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Cycling Aggregation Patterns of Cytoplasmic F-Actin Coordinated with Oscillating Tension Force Generation*

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Summary. Isometric contracting protoplasmic veins of *Physarum polycephalum* show cycling patterns of cytoplasmic F-actin, dependent on their oscillating contraction behaviour (minute rhythms). The process of fibrillogenesis represents a parallel arrangement of F-actin chains ("plasma filaments, microfilaments") during the *isometric contraction* phase. A part of the results of the present work corroborates previous results on stretch-activated veins which showed that the fibrillar form of F-actin reflects the isometric contracted state.

During *isometric relaxation* phase, a disaggregation of the fibrillar pattern takes place and is accompanied by a deparallelisation of F-actin chains. Therefore, the isometric relaxed state of cytoplasmic actomyosin is non-fibrillar in nature. Thus, the morphologically detectable fibrillar form of cytoplasmic actomyosin, according to physiological interpretation, is solely representative of the isometric contracted state.

The question whether assembly-disassembly processes, e.g. G⇌F-actin-transformation, play a role in the contraction-relaxation cycle is discussed.

Key words: Microfilaments – F-actin – Non-muscular cells – Cytoplasmic actomyosin – Tension forces – *Physarum polycephalum.*

Introduction

The function of plasmafilaments ("microfilaments") with actomyosin nature can be investigated preferably in the plasmodia of acellular slime molds as in *Physarum*

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polycephalum (Wohlfarth-Bottermann, 1964; Komnick *et al.*, 1973). The plasmodia contain veins, i.e. protoplasmic strands of which segments can be used to study the correlation of protoplasmic shuttle streaming and changing patterns of cytoplasmic actomyosin fibrils (AM fibrils) (Wohlfarth-Bottermann, 1965; Kamiya, 1972). The AM fibrils of light microscopical dimension contain as constituents F-actin filaments and oligomeric myosin (Alléra *et al.*, 1971; Alléra and Wohlfarth-Bottermann, 1972). Recently it has been possible:

1. to demonstrate AM fibrils in live observation by phase contrast- and differential interference contrast-microscopical methods (Hülsmann *et al.*, 1974) and by the freeze-etching technique (Isenberg *et al.*, 1975) and to identify the nature of the fibrils by immunofluorescence (Weber and Groeschel-Stewart, 1974; Lazarides and Weber, 1974; Lazarides, 1975),

2. to investigate the contraction physiology of cytoplasmic actomyosin (Kamiya, 1975; Fleischer and Wohlfarth-Bottermann, 1975; Schliwa and Bereiter-Hahn, 1975).

Due to the high sensitivity of a tensiometer (± 0, 01 mp), the time course behaviour of contraction in living protoplasmic strands can be measured (Kamiya, 1970; Wohlfarth-Bottermann, 1975a). The oscillating contractions could be correlated with the corresponding fine structural alterations of the strands (Kamiya, 1975; Fleischer and Wohlfarth-Bottermann, 1975). In this laboratory the reaction to strong stretch-activation was investigated and a relationship between tension force development, fibrillogenesis and ultrastructure of the AM fibrils was found. Two main results were formulated:

1. The fibrillar stage of the cytoplasmic actomyosin reflects its *isometrically* contracted state.

2. AM fibrils contracting isotonically (under 0 tension) transform into a non-fibrillar stage.

In these experiments the reaction of the protoplasmic strands within a certain time period (up to 3 mins after stretch-activation) was tested. However, a cyclic contraction behaviour of the strands is measurable over many hours and one should suppose, that after longer periods of stretching a change in structure occurs in the strands in comparison to directly after the start of stretching. Therefore, the investigations were extended so that the strands remained in the tensiometer for 18 mins, during which their rhythmic contractions were recorded. Our aim was to answer the following three questions:

1. Are there differences in the *general morphology* of the veins in *long-term* tensiometric experiments under isometric conditions?

2. Do long-time experiments confirm the thesis, that the fibrillar stage represents an *isometric contraction?*

3. What happens with the AM fibrils during *isometric relaxation?*

Material and Methods

Culture of the plasmodia, *Physarum*, the physiological measurement of contraction forces, the embedding of specimens and the microtomy and microscopy have been described previously (Fleischer and Wohlfarth-Bottermann, 1975).

Protoplasmic veins (segments of plasmodial strands) were mounted vertically on the tensiometer and stretched to 50% of their original length. After this experimental procedure the oscillating contraction activity was registrated continuously under isometric conditions of measurement. Fixation took place during tensiometric investigation (see Fig. 3) by immersion of the vein with the fixative: 2% OsO_4 + 0.5% $HgCl_2$ in 0.05 Na-cacodylate buffer, pH 7.1 (Parducz, 1952).

Results

Living protoplasmic strands of *Physarum* react to strong stretch-activation (stretch degree: 50% of their original length) by a stress relaxation phase following immediately the stretch stimulus (Fig. 1, tensiometer curve stages 1, 2). This stress relaxation phase is followed by a contraction phase, which begins 30–35 secs (average) after stretch and ends 80 secs thereafter (Fig. 1, stages 3–5). After this stretch reaction, the spontaneous oscillating contraction activity continues and is tensiometrically measurable over many hours.

In a previous investigation (Fleischer and Wohlfarth-Bottermann, 1975) we correlated under isometric conditions of measurement the development of longitudinal contraction force with the appearance of AM fibrils within the strands: during the isometric contraction phase (Fig. 1, stage 3–5), the cytoplasmic AM within the endoplasm forms fibrillar aggregates of light microscopical size containing F-actin filaments which in the following relaxation phase (Fig. 1, stage 6) disaggregate. This result suggested that the fibrillar form of AM reflects its isometric contracted state.

Stretch-activated, isometrically contracting veins (Fig. 1, stage 2–3), exposed for only 5 secs to isotonic conditions (0 external tension), react during contractile shortening by a disaggregation of the AM fibrils within a few seconds, such that the fibrils are neither light- nor electron microscopically detectable (Fig. 1, 7 and 8, arrows). From this, it was concluded that the isotonically contracted state of cytoplasmic AM is no longer fibrillar in nature, when the fibrils were in isometric contraction before they had opportunity to contract isotonically. During isotonic contraction, the parallel arrangement of F-actin chains which is characteristic of the isometric contracted state disappears. The isotonic contracted state of cytoplasmic AM, therefore, was interpreted to be non-fibrillar (Fleischer and Wohlfarth-Bottermann, 1975).

The results summarized above were gained on AM fibrils induced *de novo* in the endoplasm by stretching the veins; without application of stretch-activation, the streaming endoplasm of experimentally unaffected veins usually does not form AM fibrils (Wohlfarth-Bottermann, 1975b). We, therefore, extended the investigation to veins which remained in the tensiometer for a time interval of 15–18 mins after stretching. During this time, the strands showed normal oscillating contraction rhythms (Fig. 3). These experiments were performed to test whether correlations could be found between the structure of the veins and their tensiometrically measured behaviour during long periods of isometric contraction activity. The more precise question read: does the endoplasmic core of the strands possess AM fibrils for longer periods after stretch-activation or were the fibrils to be found again only in the ectoplasm, as is the case in experimentally unaffected veins (Wohlfarth-Bottermann, 1975b)? Fig. 2a and b show cross

sections of veins. (That in Fig. 2a was fixed *2 mins* after stretch-activation, that in Fig. 2b was fixed *15 mins* after stretching and continuous tensiometric measurement.) As can be seen by comparing these semithin sections (thickness 2–3 μm), long term isometric conditions cause an extremely strong plasmalemma-invagination system which in less pronounced form is characteristic of the ectoplasmic regions of experimentally unaffected protoplasmic veins (Wohlfarth-Bottermann, 1974). Just after stretch-activation, the ectoplasmic area with invaginations occupies an outer region, i.e. a relatively small part of the total vein. 15 mins after stretching, the invagination system was found to be extremely enlarged and thereby the endoplasm reduced to several small channels, which can be best identified in cross sections of veins (Fig. 2b). The isometric stretching of the veins for a longer period of time obviously leads to a reduction of the endoplasm by an inwards invasion of the plasmalemma. Thereby, the greater part of the previous endoplasmic volume (at least structurally) is transformed into ectoplasm. The invasion of plasmalemma invaginations can be interpreted as an adaptation of the veins to long-time isometric stretch conditions.

