PL ISSN 0065-1583

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

SYMPOSIUM ON CELL MOTILITY

Warszawa, June 26-28, 1978

VOLUME 18

Number 1

WARSZAWA 1979

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

International Journal of Protozoology

Manuscripts may be submitted to the Editorial Office: Acta Protozoologica, M. Nencki Institute of Experimental Biology, 02-093 Warszawa, 3 Pasteur Street, Poland, or to each member of the Editorial Board.

A subscription order stating the period of time, along with the subscriber's name and address, can be sent to your subscription agent or directly to Foreign Trade Enterprise Ars Polona Ruch, 00-068 Warszawa, 7 Krakowskie Przedmieście, P.O. Box 1001, Poland. Please send payments to the account of Ars Polona – Ruch in Bank Handlowy S.A., 7 Traugutt Street, 00-067 Warszawa, Poland.

ACTA PROTOZOOLOGICA appears quarterly. The indexes of previous volume will appear in No. 1 of the next volume.

Indexed in Current Contents.

INTRODUCTION

The Symposium "Cell Motility" was organized on the occassion of 60th Anniversary of the Nencki Institute of Experimental Biology (Polish Academy of Sciences) in Warsaw. The Nencki Institute has an old tradition of interest in the field of basic forms of cellular movement which goes back to pioneer studies of the early twenties by Prof. Jan Dembowski on geotaxis and cyclotic movement of food vacuoles in *Paramecium*.

The idea of organizing at the Nencki Institute international symposia on cell motility is not a new one. It should be pointed out in this connection that Symposium on "Physiology of Motor Response in Protozoa" was organized in Warsaw during the celebration of 50th Anniversary of the Nencki Institute in December 1968 (published in Acta Protozoologica, Vol. VII, fasc. 23–30, 1970) and Symposium "Motile Systems of Cells" was held in Cracow in August 1971 (published in Acta Protozoologica, Vol. 11, 1972).

In the recent years the rapid expansion of knowledge covering the physiology, biophysics and biochemistry of cell motile systems found its expression in numerous symposia, conferences and other kinds of meetings dealing with various aspects of cell motility.

The objective of the present Symposium was to bring together scientists representing various fields who have contributed to progress in various areas of cell motility. The following topics covered by three one-day sessions were emphasized:

June 26, 1978 — Excitability and Motor Response to External Stimuli.

Chairmen and Invited Speakers: Y. Naitoh (Japan), B. Diehn (USA), H. Kinosita (Japan), E. Hildebrand (GFR) and S. Dryl (Poland).

June 27, 1978 — Intracellular Organization and Coordination of Movement.

Chairmen and Invited Speakers: P. Satir (USA), W. Stockem (GFR) and A. Grębecki (Poland).

June 28, 1978 — Contractile Phenomena and Cell Motility.

Chairmen and Invited Speakers: G. K. Wohlfarth-Bottermann (GFR), E. D. Korn (USA) and L. Kuźnicki (Poland).

Each one-day session consisted of three parts: (a) Two or three introductory lectures given by chairman and invited speakers, (b) Presentation of posters and (c) Final discussion. The organizers believe that the above-mentioned organization of Symposium gave really good opportunity for scientific contact and free discussion of presented material.

The contribution of chairmen, invited speakers and all other participants of Symposium is highly appreciated by the Organizing Committee. The organizers of Symposium express their sincere thanks to the Polish Academy of Science, to the Nencki Institute and to the International Union of Biological Sciences for their generous support of the Symposium.

Organizers of Symposium

Stanisław DRYL, Andrzej GRĘBECKI
Leszek KUŹNICKI, Jerzy SIKORA

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31. III. 1979 pp. 1–6

Institute of Biological Sciences, University of Tsukuba, Ibaraki 300-31, Japan

Yutaka NAITOH

Membrane Currents in Voltage-Clamped *Paramecium* and Their Relations to Ciliary Motion*

Synopsis. General behavior of the membrane currents in *Paramecium* associated with a depolarization under voltage-clamp condition are essentially similar to those in other excitable membranes. Hodgkin and Huxley-type kinetics is applicable to the membrane currents. Possible role of the membrane currents in the control of ciliary motion was discussed.

Electrophysiological studies on ciliate protozoans have revealed that changes in their ciliary motion in response to the external stimuli are closely related to the electric responses of their membrane to the stimuli (Eckert et al. 1976).

In order to elucidate the mechanism underlying the control of ciliary motion by the electric events in the membrane, it is primarily important to understand precise ionic behaviors across the membrane during the events.

The present paper will deal with quantitative description of the membrane currents associated with a depolarization under voltage-clamp condition. The relation between the membrane currents and ciliary reversal will be discussed.

Material and Methods

Specimens of *Paramecium caudatum* (Syngen 1 of the Mating type I) equilibrated in a saline solution (4 mM KCl + 1 mM $CaCl_2$ pH 7.2 by 1 mM Tris-HCl) were subjected to the voltage-clamping experiments under room temperature (17°C ±3°C). Details of the experimentations will appear elsewhere (Naitoh 1979, see also Naitoh and Eckert 1972).

Results

(1) Membrane Current Associated with One Step Depolarization

An outward current just after the capacitive current (the initial outward current) decreased with time to become inward-going current. The inward current increased to its peak value (the early inward current), then decreased to become outward-going

*Paper presented at Symposium on Cell Motility, Warszawa, June 26-28, 1978.

current. The outward current continued to increase to its final steady level in 100–150 ms (the late steady outward current).

The initial outward current increased linearly with increasing the magnitude of depolarization. The current presumably corresponds to the resting membrane conductance.

The peak value of the early inward current showed its maximum at 35 mV depolarization. Its sign reversal took place when the depolarization was over 45 mV.

The late steady outward current became conspicuous when the membrane was depolarized over 20 mV, increasing with further increase in depolarization.

The tail current after cessation of a depolarization was inward when the depolarization was less than 20 mV, and it became outward for larger depolarization. The time-decay of the tail current was nearly exponential with time constant of 3–5 ms.

(2) Instantaneous I–V Relationship

Conductance of the membrane after its subjection to a depolarization was examined by plotting the I–V relationship at the end of depolarizations (70 ms duration) of different magnitudes (0–40 mV). The instantaneous I–V relationships thus obtained were almost linear and converged into a certain point which corresponds to E_k (potassium equilibrium potential) and I_{Ca} (calcium current). The slope of the plot was steeper with stronger depolarization. These facts indicate that the potassium conductance of the membrane (g_k) becomes high after 70 ms depolarization, but calcium conductance of the membrane (g_{Ca}) is as low as that of the resting membrane. The value of g_k, which corresponds to the current intensity at E_{Ca} (calcium equilibrium potential), increases with increasing the magnitude of depolarization.

The instantaneous I–V relationships were then determined after depolarizations of different durations (5 ms, 10 ms and 70 ms) but of constant magnitude (34 mV). The instantaneous inward current at E_k after 5 ms depolarization was very large, but its value at 10 ms depolarization was as small as that at 70 ms depolarization. These facts indicate that in response to a depolarization g_{Ca} increases and reaches its maximum value in 5 ms, then decreases to its original resting value in less than 10 ms. The early inward current and the late steady outward current associated with a depolarization correspond to the g_{Ca} increase and g_k increase respectively.

(3) Inactivation of the Early Inward Current

The effects of both duration and magnitude of initial conditioning depolarization on the early inward current associated with the second depolarization with a definite magnitude (34 mV) were examined to know time course of Ca-inactivation during a depolarization.

The ratio (R) of the value of the early inward current after a conditioning depo-

larization to that without conditioning depolarization, which corresponds to the rate of inactivation, could be formulated as

$$R = 1-(1-R_\infty)\ \{1-\exp(-t/\tau)\}^5, \tag{1}$$

where R_∞ is the value of the ratio at infinite time and τ is a time constant.

(4) Time-change in the Late Steady Outward Current

Since the early inward current was found to be fully inactivated by a depolarization of 35 mV with a duration of 70 ms, the time-change in the outward current associated with the following second voltage step is thought to be entirely due to the time-change in g_k. Thus obtained time-change in g_k could be formulated as

$$g_k = g_{k\infty} - (g_{k\infty} - g_{ko}) \exp(-t/\tau_n) \tag{2}$$

where $g_{k\infty}$ is the final steady value of g_k at infinite time and g_{ko} is the g_k at the beginning ginning of a depolarization. τ_n is a time constant.

(5) Time-change in the Early Inward Current

The value of I_{Ca} was obtained by subtraction of a leakage current and I_k, which was calculated from the equation (2), from the value of the membrane current associated with a depolarization.

g_{Ca} increased with time to attain its maximum value, then decreased to 0. The maximum g_{Ca} increased with increasing depolarization and tended to attain its highest saturated value with further increase in the magnitude of depolarization. The time to reach the maximum g_{Ca} became shorter with stronger depolarization. The time to come to 0 current was also shorter with stronger depolarization.

(6) Minor Current Components

It was found that there were some minor kinetic components in the membrane current associated with a depolarization. (1) A small increase in calcium conductance without inactivation. (2) Slow increase in potassium conductance (time constant = 60–70 ms). (3) Slow inactivation of potassium conductance (time constant = 300 ms or more).

Discussion

(1) Calcium Inactivation

If a calcium channel is assumed to close only when all five inactivation particles occupy the channel, probability in which the channel is kept open (P) can be formulated as

$$P = 1-(1-h)^5. \tag{3}$$

According to Hodgkin and Huxley (1952), h can be formulated as

$$h = h_\infty - (h_\infty - h_0)\exp(-t/\tau_n) \tag{4}$$

h_0 is thought to be 1. P_∞, which is the value of P at infinite time, can be formulated as

$$P_\infty = 1-(1-h_\infty)^5. \tag{5}$$

By introducing equations (4) and (5) into equation (3), P can be rewritten as

$$P = 1-(1-P_\infty)\{1-\exp(-t/\tau_n)\}^5. \tag{6}$$

Since P and P_∞ correspond to R and R_∞ in equation (1) respectively, equation (6) fully accords with the experimental equation (1) which shows time course of calcium inactivation.

(2) Calcium Activation

If calcium inactivation does not occur during a depolarization ($h = 1$; constant), calcium conductance without inactivation (g'_{Ca}) should increase with time to attain a certain level ($g'_{Ca\infty}$). Experimentally obtained value of I_{Ca} was divided by the corresponding value of P which was calculated from equation (6). Our calculation showed that if an appropriate value was chosen for τ_h, later I_{Ca} tended to become a constant value. The time change in g'_{Ca} thus obtained could be formulated as

$$g'_{Ca} = g'_{Ca\infty}\{1-\exp(-t/\tau_m)\}^5. \tag{7}$$

If a calcium channel is assumed to open when all five activation particles are outside the channel, g'_{Ca} can be formulated as

$$g'_{Ca} = \bar{g}_{Ca} m^5, \tag{8}$$

where $\bar{g}_{Ca}$ shows the value of g_{Ca} when all the calcium channels open. According to Hodgkin and Huxley (1952) time change in m is as follows:

$$m = m_\infty - (m_\infty - m_0)\exp(-t/\tau_m). \tag{9}$$

Since m_0 is thought to be negligible, equation (8) can be written as

$$g'_{Ca} = \bar{g}_{Ca} m_\infty^5 \{1-\exp(-t/\tau_m)\}^5 \tag{10}$$

$g'_{Ca\infty}$ can be written as

$$g'_{Ca\infty} = \bar{g}_{Ca} m_\infty^5. \tag{11}$$

Thus equation (10) becomes

$$g'_{Ca} = g'_{Ca\infty}\{1-\exp(-t/\tau_m)\}^5. \tag{12}$$

Equation (12) is exactly same with equation (7). Thus the time change in g_{Ca} during depolarization can be formulated as

$$g_{Ca} = \bar{g}_{Ca} m^5\{1-(1-h)^5\}. \tag{13}$$

(3) Relation of I_{Ca} to Ciliary Reversal

It has long been known that a depolarization of the membrane by an outward current induces a calcium dependent depolarizing action potential which is always associated with ciliary reversal (Naitoh et al. 1972). On the other hand, chemically skinned *Paramecium* was found to show ciliary reversal in the presence of calcium ions and ATP in the reactivation medium (Naitoh and Keneko 1972). It was, therefore, concluded that an increase in the intracellular (or intraciliary) calcium ion concentration due to an inflow of external calcium ions into the cell (or cilium) during the action potential is responsible for the ciliary reversal by an outward current stimulation (Eckert 1972, Eckert and Naitoh 1972, Naitoh and Eckert 1974, Naitoh 1974, Eckert et al. 1976).

Present experiments clearly demonstrated that a membrane depolarization brings about a temporary increase in g_{Ca}. However, duration of g_{Ca}-increase is far shorter (less than 10 ms) than that of the associated ciliary reversal (more than scores of seconds, depending on the magnitude of depolarization) (Machemer and Eckert 1975, Oertel et al. 1977).

It was recently demonstrated that depolarization-sensitive calcium channels are located exclusively in the ciliary membrane (Ogura and Takahashi 1976, Dunlap 1977). Since the ciliary volume is minute, it is presumable that inward flow of calcium ions into the cilium due to increase in g_{Ca} brings about rapid increase in the intraciliary calcium concentration, which consequently limits further inflow of calcium ions into the cilium even though g_{Ca} is maintained high (Eckert 1977). This assumption well explains the discrepancy between the time courses of ciliary reversal and g_{Ca}. However, the instantaneous I–V relationship obtained in the present study clearly shows that I_{Ca} at E_k becomes very small even after 10 ms depolarization. This indicates that g_{Ca} might truly inactivate in less than 10 ms.

Non-inactivating small calcium current associated with a membrane depolarization might be responsible for the long-lasting ciliary reversal, though we do not have any direct evidence to show that the small current can maintain the intraciliary calcium concentration high enough to induce ciliary reversal.

The long-lasting ciliary reversal is also explainable if we assume that calcium pumping out system is inactivated by a depolarization. The inactivation of the system might result in keeping the intraciliary calcium concentration high after cessation of g_{Ca}-increase.

ACKNOWLEDGEMENTS

I wish to express my hearty thanks to the Polish Academy of Sciences for the financial support during my stay in Poland. I am also greatly indebted to the Kajima Foundations for the support of my travel expenses to Poland.

REFERENCES

Dunlap K. 1977: Localization of calcium channels in *Paramecium caudatum*. J. Physiol., 271, 119–133.

Eckert R. 1972: Bioelectric control of ciliary activities. Science, Wash., 176, 473–481.

Eckert R. 1977: Genes, channels and membrane currents in *Paramecium*. In: News and Reviews, Nature, London 268, 104–105.

Eckert R. and Naitoh Y. 1972: Bioelectric control of locomotion in the ciliates. J. Protozool., 19, 237–243.

Eckert R., Naitoh Y. and Machemer H. 1976: Calcium in the bioelectric and motor functions of *Paramecium*. In: Calcium in Biological Systems, (ed. Duncan C. T.) Cambridge Univ. Press., London, p. 233–255.

Hodgkin A. L. and Huxley A. F. 1952: A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol., London, 117, 500–574.

Machemer H. and Eckert R. 1975: Ciliary frequency and orientational responses to clamped voltage steps in *Paramecium*. J. Comp. Physiol., 104, 247–260.

Naitoh Y. 1974: Bioelectric basis of behavior in protozoa. Am. Zool., 14, 883–893.

Naitoh Y. 1979: Membrane currents in voltage-clamped ciliate protozoa, *Paramecium caudatum* (in manuscript).

Naitoh Y. and Eckert R. 1972: Electrophysiology of the ciliate protozoa. In: Experiments in Physiology and Biochemistry. Vol. V, (ed. Kerkcut G. A.) Academic Press, New York, London, p. 17–31.

Naitoh Y. and Eckert R. 1974: The control of ciliary activity in protozoa. In: Cilia and Flagella, (ed. M. Sleigh), Academic Press, London, p. 305–352.

Naitoh Y. and Keneko H. 1972: Reactivated triron-extracted models of *Paramecium*: Modification of ciliary movement by calcium ions. Science, Wash., 176, 523–524.

Naitoh Y., Eckert R. and Friedman K. 1972: A regenerative calcium response in *Paramecium*. J. exp. Biol., 56; 667–681.

Oertel D., Schein S. J. and Kung C. 1977: Separation of membrane currents using a *Paramecium* mutant. Nature, 268, 120–124.

Ogura A. and Takahashi K. 1976: Artificial deciliation causes loss of calcium-dependent response in *Paramecium*. Nature, 264, 170–172.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 7–16

Department of Chemistry, The University of Toledo, Toledo, Ohio 43606, USA

Bodo DIEHN

The Interactions of Photic and Chemical Stimulus/Response Systems in *Euglena gracilis* *

Synopsis. The unicellular alga *Euglena gracilis* exibits characteristic behavioral responses (strophophobic responses) upon changes in illumination intensity. The same type of response can also be triggered by temporal changes in oxygen concentration. The photo- and chemosensory transduction systems, operating on the same motor apparatus, interact in a complex fashion. Studying the responses exhibited by *Euglena* in the presence of competing photic and chemical stimuli yields information on the manner in which *Euglena* processes sensory inputs.

The ultimate aim of the study of sensory transduction processes in single cells is, in our laboratory as in others, a description of cellular behavior in molecular terms. Photosensory systems are particulary suitable for such studies because of the ease with which the stimulus can be manipulated, and that of *Euglena* is probably the best understood of any microorganism. Recently, mechanosensory (Mikołajczyk and Diehn 1976) and chemosensory (Colombetti and Diehn 1978) stimulus/response systems have been discovered in *Euglena,* and the first step toward a molecular description of the latter has been taken with the tentative identification of its receptor molecule (Miller and Diehn 1978). Since the outputs of their sensory systems converge at least in part upon the flagellar motor, a new tool has become available to us for investigating the manner in which the cell processes its sensory information: the study of how *Euglena* handles parallel or contradictory commands to its motor apparatus from the sensory receptors.

In the present contribution, I will discuss what we know thus far

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

about the interdependence of the photo- and chemosensory response systems. Since we are just beginning to study these interactions, we do not yet have much of an indication of their biochemical basis. However, we will be able to draw some preliminary conclusions about the operation of the cell's "signal processor" from our observations of cell behavior.

It seems appropriate to preface this paper with a description of the stimulus-induced motor responses that *Euglena*, and for that matter motile organisms in general, can exhibit. In the context of that introduction, I will also touch upon the terminology that has recently been proposed by a group of workers active in this field of study (D i e h n et al. 1977).

Stimulus-Induced Motor Responses

Three types of movement responses of the organism as a whole ("gross motor responses", GMR) must be considered, together with the effector responses ("elementary motor responses", EMR) of their motor organelles:

(1) A change in linear velocity (GMR), caused in *Euglena* by a change in the frequency or amplitude of flagellar beating (EMR).

(2) A change in the direction of the organism's movement (GMR), in *Euglena* effected by an EMR consisting of flagellar reorientation. The term "elementary motor response" was coined by C h e c c u c c i (1976) for this specific reaction.

(3) A change in the body shape or outline. In *Euglena*, this GMR is brought about by an EMR of the body contraction system.

In this paper, I will confine my discussion to a class of responses in which the above GMR's and EMR's are induced by an intensity change (step-up or step-down) of the stimulus. This response class, termed "Phobic Responses", is characterized by the fact that the organism's sensory system eventually adapts to the new stimulus intensity, thus terminating the response.

The GMR or EMR type can be indicated by a prefix. "Ortho-" is accepted for a change in linear velocity (type 1, above) and I propose to use "stropho-" for a change of direction (2), and "morpho-" for a change of shape (3). Thus, the term "step-up chemo-strophophobic response", seemingly a long expression would mean "a transient change in direction induced by a temporal increase in the concentration of a chemical stimulus" — a description requiring three times as many words. Since both of these terms are uncomfortably long in repeated

usage, one customarily speaks simply of a "step-up response" once the class and type of response, and the nature of the stimulus, have been identified.

The Strophophobic Response of *Euglena*

We have studied this reaction, which in *Euglena* is the main response mediating accumulation in or dispersal from a stimulated region, in detail with light as the stimulus (the strophophobic response appears identical when triggered by mechanical or chemical stimuli).

Upon application of the photic stimulus, the flagellum continues to beat normally for approximately 200 ms (D i e h n et al., 1975). This is the "transduction time", during which stimulus reception, signal processing, and transmission of the command to the flagellum take place. Within the time required for one flagellar beat (~30 ms), the regular 180° bend in the normally trailing flagellum then changes to 90°, such that the propulsive force of the flagellum now acts on the anterior end of the organism at right angles to the cell's long axis (Fig. 1). Forward movement ceases as a result, and the cell begins to turn toward its stigma-bearing side.

With a strong stimulus, i.e., upon a large change in stimulus intensity, the phobic response continues for many seconds, at about 1 turn per second. When adaptation to the stimulus occurs, the turning response is interrupted by periods of straight swimming that increase in length. If the stimulus is removed before adaptation has taken place, the flagellum will reorient to the normal trailing position, within ~30 ms, after a transduction time that again approximates 200 ms.

Having a transduction time of this magnitude has behavioral advantages for *Euglena*. While it is the t e m p o r a l intensity gradient of the stimulus that triggers the phobic response, such gradients are rarely encountered in nature. Instead, a cell often finds itself in a s p a t i a l stimulus gradient, i.e., in a situation where the stimulus distribution is nonuniform in its surroundings. It is the cell's movement which translates the spatial gradients into temporal gradients perceived by its receptor. I have called this method of detecting the sign and magnitude of a spatial gradient the "movement-modulated detection mode" (D i e h n 1978).

The strophophobic responses are most pronounced at a stimulus interface, i.e., where there is a strong stimulus gradient, such as a light/dark border. As all stimulus receptors appear to be located at the cell's anterior end (see below), a cell in encountering such an inter-

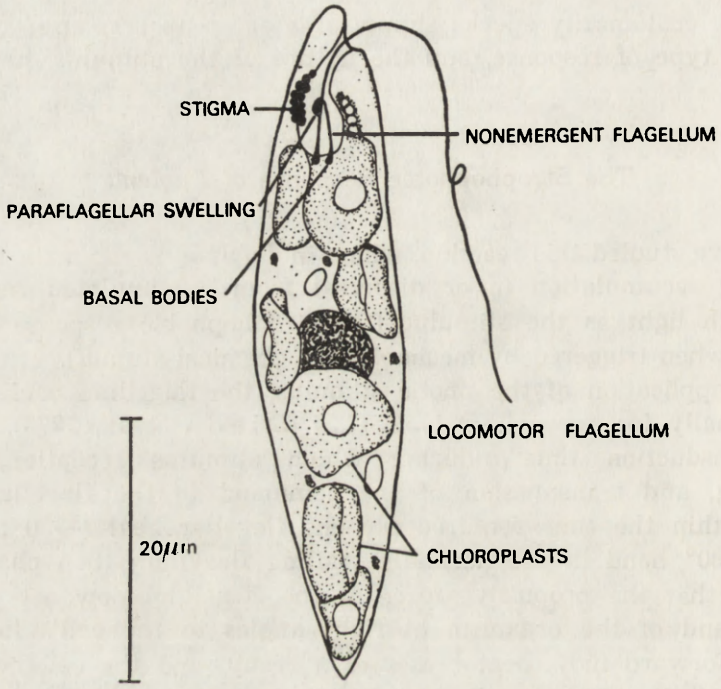


Fig. 1. The morphology of *Euglena gracilis*

face will during the transduction time enter the region of changed stimulus intensity to about a quarter of its own length. For this reason, stimulation persists long enough to drive the biochemical reactions occurring in the receptor to their new steady-state value (C r e u t z et. al 1978): since the cell has to move back out, the stimulation time will be approximately twice the transduction time. When the anterior end of the cell turns out of the stimulated region, the persistence of the response for another 0.2 s ensures that at its termination, *Euglena* will point approximately 180° away from the stimulus interface, and thus is not likely to encounter it again.

The Photic Stimulus Transduction System

I have postulated that in addition to the "motor" which e x p r e s s e s a given response, a transduction system consists of a receptor, a "signal processor", and an effector (D i e h n 1973). These are functional components that are defined operationally, and may or may not exist as distinct organelles in a given organism. The receptor (in the photosensory system of *Euglena* this is the paraflagellar body, Fig. 1)

converts the stimulus to a physiological signal. This signal is then conditioned in the processor, where such functions as differentiation and comparison with threshold and reference levels are carried out. Under the proper conditions, the processor then passes a command to the effector. In the case of the strophophobic response system of *Euglena*, the effector in turn alters the orientation of the motor without affecting the activity otherwise.

Both step-up and step-down phobic stimuli can induce the phobic response, but a step-up in light intensity will trigger the response only if the final intensity is above a "reference level" which in *Euglena* is roughly equivalent to the intensity of sunlight. Conversely, a step-down stimulus is effective only if the final intensity is below the reference level. In the photic system, there appears to be only one reference level. Figure 2 shows under what conditions of temporal light intensity change

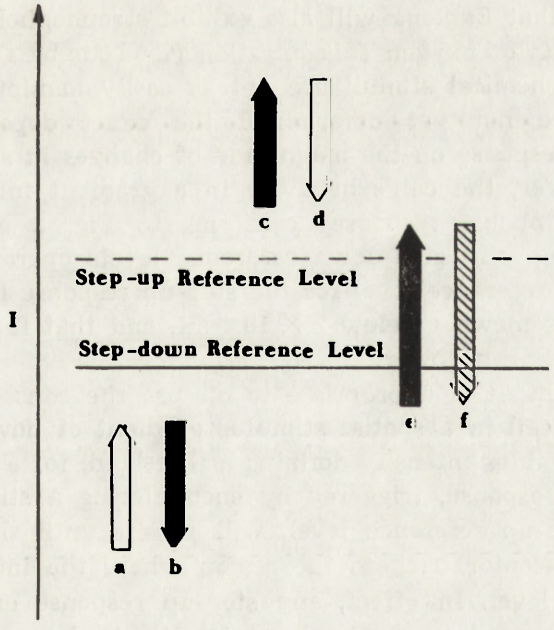


Fig. 2 Characteristic of stimulus intensity changes that induce, or fail to induce, strophophobic responses in *Euglena gracilis*. The tail of an arrow indicates the initial stimulus intensity, the head the final stimulus intensity. A solid arrow denotes that a phobic response occurs, an open arrow that there is no overt response. The striped arrow (f) indicates that upon an intensity decrease from above the step-up reference level to below the step-down reference level, a step-down response occurs that is delayed and attenuated with respect to an intensity decrease that does not cross the reference levels (b). For the phototransduction system, the step-up and step-down reference levels have an identical value (solid line) whose magnitude depends upon certain external conditions (Mikołajczyk and Diehn 1975, 1978). The chemotransduction system operates with two distinct reference levels, for the step-up response (broken line) and for the step-down response (solid line)

a photophobic response will be executed (D i e h n 1969). As seen in this figure, the reference level acts as a bidirectional response threshold, with asymmetric characteristics of step-up and step-down responses if the light intensity change crosses this level ("e" vs. "f"; see footnote to p. 12). The complexity of this behavior is one of the strongest arguments for postulating the presence of a signal processing function in *Euglena* (D i e h n 1974). In situations (a) and (d), no phobic responses occur. However, these stimulus regimes restore responsiveness of the cells to the same change in the opposite direction, (b) and (c) respectively. I have called this cancellation of the system's adaptation the "re-setting" phenomenon.

The Chemosensory System

We found that *Euglena* will also exhibit strophophobic responses in spatial gradients of oxygen concentration (C o l o m b e t t i and D i e h n 1978). Sience chemical stimuli are not as easily manipulated as photic ones, we have not yet determined the exact dependence of the chemophobic response on the magnitude of changes in stimulus concentration. However, the cell's behavior in a gradient indicates that the expression of phobic responses conforms to Fig. 2 a to 2 d, with separate step-up and step-down reference levels operative. It appears that *Euglena*'s reference lovel for the step-up response is at an oxygent concentration somewhat below 2×10^{-4} M, and that for the step-down response significantly lower.

At this point, it is appropriate to discuss the consequences for the behavior of a cell in a spatial stimulus gradient of having a reference level (i.e., a stimulus intensity defining a threshold) for a given response:

A step-up response, triggered by encountering a stimulus intensity above the step-up reference level, will persist until the response has brought the receptor back to the region where the intensity is below the reference level. In effect, any step-up response ensures that the cell remains exposed to a stimulus intensity b e l o w the s t e p - u p threshold. Similarly, a step-down response has the result of ensuring that the receptor remains stimulated at a level a b o v e the s t e p - d o w n threshold. Thus, both responses together act as a mechanism for keeping a cell within a given range of presumbly favorable stimulus intensity. [1]

[1] The asymmetry of the cell's step-up and step-down photophobic responses when crossing the reference level is important behaviorally in a light intensity gradient: Since both reference levels are identical, symmetric responses would keep all cells on the high intensity side that are there initially, and keep those cells

In a spatial gradient, the result is an accumulation pattern which displays the boundaries of the step-up and step-down reference levels. A dramatic example of such a pattern around an air bubble is shown in Pl. I.

Responses to Simultaneous Photic and Chemical Stimulation

It is fairly easy to subject a *Euglena* population to light and chemical stimuli at the same time. Cells in a chemoaccumulation pattern such as the one shown in Pl. I are kept within this pattern by constant chemophobic responses at the pattern boundaries. Photic stimuli can be added by varying the illumination of the cell suspension (ordinarily, chemoresponses are studied under yellow illumination of 577 nm, which is a wavelength to which the cell's photosensory system is not responsive).

If a photic stimulus is applied at an intensity below the reference level, the cells will, as one would expect, not exhibit any photophobic responses. However, the cells immediately begin to leave the chemoacumulation ring, apparently because they no longer respond with chemophobic reactions to the chemical stimulus gradients. As a consequence, the ring pattern disappears within approximately 10 s. This effect is transient: upon continuing illumination, the pattern forms again within another 30 s or so. If the light stimulus is removed at this time, the cells execute normal-appearing step-down photophobic responses. Simultaneously, the ring pattern boundaries sharpen perceptibly.

At this time, any interpretation of this behavior must of necessity be speculative. Clearly, the photic and chemical stimuli do not simply compete for the flagellar response system. The light stimulus, when given by itself, would only cause a re-setting of the photosensory transduction system, but no overt response whatever in the absence of the chemical stimulus.

One must conclude from the dispersal of the chemoaccumulation pattern that this photic stimulus causes a temporary erasure of chemosensory transduction. Apparently, control of the flagellar response system is transiently switched from the chemosensory to the photosensory transduction system. We might speculate that this occurs in anticipation of the step-down stimulus that w o u l d be presented if the light w e r e to be turned off again. If this stimulus does not materialize within approximately 30 s, the chemosensory apparatus resumes control over

on the low intensity side that start out on that side. In actuality, *Euglena* cells will promptly leave a region of high intensity surrounded by darkness. The reason for this is that when crossing the reference level intensity, the step-down response (unlike the step-up response) occurs with a greatly increased transduction time, and thus will not return the cell to the region of high intensity. The advantages of such response characteristics are easy to see: too high a light intensity is, of course, more dangerous than too low an intensity.

the strophic response system. Nevertheless, the photic transduction system remains reset, and will again take control of the strophic effector if the step-down stimulus is given now. At this time, the control is no longer absolute: The ring pattern sharpens during the photophobic response, indicating that chemophobic responses occur in addition to the photophobic responses, and moreover that the range between their step-up and step-down thresholds has been reduced.

The above conjectures call for further experimental tests. For instance, if it is the "resetting" characteristic of the stimulus that causes the cell to switch from one sensory system to another, then resetting for the step-up photophobic response should have the same effect as resetting for the step-down response. In other words, turning off high intensity illumination should have the same effect as turning on low intensity light.

There are two problems if one attempts such an experiment with ordinary light-grown *Euglena:* Firstly, the cells are photosynthetic and will evolve oxygen upon high intensity illumination. This, in turn, will destroy the chemoaccumulation pattern by raising the stimulus concentration above the step-up reference level. Secondly, turning off the light will cause an, albeit delayed and attenuated, step-down response (Fig. 2 "f"). Both problems can be overcome by using dark-grown cells. Without a functioning photosynthetic system, these cells neither evolve oxygen, nor are they capable of step-down responses (Diehn and Tollin 1967, Diehn 1973). They do, however, form chemophobic accumulation patterns.

The results of experiments with etiolated cells were exactly as predicted: increasing the illumination above the step-up reference level triggered photophobic responses and at the same time sharpened the chemophobic pattern. When the light was turned off, we indeed observed dissolution of the ring within a few seconds.

We are now engaged in studying the erasure of chemotransduction as a function of the magnitude of the resetting stimulus. These experiments should yield information on the characteristics of *Euglena*'s signal processor. Other problems that will be investigated include the question of whether resetting for the chemoresponses in turn inactivates the phototransduction system, or whether the latter is assigned an overriding importance in the hierarchy of sensory systems.

With the studies just described we are utilizing a novel means of investigating sensory transduction in a microorganism. While the results are strictly descriptive at this time, we hope that they will lead us to the design of experiments for probing these sensory processes at the molecular level.

REFERENCES

Checcucci A. 1976: Molecular sensory physiology of *Euglena*. Naturwiss., 63, 412–417.

Colombetti G. and Diehn B. 1978: Chemosensory responses toward oxygen in *Euglena gracilis*. J. Protozool., 25, 211–217.

Creutz C., Colombetti G. and Diehn B. 1978: Photophobic behavioral responses of *Euglena* in a light intensity gradient, and the kinetics of photoreceptor pigment interconversion. Photochem. Photobiol., 27, 611–616.

Diehn B. 1969: Phototactic response of *Euglena* to single and repetitive pulses of actinic light: Orientation time and mechanism. Exp. Cell. Res., 56, 375–381.

Diehn B. 1973: Phototaxis and sensory transduction in *Euglena*. Science, 181, 1009–1015.

Diehn B. 1974: Cybernetics and the behavior of microorganisms. In: Aneural Organisms in Microbiology. Plenum, New York.

Diehn B. 1978: Photic responses and photosensory transduction in motile protists. In: Handbook of Sensory Physiology VII/6A. Springer, Berlin/Heidelberg/Hew York, (in press).

Diehn B., Feinleib M., Haupt W., Hildebrand F., Lenci F. and Nultsch W. 1977: Terminology of behavioral responses of motile microorganisms. Photochem. Photobiol., 26, 559–560.

Diehn B., Fonseca J. and Jahn T. L. 1975: High speed cinemicrography of the direct photophobic response of *Euglena*, and the mechanism of negative phototaxis. J. Protozool., 24, 492–494.

Diehn B. and Tollin G. 1967: Phototaxis in *Euglena*. IV. Effect of inhibitors of oxidative and photophosphorylation on the rate of phototaxis. Arch. Biochem. Biophys., 121, 169–177.

Mikołajczyk E. and Diehn B. 1975: The effect of potassium iodide on photophobic responses in *Euglena*: Evidence for two photoreceptor pigments. Photochem. Photobiol., 22, 269–271.

Mikołajczyk E. and Diehn B. 1976: Light-induced body movement of *Euglena gracilis* coupled to flagellar photophobic responses by mechanical stimulation. J. Protozool., 23, 144–147.

Mikołajczyk E. and Diehn B. 1978: Morphological alterations in *Euglena gracilis* induced by treatment with CTAB (Cetyltrimethylammonium Bromide) and Triton X-100: Correlations with effects on photophobic behavioral responses. J. Protozool., 25, 461–470.

Miller S. and Diehn B. 1978: Evidence for cytochrome c oxidase as the receptor molecule for chemoaccumulation ("Chemotaxis") of *Euglena gracilis* toward oxygen. Science, 200, 548–549.

EXPLANATION OF PLATE I

3: Chemoaccumulation pattern of *Euglena gracilis* around an air bubble in a 0.1 mm thick suspension. The ring boundaries delineate oxygen concentrations corresponding to the step-up reference level (toward the bubble) and step-down reference level (opposite the bubble) for the chemophobic response

B. Diehn auctor phot.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 17–21

Department of Cell Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, 02-093 Warszawa, Poland, and Institute of Neurobiology, Nuclear Research Center, 5170 Jülich, Federal Republic of Germany

Stanisław DRYL and Eilo HILDEBRAND

Modifying Effects of Chemical Factors on Behavior and Excitability of Ciliate *Protozoa* *

Synopsis. Short-lasting exposure of *Paramecium* to higher concentrations of potassium ions (ca. 16 mM KCl) causes ciliary reversed beat while longer exposure to potassium reach medium results in complete or partial inhibition to ciliary response to various stimuli. This response-inhibiting effect of potassium ions may last for several minutes even after replacement of ciliates to external medium devoid of potassium. The possible mechanism of modifying effects of potassium and other external factors (e.g. ionic detergents) on the ciliary response of paramecia and other protozoa is discussed.

