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OSCILLATING CONTRACTIONS IN PROTOPLASMIC STRANDS OF *PHYSARUM*: SIMULTANEOUS TENSIOMETRY OF LONGITUDINAL AND RADIAL RHYTHMS, PERIODICITY ANALYSIS AND TEMPERATURE DEPENDENCE

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SUMMARY

1. The construction of a 'twin-tension transducer' allows the simultaneous measurement of the same or different contraction rhythms at any selected sites of living plasmodia of *Physarum polycephalum*. This method has been used to analyse the relation of longitudinal and radial contraction activity within migrating plasmodia and plasmodial veins, under isometric as well as under isotonic conditions of measurement.

2. A periodicity analysis of the oscillating contraction rhythms revealed average period values for the longitudinal contraction cycle of 2·1 min and for the radial contraction cycle of 1·3 min at a temperature of 22 °C.

3. The periods of longitudinal contraction depend on the environment of the strands. The mean value under submerged conditions was 2·9 min.

4. The temperature dependences for both longitudinal and radial contraction cycles were determined to provide reliable values for the normal reaction range of the contractile system (cytoplasmic actomyosin). The values for radial contraction activity are 2·0 min at 16 °C, 1·5 min at 20 °C, and 1·2 min at 24 °C. The range between 16° and 24 °C can be regarded as physiological.

5. The possibility is discussed that only one 'genuine' contraction frequency of cytoplasmic actomyosin exists in *Physarum*.

INTRODUCTION

Plasmodia of *Physarum polycephalum* are multinuclear masses of cytoplasm without cellular organization. Their conspicuous locomotory behaviour has been studied to analyse basic problems of cellular motility in non-muscle cells. The plasmodia show a characteristic protoplasmic streaming within plasmodial strands. Because the streaming direction changes periodically, within minutes, this type of cytoplasmic mass transport was called 'shuttle streaming'. Plasmodial strands ('veins'), with a diameter of between 50 μm and 1–2 mm, are differentiated into a tube-like ectoplasmic wall surrounding the streaming endoplasm. The endoplasm flows along a hydrostatic pressure gradient (Kamiya, 1959). Different pressures in different parts

of the plasmodia are generated and controlled by oscillating contractile activities of the plasmodial strands.

The general biological interest in plasmodia lies in the fact that contraction activity results from contraction–relaxation cycles of cytoplasmic actomyosin. Cytoplasmic actomyosins are widely distributed in eukaryotic cells and are responsible for many phenomena of cell motility. Thus, the contraction phenomena of plasmodial strands can be regarded as a model system for studying the contraction physiology of cytoplasmic actomyosin (Hatano, 1973; Komnick, Stockem & Wohlfarth-Bottermann, 1973; Pollard & Weihing, 1974; Wohlfarth-Bottermann & Fleischer, 1975).

Recently, it has become possible to analyse the physiology of contraction of protoplasmic strands by tensiometric methods (Kamiya, 1970, 1972). Using electronically equipped electrobalances with a resolution of 0·01 milliponds (mp) 1 pond $\approx$ 9·8 Newtons), longitudinal as well as radial contraction activities can be registered continuously (Wohlfarth-Bottermann, 1975*a*). Furthermore, it is possible to study the action of different drugs on the oscillating contraction activity (Ueda *et al.* 1975). To evaluate the results of such experiments, the normal contraction behaviour (e.g. the frequency of the rhythms) has to be known. Comparable slow contraction rhythms have been described in smooth muscle (Golenhofen, 1970) and smooth muscle actomyosin has been compared with cytoplasmic actomyosin (Komnick, Stockem & Wohlfarth-Bottermann, 1973).

At the present time, the biochemistry of the oscillating contraction rhythms are not understood and the metabolic oscillator has yet to be found (see Daniel, 1970; Hess & Boiteux, 1971). Oscillating concentrations of ATP and free Ca^{2+} have to be considered (see Braatz, 1975; Ridgway & Durham, 1976).