Plasmodial segments which are used over many hours for tensiometric registration of longitudinal contraction activities grow out to small plasmodia when they are placed back into the culture vessel. This shows that the experimental conditions within the tensiometer, even during strong stretch-activation, are clearly within the physiological range of the object.

In order to differentiate structurally between contraction and relaxation, the strands were fixed during their tensiometric measurements either at the beginning of a contraction phase (Fig. 3b) or at the beginning of a relaxation phase (Fig. 3a). The exact points of fixation within the contraction-relaxation cycle can be seen clearly in the original tensiometer curves. Fast-acting fixatives cause an immediate decline in the tensiometer curves when the strands are immersed with osmium or mixtures of osmium [Fig. 3, *arrows* (Fixation)]. The curves shown in Fig. 3 deal with two ends of one strand, which was mounted as a loop in the tensiometer. Thus, both ends of one strand were simultaniously measured and fixed.

From objects fixed under conditions shown in Fig. 3, longitudinal and cross sections of veins were analyzed. Fig. 4 demonstrates a longitudinal section of a strand from the tensiometric curve shown in Fig. 3a. A small longitudinally sectioned endoplasmic channel can be seen in the center of this picture. In addition to the extensive plasmalemma invaginations, thick AM structures run longitudinally through the vein, close to the walls of the plasmalemma invaginations (Fig. 4, *arrows*). As shown by the corresponding tensiometer curve (Fig. 3A), this strand was fixed at the *beginning of an isometric relaxation phase*.

Fig. 1. Schematic drawing of the assembly and disassembly of AM fibrils dependent on the stretch-induced contraction state of living protoplasmic veins of *Physarum*. Isometrical conditions of measurement. The tensiometer curve shows a stress relaxation phase of the vein after experimental stretching (0 sec) from 0 to 35 secs. Thereafter, a contraction phase begins at 35 secs lasting until 80 secs after the stretch stimulus. A second relaxation phase begins 90 secs after the time of stretching. Note the appearance of AM fibrils of light microscopical dimensions during the stretch-induced contraction phase (*stages 3–5*). The experimental application of isotonic conditions, giving the veins the opportunity to contract under 0-tension, leads to an immediate disassembly of the fibrils (*stages 2 and 3, arrows stages 7 and 8*). For detailed explanation, see text

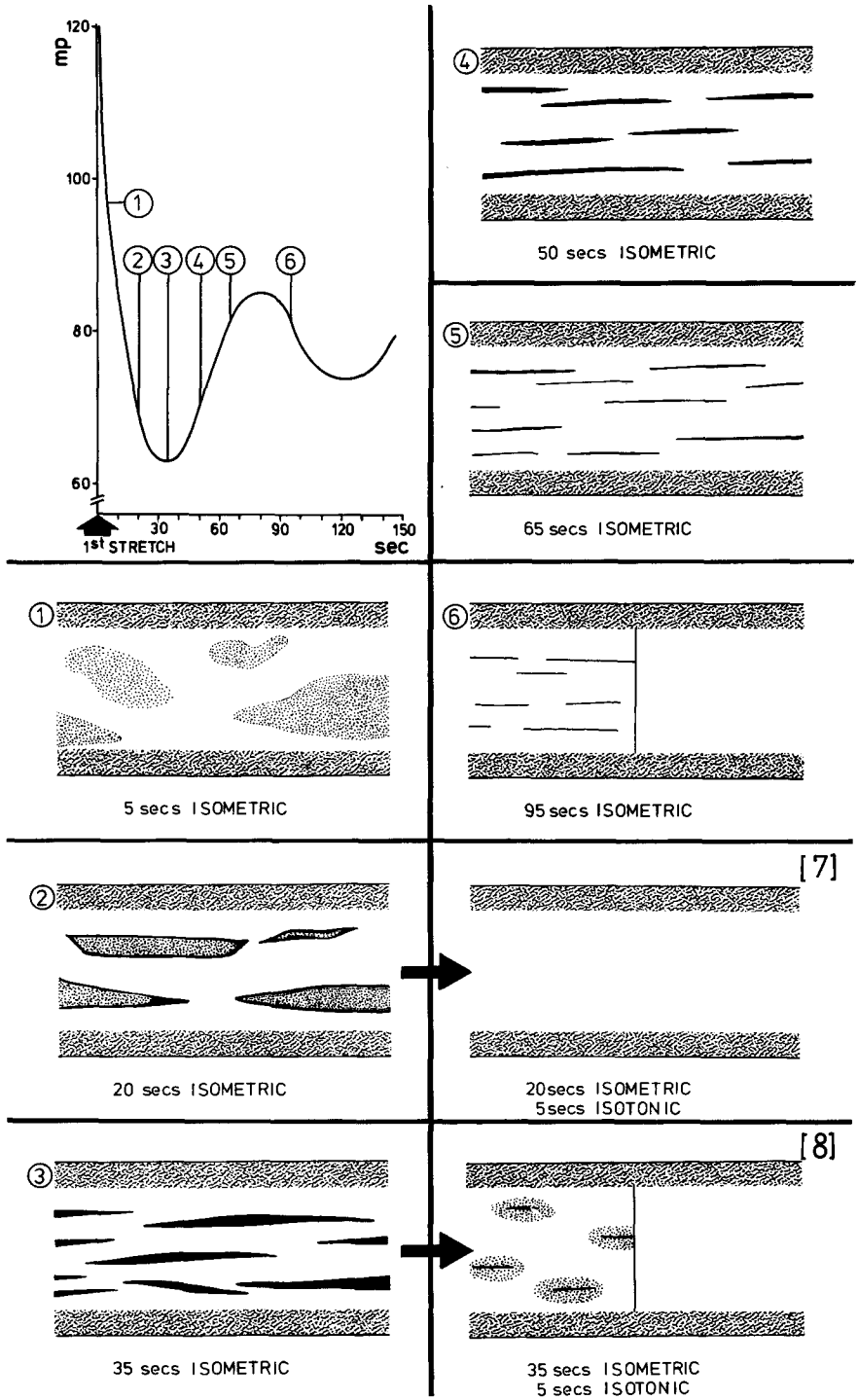
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Fig. 1

