DIK according to Nomarski)

techniques. Microcinematographical exposures were made with a Beaulieu R 16 or a Beaulieu-Euratom-Camera coupled with a Zeiss photomicroscope. Exposure speed was 2 exp/sec. Photographic material included (a) Ilford Pan-F and (b) Kodachrome 40, 16 mm.

To avoid crushing the cells, small observation-chambers were prepared by inserting plastic strips of 100 µm thickness between the microslide and the coverglass.

*c) Cinematographical Analysis*

Single-frame analysis (Kuhl 1953) of the movies was performed with a photo-optical-data-analyzer (LW-International). The interpretations were carried out under three different points of view:

The *first series of experiments* centered on the changes in outline of cells in correlation to the streaming pattern of the protoplasm (see Stockem et al. 1969). Diagrams were drawn from the cells at the points of reversal of the streaming protoplasm. Outline changes were made visible by superimposing one diagram upon another in sequence (Figs. 2b, 4b).

The *second series* dealt with the quantification of these changes in outline. The diagrams were subdivided in areas of contour-loss and contour-gain and surveyed. The data observed from all corresponding areas in each single cell were added, thus indicating the contour-loss or contour-gain in correlation with the direction of streaming of the protoplasm.

The *third series* of experiments utilized dumbbell-shaped microplasmodia only (Fig. 1h). According to a simple method described elsewhere (Gawlitta 1977), the planimetric values of changes in outline in the diagrams were transformed to volume units. The aim of this investigation was to correlate volume changes in the spherical heads with the quantity of protoplasm that is transported through the connecting tube during one period of streaming. The volume of the transported protoplasm was measured by the assumption that the connecting tube can be seen as a cylinder. Streaming protoplasmic particles that could be followed within the connecting tube made it possible to survey the volume of transported protoplasm: The cross section of the connecting tube was multiplied by the distance that the particles traversed. The velocity of particles and protoplasm was assumed to be equal. Only the inner part of the cylinder, where the velocity of all particles was nearly the same, was considered. Therefore, the actual transported volume was always greater than the measured values. However, this error did not falsify the results.

*d) Electron Microscopy*

For *scanning electron microscopy* (*SEM*) microplasmodia were frozen in liquid propane without previous chemical fixation followed by freeze-drying in a Leybold-Heraeus GT-1 apparatus (Klein and Stockem 1976). Subsequently, ion-etching in a dc-sputter-instrument and gold-coating in the same apparatus were performed (Kontron LWU-type Hummer; technical parameters for both etching and coating: 3 min, 10–12 mA, 1 kV, 130–150 mTorr). The specimens were photographed on a Philips 300 electron microscope with scanning equipment. A Leica-camera and Ilford Pan-F films were used (Fig. 5b).

For *transmission electron microscopy* (*TEM*) microplasmodia were fixed with 2.5% glutaraldehyde/0.01 M spermidine phosphate in 0.05 M s-collidine buffer (pH 7.2) according to Hauser (1978), postfixed in 1% $OsO_4$ and embedded in a low-viscosity epoxy resin (Spurr 1969) (Figs. 2a, 4a). Other cells were fixed simultaneously with glutaraldehyde/$OsO_4$ (Danneel and Weissenfels 1965; Daniel and Järlfors 1972), buffered with 0.05 M cacodylate (pH 7.2), followed by embedding in styrene methacrylate (Figs. 3, 6). Before fixation some microplasmodia were incubated in 0.5 mM DNP (dinitrophenol) or in 0.5 mM NEM (n-ethylmaleimide) for 10 min.

Thin sections prepared with an LKB Ultrotome III were observed in a Philips 301 electron microscope after poststaining with lead citrate (Venable and Coggeshall 1965).

## Results

### *Terminology of Microplasmodia*

As microplasmodia are of different shapes and show different characteristic patterns of protoplasmic streaming, it is useful to separate them into three groups:

1) Spherical- (100–150 µm in diameter) and rod-shaped (10 µm in diameter, 50 µm in length) types, which show no or little protoplasmic streaming and appear sporadically (Fig. 1a, b)

2) Ameboid types with irregular shape (50–100 µm in diameter) and streaming pattern. These microplasmodia are rarely found (Fig. 1d, e)

3) Symmetrical microplasmodia (200–400 µm in length), which exhibit regular shuttle streaming. These forms are the most frequent types found under the present culture conditions (Fig. 1g–l).

Intermediate stages between these three groups (Fig. 1c, f) and between macro- and microplasmodia (Fig. 1m) are also detectable.

The component level under which the single types appear in the stock is dependent on the shuttle frequency of the culture flasks. Under conditions of high shuttle frequencies (2.5 Hz = 150 vibrations per minute) nearly all microplasmodia were rounded up (Fig. 1b), whereas low frequencies (1 Hz = 60 vib./min) allow the microplasmodia to form small networks of fused plasmodia, even under submersed conditions. The more primordial types, which are described above, are rarely detected under these conditions.

The following description of fine structure and kinematics of moving activities are focussed on the ameboid and dumbbell-shaped types of microplasmodia.

### *a) Ameboid Type of Microplasmodia*

*Fine Structural Organization.* The cells are irregular in shape, display protuberant lobes, and are filled with numerous vacuoles (Fig. 2a). Plasmalemmal invaginations, described as typical constituents of the ectoplasm in protoplasmic veins of macroplasmodia (see Wohlfahrt-Bottermann 1974) appear very sporadically (Fig. 2a, *arrows*); however, ameboid microplasmodia form numerous broad, short plasma membrane indentations (Figs. 2a, 5d, *stars*). A continuous layer of thin filaments (5–6 nm in diameter) probably consisting of actin (Allera et al. 1971) is associated with the plasma membrane. Its thickness is approximately 0.2 µm (Fig. 3a, *cfl*). After treatment with DNP this filamentous cortex shows condensation (Fig. 3b, *cfl*), and after NEM treatment the filaments exhibit a parallel orientation (Fig. 3c, *cfl*).

