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Studies on contraction rhythm of the plasmodial strand

by

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I  Synchronization of local rhythms

SUMMARY

To express the process of attaining synchrony of local rhythms in a segment of plasmodial strand excised from a network of Physarum plasmodium under isotonic conditions, the entire segment of the isolated strand was divided into subsegments with particles attached as index markers and their cyclic changes in length were registered in a set of minor wave trains. Thus entire vista of local contraction rhythms was obtained. For convenience sake, one wave range was separated from the adjacent ones with the time coordinates of the peaks of the major waves, or of the maximal contractions of the whole strand. Then the standard deviation of the time coordinates, when the individual subsegments reached their maximal length, was calculated for each wave range. This value decreases with time reaching after 30 min as small as 3-5 sec or 3% of the period of the major waves. Under isometric conditions, the method using index markers was also useful, but we demonstrated the synchrony also by the fact that the amplitude, period and phase of the tension waves became independent of the length of the strand. Once the contraction-relaxation cycle of each segment in the strand is synchronized, it is maintained under isotonic as well as isometric conditions. The facts presented here raise a new problem as to how information about period and phase is transmitted so that local contractile oscillations can be unified.
INTRODUCTION

A segment of strand excised from the network of myxomycete plasmodium contracts and relaxes with a certain rhythm. KAMIYA and SEIFRIZ (1954) measured twisting of the Physarum strand simultaneously with changes in length under isotonic conditions and demonstrated directly rhythmic contractile activities of the material. In order to gain further insight into dynamic characteristics of the plasmodial strand, KAMIYA et al. (1970, 1972ab, 1973) constructed a sensitive electromagnetic tensiometer which can record contractile activities of the strand either under isometric or under isotonic conditions. Some important dynamic properties of the slime mould strand, such as activation caused by loading or stretching, were already described in some detail. Rhythmic contraction of the strand is closely related with cyclic structural changes in microfilaments composed primarily of F-actin (NAGAI et al. 1975, 1976, FLEISCHER and WOHLFARTH-BOTTERMANN 1975, WOHLFARTH-BOTTERMANN and FLEISCHER 1976).

Still unknown is how local contraction rhythms are gradually unified into a regular contraction rhythm. In the present report, an attempt was made to describe the process in which rhythms in different parts in a segment of plasmodial strand gain synchrony under isotonic as well as isometric conditions.
MATERIAL

The plasmodium of *Physarum polycephalum* was used exclusively for the present experiments. The material was cultured with compressed oats on sheets of wet filter paper lining the bottom and side wall of a plastic bucket. Outgrowth of the plasmodium was detached together with filter paper from the side wall of the bucket and dried slowly for one day. Sclerotia obtained with this procedure were stocked in the desiccator.

For preparing experimental materials, a sheet of the sclerotium of a size of ca. 10 cm² was placed on one edge of the rectangular plate (20 x 30 cm) of 1.5% agar prepared with tap water and left there overnight. During 15-20 hr, the sclerotium on the agar plate developed an active plasmodium. Except the advancing front zone, the plasmodium took the form of a network with many ramifying strands.

A segment of smooth plasmodial strand, 10-40 mm in length and 0.5-0.9 mm in diameter, was excised carefully from the network and served as material.

METHODS

Using a specially constructed vertical type tensiometer with a sensitivity of ca. 0.1 mg (Fig 1), rhythmic contraction of a segment of plasmodial strand was recorded according to the procedure stated before (KAMIYA et al. 1970, 1972ab, 1973). The upper end of the plasmodial segment
was hung around a tiny cross bar attached to the stalk of the fine glass hook. The terminal region of the strand soon fused into a tiny mass of the plasmodium surrounding the cross bar on the hook. The cross bar helped prevent slippage between the strand and the hook. The hook was connected to the tensiometer by a piece of glass fiber. The lower end of the plasmodial strand was attached to another hook affixed to the bottom of the moist chamber. Either isometric tension production or isotonic shortening and elongation of the strand was recorded by setting a compound switch (S) of the tensiometer at the appropriate position.

**Isotonic contraction**

In order to record local changes in length of the strand under isotonic conditions, plastic (acrylonitrile-styrene resin) particles, 0.2-0.5 mm in diameter, were attached to one side of the strand as index markers at a nearly equidistant interval of 2-5 mm (Fig 2). While isotonic length changes of the whole strand were recorded on a chart of the pen-recorder, positions of these particles were recorded photographically at every 10 sec. Successive changes in length of each segment (a-f, Fig 2) between two adjacent index particles were measured by serial photographs thus taken.

**Isometric contraction**

Besides the above mentioned method of attaching index markers, we used also the following means to check synchrony of tension force production in different parts of the strand under isometric conditions.
A smooth plasmodial strand was held in the moist chamber equipped with one or two horizontal sliding rods (Fig 3). To the end of the sliding rod (SR) facing the plasmodial strand was cemented a small piece of elder tree pith (P). The rod could be shifted manually through the side wall of the chamber so that the tip of the rod, 1-2 mm wide, came in contact with the strand. Once the rod end with a piece of dry pith was attached to the strand, no slippage occurred any longer at the attached area with the tension force developed in the strand. If the plasmodial strand was clamped on both sides gently with a pair of similar sliding rods, the strand could be held firm enough even when the upper part of the strand was stretched. Since the strand was mechanically fixed at the contact region, the tension recorded after the contact represented only that developed by the portion of the strand above the fixed region.

If cyclic tension production in every part of the strand was perfectly synchronized under isometric conditions, the amplitude of cyclic tension changes must be independent of the length of the strand. Hence, no changes in amplitude was expected to occur when the effective length of the strand was made shorter through mechanical holding. Were there local discrepancies in phase, however, the amplitude of tension waves would be larger the shorter the strand and there would also be a shift of phase of the waves before and after the mechanical clamping.
RESULTS

Isotonic contraction

After a segment of smooth plasmodial strand was isolated from the network and set to the tensiometer, 5-10 plastic particles were attached with a nearly equal interval to one side of the strand (Fig 2). Within 5 min after a segment of the strand was excised, electronic registration of isotonic changes in length of the strand was started and at the same time, positions of the particles attached to the strand were recorded photographically as described in the Methods section.

There was a quiescent stage lasting 10-20 min for a segment of the plasmodial strand after it was isolated from the network of the mother plasmodium. This quiescent stage is referred to in the present paper as "stage I". When the stage I elapsed, the strand gradually began to contract and relax with wave patterns which were still irregular. This irregular stage is designated as stage II. It lasted for 10-20 min. Then the plasmodial strand entered the next stage where the contraction-relaxation waves manifested regular periodicity and enhanced amplitude. This regular stage is called stage III.

Fig 4 shows an example of isotonic length changes of all the subsegments (a-f), each ca. 3 mm long, of a piece of strand ca. 20 mm long. The total contraction was represented by the sum of minute contractions of all the segments. This coincided with length changes of the strand recorded electronically on a chart of the tensiometer.
In the stage I of isotonic contraction, appreciable rhythmicity was absent also in minor local segments (Fig 4-I). Thus lack of rhythmicity in total contraction may not be interpreted as a result of statistical cancellation of local oscillations which are not in phase on the visual level.