The cinematographic documentation and analysis of contraction phenomena has some methodological shortcomings. Baranowski (1976) analysed the radial contraction rhythms of veins and continuous protoplasmic layers by laser interferometry which also has some methodological shortcomings. In contrast to interferometric methods, tensiometric measurements are not restricted to thin objects but can be applied irrespective of the thickness of channels and layers. Moreover, as will be shown in a following paper, the electrobalance used as tension transducer also allows the measurement of direction and velocity of protoplasmic flow and contraction phenomena simultaneously (N. Hülsmann and K. E. Wohlfarth-Bottermann, unpublished).

The present investigation was undertaken to answer the following questions.

(1) Is it possible to measure, simultaneously, different contraction activities in *one* protoplasmic strand?

(2) Are there differences in the frequencies of longitudinal and radial contraction rhythms?

(3) Are there differences in the frequency of longitudinal contraction rhythms when the strands are maintained in a humid atmosphere or submerged in physiological solutions?

(4) What is the physiological temperature range and how are the contraction rhythms influenced by temperature changes?

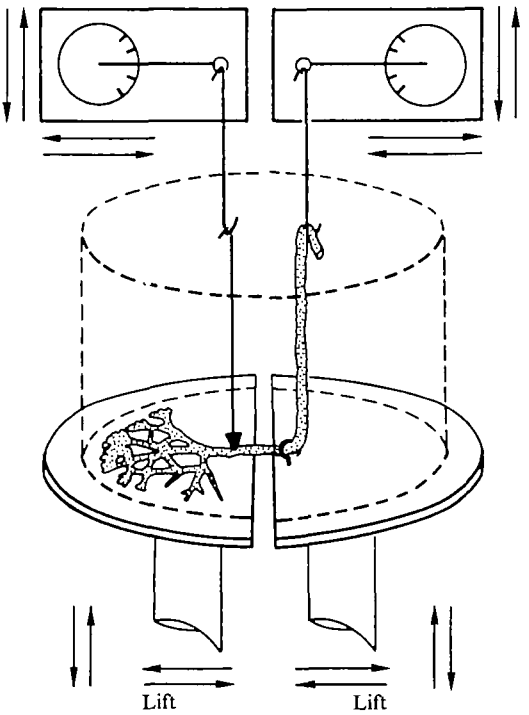

Fig. 1. Diagram illustrating the method of simultaneous measurement of radial (left) and longitudinal (right) contraction activities in *one* protoplasmic strand of *Physarum*. The twin-tension transducer construction allows the adjustment of both electrobalances as well as their lifts in the vertical *and* in the horizontal direction. For further explanation see text.

METHODS

The construction principle of the tension transducer has been described elsewhere (Kamiya, 1970; Wohlfarth-Bottermann, 1975*a*). This home-made instrument has a sensitivity of 0·01 mp. A second instrument (Fig. 1) was used in this study to make simultaneous measurements on a single strand. For such measurements it is necessary that each electrobalance, as well as each appropriate lift, can be adjusted independently in the vertical and horizontal directions. The main difficulty with such an adjustable arrangement is mechanical precision. The apparatus allows the measurement of contraction forces, in mp, in isometric conditions, and shortening and elongation of strands (in mm) under isotonic conditions. During isotonic measurement, the lift is automatically controlled and driven by a servo motor. For continuous registration of contraction forces and lift movements, respectively, two independent 2-channel pen-recorders are used.

Cultures of *Physarum polycephalum* were grown according to the method of Camp (1936). Tensiometric measurement was performed on living strands of the plasmodium, without any pretreatment. Radial activity was registered *in situ* (i.e. with the strands on their growth substrate) (Fig. 1, left side); for measurement of longitudinal activity, the strands were separated from the growing substrate and

mounted vertically in the tension transducer (Fig. 1, right side). Both procedures do not influence the vitality of the protoplasmic strands. After a period of more than 8 h, the measured strands grow out to a new plasmodium and can be further cultured.