*Kinematics of Motility.* The protoplasmic flow in ameboid microplasmodia shows irregular streaming patterns (Fig. 2b, *arrows*) causing constant changes in the contour of the cell (contour-gain is indicated by *black areas* in the diagrams, contour-loss is indicated by *white areas between thin and thick lines;* Fig. 2b).

The inflow of protoplasm into a certain cell region is correlated with contour-gain in this area in 79–84% of all investigated samples. The outflow of protoplasm from a certain cell region is correlated with contour-loss, to the same extent.

The cell membrane becomes folded during contour-loss (Fig. 2b; stages *5′24″* up to *6′25″*), whereas in regions of contour-gain the surface becomes smooth and occasionally assumes a balloon-like appearance (Fig. 2b; stages *4′1″* up to *5′24″*).

In spite of the marked variability in cell shape, ameboid microplasmodia show only negligible locomotor activity.

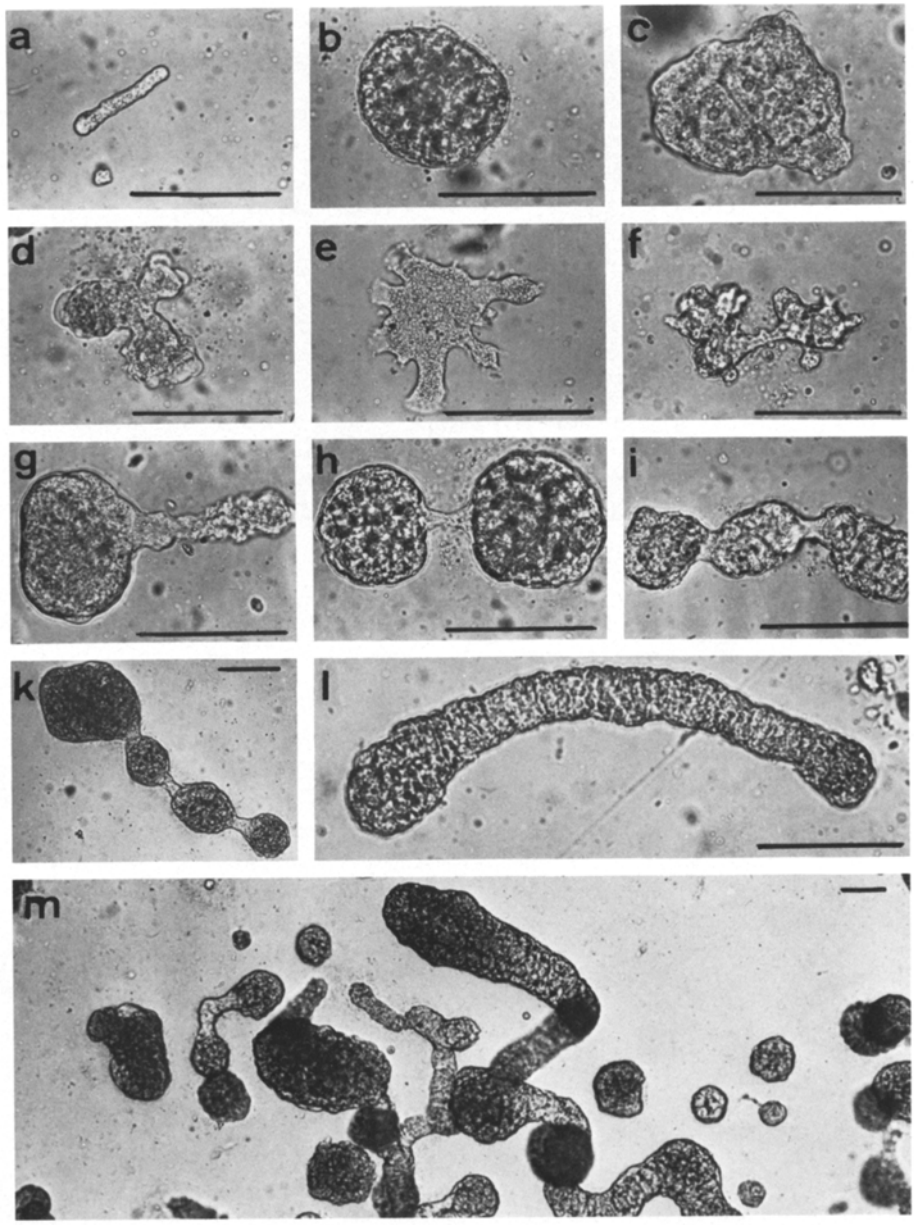


**Fig. 1 a–m.** Classification of microplasmodia of *Physarum polycephalum* in the bright-field microscope. **a** Rod type, **b** spherical type, **c** intermediate stage between spherical and ameboid types, **d** ameboid type with hyaline caps, **e** flattened ameboid type, **f** intermediate stage between ameboid and symmetrical shaped types, **g** club type, **h** dumbbell-shaped type, **i, k** chain type, **l** snake type, **m** survey micrograph of different types of microplasmodia in the submerged culture. Bars = 100 μm