In ciliate protozoa the pattern of ciliary beat and its frequency are the basic features of their behavior. In dependence on the strength of applied stimulus paramecia may respond by the longer lasting ciliary reversal (CR) or by "Avoiding reaction" (Jennings 1906) which is characterized by change of direction of effective beat of cilia or even by short lasting CR, followed by pivoting movement of ciliate and finally by the further forward swimming in the new direction.

Jennings (1906) in his famous monograh "Behavior of Lower Organisms" mentioned already that motor response of protozoa may depend on physiological state of organism due to effects of physico-chemical factors of external medium. The pioneer work by Kamada and Kinosita (1940) revealed the significance of external calcium for response of *Paramecium* towards potassium ions. The japanese authors found that duration of K-induced CR incereases when level of external calcium decreases, but no response was detected if calcium ions were no more present in surrounding medium.

After recalculating and analyzing in detail the data of Kamada and Kinosita (1940) and Jahn (1962) suggested the presence of ions

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

exchange system in the cell membrane of *Paramecium* and he suggested that duration of K/Ca-induced CR depends on the ratio $[K^+] : [Ca^{2+}]^{1/2}$ which reflects competing action of potassium (or other external cations) on the cell membrane bound calcium. Jahn's view was supported by experimental results achieved by Grębecki (1964) and Kuźnicki (1966) who also assumed that external cations may release calcium ions from hypothetical ions exchange system within ciliate cell membrane. Naitoh (1968) in his "calcium hypothesis" postulated that calcium ions released from their binding sites in the cell membrane are acting directly on the intraciliary motile elements, causing a change of direction of effective beat of cilia, expressed in form of CR and beckward swimming of animals.

The important role of calcium and other external cations in response of ciliates to external stimuli is evident from results obtained by Kinosita et al (1964) who brought evidence that both graded response and all-or-none responses of the cell membrane in form of depolarizing spikes could be induced in *Paramecium cadatum* exposed to appropriate concentrations of barium and calcium ions. In this way it was proved that paramecia possess capacity of generating action potentials and so they represent excitable cells in terms of generally accepted theory by Grundfest (1966).

The next important step in our present knowledge of excitability in ciliates was the concept of transmembrane current of calcium ions from external medium (Eckert 1972, Eckert and Naitoh 1972) as a result of changes in the cell membrane permeability induced by cations or other external factors. According to this theory CR would appear due to direct action of external calcium ions on the motile elements within cilium. The possibility of occurrence of hypothetical voltage sensitive calcium channels in the cell membrane of *Paramecium* was strongly supported by studies of Kung (1971, 1973) on the behavioral mutants of *Paramecium tetraurelia*. Our present view on the mechanism of reception of external stimuli in *Paramecium* can be described briefly as follows:

(1) External stimulus acts on the reception portion of the cell membrane, causing opening of voltage sensitive calcium gate.
(2) Passive transmembrane current of external calcium through the opened channel occurs along electrochemical gradient and causes CR due to direct action of increased concentration of calcium ions on the motile elements within cilium. The depolarized state of membrane is increased.
(3) Potassium channels become opened and potassium ions flux out from the cell interior along its electrophysiological gradient.

(4) Calcium channels are closed and the level of intraciliary calcium returns to its initial low level, as response disappears.

It is clear from the above, presented sequence of events that the external stimuli may change properties of the cell membrane by action on the hypothetical calcium gates (p. 1) while other stages (p. 2, 3, 4) are triggered by the first one. Eckert (1972) disregarded the significance of Jahn's hypothetical ions exchange system within cell membrane of *Paramecium* by assumption that changed ratio $[K^+]$: $[Ca^{2+}]^{1/2}$ is accompanied by corresponding changes of gCa (Calcium conductance) and gK (potassium conductance). It should be emphasized that this view is not generally accepted and it enhanced Dryl to suggest (Dryl and Jahn 1974) that external cations — in accordance with Jahn's hypothesis — release calcium ions from their binding sites within receptor portion of excitable membrane, causing in this way conformational changes in the membrane which in turn result in an increased permeability of cell membrane for external calcium. This two-steps hypothesis connects in one logical unit Jahn's ions exchange system in the cell membrane with Eckert's concept of calcium transmembrane current. It should be pointed out that similar view was presented by Hildebrand (1975) who on the basis of his studies on the marine ciliate *Euplotes* calculated that the amout of the membrane bound calcium may directly determine gCa.

It is known from the early studies of Dryl (1952, 1959) on effects of potassium ions on the sensitiveness of paramecia towards external stimuli that after longer lasting exposure (1 h or more) of ciliates towards medium containing higher concentrations of K^+ the animals — after washing them in the medium devoid of potassium — cease during some period of time to respond with CR not only to potassium ions but also to other chemical stimuli. The response-inhibiting effects of potassium ions can be detected even after 15–20 min since the beginning of washing. The resting potential comes immediately after washing to more or less initial value (Kinosita et al. 1964) or is so little changed that completely loss of excitability can not be explained by changes of cell membrane polarity (Dryl and Kubalski, unpublished). In more recent studies on these lines Dryl and Hildebrand (1975 and some unpublished data) showed that also responsiveness of *Paramecium caudatum* to Ba/Ca factor is inhibited by previous exposure of ciliates to higher concentrations of potassium and that this effect depends not only on the concentrations of applied cations but also on the duration of exposure of ciliates to adaptation medium. In other series of experiments Hildebrand and Dryl (1976) found a critical value of external calcium (pCa 6.8–7.2) at which paramecia ceased to

respond with CR to various chemical agents. Recently analogous results were achieved in studies on chemotactic response of *Paramecium* (Dryl and Kurdybacha 1978) and on response of *Stylonychia mytilus* to chemical and mechanical stimuli (Dryl et al. 1978). It was found that the threshold for response to chemical stimuli in *Paramecium* (Quinine sol.) and *Stylonychia mytilus* (KCl sol.) decreases paralell to lowering of the level of free calcium ions in surrounding medium within range of pCa 2.0–5.0. The response to chemical stimuli was absent at very low levels of calcium (pCa 7.0–8.0). However, unlike in the case of potassium-adaptation, the ciliates exposed to EGTA/Ca buffer solutions (pCa 7.0–8.0) regain immediately their normal response capacity when replaced from the medium of low calcium ions content to solution with normal concentration of Ca^{2+} (pCa 3.0), provided that the initial exposure time to medium with low concentration of calcium did not exceed 50–60 s. These observations seem to point out again the great importance of external calcium in those processes of cell excitability which are related to activation and disactivation of hypothetical calcium channels within cell membrane of ciliates. It should be added in this connection that at low levels of external calcium (pCa 7.0-8.0) the simultaneous exposure of paramecia to high concentrations of potassium ions (ca 16 mM KCl sol.) does not induce any detectable effect on the response of ciliates to external stimuli after replacement of animals to medium of normal calcium ions content, but devoid of potassium (Dryl and Hildebrand, unpublished). This interesting finding is in good agreement with hypothesis of Hildebrand and Dryl (1976) that K-induced long lasting response-inhibiting effects on *Paramecium* (in presence of external calcium ions in concentrations higher than 10^{-6}M) may result from binding of calcium ions at the inner face of the ciliary membrane; consequently no inhibition of response should occur in ciliates preadapted in medium with very low level of external calcium.

Besides cations also ionic detergents may affect the receptor properties of the cell membrane in the ciliates as it was shown in extensive studies by Dryl and Bujwid-Ćwik (1972 a, b). The cationic detergent CTAB (Cetyl trimethyl ammonium bromide) proved to decrease the duration of K-induced CR in *Paramecium caudatum*, while the anionic detergent SDS (Sodium dodecyl sulfate) exerted an opposite effect, i.e., caused increase of duration of K-induced CR (Bujwid-Ćwik and Dryl 1975). The inhibiting effect of CTAB on excitability of *Paramecium* persisted during 15–30 min after washing ainmals in medium devoid of detergent (Dryl and Bujwid-Ćwik

1972 a) but it is not clear whether the reported effect of CTAB is due to the presence of detergent substance in the cell membrane or it should be explained by replacement of calcium from the outer to the inner face of the ciliary cell membrane.

It appears highly desirable to extend the experimental studies on modifying effects of various external agents on excitability of ciliate protozoa, since this may contribute in very essential way to our better understanding of receptor properties of the cell membrane.

REFERENCES

Bujwid-Ćwik K. and Dryl S. 1975: Effects of sodium dodecyl sulphate on motor response of *Paramecium caudatum* induced by K+ ions. Acta Protozool., 13, 267–274.

Dryl S. 1952: The dependence of chemotropism in *Paramecium caudatum* on the chemical changes in the medium. Acta Biol. Exp., 16, 23–53.

Dryl S. 1959: Effects of adaptation to environment on chemotaxis of *Paramecium caudatum*. Acta Biol. Exp., 19, 83.

Dryl S. and Bujwid-Ćwik K. 1972 a: Effects of detergent cetyl trimethyl ammonium bromide on motor reactions of *Paramecium* to potassium/calcium factor in external medium. Bull. Acad. Pol. Sci., Cl. II 20, 551–555.

Dryl S. and Bujwid-Ćwik K. 1972 b: Effects of detergents on excitability and motor response in protozoa. Acta Protozool., 11, 367–372.

Dryl S. and Hildebrand E. 1975: Long lasting potassium effect on excitability of *Paramecium* (*Protozoa, Ciliata*). Vth Int. Biophys. Congress, Copenhagen, August 4–9, 1975.

Dryl S. and Jahn T. L. 1974: Ciliary and flagellar movement. In: Actualites Protozoologiques. Resume des discussions des tables rondes du 4e Congress Int. Protozool., Clermont-Ferrand (eds. P. de Puytorac et J. Grain), Vol. I, 119–127.

Dryl S. and Kurdybacha J. 1978 a: The influence of calcium ions in external medium on chemotactic response in *Paramecium caudatum* and *Paramecium aurelia*. Acta Protozool., (in press).

Dryl S., Kurdybacha J. and Totwen-Nowakowska I. 1978 b: Galvanotactic response in *Paramecium caudatum* in various levels of external calcium ions. Acta Protozool., (in press).

Eckert K. 1972: Bioelectric control of ciliary activity in *Paramecium*. Science, 176, 472–481.

Eckert K. and Naitoh Y. 1972: Bioelectric control of locomotion in ciliates. J. Protozool., 19, 237–243.

Grundfest H. 1966: Comparative electrobiology of excitable membranes. Advanc. Comp. Physiol. Biochem., 2, 1–116.

Grębecki A. 1964: Role des ions K+ et Ca++ dans l'excitabilité de la cellule des protozoaires. I. Equilibrement des ions antagonistes. Acta Protozool., 2, 69–79.

Hildebrand E. 1975: Significance of the competition between calcium and other cations for the conductivity control in sensory membranes. Verh. Dtsch. Zool. Ges., 1974, 24–28.

Hildebrand E. and Dryl S. 1976: Significance of $Ca^{2}+$ and K+ ions for the excitation of the protozoan membrane. Bioelectrochem. Bioenergetics, 3, 543–544.

Jahn T. L. 1962: The mechanism of ciliary movement. II. Ciliary reversal and ion antagonism. J. Cell. Comp. Physiol., 60, 217–228.

Jennings H. S. 1906: Behavior of the Lower Organisms. New York.

Kamada T. and Kinosita H. 1940: Calcium-potassium factor in ciliary reversal of *Paramecium*. Proc. imp. Acad. Tokyo, 16, 125–130.

Kinosita H., Dryl S. and Naitoh Y. 1964: Changes in the membrane potential and the responses to stimuli in *Paramecium*. J. Fac. Sci. Univ. Tokyo, Sec. IV, 10, 291–301.

Kung C. 1971: Genic mutants with altered system of excitation in *Paramecium aurelia*. I. Phenotypes of the behavioral mutants. Z. Vergl. Physiol., 71, 142–164.

Kung C. 1973: Behavioral mutants of *Paramecium aurelia* In: Behavior of Microorganisms. (ed. A. Perez-Miravete), Plenum Press, New York.

Kuznicki L. 1966: Role of Ca^{2+} ions in excitability of protozoan cell. Calcium factor in the ciliary reversal induced by inorganic cations in *Paramecium caudatum*. Acta Protozool., 4, 241–256.

Naitoh Y. 1968: Ionic control of the reversal response of cilia in *Paramecium caudatum*. A calcium hypothesis. J. Gen. Physiol. 51, 85–103.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 23–24

Department of Biology, Saitama Medical School, Kawakado, Moroyama-cho Iruma-gun, Saitama, 350-04 Japan

Haruo KINOSITA

Development of Electrophysiological Studies of Ciliate *Protozoa* in Japan *

The first Laboratory of Animal Physiology in Japan was opened in 1934 at the Zoological Institute, Faculty of Science, Tokyo Imperial University, or the University of Tokyo, as it is called now. The Laboratory was chaired by Prof. T. Kamada, my teacher. From his childhood he has been strongly attracted by the homing behavior of animals, and this caused him choose the simplest and seemingly the most easily interpretable type of behavior; galvanotaxis of seemingly the simplest type of organism, *Paramecium* as the first subject of study in his research carrier.

With the progress of the researches of this line by himself (1928–31) and by me (1936–39), it became clear that the resemblance of the electric polar effect in the ciliary response of protozoa with that in the excitation of nerve and muscle, as was pointed out by Bancroft (1905), was very close, especially with respect to the intensity effect and to the time course of growing up. This inevitably led him and his pupils to push their way to the measurement of various electric characteristics of ciliates in relation to the ciliary activity.

The first measurement of the resting membrane potential by means of intracellular microelectrode was done by Kamada during his stay at the Zoological Laboratory, Cambridge, England in 1934, using *Paramecium*, five years before the famous work of Hodgkin (1939) on giant axon. Kamada pointed out the dependence of the resting membrane potential on external cationic concentration, which was later confirmed and studied in more details mainly by the japanese investigators, such as Yamaguchi (1960), Kinosita et al. (1964) and Naitoh et al. (1968), who tried to explain the resting membrane potential of *Paramecium* in terms of K-potential.

---

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

Professor Kamada died of cancer on 1946 during the War. And it was not until 1954 that Kinosita, his successor, first demonstrated the active membrane potential, appearing in close association with both the experimentally induced and the spontaneously occurring ciliary response of *Opalina*. Similar results were obtained with *Paramecium* by Yamaguchi (1960), Naitoh (1964), and Kinosita et al. (1964).

On the other hand, Kamada (1938, 40) had postulated that decrease of intracellular free Ca-ions brought about by electrical, chemical or any other stimuli may be responsible for ciliary reversal. Furthermore, it was pointed out by Kinosita (1954) that membrane potential change of *Opalina* appearing as a result of K-stimulation consisted of two components: one being depolarization due to K, and the other being Cahyperpolarizing potential correlated with reversal response. It is quite natural that after closer analysis of Ca-effect Naitoh (1968) and Prof. Eckert of UCLA (1972) put forward the new calcium hypothesis to account for the ciliary reversal.

Conduction of metachronal wave on the ciliated cell surface was accounted for, not by the ectoplasmic fibers as before, but by the regional differences in sensitivity and in reactivity of cilia in *Opalina* (Okajima, 1953). Even the well-known neuro-motor fibers of *Euplotes* was shown to play no part in the ciliary coordination. It is the electrotonic spread of active membrane potential change that causes coordination between anterior and posterior cirri (Okajima and Kinosita 1966).

Finally I should like to show you what kind of research activities have been conducted at the Laboratory of Animal Physiology, Zoological Institute, the University of Tokyo. Among 315 printed papers by the Laboratory members from the year 1934 to 1977 (44 years), 214 papers (68%) dealt with Cell Physiology, 162 papers (51%) with Electrophysiology, 111 papers (35%) with *Protozoa* and 98 papers (31%) with ciliary movement.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 25–31

Department of Anatomy, Albert Einstein College of Medicine Bronx, New York 10461, U.S.A.

Peter SATIR

Regulation of Microtubule Sliding in Cilia *

Synopsis. New information regarding the basic dynein, microtubule interaction that produces sliding of the axonemal doublets, thereby powering motility of protozoan and other cilia, is summarized in this report. Active sliding requires the interaction of two doublets and has a constant polarity such that the doublet with the active arms (No. n) pushes the adjacent doublet ($n + 1$) relatively tipward. Within the axoneme, the doublet microtubules do not all actively slide in unison. The dynein arms themselves consist of ca. 3 subunits and appear in two different forms which may correspond to stages in the arm work cycle related to sliding. The basic dynein, tubulin interaction responsible for sliding *per se* does not seem affected by increases in Ca^{2+}. The hierarchy of regulatory processes between the basic sliding event and ciliary motility *in vivo* therefore includes: (1) regulation of the dynein arm cycle (2) regulation of sliding synchronization and (3) behavioral control of ciliary motion.

The source of force generation in beating cilia and eukaryotic flagella is now well known to depend upon an interaction between the doublet microtubules of the 9 + 2 axoneme mediated by dynein arms that cause the doublets to slide relative to one another. Summers and Gibbons (1971) demonstrated sliding directly in trypsin-treated axonemes of sea urchin sperm. Upon ATP addition, such axonemes do not reactivate as do untreated controls; instead, they telescope apart. It is postulated that trypsin treatment disrupts one or a number of systems within the axoneme that lead to the conversion of such doublet sliding into the coordinated generation and propagation of bends necessary for normal movement. In the past year, new information regarding the basic dynein, microtubule interaction and its regulation has emerged. This is summarized and extended in this report, and current hypotheses stemming from the new findings are discussed.

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

Polarity of Microtubule Sliding

Sale and Satir (1977) have shown that ATP induces disintegration of trypsin-treated *Tetrahymena* axonemes by sliding. Together with a similar demonstration in mussel gill cilia (Walter and Satir 1977), this confirms that the sliding microtubule mechanism of motility is generally applicable to all classes of 9 + 2 axonemes including protozoan and metazoan cilia. With electron microscopy, in *Tetrahymena*, Sale and Satir (1977) have been able to identify configurations of axonemes where sliding has occurred. An example is shown in PI. I 1. Apparently, all nine doublets are capable of sliding and highly extended axonemes may be found. During active sliding, doublets can be ejected either from the tip or the base of the axoneme; once disintegration has occurred the doublets remain overlapped, but no further movement takes place. Such images do not occur in preparations when axonemes are prepared for microscopy prior to addition of ATP.

Active sliding requires the interaction of two doublets, which are by convention doublet No. n and doublet No. $n + 1$, where $n = 1–9$. Doublet n contributes the active dynein arms which bind to as yet unspecified sites on subfiber B of doublet $n + 1$. In whole mount microscopy and negative stain, Sale and Satir (1977) have demonstrated that there is a unique and constant polarity to microtubule sliding in the trypsin-treated *Tetrahymena* axonemes. After ATP addition, the arms always generate force such that doublet $n + 1$ is displaced tipward relative to doublet n.

Dynein Arm Morphology

In negative stain of axonemes induced to slide, as well as in controls, the dynein arms can be visualized. Each arm as seen consists of 3 cylindrical subunits whose morphology corresponds to the ca. three subunit aggregates of isolated dynein seen by Warner et al. (1977). As now characterized by gel electrophoresis, a complex series of 6–10 bands in the high molecular weight region corresponds to the original extraction of dynein; 2–3 of these bands possess ATPase activity (cf. Gibbons et al., 1976). Salt extraction of the outer dynein arm removes half of the band now designated as dynein-1, which is the major axonemal ATPase (Kincaid et al., 1973). Since the inner arm remains in this preparation, a likely assumption is that dynein-1 is a major component of both arms. How the other dynein-associated proteins are related to the inner and outer arms is still unclear.

In our preparations of *Tetrahymena* axonemes, the arms have two

appearances: extended and flattened (Pl I 2). In the extended form, the arms point toward the base of the cilium and they are long enough to bridge the interdoublet distance. Normally, such arms can be seen in cases where doublets are not adjacent to one another, so that no bridges can possibly be formed. However, W a r n e r and M i t c h e l l (1978) have observed that extended arms can also appear rather firmly attached to subfiber B of an adjacent doublet, an attachment that apparently requires Mg^{2+} and is released by addition of ATP. In the flattened form, the arms are more highly tilted and appear shorter, too short to bridge the interdoublet distance. W a r n e r and M i t c h e l l have also confirmed this appearance. Three possibilities exist as to the significance of these different appearances:

(1) The two forms may represent intrinsic differences in outer vs. inner arm morphologies. This is the explanation favored by W a r n e r and M i t c h e l l (1978).

(2) The differences in form may only be apparent, caused by some artifact of negative stain penetration and tilt angle or foreshortening.

(3) The differences may be indicative of different stages in the dynein arm work cycle related to sliding.

Our present preliminary work supports the last possibility.

Both configurations can be found along the same doublet after sliding, and there is the suggestion that the appearance is consistent with position. Most arms found between the sliding doublets are in the flattened configuration, while, as mentioned previously, where sliding has passed the arms are usually (always?) extended. More convincing perhaps is the fact that two corresponding images of the arms can be observed in cross-sections of fixed and embedded axonemes. These are (1) the standard image of the arms, possibly corresponding to the flattened configuration, and (2) the so-called rigor image (G i b b o n s 1975), possibly corresponding to the extended configuration. In mussel gill cilia, both images occur in the same preparation (PI I 2). Where axonemes are treated with detergent before fixation, membrane-bounded cross-sections may show the standard image of unattached arms, (Pl. I 2 a) while adjacent membraneless axonemes show the rigor image of attached arms (Pl. I 2 b).

One possible explanation of Pl. I 2 is that in the standard image, the arm is captured when ATP is bound to the dynein, while in the rigor position, no ATP is available. A scenario of the mechanochemical cycle of dynein arm activity is that the flattened configuration extends when ATP is converted to ADP-P_i at the enzymatic site. In this extended form the arm can bind to the tubulin lattice of subfiber B and perform the work of sliding; to produce the rigor position, the products of

ATP hydrolysis are released and the dynein-tubulin interactions is set. Nonetheless, ATP will plasticize this interaction and reconvert the attached extended arm to the standard free flattened form. Similar scenarios have been proposed by Bloodgood (1975) and Sleigh (personal communication). It must, however, be remembered that all such explanations are still highly speculative. We are presently seeking ways to recover the extended vs. flattened configuration and the standard vs. rigor image of the arms reproducibly under various known conditions, where negative stain and thin section images can be closely compared.

Asynchronous Activity of Ciliary Microtubules

The finding of a constant polarity of force generation for all nine doublets in the sliding *Tetrahymena* axonemes implies that the doublets do not act in complete unison to produce ciliary motion. This implication has been explored further by Satir and Sale (1977), who have examined splayed tips of *Tetrahymena* cilia to illustrate asynchronous activity of opposite halves of the axoneme. Plate I 3 shows one such tip whose doublets can be numbered. Displacements of subfiber B are presumably due to active sliding of microtubules (cf. Sale and Satir 1976 for further discussion). Note that where $n = 3$ and $n + 1 = 4$, subfiber B of doublet No. 4 is displaced tipward relative to No. 3 precisely as would be predicted if the arms of doublet No. 3 were active and the cilium behaved identically to he trypsin-treated sliding axoneme. The same situation applies where $n = 4$ and $n + 1 = 5$. Clearly, however, in Pl. I 3 where $n = 7$, 8, 9 and 1, this is not the case — i.e., when $n = 8$ and $n + 1 = 9$, doublet No. 9 is displaced baseward relative to No. 8. This suggests that the arms of doublets 7, 8, 9, and 1 are not active at this time in the cilium, at least not in a way which is preserved in the trypsin-treated axonemes. In the model constructed by Satir and Sale (1977) such tip displacements are consistent with doublets 1–4 actively sliding during the ciliary effective stroke, while doublets 6–9 are active during the recovery stroke. In each case, the passive doublets presumably move in response to the geometrical considerations of bend formation. What turns the active sliding on and off? We know only that such regulation is destroyed by brief treatment with trypsin. We must remember that trypsin as used to produce the sliding models degrades several components of the axoneme including the radial spokes, interdoublet links and possibly parts of the arms themselves. One speculation looks promising: the two halves of the axoneme might be regulated by different members of the

central pair of microtubules, since radial spokes of doublets 2-4 and 6-9 abut against different sides of the central complex, that is sheath projections of cm 3 and cm 8, respectively (Warner and Satir 1974).

Calcium Control of Sliding

Many cilia show stoppage when the calcium ion concentration surrounding the axoneme rises to greater than 10^{-6}M. The lateral cilia of the freshwater mussel gill epithelium, for example, are arrested in one particular position approximately equivalent to the end recovery stroke (R-pointing; see Satir 1968) by this calcium ion concentration. This arrest seems to involve differential microtubule sliding — i. e., activity of one half of the axoneme (doublets 6–9?) and not the other. In protozoa, arrest of beat is not a necessary consequence of elevation of axonemal Ca^{2+} (e. g., *Paramecium:* Naitoh and Kaneko 1972; Machemer 1976); instead, the direction of the effective stroke, direction and bend propagation or parameters of stroke form may change. These changes require continued microtubule sliding in the presence of elevated Ca^{2+}.

In *Tetrahymena,* detergent treated axonemes subjected to trypsin treatment and induced to slide as in PI. I 1 alter neither extent nor direction of sliding when treated with millimolar Ca^{2+} (cf. Sale 1977). The basic dynein, tubulin interaction responsible for sliding *per se* does not seem to be significantly affected by increases in Ca^{2+}. Consequently Ca^{2+} must act on the systems which regulate the conversion of microtubule sliding into propagating, localized bending.

Conclusion: Hierarchies of Regulation

In this report, I have briefly explored present knowledge of regulation of microtubule sliding in the ciliary axoneme at several levels:

(1). Regulation of the dynein arm cycle. This affects the basic mechanochemistry of sliding.

(2). Regulation of sliding synchronization. This is related to the conversion of sliding into true ciliary motility.

(3). Regulation of ciliary beat by Ca^{2+}. This is a behavioral control superimposed on the production of propagated bends. This hierarchy of regulatory processes suggests the complexity of genetic and biochemical events involved in the production and control of ciliary motility. As I have indicated, despite the present important success of the sliding microtubule model, at every level fundamental questions remain.

ACKNOWLEDGEMENTS

This work was supported by a grant from the United States Public Health Service (HL 22560). Portions of this work were done in collaboration with Dr. Winfield Sale and Dr. Jacobo Wais. I thank these individuals for their permission to use both published and previously unpublished data from these collaborative studies. Mr. Stephen Lebduska provided important photographic assistance with this work.

REFERENCES

Bloodgood R. A. 1975: Biochemical analysis of axostyle motility. Cytobios., 14, 101–120.

Gibbons I. R. 1975: The molecular basis of flagellar motility in sea urchin spermatozoa. In: Molecules and Cell Movement. (Inoue S. and Stephens R. E. eds.) Raven Press, N. Y. p. 207–232.

Gibbons I. R., Fronk E., Gibbons B. H. and Ogawa K. 1976: Multiple forms of dynein in sea urchin sperm flagella. In: Cell Motility. (Goldman R. D. et al. eds.) Cold Spring Harbor Lab., New York p. 915–932.

Kincaid H. L. Jr., Gibbons B. H. and Gibbons I. R. 1973: The salt extractable fraction of dynein from sea urchin flagella: an analysis by gel electrophoresis and adenosine triphosphatase activity. J. Supramol. Struct. 1, 461–470.

Machemer H. 1976: Motor activity and bioelectric control of cilia. Symp. "Bewegungsphysiologie-Biomechanik" (Nachtigall W. ed.) Akademie der Wissenschaften, Mainz (in press).

Naitoh Y. and Kaneko H. 1972: Reactivated triton extracted models of *Paramecium*: modification of ciliary movement by calcium ions. Science, 176, 523–524.

Sale W. S. 1977: Fine structure and direction of active microtubule sliding in the cilia of *Tetrahymena*. Ph. D. thesis, Univ. of California, Berkeley.

Sale W. S. and Satir P. 1976: Splayed *Tetrahymena* cilia J. Cell Biol., 71, 589–605.

Sale W. S. and Satir P. 1977: The direction of active sliding of microtubules in *Tetrahymena* cilia. Proc. Natl. Acad. Sci. USA, 74, 2045–2050.

Satir P. 1968: Studies on Cilia III. Further studies on the cilium tip and a "sliding filament" model of ciliary motility. J. Cell Biol., 39, 77–94.

Satir P. and Sale W. S. 1977: Tails of *Tetrahymena*. J. Protozool., 24, 498–501.

Summers K. E. and Gibbons I. R. 1971: Adenosine triphosphate induced sliding of tubules in trypsin-treated flagella of sea urchin sperm. Proc. Natl. Acad. Sci. USA, 68, 3092–3096.

Walter M. F. and Satir P. 1977: Calcium does not inhibit sliding of microtubules from mussel gill cilia. J. Cell Biol., 75, 287 a.

Warner F. D. and Mitchell D. R. 1978: Structural conformation of ciliary dynein arms and the generation of sliding forces in *Tetrahymena* cilia. J. Cell Biol., 76, 261–277.

Warner F. D. and Mitchell D. R. 1978: Structural conformation of ciliary mation of the ciliary ATPase dynein. J. Mol. Biol., 114, 367–384.

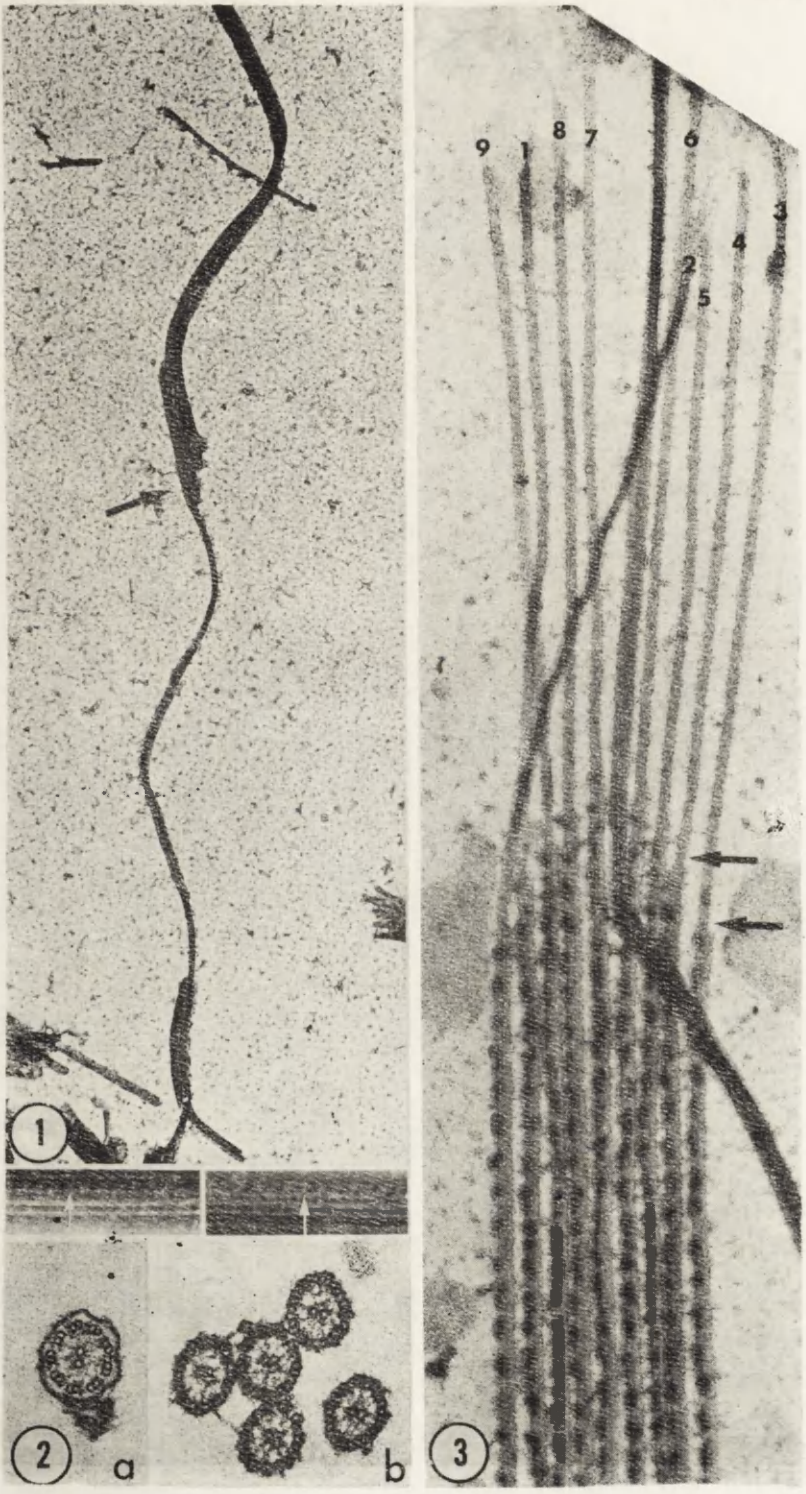
Warner F. D. and Satir P. 1974: The structural basis of ciliary bend formation. J. Cell Biol., 63, 35–63.

EXPLANATION OF PLATE I

1: *Tetrahymena* axoneme which has telescoped apart by sliding. Arrow indicates region of overlap. (Previously unpublished micrograph from Sale and Satir 1977) 22 500×

2: Dynein arm images. Above: Negative stain: regions along a single *Tetrahymena* axoneme (Previously unpublished micrograph from Satir and Sale 1977). 92 000 ×. Below: Thin sections: regions from a single mussel gill preparation (courtesy J. Wais) 54 000 ×. (a) Flattened configuration (arrow) and standard image. Note that in these micrographs the arms do not bridge the interdoublet space (b) Extended configuration (arrow) and rigor image

3: Splayed *Tetrahymena* axoneme showing microtubule displacements. Doublets numbered according to Sale and Satir (1976). Termination of subfiber B of doublets No. 3 and 4 marked by arrows. Note tipwards displacement of No. 4 vs. 3. See text for further explanation (from Sale and Satir 1976, courtesy W. Sale and the J. Cell Biol.,) 48 000 ×



P. Satir auctor phot.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 33–41

Institute for Cytology, University of Bonn, Ulrich-Haberland-Str. 61 a, Bonn 1, Fed. Rep. Germany

W. STOCKEM

Cell Surface Morphology and Activity in *Amoeba proteus* and *Physarum polycephalum* *

Synopsis: The results presented here on *A. proteus* and two different types of microplasmodia of *Physarum polycephalum* elucidate the important role of membrane-associated contractile proteins in cell movement phenomena and furthermore demonstrate the correlation that exists between the three-dimensional organization and activity of these structures on the one hand and the resulting type of movement on the other.

Recent progress in cell surface research (Poste and Nicolson 1977) has revealed that the plasma membrane is a rather complicated structure consisting of several layers (Pl. I). The external cell surface is coproteins and glycolipids. The macromolecules of the mucous layer are covered by a mucous layer (Pl. I $1_{,1}$) rich in mucopolysaccharides, glyinsserted into a lipid bilayer containing most of the integral protein components (Pl. I $1_{,2}$). Peripheral proteins like α-actinin, spectrin or filamin are attached to the inner surface of the integral layer (Pl. $I_{,3}$) and seem to function as a connecting link for a membrane-associated zone of microfilaments or microtubules (Pl. $I_{,4}$). The fine structure and celll physiological significance of the membrane-associated proteins has become more important since numerous investigations could demonstrate the participation of this layer in different movement phenomena (Comly 1973, Gruenstein et al. 1975, Korn and Wright 1973, Korohoda and Stockem 1975, Pollard 1975, Pollard and Korn 1973, Schroeder 1975, Taylor 1976, Tilney 1975, Tilney and Mooseker 1971). According to these results the membrane-attached components are involved in

(1) Changes in cell form and cell surface morphology,

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

(2) generation of motive force for cytoplasmic streaming and cell locomotion,

(3) cytotic membrane flow processes,

(4) cell division and

(5) cell body stabilization.

In a series of experiments we studied the fine structure, dynamics and function of a cortical filament layer present in close proximity to the plasma membrane of *Amoeba proteus* and *Physarum polycephalum* (PI. I 2). The most important results of these experiments are summarized in the following.

Amoeba proteus

(a) The Influence of Phalloidin

The phallotoxins have been used as specific drugs for the investigation of the actomyosin system (Wieland 1975). Phalloidin reacts stoichiometrically with actin, induces the polymerisation of G- to F-actin and stabilizes F-actin filaments (Lengsfeld et al. 1974). Because of these properties it was expected that locomotory phenomena based on a control of the G-F-actin equilibrium could be influenced by phalloidin. Since the external application of phalloidin proved to be ineffective the drug was microinjected in different concentrations (Wehland et al., 1978, Stockem et al., 1978). The injection of a 2×10^{-4} M phalloidin solution resulted in a temporary cessation of amoeboid movement: most of the cells returned to normal movement 30–60 min later. After increasing the concentration of phalloidin to 10^{-3} M the cells lost their ability for active locomotion in a irreversible manner. Simultaneously a separation of hyaloplasm and granuloplasm was observed.