Temperature control was performed electrically by two Peltier cells. The Peltier effect allows rapid heating or cooling by using a controlled reversal of an electrical current flow between two different chemical elements. The actual temperature of the object was registered by a pen-recorder. For determining the temperature dependence of longitudinal contraction activity, the strands were mounted vertically and submersed with physiological solutions.

RESULTS

Longitudinal and radial contraction cycles

Fig. 2 shows the tensiometric curves of simultaneous measurements of radial and longitudinal contraction activities in a single strand. The left part of the strand (cf. Fig. 1) remained *in situ* (i.e. within the plasmodium on its substrate, filter paper). The right part of the strand was detached and was fastened to the right lift and mounted vertically on the right electrobalance of the second tension transducer unit. Measurement of radial activity was performed by placing a lightly weighed measuring rod onto the surface of the vein (Wohlfarth-Bottermann, 1975*a*) and measuring the periodic weight increase and decrease with the electrobalance of the first tension transducer (see Fig. 1).

In Fig. 2 the average time period of the longitudinal activity is 2·0 min, as compared with the radial activity period of 1·5 min. In other corresponding simultaneous measurements of radial and longitudinal activities, however, nearly identical time periods were measured. An investigation was, therefore, undertaken to determine whether radial and longitudinal activity possess identical or different time periods in their oscillating contraction behaviour.

Altogether 461 different strands with regular sinusoidal oscillations (e.g. Fig. 2) were measured, each for a time period from at least 20 min up to several hours. The average time period of contraction activity per strand was calculated. Fig. 3 shows the distribution of all average values, separated according to four different experimental conditions. In Table 1 the mean average values are listed. The longitudinal time periods of 2·1 and 2·9 are markedly different from the radial periods of 1·3 and 1·1.

The distribution of average values of longitudinal contraction periods, in comparison to radial contractions, shows a conspicuous deviation for the longitudinal contraction activities (from 1·2 to 4·1) (Fig. 3). In contrast, the corresponding range for radial contractions is only 1·0 to 2·2. Even the radial measurements, at higher temperatures, showed that the deviation range of average values for radial contraction does not increase significantly, but remains small in comparison to that of longitudinal average values.

The distribution of average values (Fig. 3) indicates that there is a pronounced difference between the mean values of time periods of longitudinal contraction which depends on the environment of the strands during the measurement. The

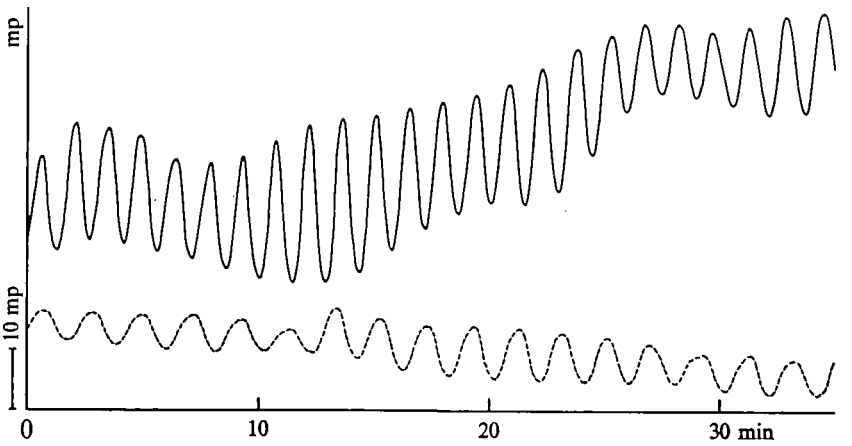

Fig. 2. Cycling contractions registered by the experimental arrangement as shown in Fig. 1. Average time period of longitudinal (----) activity = 2·0 min, of radial (——) activity = 1·5 min. Temperature 22 °C. Length of the longitudinal measured part of the strand = 22 mm. Distance of the point of radial measurement from the longitudinal measured portion = 20 mm. Isometric conditions of measurement.