Following the stage I, i.e. about 15 min after the strand was isolated, subsegments of the strand began to contract and elongate rhythmically, although durations of the stage I differed slightly according to individual subsegments. In the subsequent stage, or stage II, contractile rhythms in individual subsegments were still irregular, and time coordinates of their peaks diverged (Fig 4-II). Contraction of the whole strand became, however, increasingly regular by 30 min. Contraction-elongation rhythms of subsegments became also regular and synchronous (Fig 4-III). The synchrony of local activities is shown by convergence of times of "elongation maxima" or trough of the waves. Their positions are indicated with short vertical lines in Fig 4.

As a measure to quantitize objectively how these contraction waves were gradually synchronized with time after stage I, we adopted standard deviation (σ) of time coordinates of elongation maxima of the component waves. In Fig 5, the time course of acquiring higher synchrony of contraction waves (a-f) is represented with decrease in σ value. In Fig 5, σ was 19 sec, or 13% of the period (142 sec) in the early part of the stage II (18 min after isolation) showing that the time coordinate of elongation maxima diverged to a considerable extent. Although there was a slight difference in length of the subsegments, we did not make
correction for it in calculating the standard deviation in the present case. The standard deviation diminished rapidly at first until it reached stage III, where $\sigma$ was kept nearly constant at 4 sec in average or 3% of the period (137 sec in average). This fact indicates distinctly that contractile waves generated in different loci in one and the same strand became highly but not completely synchronous with one another as time elapsed after the strand was isolated.

**Isometric contraction**

If every part of an evenly thick segment of the plasmodial strand changed the tension force rhythmically under isometric conditions with the same period and in the same phase, it is expected that there are no local changes in length and that pattern and magnitude of tension production must be independent of the length of the strand. Experiments were done in two different ways.

Experiment I. In one group of the experiments, changes in length of subsections of the strand separated with index markers were measured at first under isotonic conditions as was the case in the foregoing experiment. After a while, the isotonic condition was converted into isometric condition and the recording of index markers was continued. Later the strand was brought back again to the isotonic condition.

One of the results of this kind of experiments is shown in Fig 6. Under the isotonic conditions in stage III, the plasmodial strand contracted and relaxed with regular periodicity. Changes in length of each subsegment of the strand (a-f) were sufficiently synchronized with one another.
The peak-to-peak amplitude of length changes of the whole strand were 21% of the average length. On the other hand, changes in total length of the strand under isometric conditions were practically null and not detectable (less than 10 μm for a load of 100 mg), showing that tensiometry of the whole strand was performed under an exactly isometric condition.

Under this condition, segments a-f, which had changed their lengths by more than 20% under isotonic conditions, showed no longer any conspicuous changes in length, but moderate changes in length less than 5% were visible locally. One might think from this record that synchrony was not satisfactory as was supposed from the preceding isotonic record, but it is to be noted in this case, that when a local shortening occurs by a slight unbalance in tension force production, the strand must be passively stretched elsewhere as the total length is fixed. This situation has an effect to exaggerate a slight discrepancy in phase. After converting isometric condition to isotonic condition again, we realize that the six component waves were highly synchronized as far as their elongating maxima were concerned.

Experiment II. Another method to confirm synchrony of periodic tension force production over the strand under isometric conditions is to clamp a part of the strand mechanically and to make the effective length of the strand shorter. For this purpose, special chambers with a pair of slide arms were made as described in the section of Methods (Fig 3).
An example of the results obtained was shown in Fig 7 where the original length of the whole strand was represented by \( a \). The length of the upper portion of the strand proximal to the tensiometer from the region held, or the length of the strand effective for tensiometry, was designated as \( c \), and the lower portion including the area attached to the tip of the sliding rod as \( b \). The time when the strand was mechanically fixed is indicated by the arrow. After mechanical fixation, isometric tension force produced in the strand at and beyond the fixed region was no longer measured. Tension waves after clamping in Fig 7 represent those produced only by portion \( c \), which was 1/5 the length of the whole strand \( a \).

It is shown in Fig 7 that there were no changes in the tension waves after the mechanical clamp both in respect to amplitude and period. Further no shift in phase was observable after the clamping. Were there any local discrepancies in phase, the amplitude of tension changes measured under an isometric condition would become larger by making the length of the strand shorter. Fig 8 shows an example of such cases. If the strand is in full synchrony, no changes in amplitude are expected after it was clamped at whatever site and whatever phase of a contraction cycle. The reverse must also be true. Namely, when there are no change in amplitude and phase of the tension waves before and after clamping, it will serve as evidence for synchronization.
DISCUSSION

A segment of plasmodial strand excised from a network of the mother plasmodium exhibited no appreciable periodicity soon after it was isolated. We have never found the strand which showed the periodicity immediately after isolation. As will be discussed in Chapter IV, there are reasons to believe that the absence of periodicity after isolation is not solely due to the injury caused by the operation, but to the intrinsic characteristics of the mother plasmodium's locus from where the segment of the strand has originated. According to our experiments to be described later, it is likely that the periodic activity of the migrating plasmodium resides mostly in the front zone. If this is the case, the above fact implies that the strand had no active periodicity in situ when it was a part of the caudal network of the mother plasmodium and that rhythmicity is initiated only after a segment of the plasmodium becomes an independent whole detached from the mother plasmodium. Certainly this is an intriguing biological problem for which further analysis is needed.

When the lag stage ( stage I ) lasting for 10-20 min was over after the strand was isolated from the network, it began to gain periodical activity locally ( stage II ). Concomitantly, the cyclic contraction in each part of the strand became gradually synchronized. Twenty to forty min later, the local cyclic contractions in the strand became sufficiently, if not perfectly, synchronous both under isotonic and isometric conditions ( stage III ). We are
interested to know how such synchronization is realized. There must be some mechanism with which different loci in the strand are mutually informed about period and phase of oscillation. We shall consider this problem further in the next chapter.

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A diagram of the vertical type tensiometer. Either isometric or isotonic contraction of the plasmodial strand can be measured by placing a compound switch (S) at the upper or lower position. The plasmodial strand (PS) was held between the upper hook connected to the electrobalance (EB) and the lower hook affixed to the bottom of the moist chamber (MC). L: light source, PR: photoresistor, P₁, P₂: potentiometers, RP: rack and pinion, C: clutch, WG: worm gear.
A segment of plasmodial strand (PS) set in position for tensiometry. For measuring isotonic contraction of the whole strand, the lower end of the strand connected with the lower hook affixed to the bottom of the moist chamber moves up and down automatically so that the tension of the strand is kept always constant. This movement is registered on a chart electronically. To record local changes in length small plastic particles serving as index markers were attached to the strand at nearly equidistant interval. Changes in length of subsegments (a–f) divided by the markers were measured by taking photographs every 10 sec.
The moist chamber equipped with the horizontal sliding rods (SR) for holding the plasmodial strand (PS) to make the effective length shorter. To one end of the sliding rod facing the strand is cemented a small piece of pith (P). The pith helps prevent slippage between the strand and the rod when the two come in contact.
Fig 4  Isotonic length changes of subsegments (a-f) and of the whole strand. The figure represents records covering 5-60 min after the strand was isolated. According to the degree of synchronization, the process was divided into stages I, II, III. Solid vertical lines on the waves represent time coordinates of maximal lengths of the subsegments and of the whole strand.