The light microscopical observations could be correlated with electron microscopical studies on cells at corresponding times after the microinjection of phalloidin. According to these studies the application of the drug induces the display of a 0.5–1.0 μm thick filamentous layer at the cell membrane (c. f. Pl. II 3 and 4). This layer consists of an unordered meshwork of 6 nm filaments (probably actin) and is partially connected with the inner surface of the membrane (PI. II 5 and 6). Later the layer separates from the cell membrane by a slow contraction and moves into the cell interior (Pl. III 7 and 8). During the contraction thick filaments (probably myosin) are formed with diameters of 10–30 nm and lengths of 300–500 nm. The same observations were made by D'Haese and Komnick (1972 a and b) and Hinssen and D'Haese (1976) in actomyosin gels of striated muscle and *Physarum polycephalum*.

The filamentous layer can be compared to a closed capsule with a porous wall that acts as a sieve on the cytoplasm during contraction (Pl. III 8): Smaller particles and fluid are pressed towards the cell periphery whereas bigger cellular structures are condensed within the interior of the capsule.

Summarizing the results obtained with phalloidin in *A. proteus*, two conclusions can be drawn: (1) The controlled assembly and disassembly of membrane-associated contractile filaments seem to play an important role for the normal locomotion behaviour of this organism and (2) the often observed separation of hyaloplasm and granuloplasm, also typical for other species of amoebae, can be explained by the display and action of a capsule or sheath of contractile filaments (c.f. also Haberey 1973).

(b) The Influence of Pinocytosis-inducing Substances.

It is well established that a variety of different substances induce membrane flow processes during endocytotic food ingestion in *A. proteus* (Chapman-Andresen 1973, Stockem 1977). This phenomenon represents an excellent model for the study of cell surface movements caused by the activity of a membrane-associated layer of contractile filaments (Klein and Stockem, in press). Such a layer is very well preserved after stimulation of pinocytosis with NaCl and prolonged fixation with osmium tetroxide (Pl. IV 9–11). With regard to the dimension and fine structural organization it is comparable to the membrane-attached filament layer induced by microinjection of phalloidin into the same organism. Cinematografic single frame analysis and electron microscopy have shown that pinocytotic channel elongation, vesiculation and shortening can be retraced to both the alternative contraction and relaxation of this layer and the formation and retraction of small pseudopodia in the cell periphery (Pl. IV 12).

Pinocytosis is initiated by changes in permeability of the plasma membrane due to the addition of an inducing substance into the culture medium (Pl. IV 12 a and b, dots) and substitution of membrane-bound ions (probably calcium) by the inducer (Pl. IV 12 a and b, stars). In consequence of this exchange membrane permeability increases and medium (Pl. IV 12 a and b, dots) and substitution of membrane-bound external calcium or other ions can enter the cell interior. Calcium ions are necessary for a local contraction of the cortical filament layer and the formation of a short membrane invagination (Pl. IV 12 c and d). The basis of this channel is then anchored by the contracted filament sheath and further elongation of the channel occurs by the extension of a small pseudopodium (Pl. IV 12 e and f). Channel vesiculation and hence formation of pinocytotic vacuoles is the consequence of a constriction of

the filament layer within a limited channel region and membrane disruption by simultaneous pseudopodium extension (Pl. IV 12 h–k).

Although this model is still hypothetically in some respect, our results have demonstrated that the cortical filament layer in *A. proteus* is responsible not only for cytoplasmic streaming and cell locomotion but also for plasma membrane movement phenomena during pinocytotic food ingestion.

Physarum polycephalum

The acellular slime moulds can adapt to different culture methods by the development of special growing forms. In axenic cultures (Daniel and Rusch 1961) *Physarum polycephalum* forms so-called microplasmodia which may be spherical or elongated. They differ in diameter (50–500 μm) and show various protoplasmic streaming patterns. Microplasmodia are therefore interesting models to study the dynamics and fine structure of cell differentiations involved in the generation of motive force for protoplasmic streaming and amoeboid movement.

Two different forms of microplasmodia were chosen for the cinematografic and electron microscopic analysis: (1) amoeboid microplasmodia (Pl. V 13) and (2) dumbbell shaped microplasmodia (Pl. VI 15).

(a) Amoeboid Microplasmodia

The size, form and moving behaviour of amoeboid microplasmodia of *Physarum polycephalum* show certain similarities to rhizopod organisms. Single frame analysis of locomoting individuals recalls to similar sequences obtained from cinematografic drawings of *Amoeba proteus* (c.f. Fig. 14 with Abb. 4 from Stockem et al. 1969). The evaluation of more than 160 sequences revealed a very clear correlation between the direction of protoplasmic streaming on the one hand and outline changes on the other. Although the protoplasm in amoeboid microplasmodia shows an irregular streaming pattern without any preferential streaming direction (Pl. V 14), an increase in outline area (Pl. V 14, black regions) was normally due to an influx of protoplasm, whereas a decrease in outline area (Pl. V 14, white regions between the thin and thick lines) corresponded to an efflux of protoplasm from these cell regions (in 79–84% of all cases analysed by Gawlitta 1978). Comparatively few individuals showed no clear correlation between the direction of protoplasmic streaming and outline changes of the cell body (21–16%). In addition, investigations with the differential interference contrast microscope and the scanning electron microscope revealed that cell regions from which protoplasmic streaming originated were always

characterized by a high degree of folding of the cell surface, whereas regions of protoplasmic influx were characterized by a smooth cell membrane (c.f. also Stockem et al., 1969 for *A. proteus*).

According to transmission electron microscopic investigations the cell surface activity of amoeboid microplasmodia seems to be caused by the contractile behaviour of a membrane-associated layer of thin filaments (c.f. Pl. I 2). This layer is of similar fine structure and extension as described for the corresponding layer in *A. proteus*; it turned out to be sensitive to substances which change the morphology of cytoplasmic actomyosin systems: DNP (dinitrophenol) and NEM (N-ethylmaleimide). Both substances induced an increase in thickness and density of this layer in the investigated individuals (Hoffmann 1977).

Cytoplasmic fibrils known as a characteristic component in veins of macroplasmodia (c.f. Wohlfarth-Bottermann 1974, 1975) are lacking in this type of microplasmodia of *Ph. polycephalum.*

(b) Dumbbell Shaped Microplasmodia

The morphological appearance of dumbbell shaped microplasmodia differs from that of amoeboid individuals (c.f. Pl VI 15 with Pl V 13). Dumbbell shaped microplasmodia consist of two spherical heads which are connected by a small tube. Their protoplasmic streaming pattern is very regular and corresponds to the shuttle streaming in isolated veins of *Ph. polycephalum.* In contrast to the amoeboid type the dumbbell shaped microplasmodia possess a very constant shape. This can be concluded from single frame analysis (Pl. VI 16) of more than 150 sequences performed in the same way as described before (c.f. Pl. V 14).

A correlation between the direction of protoplasmic streaming and outline changes of the microplasmodia is largely lacking. Volume increases and decreases in the two heads occur not always alternatively and with the same range as it must be postulated in the case of a simple hydraulic pressure flow (c.f. the hypothetic model in Pl. VIII 20). The observed outline changes indicated by the black and white areas between the two successive stages in the analysed microplasmodium are due to a slight rotation of the whole individual perpendicular to the long axis rather than to true volume changes of the heads or the connecting tube. Moreover, morphometric measurements have clearly demonstrated that the slight volume changes observed in the two heads cannot explain the relatively large amount of protoplasm transported through the tube from one head into the other within one streaming period, i.e., the motive force for the hydraulic flow of protoplasm is probably not caused by the

exclusive action of a cortical layer of membrane-associated filaments as it seems to be the case for amoeboid types.

A possible explanation for these apparently inconsistent observations resulted from the structural analysis of the internal morphology of the dumbbell shaped microplasmodia. In contrast to amoeboid forms the dumbbell shaped types have already developed a cell membrane invagination system (c.f. Pl. VIII 19 a and b). This system is more extensive but less organized (Pl. VII 17) than the same system in veins of microplasmodia (c.f. Pl. VIII 19 and c). In agreement with investigations on macroplasmodia of *Ph. polycephalum* (Wohlfarth-Bottermann 1974, 1975) it could be shown that the invaginations are connected with one another by membrane attached fibrils.

The cinematografic analysis of single invaginations in the differential interference contrast microscope revealed rhythmic volume changes which are correlated with the influx and efflux periods of the protoplasm into the observed head (Pl. VII 18): during the period of protoplasmic influx the invaginations decreased in volume (Pl. VII 18, dotted stages), whereas during protoplasmic efflux the volume of the invaginations increased again (Pl. VII 18, white stages). These results allow the preliminary conclusion that the generation of motive force in dumbbell shaped microplasmodia is caused by changes both in the external and internal outline of the microplasmodia (Pl. VIII 21). The activity of a membrane-associated filament layer can effect volume changes in the external outline of the heads, whereas alternative contraction and relaxation (or desintegration) of the cytoplasmic fibrillar system between the cell membrane invaginations can induce volume changes in the internal membrane system (c.f. Pl. VIII 22).

Further investigations should prove whether this model — which is in agreement with all morphological and physiological data so far obtained in dumbbell shaped microplasmodia — can be of any use for the interpretation of the more complicated protoplasmic streaming pattern in macroplasmodia of *Ph. polycephalum*. In this connection it can be stated that microplasmodia are very useful objects to study the development of both the architecture and protoplasmic movement phenomena of variously differentiated stages of the acellular slime mould.

REFERENCES

Chapman-Andresen C. 1973: Endocytotic processes. In: The Biology of *Amoeba* (ed. Jeon K. W.), 319–348, Academic Press, New York.

Comly L. T. 1973: Microfilaments in *Chaos carolinensis*: membrane association, distribution and heavy meromyosin binding in the glycerinated cell. J. Cell Biol., 58, 230–237.

Daniel J. W. and Rusch H. P. 1961: The pure culture of *Physarum polycephalum* on a partially defined soluble medium. J. Gen. Microbiol.. 25. 47–59.
D'Haese J. and Komnick H. 1972 a: Fine structure and contraction of isolated muscle actomyosin 1. Evidence for a sliding mechanism by means of oligomeric myosin. Z. Zellforsch., 134, 411–426.
D'Haese J. and Komnick H. 1972 b: Fine structure and contraction of isolated muscle actomyosin 2. Formation of myosin filaments and their effect on contraction. Z. Zellforsch., 134, 427–434.
Gawlitta W. 1978: Cytologische Untersuchungen zum Bewegungsverhalten von verschiedenen Mikroplasmodientypen des azellulären Schleimpilzes *Physarum polycephalum*. Dipl. Arbeit Math.-nat. Fak Univ. Bonn.
Gruenstein E., Rich A. and Weihing R. R. 1975: Actin associated with plasma membranes from 3T3 and Hela cells. J. Cell Biol., 64, 223–234.
Haberey M. 1973: Räumliche Anordnung von Plasmafilamenten bei *Theacamoeba sphaeronuceolus*. Cytobiologie, 8, 61–75.
Hinssen H. and D'Haese J. 1976: Synthetic fibrils from *Physarum* actomyosin — self assembly, organization and contraction. Cytobiologie, 13, 132–157.
Hoffmann H. U. 1977: Methodische Untersuchungen zur Feinstruktur von Mikroplasmodien des azellulären Schleimpilzes *Physarum polycephalum*. Dipl. Arbeit Math.-nat. Fak. Univ. Bonn.
Klein H. P. and Stockem W.: Pinocytosis and locomotion of amoeba: XII. Dynamics and motive force generation during induced pinocytosis in *Amoeba proteus* Cell Tiss. Res. (in press).
Korn E. D. and Wright P. L. 1973: Macromolecular composition of an amoeba plasma membrane. J. Biol. Chem., 248, 439–447.
Korohoda W. and Stockem W. 1975: On the nature of hyaline zones in the cytoplasm of *Amoeba proteus*. Microscopica Acta, 77, 129–141.
Lengsfeld A., Low I., Wieland T., Dancker P. and Hasselbach W. 1974: Interaction of Phalloidin with actin. Proc. Acad. Nat. Sci., 71, 2803–2807.
Pollard T. D. 1975: Functional implications of the biochemical and structural properties of cytoplasmic contractile proteins. In: Molecules and Cell Movement (eds. Inoue S. and Stevens R. E.), 259–286, Raven Press, New York.
Pollard T. D. and Korn E. D. 1973: Electron microscopic identification of actin associated with isolated amoeba plasma membranes. J. Biol. Chem. 248, 4682–4690.
Poste G. and Nicolson G. L. 1977: Dynamic aspects of cell surface organization. In: Cell Surface Reviews 5, North-Holland-Publ. Com., Amsterdam, New York, Oxford.
Schroeder T. E. 1975: Dynamics of the contractile ring. In: Molecules and Cell Movement (eds. Inoue S. and Stevens R. E.), 305–334, Raven Press, New York.
Stockem W. 1977: Endocytosis. In: Mammalian Cell Membranes 5, (eds. Jamieson G. A. and Robinson D. M.), 151–195, Butterworths, London.
Stockem W., Wohlfarth-Bottermann K. E. und Haberey M. 1969: Pinocytose und Bewegung von Amöben. V. Konturveränderungen und Faltungsgrad der Zelloberfläche von *Amoeba proteus* Cytobiologie, 1, 37–57.
Stockem W., Weber K. and Wehland J. 1978: The influence of microinjected phalloidin on locomotion, protoplasmic streaming and the organization of the cytoplasma in *Amoeba proteus* and *Physarum polycephalum*. Cytobiologie, 18, 114–131.
Taylor D. L. 1976: Motile model systems of amoeboid movement. In: Cell Motility. Actin, Myosin and Associated Proteins (eds. Goldman R., Pollard T. and Rosenbaum J.), 797–821, Cold Spring Harbor Symposium.
Tilney L. G. 1975: The role of actin in nonmuscle cell motility. In: Molecules and Cell Movement (eds. Inoue S. and Stevens R. E.), 339–388, Raven Press, New York.
Tilney L. G. and Mooseker M. 1971: Actin in the brush border of epithelial cells of the chicken intestine. Proc. Nat. Acad. Sci. USA, 68, 2611–2615.
Wehland J., Stockem W. and Weber K. 1978: Cytoplasmic streaming in *Amoeba proteus* is inhibited by the actin specific drug phalloidin. Exp. Cell Res., 115, 451–454.

Wieland T. 1975: Phallotoxins and microfilaments (eds. Heilmeyer L. H. G., Rüegg J. C. and Wieland T.), 203–241, Springer Berlin, Heidelberg, New York.

Wohlfarth-Bottermann K. E. 1974: Plasmalemma-invaginations as characteristic constituents of plasmodia of *Physarum polycephalum*. J. Cell Sci., 15, 23–37.

Wohlfarth-Bottermann K. E. 1975: Weitreichende fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. X. Die Anordnung der Actomyosin - Fibrillen in experimentell unbeeinflussten Protoplasmaadern von *Physarum polycephalum in situ*. Protistologica, XI, 1, 19–30.

Wolf K. 1978: Cytologische Untersuchungen zur Nahrungsaufnahme bei Mikroplasmodien von *Physarum polycephalum*. Dipl. Arbeit Math.-nat. Fak. Univ. Bonn.

EXPLANATION OF PLATES I–VIII

Plate I. General morphology of the cell surface

1: Schematic drawing of the cell membrane complex
1_1 — mucous layer, 1_2 — plasmalemma, 1_3 — peripheral proteins and 1_4 — associated proteins
2: Electron micrograph of the cell membrane complex of *Physarum polycephalum;* mL — mucous layer, pm — plasmalemma, cLf — cortical filament layer, cp — adjacent cytoplasm, 200 000 × (From Hoffmann 1977, unpublished)

Plate II and III. Action of phalloidin on *Amoeba proteus*

3: Fine structure of the cell periphery of a control amoeba
4: Formation of a cortical filamentous layer (cLf) a few minutes after injection of a 10^{-3} M phalloidin solution
5, 6: Beginning contraction of the filamentous layer and local discharge from the plasmalemma
7: Contracted filamentous layer (fL) and resulting separation of peripheral hyaloplasm (hp) and central granuloplasm (gp)
8: Schematic drawing summarizing the effect of phalloidin,
m — mitochondria, cv — crystal vacuole 3,4 = 8500 ×; 5,6 = 17 000 ×; 7 = 6300 × (From Stockem et al., in 1978)

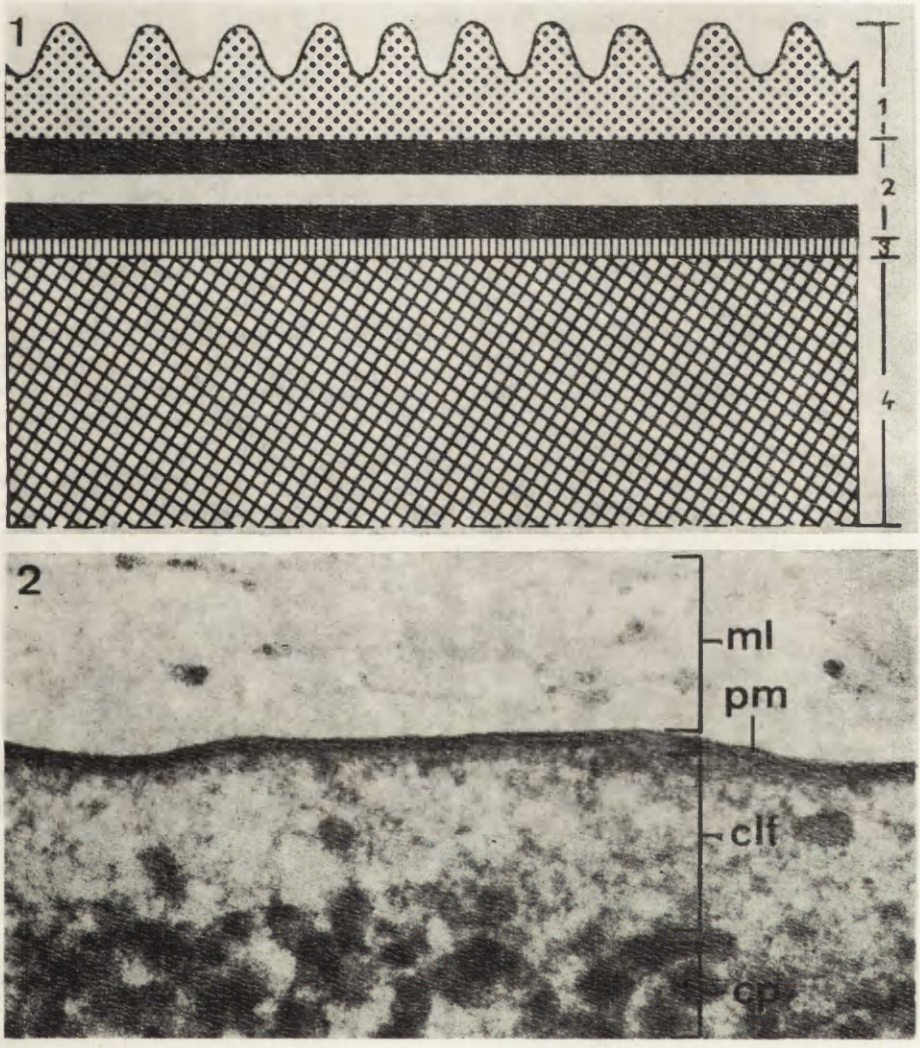
Plate IV. Induced pinocytosis in *Amoeba proteus*

9–11: Cortical filament layer of varying thickness and density after induction of pinocytosis with NaCl
12 a–k: Schematic drawing summarizing the activity of the cortical layer during formation, elongation and vesiculation of a endocytotic channel.
9–11 = 25 000 × (From Klein and Stockem, in press)

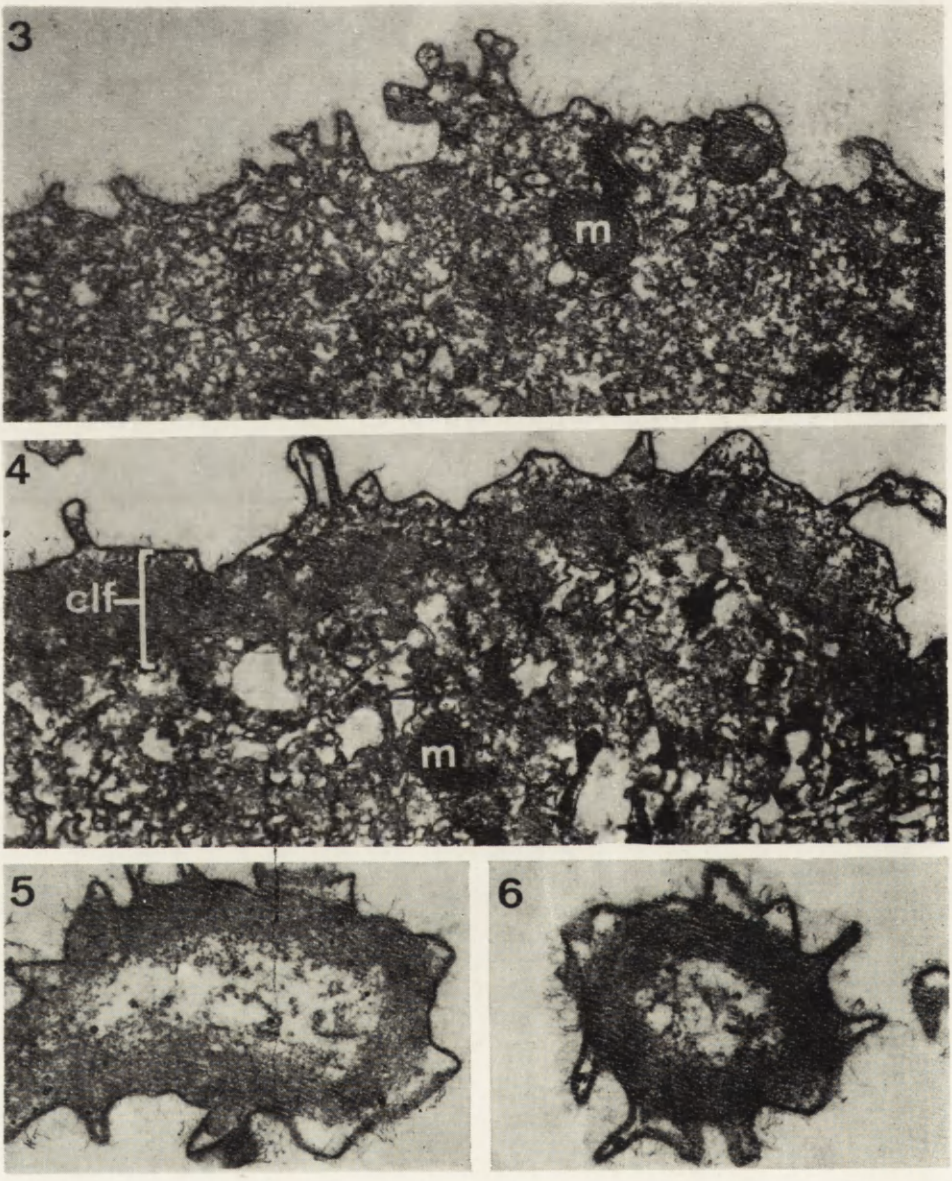
Plate V–VIII. Structure and protoplasmic streaming activity of microplasmodia of *Physarum polycephalum*

13: Amoeboid microplasmodium of *Physarum polycephalum.* 900 × (From Gawlitta 1978)
14: Single frame analysis demonstrating the moving behaviour of an amoeboid microplasmodium. Two successive stages are always compared within one picture in order to demonstrate outline increases (black areas) and outline decreases (white areas between the thin and thick lines). The first time stated refers to the thin outline, the second to the thick outline. The arrows indicate the streaming direction of the protoplasm (From Gawlitta 1978)
15: Dumbbell shaped microplasmodium of *Physarum polycephalum.* 900 × (From Gawlitta, unpublished)
16: Single frame analysis of a dumbbell shaped microplasmodium. The technique used is the same as described in fig. 14 (From Gawlitta 1978)
17: Schematic drawing of a median cross section through a head of a dumbbell shaped microplasmodium showing the expansion of the cell membrane invagination system. 800 × (From Wolf 1978)
18: Single frames from a differential interference contrast film showing the dynamics of four different cell membrane inveaginations in a head of a shaped shaped microplasmodium during maximal efflux (white) and influx (dotted) of protoplasm into the head (From Gawlitta 1978)
19: Development and organization of the cell membrane invagination system in (a) an amoeboid microplasmodium (c. f. fig. 13), in (b) a dumbbell shaped microplasmodium (c. f. fig. 15) and (c) in a plasmodial vein (cross view); (From Gawlitta 1978)
20: Schematic demonstration explaining the generation of hydraulic pressure in a dumbbell shaped model by correlated volume changes of the two heads (From Gawlitta 1978)
21: Schematic demonstration explaining the generation of hydraulic pressure in a dumbbell shaped model by both correlated volume changes of the two heads

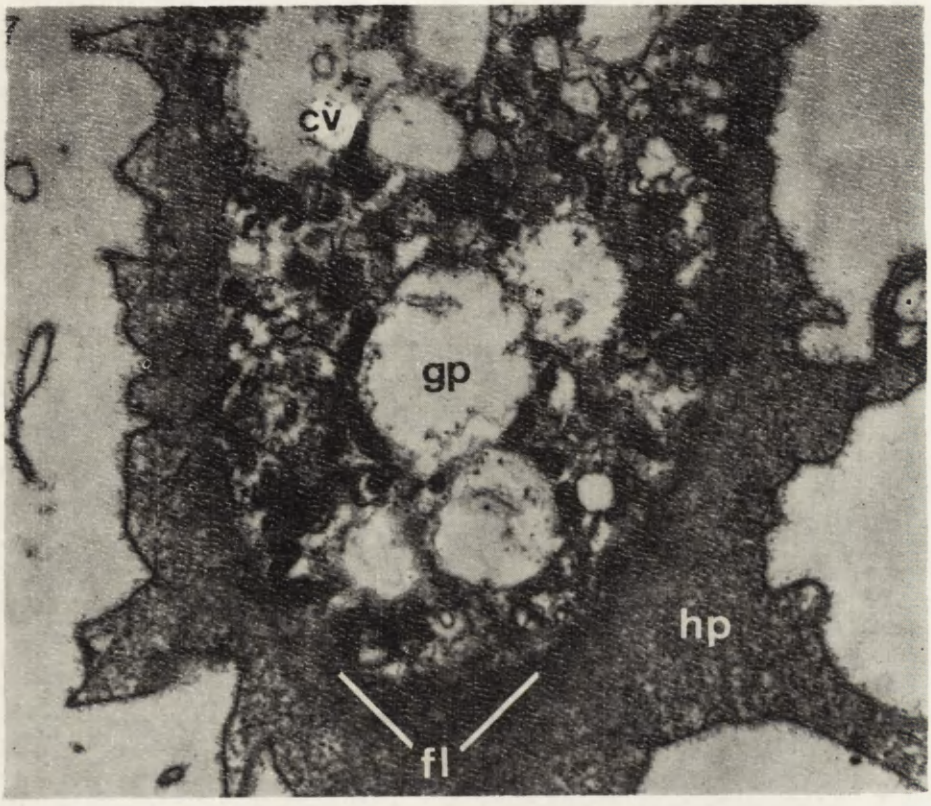
and an invaginated system (From G a w l i t t a 1978)
22.: Schematic diagram explaining the generation of the motive force for protoplasmic streaming in a dumbbell shaped microplasmodium by the action of both fibrils connected with the membrane of the invagination system as well as volume changes of the whole head caused by the contraction and relaxation of a membrane-associated filament layer (From G a w l i t t a 1978)