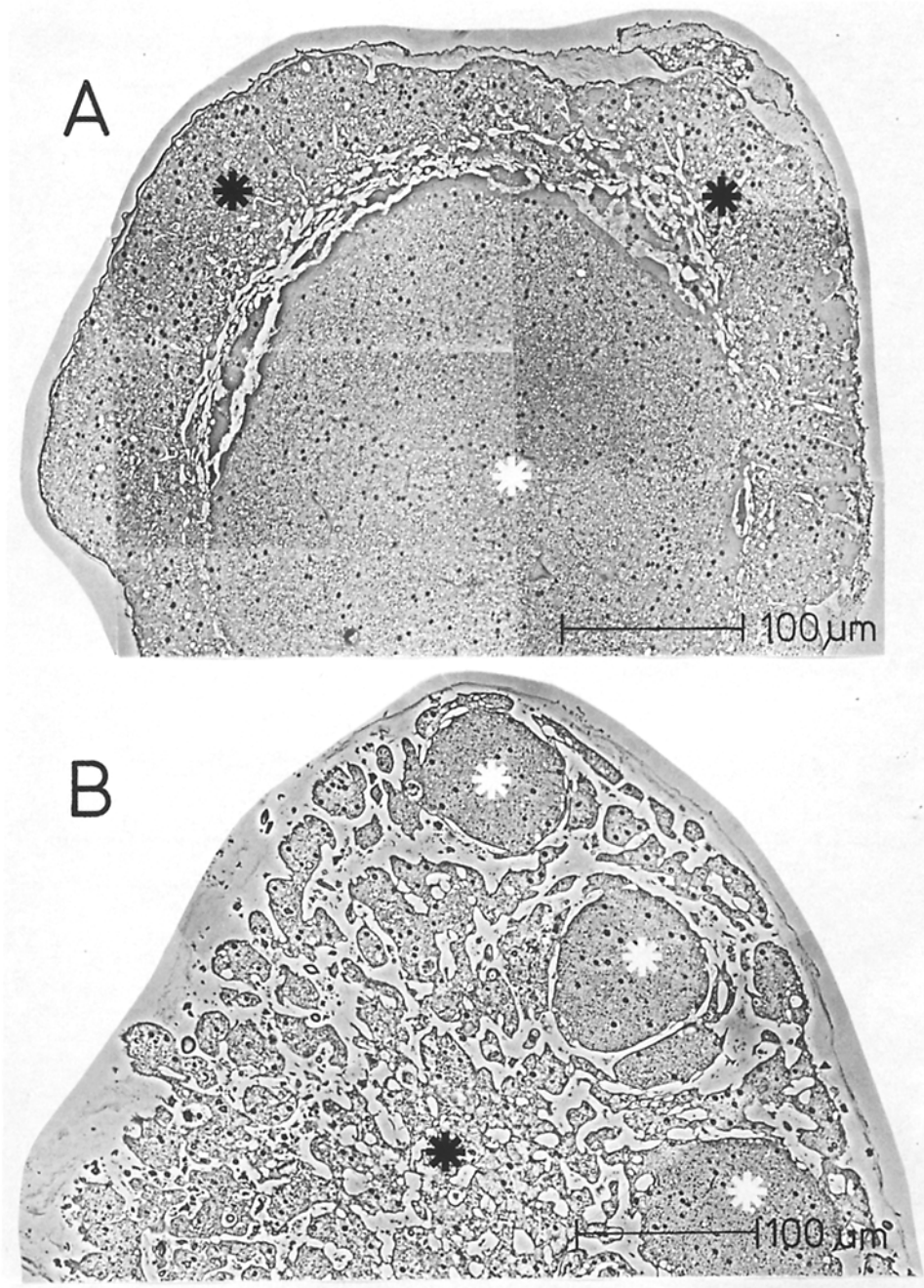

Fig. 2 A and B. Phase contrast survey micrographs of cross sectioned veins. *Black asterisk:* ectoplasm; *white asterisk:* endoplasm. *Upper micrograph* (A): fixed approximately 2 mins after stretching; *lower micrograph* (B): fixation 16 mins after stretching in the tensiometer. Note the invasion of plasmalemma invaginations into the center of the vein, thereby reducing the volume of the endoplasm to several small channels. 240:1

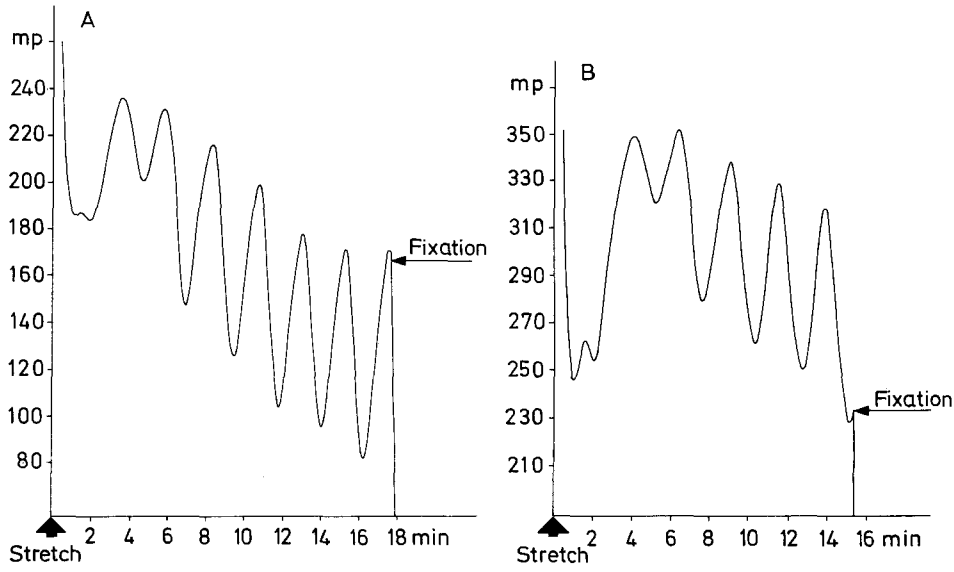

Fig. 3A and B. Tensiometer curves from two veins both of which were mounted as a loop vertically arranged in the tensiometer. Isometric conditions. The stretch occurred at 0 mins. Thereafter, the rhythmic contraction activity was measured for 15–18 mins. Vein *A* was fixed at the beginning of a relaxation phase, vein *B* at the beginning of a contraction phase (*arrows*)

Comparative analysis of veins which were fixed at the *beginning of a contraction phase*, shows that the above-described AM structures lining the plasmalemma invaginations were also present. As in the relaxation phase (Fig. 4), a characteristic layering of AM on the walls of the invaginations is found in the contraction phase as well (Fig. 5, *arrows* pointing to the left). In addition, however, fibrils lying free in the cytoplasm appear at the beginning of a contraction phase (Fig. 5, *arrows* pointing to the right). These AM fibrils appear to lie free in the ground cytoplasm, but actually all insert on plasmalemma invaginations. The points of plasmalemma insertion are, however, not found in each level of section, but can always be demonstrated in serial sections.

Fig. 6 represents an electron microscopic survey picture of the AM fibrils shown in Fig. 5. In several areas branching points of the fibrils can be seen. The individual fibrils are arranged predominantly longitudinally, that means parallel to the long axis of the vein.

The fine structural investigation of veins fixed during different stages in the contraction-relaxation cycle revealed the following results: veins fixed during increasing tension development (curve, Fig. 3B and Fig. 6) demonstrate within their AM fibrils a nearly complete parallel arrangement of filaments which have an average diameter of 50–60 Å (Fig. 7). There is no doubt, that the 50–60 Å-filaments represent mainly F-actin (Alléra *et al.*, 1971). This parallelism of F-actin chains is evident in all longitudinally sectioned fibrils. There are no indications that a folding of F-actin (Mg^{++}-polymer) is involved in isometric contraction.