Fig 5  Standard deviation in time coordinates of elongation maxima (troughs) of subsegments shown in Fig 4 (a-f). The time course of attaining a higher degree of synchronization is represented as a decrease of σ.
Fig 6 Local and total changes in length of the strand under isotonic and isometric conditions. Vertical lines on the waves show time coordinates of troughs (elongation maxima) of respective waves. Arrows at the top indicate the times of conversion from isotonic to isometric or from isometric to isotonic conditions. Waves with a solide ine at the bottom show isometric tension changes.

Fig 7 Isometric tension changes before and after a part of the strand was clamped to make the effective length shorter. The strand was already in stage III. a: length of the whole strand, b: length of the lower part of the strand including the region attached to the rod end, c: length of the upper free part of the strand. After the strand was clamped at the time indicated by the horizontal arrow, tension recorded represents only that produced in the upper part (c) of the strand. Note no changes in period and phase.
Isometric tension changes under the condition similar to Fig 7 except that the strand was in the stage (stage II) where tension production was still irregular. Note appearance of regular waves after the effective length of the strand was made 1/5 the original length. Time 0 on the figure represents 12 min after the strand was isolated.
II  Effect of externally applied forces

SUMMARY

Rhythmic contraction of a segment of plasmodial strand was recorded under either isometric or isotonic conditions. When the strand was stretched during isometric tension force measurement, or when the strand was loaded with a higher tension during isotonic length measurement, the amplitude of the oscillation is augmented. But these treatments neither change the period nor shift the phase of the cycle. When the strand was stretched instantaneously by as much as 30-50% of the whole length, the oscillation in tension production became irregular or even lost for a while. Nevertheless the waves recovered subsequently are found in such a phase as to be expected through extrapolation from the waves before stretching. These facts suggest the presence of the latent cycle, the phase of which advances independently of the externally applied forces.

INTRODUCTION

As reported in chapter I, a segment of plasmodial strand of *Physarum polycephalum* showed no oscillatory activity soon after it was isolated from the mother plasmodium. In the lapse of 20-40 minutes when local contraction-relaxation activities start oscillation and are brought in phase with one another, the whole strand began to contract and relax regularly both under isometric and isotonic conditions. To understand this phenomenon it may be necessary
to postulate the presence of a mechanism with which information about phases of local rhythms are transmitted. It is unknown that tension force *per se* can act as the transmitting agent of phase information. What has been made clear so far is that the amplitude of tension or length waves of the strand is increased when the tension level is made higher by stretching under isometric conditions or by additional loading under isotonic conditions (KAMIYA et al. 1970, 1972ab, 1973). Further it is known that the period of the wave is not affected in these cases (KAMIYA and YOSHIMOTO 1972). In the present paper we studied the response of the slime mold strand under several selected mechanical conditions at an attempt to rule out the possibility of tension force to be a candidate for a transmitter of phase information.

**MATERIALS and METHODS**

Segments of smooth plasmodial strand of *Physarum polycephalum*, 10-40 mm in length and 0.5-0.9 mm in diameter, served as material. They were excised from a network of a huge plasmodium developed overnight from a sufficient amount of sclerotia on the plain 1.5% agar plate as reported previously.

The method for measuring and recording isometric tension changes or isotonic length changes was also the same as before. Further descriptions of methods for different experiments will be made later in respective places.
RESULTs

Effect of stretching on rhythmic tension force development under isometric conditions

After recording spontaneous tension changes under isometric conditions, we increased the tension of the strand by stretching it quickly by 10-20% of the whole length and continued to record the subsequent tension force generation.

Stretching of the plasmodial strand by 10-20% of the whole length was accompanied with two- to tenfold increase in tension force and simultaneously with marked augmentation of the amplitude of isometric tension waves. Increase in non-periodic tension level (the axis of the waves) relaxed rapidly at first and gradually later. The period of tension waves, however, did not change after stretching. This response of the strand on stretching was reported before (KAMIYA and YOSHIMOTO 1972).

Another important characteristics of the response on the part of the strand was that no shift of the phase of waves was observed after stretching. Fig 9 shows an example of the results. The strand was stretched twice, each time by 20% of the whole length. Open circles and closed circles before stretching indicate positions of "contraction maxima" and "relaxation maxima" of the tension waves respectively. Open and closed circles on the waves after stretching indicate presumptive time coordinates of the contraction and relaxation maxima extrapolated from the waves before stretching. As is shown in Fig 9, time coordinates of the open and closed circles
well coincide with those of the maxima and minima of the measured waves after stretching. This fact shows that the stretching of the strand caused no shift in phase of the contraction-relaxation cycle.

Effect of loading on rhythmic contraction and elongation under isotonic conditions

In another series of experiments where cyclic contractile activities were measured and recorded under isotonic conditions, we increased the applied load stepwise, each time by 5-20 mg. Such increase in load caused increase in amplitude of cyclic changes in length up to a critical level (KAMIYA and YOSHIMOTO 1972). Under isotonic conditions the applied tension is kept constant there being no tension relaxation such as seen after stretching under isometric conditions. Augmentation of amplitude of the isotonic waves did not decrease in this case (Fig 10).

The period of the waves remained constant after additional loading. As is seen in Fig 10, when the load was increased as high as 50 mg, the strand could no longer hold it and was elongated irreversibly being snapped eventually. Even in this process of elongation, there was neither change in period of the waves nor shift in phase.

The foregoing results show that the amplitude of the waves representing isometric tension changes or isotonic length changes depend clearly upon the applied tension. At the same time they show that the oscillatory function, or pacemaking, of contractile activity is regulated independently of the external force. This conclusion is further supported
Conversion between isometric and isotonic contractions

The tensiometer which we constructed made it possible to convert the measuring system for isometric contraction into that for isotonic contraction, or vice versa isotonic contraction into isometric contraction, instantaneously with a single switch at an arbitrary time (KAMIYA et al. 1970, 1972ab, 1973). Hence, it is possible with this setup to study the phase relation between the two waves, one representing isometric tension changes and the other isotonic length changes of the strand.

An example of the records is shown in Fig 11. The two measuring conditions were converted from one to the other three times at different phases of waves. The length of the strand under isotonic conditions was shown to increase downward and decrease upward on the ordinate in this case. This is because changes in tension and those in length are opposite in sign. First, it should be noted that the phase of maximal tension in isometric contraction wave coincides with the shortest phase in isotonic contraction wave and not with the phase where the contraction rate is maximal. Although this is a point extremely important for gaining insight into the mechanism of contraction of this material, we should like to restrict our consideration here to the facts relevant to the present context.

Fig 11 and many other records of similar experiments reveal that 1) there was no difference in period between the two waves, isometric and isotonic, and 2) there was
no shift of phase after conversion. No matter whether the length of the strand was kept constant under isometric conditions or tension was kept constant under isotonic conditions, the period and phase of cyclic contractile activities were the same indicating that the oscillatory mechanism is operated independently of tension changes as well as length changes.