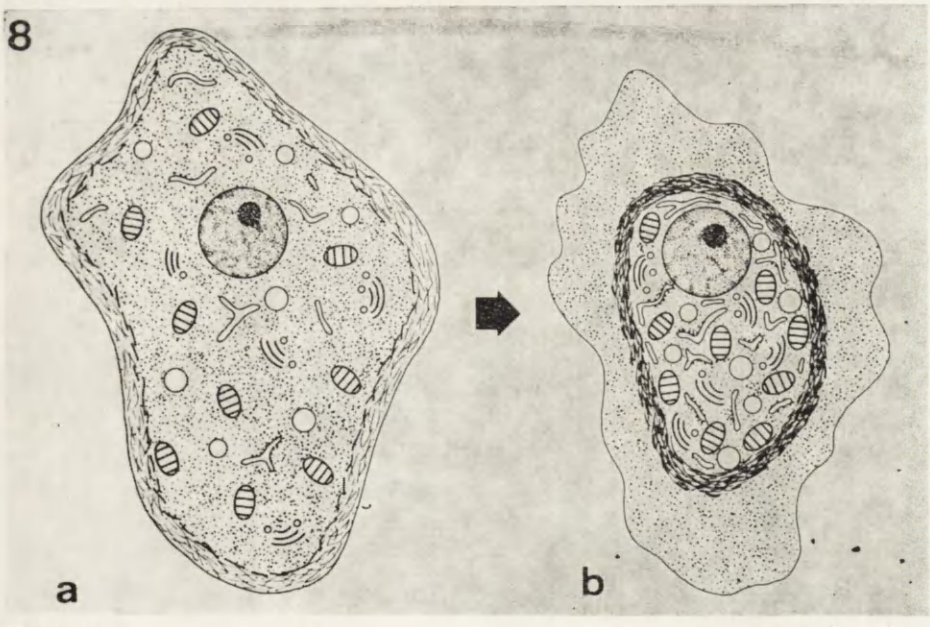


W. Stockem auctor phot.

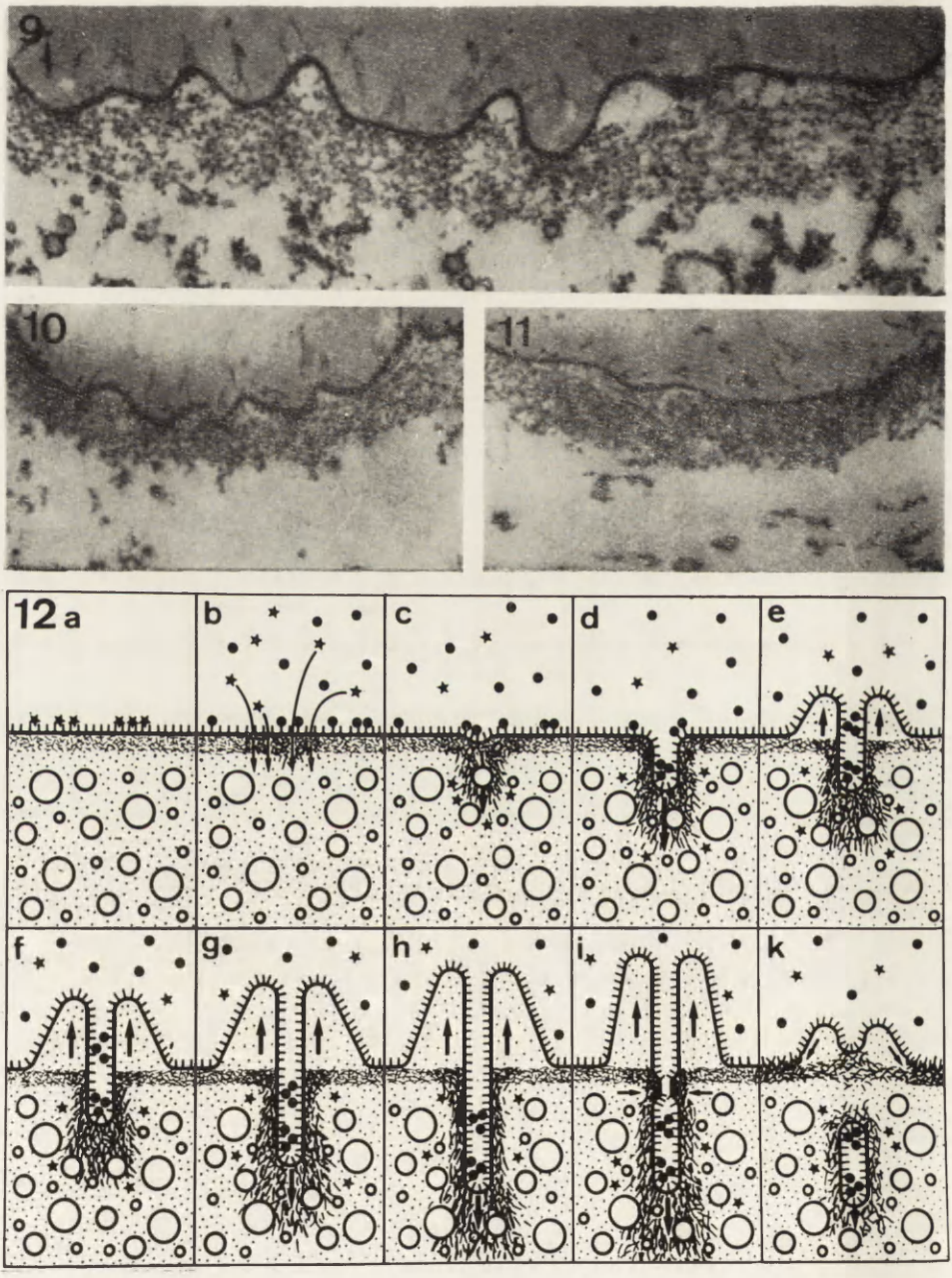


W. Stockem auctor phot.

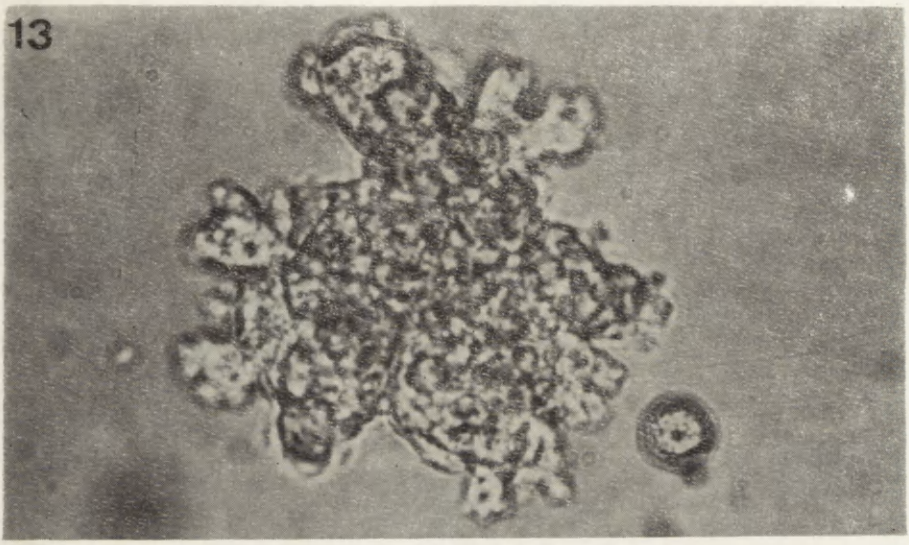


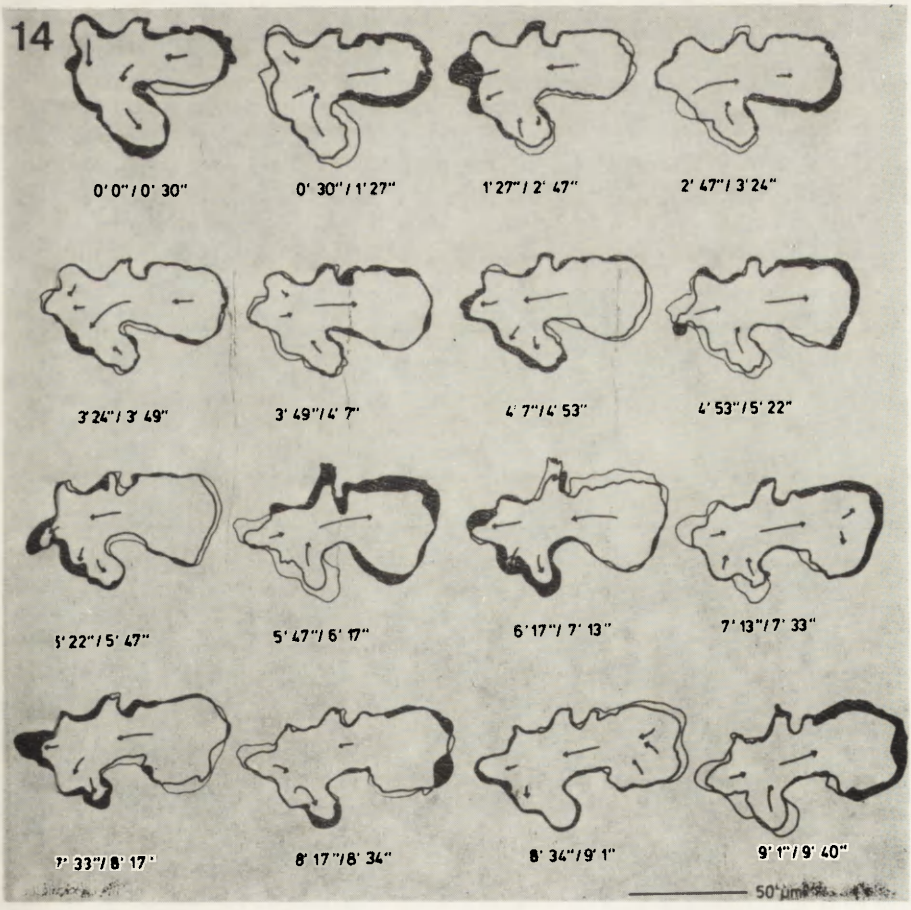


W. Stockem auctor phot.

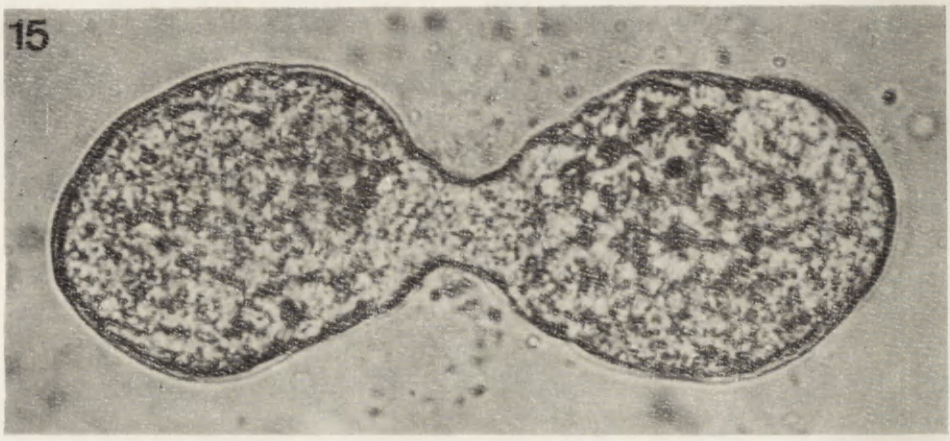


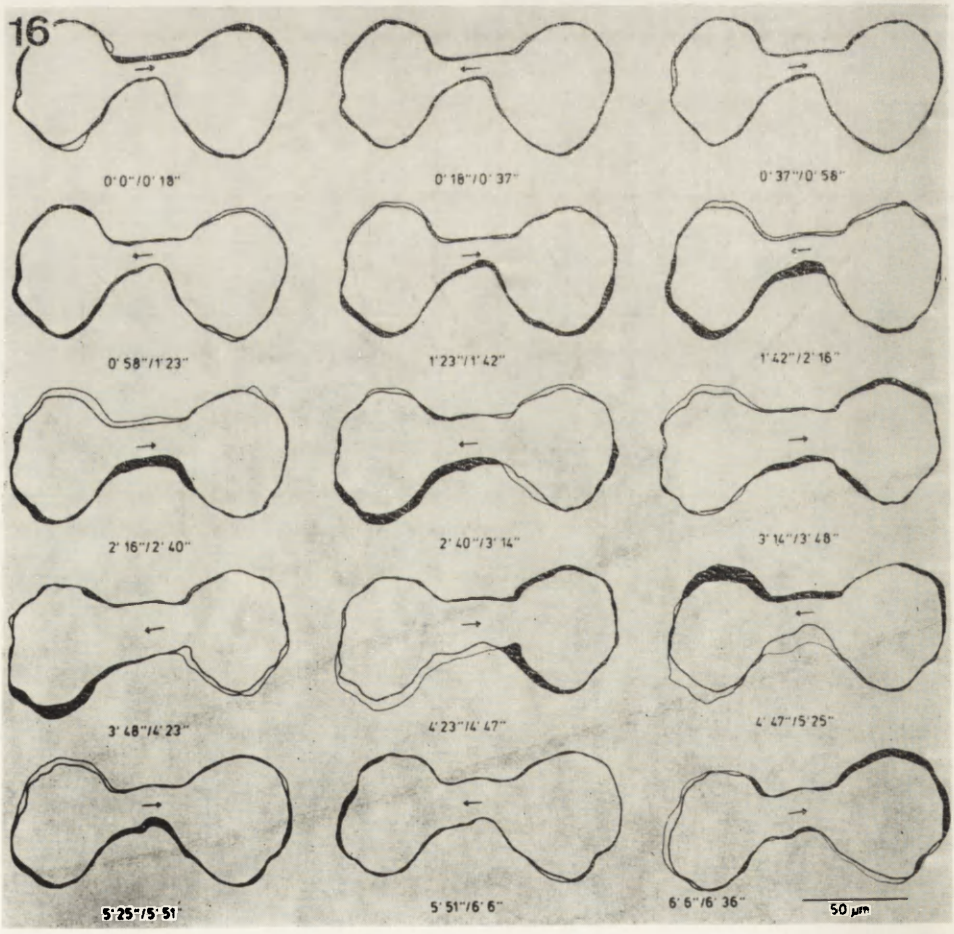
W. Stockem auctor phot.



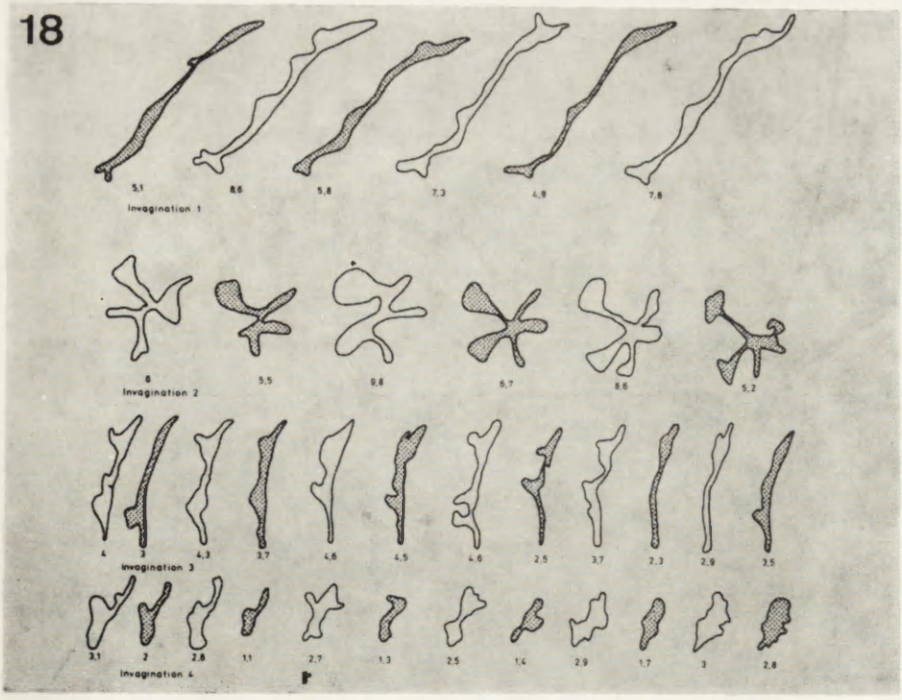


W. Stockem auctor phot.

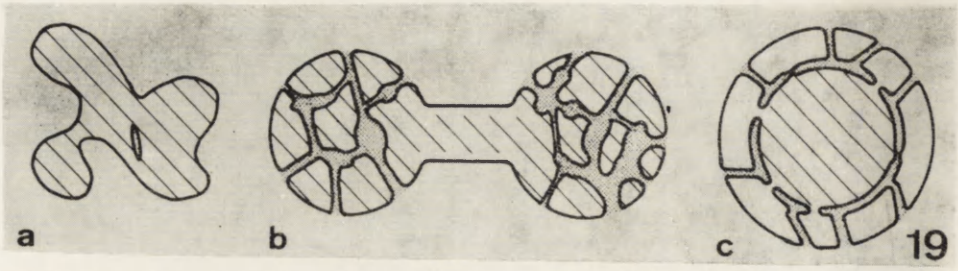


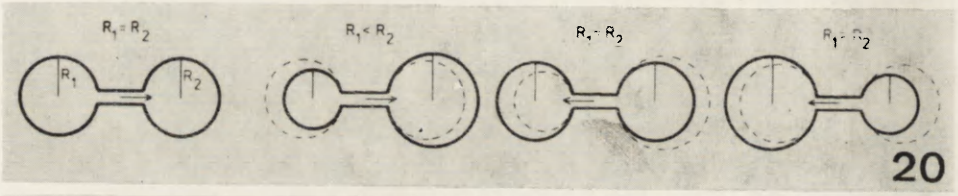


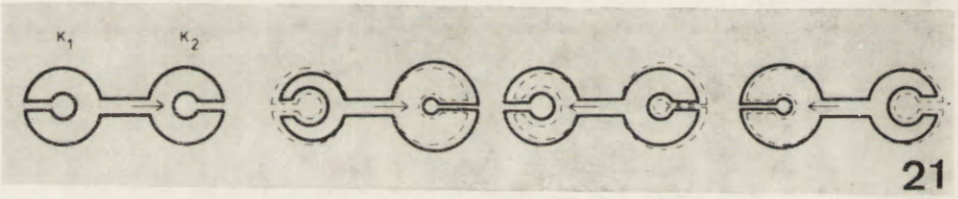
W. Stockem auctor phot.

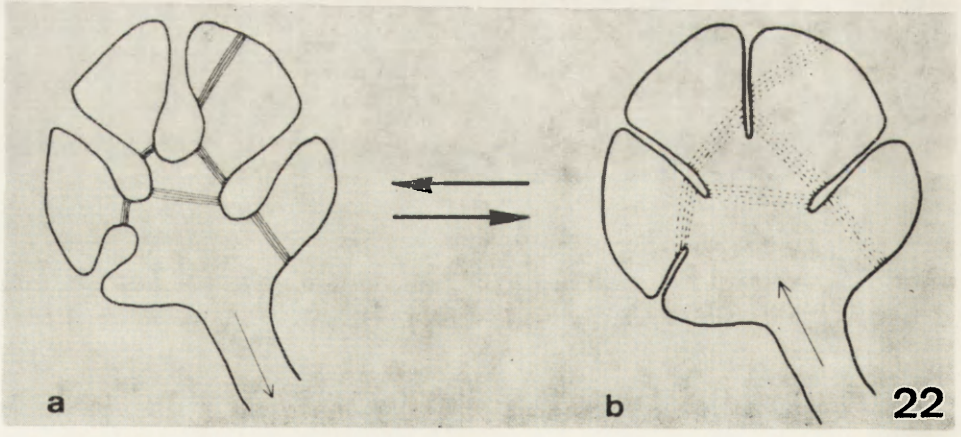


W. Stockem auctor phot.









W. Stockem auctor phot.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 43–58

Department of Cell Biology, M. Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3, Poland

Andrzej GRĘBECKI

Organization of Motory Functions in Amoebae and in Slime Moulds Plasmodia *

Synopsis: It is postulated to abandon the restrictive theories of tail or front contraction in amoebae, because of a number of new facts indicating that the contractile cortical layer may exert and maintain pressure along the whole length of the cell, and that the endoplasm is forced to flow toward the breaches arising in the cortex. In the slime mould plasmodia as well, the motory force is not generated at separate active centres, but it is produced by synchronous contractions of the whole network of veins, which result in simultaneous step-wise progression of the frontal edge.

This article is oriented to review the topics of main interest of the Laboratory of Morphodynamics of the Primitive Motile Systems, of the Nencki Institute of Experimental Biology, for the period of last 5 years. From that time all our efforts concentrated on the main question of the nature of motory polarity manifested by moving cells. Theoretically, we limit our field of interest to the cell systems capable of the amoeboid mode of movement and/or of the intracellular streaming. In the practice, we restrict it to the large fresh water amoeba: *Amoeba proteus*, and to plasmodia of the acellular slime mould: *Physarum polycephalum*, which both are the classical subjects of cell biology, physiology, and biochemistry.

One peculiarity of this material should be mentioned at the very beginning, to demonstrate that some recent results and concepts concerning the motory polarity of various animal tissue cells are not applicable in full extent to our subjects. In many migrating cells investigated in tissue cultures, the motility is related to the presence of both microtubules and microfilaments. The evidence is accumulating that,

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

while the motory force generation is depending on microfilaments, the microtubules maintain the semi-rigid condition of the cell, their arrangement defines the cell shape during locomotion, and as result they play very important role in producing the motory polarity (cf. Porter 1976, Vasiliev and Gelfand 1976).

There are no microtubules present, neither in free-living amoebae nor in slime moulds, to determine the shape of moving structures and to enable anchoring the contractile material. The analogous functions in these organisms are secured by the presence of peripheral ectoplasmic cylinder, traditionally described as the plasmagel layer, which forms the semi-rigid frame of moving cell. Recently, the role of ectoplasmic cylinder in *Amoeba proteus* was stressed by several authors: in moderating the shape transformations during locomotion (Grębecka and Grębecki 1975), in transmitting tension between the contracting regions and the sites of cell adhesion to the substrate (Grębecki 1976, 1977), in maintaining the shape of growing pseudopodia (Kalisz 1978), and in anchoring the contractile filaments active during endocytosis (Stockem and Klein 1977).

While the cytoskeletal role of the ectoplasmic cylinder seems unquestionable, there is a difference of opinions as to its role in contraction. Over 50 years ago Mast (1926) in his pioneer paper localized contraction in the plasmagel walls of the cylinder, but he was incapable at that time to be very specific in formulations. In the fifties Goldacre developed this theory in more uncompromise manner by postulating that the contractile activity of amoeba is confined to the tail region (Goldacre and Lorch 1950, Goldacre 1956, 1961, 1964). Allen (1961 a, b) put forward the opposite theory, postulating that contraction is localized in the frontal fountain zone, i.e., in the loop formed at the tips of advancing pseudopodia by the streaming endoplasm core and the reconstituting ectoplasmic cylinder. The tail contraction theory was supported by Jahn (1964 a), Rinaldi and Jahn (1963), Rinaldi et al. (1975), and in our earlier papers (Grębecki 1964, Grębecka and Grębecki 1975), in a rather orthodox way. It seems preferable to avoid, at the time being, a purely argumentative confrontation of the historical versions of the tail and frontal contraction theories, because seventeen years spent on polemics proved to be in no way conclusive. Moreover, there is a chance for both points of view to become perhaps less dogmatic than in past.

In our group, though we persist to think that contraction squeezes the endoplasm forwards, we are inclined to abandon the simplistic concept restricting the site of contraction to the tail region of amoeba. Already

Marsland (1964) proposed the idea of a "general tube-wall contraction". Some observations of Korohoda (1970) also spoke against attributing the principal role to the contraction of the uroid. We decided to study moving amoebae morphometrically (Grębecka and Grębecki 1975), and we found that the uroid contributes to the total contraction only in 30%, contracting pseudopodia contribute at nearly 40%, and the remaining 30% is due to the trunk which shows in its successive sub-regions a distinct contraction gradient decreasing in the posterio-anterior direction. In total, about 67% of amoeba's body do visibly contract during locomotion. These evaluations are based on the decreasing size of the contracting body regions, i.e., they concern only the contraction component effected under predominantly isotonic conditions. We think now that probably also the more anterior 1/3 of the peripheral cylinder may contract under isometric conditions, i.e., to exert force maintaining the overpressure inside the cell, in spite of its distending by the massive inflow of the endoplasm. The posterio-anterior collapsing/expansion gradient established by us before (Grębecka and Grębecki 1975) should be therefore re-interpreted as gradient of conditions changing from almost isotonic at the rear, to nearly isometric at the middle-anterior body part.

The isometric character of contraction, at least in its longitudinal component, may be obviously imposed by cell adhesion to the substrate. The dynamics of adhesion during locomotion of amoeba has been studied in this laboratory by the side-view techniques (Grębecki 1976). The advancing pseudopodia attach laterally behind their tips (as demonstrated earlier by Bell and Jeon 1963), and the region of the most stable adhesion was defined as lying roughly 1/3 of body length behind the front. More posterior adhering knobs glide against the substrate and gradually detach. So, the topography of adhesion corroborates the view that in the posterior 2/3 of the peripheral cylinder the visually recorded isotonic contraction may dominate, whereas in the anterior 1/3 it should be largely restricted to its isometric manifestation.

In the beautiful series of papers by Taylor, Allen, and their coworkers (Taylor et al. 1973, 1975, 1976 a, b, Condeelis et al. 1976) arguments are accumulated that contraction probably begins at the frontal rim of the ectoplasmic cylinder, at the moment of endoplasm-ectoplasm conversion, and this is fully accepted in our present interpretation of the cylinder contraction theory. But, is the contraction strictly limited to that frontal area? Could not the ectoplasmic cylinder continue to contract during all its life-span, and press the endoplasm forwards? This idea seems no more to be firmly dismissed in the modern model of the frontal contraction theory: Allen and Taylor (1975) write that

"it has not been experimentally demonstrated where the contraction ends and relaxation begins". Taylor (1976) is even more explicit in the statement that "the cytoplasm becomes maximally contractile during and after the conversion of endoplasm to ectoplasm, and the ectoplasm remains in this contracting or contractile state throughout the length of the cell". We accept these ideas as concurrent with our present conviction that not the restrictive theory of tail contraction, but the broader concept of generalized contraction of peripheral cylinder, should be choosen as working hypothesis. According to it, the pressure gradient needed to promote the endoplasm flow would not be created by the strictly posterior localization of contraction, but by the localization of the openings in the contracting cell envelope.

The classical theory of ectoplasm contraction underwent in the last few years another important evolution. Wohlfarth-Bottermann (1964) proposed a terminological distinction between the outer 1–2 μm thick hyaline ectoplasm and the inner much thicker layer of the granular ectoplasm. Later on, Korohoda and Stockem (1975, 196) revealed that the optically empty hyaline layer is electron dense: it is packed with the filamentous material. Soon, the rather unprecise concept of the contractile ectoplasmic cylinder has been replaced by much more specific notion of the contractile cell cortex. The definition of the cortex still remains somewhat ambiguous, because bundles of filaments are often seen to penetrate inside the granular ectoplasm layer. Probably, the filaments may be anchored as well to the cell membrane as to the ectoplasmic cylinder. Their anchoring to the semi-rigid cell frame is the obvious condition to make the contraction mechanically effective. Anyhow, it was proved that in fact the organized contractile material is abundant just in the most peripheral layer of amoeba, where it may be anchored.

If the contractile activity is localized in the peripheral cell cortex, the cortex should manifest a certain degree of motory autonomy, in respect to the endoplasm streaming and endoplasm-ectoplasm interconversion cycle. We concentrated on this question 3 years ago, and a number of co-axial and non-axial cell frame movements were described and analyzed. The co-axial movements (Grębecki 1976) are expressed in that the whole cell, together with its ectoplasmic cylinder, cortex and cell membrane, moves as a block along the main axis of locomotion and of the endoplasm streaming. The cell most often, when attached as usually at its middle-anterior regions, moves as a whole forwards. It may be demonstrated either by superposing the cell contours corresponding to the successive locomotion stages, or by recording the forward movemet of the ectoplasmic granules. The ectoplasm remains stationary only for a short time in the limited attachment zone, which itself continuously

changes its position. The result is a real crawling movement of the whole semi-rigid cell frame. This crawling cannot be explained by any restrictive theory of contraction confined to the tail or to the front of amoeba. As well the contraction of the unattached tail as the contraction of frontal streaming endoplasm could explain the intracellular flow phenomena, but not the movements of the cell as a whole. On the contrary, if the contractility is localized at the whole length of cortex, and if the attachment sites are changing, amoebae could crawl as it was demonstrated.

The general contractility of cortex in amoebae and the dynamics of cell adhesion permit also a number of non-axial movements: side shifts of amoeba, turning on the spot, bending of the whole body or of separate pseudopodia (Grębecki 1977). All these phenomena are very common, and the substrate oriented bending of a growing new pseudopodium, enabling its attachment, is a necessary component of locomotion (Bell and Jeon 1963, Grębecki 1977) which was demonstrated to be an active movement independent of gravitation (Nowakowska and Grębecki 1978). A monotactic amoeba suspended upside-down by its tail, rotates about its axis (Nowakowska 1978). All such non-axial movements have no relation to the direction of endoplasm streaming, and any attempt to explain them by the tail contraction or by the frontal contraction seems to be hopeless.

Independently of these studies Kalisz and Korohoda (1976) provided an excellent experimental evidence of motory functions of the cell cortex. They obtained by centrifugation fragments of amoebae from which the most part of the granular cytoplasm has been evacuated. Such fragments are incapable of the organized cytoplasm streaming, of the endoplasm-ectoplasm conversion cycle, have no frontal fountain zone and no differentiated tail, but they do locomote.

Going back to the primary function of the cortical envelope, that of pressing forward the mass of endoplasm, it should be stressed again that, according to our present views, the whole cortex exerts a more or less uniform pressure, and the outflow should be oriented to areas where there is no cortex, no contraction, and — as a result — no active resistance. Such a discontinuity of cell cortex remained until now a pure hypothesis. Recently, Grębecka and Hrebenda (in preparation)[1] studied the topography of cortex as depending on the dynamic morphology of moving amoeba. Individual moving amoebae were fixed, and dissected after fixation, in order to isolate and to study separately the well defined body

[1] For illustrations cf. the communication by Grębecka and Hrebenda in the same volume, pp. 143–144.

regions. The cortex is found all along the sides of body trunk and of main pseudopodia, where it is well organized in the form of a dense layer of longitudinal filaments, which in some places are arranged in thicker bundles. Similar bundles are present in the tail. But there is no organized contractile material under the frontal membrane of the tips of advancing pseudopodia. At those places the cytoplasm underlying the cell membrane is either electron empty, or it contains granules and some vacuoles but no ordered filaments. So, the postulated discontinuity of the contractile cortex does exist in the reality and, more so, it is found where it was expected.

In a parallel light microscopic study Kalisz (1978) demonstrated that the formation of a new pseudopodium is initiated by changes in the most peripheral cell layer, expressed by arising of an optically empty spot, and changes in the streaming pattern intervene at the later stages.

Slightly earlier we found in this laboratory other arguments to support the view that the endoplasm is forced to flow toward the gaps in the contractile cell cortex. These arguments were provided by morphologic studies and experimentation on the natural and artificial monotactic forms of amoeba (the monotactic amoeba is one of the two morphodynamic types earlier called "monopodial")[2]. Such amoebae manifest a vigorous streaming steadily directed toward the prominent frontal cap. Korohoda and Stockem (1976) found that this type of cap is vacuolar in nature. We established that in fact it is a huge vesicle surrounded by a membrane-like envelope which can be made visible by microdissection experiments (Grębecka 1978 b) and in the electron microscope (Hrebenda and Grębecka 1978). There is no cell cortex neither between the cap and the outer membrane nor behind the cap, and thus the endoplasm is steadily pressed toward the gap. It was demonstrated by Grębecka (1978 a) that such a vesicular cap arises by dense packing and fusion of smaller cytoplasmic vacuoles, and the streaming becomes irreversibly unidirectional when they distend and disrupt the cortical layer.

The same phenomenon was produced artificially by injecting an oil droplet from inside the cell body against the inner face of the cell membrane. The oil locally disrupts the cortex, immediately orients the streaming toward the gap, and reshapes amoeba into monotactic form. This technique was applied in *A. proteus* earlier by Goldacre (1961)

[2] It was proposed by Grębecki and Grębecka (1978) to call: monotactic — the amoebae with prominent frontal caps and steady polarity, insensitive to stimuli; orthotactic — amoebae with unidirectional but facultative stimuli-dependent polarity; polytactic — the common forms with regular succession of leading pseudopodia; heterotactic — the non-locomotive forms with very long pseudopodia arranged radially.

and by us (Czarska and Grębecki 1966). Two years ago Grębecka (1977 b) studied this phenomenon by provoking competition of two oil droplets injected into polytactic amoebae, and the competition between oil drop and the natural frontal cap in monotactic amoebae. The streaming is instantly and invariably oriented toward the breaches formed by the oil, and the dominating direction is determined by this droplet which is larger or which was earlier injected. It has been demonstrated in the electron microscope (Hrebenda and Grębecka 1978) that in fact there is nothing more than the naked cell membrane between the injected oil and the external medium.

The former conclusion must be repeated again, that the endoplasm is squeezed by the general contraction of the cortex toward the areas where the latter is absent and any counter-contraction cannot be produced.

The problem of the motory polarity of amoeba may be put also in a different way: What maintains the relative stability of the posterio-anterior motory axis during spontaneous migration of an unstimulated cell? A link has been recently established between this issue and the old puzzling question why enucleated amoebae immediately cease movement, and instantly recover it after renucleation. Proteins rapidly and bidirectionally migrating between the nucleus and the cytoplasm of amoeba, discovered by Goldstein and his group (cf. Goldstein 1973 for review), may play a role in regulating the motility phenomena. The hypothesis that the nuclear factor involved in the motility of amoeba has relaxing properties was put forward simultaneously by Korohoda (1977) basing on the possibility to re-activate the anucleate fragments of amoebae by local external application of relaxing agents, and by Grębecka (1977 a) who found that the anucleate posterior fragments cut upstream in respect to the nucleus instantaneously stop and shrink, whereas the anucleate anterior fragments cut downstream continue to move for many minutes and remain smooth. She postulated that an unstable regulatory factor of nuclear origin is distributed across the cell by the cytoplasm streaming, and therefore its abundance in the frontal region moderates the contractility, and its defficiency behind the nucleus permits the uninhibited contraction. If so, a positive feedback would be established and the tendency to maintain the pre-existing direction of cytoplasm flow could find an easy explanation.

This concept was supported by further experiments by Grębecki et al. (1978) in which the enucleated amoebae were used as well as specimens with implanted supernumerary nuclei. Both forms were expected to be incapable of building up the appropriate gradient of the nuclear regulatory agent. In fact, they were apt to general contraction but not to co-ordinated locomotion, but it was enough to apply an

external polarizing factor to produce normal migration. The enucleated and polynucleated cells, when unevenly illuminated so as to obtain a sharp light-shade borderline across the cell, migrate to the shaded zone.

The conclusion that the cell nucleus is not necessary for the contraction in amoeba, but it secretes a regulatory factor and is therefore needed to maintain the motory polarity, completes our present views on the organization of motory functions in this cell.

The situation might seem at the first sight easier when mechanism of movement in the slime moulds plasmodia is considered. It is less confuse because the so-called contraction-hydraulic theory put forward by Kamiya (1959) and based on his classical pressure experiments in double chambers, was supported by many authors (e.g., Jahn 1964 b, Stewart 1964, Wohlfarth-Bottermann 1964, Nakajima and Allen 1965) and never seriously objected. Its basic principle consists in the assumption that contractions of the ectoplasmic walls of plasmodial veins create hydrostatic pressure which forces the more fluid endoplasm to flow through the central channel down the pressure gradients. As a matter of fact, two systems of filaments: one circular and one longitudinal, were soon described in the walls of veins *in vivo* (Nakajima and Allen 1965) and in the fixed material (Wohlfarth-Bottermann 1974, 1975 b, v. Kortzfleisch 1976). Tensiometric methods were applied and developed by the schools of Kamiya (Kamiya 1970, Kamiya and Yoshimoto 1972, Kamiya et al. 1972) and of Wohlfarth-Bottermann (1975 a) to measure contraction in isolated veins, and they are gradually adapted to study its radial and longitudinal components *in situ* (Wohlfarth- Bottermann 1977, Hülsmann and Wohlfarth-Bottermann 1978).

However, at least two specific features of plasmodia complicate the full understanding of the mechanism of their movement: their very large size reaching sometimes several decimeters in diameter, and the fact that the contraction-relaxation process in the walls and the protoplasmic streaming are not steady, as in amoeba, but alternating. That creates two big problems: (1) how the net gain in the mass transport of protoplasm is obtained for the frontal direction, in the course of successive alternating phases of progressive and recessive flow, and (2) how are organized in time and in space the contraction-relaxation cycles across the whole plasmodium.

The obvious preliminary step to approach the solution of the first question should be done by discriminating between three distinct, though not mutually exclusive, possibilities: the forward flow of protoplasm

being either longer, quicker, or correlated with the stage of veins dilatation. Such a discrimination has never been empirically made in a statistically significant material. Baranowski (1976) introduced "the polarity parameter A" and in its physico-mathematical description gave priority to the concept of enlarged vein diameter at the forward streaming phase. We started to approach this question by checking first in what extent one can judge about the internal diameter of the channel accessible for flow, from measuring the external diameter of the vein (Grębecki and Cieślawska 1978 b). In other words: what is the dynamics of ectoplasmic walls during the contraction-relaxation cycle? We found optical conditions producing a bright line at the ectoplasm-endoplasm interface, and we applied the time-lapse filming. The films were projected on the screen with a transversal slit covered with semitransparent material, and a camera with continuous film run focused upon the slit produced the pulsation curves. We found that the walls of a vein get remarkably thinner during contraction. When contracting, the veins locally lose ca. 29% of volume of their wall material. But by the same time the dimensions of the internal channel diminish as well, by 35% as calculated per volume. So, at this stage of inquiry, the diameter-dependent polarization of flow remained theoretically possible.

It should be added that independently Hülsmann and Wohlfarth-Bottermann (1978) also demonstrated the decrease of walls thickness during the contraction of plasmodial veins. Their results were obtained by morphological methods and they don't permit to evaluate quantitatively the wall dynamics, but they provide much more information about what really happens inside the ectoplasm.

In another series of experiments (Grębecki and Moczoń 1978) we tried to establish whether the forward direction of streaming is correlated in time with the stage of maximal dilatation of a vein, and the recessive streaming with its minimal diameter. However, the streaming directions recorded at the maxima and at the minima of the pulsation cycles proved to be distributed at random. The question remains yet open, but we are inclined to think that the polarizing mechanism which produces the net forward transport of volume during the shuttle streaming is time-dependent or velocity-dependent, but not diameter-dependent.

We concentrated more efforts on the second question mentioned above, that of the time and space organization of the contractile activity across the whole plasmodium. This question is technically difficult to be directly approached, because the requirement to keep under control the whole area occupied by plasmodium is incompatible with the necessity,

to follow the motory phenomena in the field of view of a microscope. As result, all the present concepts of the dynamic topography of motory events in slime moulds are not based on actual simultaneous surveying the whole plasmodium, but are deduced from experiments on isolated fragments or from observations *in situ* limited to minute areas. In such indirect way developed the almost common opinion, that the overall pattern of the contractile activity of plasmodium results from the interaction of multiple separate active centres, never localized but called "origins" by Jahn (1964 b) and "sources" by Stewart (1964), and considered as coupled oscillators by Durham and Ridgway (1976). This concept is obviously related to earlier statements of Seifriz (1953) and Kamiya (1959) who defined plasmodia as polyrhythmic systems. In addition, many authors believe that contraction is propagated across the whole plasmodium in form of peristaltic waves (Seifriz 1953, Stewart 1964, Rhea 1966, Durham and Ridgway 1976).

The expasion-collapsing waves are indeed easily observable in the time-lapse films taken at the frontal regions of plasmodia, but recently Baranowski (1978) established that they are limited to the narrow zone, a few mm behind the frontal margin. Another extremely important fact hardly compatible with the current theory has been reported by Takeuchi and Yoneda (1977): the isolated segments of veins, arranged in an artificial interconnected system, contract and relax synchronously. Earlier, some general objections against the concept of separate active centres were raised by Wohlfarth-Bottermann (1964) and by Teplov (1976).

In the study of this question we proceeded step-by-step from investigating the motory behaviour in more or less limited areas up to controlling it simultaneously over the whole freely migrating plasmodium. The first objective was to learn whether the hypothetical polyrhytmicity is manifested in any consistent phase or amplitude differences, when two different geometrical components of contraction are simultaneously recorded in a plasmodial vein at the same site. Two pairs of components were confronted: the horizontal vs. the vertical components of radial contraction, and the radial vs. the longitudinal contraction.

The first type experiments (Moczoń and Grębecki 1978 a) were run on isolated segments of veins, winded round agar rods in moist chamber. When the microscope is focused upon the rod's edge, the vein appears in its optical cross-section, and the dynamics of its profile in the course of successive contraction-relaxation cycles may be studied by time-lapse cinematography. The slight phase differences between the ridge of the vein and its lateral slopes produce undulations of the profile contour. The amplitudes are regularly more important at the lateral

slopes and, as a result, the profile becomes more convex at the expanded state and more concave in the contracted condition. Such changes of vein's geometry during the pulsation cycle were confirmed by Cieślawska and Grębecki (1978 b) *in situ*, by observation of refraction of the polarized light beam at the slime-air interface. It should be stressed however, that the frequency of pulsation is the same all along the vein's contour, and the phase differences are completely irregular. It means that all the geometrical components of radial contraction preserve the same principal common rhythm. [3]

The radial and longitudinal contractions were recently measured tensiometrically in the same vein by Wohlfarth-Bottermann (1977) and Hülsmann and Wohlfarth-Bottermann (1978). The radial contraction was recorded *in situ*, and the longitudinal contraction was measured in another segment of the same vein, detached from the substrate and suspended. We assessed only the periodicity of both events by recording their geometrical effects, but we kept both components under control at the same conditions and at the same site (Moczoń and Grębecki 1978 b). Isolated fragments of veins were horizontally suspended between two agar gel blocks, loosely enough to enable the up and down movements due to shortening and elongation of the suspended segment. The loop always moves up at the same phase at which the vein's diameter decreases, and vice versa. *In situ*, the glass wool hairs leaning against the vein's ridge and manifesting lateral oscillations, were used as markers of the longitudinal contraction, and the pulsation of the vein's edge served as indicator of the radial rhythm. The hairs oscillate in phase with the pulsation of vein. So, we came to the conviction that the longitundinal and the circular contractile systems of plasmodial veins act in unison.

At the next stage, the same component of contraction: the pulsation of vein recorded *in situ* in the horizontal plane, was compared at two sites within the microscope field of view, ca. 1 mm apart from one another (Grębecki and Moczoń 1978). In the unbranched segments of veins we expected to find regular phase differences proving the propagation of the peristaltic waves of contraction, but the pulsation curves obtained by the slit technique always demonstrated the existence of the same synchronous rhythm at both tested sites. At the anastomoses in the plasmodium network we expected symptoms of interference of two different rhythms, according to the active centres theory, but as well the

[3] Cf. the communication by Moczoń and Grębecki in this volume, p. 177–178.

main vein before and behind junction as the lateral branch pulsate again in unison. The time correlation was studied between the contraction periods recorded at these three sites. Correlation coefficients proved to be very high along the main vein on both sides of the junction, and high between major vein and its branch. It is meaningful that, in spite of this virtual synchrony of pulsation of both anastomosing veins, the correlation of streaming directions inside them is insignificant. These time and space relations may be also presented in form of the periodicity diagrams [4].

We further increased the distance between the two simultaneously tested sites by placing a spherical mirror, at the double focal distance under the migrating plasmodium (Cieślawska and Grębecki 1978 a). With the spread of 0.5–4.0 mm between the optical axes of the mirror and of the microscope we could introduce into the field of view, in parallel to the normal image, the inverted reflected image of sites lying at the distance of 1.0–8.0 mm. But the pulsation curves taken along the veins, or at different anastomosing regions, demonstrated again the pattern of a not always precise but distinct synchrony of pulsation, instead of regular phase shifts required by the theory of travelling contraction waves.

Only very recently we found (Grębecki and Cieślawska 1978 a) that it is possible to reproduce correctly the pulsation rhythm of veins without microscope, from macrocinematographic time-lapse films by recording the oscillations of current generated by a photo-element applied to different spots at the screen, during projection of the film. By macro-filming the whole plasmodium may be recorded and, moreover, the pictures are taken in very dim light (80 lux) not disturbing its motory behaviour. In the course of repeated projections, the simultaneous records of pulsation rhythms may be produced at an illimited number of sites. We used to control the rhythmicity at 20–30 sites scattered over all the network of veins, and 5–10 disposed along the frontal edge.