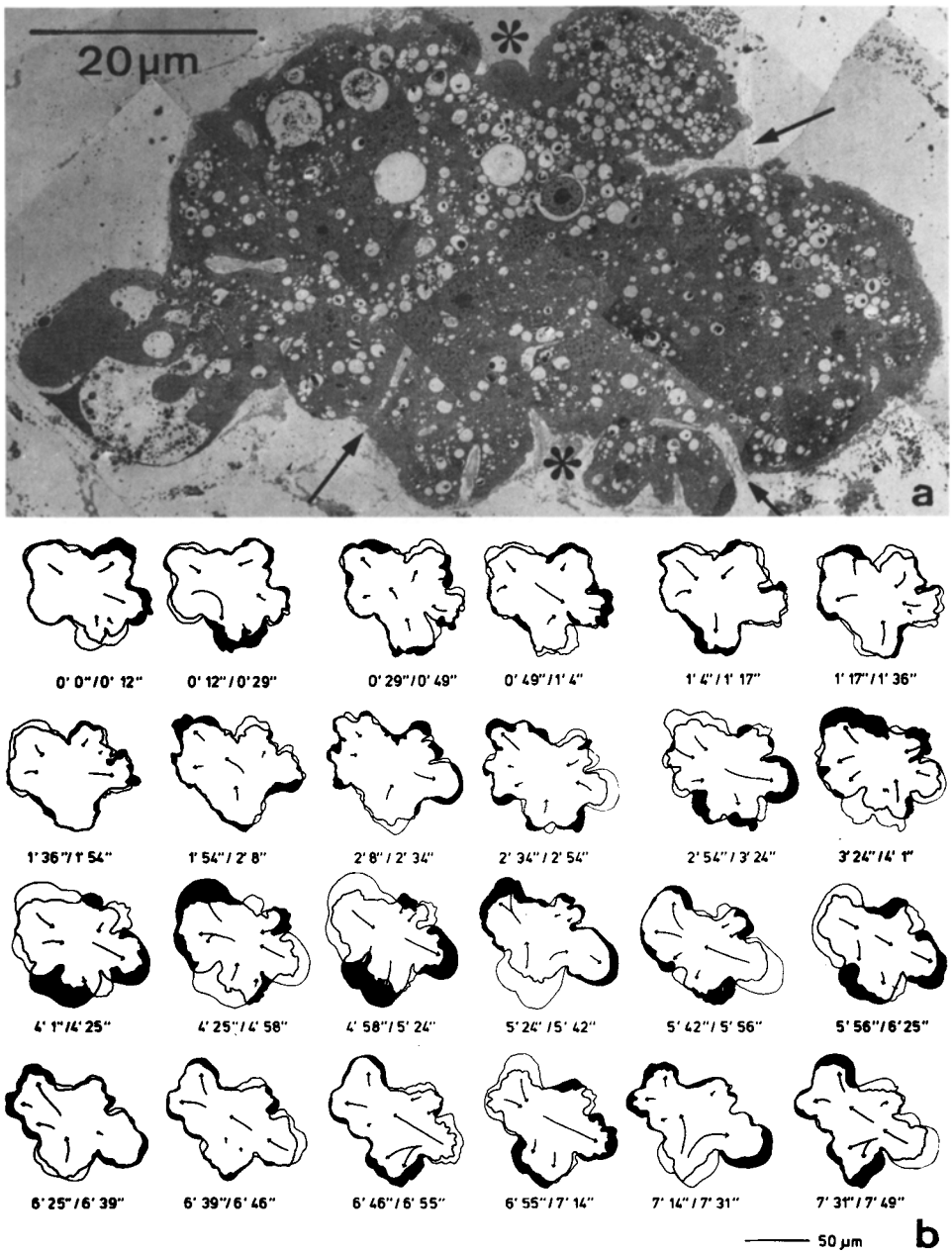


**Fig. 2a and b.** Ameboid microplasmodium. **a** Electron micrograph showing broad plasmalemmal indentations (*stars*), and short plasma membrane invaginations (*arrows*). In addition to common cell organelles (nuclei, mitochondria) the cytoplasm contains numerous vesicles. **b** Microcinematographic single frame analysis of the dynamic activity. Two subsequent pictures are superimposed upon another. *Black areas* indicate gain of contour and *white areas* between thin and thick outlines indicate loss of contour of the later stage. *Arrows* indicate irregular protoplasmic streaming. Numbers represent time course