**Tension force production of the strand elongated and shortened periodically by artificial means**

To confirm the above conclusion further, we stretched and shortened the strand periodically with a suitable device while measuring the tension force continuously. Stretching was performed as a sinusoidal function of time at various frequencies and amplitudes. Fig 12-a shows the case in which the strand was stretched by 5.5% of the total length with a period of 35 sec when the inherent period was about 120 sec. An example of stretching with a period longer than the inherent period is shown in Fig 12-b. The inherent period was about 96 sec while the period of externally applied stretching was 130 sec. In both cases, no shift of the original period to the period applied from without was observed. These results indicate that periodical changes in tension or length superposed on natural activity could not alter the intrinsic cycle of dynamic activity of the strand.
Tension force production of a system composed of two strand segments in series

To see whether or not cyclic tension changes in one part of the strand may exert some influence to the wave parameters of the other part of the strand through mechanical transmission, we connected the two different segments of the strand (PS₁, PS₂) in series intervened with a piece of delicate glass rod and measured tension production in this system (Fig 13). The glass rod was equipped with two cross bars at its both ends so that it can hold the plasmodial strand conveniently. With this setup there was no chemical or electrical transmission between the two segments except that mechanical force was transmitted from one to the other. If mechanical force is a transmitting agent of information about the phase of cyclic tension production, each of the two segments of the strand is expected to contract and relax in the same period, because there is a mechanical coupling between them by which the tension force in one segment can be conveyed to the other. If the above assumption were actually the case, the tension force of this compound system would be produced as if the system were a single segment of strand.

One of the results of this kind of experiments is shown in Fig 14. The tension of the compound system changed in an irregular pattern which a single segment of strand rarely exhibited. It may indicate that two segments of the system contracted and relaxed with their own period independently of each other. Again this is another piece of evidence
showing that changes in tension or length do not play a direct part in synchronizing contraction-relaxation rhythm.

**Effect of acute stretching**

When the strand was stretched suddenly by as much as 30-50 % of the whole length under isometric conditions, the cyclic tension change of the strand was often prevented for a while. The inhibited stage lasted usually 2-10 min. Leaving aside the cause of this phenomenon, we are interested to see whether or not the phase of cyclic contraction wave advances during the period of inhibition. If there were a clock mechanism controlling the cycle of contractile activity of the strand, the mechanism which is operated independently of tension produced, the phase of the cycle would advance during this period.

To see whether or not this is actually the case, we stretched the strand instantaneously by as much as 50 % of the whole length and followed the time course of cyclic tension changes of the strand under an isometric condition. One of the results is shown in Fig 15 where open circles and closed circles before stretching indicate respectively positions of "contraction maxima" and "relaxation maxima" of the waves. Open and closed circles after stretching represent tension levels at the moment of presumptive peaks and troughs extrapolated from the pre-stretched waves with the assumption that the clock advanced normally. As is shown clearly in Fig 15, the peaks after the irregular period were sufficiently coincident with time coordinates of the expected peaks.
This fact is a further example which supports the view that the phase of the cyclic contraction can advance even when contraction per se is inhibited.

DISCUSSION

It was shown previously that both under isometric and under isotonic conditions of the plasmodial strand the increase in tension enhanced the amplitude of the contraction waves (KAMIYA et al. 1970, 1972ab, 1973). In the present paper we demonstrated that the phase of the cyclic contraction advanced independently of tension changes or length changes. This conclusion is perfectly in conformity with the fact shown by KAMIYA and NAKAJIMA (1955) that the phase of shuttle streaming in the plasmodium advanced even when the endoplasm was set to gel through application of 10% CO₂ and the streaming was brought to a standstill. These facts suggest that there must be a physiological clock controlling the period of contraction-relaxation cycle and that neither tension changes nor length changes play an essential part in operating the clock. Transmitter of information with which the local asynchrony is supposed to be unified into a single rhythm must be sought somewhere other than the mechanical force. Recently we found some evidence showing that it is endoplasm that carries necessary information. We shall describe details of this problem in the subsequent chapter.
REFERENCES


Effect of stretching on tension production of the strand. While measuring isometric tension changes, the strand was stretched stepwise twice by 20% of the whole length. Open circles and closed circles before stretching show maxima and minima of cyclic tension production. Those after stretching represent coordinates of the waves at presumptive moments of maximal and minimal tension extrapolated from the pre-stretched waves. Note period and phase have not been changed after stretching.
Fig 10

A record of isotonic contraction under stepwise loading. Closed circles before loading indicate the coordinates of maximal length, and those after loading the coordinates of the waves at presumptive moments of maximal elongation extrapolated from the pre-loaded waves. Note these circles coincide well with peaks of the waves actually recorded.
Alternate conversions between isometric and isotonic contractions of the plasmodial strand. The ordinates for isotonic contractions are shown inverted. No shift of the phase occurred after conversions. Upper waves: isometric tension changes, lower waves: isotonic length changes.
Fig 12

Changes in tension when the strand was stretched and shortened in a sine-wave pattern with period of 35 sec (a) and 130 sec (b) while the intrinsic periods were ca. 120 sec (a) and 96 sec (b). No aftereffect of periodic stretching was detectable.
Fig 13

Two segments of the plasmodial strand (PS₁, PS₂) connected in series with a piece of fine glass rod.
Fig 14

Isometric tension changes in the compound system consisting of two segments of plasmodial strand as shown in Fig 15.
Tension waves under extreme stretching. The strand was stretched in a moment by 50% of the whole length. Open circles and closed circles before stretching indicate coordinates of maximal and minimal tension phases. Open and closed circles after stretching represent coordinates of tension levels at the moments of presumptive peaks and troughs extrapolated from the pre-stretched waves. Note that they coincide almost perfectly with actual peaks and troughs of the waves except a period after stretching where tension development was inhibited.
III Role of endoplasmic streaming in synchronization of local rhythms

SUMMARY

The periods of cyclic contraction of two separate segments of two plasmodial strands (Physarum polycephalum) are generally different, but if the two are bridged with each other with another small segment of strand so that the three segments are fused into one, the contraction cycles of the two previously independent segments become gradually unified either under isometric or under isotonic conditions. To check the possible role of endoplasm as information carrier in synchronization, we controlled its streaming between the two parts of the strand using the double-chamber technique. Contraction-relaxation cycles of the two halves of the strand, which were once in good synchrony, became out of phase with each other when the endoplasm was prevented from flowing between the two. After the endoplasm in the strand was allowed to stream freely again, the synchrony of their cyclic contraction was soon re-established. Thus it was concluded that the endoplasm flowing back and forth in a plasmodial strand must carry as yet unknown factor(s) which control the period and phase of contraction-relaxation cycle.
INTRODUCTION

We have shown in the foregoing chapters that a segment of the plasmodial strand or vein gradually acquires rhythmic contractile activity after it is excised from the network of a plasmodium of Physarum polycephalum, and that the phase of contraction-relaxation cycle advances independently of a change in tension or in length. In order to check the possibility of endoplasmic streaming as an agent carrying phase information for synchronization, we conducted several experiments in which the streaming of the endoplasm was modified by one way or other. First, we took advantage of the unique capacity of myxomycete plasmodia to be fused into a single larger plasmodium if brought in contact with one another or the capacity of a single plasmodium to be divided into smaller plasmodia by cutting without any ill effect. In another series of experiments, we controlled the endoplasmic streaming in the strand resorting to the double-chamber technique.