Table 1. *Mean average values of time periods in minutes from measurement of* 461 *different strands*

Longitudinal		Radial	
Submersed	Atmosphere	Atmosphere *in situ*	Atmosphere *in situ*
22–26 °C	22–26 °C	22–26 °C	26–30 °C
2·91	2·13*	1·34	1·15

* According to Krüger (unpublished).

mean value for strands measured in humid atmosphere is 2·1 min, as compared with 2·9 min in submersed conditions.

Table 2 shows the effects of the different media used for submerging the strands. Submersed strands do not have to support their own weight. However, this is not the reason for the observed prolongation of time periods, as can be shown by variation of the applied tension in submerged strands.

For statistical analysis, standard deviations were determined and a Student's t test was performed to evaluate the significance of differences between the time period values (i.e. longitudinal/radial; longitudinal (submerged)/longitudinal (atmosphere). The t test shows that these differences are highly significant:

	$\bar{x} \pm s$		
Longitudinal (atmosphere)	2·13 ± 0·50	(n = 167)	t = 12·33, $P < 0{\cdot}001$
Radial (atmosphere)	1·39 ± 0·23	(n = 97)	
Longitudinal (submerged)	2·91 ± 0·65	(n = 100)	t = 13·00, $P < 0{\cdot}001$
Longitudinal (atmosphere)	2·13 ± 0·50	(n = 167)	

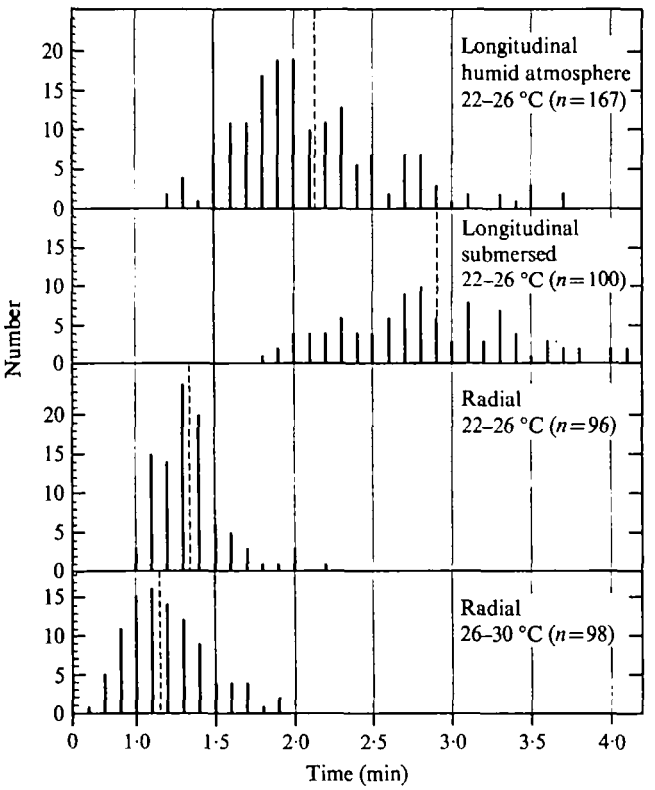

Fig. 3. Distribution of time periods (average values) of different contraction activities. n = number of experiments or measured strands, each resulting in one average value.

Table 2. *Mean values of longitudinal contraction periods of protoplasmic strands submersed in different media*

Longitudinal contraction, strands submersed in	Number of measured strands	Minimal time periods (min)	Maximal time periods (min)	Mean values of time periods (min)
Tap water	32	2·6	2·9	2·8
Phosphate buffer, pH 7·0	56	2·8	3·2	3·0
Chalkley solution	12	2·7	3·2	3·0

Temperature dependence of time periods

The periods of radial contractions became shorter with ascending temperature (Fig. 3). A Peltier cell was used to analyse this temperature dependence. This device allowed a rapid temperature control of the plasmodial environment. Fig. 4 shows the original temperature curves during two experiments with rapidly changing temperatures. Temperature varied repeatedly within 1 hr between 14 and 30 °C. The average values of time periods of longitudinal contraction (marked as empty circles in Fig. 4) were determined in time intervals of 5 min. Fig. 4 reveals the pronounced temperature dependence of the frequency of longitudinal contractions.