The periodicity diagrams are constructed basing on the recorded curves of photocurrent. They demonstrate that the lines, parallel to the time scale, which correspond to the successive serial contraction periods in the veins of the network, form distinct and well separated vertical columns appearing usually at the frequency of 90–100 s. It means that there is no wave-like propagation of contraction, and no interaction of different rhythms, but the whole network of veins contracts synchronously. The synchrony is not always strictly precise, but the occasional

[4] Ibidem.

phase shifths are never regular, and any disturbed place returns after one or two next cycles to the common rhythm.

The lines corresponding to the periods of intense expansion of the front have the same frequency and fairly well coincide in time with the contraction lines of veins. In other words, the geometrical deformations of veins and of the advancing margin remain in constant reciprocal opposition along the successive contraction-relaxation cycles of the migrating plasmodium. When the whole network of plasmodium synchronously contracts, the front makes a step forwards. The basic mechanism seems to be much simpler than could be expected before from indirect data. Plasmodium appears not to be polyrhythmic, but an imperfectly synchronized monorhythmic contractile system [5].

It may be deduced that each simultaneous general contraction of all the system of veins squeezes the protoplasm out, toward the peripheral regions. The morphology of the frontal periphery predestinates it to accept the major part of the squeezed protoplasm, and the main outflow is oriented in that direction. As a matter of fact, we established before (Grębecki and Moczoń 1978) a weak but significant positive correlation between the periods of veins contraction and the periods of forward streaming. The similar relation was recently found in the holograms obtained by Baranowski (in preparation) [6]. However, the synchrony of the shuttle streaming is much less pronounced that the synchrony of contractions. This difference may be easily understood when keeping in mind that contraction is the primary event in the motory activity of plasmodium, whereas the topography of pressure gradients and that of streamings are the after-effects modified by the very complicated geometry of plasmodium and by the elasticity of its walls.

It seems that the contraction and streaming patterns appear phenomenologically different, steady in amoeba and pulsating in plasmodium, owing to the differences in size and in morphology of these two motile systems. But we are disposed to think, at the present stage, that the basic principle of driving the protoplasm flow during the locomotion of large amoebae and of slime moulds plasmodia is common. In both cases the endoplasm seems to be forced to flow forwards by the pressure generated synchronously over the entire length of the contractile walls. In our opinion, the idea of specific contractile sites monopolising the motory force generation should be abandoned, as well in the case of plasmodium as in that of amoeba.

---

[5] Cf. the communication by Cieślawska and Grębecki in this volume, p. 125–126.

[6] Cf. the communication by Baranowski in this volume, p. 113–114.

REFERENCES

Allen R. D. 1961 a: A new theory of ameboid movement and protoplasmic streaming. Expl. Cell Res., (Suppl.), 8, 17–31.

Allen R. D. 1961 b: Ameboid movement. In: The Cell. Academic Press. 2, 135–216.

Allen R. D. and Taylor D. L. 1975: The molecular basis of ameboid movement. In: Molecules and Cell Movement. Raven Press, 239–258.

Baranowski Z. 1976: Three-dimensional analysis of movement in *Physarum polycephalum* plasmodia. Cytobiologie, 13, 118–131.

Baranowski Z. 1978: The contraction-relaxation waves in *Physarum polycephalum* plasmodia. Acta Protozool., 17, 387–397.

Bell L. G. E. and Jeon K. W. 1963: Locomotion of *Amoeba proteus*. Nature, 198, 675–676.

Cieślawska M. and Grębecki A. 1978 a: Contraction-expansion rhythms simultaneously observed in two sites of *Physarum polycephalum* plasmodium. Acta Protozool., 17, 533–541.

Cieślawska M. and Grębecki A. 1978 b: Contraction-expansion cycles in plasmodia of *Physarum polycephalum* observed in polarized light. Acta Protozool., 17, 525–531.

Condeelis J. S., Taylor D. L., Moore P. L. and Allen R. D. 1976: The mechanochemical basis of amoeboid movement. II. Cytoplasmic filament stability at low divalent cation concentrations. Exp. Cell Res., 101, 134–142.

Czarska L. and Grębecki A. 1966: Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*. Acta Protozool., 4, 201–239.

Durham A. C. H. and Ridgway E. B. 1976: Control of chemotaxis in *Physarum polycephalum*. J. Cell Biol. 69, 218–223.

Goldacre R. J. 1956: The regulation of movement and polar organisation in amoeba by intracellular feedback. Proc. 1st Intern. Congr. Cybernetics, 715–725.

Goldacre R. J. 1961: The role of the cell membrane in the locomotion of amoebae, and the source of the motive force and its control by feedback. Expl. Cell. Res., (Suppl.), 8, 1–16.

Goldacre R. J. 1964: On the mechanism and control of ameboid movement. In: Primitive Motile Systems in Cell Biology. Academic Press, 237–255.

Goldacre R. J. and Lorch I. J. 1950: Folding and unfolding of protein molecules in relation to cytoplasmic streaming, amoeboid movement and osmotic work. Nature, 166, 487–499.

Goldstein L. 1973: Nucleocytoplasmic interactions in amoebae. In: The Biology of Amoeba, Academic Press, 479–504.

Grębecka L. 1977 a: Behaviour of anucleate anterior and posterior fragments of *Amoeba proteus*. Acta Protozool., 16, 87–105.

Grębecka L. 1977 b: Changes of motory polarization in *Amoeba proteus* as induced by oil injections. Acta Protozool., 16, 107–120.

Grębecka L. 1978 a: Frontal cap formation and origin of monotactic forms of Amoeba proteus under culture conditions. Acta Protozool., 17, 191–202.

Grębecka L. 1978 b: Micrurgical experiments on the frontal cap of monotactic forms of *Amoeba proteus*. Acta Protozool., 17, 203–212.

Grębecka L. and Grębecki A. 1975: Morphometric study of moving *Amoeba proteus*. Acta Protozool., 14, 337–361.

Grębecka L. and Hrebenda B.: Topography of cortical layer in *Amoeba proteus* as related to the dynamic morphology of moving cell, (in preparation).

Grębecki A. 1964: Modern lines in the study of amoeboid movement. Acta Protozool., 2, 379–402.

Grębecki A. 1976: Co-axial motion of the semi-rigid cell frame in *Amoeba proteus*. Acta Protozool., 15, 221–248.

Grębecki A. 1977: Non-axial cell frame movements and the locomotion of *Amoeba proteus*. Acta Protozool., 16, 53–85.

Grębecki A. and Cieślawska M. 1978 a: Plasmodium of *Physarum polycephalum* as a synchronous contractile system. Cytobiologie, 17, 335–342.

Grębecki A. and Cieślawska M. 1978 b: Dynamics of the ectoplasmic walls during pulsation of plasmodial veins of *Physarum polycephalum*. Protoplasma, (in press).
Grębecki A. and Grębecka L. 1978: Morphodynamic types of *Amoeba proteus:* a terminological proposal. Protistologica, 14, 349–358.
Grębecki A. and Moczoń M. 1978: Correlation of contractile activity and of streaming direction between branching veins of *Physarum polycephalum* plasmodium. Protoplasma, 97, 153–164.
Grębecki A., Kalinina L. V. and Grębecka L. 1978: Response to light-shade difference in anucleate and polynucleate specimens of *Amoeba proteus*. Cytobiologie, 17, 343–353.
Hrebenda B. and Grębecka L. 1978: Ultrastructure of the frontal cap of monotactic forms of *Amoeba proteus*. Cytobiologie, 17, 62–72.
Hülsmann N. and Wohlfarth-Bottermann K. E. 1978: Spatio-temporal relationships between protoplasmic streaming and contraction activities in plasmodial veins of *Physarum polycephalum*. Cytobiologie, 17, 317–334.
Jahn T. L. 1964 a: Relative motion in *Amoeba proteus*. In: Primitive Motile Systems in Cell Biology. Academic Press, 279–302.
Jahn T. L. 1964 b: Protoplasmic flow in the mycetozoan, *Physarum* — II. The mechanism of flow: a re-evaluation of the contraction-hydraulic theory and of the difusion drag hypothesis. Biorheology, 2, 133–152.
Kalisz B. 1978: Experimental study on locomotion of *Amoeba proteus*. II. Reactions to some external stimuli in *Amoeba proteus* and its fragments from which a part of the cytoplasm has been removed. Acta Protozool., 17, 467–474.
Kalisz B. and Korohoda W. 1976: Experimental study on locomotion of *Amoeba proteus*. I. Movements in the nucleated and anucleated fragments of the amoebae after removal of the part of their cytoplasm. Acta Protozool., 15, 345–358.
Kamiya N. 1959: Protoplasmic streaming. In: Protoplasmatologia VIII 3a. Springer-Verlag, 1–199.
Kamiya N. 1970: Contractile properties of the plasmodial strand. Proc. Jap. Acad., 46, 1026–1031.
Kamiya N., Allen R. D. and Zeh R. 1972: Contractile properties of the slime mold strand. Acta Protozool., 11, 113–124.
Kamiya N. and Yoshimoto Y. 1972: Dynamic characteristics of the cytoplasm. In: Aspects of Cell Mol. Physiol., Tokyo University Press, 167–189.
Korohoda W. 1970: Locomotion of *Amoeba proteus* in conditions imitating its natural environment. Folia Biol., 18, 145–151.
Korohoda W. 1977: Experimental induction of locomotion in enucleated fragments of *Amoeba proteus* and its bearing on the theories of amoeboid movement. Cytobiologie, 14, 338–349.
Korohoda W. and Stockem W. 1975: On the nature of hyaline zones in the cytoplasm of *Amoeba proteus*. Microsc. Acta, 77, 129–141.
Korohoda W. and Stockem W. 1976: Two types of hyaline caps, constricting rings and the significance of contact for the locomotion of *Amoeba proteus*. Acta Protozool.. 15, 179–185.
v. Kortzfleisch D. 1976: Elektronenmikroskopische Darstellung der Topographie cytoplasmatischer Actomyosin-Fibrillen in Protoplasmaadern von *Physarum polycephalum*. Protistologica, 12, 399–413.
Marsland D. 1964: Introduction (Broad concept of the tube-wall contraction hypothesis). In: Primitive Motile Systems in Cell Biology. Academic Press, 331–332.
Mast S. O. 1926: Structure, movement, locomotion and stimulation in Amoeba. J. Morphol., 41, 347—425.
Moczoń M. and Grębecki A. 1978 a: Dynamics of the profiles of plasmodial veins of *Physarum polycephalum* during the pulsation cycle. Protoplasma, 97, 173–183.
Moczoń M. and Grębecki A. 1978 b: Time relationships between the longitudinal and radial contraction in plasmodial veins of *Physarum polycephalum*. Acta Protozool., 17, 543–550.
Nakajima and Allen R. D. 1965: The changing pattern of birefringence in plasmodia of the slime mold, *Physarum polycephalum*. J. Cell Biol. 25, 361–374.

Nowakowska G. 1978: Twisting of suspended monotactic specimens of *Amoeba proteus*. Acta Protozool., 17. 347–352.
Nowakowska G. and Grębecki A. 1978: Attachment of *Amoeba proteus* to the substrate during upside-down crawling. Acta Protozool., 17, 353–358.
Porter K. R. 1976: Motility in cells. In: Cell Motility, Book A, Cold Spring Harbor Laboratory, 1–28.
Rhea R. P. 1966: Microcinematographic, electron microscopic, and electrophysiological studies on shuttle streaming in the slime mold *Physarum polycephalum*. In: Dynamics of Fluids and Plasmas. Academic Press, 35–58.
Rinaldi R. A. and Jahn T. L. 1963: On the mechanism of ameboid movement. J. Protozool., 10, 344–357.
Rinaldi R. A., Opas M. and Hrebenda B. 1975: Contractility of glycerinated *Amoeba proteus* and *Chaos chaos*. J. Protozool. 22, 286–292.
Seifriz W. 1953: Mechanism of protoplasmic movement. Nature, 171, 1136–1138.
Stewart P. A. 1964: The organization of movement in slime mold plasmodia. In: Primitive Motile Systems in Cell Biology. Academic Press, 69–76.
Stockem W. and Klein H. P. 1977: Pseudopodium formation and retraction as a basic mechanism of endocytosis in amoebae. Abstr. Fifth int. Congr. Protozool. New York 1977.
Takeuchi Y. and Yoneda M. 1977: Synchrony in the rhythm of the contraction-relaxation cycle in two plasmodial strands of *Physarum polycephalum*. J. Cell Sci., 26, 151–160.
Taylor D. L., Condeelis J. S., Moore P. L. and Allen R. D. 1973: The Book B. Cold Spring Harbor Laboratory, 797–821.
Taylor D. L. Condeelis J. S., Moore P. L. and Allen R. D. 1973: The contractile basis of amoeboid movement. I. The chemical control of motility in isolated cytoplasm. J. Cell Biol, 59, 378–394.
Taylor D. L., Reynolds G. T. and Allen R. D. 1975: Detection of free calcium ions in amoebae by aequorin luminescence. Biol. Bull., 149, 448.
Taylor D. L., Rhodes J. A. and Hammond S. A. 1976: The contractile basis of amoeboid movement. II. Filament formation in motile extracts and plasmalemma-ectoplasm ghosts. J. Cell Biol., 70, 123–143.
Taylor D. L., Moore P. L., Condeelis J. S. and Allen R. D. 1976: The mechanochemical basis of amoeboid movement. I. Ionic requirements for maintaining viscoelasticity and contractility of amoeba cytoplasm. Exp. Cell Res., 101, 127–133.
Teplov V. A. 1976: Mechanika protoplazmaticeskich tjazej plazmodia miksomiceta. In: Nemyšecnyje Formy Podviznosti. AN SSSR, 37–46.
Vasiliev J. M. and Gelfand I. M. 1976: Effects of colcemid on morphogenetic processes and locomotion of fibroblasts. In: Cell Motility, Book A. Cold Spring Harbor Laboratory, 279–303.
Wohlfarth-Bottermann K. E. 1964: Cell structures and their significance for ameboid movement. Intern. Rev. Cytol., 16, 61–131.
Wohlfarth-Bottermann K. E. 1974: Plasmalemma invaginations as characteristic constituents of plasmodia of *Physarum polycephalum*. J. Cell Sci., 16, 23–37.
Wohlfarth-Bottermann K. E. 1975 a: Tensiometric demonstration of endogenous, oscillating contractions in plasmodia of *Physarum polycephalum*. Z. Pflanzenphysiol., 76, 14–27.
Wohlfarth-Bottermann K. E. 1975 b: Weitreichende fibrillare Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. X. Die Anordnung der Actomyosin-Fibrillen in experimentell unbeeinflussten Protoplasmaadern von *Physarum in situ*. Protistologica, 11, 19–30.
Wohlfarth-Bottermann K. E. 1977: Oscillating contractions in protoplasmic strands of *Physarum*: Simultaneous tensiometry of longitudinal and radial rhythms, periodicity analysis and temperature dependence. J. Exp. Biol., 67, 49–59.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 59–73

Institute for Cytology, University of Bonn Ulrich Haberland-Str. 61a, 5300 Bonn 1
Fed. Rep. Germany

K. E. WOHLFARTH-BOTTERMANN

Contraction Phenomena in *Physarum*: New Results *

Synopsis: Modified tensiometrical methods allow the registration of longitudinal and radial contractions of veins *in situ* and their endoplasmic transport rate simultaneously. — Endoplasm and ectoplasm are involved in mutual transformation processes along the ecto-endoplasmic borderline. — Blockage of glycolysis reduces force output by one half of the original value; anaerobiosis has the same effect. Only simultaneous application results in a complete cessation of contraction. — After replacement of endoplasm by artificial media, the ectoplasmic tube survives and allows an analysis of the physiological concentration of nucleotides and Ca^{++}-ions. Optimal force output was registered at 0.2 mM ATP and $2\text{–}4 \times 10^{-7}$ M Ca^{++}. ADP induced maximal force output at 0.5 mM, AMP at 2.5 mM.

Physarum polycephalum can be used as a model system for the study of elementary processes involved in the chemo-mechanical energy transformations by cytoplasmic actomyosin in eukaryotic cells. For the analysis of the molecular basis, a detailed knowledge of the contraction phenomena within plasmodia and their relation to motive force generation for protoplasmic streaming represents an important precondition. This lecture presents some technical improvements concerning registration and interpretation of the contraction phenomena in plasmodial veins and describes a new technique for the study of the requirements of the contractile apparatus concerning the supply with ATP and free Ca^{++}-ions.

The functional morphology, i. e., the spatial arrangement of cytoplasmic actomyosin-fibrils within a plasmodial vein can be compared with a contracting muscle tube. Fine structural analysis revealed the presence of cytoplasmic F-actin, whereas myosin seems to remain in a low aggregation stage, according to experience gained after fixation by osmiumtetroxide, as well as after application of glutaraldehyde.

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

A twin arrangement of two tensiometric devices alows the registration of radial and longitudinal contraction activities of the veins simultaneously. Both oscillating contraction activities showed different frequencies, when analyzed under these experimental conditions: the radial contraction has a mean average time period of 1.3 min, whereas the values for longitudinal contraction do not show identical time periods but lie in the range of 2.1 min (Wohlfarth-Bottermann 1977). This raised the question, whether we have to take into account different

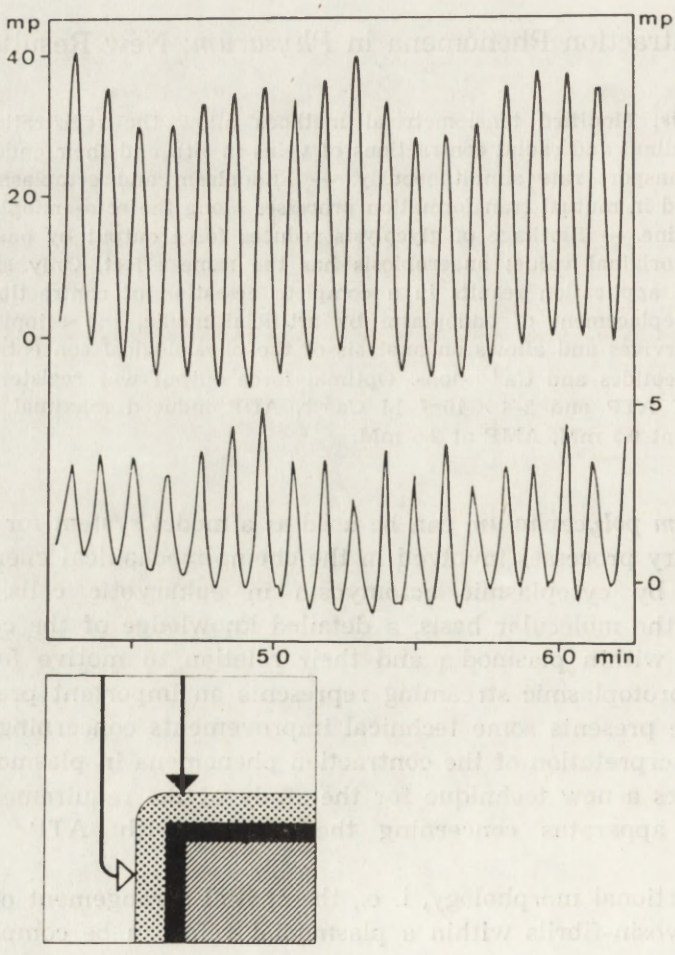


Fig. 1. Simultaneous tensiometric registration of radial (upper curve) and longitudinal contraction activities (lower curve) under *in situ*-conditions according to the diagram. Distance of the measuring points along the vein: 9 mm; diameter of the vein: 0.9 mm; room temperature: 26.5°C. Average time period of oscillation: 1.1 min. Diagram: vein — screened; filter paper — black; tensiometer lift — hatched. The black arrowhead represents the measuring stamp for the radial measurement (Hülsmann and Wohlfarth-Bottermann 1978)

time periods and thus two different contraction rhythms, or whether a genuine time period of the oscillation exists. The first assumption seems to be in accordance with the morphological evidence of spatially different arranged actomyosin fibrils within the veins, i. e., a circular and a longitudinal system, respectively. Interpretations in this direction, however, would imply more complicated regulatory phenomena in comparison to the assumption of only one, i. e., a genuine frequency, in other words a period and phase accordance of the registerable longitudinal and radial contraction phenomena.

By introducing a new technique to register longitudinal contraction activity of veins *in situ* (Fig. 1), it pointed out that both radial and longitudinal contractions are identical concerning their time periods as well concerning their phase relations. Both longitudinal and radial activities show identical frequencies when the measurements can be performed *in situ*. Obviously, there is only one genuine frequency and therefore one has not necessarily to suppose a cooperation of two oscillating systems which differ considerably in their time periods. The genuine period lies in the range of 1.3 min when investigating both contraction activities *in situ*. This means that the longitudinally measured periods of veins registered after their removal from the substrate to a certain degree have to be looked upon as an "artificial" prolongation of the more genuine radial time period of 1.3 min. This knowledge has a practical importance since the investigation of veins removed from their substrate is a method routinely used in tensiometry for other experimental approaches.

The tensiometric techniques have the advantage of delivering absolute values of the contraction forces. A shortcoming, however, lies in the

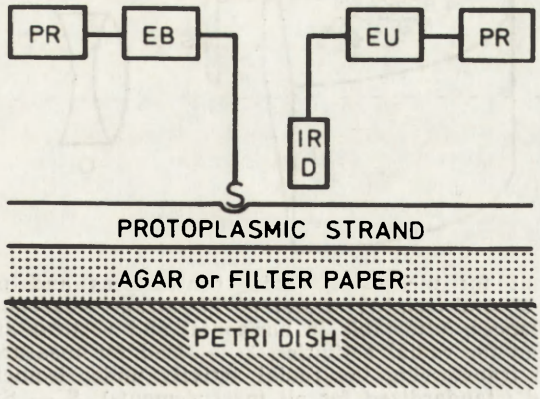


Fig. 2. Diagram comparing tensiometric (left) and infrared (right) registration. PR — pen recorder, EB — electrobalance, EU — electronic unit, S — stamp, IRD — infrared reflexion detector (reflective object sensor) (Samans et al. 1978)

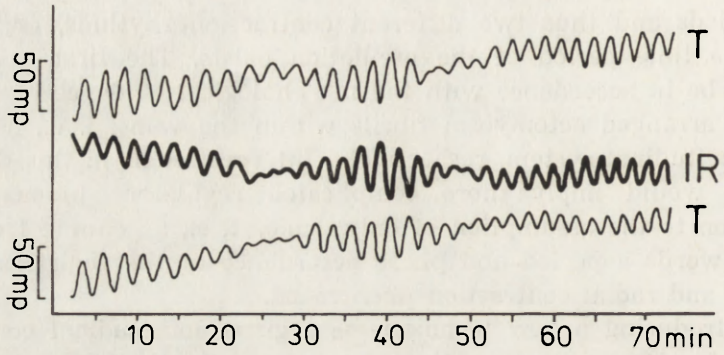


Fig. 3. Simultaneous registration of radial contraction on three immediately neighboring points of one vein. Two tensiometric (T) measuring points on each side next to the infrared registration (IR). Distance between each measuring point: 5 mm (S a m a n s et al. 1978)

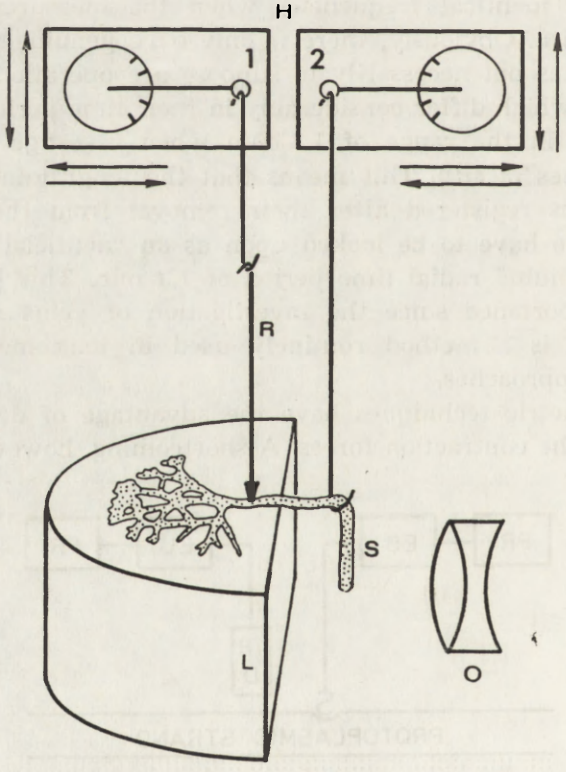


Fig. 4. Arrangement for measuring radial contractions and protoplasmic flow simultaneously on a semi-isolated strand of *Physarum polycephalum*. H — Tensiometric electrobalances, adjustable in horizontal and vertical directions (*arrows*). *1* — Measuring head for radial activities. *2* — Head for measurements of weight changes. *R* — Rod transferring the radial activity under isometric conditions to the measuring head (standardized for all measurements). *S* — Strand hanging on a hook and remaining partly in its original position on filter paper (object area within a moist chamber). *O* — Objective of monitoring system (H ü l s m a n n and W o h l f a r t h - B o t t e r m a n n, 1978).

fact that they cannot be applied without touching the veins. A newly developed infra-red reflexion technique (Fig. 2) enables to register the radial contraction rhythms without touching the strands, i. e., this technique is non-invasive and registers radial contraction activity in veins of all sizes *in situ*. The electronic device is inexpensive and employs a miniaturized infrared detector. The reliability of the infrared reflexion technique can be demonstrated when registering radial activity by the new method simultaneously and immediately beneath two radial measu-

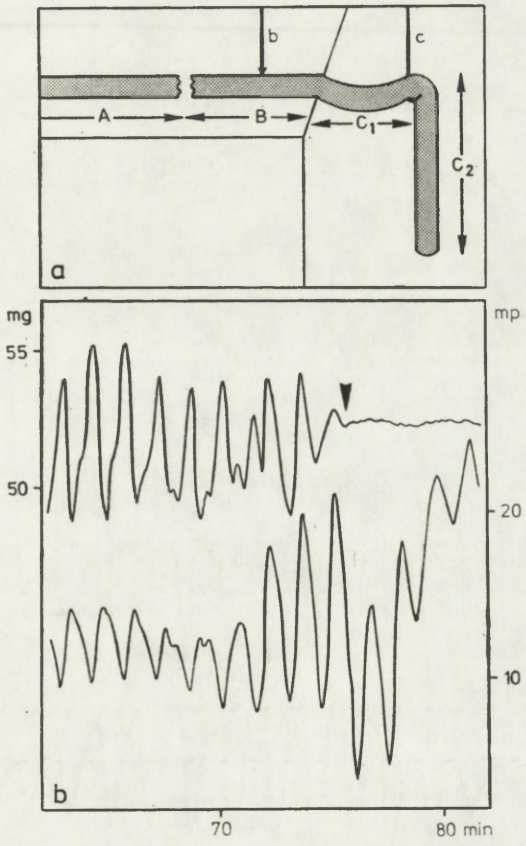


Fig. 5 a. Diagram of the simultaneous measurement of radial contraction at region *B* (by rod *b*) and of streaming phenomena between regions *B* and *C* (by weighing hook *c*). Compare Fig. 4. *A* — Region representing the rest of the plasmodium. *B* — About 10 mm of the strand responsible for generating the radial forces registered by rod *b*. C_1 — About a 4–5 mm part of the strand hanging horizontally to prevent the transfer of tension forces between substrate and hook *c*. C_2 — The end region of the suspended strand (length about 20–60 mm). — *b*. Experimental proof of the reliability of flow measurements registered by hook *c*. Ascending arms of the upper curve = inflow into compartment *C*. An amputation of the vein between rod *b* and the border of the solid substrate stops immediately the oscillation of weight changes (*arrow upper curve*), but radial contraction activity in region *B* is not sustained (*lower curve*). (Hülsmann and Wohlfarth-Bottermann, 1978)

rements by tensiometry (Fig. 3). The identical characteristics within all three curves confirms that (1) the infrared reflexion technique is reliable, and (2) radial contraction phenomena as registered by tensiometry represent spontaneous, permanent phenomenon, and are not induced (as has been argued) by the contact of the tensiometer stamp with the plasmodial vein.

A combination of two tensiometric devices ("twin arrangement") with a television monitoring system (Fig. 4), moreover, allows a registration

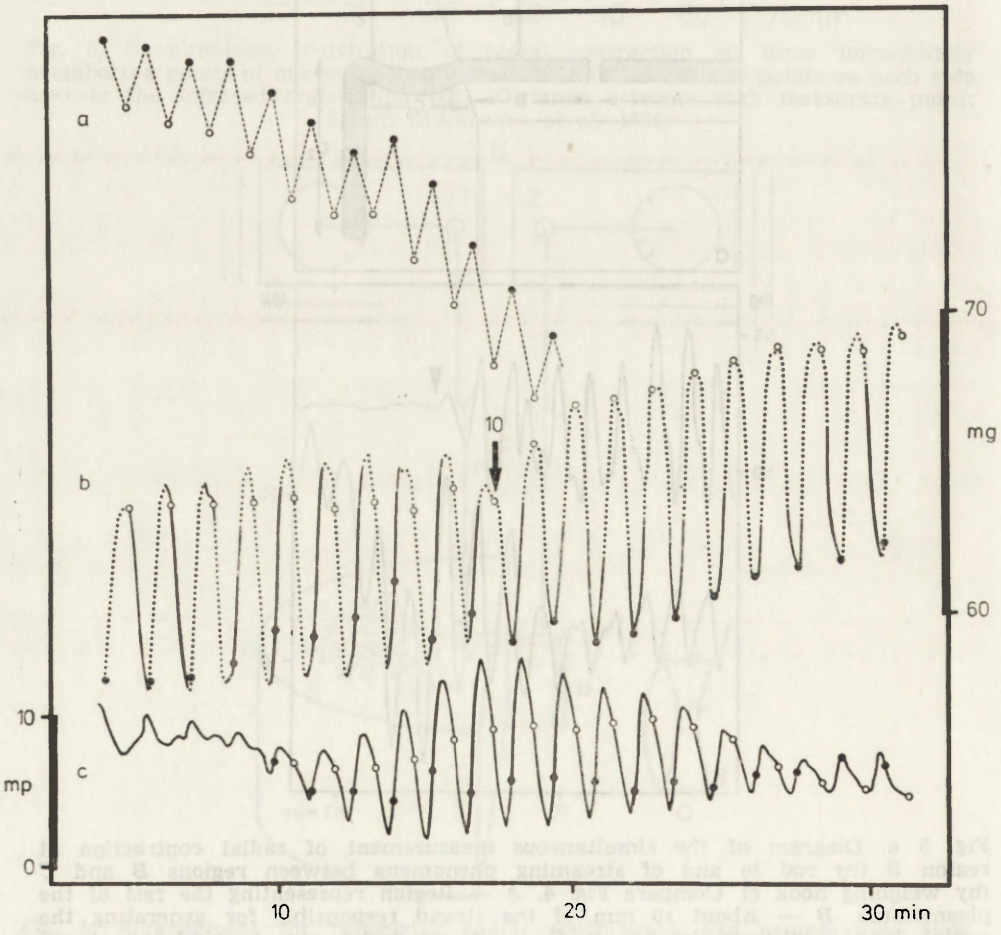


Fig. 6. Simultaneous registration of (*a*) isotonic longitudinal contraction rhythms in the suspended C_2 region registered by TV monitoring (compare Fig. 4), (b) of streaming phenomena between regions *B* and *C;* and (*c*) of radial contraction cycles in region *B*. The circles and dots represent the time points of maximal elongation (relaxation, O) and maximal shortening (contraction, ●) of the suspended part C_2. The phases of relaxation in region *B* (curve *c*) were marked by dotted lines in the streaming curve (*b*) (Hülsmann and Wohlfarth-Bottermann, 1978)

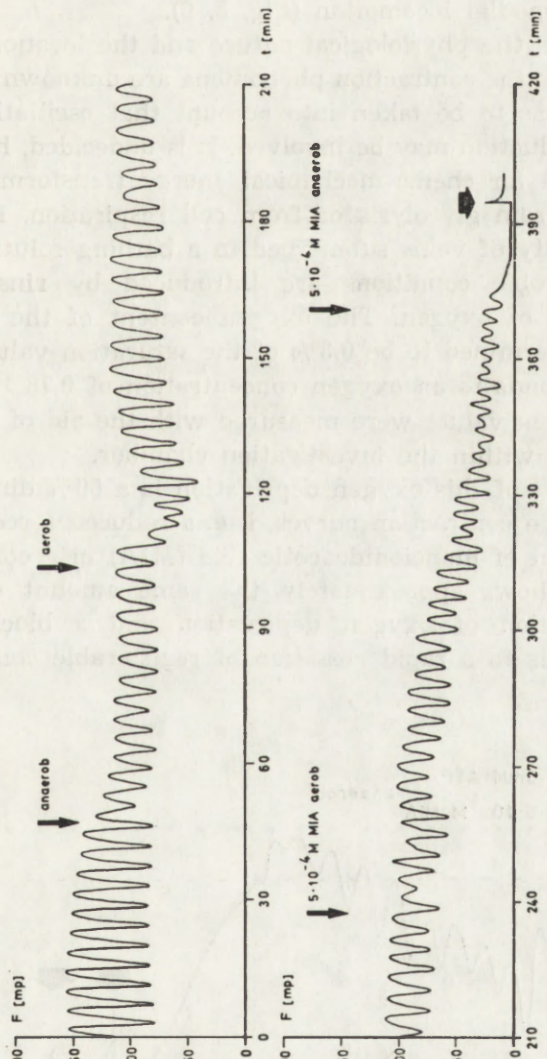


Fig. 7. Longitudinal contraction activity isometric conditions of measurement. Effects of anaerobic conditions in the external medium, the successive supply of oxygen, the effect of monoiodideacetic acid (MIA) and finally a combination of anaerobiosis and MIA (Büttner, unpublished)

of radial and longitudinal contraction activities of the veins as well as the registration of endoplasmic flow (mass transport in mg per second) simultaneously, when the second transducer is used as an electrobalance. This means that contraction phenomena of the transporting veins can be precisely correlated with the resulting shuttle streaming rates and thereby with plasmodial locomotion (Fig. 5, 6).

At the present time, the physiological nature and the location of the oscillator which governs the contraction phenomena are unknown. Among other possibilities, it has to be taken into account that oscillating processes during ATP production may be involved. It is undecided, however, whether the ATP used for chemo-mechanical energy transformation in *Physarum* is derived from glycolysis or from cell respiration. Longitudinal contraction activity of veins submerged in a bathing solution continues if strict anaerobic conditions are introduced by rinsing the veins with water free of oxygen. The oxygen content of the desoxygenated water was determined to be 0.3% of the saturation value of air in water. This corresponds to an oxygen concentration of 0.78×10^{-6} M (= 0.13 mm Hg). Oxygen values were measured with the aid of an oxygen electrode mounted within the investigation chamber.