MATERIALS and METHODS

Segments of smooth plasmodial strand (or vein) of Physarum polycephalum, which served as material for the present experiments, were 10-40 mm in length and 0.5-0.9 mm in diameter. The method of their preparation was the same as described before.

Isometric contraction

Isometric tension force production in two different segments of plasmodial strand or in two different parts of
a single segment can be measured and recorded simultaneously using two sensitive spring balances (S₁, S₂) made of steel fiber such as used previously by us (KAMIYA and YOSHIMOTO 1972). As is shown in Fig 16, each plasmodial strand (PS₁, PS₂) was held between two pairs of glass hooks. The upper hooks, each with a tiny cross bar, were connected with the two delicate spring balances (S₁, S₂) one for each by a piece of vertical glass fiber. The two lower hooks were affixed to the bottom of the moist chamber.

With this setup, the distances between the free ends of the horizontal spring (S₁, S₂) and the reference bars (R₁, R₂) were changed in proportion to the tension force developed by the respective strands. A slight change in length of the strand was mechanically magnified by 7.4 x. The terminal parts of the springs and reference bars were all brought to the same vertical plane so that changes in position of the tips of the horizontal springs could be focussed and recorded simultaneously on the same photokymograph with optical magnification of 12.5 x. Thus the total magnification amounted to 92.5 x.

The detail of mechano-optical measurement was already reported (KAMIYA and YOSHIMOTO 1972). By this method the tension development of the plasmodial strand was measured practically under isometric conditions with possible changes in length less than 1% of the total length.

When tension force production in two halves of one and the same strand was to be measured simultaneously, the strand was held in a U-shape as shown in Fig 17. The
two terminal ends of the strand were attached to the upper glass hooks, while the central region of the strand was clamped with a pair of lower hooks projecting from the bottom of the moist chamber. By this method, isometric tension changes in two segments of strand (A, B) were measured independently of each other with the two spring balances, since the middle small region of the strand (C) was held mechanically.

**Isotonic contraction**

To measure changes in length of two parts of a plasmodial strand under isotonic conditions at the same time, a strand was suspended in a moist chamber in an inverted U-shape as shown in Fig 20. The middle region of the strand was held with two glass hooks projecting from the upper cover of the chamber. A proper load (W) in the range of 5-50 mg was applied to each of the free ends of strand using a small glass hook such as used before. With this setup, the endoplasm moved back and forth freely between the two hanging segments (A, B) which are mechanically independent. Isotonic length changes of the two parts of the strand were recorded photographically at every 10 sec.

**Double chamber method**

In order to measure isotonic length changes of the plasmodial strand while controlling the streaming of endoplasm in the strand, the moist chamber divided into two compartments was constructed. As shown in Fig 24, two segments of the strand (PS₁, PS₂) were hung, one for each in the two compartments.
from the upper hook and the third segment (PS₃) penetrating the tight-fitting groove in the septum connected the two hanging strands to make an inverted U-shape strand. The construction was such that the left and right compartments were divided air-tight, while endoplasm could flow freely along the strand, i.e., the vein with the wall of ectoplasmic gel, between the two hanging strands in the different compartments. To the lower free end of each of the hanging strand was attached a glass hook equipped with the cross bar. We could apply an appropriate weight through these hooks.

When there was no difference in air pressure between the two compartments, endoplasm flowed back and forth between the two hanging strands (PS₁, PS₂) through the connecting strand PS₃. By observing the endoplasmic streaming in the connecting strand at the middle region penetrating the central septum with an aid of horizontal microscope, it was possible to keep the endoplasm there at a standstill by adjusting the air pressure in one of the two compartments either higher or lower than the atmospheric pressure in the other compartment. The counter-pressure just sufficient to stop the streaming, or in short the balance-pressure, changed spontaneously with time. Changes in balance-pressure as indicated by a manometer and changes in length of the two hanging strands PS₁ and PS₂, were recorded simultaneously and successively with photographs at every 10 sec.
RESULTS

Synchronization and asynchronization of contractile rhythms on connection and disconnection in a plasmodial strand system

Rhythmic changes in tension force produced in different loci in the same plasmodial strand under isometric conditions, or rhythmic changes in length of the strand under isotonic conditions become sufficiently synchronized with time over the whole strand as reported before. It is an interesting but still unsolved problem, however, how local regions in the same plasmodial strand can pulsate in phase with the same period. We demonstrated already by the experiments described in the foregoing chapter that tension per se plays no part in the synchronizing mechanism of local rhythms. In the present experiment, we observed as the first step the behavior of the plasmodial strand with the experimental setup shown in Fig 17, where the central region of a single segment of the strand was held with the two glass hooks affixed to the bottom of the moist chamber.

Changes in cyclic tension force produced in two different parts (A, B) of the same strand were thus recorded simultaneously on the photokymograph. If the synchrony of the contraction-relaxation cycles were brought about by way of a mechanical factor, cyclic tension changes in two segments of the strand would not pulsate in the same phase in this system, since the two parts were mechanically independent of each other. The result was shown in Fig 18.
Cyclic tension forces produced in two different parts (A, B, cf. Fig 17) of a single strand under isometric conditions were sufficiently synchronized with each other in spite of the lack of mechanical interaction between them. Further to be noticed is that there was a close resemblance in pattern of corresponding waves in the two wave trains. The synchrony is demonstrated by good coincidence in time coordinates of maximal and minimal tension phases indicated with vertical lines in Fig 18.

When, however, the strand was cut at C (cf. Fig 17) between the two hooks on the bottom, the synchrony of rhythmic contractions of two disconnected segments was gradually lost. They began to contract with different periods as shown in Fig 19. Twenty seven minutes after the two segments A and B were disconnected, they were reconnected again with another short piece of plasmodial strand at the bottom. It is shown in Fig 19 that the two segments regained their synchrony in cyclic contraction during 30-50 min. The times of disconnection and reconnection were indicated with the arrows and phases of minimal tension, or "relaxing peaks" were indicated with vertical lines in Fig 19. The time of reconnection, however, did not show the time of fusion. It took usually at least several minutes for individual segments brought in contact before they were fused with one another to make a single continuous strand.

Virtually the same results were obtained in isotonic contraction. In this case a long segment of the strand was hung from a pair of glass hooks affixed to the top of the
chamber so that the entire strand took an inverted U-shape (Fig 20). By this means, the two vertical parts of the strand were mechanically separated with each other.

Fig 21 shows that the two hanging parts of the strand (A and B, cf. Fig 20) contracted and relaxed synchronously in the first part of the graph (0-21 min). When the two parts of the strand were disconnected at a region (C, cf. Fig 20) between the two hooks, they were gradually deprived of synchrony (22.5-47 min).

The two segments of the strand were then reconnected. As is shown in Fig 21, they regained the synchrony once again just as was the case under isometric conditions.

The above results show that the synchrony of the cyclic contraction of the plasmodial strand is brought about by a factor other than the mechanical force. This is true both under isometric and isotonic conditions.