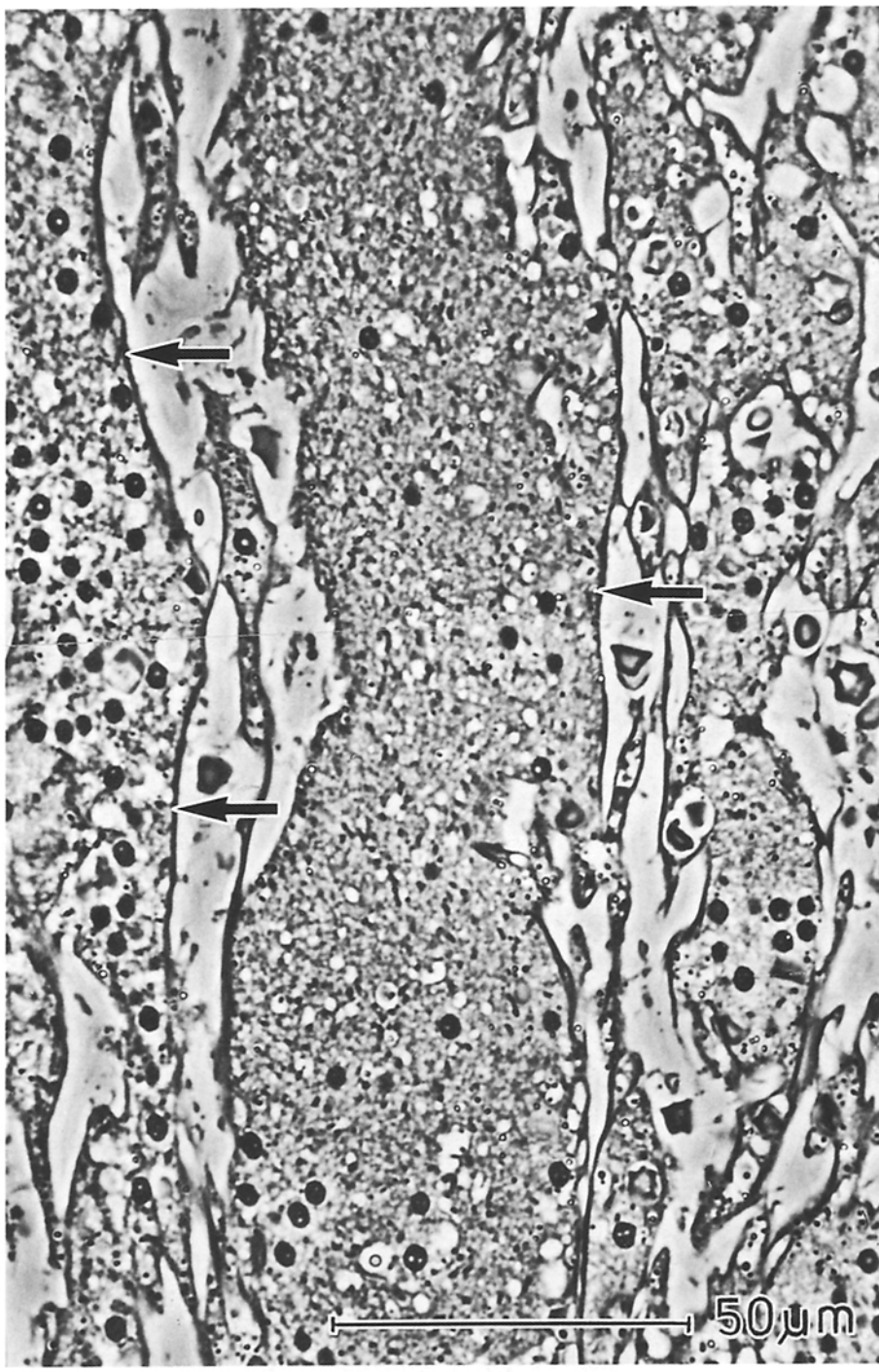

Fig. 4. Longitudinal section of the vein measured by the tensiometer curve in Fig. 3A. Phase contrast, semithin section (2–3 µm thick). The *arrows* point to AM fibrillar sheaths which adhere directly to the plasmalemma invaginations. 960:1

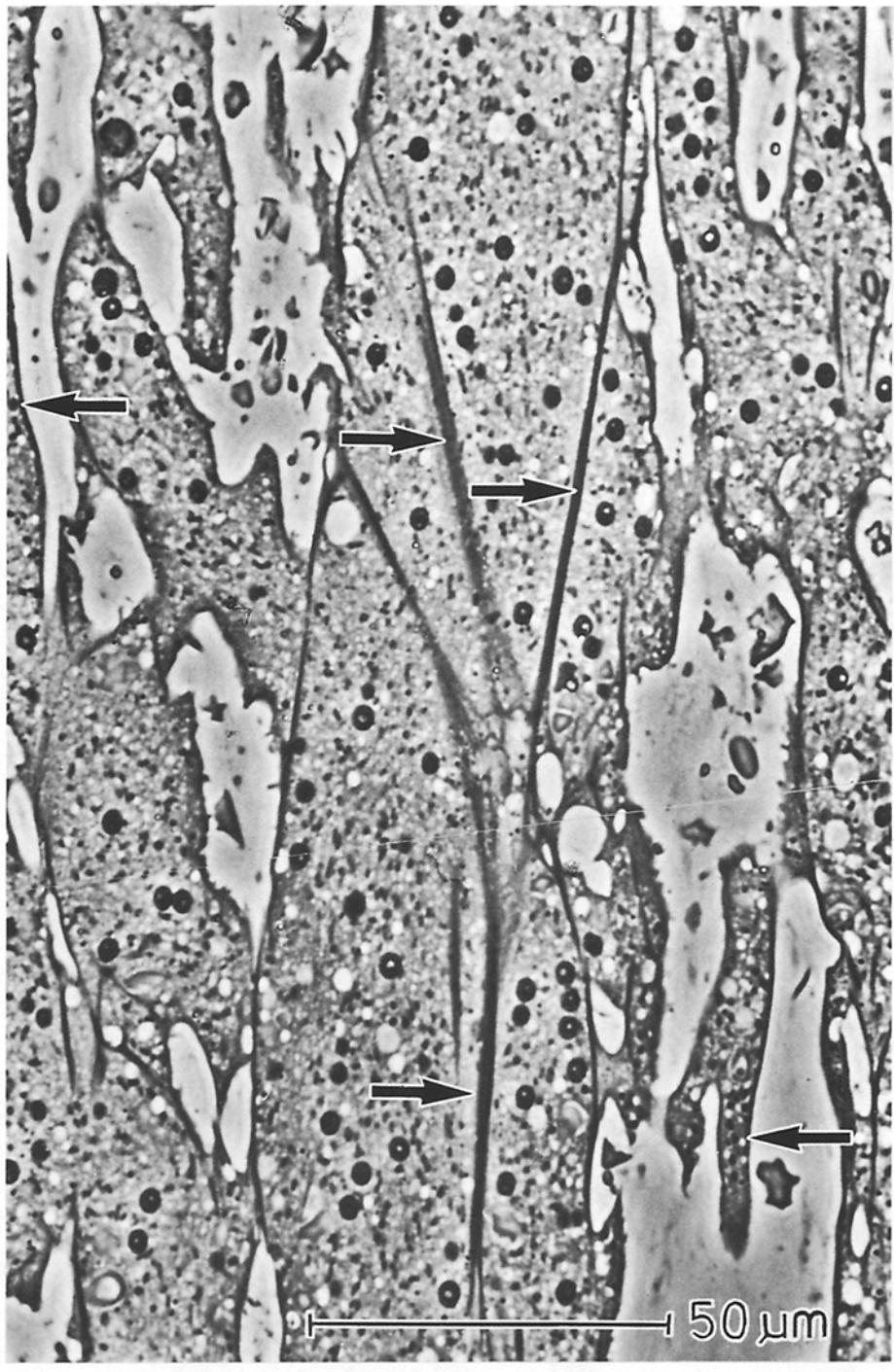

Fig. 5. Longitudinal section of the vein measured by the tensiometer curve in Fig. 3B. Phase contrast, semithin section. *Arrows* pointing to the left indicate (as in Fig. 4) AM fibrils adhering to plasmalemma invaginations. However, during contraction, in addition AM fibrils appear running through the endoplasm, but also insert on plasmalemma invaginations (*arrows* pointing to the right). 960:1