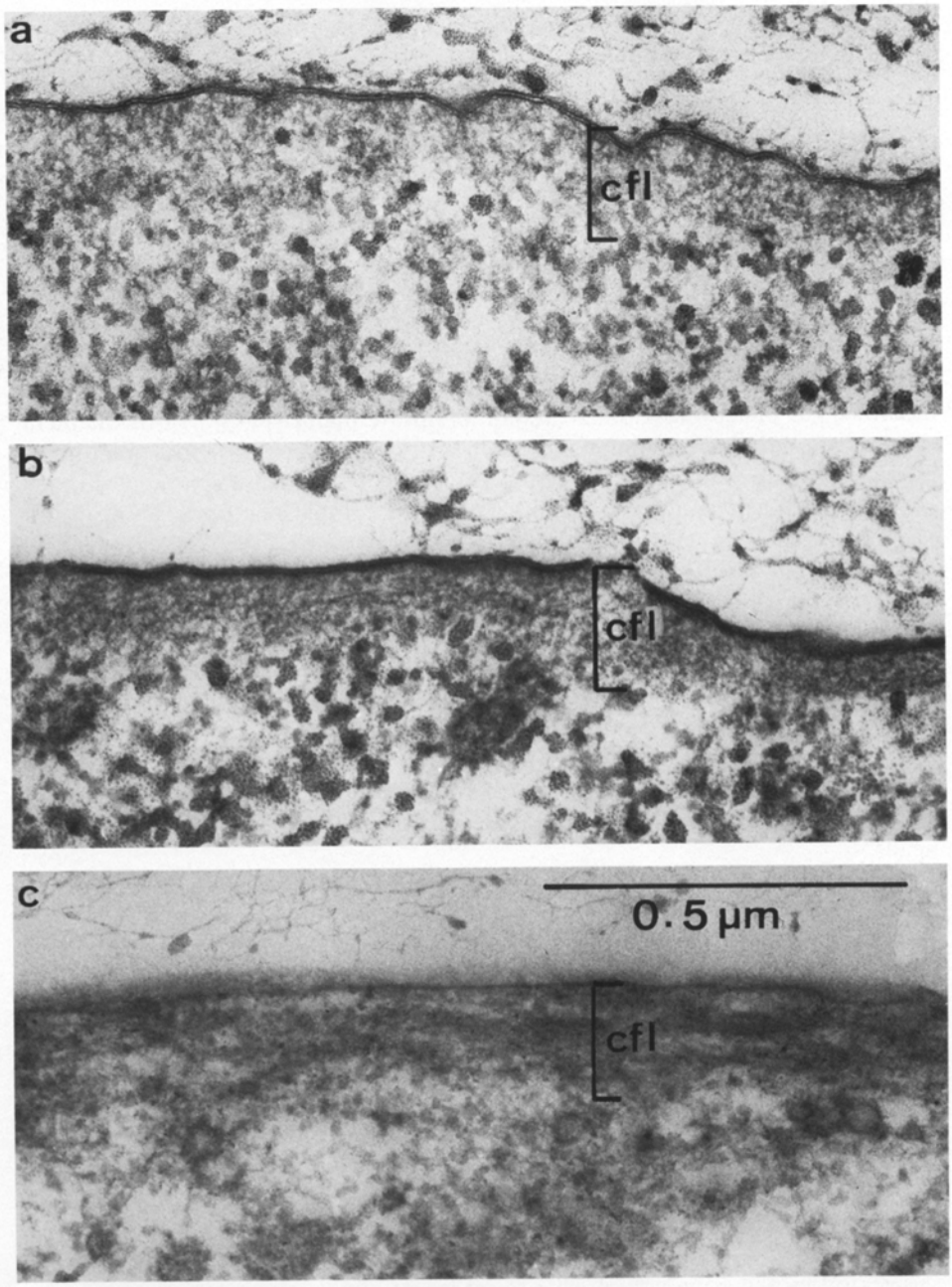


**Fig. 3a–c.** Fine structure of the filament cortex (*cfl*) of microplasmodia. **a** In untreated microplasmodia a loose network of thin filaments is found immediately beneath the plasmalemma. **b** In DNP-(dinitrophenol) treated microplasmodia the filament cortex (*cfl*) becomes more dense. **c** NEM-(N-ethylmaleimide) treatment induces parallel arrangement and aggregation of the filaments

*b) Dumbbell-shaped Type of Microplasmodia*

*Fine Structural Organization.* The dumbbell-shaped microplasmodium consists of two spheres of protoplasm (spherical heads) connected by a cylindrical tube (connecting tube) (Fig. 1h). The two spherical heads of each microplasmodium are traversed by an extended system of plasmalemmal invaginations, which are connected one with another showing differing diameters. Neither in living (Fig. 5a) nor in fixed microplasmodia of this type (Figs. 4a, 5b, c) a preferential orientation of the invaginations can be detected (*arrows*). Usually, the connecting tube is devoid of invaginations. Also in this type of microplasmodium there exists a filamentous cell cortex underlying the peripheral as well as the invaginated plasma membrane (Fig. 6a, *cfl*). In addition to this cortical layer, the cells possess a contractile fibrillar system anchored at the invaginations and running through the cytoplasm as described in macroplasmodia (Wohlfahrt-Bottermann 1975). Both filament systems are continuous. Similar to ameboid types, dumbbell-shaped microplasmodia react to DNP and NEM treatment by condensation and parallel arrangement of the cortical filaments and of the cytoplasmic fibrils (Fig. 6b, *fi*).

*Kinematics of Motility.* The protoplasmic shuttle streaming, as a typical phenomenon of macroplasmodia of *Physarum polycephalum*, is likewise a characteristic feature in dumbbell-shaped microplasmodia. This flowing mode is readily detectable in the connecting tube and results from alternative in- and efflux of protoplasm of the spherical heads (Fig. 4b): The alterations in contour of the cell surface that occur during one half period of the oscillation of the protoplasmic flow are much better correlated (90–98%) with the direction of protoplasmic streaming than in ameboid types (79–84%): Outflow of protoplasm results in decreased volume and inflow results in increased volume of the corresponding spherical heads.

As it is impossible to fix the microplasmodia in one position during the observation period, they are able to rotate around the longitudinal axis. Thus, the changes in contour in one plane (according to the two dimensional projection) are overlapped by contour changes resulting from this rotatory movement (Fig. 4b, stages *0'0"/0'25", 1'34"/2'8", 2'40"/3'17", 3'17"/3'49", 4'12"/4'37", 4'37"/5'4", 5'4"/5'33", 5'55"/6'11"*). In quantitative analyses these rotations were not considered.

Further kinematographic investigations were performed to answer the question whether the amount of volume changes in spherical heads, caused by alterations of the cell periphery, coincides with the quantity of the transported protoplasm.

In a theoretical hydraulic dumbbell-shaped system (Fig. 7a'), the total quantity of transported liquid during one halfperiod of the shuttle streaming has exactly the same volume as that which one sphere loses. The opposite sphere increases during this period by the same volume due to inflow of liquid. The same relations exist during reversals of the streaming direction.

However, microplasmodial behavior is not in accord with these simple theoretical considerations. Thus, the measured volume changes of the spherical heads (Fig. 7a, $I_r$, $I_l$, *solid line*) are always smaller than the transported volume of protoplasm (Fig. 7a, $I_r$, $I_l$, *thin line*). Even more striking inconsistencies of the