**Asynchrony of two different strands**

We measured isometric contractions of two segments of strands from different plasmodia simultaneously in the presence and absence of the connecting strand between them. At first, isometric tension changes in the two strands (PS₁, PS₂) were recorded using the setup shown in Fig 16. The two strands were then connected with a short piece of plasmodial strand (PS₃) at their lower ends as shown in Fig 22. Several minutes elapsed usually before they were fused with one another to make one U-shape strand. Having recorded autonomous changes in tension of the previously separate parts (PS₁, PS₂) of the strand for some time,
PS₁, and PS₂ was disconnected again by removing PS₃.  

Fig 23 shows a record of isometric contractions of two different strands originated from different plasmodia. They contracted and relaxed independently with different periods before they were bridged with a short piece of strand and fused with one another. The period and phase of the two series of waves were unified in 30 minutes. This synchrony in period and phase of cyclic contractions in two different strands continued as long as the two were kept connected. It is important to note, however, that there is no correlation between the two contraction waves in respect to the amplitude. The amplitude depended upon a level of the tension of each contraction wave as reported before (KAMIYA et al. 1970, 1972ab, 1973). This can be demonstrated more strikingly when one of the two strands was stretched. In this case tension level of the stretched strand was heightened and the amplitude of the contraction waves was increased immediately, but the amplitude of rhythmic contraction of the other segment was not affected (at 97 min, middle part of Fig 23).

The above facts show that some information regarding the period and phase must be transmitted from one segment to the other through the connecting strand whereas information about amplitude is not carried. It is suggested that the
oscillatory mechanism bringing about cyclic contraction in the plasmodial strand is operated independently of the strength of tension force produced in the same system.

The behavior of the plasmodial strand described above provides us with the idea that something serving as information controlling periodicity of contraction is carried by the streaming of endoplasm. In this connection it is to be added that the phases of contraction waves of the two strands had a tendency to be less satisfactorily synchronized when the connecting strand became thin or when the connecting strand made lumps in which the endoplasm was accumulated. Under such conditions, the endoplasm in the connecting strand did not flow vigorously or regularly between the two strands.

Control of endoplasmic streaming

In order to confirm a possible role of endoplasmic streaming in synchronization, we controlled the shuttle streaming of the endoplasm between the two segments taking advantage of the double chamber technique described in the Method section (Fig 24). Two segments of strand, PS, and PS₂, joined with each other via another piece of strand, were hung in the double chamber, one for each compartment as is shown in Fig 24.

Fig 25 shows isotonic length changes of PS₁ and PS₂, with or without the shuttle streaming between them. Before the streaming was brought to a standstill, they contracted synchronously as was the case described before. When the balancing counter-pressure was given to one of the compartments
so that the endoplasm was prevented from flowing, phases of cyclic length changes of PS₁ and PS₂ began to shift with one another, and continued to contract and relax in different phases. The shaded bar at the bottom indicates the period of time when the endoplasm in the connecting strand was kept immobile. When the counter-pressure was removed and the shuttle streaming between PS₁ and PS₂ was allowed to occur again, the synchrony in phase of contraction waves was regained soon.

In the above experiment, endoplasm was stopped, but the surface membrane and the ectoplasmic gel remained intact. Using a dumbbell-shaped plasmodium placed in a double chamber, KAMIYA and ABE (1950) showed that the phase of rhythmic changes in electric potential difference between the two blobs of the plasmodium advances no matter whether the endoplasm in the connecting strand is kept free to flow or brought to a standstill. Hence it is reasonable to presume that changes in electric potential difference proceeded between the two hanging segments of strand during the period in which endoplasm was held quiet. Nevertheless the synchrony of rhythmic contractions of the two segments of strand was no longer maintained if only endoplasm was prevented from flowing.

All in all, the above result shows clearly that the endoplasm plays the essential part as information carrier in the mechanism for unifying periods and phases of contraction-relaxation cycle at different parts. Electrical potential changes do not seem to have a significant role in synchronizing local rhythms.
DISCUSSION

Based on the foregoing experiments we came to the conclusion that the information necessary for unifying phases of local contraction-relaxation cycles was transmitted neither by electric signal through the membrane system nor directly by mechanical tension of the ectoplasm. It must be carried along with the streaming of endoplasm. This fact may appear to be contradictory in itself, because if the local contractions in one and the same strand were completely synchronized, there would be little difference in internal pressure and hence flow of endoplasm would hardly be brought about. The sequence of events would form the following cycle:

1. Synchronization of local contractions
2. Disappearance of internal pressure difference
3. Cessation of streaming
4. Asynchronization of local contractions
5. Restart of streaming

In other words, if phases of local contractions became completely synchronous and the flow of endoplasm were almost stopped, transmission of the phase information should be delayed, which must produce a shift of phase among local contractions and induce the endoplasmic streaming again. Hence, it is likely that slight shift in phase among local contraction cycles and intensity of streaming caused by the shift may be balanced in vivo at a certain point.

This idea well explains the fact that the speed of endoplasmic streaming in the strand is rather slow as compared
with that of mother plasmodium. Actually the motive force of endoplasmic streaming between the two synchronized strands was known to be insignificant. The motive force became gradually larger, however, as far as the endoplasm was kept at a standstill through application of the balance pressure. Concomitantly the phase shift between two strands became also greater.

In a spread out plasmodium vigorous shuttle streaming is generally observed. If all regions of the plasmodium had rhythmic contractile activities evenly and their phase were sufficiently synchronized, such vigorous streaming should not be caused. As will be shown in the Chapter IV, it is likely that the cyclic contractile activity is localized in the anterior region of a plasmodium spreading out in a fan-like shape and not in the rear network region composed of many ramifying strands. Hence it may be reasonable to suppose that the vigorous streaming in a plasmodium is caused primarily by cyclic contraction of the anterior zone while the rear region acts to maintain a certain level of static tension. We will report details about this problem in the next chapter. It remains to be revealed, however, what the entity of the messenger is that carried by the endoplasmic streaming and transmits the information leading to synchrony in phase of periodic activities.
REFERENCES


Spring balances for measuring isometric tension force production in two different plasmodial strands (PS₁, PS₂). Only the mechanical part is shown. For explanation, see text.
The plasmodial strand held in U-shape with two pairs of hooks. Tension force production of vertical strand parts, A and B, were recorded with two spring balances shown in Fig 16.
The isometric tension waves produced in two different parts of one and the same strand (A, B, cf. Fig 17). They were not only sufficiently synchronized with each other, but bear a close resemblance in wave patterns. Vertical lines on the waves indicate time coordinates of the maximal and minimal tension phases.
The isometric tension waves in two different parts of the strand (A, B, cf. Fig 17) when they were disconnected by cutting the strand (C, cf. Fig 17) between the two clamped regions and connected again with another small piece of the strand. Vertical lines on the waves show time coordinates of the minimal tension phases.
The plasmodial strand hung in an inverted U-shape. Isotonic length changes in two parts of the strand (A, B) were recorded photographically.
Fig 21

The isotonic length changes in two parts of the strand when they were disconnected for some time and reconnected later. Vertical lines on the waves indicate phases of maximal length of the waves.
Fig 22

Two separate segments of plasmodial strands (PS₁, PS₂) hung in the moist chamber are connected with each other with another segment PS₃ at the lower ends.
Fig 23