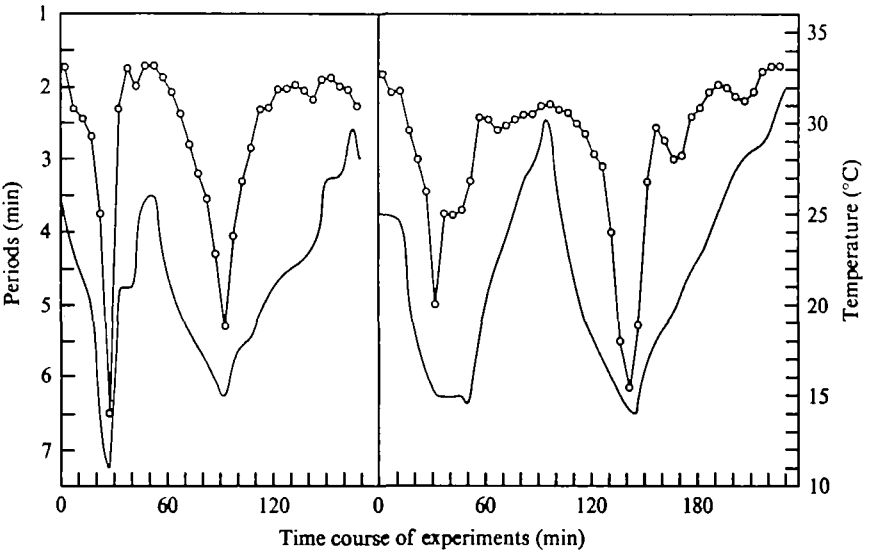

Fig. 4. Effect of rapidly changing temperatures on the duration of longitudinal contraction periods. ○, Average values of periods; lower curves, simultaneously registered temperature, which was controlled by a Peltier cell. Isometric conditions of measurement, protoplasmic strands submersed in physiological solution.

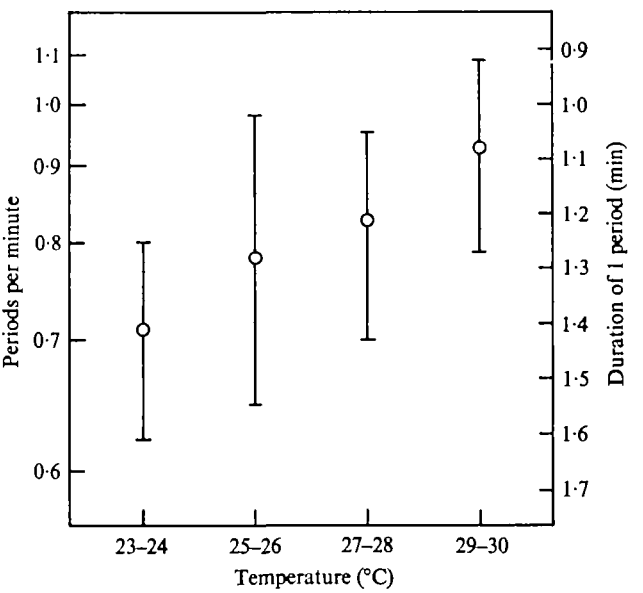

Fig. 5. Distribution of average values of radial periods depending on different constant temperatures. Isometric conditions of measurement (contact method).