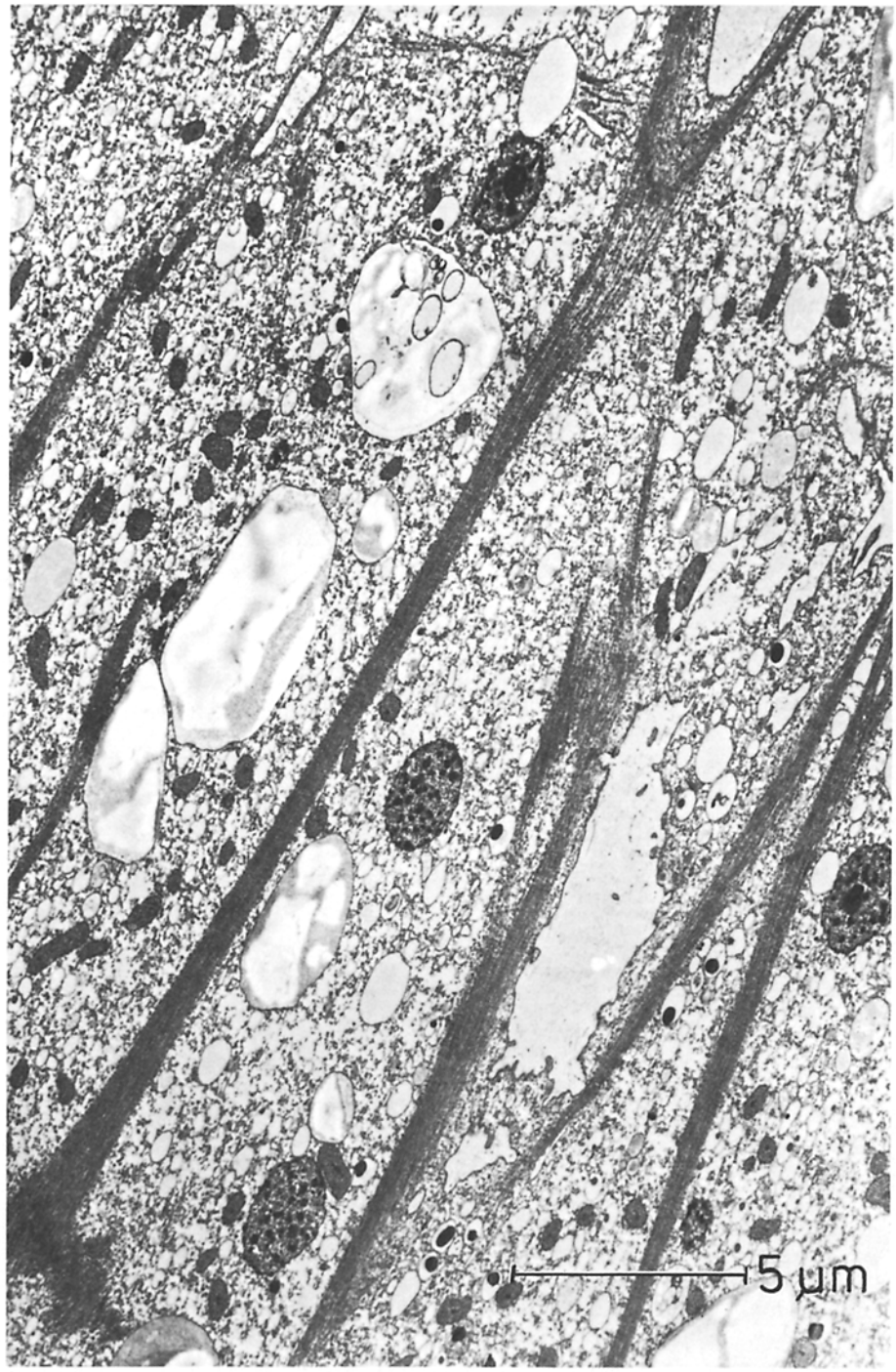

Fig. 6. Electron microscopic survey micrograph from the stage shown in Figs. 3B and 5. The fine structure of these AM fibrils is to be seen in Fig. 7. 6,000:1

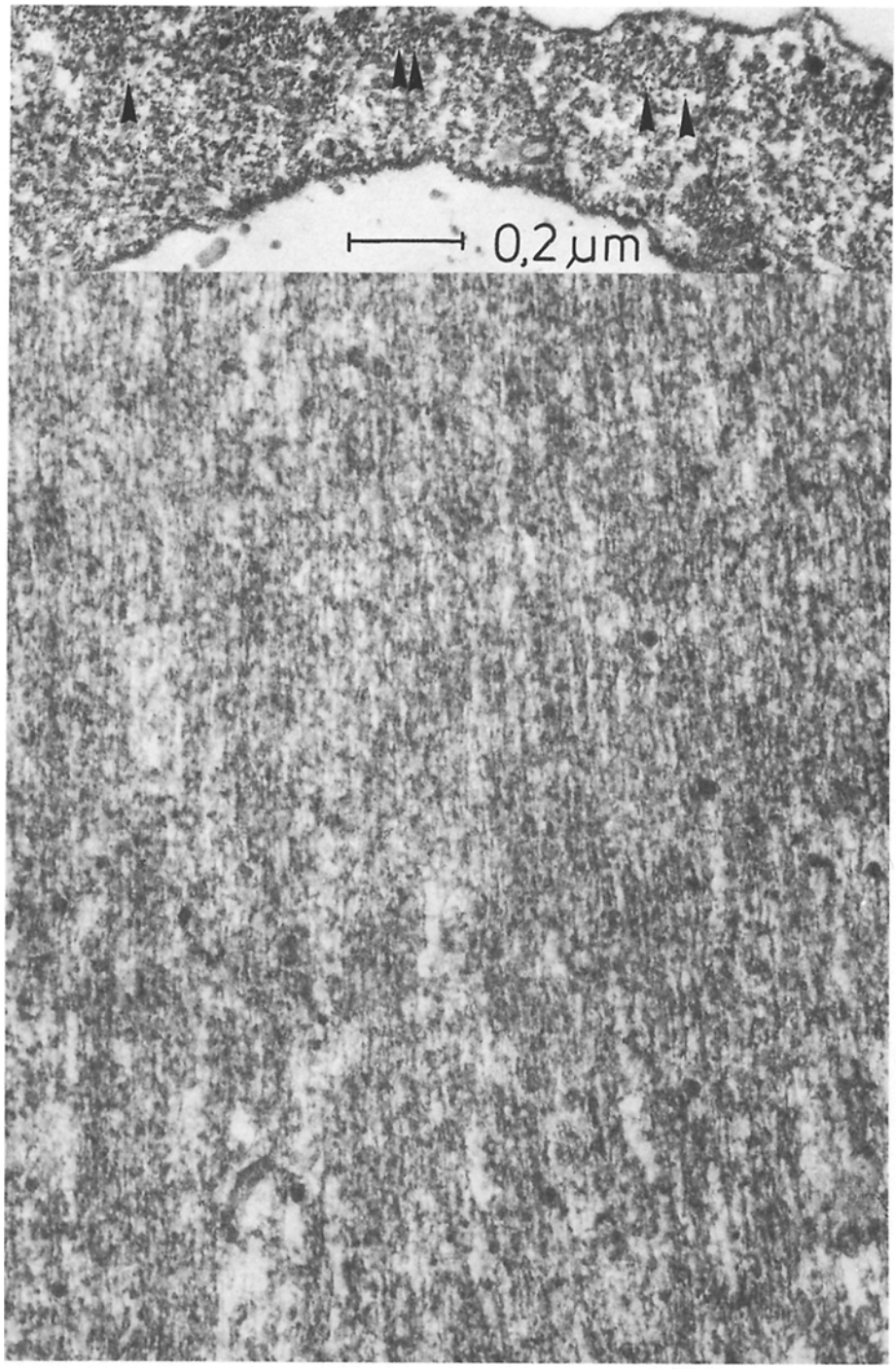

Fig. 7. Fine structure of the AM fibrils in a protoplasmic vein fixed at the beginning of a contraction phase (Figs. 3B, 5, 6). *Upper part:* cross section of a fibril. *Lower part:* longitudinal section. For explanation see text. 77,500:1