Isometric tension waves (\(PS_1, PS_2\)) of the two different strands with or without a connecting strand between them. Length of the strands were shown under respective tension waves.
The double moist chamber for regulating the endoplasmic streaming. Isotonic length changes of two different strands (PS₁, PS₂) were recorded photographically while the air pressure in the right compartment was controlled so that the shuttle streaming in the connecting strand (PS₃) was kept at a standstill.
Fig 25

Isotonic length changes of the two strands hung in the double moist chamber (Fig 9 - PS₁, PS₂) with or without the shuttle streaming between them. The shaded bar indicates the period of time when the shuttle streaming was made to stop at the connecting strand. Note the gradual shift in phase between the two wave trains during this period and re-synchronization after this period.
IV Localization of the rhythmic contractile activity in the plasmodium

SUMMARY

A thin rectangular piece of cytoplasmic gel was dissected out from the anterior region of Physarum plasmodium and its contractile properties were examined in comparison with those of a segment of strand isolated from the network region of the same plasmodium. The anterior piece, in spite of injury caused by surgical operation, began to contract and relax periodically without appreciable lag stage after it was isolated. This is a piece of evidence to show that the anterior region of a plasmodium was in the state of active rhythmic contraction. A segment of strand obtained from the network of the plasmodium showed no significant rhythmicity soon after it was isolated. Only after a lag period (stage I, cf. Chapter I) lasting for 10-20 min, the strand gradually exhibited rhythmicity both under isometric and isotonic conditions. Such behavior of the isolated strand is interpreted to show that the network structure composed of many ramifying strand behind the advancing front plays no leading part in bringing about oscillation when it is in situ.

INTRODUCTION

We demonstrated in Chapter I that when an excised segment of the strand gained a regular periodicity in contraction, the phase of local contractions became sufficiently synchronous with one another. If all the minute regions of a plasmodium in situ
would contact and relax in phase, it would be difficult to
establish efficiently a local difference in internal pressure,
and hence vigorous shuttle streaming of the endoplasm such as
observed in the normal plasmodium would not be expected.

Probably contraction-relaxation activities are not evenly
distributed over the entire plasmodium but localized to its
specific region.

The purpose of the present paper is to demonstrate that
the periodical activity is localized mainly in the front
region of a plasmodium migrating in a fan-like shape.

MATERIALS and METHODS

The preparation of the material was the same as described
before. To compare contractile properties of the anterior zone
of the plasmodium with those of the network, we dissected out a
slender piece of cytoplasmic gel (5 - 15 mm in length and 500 -
1000 μm in width) from the front zone in the direction normal to
the front (Fig 28-A). This excised segment suffered injury
of cutting along its entire length, but could be set to the
tensiometer just like a segment of plasmodial strand. We
measured tension produced by a segment of the front zone and that
produced by a segment of the plasmodial strand to make comparative
studies on their contractile properties.

To measure the lateral contraction produced by the strand in situ
we resorted to the so-called contact technique first used by
Wohlfarth-Bottermann (1975). As shown in Fig 30, a glass rod
weighing 53 mg was hung from the tension transducer in such a
way that its lower end came just in contact with the plasmodial
strand spreading on the agar surface. Changes in tension thus recorded served as a measure representing lateral oscillation.

To load and unload reversibly the longitudinal tension upon the strand during isometric contraction, we used a tensiometer of a horizontal type (KAMIYA et al. 1972, 1973), which enabled us to measure tension produced by the strand while keeping the specimen on the surface of water in a pool (Fig 37). The construction of this type of tensiometer was similar in principle to the vertical type tensiometer described before.

RESULTS

Contractile properties of anterior zone and network region of a plasmodium

a) Longitudinal contraction

As reported before (Chapter I), it takes 10-20 min for an isolated strand to start rhythmic contraction under isometric as well as under isotonic conditions (Fig 26-a,b). We have never found that the strand which exhibited rhythmic contraction immediately after it was isolated from the network of the plasmodium.

Under isometric conditions the strand contracted strongly soon after it was isolated from the network and kept a high tension level for a while before it exhibited rhythmicity (Fig 26-a). There is a possibility that the strand is kept under a high tension level when it is in the original network of the plasmodium, because a segment of strand under isometric tensiometry and the strand in situ on the agar surface share
a common condition in that both are not allowed to change their length. To check this possibility, we shortened the strand before and after it started oscillation until the tension reached 0 mg. Fig 27 shows an example of the experiments of this kind. As soon as the tension was loosened, the strand contracted strongly and regained the former tension level in a few minutes. But once the strand started to contract and relax rhythmically, it could never regain the former tension level when it was loosened. This result is in conformity with the idea that the network composed of many strands is under high tension with no periodic activity.

If the network of the plasmodium has no periodic activity in situ, the source of periodic activity, which brings forth remarkable back and forth endoplasmic streaming must be attributed to the anterior zone of the plasmodium. To prove the validity of this line of thought we examined contractile properties of the anterior zone of the plasmodium in the following way.

We excised a slender rectangular segment (Fig 28-A) of cytoplasmic gel from the anterior zone of the plasmodium, and measured its tension production under isometric conditions. Fig 29-A shows one of the results, where the rectangular piece of the anterior zone began to contract and relax periodically soon after it was excised and set to the tensiometer. But in the case of the strand (Fig 28-B) isolated from the posterior network of the same plasmodium, there was a typical non-oscillatory stage (stage I, cf. Chapter I) lasting for 14 min before tension oscillation started (Fig 29-B). This is a fact which strongly supports the view that it is mainly the anterior zone of the plasmodium that is responsible for periodic activity in situ and that the network behind the anterior zone keeps a certain level of quiescent tension.
b) Lateral contraction

Wohlfarth-Bottermann (1975) measured rhythmic radial contraction of the strand _in situ_ by combining a piece of contact glass (or metal) rod with the tensiometer. He showed that radial contraction persisted even when the strand was cut on both sides of the contact area. In the amputated segment disconnected from the mother plasmoidium there was no or little streaming of endoplasm. From this fact, he believed that radial pulsation of the strand is not the passive phenomenon caused by the shuttle streaming of the endoplasm but is a manifestation of intrinsic activity of the strand. It is, however, not known whether the rhythmic radial activity of the strand measured by Wohlfarth-Bottermann pre-existed _in situ_, or was induced as a result of contact of the rod end to the strand.

To answer this question, we performed the following series of experiments using the contact technique described in Methods section.

1) A part of the strand network was amputated at two loci leaving 5-20 mm long segment where no or little streaming was visible. We started to measure lateral oscillation _in situ_ immediately after amputation. In this case the cut segment did not show any rhythmic pattern in its lateral contraction at first. Rhythmicity appeared only 10-30 min after amputation (Fig 31).

2) Oscillation was measured _in situ_ at first under the normal condition. If the strand was cut soon after the start of measurement, oscillatory activity was lost at the moment.
of amputation and was recovered subsequently (Fig 32).

3) The strand was amputated several tens of minutes after the onset of measurement with the contact technique. In this case oscillation persisted, although in some cases the amplitude decreased to a moderate extent (Fig 33).