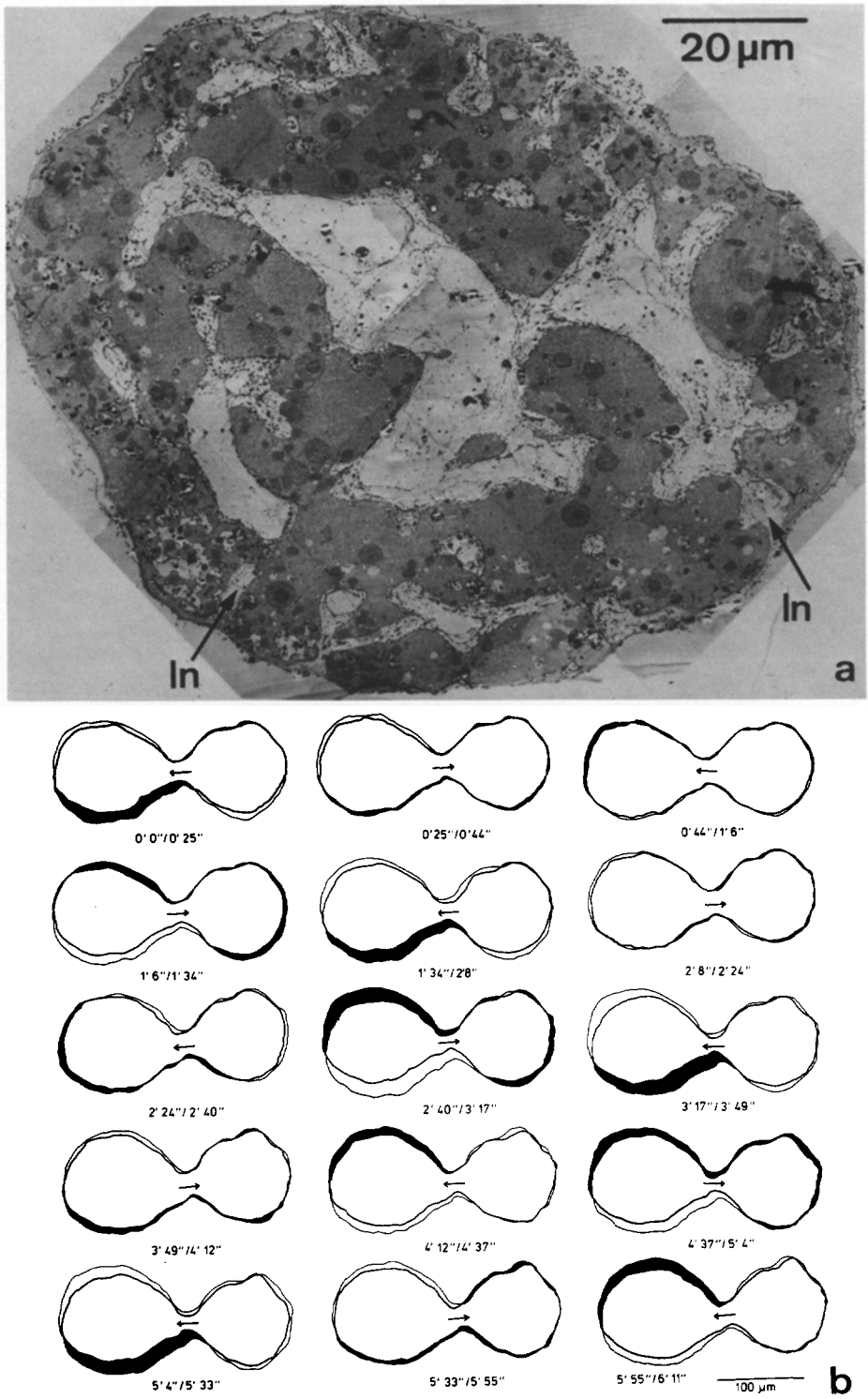


**Fig. 4a and b.** Dumbbell-shaped microplasmodium. **a** Electron micrograph of an ultrathin cross section through a spherical head. An extensive plasmalemma invagination system is formed, beginning at the surface (*arrows, In:* invagination) and running through the entire cytoplasm. The slime was stained with lanthanum hydroxide during fixation (Kogon and Pappas 1975). **b** Microcinematographic single frame analysis of the dynamic activity. The explanation of symbols is the same as in Fig. 2b. Note the regular shuttle streaming (*arrows*) from one spherical head to the other