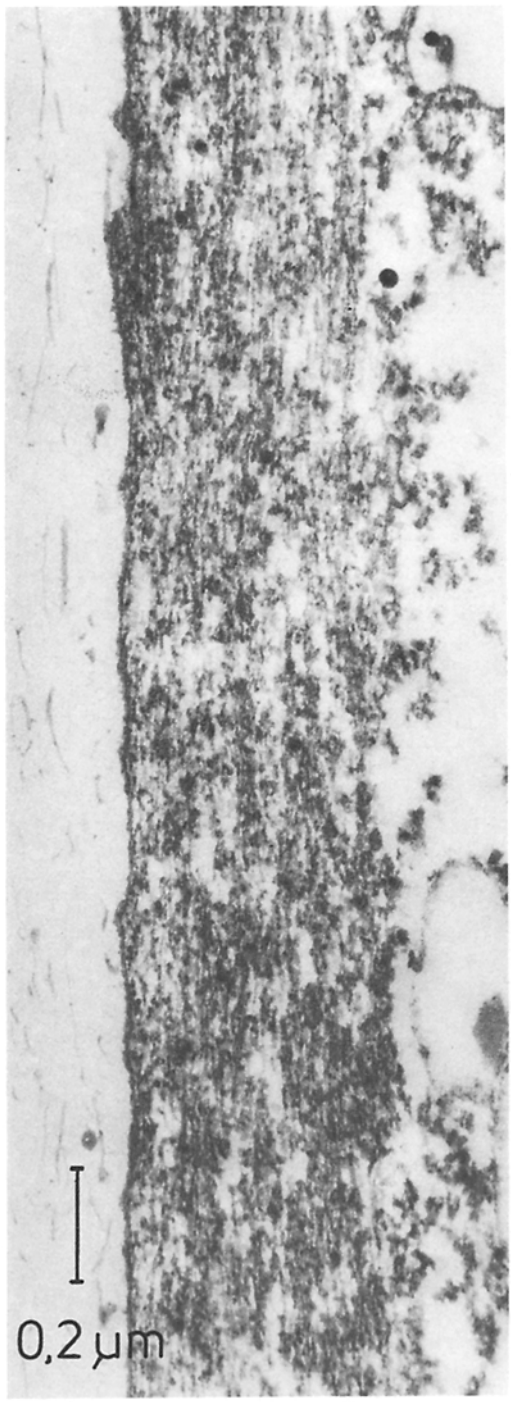

Fig. 8. Fine structure of an AM fibril in a vein fixed at the beginning of the relaxation phase (Figs. 3A, 4). For explanation see text. 77,500:1

Filaments with a diameter of 50–60 Å are also demonstrable in fibrils cut in cross section (Fig. 7, top).

As indicated earlier, most of the AM fibrils which are found during the relaxation phase (curve Fig. 3A, Fig. 4) are lining the plasmalemma invagination. In these longitudinally cut fibrils fixed during the relaxation phase, the parallel arrangement of filaments is less pronounced (Fig. 8). However, the main morphological difference between the two opposite phases of the contraction cycle lies in the fact that, in the relaxation phase, fibrils lying free in the endoplasm are scarcely found (compare Figs. 4 and 5).

Discussion

According to the results described above, assembly and disassembly of AM fibrils are correlated to contraction and relaxation, respectively, not only after short application of stretch stimulation but also after a 15 min period of isometric stretch conditions. The number of fibrils at the beginning of a contraction phase in long-time experiments (Fig. 5) is possibly smaller than in the corresponding contraction phase shortly after strong stretch-activation (Fig. 1, stage 3), but is markedly increased in comparison to comparable veins fixed in the relaxation phase (Fig. 4). A certain difficulty concerning this interpretation lies in the fact, that AM fibrils adhere to the plasmalemma invaginations in the contraction phase as well as in the relaxation phase. However, a precise analysis of phase contrast pictures reveals, that the "AM fibrils" lining the plasmalemma invaginations actually represent much more quite extended AM layers than single fibrils. This complex of the plasmalemma and the AM layer is more difficult to identify in the phase contrast microscope (Figs. 4 and 5, *arrows* pointing to the left) than the free running fibrils (Fig. 5, *arrows* pointing to the right). One has to assume that these longitudinally arranged AM layers also contribute to the isometric contraction force of the veins. During ascending tension force, the AM layers appear to increase in thickness, while during descending tension force they appear to decrease. This impression is, however, difficult to quantitate with certainty. Concerning the fibrils running through the ground cytoplasm, however, this difference between the contraction and relaxation phases can be clearly seen. Despite this clear difference (Figs. 4 and 5) the AM layers in close apposition to the invaginations may contribute to the force generation.

In earlier investigations concerning the topography of AM fibrils in isolated veins performing shuttle streaming, the pole of protoplasmic influx was found to contain relatively few fibrils, whereas the pole of protoplasmic efflux (where hydraulic over-pressure is generated by contraction of the ectoplasm) was crowded with AM fibrils (Wohlfarth-Bottermann, 1965; Kamiya, 1972). Since this topographic distribution is cyclic inversed with the rhythm of shuttle streaming direction (that means every 2 mins), there is also a correlation between AM fibrillogenesis and streaming direction.

Concerning the experiments presented here, there seems to be no doubt that the appearance of fibrils at the beginning of a contraction phase suggests a causal relationship to the augmented tension generated by the strand. There

is no evidence to support the thesis that the fibrils are the *result* of a contraction: during the contraction phase following strong stretch-activation (Fig. 1, stages 3–5), AM fibrillogenesis takes place *before* tension force begins to increase (stage 3 tensiometer curve) and not before the tension force reaches its maximum, as one should suppose if the fibrils were the passive result of a contraction. According to many earlier findings including the present ones, the fibrils seem to be at least partly the structures generating the tension force. If this interpretation is correct, our experiments lead to two statements which should have consequences for the contraction physiology of cytoplasmic AM:

1. The fibrillar form of the cytoplasmic AM represents the state of its isometric contraction in long-term experiments as well as just after stretch-activation.

2. The isometrically relaxed state of cytoplasmic AM is non-fibrillar.

In previous investigations (Fleischer and Wohlfarth-Bottermann, 1975) the isotonic contracted state was seen to be non-fibrillar (compare Fig. 1, stages 2 and 3, *arrows*). The following tabulation gives a survey of the present knowledge:

Table 1

Isometric contraction	*fibrillar* (highly parallelized F-actin aggregated to thick and long reaching fibrils)
Isometric relaxation	*non fibrillar* (F-actin deparallelized)
Isotonic contraction (0 tension)	*non fibrillar* (F-actin deparallelized)
Isotonic relaxation	*structure unknown*

Thus, the fibrillar form of cytoplasmic AM with its parallel oriented F-actin is in *Physarum* solely the isometric contracted state. Apart from the question of the molecular contraction mechanism and the problem of G⇌F-actin transformation processes during contraction and relaxation cycle this observation can be of diagnostic value: in all eukaryotic cells the presence of cytoplasmic AM is presumed; in many cells it can be revealed morphologically as parallel arranged microfilaments or even fibrils. Therefore, for the functional interpretation of phasic and local distribution of fibrils it is important to know, that their more or less ephemeric appearance (Wohlfarth-Bottermann, 1965; Fleischer and Wohlfarth-Bottermann, 1975) points to *isometric contraction processes* between the points where the fibrils are inserted. The parallel arrangement and assembly of F-actin chains to highly aggregated fibrils appears only during isometric contraction.