The registered effect of this oxygen deprivation is a 50% diminution of the amplitudes of the contraction curves, i. e. a reduced force output (Fig. 7). The application of monoiodideacetic acid (MIA) in a concentration of 5×10^{-4} M shows approximately the same amount of force reduction. A combination of oxygen deprivation and a blockage of glycolysis by MIA leads to a rapid cessation of registerable contraction

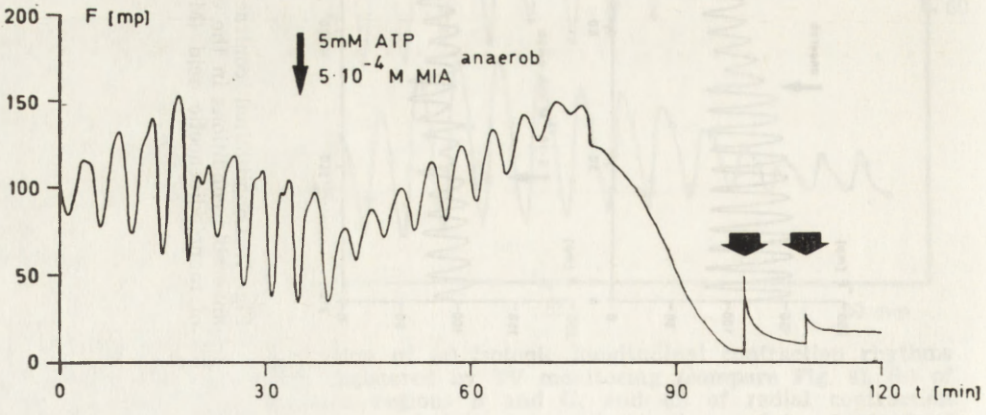


Fig. 8. Longitudinal contraction activity, isometric conditions of measurement. The outer application of ATP cannot replace the physiological energy supply, if the natural ATP production is sustained by 5×10^{-4} M monoiodideacetic acid (MIA) under anaerobic conditions (Büttner, unpublished)

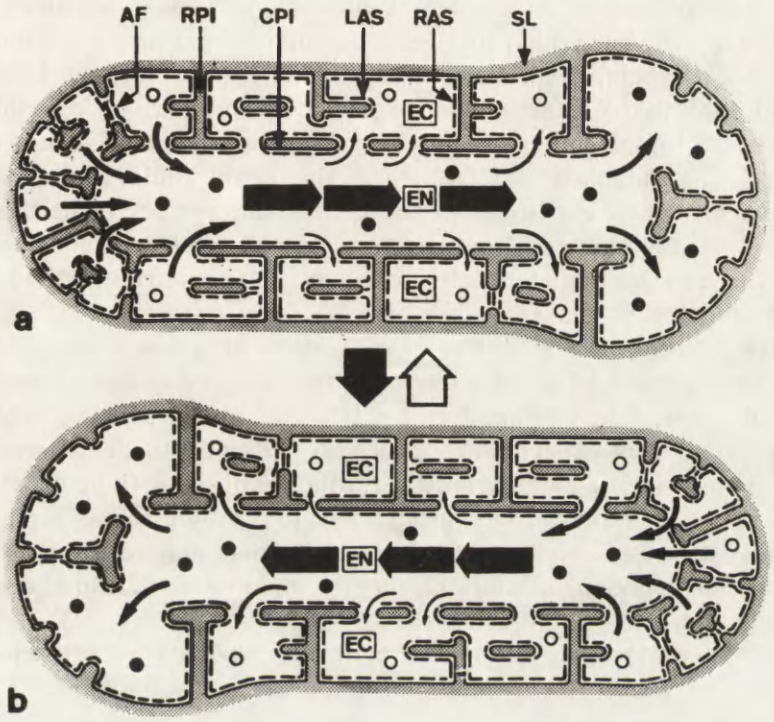


Fig. 9. Diagram representing the lateral pathways (small arrows) of the endoplasmic main stream (thick arrows) during shuttle streaming ($A \rightleftarrows B$) in a plasmodial strand of *Physarum* ("Vein model"). *EN* — endoplasmic main stream; *EC* — ectoplasmic tube; *AF* — cytoplasmic actomyosin fibril; *RPI* — radial plasmalemma invagination; *CPI* — circular plasmalemma invagination on the borderline ectoplasm/endoplasm; *LAS* — longitudinally arranged actomyosin sheet bordering a plasmalemma invagination; *RAS* — radially arranged actomyosin sheet; *SL* — outer slime layer; - - - — cytoplasmic actomyosin layers and fibrils; ● and O — inner Ca^{++} stores, regulating the level of free $Ca^{++}(i)$; ● — Ca^{++} accumulated within the stores, i. e., in areas of predominant relaxation; O — Ca^{++} partly released from the stores to the groundplasm, i.e., in areas of predominant contraction (U e d a et al., 1978)

activities. However, neither the lack of oxygen nor blockage of glycolytic energy production *per se* seem to influence the contraction frequence.

The complete elimination of energy production by blockage of respiration and glycolysis cannot be compensated by applying ATP in the external medium (Fig. 8). Although pronounced effects of externally applied ATP in motive force generation were described when using the "double chamber method", it does not seem probable that an appreciable amount of ATP extracellularly applied can penetrate the plasmalemma and thereby substitute the physiological ATP production. The plasmalemma (and the outer plasmodial slime layer) hinder the uptake of many physiologically active substances which should be tested in respect tc their influence on the contraction automaticity.

To overcome this experimental limitation, a new injection technique was introduced which implies a complete replacement of endoplasm by artificial media, e. g., air, oil, or physiological solutions. Because we learned that there is an exchange of ectoplasm and endoplasm in the form of "protoplasmic streamlets" crossing the borderline (Fig. 9) between the endoplasmic stream and the ectoplasmic tube, we anticipated to influence via injection the contractile system from the side of the endoplasmic channel. The effects of injection of artificial media into the veins can be registered by different tensiometric methods and/or by infrared reflexion.

Plate I presents a semithin cross section of a vein before, Plate II after the injection of air. To our surprise, the endoplasmic core can be replaced more or less completely by artificial media and the ectoplasmic tube survives this experimental procedure: Figure 10 demonstrates that even its contraction automaticity continues. This is a final proof for the thesis that (1) motive force generation for protoplasmic streaming is located within the ectoplasmic tube, and (2) that motive force generation is widely independent of the presence of endoplasm within the endoplasmic channel.

The experience that endoplasm could be replaced by artificial media

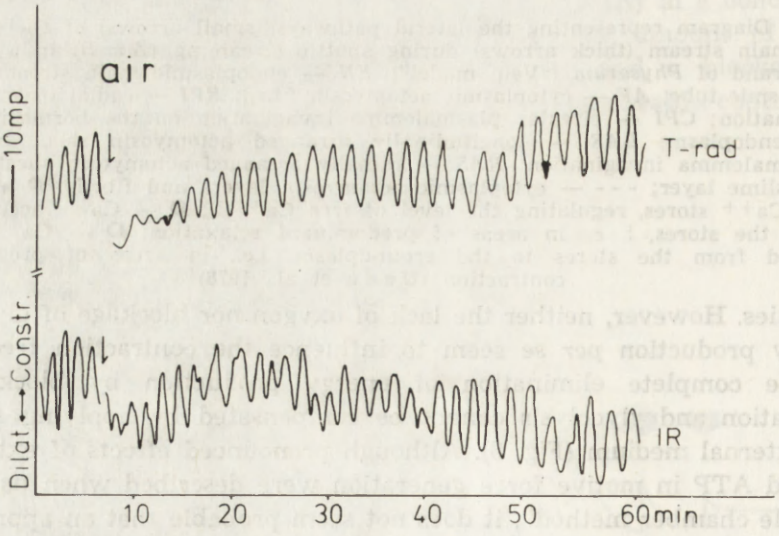


Fig. 10. The effect of replacement of endoplasm by air upon radial contraction activity. The continued contraction activities of the veins were registered by the tensiometrical contact technique [upper curve (*T*-rad), contraction force in *mp*], or by the infrared reflexion technique [lower curve (*IR*), ordinate arbitrary units]. In all original curves, the arrows mark the time point of the injection. Dilat — Dilation of the vein, Constr. — constriction (U e d a et al., 1978)

and these effects on contraction automaticity can be registered before, during and after the injection offered new possibilities to evaluate the requirements of the contractile machine, e. g., concerning the regulatory function of Ca^{++}-ions and the effects of ATP and other adenosine nucleotides. We found that a variation of the internal content of Ca^{++}-ions and nucleotides can control contraction force.

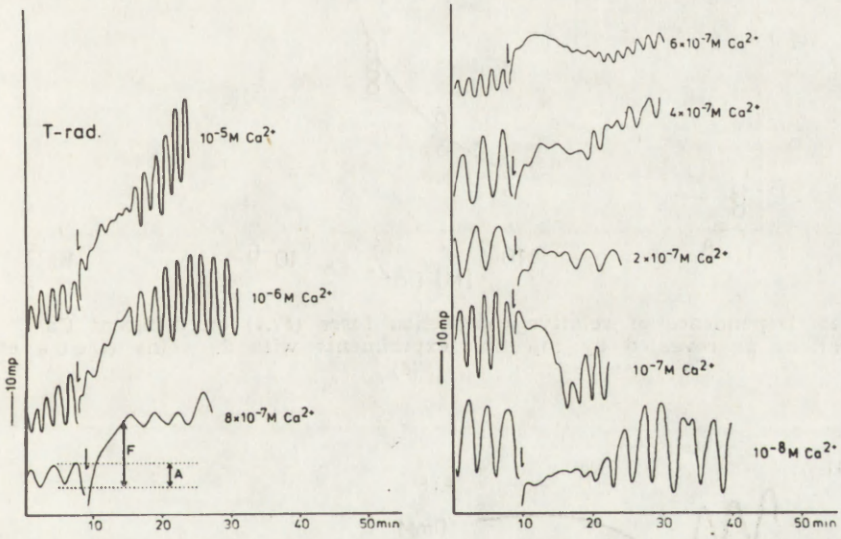


Fig. 11. Effects of different Ca^{++} concentrations within the injected media upon. force generation (quantitated as *F/A*) of the surviving ectoplasmic tube. Tensiometric registration of radial contraction activities — (*T*-rad). Original tensiometric curves (U e d a et al., 1978)

Figure 11 presents the effects of different Ca^{++} concentrations applied by injection of Ca^{++}-EGTA buffers. For a quantitation of the effects of Ca^{++} on force output, the values *F/A* i. e., the differences in the level of contraction force, were calculated from the original contraction curves. When plotting these relative values of force output for different Ca^{++} concentrations (Fig. 12), it could be shown that, beginning from a threshold concentration of 2×10^{-7} M Ca^{++}, half maximum contraction response was reached at 4×10^{-7} M Ca^{++} and a saturation at 6×10^{-7} M Ca^{++}. These results are in good agreement with findings derived from biochemical investigations, according to which the concentration of free Ca^{++}-ions for the regulation of cytoplasmic actomyosin should lie in the range to 10^{-7} M Ca^{++}.

For evaluating the effects of injection of different concentrations of ATP (Fig. 13), a modified method of quantitation of force output was used (*S/So* represents a square relation of the oscillation curve before

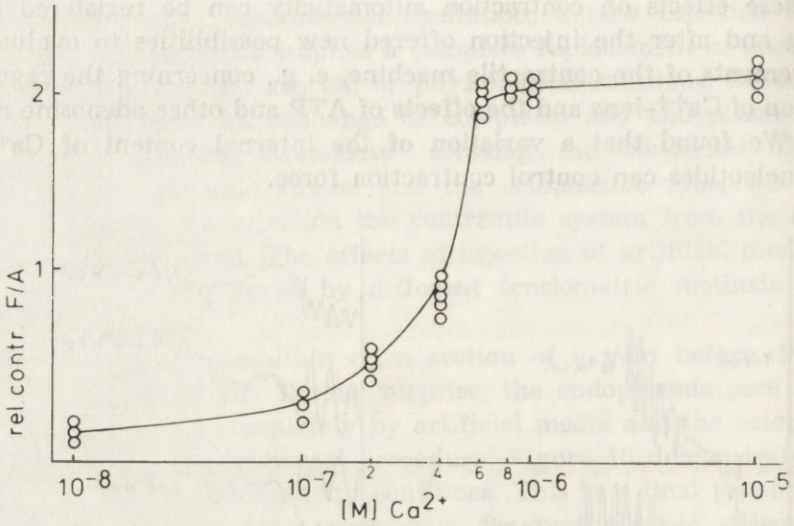


Fig. 12. Dependence of relative contraction force (F/A) on different Ca^{++} concentrations as revealed by injection experiments with 29 veins (U e d a et. al., 1978)

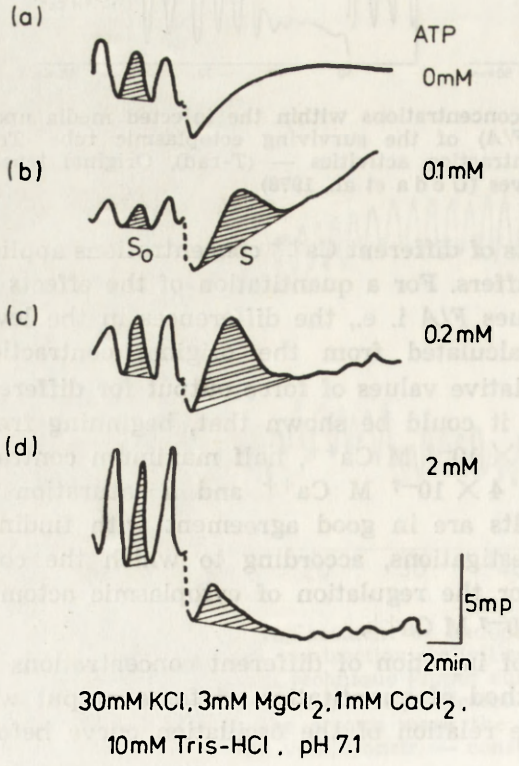


Fig. 13. Effects of different ATP concentrations within the injected media upon force generation (quantitated as S/So) of the surviving ectoplasmic tube. Tensiometric registration of radial contraction activities, original curves (U e d a et al., 1978)

and after the injection. Thus, this value implies amplitude and additionally the duration of a contraction following the injection of a nucleotide). Figure 14 shows as a summary of these results that a maximal force output is produced by 0.2 mM ATP and that higher concentrations again lead to a decrease of force production. For ADP, maximal force output was produced at 0.5 mM and for 5'AMP at 2.5 mM. The contraction response induced by 0.2 mM ATP depends strongly on the concentration of Ca^{++} within the injected ATP solution.

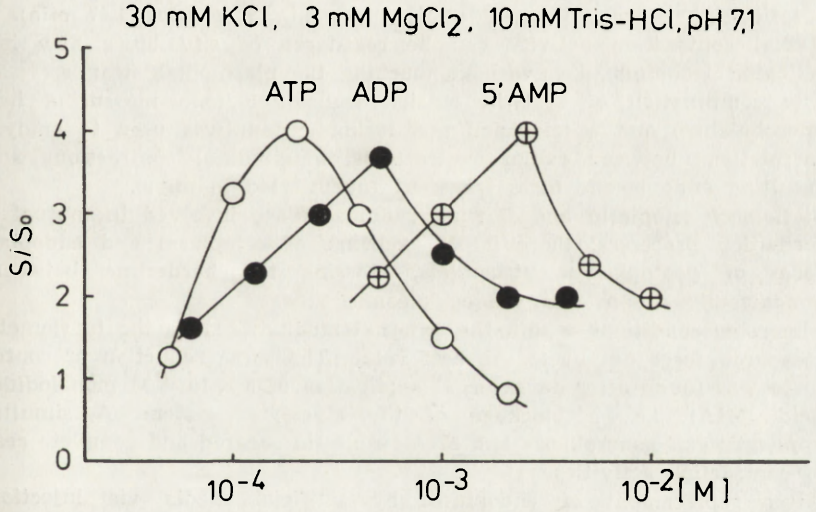


Fig. 14. Concentration dependence of different nucleotides upon force generation (*S/So*). Results from tensiometric registrations of radial contraction activities. Ca++ concentration 1 mM for ATP, 4×10^{-7} M for ADP, and 5'AMP. Maximal concentration responses for ATP: 0.2 mM, ADP: 0.5 mM, and AMP: 2.5 mM (Ueda et al., 1978).

Whereas the concentration dependent effects of ATP are easy to interprete in the sense that an ATP concentration of approximately 0.2 mM is near the internal physiological ATP level and that higher concentrations of ATP lead to a decreased actin-myosin binding affinity and thus to a decreasing force output, the interpretation of the effects of ADP and 5'AMP are more difficult and must remain widely a matter of speculation at the present time. Plate III presents a cross section of a vein after an injection of a solution which may meet approximately the physiological requirements of the cytoplasmic actomyosin in *Physarum* (0.2 mM ATP and $2–4 \times 10^{-7}$ Ca^{++}).

Surveying the present possibilities for the study of the physiology of cytoplasmic actomyosin, the *Physarum* system, in comparison to other

objects, offers paramount experimental advantages for biochemical as well as for combined biophysical techniques. It is difficult to understand why, up to now, these possibilities are not used to a larger extent.

SUMMARY

(1) Modified tensiometric methods allow the registration of longitudinal and radial contraction phenomena in plasmodial *Physarum* veins, *in situ* and simultaneously. Both activities show nearly identical time periods and identical phase relations (contraction 1.34 min, streaming 1.47 min).

(2) Radial contraction activity can be registered *in situ by* a new infrared reflexion technique, i.e., without touching the plasmodial strands.

(3) The combination of a twin tension transducer (one measuring head as microbalance) and a television monitoring system was used to analyze the correlations between radial contractions, longitudinal contractions and the resulting endoplasmic mass transport (quantitated in mg/s).

(4) Stationary ectoplasm and flowing endoplasm are involved in mutual transformation processes: there is an exchange of ectoplasm and endoplasm in form of protoplasmic streamlets, crossing the borderline between the endoplasmic stream and the ectoplasmic tube.

(5) Anaerobic conditions within the external medium reduce the tensiometrically measured force output to the half value. The same reduction of contraction force was found after an external application of 5×10^{-4} M monoiodideacetic acid (MIA), i.e., a blockage of the glycolytic system. A simultaneous application of anaerobiosis and MIA results in a rapid and complete cessation of contraction activities.

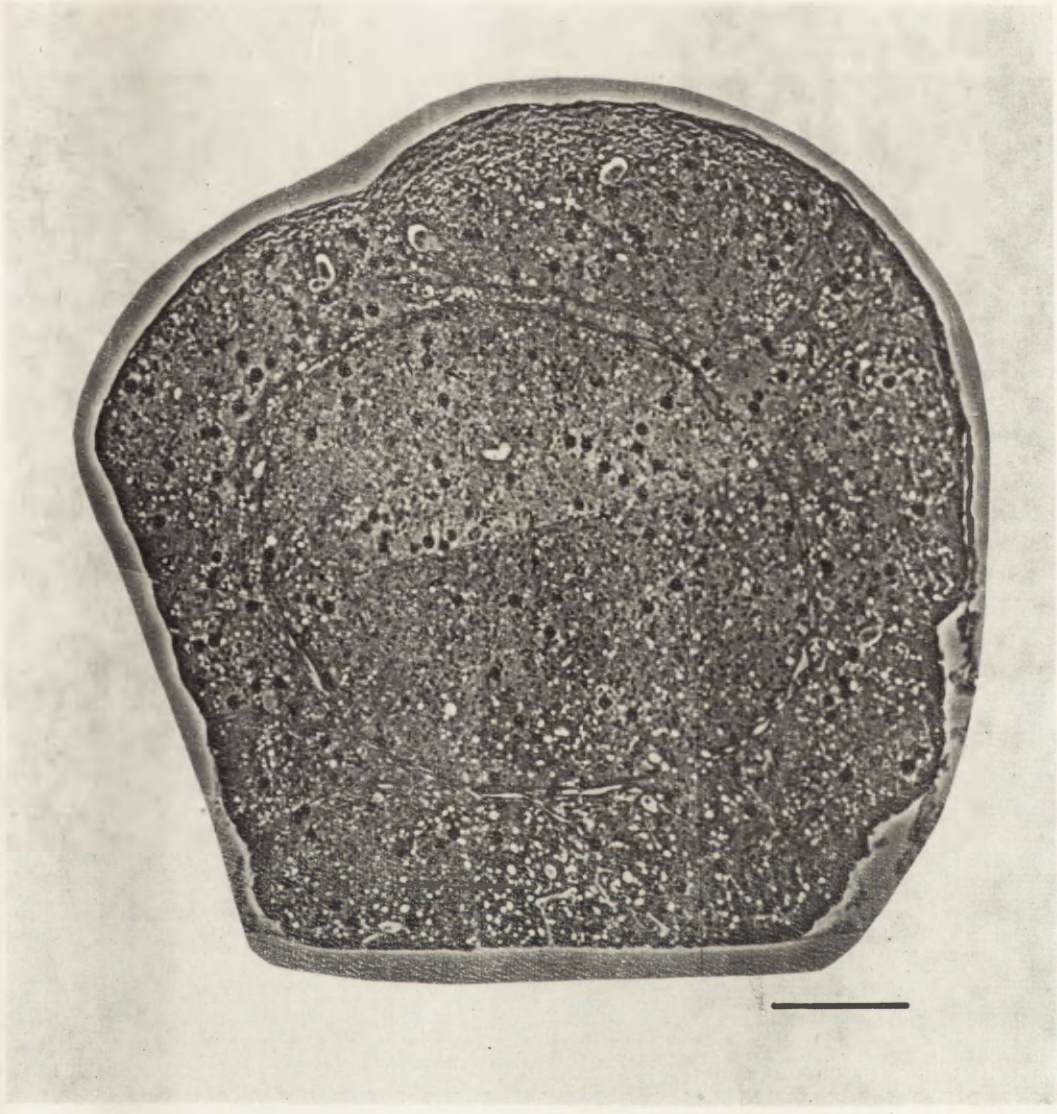
(6) After replacement of endoplasm by artificial media via injection the ectoplasmic tube survives and radial contraction activity continues. This shows that force generation for protoplasmic streaming is widely independent of the presence of endoplasm and that the ectoplasmic tube represents the force generating system.

(7) The replacement of endoplasm by Ca++-EGTA buffers (10^{-5}—10^{-8} M Ca++) resulted in an increased force output, beginning with a threshold concentration of 2×10^{-7} and reaching a saturation at 6×10^{-7} Ca++. Half maximum contraction responses were registered at 4×10^{-7} M Ca++.

(8) Injection experiments with solutions containing different concentrations of ATP revealed an increasing force output up to a concentration of 0.2 mM ATP. Higher concentrations of ATP resulted in decreasing contraction responses. The reaction to injected ATP is Ca++-dependent.

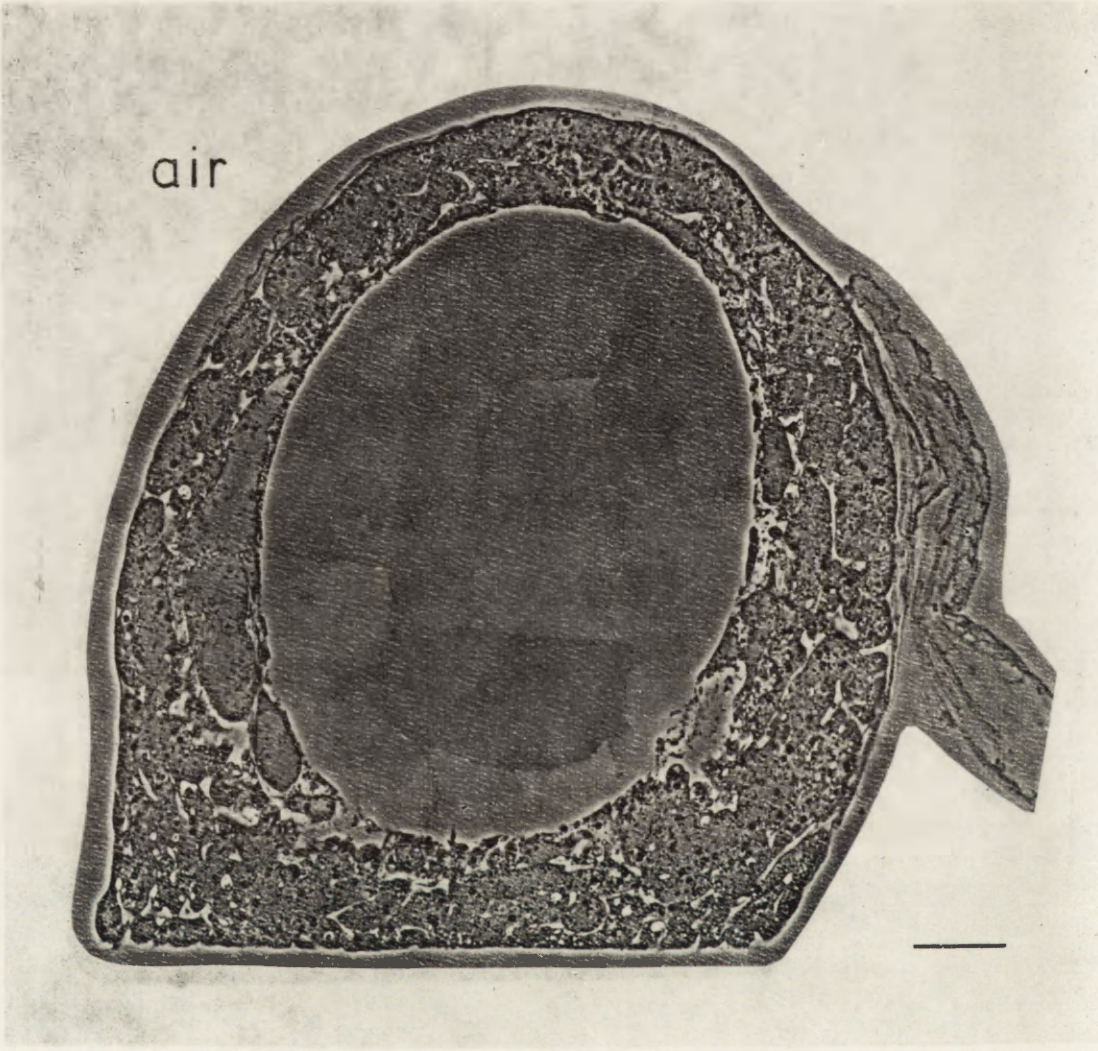
(9) ADP induced a maximal force output at a concentration of 0.5 mM, AMP at a concentration of 2.5 mM.

(10) It is concluded that $2\text{–}4 \times 10^{-7}$ Ca++ and 0.2 mM ATP approximately represent the physiological concentrations required for the cytoplasmic actomyosin system of *Physarum in vivo*.



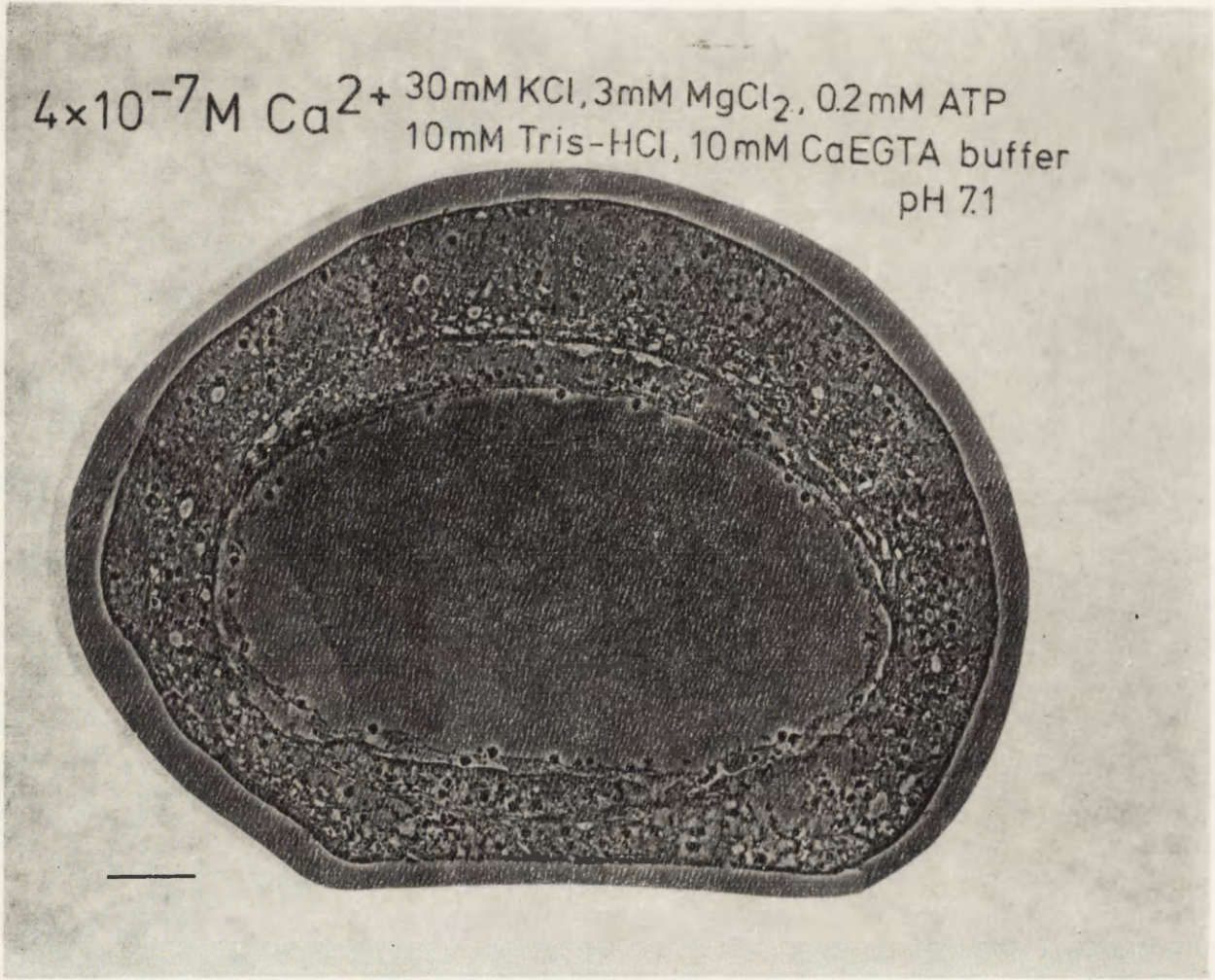
K. E. Wohlfarth-Bottermann auctor phot.

Phase contrast microscopic picture of a semi-thin cross section of a vein before injection. Bar — μm (U e d a et al., 1978)



K. E. Wohlfarth-Bottermann auctor phot

A vein after replacement of endoplasm by air. Bar — 50 μm (Ueda et al., 1978)



K. E. Wohlfarth-Bottermann auctor phot.

Phase contrast microscopic picture of a semi-thin cross section of a vein after replacement of endoplasm by a physiological solution. Bar — 50 μm (Ueda et al., 1978)

REFERENCES

Büttner A. 1978: Einfluß von Stoffwechselinhibitionen auf die oszillierende Kontraktionsaktivität von *Physarum polycephalum*. Diplomarbeit der Math.-Naturwiss. Fakultät der Univ. Bonn, (unpublished).

Hülsmann N. and Wohlfarth-Bottermann K. E. 1978: Spatio-temporal analysis of contraction dependent surface movements in *Physarum polycephalum*. Cytobiologie, 17, 23–41.

Hülsmann N. and Wohlfarth-Bottermann K. E. 1978: Spatio-temporal relationships between protoplasmic streaming and contraction activities in plasmodial veins of *Physarum polycephalum*. Cytobiologie, 17, 317–334.

Samans K. E., Götz von Olenhusen K. and Wohlfarth-Bottermann K. E. 1978: Oscillating contractions in protoplasmic strands of *Physarum*: Infrared reflexion as a non-invasive registration technique. Cell Biol. Int Reports, 2, 271–278.

Ueda T., Götz von Olenhusen K. and Wohlfarth-Bottermann K. E. 1978: Reaction of the contractile apparatus in *Physarum* to injected Ca^{++}, ATP, ADP and 5'AMP. Cytobiologie, 18, 76–94.

Wohlfarth-Bottermann K. E. 1977: Oscillating contractions in protoplasmic strands of *Physarum*: Simultaneous tensiometry of longitudinal and radial rhythms, periodicity analysis and temperature dependence. J. exp. Biol., 67, 49–59.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 75–90

Laboratory of Cell Biology, Department of Health, Education and Welfare, Public Health Service, National Institute of Health, Bethesda, Maryland 20014, USA

E. D. KORN

Regulation of the Form and Function of Actin and Myosin of Non-Muscle Cells *

Synopsis. This article describes the properties of actin, four myosin isoenzymes and a myosin heavy chain kinase that have been purified from *Acanthamoeba castellanii*. In addition, *Acanthamoeba* contains at least one protein that interacts with G-actin to prevent its polymerization and four low molecular weight proteins that interact with polymerized F-actin to cause gelation. These proteins, and other proteins that have not yet been identified, interact to regulate the form and function of actin and myosin in the amoeba. The data are presented as one specific illustration of the complexity of the biochemistry of actomyosin-dependent motile processes in non-muscle cells in general.

It is now certain that actin and myosin provide the molecular basis for many different motile processes in all eukaryotic cells. These processes are as diverse as endocytosis and exocytosis, cytokinesis and possibly chromosome movement, topographical distribution of surface membrane proteins and possibly transmembrane signalling, cytoplasmic streaming and saltatory movements, formation and movement of microvilli and cytoskeletal functions, and, of course, ameboid movement. In some of these activities actin may function without myosin but in most instances the functional entity must be the actomyosin complex. However, as is also true for the most highly evolved actomyosin systems, muscles, many other proteins regulate the organizational form and the functional expression of the actin and actomyosin complexes. The important problems now facing those of us who are studying cell motility are to identify all of the proteins in these highly integrated systems, as they occur in any one individual cell, and to work out their complex interactions at the molecular level.

The biochemical information available several years ago was orga-

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

nized into a very useful review by Pollard and Weihing (1974). More recently, Clarke and Spudich (1977) reviewed the data gathered in the last few years and I (Korn 1978) have recently critically analyzed our present understanding of the biochemistry of cell motility and suggested possible directions for future research. In this article, I will concentrate more specifically on the research currently in progress in my own laboratory which, although it includes comparative studies of actins from muscle and non-muscle cells, is mostly concerned with the biochemical characterization of the motility system in the soil amoeba, *Acanthamoeba castellanii*. However, I will attempt to discuss the implications of our research within the more general framework that has been provided by many excellent investigations by many other scientists.

Polymerization of Actin

Our first attempt to isolate actin from *Acanthamoeba* (Weihing and Korn 1971) depended on its ability to form a readily sedimentable complex with added muscle myosin. Although we did obtain *Acanthamoeba* actin in reasonable purity and in sufficient amounts to begin its characterization (Weihing and Korn 1971, 1972), it later became evident that we had isolated probably less than 1% of the total actin of the amoeba. This was not because *Acanthamoeba* contains more than one kind of actin. Rather, the major cause of the low yields was the inability of the actin to polymerize in the crude extracts. High yields were obtained by introducing a novel step, purification by chromatography on DEAE-cellulose, after which the actin was readily polymerized and purified by cycling through its polymerized and depolymerized forms (Gordon et al. 1976 a). With high yields of highly purified actin, the properties of *Acanthamoeba* actin could be compared in detail to those of rabbit skeletal muscle actin, the best characterized actin available. The two actins proved to be qualitatively very similar although there are significant quantitative differences.

The amino acid compositions of *Acanthamoeba* actin (Weihing and Korn 1971, 1972; Gordon et al. 1976) and muscle actin are similar, although not identical, including the presence of 1 mole of N^{τ}-methylhistidine per mole of actin. It is now known, through the excellent work of Elzinga and his colleagues (1976), that the amino acid sequence of *Acanthamoeba* actin, and other non-muscle actins, are very similar to that of rabbit skeletal muscle actin; *Acanthamoeba* actin differs from muscle actin in only about 6% of its residues. Despite this extensive homology, the isoelectric point of *Acanthamoeba* actin (which has only

one species on isoelectric focussing gels) is significantly more alkaline than that of rabbit muscle actin (Gordon et al. 1977).

As a result of its largely identical amino acid sequence, the physical properties of *Acanthamoeba* actin are very similar to those of muscle actin. Both actins exist as monomeric G-actin in buffers of low ionic strength and are induced to polymerize to F-actin by addition of 1 to 2 mM $MgCl_2$ or by increasing the ionic strength to 0.1 M KCl (Gordon et al. 1976 a, b). Equal concentrations of *Acanthamoeba* and muscle F-actin have the same viscosities indicating that the number and length of the filaments are the same. The filaments are indistinguishable in appearance (Pollard et al. 1970). For both actins, polymerization is a two-step process: a slow nucleation step followed by a rapid elongation step. Polymerization is concentration dependent occurring only at actin concentrations greater than the critical concentration, which is the concentration of G-actin in equilibrium with F-actin. This critical concentration varies with the ionic conditions and under some circumstances is significantly greater for *Acanthamoeba* actin than for muscle actin. However, at 25° and in the presence of 2 mM Mg^{2+}, the critical concentrations of the two actins are indistinguishable.

Essentially all of these features are also descriptive of actin from vertebrate non-muscle cells including human platelets, embryonic chick brain and rat liver. However, the vertebrate, non-muscle actins consist of two isoelectric species with isoelectric points intermediate between those of muscle and *Acanthamoeba* actins (Gordon et al. 1977). Nevertheless, under ionic conditions likely to resemble those in the cell, the critical concentrations for polymerization of all the non-muscle actins that have been studied are very similar to those of rabbit skeletal muscle actin (Table 1). The differences between muscle and non-muscle actins

Table 1

Comparison of the properties of nonmuscle and muscle actins

Source of Actin	Critical concentration mg/ml					
	2 mM $MgCl_2$		0.1 M KCl		0.5 M KCl/ 1 mM $CaCl_2$	Activation of HMM-ATPase
	25°	5°	25°	5°	25°	K_{app}, μM
Rabbit skeletal muscle	0.03	0.03	0.03	0.1	0.15	7.1
Human platelet	0.03	0.03	0.09	0.51	0.32	9.6
Rat liver	0.02	0.02	0.08	0.48		9.6
Chick embryo brain	0.02	0.02	0.07	0.5	0.22	9.6
Acanthamoeba castellanii	0.06	0.06	0.09	0.45	0.39	21.7

are somewhat greater under poor polymerization conditions such as 0.5 M KCl, or in 0.1 M KCl at 5°.