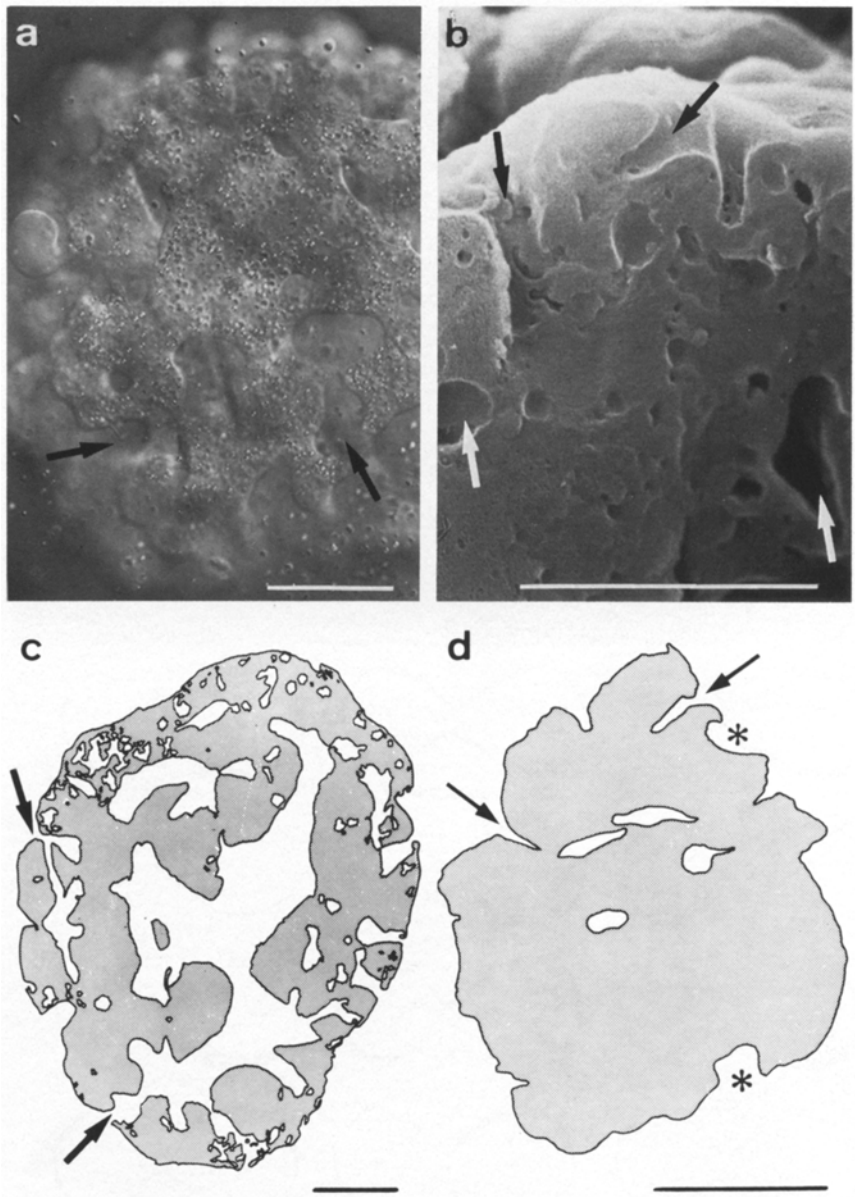


**Fig. 5a–d.** Plasma membrane differentiations of dumbbell-shaped (**a, b, c**) and ameboid microplasmodia (**d**). **a** The differential interference-contrast (DIK) microscope shows the plasmalemma invagination system (*arrows*) in the spherical head of a living microplasmodium in a very limited plane of focus. **b** View of the protoplasm of a spherical head of a freeze-dried, broken and ion-etched cell with the scanning electron microscope. *Black arrows* demonstrate the outer part of the invagination system, where the plasmalemmal surface is changing to plasmalemmal channels. The inner part of the invagination system is also displayed (*white arrows*). **c** Schematic drawing of an electron micrograph of an ultrathin section of a spherical head (*arrows*: plasmalemmal invaginations) and (**d**) of an ameboid microplasmodium (*arrow*: short invagination; *stars:* cell indentation). Note the highly differentiated plasmalemma invagination system in the dumbbell types, which is almost completely lacking in ameboid microplasmodia. Bars = 20 μm

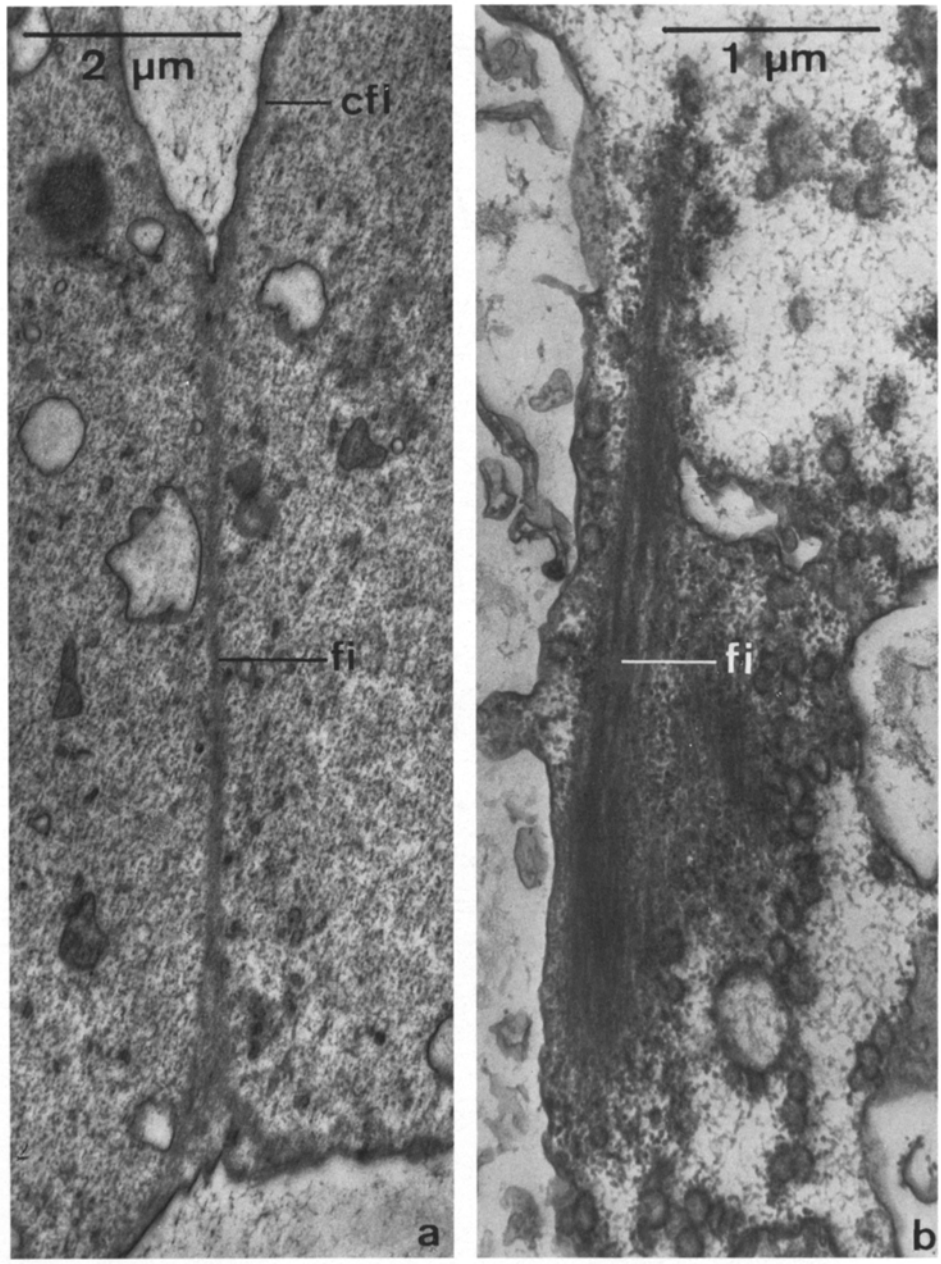


**Fig. 6a and b.** Fine structure of a spherical head of (**a**) DNP- and (**b**) NEM-treated dumbbell-shaped microplasmodia. **a** Cytoplasmic fibril (*fi*) connecting the basal region of two plasmalemmal invaginations. The filament cortex (*cfl*) and the fibrils (*fi*) are continuous structures. **b** After NEM-treatment the filaments exhibit parallel aggregation within the fibrils (*fi*)