Two examples should be mentioned for a tentative interpretation of the physiological state of AM fibrils in other non-muscle cells:

I. Pollack *et al.* (1975) used the immunofluorescence technique (with antibodies to actin and myosin) and described a correlation between the "anchorage dependence" of normal and transformed culture cells and the pattern of their actomyosin distribution.

Though normal and transformed cells do not differ in the actin- or myosin-content, the transformed cells show pronounced differences in their actual tendency to aggregate their AM-pool in form of light microscopically visible fibrils and sheaths. "The anchorage dependence is correlated with the loss of actin containing sheaths" and "appears to require a change in the internal architecture

of the cell". This interpretation seems to be in accordance with our finding, that the fibrillar state reflects isometric contraction: changes in the adhesion requirement (inability for cell division without a solid substrate) as well as changes in cell architecture must be correlated with transformations in the fibril pattern and fine structure, if the fibrils solely represent the isometric contracted state of the AM. Furthermore, the topographic course of the AM fibrils in flattened tissue culture cells suggests that they are predominantly responsible for isometric tension force transmission within the cell body: the fibrils extend between focal points of substrate adhesion. If this interpretation is right, and there is much circumferential evidence for it, the function of many AM fibrils in cultured cells could be predominantly static, but simultaneously dynamic. The following functions may be ascribed:

1. Preservation of the anisodiametric cell shape by transmission of unidirectional, isometric tension over long distances within the cell body.

2. Motive force generation for a very slow cell locomotion by partial or temperal isotonic contraction of the fibrils. This may result in a pulling force exerted to the cell body in the direction of the leading edge of the culture cell.

It should be possible to test these physiological interpretations at least phenomenologically by long-time cinematographic analysis of cultured cells.

II. Another example is the so-called "contractile ring", a dynamic structure responsible for furrow-formation and cell division of the cleaving egg (Schroeder, 1972). The ephemeric nature of this cytoplasmic AM differentiation is very similar to the dynamic nature of the *Physarum* AM fibrils and their assembly and disassembly behaviour. Cell division begins immediately when the contractile ring appears. This "highly transitory" structure can be seen only for 6–7 mins, then it disappears when cytokinesis is accomplished.

In the light of our contraction physiological findings, the contractile ring represents, during the beginning of furrow formation, predominantly an isometric contraction. Then, the contraction becomes more and more isotonic which leads to the disappearance of the contractile ring when cell cleavage is nearly accomplished and isotonic conditions prevail.

The problem of the visualization of myosin filaments has been analyzed and discussed in previous papers: myosin in the fibrils cannot be clearly distinguished due to the fact that it is most probably found *in situ* in the oligomeric stage (Alléra and Wohlfarth-Bottermann, 1972; Fleischer and Wohlfarth-Bottermann, 1975).

As well, during isotonic contraction of the AM fibrils as during their isometric relaxation the cytoplasmic AM disassembles into a non-fibrillar form. At the present time it can only be stated that this occurs by a deparallelization of the F-actin chains. The straight figuration of F-actin during isometric contraction and the fact that the actin filaments show no variation in their diameter are not in favor of a thesis (Hatano, 1973) that conformational changes of actin (Mg-polymer) are involved in the molecular contraction mechanism. However, this is only valid for isometric contraction. Since isotonically contracting AM fibrils disaggregate, the configuration of actin cannot be analyzed in thin sections: single F-actin chains cannot be identified in thin sections when cut obliquely or in transverse section; the visualization of the Mg-polymer is difficult and the demonstration of G-actin molecules in thin sections is not yet possible.

Nagai *et al.* (1975) fixed living protoplasmic strands under *isotonic conditions* of measurement (constant tension 10 mg) within a tensiometer and described a dependence of fibrillar fine structure on the corresponding state of tensiometrically measured contraction. However, these results cannot be compared with our findings due to the fact that: (1) these authors measured under isotonic conditions with nearly unstretched veins (load 10 mg) and (2) fixed the strands with osmium vapour (followed by glutaraldehyde after taking the strands out of the tensiometer). In our opinion, osmium vapour does not lead to an immediate

fixation (compare tensiometer curves). However, in consideration of the highly dynamic capacities of AM fibrils, a very fast fixation is desirable in order to stabilize clearly defined stages within the contraction relaxation cycle. Nagai *et al.* (1975) describe a deparallelization of F-actin chains ("felt-like structure", "skein body") during isotonic contraction, i.e. a structural transformation of the AM fibrils lying in the ectoplasm. These results may correspond to our observations under isotonic conditions (compare Fig. 1, stage 8, *arrow*) in so far as here the deparallelization of F-actin, i.e. the disaggregation of the fibrillar form and the disappearance of the fibrils occur by a transformation of the fibrils into "plaques" (Fleischer and Wohlfarth-Bottermann, 1975), which show non-oriented F-actin. A possible corresponding interpretation would be that these plaques in our investigation and the "felt like structures" of Kamiya are identical in nature, representing a first stage of deparallelization of F-actin chains during isotonic contraction of the AM fibrils.

The disadvantage of long-term tensiometry under isometric conditions lies in the fact that both free running fibrils as well as actomyosin layers beneath the plasmalemma seem to contribute to the measurable contraction force. At the present time it is not possible to discriminate, which quantitative portion of the motive force each system is bearing to the total longitudinal contraction force measured. In contrast to this, in the first contraction phase just after strong stretch-activation, the *de novo* generated AM fibrils within the endoplasm (Fleischer and Wohlfarth-Bottermann, 1975) have the advantages:

1. of being morphologically more clearly identifiable, and
2. of having a definite age (*de novo* generated at 35 secs after stretch-activation).

For experimental reasons, however, long time tensiometry will become important for physiological investigations in the future: the tensiometric method allows one to measure the contraction force of living protoplasmic strands in a perfusion chamber where the influence of changing ions, chemicals and drugs over longer time periods on the contraction activity of cytoplasmic AM can be tested (Wohlfarth-Bottermann, 1975a).

Therefore, both short-time experiments (strong stretch-activation) as well as long-time tensiometry most probably will be used in future for different experiments analyzing the physiology and the molecular contraction mechanism of cytoplasmic AM.

Our results show clearly that the oscillating tension-force development of the veins, i.e. their periodical longitudinal contractions, is accompanied by a cycling assembly and disassembly of AM fibrils when isometric conditions prevail. Isometric condition may prevail also for protoplasmic strands *in situ* adhering more or less strongly to their growing substrate (agar or filter paper). Under these circumstances for longitudinal contractions of the veins, isometric conditions should be present for most parts of the veins within the intact plasmodium.

According to biochemical investigations (see Hatano, 1973; Pollard and Weihing, 1974), the cytoplasmic actin content of *Physarum* makes up 5 to 20% of the total protein of the plasmodium: myosin content lies between 2 and 10%. As in muscle, G-actin has a molecular weight of approximately 42–48,000 and polymerizes by binding one Mol of ATP and Ca^{++} to F-actin. F-actin is made of a double helix of G-actin molecules. This F-actin chain (actin filament) may

reach a length of approximately 2.2 μm. When ATP and the divalent cation are removed, G-actin looses its ability to polymerize to F-actin.