The absolute values for the critical polymerization concentrations of the non-muscle actins are of more interest than just as evidence for the essential similarity of all actins. In extracts of non-muscle cells, the concentration of non-polymerized actin can be 10 mg/ml or greater, even in 2 mM $MgCl_2$ (Gordon et al. 1977). Since this is well above the concentration at which the purified actins polymerize, it must be assumed that there are other proteins present that interfere with the polymerization of actin, hence, our earlier difficulties in preparing F-actin until partially purifying it by chromatography on DEAE-cellulose. At least two proteins with the ability to interact with monomeric G-actin and inhibit its polymerization have been described: pancreatic DNase I (Lazarides and Lindberg 1974) and profilin (Carlsson et al. 1976). If interaction of actin with a cellular DNase has biological significance it might as well be to regulate function of the DNase as to regulate actin polymerization. But the interaction of profilin, a protein isolated from spleen but present in at least several mammalian tissues (Carlsson et al. 1977), with actin is presumably a mechanism for regulating the state of polymerization of actin in non-muscle cells.

Briefly, I will mention other means by which actin polymerization may be regulated and by which the state of organization of actin filaments in cells may be controlled. We have isolated from *Acanthamoeba* four low molecular weight proteins (gelation factors or "gelactins" I–IV) with native molecular weights of 23 000, 55 000, 64 000 and 78 000, that interact with filaments of F-actin to form gels (Maruta and Korn 1977 a). The three larger gelactins may be dimers. Similar behavior was observed previously for a high molecular weight protein, actin-binding protein (Hartwig and Stossel 1975), that is present in vertebrate non-muscle cells and which is similar or identical to filamin, a protein isolated from vertebrate smooth muscle (Wang et al. 1975). Filamin is a dimer of 250 000-dalton polypeptides (Shizuta et al. 1976; Wang 1977).

No information is available about the molecular mechanisms by which any of the proteins interact with G-actin and F-actin. However, these phenomena will be a rich source for future research for almost certainly they are important mechanisms for regulating the organization of the cytoplasm of non-muscle cells and controlling the kind and location of motile activity that is to occur. This is one major aspect of motility in non-muscle cells that has no counterpart in striated muscle cells.

Copolymerization of *Acanthamoeba* and Muscle Actins

We have observed that a mixture of muscle and *Acanthamoeba* actin will polymerize when each is below its critical concentration (Gordon et al. 1976 b). Since, by definition, neither actin alone will form a homopolymer under these conditions, it is necessary to conclude that these two actins, from very distant species, can form copolymers and that the monomeric subunits in equilibrium with the actin copolymer have their own unique critical concentration. More recent work, as yet unpublished, has been interpreted by us to mean that these *Acanthamoeba* actin-muscle actin copolymers are random copolymers with respect to the distribution of the actin subunits. These conclusions derive from the effect of Mg^{2+} on the binding of muscle tropomyosin to polymers of F-actin.

Tropomyosin binds to muscle F-actin in the fixed stoichiometry of one molecule of tropomyosin to 7 actin subunits. Each tropomyosin molecule has 7 subsites each of which interacts with one of 7 neighboring actin subunit in the F-actin filament. Although it is probable that *Acanthamoeba* does not contain tropomyosin, nonetheless *Acanthamoeba* F-actin also interacts with muscle tropomyosin with the same stoichiometry. There is, however, one experimentally useful difference between the interactions of muscle tropomyosin with muscle F-actin and *Acanthamoeba* F-actin. Both processes are very dependent on the Mg^{2+} concentration but whereas the binding of tropomyosin to muscle F-actin begins at about 4 mM Mg^{2+} and is complete at about 4.5 mM Mg^{2+} the interaction of tropomyosin with *Acanthamoeba* F-actin does not occur until 6 mM Mg^{2+} and is not maximal until 9 mM Mg^{2+} (Fig. 1). Copolymers of muscle actin and *Acanthamoeba* actin, on the other hand, behave entirely differently than equivalent mixtures of the two homopolymers; i.e., binding of tropomyosin is initiated, and is saturated, at Mg^{2+} concentrations unique to each copolymer and different from what would be predicted if the copolymers had the properties of an equivalent mixture of homopolymers. Stated differently, all of the actin subunits in the copolymer behave similarly; muscle actin subunits and *Acanthamoeba* actin subunits are indistinguishable in the copolymer and both are different than they are in their respective homopolymers.

The significance of these observations becomes apparent when one remembers that vertebrate non-muscle cells, in contrast to rabbit skeletal muscle and *Acanthamoeba*, contain two or more species of actins (Gordon et al. 1977). Since these isoactins, occurring in the same cell, are more similar in amino acid composition and in isoelectric points than are *Acanthamoeba* and muscle actins, it must be assumed that the actin

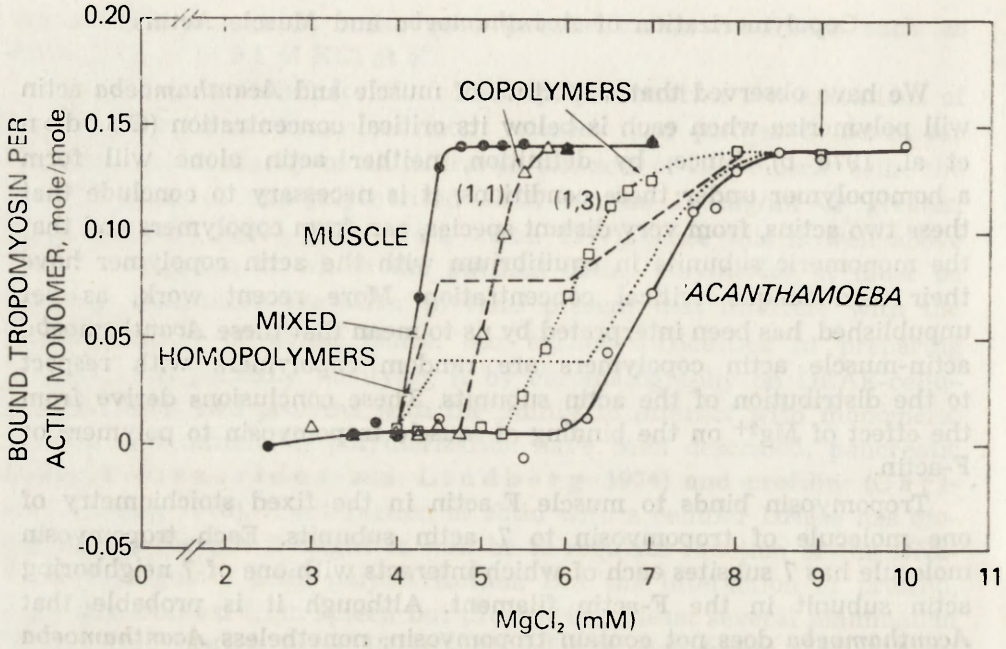


Fig. 1. Mg^{2+}-dependent tropomyosin binding to homopolymers and copolymers of muscle actin and *Acanthamoeba* actin. The two multiphasic curves for mixed homopolymers are theoretical binding curves. The 1,3 polymers (dotted lines) contain 1 part muscle and 3 parts *Acanthamoeba* actin; the 1,1 polymers (dashed lines) contain equal parts of the two actins. The binding reactions were carried out with 5 μM actin and 1.25 μM tropomyosin in 2 mM ATP, 1 mM EGTA and 2 mM imidazole, pH 7.0, at 25°

filaments in a single vertebrate non-muscle cell will be copolymers of the isoactins unless there are specific regulatory mechanisms in the cell to prevent it. Certainly, this will be the case in all experiments *in vitro*, such as those I have described above, in which the polymerization of mixtures of isoactins are studied.

Activation of Heavy Meromyosin ATPase by F-actin

Perhaps the most significant biological property of F-actin is its ability to activate the Mg^{2+}-ATPase activity of myosin. In skeletal muscle, the cyclical interaction of myosin heads with actin thin filaments is the mechanical basis of the sliding filament model (Korn 1978, Eisenberg and Hill 1978). The energy for the movement of the filaments derives from the hydrolysis of one molecule of ATP each time one myosin head interacts with one actin subunit in the thin filament (Korn 1978, Eisenberg and Hill 1978). All models of actomyosin-dependent cell motility assume a similar interaction between non-muscle

actins and myosins. Experimentally, this interaction is most readily studied by using either heavy meromyosin or heavy meromyosin subfragment 1, proteolytic fragments of myosin which retain most of its properties but are more soluble under the ionic conditions usually employed. The interaction of F-actin with heavy meromyosin (HMM) can be observed in the electron microscope by the formation of characteristic "arrowhead" complexes or, more quantitatively, by the activation of the Mg^{2+}-ATPase activity of HMM which occurs when it interacts with F-actin.

All non-muscle F-actins form arrowhead complexes with muscle HMM and all non-muscle F-actins activate the Mg^{2+}-ATPase activity of muscle HMM. The enzyme activation can be characterized by two constants; the V_{max} of the ATPase, i.e., the ATPase activity of HMM at infinite actin concentration, and the K_{app}, i.e., the actin concentration required to reach 50% of V_{max}. Experimentally, the V_{max} extrapolated for *Acanthamoeba*, human platelet, embryonic chick brain and rat liver F-actins are all identical to the value for rabbit skeletal muscle F-actin (Gordon et al. 1977). There are, however, differences in the K_{app} values (Table 1): the K_{app} for *Acanthamoeba* actin is about three times greater than for muscle actin while the values for the three vertebrate non-muscle actins are only slightly greater than that of muscle actin (Gordon et al. 1977). Other than again indicating the remarkable homology among actins of very different sources, these values have no intrinsic importance because the biologically relevant numbers are those for the interaction of F-actin and myosin from the same source. These studies do have a certain heuristic importance, however, as is often the case for comparative biochemical studies.

Activation of HMM ATPase by Copolymers of *Acanthamoeba* and Muscle Actins

In our first studies of the activation of the Mg^{2+}-ATPase of muscle HMM by copolymers of *Acanthamoeba* and muscle actin (Gordon et al. 1976 a), we saw no difference in the behavior of a 1 : 1 copolymer and a 1 : 1 mixture of the corresponding homopolymers. This has now been extended to a series of copolymers varying in composition from 4 : 1 to 1 : 4, muscle actin: *Acanthamoeba* actin. Because of the difference in their K_{app} values, the Mg^{2+}-ATPase of HMM is activated more by muscle F-actin than by *Acanthamoeba* F-actin. Activation of HMM ATPase by the copolymers of the two actins decreased in direct proportion to the increase in content of *Acanthamoeba* actin in the copolymers. This is the expected result if each actin subunit in the

copolymers interacted independently with an HMM head and, in that interaction, retained the properties it would have had in its corresponding homopolymer. I have already presented evidence that the *Acanthamoeba* and muscle actin subunits are randomly distributed in the copolymers. This must mean, then, that adjacent muscle and *Acanthamoeba* actin subunits in the same copolymer do not affect the nature of each other's interaction with HMM.

More evidence on this point was obtained by studying the effect of tropomyosin on the activation of HMM ATPase by F-actin homopolymers and copolymers. Although the binding of tropomyosin to *Acanthamoeba* F-actin is weaker than its binding to muscle F-actin, in 9 mM $MgCl_2$ it binds with the same saturating stoichiometry to both actin homopolymers and to copolymers of all compositions, one tropomyosin molecule binds to 7 actin subunits. Under these conditions, 9 mM $MgCl_2$, tropomyosin inhibits by about 85% the ability of muscle F-actin to activate the Mg-ATPase activity of HMM but has no effect on the ability of *Acanthamoeba* F-actin to activate HMM ATPase. The effect of bound tropomyosin on the activation of the Mg-ATPase activity of HMM by copolymers of muscle and *Acanthamoeba* actin is essentially identical to its effect on corresponding mixtures of homopolymers of the two actins. Since these are random copolymers, this is clear evidence that one tropomyosin molecule bound to adjacent muscle actin and *Acanthamoeba* actin subunits can interfere with the interaction of the muscle actin subunit with HMM but have no effect on the interaction of the *Acanthamoeba* actin subunit with HMM. Moreover, under somewhat different ionic conditions, 80 mM KCl and 5 mM $MgCl_2$, bound tropomyosin still inhibits activation of HMM ATPase by muscle actin subunits but now actually enhances the activation of HMM ATPase by *Acanthamoeba* actin subunits, both in homopolymers (Y a n g et al. 1977) and in the copolymers. The *Acanthamoeba* actin-HMM ATPase activity can be inhibited, however, in homopolymers and copolymers, and to the same extent as muscle actin-HMM ATPase activity, by the addition of troponin as well as tropomyosin.

These experiments have several interesting implications. They are the first positive evidence that actin subunits in the same polymer can act independently and express different behaviors. Thus, while adjacent muscle and *Acanthamoeba* actin subunits interact cooperatively in their polymerization and in their binding of tropomyosin (and each differently than in homopolymers), in their interaction with HMM and in the effect of tropomyosin on that interaction, the muscle and *Acanthamoeba* actin subunits in the copolymers each continues to express its unique properties characteristic of its homopolymer. From these model

experiments, we can reach the important biological conclusion that any functional differences that may exist among the isoactins of vertebrate non-muscle cells will be expressed *in situ* equally in copolymers as in homopolymers of the several actins.

These experiments also illustrate one other potential in the comparative biochemical study of actins and myosins. The nature of the regulation of muscle actomyosin ATPase by tropomyosin-troponin is a topic of great interest. Two hypotheses have been proposed: tropomyosin, or tropomyosin-troponin (in the absence of Ca^{2+}), physically blocks the interaction of HMM with actin subunits (Nakabayashi et al. 1973) or, alternatively, bound tropomyosin, or tropomyosin-troponin, induce a conformational change in the actin subunit so that it cannot bind HMM (Poo and Hartshorne 1976). Our data suggest that the physical blocking hypothesis cannot be correct because it would require that a tropomyosin molecule bound to adjacent actin subunits in the copolymers would block access of HMM to the subunit if it were derived from muscle but not if it were derived from *Acanthamoeba*. Furthermore, the enhancement of the interaction of HMM and *Acanthamoeba* actin subunits observed under some ionic conditions is very difficult to rationalize within the blocking hypothesis. It is easier to imagine that the conformational alteration induced by bound tropomyosin might be different for the different actins and that, in some circumstances, this conformational change might result in an activation, rather than an inhibition, of the interaction of the actin with HMM.

Acanthamoeba Myosins

Myosins isolated from most non-muscle cells physically resemble muscle myosins, especially smooth muscle myosin, in that they are all two-headed molecules of about 500 000 molecular weight consisting of a pair of heavy chains of molecular weight about 200 000 to 225 000 and two pairs of light chains of approximate molecular weights of 20 000 and 17 000 (Korn 1978). With some exceptions, these non-muscle myosins have low Mg^{2+}-ATPase activities and higher Ca^{2+}-ATPase activities which are about equal to their K^+, EDTA–ATPase activities. I will discuss later the effect of F-actin on their Mg^{2+}-ATPase activities.

We have found a markedly different situation with purified *Acanthamoeba* myosin activities and are continuing to investigate the extent to which these interesting differences may reflect the true intracellular situation.

The first myosin-ATPase we isolated from *Acanthamoeba* (Pollard and Korn 1973 a, b) was a single-headed enzyme of native molecular

weight about 180 000 consisting of one heavy chain and two different light chains. It was enzymatically very similar to rabbit skeletal muscle myosin, in that its K^+, EDTA–ATPase activity was about 8 to 10 times greater than its Ca^{2+}-ATPase activity, despite its great physical difference. More recently, we have extended these observations and the situation has become more complex.

Table 2

Comparisons of *Acanthamoeba* myosins

Myosin	Native Mol. Wt.	Subunit Composition	ATPase Activity (μmol $min^{-1}mg^{-1}$)				
			K^+, EDTA	Ca^{2+}	Mg^{2+}		
					-Actin	+Actin	Phosphorylated +Actin
IA	180 000	130 000 (1) 17 000 (1) 14 000 (< 0.5)	2.8	0.36	0.06	0.12	1.8
IB	180 000	125 000 (1) 27 000 (1) 14 000 (< 0.5)	2.4	0.43	0.09	0.26	1.5
IC	180 000	130 000 (1) 18 000 (1) 17 000 (1) 14 000 (< 0.5)	0.93	0.02	0.004	0.13	0.68
II	400 000	170 000 (2) 17 500 (1.5) 17 000 (2)	0.12	0.08	0.04	0.07	

We now can identify three forms of this relatively low molecular myosin which we refer to as myosins IA, IB and IC (Table 2). All three activities are eluted at the same position when crude extracts are separated by DEAE-cellulose chromatography and all three have the same native molecular weight within the resolving capacity of gel filtration columns. But myosin IC elutes from an affinity column of ADP-agarose at a lower concentration of KCl than is required to elute myosins IA and IB. Myosins IA and IB can be separated by adsorption chromatography on Biogel agarose and myosins IA, IB and IC each are eluted at a slightly different position from a column of hydroxylapatite by a gradient of phosphate. By a combination of these chromatographic procedures it is possible to purify myosins IA, IB and IC from each other and each to a high state of purity. Myosin IA has a heavy chain of 130 000 daltons and light chains of 17 000 and 14 000 daltons; myosin IB has a heavy chain of 125 000 daltons and light chains of 27 000 and 14 000 daltons;

myosin IC has a heavy chain of 130 000 daltons and light chains of 18 000 and 17 000 daltons with a trace of a 14 000-dalton light chain (Table 2). In each case there is close stoichiometry, as judged from staining on electrophoretic gels, between the heavy chains and light chains except that the 14 000-dalton light chain varies in amount from preparation to preparation and is always present in less than equimolar ratio. Enzymatically, the three myosins are very similar each having higher K^+, EDTA–ATPase activity than Ca^{2+}-ATPase activity and very low Mg^{2+}-ATPase activity (Table 2).

At this time, we assume that the three myosins I are related to each other and presumably originate by proteolytic cleavage of a common parent molecule. We do not know the nature of the putative parent molecule but, from the data given above, it would have to consist minimally of one heavy chain of 130 000 daltons and light chains of 27 000 and 17 000 daltons, if the 27 000, 18 000, 17 000, and 14 000 molecular weight polypeptides are all either true light chains or degradation products of light chains.

The situation has become further complicated by discovery of an *Acanthamoeba* myosin II (Maruta and Korn 1977 a, b). This molecule is more typical of other myosins being a two-headed enzyme of molecular weight about 400 000 consisting of two heavy chains of about 170 000 daltons and two pairs of light chains of about 17 500 and 17 000 daltons each (Table 2). This is the myosin that is responsible for the syneresis ("contraction") of gelled *Acanthamoeba* extracts and of reconstituted systems consisting of F-actin and one of the four *Acanthamoeba* gelation factors (gelactins I–IV) described above (Maruta and Korn 1977 a). *Acanthamoeba* myosin II differs from all the myosins I in its enzymatic properties (Table 2). It has a higher Ca^{2+}-ATPase activity than K^+, EDTA–ATPase activity. Before discussing whether myosin II and the myosins I are related, it is necessary to discuss the actin-activation of their Mg^{2+}-ATPase activities.

Actin-activation of Mg^{2+}-ATPase Activities of *Acanthamoeba* Myosins I and II

We must assume that in their major roles in cell motility actin and myosin function by exerting force through their interaction in the form of actomyosin and that the chemical energy necessary to generate this force derives from the hydrolysis of ATP (Korn 1978, Eisenberg and Hill 1978). The corresponding biochemical observation *in vitro* is that under appropriate conditions, the ATPase activities of myosins from non-muscle cells should be activated by interaction with F-actin.

Published observations for different non-muscle myosins vary in this regard (Korn 1978).

The Mg^{2+}-ATPase activity of *Acanthamoeba* myosin I, as we reported several years ago (Pollard and Korn 1973 a, b), is activated by F-actin but only in the presence of a cofactor protein that was partially purified and was free of both *Acanthamoeba* actin and myosins. Functionally similar "cofactor" activity was later described for actin-activation of pulmonary macrophage myosin (Stossel and Hartwig 1975). More definitive results were obtained for platelet myosin which, it was found (Daniel and Adelstein 1976), could be actin-activated only after phosphorylation of its 20 000-dalton light chain by a specific protein kinase also present in platelets. This has now become a general observation for vertebrate myosins from non-muscle and smooth muscle cells (Korn 1978, Adelstein 1978). In many cases, actin-activation occurs only for myosins that have been phosphorylated (1 mol phosphate per mol light chain) in the 20 000-dalton light chain. The presence of specific phosphatases that catalyze the hydrolytic removal of the phosphate then allows for a regulatory mechanism that, in smooth muscle and probably also in non-muscle cells, is Ca^{2+}-dependent (Adelstein 1978).

Upon reinvestigation of the *Acanthamoeba* myosin cofactor protein, we found (Maruta and Korn 1977 c) that it also was a kinase that phosphorylates *Acanthamoeba* myosin I but, in contrast to the situation with vertebrate myosins, the *Acanthamoeba* kinase catalyzes specifically the phosphorylation of the heavy chain of *Acanthamoeba* myosin I. *Acanthamoeba* myosin I heavy chain kinase is equally active with myosins IA, IB and IC but does not catalyze detectable phosphorylation of *Acanthamoeba* myosin II. The phosphorylated myosins IA, IB and IC are highly actin-activated (Table 2) with specific activities much higher than those of actin-activated smooth muscle myosin or vertebrate non-muscle myosins (Korn 1978, Daniel and Adelstein 1976). In no case, do we yet understand why the Mg^{2+}-ATPase activity of actophosphomyosin is so much greater than the activity of actomyosin but the phosphorylation-dephosphorylation reactions obviously provide a major regulatory mechanism for the force-generating systems and, in some instances, a place for Ca^{2+} to exert a regulatory effect.

Possible Relationship between *Acanthamoeba* Myosins I and II

As mentioned above, I assume that myosins IA, IB and IC are products of the same gene and are modified post-translationally, possibly for functional reasons within the cell or possibly non-functionally either

intracellularly or as an artifact of their preparation. These several alternatives are actively under study in our laboratory. The related, and in many ways more interesting, questions are whether the myosins I and myosin II are different gene products and, if so, whether myosin I is a single-headed enzyme in the cell. If the latter is true, it is the first example of a single-headed functional myosin. Neither of these questions can be given an unequivocal answer now.

We (Maruta and Korn 1977 a, b), and independently Pollard et al. (1978), have been unable to find any evidence for the conversion of myosin II to myosin I in crude extracts of *Acanthamoeba*. If such a conversion does occur, it must be very rapid and consistently cease when a fixed amount of myosin I is formed because the absolute amounts of myosin I and II recovered in extracts has been very constant for several years. Moreover, the molecular weights of the subunits of myosin II and myosins IA, IB and IC make it highly unlikely that the former can be the source of the latter. Although the heavy chain of myosin II is large enough to be the precursor of the heavy chains of all of the myosin I, one of the light chains of myosins IB and IC is larger than either light chain of myosin II. If these putative light chains of myosins IB and IC (27 000 and 18 000 daltons, respectively) are true light chains, and not degradation products of heavy chains or contaminants, then the myosins I cannot be derived directly from myosin II.

This leaves as the only possibility for a common origin for myosins I and II that all these molecules might be derived from a common precursor, perhaps the true *Acanthamoeba* myosin, that we have yet to isolate. If it were to exist, this precursor myosin would have to be a two-headed molecule with heavy chains of 170 000 daltons or greater and two pairs of light chains of minimal size 27 000 and 17 000 daltons. One must further postulate that this putative precursor molecule has a phosphorylatable site that is a substrate for the *Acanthamoeba* heavy chain kinase and that this site is lost upon degradation of the precursor to myosin II but retained when it is converted to the smaller, single-headed myosins IA, IB and IC. Since myosin II has a longer rod portion than the myosins I, and, therefore, can form bipolar filaments (Pollard et al. 1978), the phosphorylation site would have to be in a peptide at the N-terminal end of the molecule. The myosins I then might have the true N-terminal region of the original precursor molecule which would be partially removed in conversion to myosin II. Myosins I, on the other hand, would have lost more of the C-terminal region of the original molecule than would myosin II. Both myosins I and myosin II would have retained the regions of the heavy chain involved in actin-binding, ATP-binding and the catalytic ATPase site. Of course, the ionic specificity

of the catalytic site would have to be significantly modified in its conversion to myosin I or II, or both, because the isolated myosins differ greatly in the absolute and relative activities of their Ca^{2+}- and as K^{+}, EDTA–ATPases. The necessary postulation that the putative myosin precursor must have a heavy chain greater in size than either myosin I or myosin II heavy chain is quite compatible with the size of the heavy chains of other known myosins.

We are actively studying the peptides produced by controlled chemical and enzymatic cleavages of myosins I and II to determine if they are sufficiently alike to allow for the possibility of a common origin. At the time of this writing, we have insufficient data to reach a definitive conclusion. What can be said is that we have not yet been able to convert myosin II into an active enzyme with properties resembling those of myosin I. We can degrade myosin II to a single-headed enzyme as small, and smaller, than any of the myosins I but the myosin II-product still retains the catalytic properties characteristic of myosin II and is not a substrate for the heavy chain kinase. The simplest interpretation of all of our data is that myosin I and myosin II are products of different genes.

ACKNOWLEDGEMENT

The early work in this laboratory on the purification of *Acanthamoeba* actin was carried out with Robert Weihing; more recent procedures for actin purification and our polymerization studies of non-muscle actins were developed with David Gordon; the binding of tropomyosin to copolymers is work with Yey-Zu Yang and Evan Eisenberg; *Acanthamoeba* myosin I and cofactor protein were discovered with Tom Pollard; discovery of *Acanthamoeba* myosin II, the four gelation factors, the multiple forms of myosins I and that cofactor is an *Acanthamoeba* myosin I heavy chain are the result of work with Hiroshi Maruta; our present studies on the *Acanthamoeba* myosins and myosin heavy chain kinase are collaborative efforts with Hiroshi Maruta, Jimmy Collins, and Hana Gadasi. The continued advice of Evan Eisenberg has been invaluable.

REFERENCES

Adelstein R. S. 1978: Myosin phosphorylation, cell motility and smooth muscle contraction. Trends Biochem. Sci., 3 27–30.

Carlsson L., Nyström L.-E., Lindberg U., Kannan K. K., Cid-Dresdner H., Lövgren S. and Jörnvall H. 1976: Crystallization of non-muscle actin. J. Mol. Biol., 105, 353–366.

Carlsson L., Nyström L.-E., Sundkvist I., Markey F. and Lindberg U. 1977: Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. J. Mol. Biol., 115, 465–483.

Clarke M. and Spudich J. A. 1977: Nonmuscle contractile proteins: The role of actin and myosin in cell motility and shape determination. Ann. Rev. Biochem., 46, 797–820.

Daniel J. C. and Adelstein R. S. 1976: Isolation and properties of platelet myosin light chain kinase. Biochemistry, 15, 2370–2377.
Eisenberg E. and Hill T. L. 1978: A cross-bridge model of muscle contraction. Prog. Biophys. Molec. Biol., 33, 55–82.
Elzinga M. and Lu R. C. 1976: Comparative amino acid sequence studies on actins. In: Contractile Systems in Non-Muscle Tissues, (eds. Perry S. V., Margreth A. and Adelstein R. S.) North-Holland, Amsterdam. pp. 29–37.
Gordon D. J., Boyer J. L. and Korn E. D. 1977: Comparative biochemistry of non-muscle actins. J. Biol. Chem,. 252, 8300–8309.
Gordon D. J., Eisenberg E. and Korn E. D. 1976 a: Characterization of cytoplasmic actin isolated from *Acanthamoeba castellanii* by a new method. J. Biol. Chem., 251, 4778–4786.
Gordon D. J., Yang Y.-Z. and Korn E. D. 1976 b: Polymerization of *Acanthamoeba* actin. Kinetics, thermodynamics, and co-polymerization with muscle actin. J. Biol. Chem., 251, 7474–7479.
Hartwig J. H. and Stossel T. P. 1975: Isolation and properties of actin, myosin, and a new actin-binding protein in rabbit alveolar marcrophages. J. Biol. Chem., 250, 5595–5905.
Korn E. D. 1978: Biochemistry of actomyosin-dependent cell motility (a review). Proc. Natl. Acad. Sci. USA, 75, 588–599.
Lazarides E. and Lindberg U. 1974: Actin is the naturally occurring inhibitor of deoxyribonuclease I. Proc Natl. Acad. Sci. USA, 71, 4742–4746.
Maruta H. and Korn E. D. 1977 a: Purification from *Acanthamoeba castellanii* of proteins that induce gelation and syneresis of F-actin. J. Biol. Chem., 252, 399–402.
Maruta H. and Korn E. D. 1977 b: *Acanthamoeba* myosin II. J. Biol. Chem., 252, 6501–6509.
Maruta H. and Korn E. D. 1977 c: *Acanthamoeba* cofactor protein is a heavy chain kinase required for actin activation of the Mg^{2+}-ATPase activity of *Acanthamoeba* myosin I. J. Biol. Chem., 252, 8329–8332.
Pollard T. D. and Korn E. D. 1973 a: *Acanthamoeba* myosin. Isolation from *Acanthamoeba castellanii* of an enzyme similar to muscle myosin. J. Biol. Chem., 248, 4682–4690.
Pollard T. D. and Korn E. D. 1973 b: *Acanthamoeba* myosin. Interaction with actin and with a new cofactor protein required for actin activation of Mg^{2+} adenosine triphosphatase activity J. Biol. Chem., 248, 4691–4697.
Pollard T. D., Shelton E., Weihing R. R. and Korn E. D. 1970: Ultrastructural characterization of F-actin isolated from *Acanthamoeba castellanii* and identification of cytoplasmic filaments as F-actin by reaction with rabbit heavy meromyosin. J. Mol. Biol., 50, 91–97.
Pollard T. D., Stafford W. F. and Porter M. E. 1978: Characterization of a second myosin from *Acanthamoeba castellanii*. J. Biol. Chem., 253, (in press).
Pollard T. D. and Weihing R. R. 1974: Actin and myosin and cell movement. CRC Crit. Rev. Biochem., 2, 1–65.
Poo W.-J. and Hartshorne D. J. 1976: Actin crosslinked with glutaraldehyde: Evidence to suggest an active role for actin in the regulatory mechanism. Biochem. Biophys. Res. Commun., 70, 406–412.
Shizuta Y., Shizuta H., Gallo M., Davies P., Pastan I. and Lewis M. S. 1976: Purification and properties of filamin, an actin binding protein from chicken gizzard. J. Biol. Chem., 251, 6562–6567.
Stossel T. P. and Hartwig J. H. 1975. Interactions between actin, myosin, and an actin-binding protein from rabbit alveolar macrophages. Alveolar macrophage myosin Mg^{2+}-adenosine triphosphatase requires a cofactor for activation by actin. J. Biol. Chem., 250, 5706–5712.
Wakabayashi T., Huxley H. E., Amos L. A. and Klug A. 1975: Three-dimensional image reconstruction of actin-tropomyosin complex and actin-tropomyosin- troponin T-troponin I complex. J. Mol. Biol., 93, 477–497.
Wang K. 1977· Filamin, a new high-molecular-weight protein found in smooth muscle and nonmuscle cells. Purification and properties of chicken gizzard filamin. Biochemistry, 16, 1857–1865.

Wang K., Ash J. F. and Singer S. J. 1975: Filamin, a new high-molecular-weight protein found in smooth muscle and non-muscle cells. Proc. Natl. Acad Sci. USA, 72, 4483-4486.
Weihing R. R. and Korn E. D. 1971: *Acanthamoeba* actin. Isolation and properties. Biochemistry, 10, 590–600.
Weihing R. R. and Korn E. D. 1972: *Acanthamoeba* actin. Composition of the peptide that contains 3-methylhistidine and a peptide that contains N^6-methyllsine. Biochemistry, 11, 1538–1543.
Yang Y.-Z., Gordon D. J., Korn E. D. and Eisenberg E. 1977: Interaction between *Acanthamoeba* actin and rabbit skeletal muscle tropomyosin. J. Biol. Chem., 252, 3374–3378.

Addendum — June 1978

Data recently obtained have definitely established that myosin II is a different gene product than either myosin IA, IB or IC. Myosin IA and IC are products of the same gene but myosin IB may by the product of yet a different gene. Thus, *Acanthamoeba* expresses at least two, and possibly three, myosin genes. These conclusions were reached by comparing the peptides produced from the four myosins by cleavage with proteases, cyanogen bromide and by cyanylation reactions. Recent evidence has also shown that the heavy chain of myosin IB (and presumably also the heavy chains of myosins IA and IC) contains the ATPase site, the actinobinding site and the phosphorylation site. The light chains of myosin IB can be removed by treating the enzyme with 2M LiCl and the isolated heavy chain is enzymatically identical to the intact molecule under all the usual assay conditions. This is the first proof that all of the activities of any myosin can reside solely in its heavy chain. Furthermore, the 20 000-dalton light chain can be removed from myosin IC by 2M LiCl leaving a molecule that is now identical enzymatically and physically to myosin IA. The 20 000-dalton peptide is a regulator protein that, when added back to myosin IA or to IC from which it has been removed, inhibits strongly their Ca^{2+}- and Mg^{2+}-ATPase activities and less extensively their (K^+, EDTA)- and actin-activated Mg^{2+}-ATPase activities. The enzymatic differences between myosins IA and IC, as isolated, are, therefore, due to the presence of the 20 000-dalton peptide in IC. This is the first example of a peptide that regulates all of the ATPase activities of a myosin.

ACTA PROTOZOOLOGICA
VOL. 18 (No. 1) WARSZAWA, 31.III.1979 pp. 91–107

Department of Cell Biology and Department of Biochemistry of Nervous System and Muscle, M. Nencki Institute of Experimental Biology, Warszawa 02-093, Pasteura 3, Poland

Leszek KUŹNICKI and Jacek KUŹNICKI

Speculations on Evolution of Mechanisms Regulating Actin and Myosin Interaction *

Synopsis. The purpose of this paper is to collect data concerning evolution of actin, myosin and Ca^{2+}-binding proteins modulating their interaction and to give some ideas what types of regulation might be expected in other systems than muscle, including *Protozoa* and *Myxomycete*.

One of the feature highly characteristic for living organisms is movement, which has its evolutionary background and aspects as every topic in biology. Among various molecular mechanisms involving diverse type of cell movements the largest evolutionary potentiality to open out interaction of actin and myosin molecules. Movements of parts or whole of animals above the level of sponges (*Parazoa*) are generally the result of muscular motion. In majority *Metazoa* exists distinction between muscles that produce the sorts of motion and those involved only in internal processes.

Several biochemical, physiological and ultrastructural studies on some species of *Protozoa and Myxomycete* have provided convincing evidence that actomyosin complex is responsible for generating the force in many different motile phenomena, especially for amoeboid movement of soil and giant amoebas and shuttle streaming of *Physarum*. Actin and myosin associated with a regulatory proteins are probable present in all eukaryotic cells (Pollard and Weihing 1974, Clarke and Spudich 1977) and the cytoplasm of all cells is capable of contraction to some extent (Fig. 1). In muscle cells this capacity is fully developed. The common presence of actin and myosin suggest that they must have arisen with all key functional properties and satisfactory mechanism of

* Paper presented at Symposium on Cell Motility, Warszawa, June 26–28, 1978.

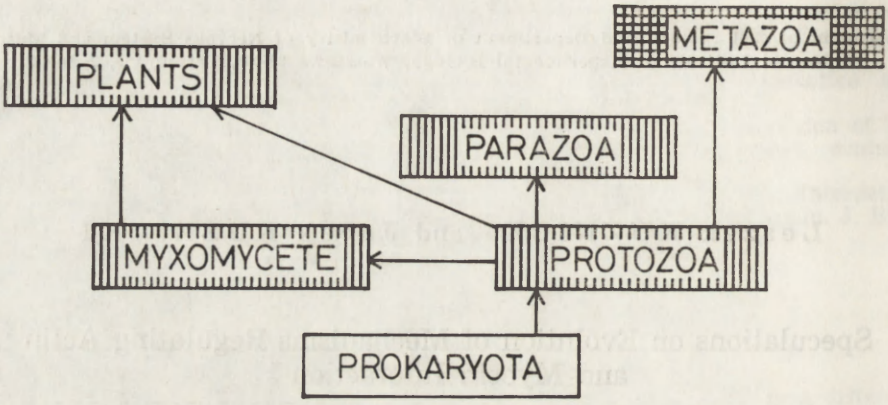


Fig. 1. Distribution of actomyosin complexes and muscle tissues in the living world. Arrows indicate probable phylogenetic relationships. Vertical lines — presence of actomyosin complex. Horizontal lines — presence of muscle tissues

contraction in the earliest eukaryotic cells and then have developed in some directions.