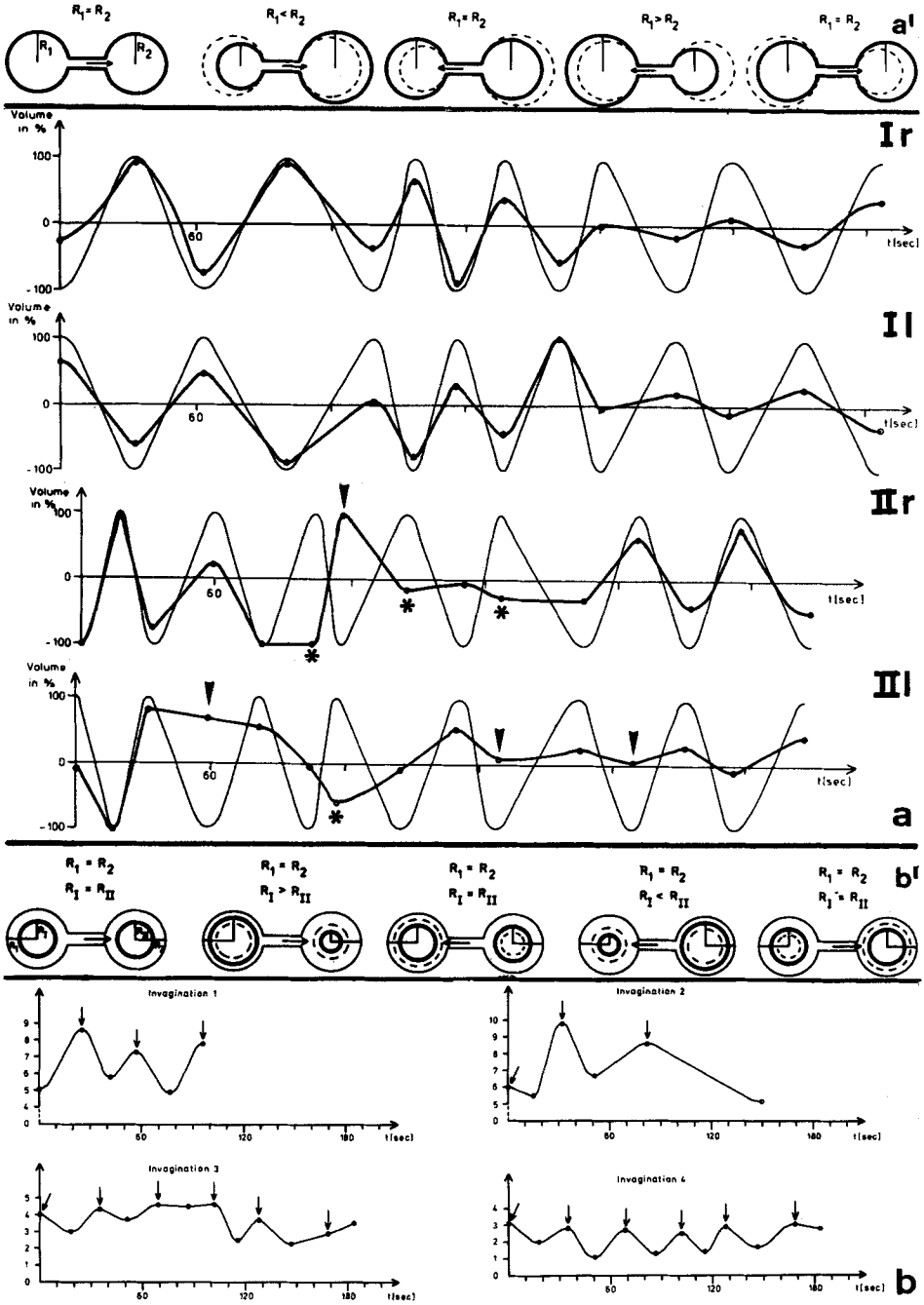


**Fig. 7 a and b.** Morphometric data obtained from single frame analysis of dumbbell-shaped microplasmodia. **a** Volume changes (*thick lines*) in the left ($I_l$ and $II_l$) and in the right ($I_r$ and $II_r$): spherical heads of two microplasmodia (*cell I* and *II*) were compared with the volume of protoplasm (*thin line*) transported through the shaft between the two heads. **a'** Theoretical hydraulic dumbbell-shaped system. Changes in the surface cause streaming of the fluid within the system. **b** Volume changes of single invaginations measured morphometrically. The markers on the ordinate give relative values for the invagination planes. **b'** Theoretical hydraulic dumbbell-shaped system. Changes in the inner surfaces (*thick lines*) cause streaming of the fluid within the system

simple hydraulic system are shown in cases where a spherical head receiving an inflow of protoplasm actually decreases in volume (Fig. 7a, *stars*) and *vice versa*, a spherical head showing outflow of protoplasm increases in volume (Fig. 7a, *arrowheads*). When the two heads of the same cell are observed simultaneously, it can be seen that they are somewhat independent of each other regarding volume changes during protoplasmic flow (Fig. 7a, *solid lines*, $I_r$ and $I_l$, right and left heads, respectively of *cell I*; $II_r$ and $II_l$, right and left heads respectively of *cell II*). It must be mentioned here that dumbbell-shaped microplasmodia do not possess membrane foldings correlated with the protoplasmic streaming pattern as described for ameboid-like types.

Additional investigations were focussed on the question whether the invagination system of the plasma membrane in spherical heads (Figs. 4a, 5a, b, c) shows dynamic behavior. For this purpose portions of invaginations were examined light microscopically (Fig. 5a). All invaginations showed maximum extension (Fig. 7b, *arrows*) during maximum outflow of protoplasm from the corresponding spherical head, and collapsed (Fig. 7b, *minima*) during inflow of protoplasm.

A second theoretical hydraulic model (Fig. 7b′) may be envisioned that exhibits a solid peripheral envelope (*thin lines*, showing a dumbbell) together with flexible surfaces inside this system (*thick lines*). In this model the fluid, which fills the space between the outer surface and the inner elastic spheres (*thick lines*), can be transported without altering the surface outline. Only volume changes of the inner elastic surfaces in the spherical heads are required. This model can also be considered in the analysis of microplasmodial protoplasmic streaming activity.

## Discussion

Ameboid microplasmodia of the acellular slime mold *Physarum polycephalum* show striking similarities to *Amoeba proteus* regarding fine structural organization and movement activity. Cinematographic investigations demonstrate that membrane folding in microplasmodia is closely correlated with outflow of protoplasm from these cell regions, which therefore can be compared with the uroid of amebae. On the other hand, unfolding of the plasma membrane occurs in regions into which protoplasm is streaming. Consequently, these regions can be compared with amebial pseudopods.