Consequently the radial activity of the strand shown by Wohlfarth-Bottermann soon after the onset of measurement is interpreted to represent a passive phenomenon caused by the shuttle streaming. It is also reasonable to suppose that radial activity recorded several tens of minutes later represents the oscillatory activity newly acquired by the strand through its contact with the glass rod. If we detach the tip of the glass rod from the strand after 1 hr or later, we find, as a matter of fact, a small newly developed advancing front at the very region with which the glass was in contact.

Using the same contact method, we measured the oscillation also at the front area. If we dissected out a small piece of plasmodial gel from the front area and immediately measure the "lateral" activity, we could get vigorous oscillation without a lag phase (Fig 34). Even when the specimen was cut into smaller pieces, oscillation did not disappear.

Based on the above observations, we have come to the conclusion that the strand forming the network at the rear region is devoid of active contractile rhythm in both radial and longitudinal directions.
Acquirement and maintenance of periodic activity in a plasmodial strand under the zero-tension condition

To investigate whether or not the tension is necessary for the strand to develop rhythmicity after isolation, we performed the following experiments. After having excised a segment of the plasmodial strand from the network, we left it on the surface of the agar plate which was slightly flooded with water. The thin film of water on the agar surface helped the segment prevent from being attached to the agar and hence prevent from establishing the stress in itself. After 40-60 min, the segment of strand was suspended in the moist chamber and its isometric contraction or isotonic contraction was recorded. The result was shown in Fig. 35. The strand segment, which had been left on the agar surface, started periodic contraction and relaxation without appreciable lag stage under isometric as well as under isotonic conditions (Fig. 35-a,b). This is a fact to show that the initiation of physiological rhythm is not triggered by tension imposed upon the strand but by some other conditions accompanying isolation of the segment from the mother plasmodium.

Next we conducted two series of experiments at an attempt to see whether or not the tension is necessary for the strand to maintain once started oscillation.

The first series of experiments were done as follows. While cyclic tension changes of the strand were being recorded, we disconnected the strand from the tensiometer and laid it onto the agar surface covered with thin water film. The disconnected piece of strand did not adhere the agar
surface and the strand was kept under zero tension under this condition. After 30-60 min, we set the strand again to the tensiometer and restarted the recording of isometric tension changes of the strand. The result is shown in Fig 36. The strand began to contract and relax periodically without lag period when the recording was resumed after 45 minutes' stay of the strand on the agar surface. Thus it may be concluded that the tension is not necessary for the strand to continue the cyclic contraction.

To confirm this conclusion further, the second series of experiments were done using the horizontal-type tensiometer (KAMIYA et al. 1972, 1973), which enable to measure contraction and relaxation of the strand floating on the surface of the tap water in a small vessel (Fig 37). While rhythmic tension changes of the strand were recorded on the chart, we brought one terminal end of the strand closer to the other so that the strand was bent aside and longitudinal tension was nullified. After 10-30 min we brought the span of the two strand terminals to the original length and resumed the recording of isometric tension changes. As (is) shown in Fig 38, the strand began to contract and relax periodically without any lag as soon as the strand was stretched to the former length. Again this is another piece of evidence demonstrating that the strand did not lose the periodic activity when the strand was kept under zero tension.
DISCUSSION

It was shown that the cyclic contractile activity of an advancing plasmodium is localized at the anterior front. The network structure composed of many ramifying strands behind the anterior zone has no oscillatory activity. In other words, the anterior part of a plasmodium is mainly responsible for giving rise to periodicity to the endoplasmic streaming. If the internal pressure in the posterior network is higher than the time averaged internal pressure in the anterior zone in situ, endoplasm may flow to the anterior zone and a part of it will be converted into the ectoplasm forming a new anterior zone, when the plasmodium advances.

KAMIYA (1973) showed with a highly sensitive polarizing microscope that birefringence of cytoplasmic fibrillar structure in the anterior zone of a typical fan-like expanse of the plasmodium changes rhythmically. In the phase in which streaming takes place away from the front, these birefringent fibrils appear clearly and in the opposite phase in which the streaming takes place toward the front these fibrils almost disappear. This observation agrees well with our conclusion.

We reported before that the mechanical tension regulates the amplitude of cyclic contraction of the plasmodial strand, but, as was shown by the present work clearly, tension does not participate directly in regulation mechanism of pace-making. Thus it may be concluded that the regulation of the period and phase and regulation of the amplitude are performed independently of each other.
How active rhythmicity is localized in the front region of the plasmodium and not in the network behind it, and why isolation of a small segment of strand from the network gives the segment the motive for starting oscillation in contraction are intriguing problems which await further studies.

REFERENCES


Acknowledgment

I wish to express my thanks to Professor Noburo Kamiya for suggesting this investigation as well as for constant guidance in the course of the work.
Time courses of isometric (a) and isotonic (b) contractions of segments of strand soon after they were isolated from the network of a plasmodium. Isolation of each of the strands was done about 1 min before time zero, because it took this much time for preparation of the material to be ready for measurement. Note the presence of lag phase lasting for 15-20 min before active oscillation was started.
Tension of the strand in response to sudden shortening during quiescent stage (stage I, cf. Chapter I) and regular stage (stage III). In each stage, one end of the strand was brought closer to the other until the tension force reached null.
Fig 28

A sketch of a plasmodium spreading in a fan-like shape on agar surface. A: a slender rectangular segment to be isolated from the anterior zone, B: a segment to be excised from the strand region.
Isometric contractions of the two different plasmodial parts excised from the anterior zone (A) and from the posterior network (B). It took less than 1 min before recording was started after the samples were isolated from the mother plasmodium. Note oscillation started without delay in (A) while there was a quiescent stage in (B) before rhythmicity appeared.
Fig 30

Diagrammatic representation of the method for measuring lateral contraction of the strand in situ. The glass rod (R), the lower end of which is brought in contact with a dorsal part of the strand, is connected to the tension transducer of a vertical type.
Fig 31

Lateral oscillation of a segment of strand. Registration started as soon as the strand was cut on both sides of the contact area. Length of the segment: ca. 20 mm.
Lateral oscillation of the strand in situ.

At the time marked with the arrow the strand was cut on both sides of the contact area. Note the presence of lag stage before regular waves appeared.
Lateral oscillation of the strand *in situ*. At the time marked with the arrow (35 min after the measurement was started) the strand was disconnected from the mother plasmodium. Note there was no longer any lag stage.
Fig 34
Lateral oscillation of the advancing front area as measured by the contact method. Note there was no lag stage.
Fig 35

Isometric (a) or isotonic (b) contractions of segments of strand before the specimens were left free on the wet agar surface for 40-60 min. Note there was no lag phase.
Isometric contraction before and after the strand was laid onto the agar surface. During the period of 20-70 min lasting 50 min, the strand was disconnected from the tensiometer and laid onto the agar surface.
Fig 37

A setup for measuring the cyclic contraction of the strand floating on the surface of the tap water. T: Horizontal-type tensiometer, PS: plasmodial strand.
Isometric contraction of the strand floating on the tap water. During the measurement, the strand was loosen for 17 min to remove the tension. The broken line represents the distance between the two terminal ends of the strand, not the actual length of the strand which was bent aside.