Table 3. *Mean average values of time periods of radial contraction activity at different temperature ranges*

Temperature ...	23–24 °C	25–26 °C	27–28 °C	29–30 °C
Time periods (min)	1·41	1·28	1·21	1·08

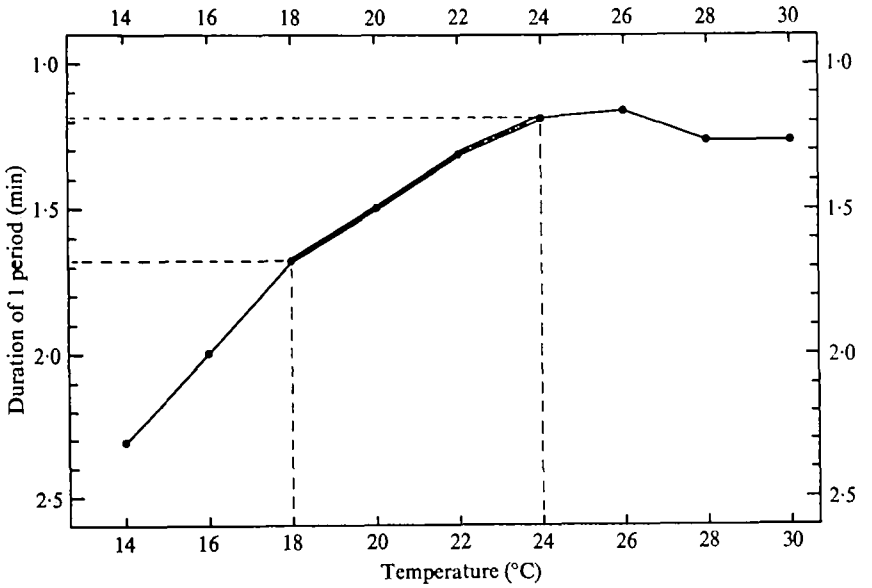

Fig. 6. Effect of rapidly changing temperatures on the duration of radial contraction periods. Temperature control by a Peltier cell. Isometric conditions of measurement (contact method).

Table 4. *Mean average values of time periods of radial contraction activity of* 131 *measured strands at different temperatures*

t (°C) ...	14	16	18	20	22	24	26	28	30
Periods (min)	2·31	2·00	1·68	1·50	1·31	1·19	1·16	1·26	1·26

The original tensiometer readings show distinct irregularities of the sinusoidal contraction curves at temperatures below 14 °C and above 28 °C.

The temperature dependence of radial contraction activities measured *in situ* and the mean average values of time periods at different temperature ranges are summarized in Fig. 5 and Table 3, respectively. In these experiments the plasmodia were acclimated to the relative high temperatures for several hours.

In further radial measurements, temperature was rapidly controlled by a Peltier cell. Fig. 6 represents a temperature-dependence curve gained from the mean values of 131 measurements of radial contraction activity, each measurement with a duration of at least 15 min. Temperatures above 28 °C induce irregularities in the sinusoidal wave form of the contraction curves, comparable to those irregularities observed in the high temperature range during longitudinal measurements. Table 4 summarizes the mean values for varying temperatures between 14° and 30 °C. Within the room temperature range from 18° to 24 °C, the average radial contraction periods change between 1·7 min and 1·2 min, respectively.

DISCUSSION

Advantage of the 'twin-tension transducer'

The reliability of the tensiometric measurement does not need to be discussed, the different contraction activities can as well be registered by cinematographic methods. The registration by a tensiometric device arranged as a 'twin-tension transducer' offers new possibilities for analysing the relation between different contraction phenomena in both protoplasmic channels and continuous layers of the plasmodia. In particular, the following measurements provide information about the relation, phase differences and wave propagations of the different oscillating contraction activities.

(1) Simultaneous measurement of longitudinal and radial activity on a single strand (phase relations).

(2) Two simultaneous measurements of radial activity on a single strand, *in situ*, with any desired distance between the measuring points (occurrence of peristaltic waves).

(3) Independent longitudinal measurement of contraction activities of two different parts of one strand, either both isometric or one isometric and one isotonic.

(4) Independent longitudinal measurement of a single strand: one part in humid atmosphere, the other submerged.

Other arrangements enable studies to be made of the control mechanism underlying oscillating phenomena (e.g. the space maker and its modulation). The modulation of the contraction activity is of considerable interest in the study of chemotactical responses of cells (see Ueda *et al.* 1975).