Membrane foldings in *A. proteus* have been described by Czarska and Grebecki (1966), Haberey et al. (1969) and Stockem et al. (1969). These authors have shown that the folding process of the plasma membrane results from contraction and relaxation activities of the contractile material underlying these membrane areas as a filamentous cortex. This structure consisting of actomyosin filaments exists in *Amoeba proteus* (Korohoda and Stockem 1975; Hauser 1978; Wehland et al. 1978; Stockem et al. 1978; Wehland et al. 1979; Gawlitta et al. 1980) as well as in ameboid microplasmodia (Fig. 3). Alternating contractions (Fig. 8a, *thick broken line*) and relaxations in distinct cell regions (Fig. 8a, *thin broken line*) of this peripheral cortex are responsible for disoriented protoplasmic flow along pressure gradients.

Dumbbell-shaped microplasmodia are similar to macroplasmodia of *Physarum polycephalum* with respect to fine structural aspects and flow of protoplasm

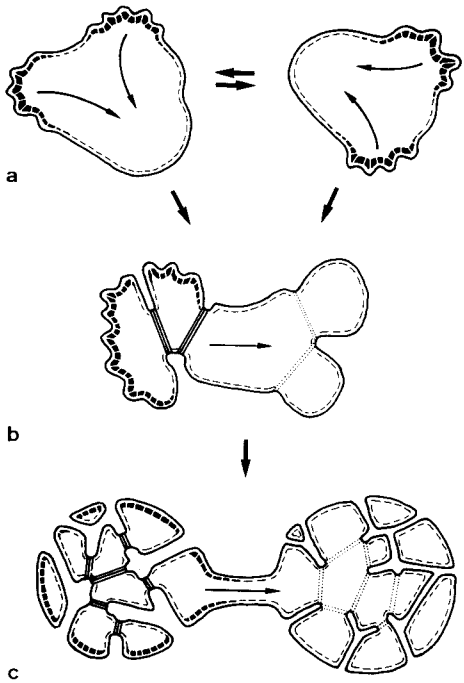


**Fig. 8a–c.** Development and organization of contractile structures and of plasmalemmal invaginations from ameboid microplasmodia (**a**) via intermediate stages (**b**) to dumbbell-shaped microplasmodia (**c**). *Thick broken lines:* contracting filament cortex; *thin broken lines:* relaxed filament cortex; *straight lines:* contracting fibrils: *dotted lines:* relaxed fibrils. *Arrows* within the cells: direction of protoplasmic streaming

(Wohlfahrt-Bottermann 1962, 1974, 1975). They show a regular shuttle streaming from one spherical head to the other (Fig. 4b), but membrane foldings in course of the protoplasmic streaming do not occur in this type of microplasmodia. Plasmalemmal invaginations are characteristic differentiations running through the entire spherical heads (Figs. 4a, 5a, b, c; Wolf et al., in preparation). The contractile material underlies the plasma membrane in the cell periphery and along the invaginations (Fig. 6) in the form of a distinct cell cortex. Additional cytoplasmic fibrils, continuous with the cortical layer, traverse the spherical heads (Fig. 6a, b). The connecting tube possesses a cortical layer only, which seems to serve as a stabilizing structure between the two spherical heads.

In both ameboid and dumbbell-shaped microplasmodia the cortical filament layer as well as the cytoplasmic fibrils connecting adjacent plasmalemmal invaginations consist of thin filaments only, whereas thick filaments are completely lacking. In agreement with extensive investigations on macroplasmodia, this does not mean, however, that myosin is absent in these structures (Fleischer and Wohlfahrt-Bottermann 1975; Kortzfleisch 1976; Wohlfarth-Bottermann and Fleischer 1976; Wohlfarth-Bottermann 1977). Myosin apparently exists in a low-polymerized state that is impossible to identify morphologically. Exclusively under special experimental conditions, myosin filaments are formed and preserved during fixation and embedding (Allera et al. 1971).

The shuttle streaming in dumbbell-shaped types must be regarded as a combined result of the activities of the two filamentous systems leading to alterations of the invaginations and the cell periphery (Fig. 8c):

1) Volume changes of the cell outline mainly result from contractions and relaxations of the membrane-associated filament cortex (Fig. 8c, *broken lines*).

2) Volume changes of the invagination system are mainly caused by contractions and relaxations of the cytoplasmic fibrils connecting the basal region of these invaginations (Fig. 8c, *straight lines*).

Both mechanisms of volume changes establish a hydraulic pressure gradient. The shuttle streaming is a result of such alternating gradients: In one spherical head the hydraulic pressure is established by contractions which cause the protoplasm to stream into the opposite relaxing head. Thus, the cortical layer of the connecting tube must only transduce the hydraulic pressure into dynamic pressure.

The microplasmodium shown in Fig. 8b represents the transitional form between ameboid and dumbbell-shaped types. Differentiations characteristic of the ameboid types (such as membrane foldings) as well as structural features of dumbbell-shaped types (cytoplasmic fibrils, shuttle streaming, higher amount of protoplasm) are visible. It is suggested that the formation of invaginations, which occurs during the transition from ameboid to dumbbell-shaped types, is mainly based on one mechanism: namely, the active pulling of the plasma membrane into the cell interior by contractile fibrils (Figs. 6a, 8b, *left area*). Any indications for the participation of other processes, such as the elevation of additional pseudopodia surrounding invaginated channels formed by membrane flow or the fusion of membrane vesicles with each other and with the plasma membrane, as has been discussed for plasmodial veins (Wohlfahrth-Bottermann 1974) and protoplasmic drops (Achenbach et al. 1979), respectively, were not found in microplasmodia.

The general difference between the two types of microplasmodia investigated in the present paper can be summarized by the fact that the motive force generated solely by the membrane-associated cortex is strong enough to cause the flow of such small amounts of protoplasm as occurring in ameboid microplasmodia. However, in dumbbell-shaped microplasmodia and in macroplasmodia, much higher quantities of protoplasm must be translocated. In these last two growing types, additional cytoplasmic fibrils, which are connected with plasmalemmal invaginations, are required.

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