Connected with the rapid translocation processes of protoplasm during shuttle streaming in *Physarum* and considering the ephemeric nature of the cytoplasmic AM fibrils, the question was raised whether a transformation of G⇌F-actin plays a role in the contraction cycle of cytoplasmic AM. Hinssen (1972) was able to produce paracrystalline F-actin from a fraction of the *Physarum* ground cytoplasm containing no actin filaments: the formation of paracrystalline actin was accomplished by addition of 50 mM $MgCl_2$ to an original fraction of ground cytoplasm containing no F-actin. From this finding it can be concluded that the ground cytoplasm of *Physarum* contains a large portion of G-actin or short chained F-actin, which can be polymerized to F-actin under certain conditions. Therefore, at least the possibility has to be considered that a part of the cytoplasmic actin within the plasmodium is transported as G-actin during locomotory translocation processes, e.g. during shuttle streaming within the endoplasm. Shuttle streaming leading to locomotion and contraction rhythms are oscillatory processes working in minute rhythms. It has to be taken into account that in both cases a G⇌F-actin transformation may be involved. In this connection it is interesting to note that blood platelet AM (thrombostenin) in the resting platelet seems to be present in a non-polymerized form and is first transformed into a polymerized, i.e. filamentous form, after activation of the platelets (Lüscher, 1975). Here again it appears that the activation of the contractile protein is bound to a polymerization, i.e. an assembly of F-actin.

In order to make further statements concerning the question whether a G⇌F-actin transformation plays a role in the relaxation-contraction cycle of *Physarum*, the G-F-actin ratio must be determined, comparatively in flowing endoplasm (presumably a state of relaxation) and in the stationary ectoplasm (predominantly a state of contraction).

While the results of our present experiments concerning the cycling patterns of AM fibrils allow a clear correlation between tension force increase and AM fibrillogenesis, for the disaggregation of AM fibrils during isometric relaxation two possibilities exist: (1) there is only a deparallelization of F-actin or (2) this deparallelization is accompanied (or brought about) by depolymerization of F- to G-actin (actin disassembly). The elucidation of this question is probably not possible using the normal sectioning technique due to the fact that G-actin molecules can not be visualized in ultrathin sections. In our laboratory biochemical techniques which require cell homogenization proved to be inadequate due to the fact that homogenization without exception resulted in a transformation of the AM fibrils. Cryo-ultramicrotomy in connection with the negative-staining procedure should be applied to analyze semi-quantitatively whether in defined isometric contraction stages more F-actin can be demonstrated as in the flowing endoplasm, which can be regarded as a predominantly relaxed state.

References

Alléra, A., Beck, R., Wohlfarth-Bottermann, K.E.: Weitreichende fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. VIII. Identifizierung der Plasmafilamente von *Physarum polycephalum* als F-Actin durch Anlagerung von heavy meromysin *in situ*. Cytobiologie **4**, 437–449 (1971)

Alléra, A., Wohlfarth-Bottermann, K.E.: Weitreichende fibrilläre Protoplasmadifferenzierungen und

ihre Bedeutung für die Protoplasmaströmung. IX. Aggregationszustände des Myosins und Bedingungen zur Entstehung von Myosinfilamenten in den Plasmodien von *Physarum polycephalum*. Cytobiologie **6**, 261–286 (1972)

Fleischer, M., Wohlfarth-Bottermann, K.E.: Correlation between tension force generation, fibrillogenesis and ultrastructure of cytoplasmic actomyosin during isometric and isotonic contractions of protoplasmic strands. Cytobiologie **10**, 339–365 (1975)

Hatano, S.: Contractile proteins from the myxomycete plasmodium. Advan. in Biophys. **5**, 143–176 (1973)

Hinssen, H.: Actin im isolierten Grundplasma von *Physarum polycephalum*. Cytobiologie **5**, 146–164 (1972)

Hülsmann, N., Haberey, M., Wohlfarth-Bottermann, K.E.: Vitalmikroskopische Darstellung cytoplasmatischer Actomyosin-Fibrillen. Microscopica Acta **76**, 38–47 (1974)

Isenberg, G., Giesbrecht, P., Wecke, J., Wohlfarth-Bottermann, K.E.: Demonstration of cytoplasmic actomyosin fibrils by the freeze-etching technique. Microscopica Acta **77**, 30–36 (1975)

Kamiya, N.: Contractile properties of the plasmodial strand. Proc. Japan Acad. **46**, 1026–1031 (1970)

Kamiya, N.: Contractile characteristics of the myxomycete plasmodium. Proc. IV. Internat. Biophysics Congress Moscow, 1972 (Symposium III, in press)

Komnick, H., Stockem, W., Wohlfarth-Bottermann, K.E.: Cell Motility: Mechanisms in protoplasmic streaming and ameboid movement. Int. Rev. Cytol. **34**, 169–249 (1973)

Lazarides, E.: Tropomyosin antibody: The specific localisation of tropomyosin in non-muscle cells. J. Cell Biol. **65**, 549–561 (1975)

Lazarides, E., Weber, K.: Actin antibody: The specific visualisation of actin filaments in non-muscle cells. Proc. Nat. Acad. Sci. USA **71**, 2268–2272 (1974)

Lüscher, E.F.: Microfilaments in blood platelets. Hoppe-Seyler's Zeitschr. Physiol. Chemie **356**, 382 (1975)

Nagai, R., Yoshimoto, Y., Kamiya, N.: Changes in fibrillar structures in the plasmodial strand in relation to the phase of contraction-relaxation cycle. Proc. Japan Acad. **51**, 38–43 (1975)

Parducz, B.: Eine neue Schnellfixierungsmethode im Dienste der Protistenforschung und des Unterrichts. Ann. Mus. Nat. Hung. **2**, 5–12 (1952)

Pollack, R., Osborn, M., Weber, K.: Patterns of organization of actin and myosin in normal and transformed cultured cells. Proc. Nat. Acad. Sci. USA **72**, 994–998 (1975)

Pollard, T.D., Weihing, R.R.: Actin and myosin and cell movement. CRC Critical Rev. Biochem. **2**, 1–65 (1974)

Schliwa, M., Bereiter-Hahn, J.: Pigment movement in fish melanophores: Morphological and physiological studies. Cell Tiss. Res. **158**, 61–73 (1975)

Schroeder, T.E.: The contractile ring. II. Determining its brief existence, volumetric changes, and vital role in cleaving *Arbacia* eggs. J. Cell Biol. **53**, 419–434 (1972)

Weber, K., Gröschel-Stewart, U.: Antibody to myosin: The specific visualization of myosin-containing filaments in non-muscle cells. Proc. Nat. Acad. Sci. USA **71**, 4561–4564 (1974)

Wohlfarth-Bottermann, K.E.: Cell structures and their significance for ameboid movement. Int. Rev. Cytol. **16**, 61–131 (1964)

Wohlfarth-Bottermann, K.E.: Weitreichende fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. III. Entstehung und experimentell induzierbare Musterbildungen. Roux' Archiv f. Entwicklungsmechanik **156**, 371–403 (1965)

Wohlfarth-Bottermann, K.E.: Plasmalemma invaginations as characteristic constituents of plasmodia of *Physarum polycephalum*. J. Cell Sci. **16**, 23–27 (1974)

Wohlfarth-Bottermann, K.E.: Tensiometric demonstration of endogenous oscillating contractions in plasmodia of *Physarum polycephalum*. Zeitschr. Pflanzenphysiol. **76**, 14–27 (1975a)

Wohlfarth-Bottermann, K.E.: Weitreichende fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. X. Die Anordnung der Actomyosin-Fibrillen in experimentell unbeeinflußten Protoplasmaadern von *Physarum in situ*. Protistologica **11**, 19–30 (1975b)

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