Interpretation of different frequencies

Changes in contraction frequency, in response to alterations in temperature, are completely reversible at least within the range between 10° and 28 °C (Fig. 4). Low temperatures (< 13 °C) induce strong contractures in radial activity of the strands with a pronounced plateau phase of the contraction curve. The aim of this study was restricted to the analysis of the temperature dependence of frequency near to the room temperature range. Therefore, the temperature characteristics of different contraction activities cannot be discussed. Obviously, room temperature (22 °C) is within the physiological range of plasmodia (16°–24 °C).

From the frequency differences between the radial and the longitudinal contractions raises the question whether these phenomena are controlled by two different oscillating systems. Under identical external conditions (humid atmosphere and room temperature), the mean average values for longitudinal and radial contractions are markedly different: longitudinal 2·1 min and radial 1·3 min. At first sight this seems to accord with the morphological architecture of the strands. There is a longitudinal system of actomyosin fibrils within the periphery of the strand and a circular system surrounding the endoplasmic channel (Nakajima & Allen, 1965; Wohlfarth-Bottermann, 1965, 1975*b*).

Although there is no reason to suppose a difference in their biochemical composition, it could be argued that both morphological systems work with different frequencies. Furthermore, this accords with the theoretical interpretation of Kamiya

& Yoshimoto (1972) and Kamiya, Allen & Zeh (1972), who suggest that the frequently visible 'shoulders' in the ascending part of the longitudinal contraction curves could result from a 'coexistence of at least two periods of contraction–relaxation cycles, of which one is about half the length of the other'. This corresponds to the ratio of longitudinal:radial contractions demonstrated in this investigation: 2·1:1·3 min = 1:0·6. Thus, from a theoretical point of view, an interference of both frequencies would explain the irregularities ('shoulders') observed by Kamiya (1970) in the sinusoidal form of the contraction curves.

However, the assumption of a co-operation of two oscillation systems (with genuinely different frequencies), would complicate the unknown control system governing the oscillating phenomena. The observations that values for radial activity show a much smaller deviation than longitudinal ones (Fig. 3), as well as the prolongation of the longitudinal periods (when the strands are measured in submerged conditions) point to another possibility. This is that only *one* genuine frequency exists (e.g. a period of the radial rhythm of 1·3 min) which could be prolonged during longitudinal contraction. The question cannot be resolved at the present time. It should be borne in mind, however, that *in situ*, the plasmodial strands adhere more or less firmly to the substrate. Thus, while the radial contraction activity can be predominantly expressed in isotonic, the longitudinal activity is largely restricted to isometric conditions (N. Hülsmann and K. E. Wohlfarth-Bottermann, unpublished). This illustrates the complexity of the interactions of the different demonstrated frequencies within the plasmodial polyrhythmic system (see Baranowski, 1976).

Irrespective of the question as to whether there are one or two genuine frequencies, the periodicity analysis for longitudinal and radial contraction activity can be regarded as a reliable basis for the evaluation of future experimental work. Radial contraction frequency is likely to be a more reliable parameter as it may be more directly related to the genuine oscillator and shows less irregularity than longitudinal contraction. Longitudinal activity can be used as a criterion for evaluating the responses of the living system to different experimental influences. The study of externally applied substances will give information on the following questions.

(1) Is there an oscillating Ca^{2+} influx and efflux across the plasmalemma (compare smooth muscle)?

(2) Is there a correlation of ion pumps and oscillating differences in membrane potentials of the plasmodial surface membranes?

(3) Is there a convincing mechanism transforming chemotactical signals received by the plasmalemma to a modulating effect of the contractile apparatus (chemotactical response)?

The answers to these questions are not only of interest for the movement phenomena of plasmodia (e.g. the localization of the postulated oscillator), but also in the contraction physiology of cytoplasmic actomyosins in non-muscle cells and, thereby, the general physiology of cell locomotion.

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