Therefore, we believe that a major evolutionary alternation in the molecular mechanism of contraction has occurred first of all in the various proteins which interact with both molecules in process of force generation.

The majority of such variation must have originated hand by hand with evolution and differentiation of muscle tissue and on the cellular level of biological organization as a specialization to different types of amoeboid locomotion, body cell contraction, rotationaly cytoplasmic streaming within cell, etc.

During all types of amoeboid movement of amoebe and slime molds there are involved at least three separate events (H i t c h c o c k 1977):

(1) polymerization of actin to form filaments
(2) gelation of actin filaments,
(3) contraction of the gel reticulum which requires actin and myosin interaction.

For each step of a contraction–relaxation cycle separate regulatory mechanism exist. In this article we will not discuss polymerization of actin and gelation of actin filaments, since they have no equivalent in muscle cells. We will concentrate only on these processes in which during interaction between actin and myosin transformation of chemical energy derivating from the hydrolysis of ATP into mechanical work occurs.

In the last decade the regulation of actin and myosin interaction in muscle cells and primitive motile systems remains one of the most active research areas. Recently several important experimental data of

distribution of actin- and myosin-linked regulation in the *Metazoa* muscles have appeared (Lehman and Szent-Györgyi 1975, Lehman 1976), as well as very thoughtful reviews on actin, myosin and regulation of motility in nonmuscle cells (Pollard and Weihing 1974, Hitchcock 1977 and Korn 1978). These papers offer some reasonable ideas, but the evolutionary relationship between known and postulated actin-myosin types of regulation still remains an obscure question.

The main cause of our speculations on evolution of actin-myosin interaction is to collect data concerning these problems and to give some ideas what types of regulation might be expected in other systems than muscle including *Protozoa* and *Myxomycete*. We recognize that these speculations are meaningless unless they arise from specific data and can be applied to particular problems. So we have tried to underpin the premises with supporting facts to show how they do arise from these facts.

Even so, our thesis can be very easy criticized, because until recently, actin and myosin of many group of organisms have never been study and the data available on the factors controlling interaction between actin and myosin in nonmuscle cells are still very fragmentary.

Considering the difficulties which have been encountered in these speculations we like offer some new approach to the problem how interaction between actin and myosin may have evolved in unicellular and multicellular organisms.

Generalization about some Major Tendencies in Evolution of Actin and Myosin Molecules

In recent years distinct advances in our knowledge of comparative biochemistry of actin and myosin from muscle and nonmuscle cells have been achieved.

All the actins which have been examined show extensive homology in chemical and physical properties (Pollard and Weihing 1974, Elzinga et al. 1976). We can reach some evolution conclusion especially from recent comparative studies on actins of *A. castellanii* and mammals muscle and nonmuscle cells. The amino acid composition of *Acanthamoeba* actin is remarkable similar to rabbit skeletal muscle actin, what more both actins differs in only ±6% residues (Elzinga and Lu 1976, Weihing and Korn 1971, 1972, Gordon et al. 1976 a).

One of the key function of cytoplasmic actin is the ability to reversible polymerization from the monomeric G-actin to the F-actin filaments,

because probably only in filamentous form actin can interact with myosin. *In vitro* all the physical properties of *Acanthamoeba* actin are qualitatively similar to rabbit muscle actin. These include molecular weight of G-actin (Weihing and Korn 1971, 1972) polymerize properties, length of F-actin filaments and content of bound nucleotide per mol G-actin monomer (Gordon et al. 1976 a, b, Gordon et al. 1977). Besides their homology actins show the quantitative differences, which give possibilities for speculations on general trends of their specialization during evolution of motile system on the molecular level. Alkaline isoelectrofocusing data have shown that at least four isoactins can be distinguished: α which exists in rabbit skeletal muscles, β and γ present in embrionic chicken brain, human platelets and rat liver and δ found in *Acanthamoeba castellani*. In respect to isoelectric points of these isoactins the following sequence can be drawn:

$$\delta > \gamma > \beta \gg \alpha \qquad (1)$$

in which form α represents most acidic protein.

The ability of all actins to activate Mg^{2+}-ATPase activity of rabbit skeletal heavy meromyosin characterized by the V_{max} activity are identical. However, the muscle HMM activation characterized by K_{app} constant gave the sequence:

Skeletal muscle actin	<	Vertebrate nonmuscle actin	⋘	*Acanthamoeba* actin	(2)
7.1		9.6		21.7	

It means that 1.3 times more of the vertebrate nonmuscle actins and 3 times more of *Acanthamoeba* actin than rabbit actin is required to reach half-maximal activation of the Mg^{2+}-ATPase of rabbit skeletal muscle heavy meromyosin.

Both above sequences we can interpret that way: the first one although is not real ancestor — descent sequence, gave general idea about tendency in the direction changes of actin during evolution. The values in the second sequence illustrate a degree in functional divergence between protozoan actins — mammals nonmuscle actins and muscle skeletal actins.

All muscle and cytoplasmic myosins except myosin I from *Acanthamoeba castellani* consist of two heavy chains and two pairs of light chains. However, in contrast to small variability of actins, which means extensive evolutionary conservatism, the diversity in chemical and physical properties of myosin is greater. According to Pollard

and Weihing (1974) term "myosin" concerns a type of enzymes with actin activated ATPase activity and which bind reversible to F-actin filaments. The authors distinguished five classes of myosin: skeletal muscle, smooth muscle, vertebrates nonmuscle, *Physarum* and *Acanthamoeba*.

Undoubtedly, myosin — show species and tissues variation but at present time only two distinctly different myosins have been categorized: two-headed and single-headed molecules.

The single-headed myosin consisting of one heavy chain and two different light chains was discovered in *A. castellanii* by Pollard and Korn (1973 a, b). Four years later Maruta and Korn (1977) in the same species found "myosin II", typical two-headed enzyme with two pairs of light chains.

In contrast to the single-headed myosin which has very peculiar properties, *Acanthamoeba* myosin II has generally more typical properties to other nonmuscle and muscle myosins. We assume that the single-headed myosin is either an artifact or a molecule existing *in vivo* but without significant function in *Acanthamoeba* motility. In all protozoan and myxomycetes species myosins responsible for contraction phenomena are probably two-headed molecules consisting of a pair of heavy chains and two pairs of light chains. The same general structure must have a myosin in all eukaryotic cells.

We can conclude that because:

(1) Two-headed myosin as Mg^{2+}-ATPase provides the energy for the motility in similar circumstance in all cells from *Protozoa* to mammals.

(2) For high ATPase activity myosin has to interact with actin which is a very conservative protein and actin — myosin interaction requires contact in the strict regions of myosin heads. It is well known that the chemical bond constraints have prevented major changes of any molecular complex which was used for only one function throughout evolution.

(3) Only two-headed myosins are able to form bipolar filaments.

We assume that two-headed myosin is an example of a molecule of the rigid specification appearing on the early stages of eukariota evolution and can not originate from a single-headed one. What more, in contrast to actin which also separately execute various functions within cells all significant evolutionary innovations of two-headed myosin have occurred if are necessary for functional adaptation of actomyosin complex as an effective unit of motility. Although the comparative studies on myosins are less extensive than the research on actins, the striking features have already emerged from these studies.

We could assume that some regions of myosin are conservative i.e., the rod and the head with actin binding site but other regions of the head are rather mutable (Fig. 2).

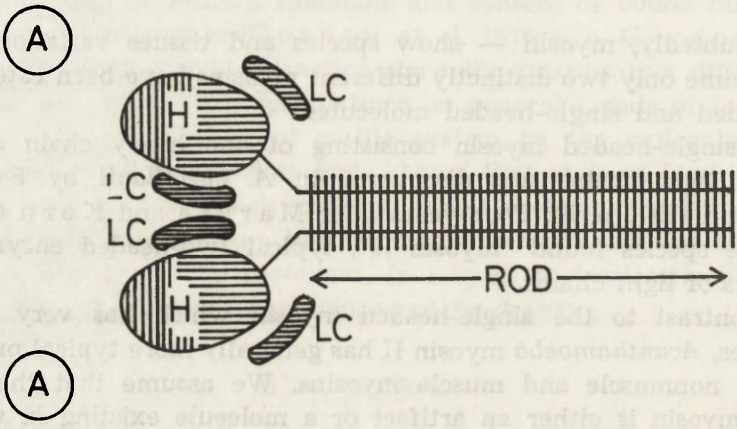


Fig. 2. Schematic structure of myosin with possible conservative (vertical lines) and mutable (horizontal lines) regions A — actin, H — head of myosin, LC — light chains

Tropomyosin is associated with actin filaments also in muscle with myosin–linked regulation. According to Fine et al. (1973) two classes of tropomyosin can be distinguished: muscular and non-musclar. The molecular weight as well as the length of the non-muscle tropomyosin from brain, platelets and fibroblasts is smaller than those of skeletal muscle tropomyosin. Except that, tropomyosin seems to be also rather conservative protein as it was shown by peptide map of brain and muscle tropomyosin (Fine et al. 1973). McLachlan and Stewart (1976) suggested that non-muscle tropomyosin has six and muscle tropomyosin seven 42-amino acid residue segments which represent different steps of gene duplication.

Hayashi and Hirabayashi (1978) showed recently that tropomyosin from muscle of chickens, rabbits, frogs, shrimps and shellfish mediate in the absence of calcium the inhibitory activity of rabbit troponin toward rabbit actomyosin ATPase activity. Some variations of these properties reflect the evolutionary course of tropomyosin but ability to bind to rabbit skeletal troponin and actin suggest that in general these properties have been conserved during evolution.

The conservatism of tropomyosin and its presence in non-muscle systems does not implicate its involvement in regulation of actin–myosin interaction via troponin–tropomyosin system typical for skeletal muscle. It can be suggested that first biological function of tropomyosin was

stabilization of actin filaments and its role in regulation was a function developed during evolution.

It appears that hand in hand with evolution and differentiation of *Metazoa* muscle significant evolutionary innovations of actomyosin complex was related with a structural stabilization of actin and myosin filaments and improvement regulation of their interaction during contraction-relaxation cycle.

Informational Role of Ca^{2+}ions and Possible Evolutionary Relationship between Calcium Modulated Proteins Associated with Myosin ATPase

First and most exciting question arises why calcium was choosen to play the informational role in molecular processes of motility? And second, how calcium ion is involved in these processes — directly with actomyosin or indirectly via other calcium sensitive system? The answer for these questions was proposed by K r e t s i n g e r (1977).

Within the cell which uses ATP as a main source of energy, the concentration of phosphate is about 10^{-2} M. Since magnesium phosphate is soluble but calcium phosphate not cells developed a system which pumped out or stored, anyway which decreased, concentration of free calcium to at least 10^{-5} M. Actomyosin ATPase activity is observed only in the presence of high (10^{-3} M) concentration of magnesium. Also other enzymes which use ATP or other phosphorylated substrates require magnesium for their activity, although for regulation of their activity a change of concentration of calcium in range 10^{-5}–10^{-7} M is used.

It became clear that only proteins could build a structure which let them bind calcium specifically and with high affinity in the presence of physiological level of magnesium. If calcium has to be a messenger which under certain condition switch on the motile system there must be a Ca^{2+} — sensitive target: the actomyosin itself or Ca^{2+} — modulated factor which sensitizes this enzyme for calcium.

It is well established that concentration of calcium within the resting muscle cell is maintain in low although definited level, near 10^{-7}–10^{-8} M. After an appropriated stimulus concentration of calcium increases to 10^{-4}–10^{-5} M. At this concentration proteins which Ca^{2+}-binding constant lies between 10^{4}–10^{7} M^{-1} interacts with Ca^{2+}, so they are modulated by this cation. Another word these proteins have two states: the relaxing one when level of calcium is lower than 10^{-7} M and activated state when concentration of free calcium achieves about 10^{-4}–10^{-5} M, in the latter, i.e., after binding of calcium these proteins undergo the significant changes of their conformation.

In respect to one of fundamental features of life — motion we can

conclude that at least two-independent systems are involved in calcium regulation. The first which in rest decreases concentration of calcium and increases in active state. And the second, which could be a younger evolutionary or evolved at the same time, consists of proteins modulated by calcium connected with actomyosin complex. To the first system which regulate level of calcium in *Eukaryota* belong membrane ATPase including sarcoplasmic reticulum ATPase and probably also ATPase pump of calcium in *Prokaryota*. To the second belong mainly homologous Ca^{2+}-modulated proteins which as it was suggested by Kretsinger (1975, 1976) contain a characteristic EF-hand structure (Fig. 3). This point we would like to consider a bit more since these Ca^{2+}-binding proteins were found and characterized in various tissues and organisms.

To the group of homologous Ca^{2+}-modulated proteins with EF-hand structure belong: light chains of myosin, parvalbumins, troponin C and modulator protein of 3' : 5'-cyclic nucleotide phosphodiesterase. The EF-hand conformation consists of α-helical region, loop containing Ca^{2+} coordinated ligands and again region of α-helix. It is proposed that this structure can be shown by the forefinger, middle finger and thumb of a hand (Kretsinger 1975). Parvalbumins contain three and other proteins from these group four EF-hand structures (Fig. 3), however, their capacity varies from 0 to 4 ions of bound calcium per molecule and the affinity constant varies between 10^4 to 10^7 M^{-1}.

Evolution of all these homologous calcium binding proteins may be considered in two ways. The first as an evolution of a whole group from a common ancestor and as a subsequent divergation. The most important point in these events is when the divergences appeared and which proteins are closely related and which further. The second is a subsequent evolution within a subgroup of troponins C, parvalbumins and light chains of myosin, where consideration is concerned with their calcium binding capacity (which could be developed or lost).

We will discuss both aspects of evolution of these calciproteins in order to answer the question: which of them could be historically first having its function in motility and this can precise what was the calcium binding properties of this protein?

Historical order of divergence of EF-hand calcium binding proteins is mainly estimated by Pechère (1977). The common ancestor was probably a peptide with single EF-hand structure (Maximov et al. 1978). After duplication of its gene a peptide with double EF-hand structure appeared. It already could strongly bind calcium, since many results done on peptides of parvalbumins or troponin C show that a single EF-hand structure has bigger Ca^{2+}-binding properties in a double than in a single form. Next, subsequent duplication gave a gene of

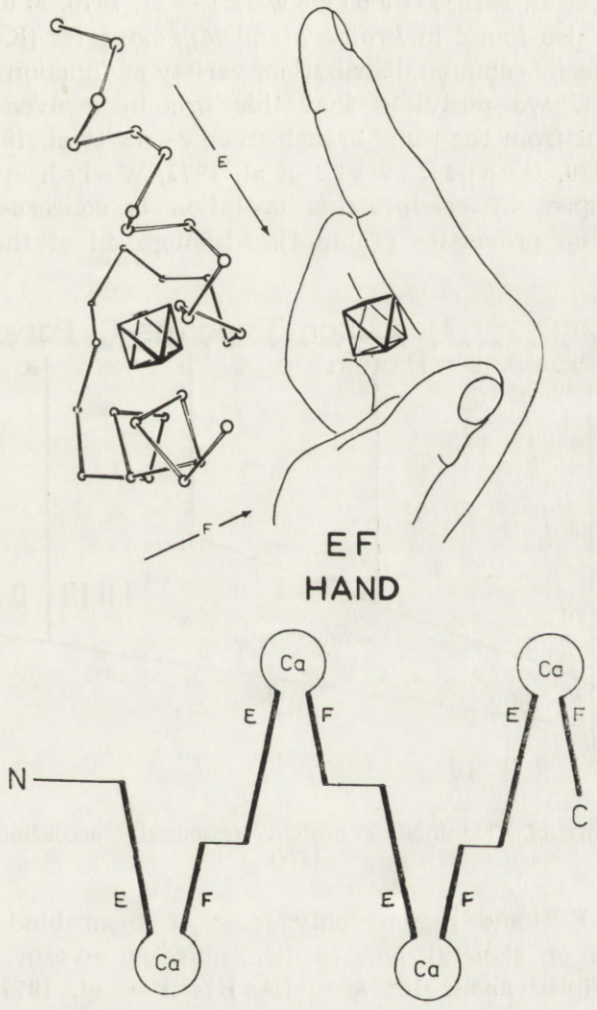


Fig. 3. EF-hand structure according to Kretsinger (1977) and schematic presentation of protein with four calcium binding sites

a protein with four EF-hand structures. This was a real ancestor of present found calciproteins. Figure 4 shows the phylogeny of these proteins. From Fig. 4 it is concluded that parvalbumins are closer related to troponins C and alkali light chains than to EDTA and DTNB light chains, because the first divergence gave an EDTA and DTNB LC, the next the branches of parvalbumins evolved, finally there was a divergence which gave alkali light chains and troponins C.

It is difficult to estimate the real evolutionary relationship between all mentioned calciproteins and modulator protein. The latter seems to be an ubiquitous one being present in all animal phylla and tissues

(Waisman et al. 1975, Drabikowski et al. 1978, Muszbek et al. 1977). It was also found in *Protozoa* and *Myxomycetes* (Kuźnicki et al. 1977). From its common distribution, variety of function and similarity to troponin C we postulate that this protein evolved earlier than troponin C but from the same branch (Stevens et al. 1976, Watterson et al. 1976, Drabikowski et al. 1977, Walsh et al. 1977).

Second aspect of calciproteins evolution is concerned with their calcium binding properties (Table 1). Although all of the light chains

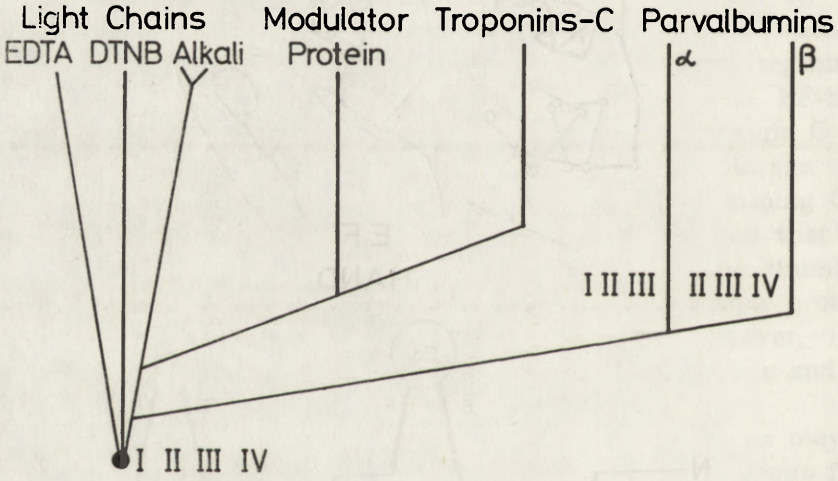


Fig. 4. Phylogeny of Ca^{2+}-binding proteins (essentially according to Pechère 1977)

contain four EF-hand regions only some of them bind calcium, i.e., P-light chain of skeletal and cardiac muscles myosin (two calcium ions with affinity about 10^5 M^{-1}) (Fabian et al. 1977), and EDTA light chain of scallop (about two ions of calcium) (Szent-Györgyi et al. 1973). Contrary to P-light chain from skeletal muscle the latter protein losts ability to bind calcium after removal from its myosin. Modulator protein from brain as well as troponin C from skeletal muscles binds four calcium ions with affinity constant about 10^7 M^{-1} one pair of sites and 10^5 M^{-1} for another pair of sites (Klee 1977, Potter and Gergely 1975, respectively).

Troponin C isolated from other sources than mammalian skeletal muscle binds different amount of calcium: from lobster tail-one ion, from crayfish tail two, as well as dogfish and varan, and hake's and python's — three. However, even in mammalian tissues as cardiac muscle troponin C binds only three calcium ions (Eerd and Takahas-

hi 1975, Burtnick and Kay 1977). Parvalbumins bind two calcium with affinity about 10^7 M^{-1} (Pechère 1977). It can be concluded that a basic group of calciproteins is a light chains group (Fig. 4). The first

Table 1

Calcium Binding Proteins

Ca^{2+}-Binding Protein	Amount of EF-hand Structures	Moles of Bound Calcium	Affinity Calcium Binding Constant pCa	References
LCH–EDTA (Scallop)	~4	1–2	~6	Szent-Gyorgyi, Szent-kiralyi and Kendrick-Jones 1973
LCH–DTNB (Skeletal muscle)	4	2	5	Fabian, Mason and Wikman-Coffelt 1977
P–LCH				
LCH–Alkali –„–	4	0	–	
Troponin C –„–	4	4	~5–7	Potter and Gergely 1975
Troponin C (Cardiac)	4	3	~6	Burtnick and Kay 1977
Troponin C(Lobster Tail)	4	1		
(Dogfish)	4	2		Pechere 1977
(Hake)	4	3		
Modulator Protein	4	4	~5–7	Klee 1977
Parvalbumins	3	2	~7–8	Pechere 1977

light chains had low calcium capacity and bound only one or two calcium per molecule. This calcium binding properties could be a properties of light chain itself as a DTNB light chain or could be connected with whole complex of myosin (as EDTA light chain of scallop). Further evolution of light chains caused that they lost their calcium binding capacity, however, in the same time specific calcium binding proteins appeared. Since myosin appeared early in evolution we can expect that its light chains have properties resembling those of DTNB or EDTA light chains. Further evolution based on the same structure gave more particular proteins which function was more (troponin C) or less (modulator protein) precised.

The Types of Actin-Myosin Regulation that have been Found or Might be Expected

Lehman and Szent-Györgyi (1975) and Lehman (1976) in their comparative studies on muscles of about 100 Metazoa species have shown that actin-linked and myosin-linked regulation are widespread among animals. Furthermore, both controlling systems operate simulta-

neuosly in many invertebrates including phyla which appeared early in evolution.

The results let authors suggest that both regulatory systems have evolved also simultaneously and independently. The genome of most, possible of all living animals contains information for myosin-linked, as well as actin-linked regulation, although the first one is not expressed in species which belong to molluscs, brachiopods, echinoderms, and enchiuroids, and a second one in vertebrates and some invertebrates.

Recent knowledge let us say that at least three different types of regulation exist in muscle (Fig. 5).

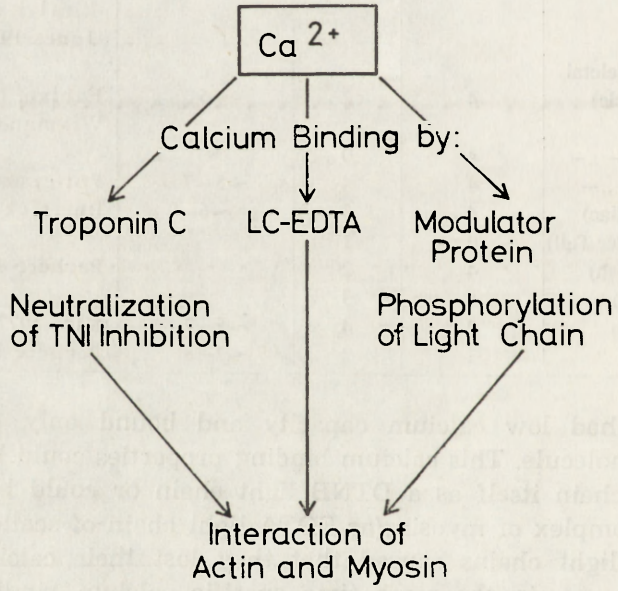


Fig. 5. Types of regulation of actomyosin ATPase in muscle

(1) The well known troponin–tropomyosin system linked with actin filaments.

(2) Myosin-linked system present in certain molluscan muscles which does not require any additional proteins.

(3) Myosin-linked system of smooth muscle in which the key reaction is reversible phosphorylation and dephosphorylation of myosin light chain.

The majority of our knowledge on the Ca^{2+}-regulatory system of actin-myosin interaction originated from studies of the rabbit skeletal muscles (E b a s h i and E n d o 1968, G e r g e l y 1976). In striated muscle of vertebrates and some muscle of invertebrates actomyosin ATPase is regulated via proteins connected with thin filaments. These are the

tropomyosin and troponin complex. The thin filament of striated muscle is a complex system in many aspects. According to Perry (unpublished) five structural genes determine functional I filament and each protein component has polymorphic forms (Table 2). Ca^{2+}-binding induces

Table 2

Structural Genes and 3 Filament Proteins of Striated Muscle (Skeletal and Cardiac)

	Polymorphic Forms	Structural Genes
Troponin I	3	3
Troponin T	3	3
Troponin C	2–3	2–3
Tropomyosin	2	2
Actin	2–3	2–3
Total	12–14	12–14
Functional I Filament	1 of Each Protein	5

According to S. V. Perry.

conformational changes of troponin C (one of the troponin subunits) which afterwards neutralizes inhibition of the Mg^{2+}-stimulated ATPase of actomyosin by the second subunit of troponin-troponin I.

In certain primitive molluscs the troponin complex is absent and Ca^{2+}-regulation of actin-myosin interaction is mediated directly through one of the light chains of myosin.

Its removal leads to the losting of Ca^{2+}-sensitivity of actomyosin and decreasing of amount of bound calcium (Szent-Györgyi et al. 1973).

In smooth muscle regulation of Mg^{2+}-stimulated actomyosin ATPase seems to be connected with Ca^{2+}–dependent phosphorylation of P-light chain (Sobieszek and Small 1977, Górecka et al. 1976, Adelstein 1978). Specific kinase consists of two subunits one of which is a Ca^{2+}-binding protein similar to troponin C called so far modulator protein (Dąbrowska et al. 1978 a, b). It has to be mention that some authors believe that phosphorylation is not required for actin-myosin interaction in smooth muscles (Mikawa et al. 1977) and other who suggest the presence of additional to phosphorylation process of regulation in these tissue (Chacko et al. 1977).

In all mentioned systems homologous Ca^{2+}–binding proteins operate: troponin C, light chains of molluscan myosin or modulator protein.

In the last three years studies on troponin C-like proteins and regulation of actin and myosin interaction rapidly progressed. Series of

experiments have shown that only skeletal and cardiac muscle possess troponin C while smooth muscle and mammalian nonmuscle cells (platelets, brain, adrenal medulla) have not troponin-C (TN-C) but only modulator protein (Drabikowski et al. 1978). At this time there is no convincing evidence that the TN-C exists in *Protozoa* and *Myxomycete*, but in *Amoeba proteus*, *Euglena gracilis* and *Physarum polycephalum* plasmodia modulator protein (MP) was found (Kuźnicki et al. 1977).

This could indicate that either MP is a counterpart of troponin C in non-muscle regulatory systems as it was suggested earlier (Perry 1976, Amphlett et al. 1976) or as recent results indicate it could be involved in myosin-linked regulation via Ca^{2+}-dependent phosphorylation (Dąbrowska and Hartshorne 1978, Baryłko et al. 1978). However, it was shown that *Physarum* actomyosin ATPase has two regulatory systems: actin-linked and myosin-linked (Kato and Tonomura 1975, Nachmias and Asch 1976). Modulator protein could be involved in both system but probably operates only in the latter. So we have to assume the presence of another actin-linked system in *Physarum* to that operating in striated muscles. This regulation could be based for instance on Ca^{2+}-sensitive reversible polymerization of actin into filaments.

Concluding Remarks

In previous parts we considered some aspects of evolution of actin, myosin and Ca^{2+}-binding proteins regulating their interaction. And now we would like to summarize all these considerations to present possible general scheme of evolutionary events. So we propose following thesis:

(1) The all types of motility in living world where actin, myosin are involved are based on active sliding of the actin filaments past to the myosin filaments (or less organizing myosin agreggates).

(2) Ca^{2+}-sensitivity of actin-myosin interaction is as old as actin and myosin themselves. So, calcium independent actomyosin ATPase found *in vitro* does not resemble the physiological situation and in this sense reflects an experimental artefacts.

(3) All proteins which sensitize actin and myosin interaction for calcium contain EF-hand structure typical for homologous Ca^{2+}-binding proteins.

(4) It is generally believed that in non-muscle cells including *Protozoa* and *Myxomycetes* all known in muscles regulatory mechanisms may exist. However, in our opinion the troponin complex similar to that found in striated muscle if present, does not operate there.

(5) The *Metazoa* have probably evolved from a line of multicellular flagellate-descendants in which the problems of rapid coordinated movement was solved through the agency of muscle cells. All myosin systems regulating actin-myosin interaction and existing in unicellular organisms were used in muscle of *Metazoa*. In striated muscles an additional process which does not operate in primitive motile systems was developed, i.e., troponin-tropomyosin system.

REFERENCES

Adelstein R. S. 1978: Myosin phosphorylation, cell motility and smooth muscle contraction. Trends Biochem. Sci., 3, 27–30.

Amphlett G. W., Vanaman T. C. and Perry S. V. 1976: Effect of troponin-C like protein from bovine brain (brain modulator protein) on the Mg^{2+}-stimulated ATPase of skeletal muscle actomyosin. FEBS Lett., 72, 163–168.

Baryłko B., Kuźnicki J. and Drabikowski W. 1978: Identification of Ca^{2+}-binding subunit of myosin light chain kinase from skeletal muscle with modulator protein. FEBS Lett. 90, 301–304.

Burtnick L. D. and Kay C. M. 1977: The calcium binding properties of bovine cardiac troponin C. FEBS Lett., 75, 105–110.

Chacko S., Conti M. A. and Adelstein R. S. 1977: Effect of phosphorylation of smooth muscle myosin on actin activation and Ca^{2+}-regulation. Proc. Natl. Acad. Sci. USA., 74, 129–133.

Clarke M. and Spudich J. A. 1977: Nonmuscle contractile proteins: The role of actin and myosin in cell motility and shape determination. Ann. Rev. Biochem., 46, 797–820.

Dąbrowska R., Sherry J. M. F., Aromatorio D. and Hartshorne D. J. 1978 a: Modulator protein as a component of the myosin light chain kinase from chicken gizzard. Biochemistry, 17, 253–257.

Dąbrowska R., Aromatorio D., Sherry J. M. F. and Hartshorne D. J. 1977: Composition of myosin light chain kinase from chicken gizzard. Biochem. Biophys. Res. Commun., 78, 1263–1272.

Dąbrowska R. and Hartshorne D. J. 1978: Ca^{2+}-dependent myosin light chain kinase from non-muscle tissues. Cold Spring Harbor, New York, May 1978.

Drabikowski W., Kuźnicki J. and Grabarek Z. 1977: Similarity in Ca^{2+}-induced changes between troponin C and protein activator of 3' : 5'-cyclic nucleotide phosphodiesterase and their tryptic fragments. Biochim. Biophys. Acta, 485, 124–133.

Drabikowski W., Kuźnicki J. and Grabarek Z. 1978: Distribution of troponin C and protein activator of 3' : 5'-cyclic nucleotide phosphodiesterase in vertebrate tissues. Comp. Biochem. Physiol., 60 C, 1–6.

Ebashi S. and Endo M. 1968: Calcium ion and muscle contraction. Prog. Biophys. Mol. Biol., 18, 123–183.

Eerd J. P. and Takahashi K. 1975: The amino acid sequence of bovine cardiac troponin C comparison with rabbit skeletal troponin C. Biochem. Biophys. Res. Commun., 64, 122–127.

Elzinga M. and Lu R. C. 1976: Comparative amino acid sequence studies on actins. In: Contractile Systems in Non-Muscle Tissues, (eds. Perry S. V., Margreth A. and Adelstein R. S.) North-Holland, Amsterdam, pp. 29–37.

Elzinga M., Maron B. J. and Adelstein R. S. 1976: Human heart and platelets actin are products of different genes. Science, 191, 94–95.

Fabian F., Mason D. T. and Wikman-Coffelt J. 1977: Calcium binding properties of cardiac and skeletal muscle myosins. FEBS Lett., 81, 381–385.

Fine R. E., Blitz A. L., Hitchcock S. E. and Kaminer B. 1973: Tropomyosin in brain and growing neurones. Nat. New Biol., 245, 182–186.

Gergely J. 1976: Troponin–Tropomyosin-dependent regulation of muscle contraction by calcium. In: Cell Motility. Cold Spring Harbor Laboratory, 137–149.
Gordon D. J., Boyer J. L. and Korn E. 1977: Comparative biochemistry of non-muscle actins. J. Biol. Chem., 252, 8300–8309.
Gordon D. J., Eisenberg E. and Korn E. D. 1976 a: Characterization of cytoplasmic actin isolated from *Acanthamoeba castallanii* by a new method. J. Biol. Chem., 251, 4778–4786.
Gordon D. J., Yang Y.-Z. and Korn E. D. 1976 b: Polymerization of *Acanthamoeba* actin. Kinetics, thermodynamics, and co-polymerization with muscle actin. J. Biol. Chem., 251, 7474–7479.
Górecka A., Aksoy M. O. and Hartshorne D. J. 1976: The effect of phosphorylation of gizzard myosin on actin-activation. Biochem. Biophys. Res. Commun., 71, 325–331.
Hayashi J. and Hirabayashi T. 1978: The functional characteristics conserved in tropomyosin. J. Biochem., 83, 341–348.
Hitchcock S. E. 1977: Regulation of motility in nonmuscle cells, J. Cell Biol., 74, 1–15.
Kato T. and Tonomura Y. 1975: Ca^{2+}-sensitivity of actomyosin ATPase purified from *Physarum polycephalum*. J. Biochem., 77, 1127–1134.
Klee C. B. 1977: Conformational transition accompanying the binding of Ca^{2+} to the protein activator of 3', 5'-cyclic adenosine monophosphate phosphodiesterase. Biochemistry, 16, 1017–1024.
Korn E. D. 1978: Biochemistry of actomyosin-dependent cell motility (a review). Proc. Natl. Acad. Sci. USA, 75, 588–599.
Kretsinger R. H. 1975: Hypothesis: Calcium Modulated Proteins Contain EF–hands. In: Calcium Transport in Contraction and Secretion (E. Carafoli, ed.) North Holland Publishing Co., pp. 469–478.
Kretsinger R. H. 1976: Evolution and function of calcium-binding proteins. Ann. Rev. Cytol., 46, 323–393.
Kretsinger R. H. 1977: Evolution of the informational role of calcium in *Eukaryotes*. In: Calcium Binding Proteins and Calcium Function (eds. R. Wasserman et al.) Elsevier, North Holland, New York, pp. 63–72.
Kuźnicki J., Kuźnicki L. and Drabikowski W. Ca^{2+}-regulation of motility and troponin C-like proteins in *Protozoa* and *Myxomycete*. Proc. of 6th Meeting of European Muscle Club. Saclay 1977, 67–68.
Lehman W. and Szent-Györgyi A. G. 1975: Regulation of muscle contraction. Distribution of actin control and myosin control in the animal kingdom. J. Gen. Physiol., 66, 1–30.
Lehman W. 1976: Phylogenetic diversity of the proteins regulating muscular contraction. Int. Rev. Cytol., 44, 55–92.
Maruta H. and Korn E. D. 1977: *Acanthamoeba* myosin II. J. Biol. Chem., 252, 6501–6509.
Maximov E. E., Zapevalowa N. P. and Mitin Y. V. 1978: On the calcium binding ability of the synthetic evolutionary ancestor of calcium binding proteins. FEBS Lett., 88, 80–82.
McLachlan A. D. and Stewart M. 1976: The 14-fold periodicity in tropomyosin and the interaction with actin. J. Mol. Biol., 103, 271–298.
Mikawa T., Nonomura Y., Ebashi S. 1977: Does phosphorylation of myosin light chain have direct relation to regulation in smooth muscle? J. Biochem., 82, 1789–1791.
Muszbek L., Kuźnicki J., Szabo T. and Drabikowski W. 1977: Troponin C like protein of blood platelets. FEBS Lett., 80, 308–312.
Nachmias V. T. and Asch A. 1976: A calcium-sensitive preparation from *Physarum polycephalum*. Biochemistry, 15, 4273–4278.
Pechère J. F. 1977: The significance of parvalbumins among muscular calciproteins. In: Calcium Binding Proteins and Calcium Function, (eds. R. H. Wasserman et al.) Elsevier, North Holland, pp. 213–221.
Perry S. V. 1976: Regulation of contraction in muscle. In: Contractile Systems in Non-Muscle Tissues (eds. Perry S. V. et al.) Elsevier, North Holland, Biomedical Press, Amsterdam., pp. 141–151.
Perry S. V. (unpublished). Lecture on the 7th European Conference on Muscle and Motility, Warsaw, June 29-July 1, 1978.

Pollard T. D. and Korn E. D. 1973 a: *Acanthamoeba* myosin. Isolation from *Ascanthamoeba castellanii* of an enzyme similar to muscle myosin. J. Biol Chem., 248, 4682–4690.

Pollard T. D. and Korn E. D. 1973 b: *Acanthamoeba* myosin. Interaction with a new cofactor protein required for actin activation of Mg^{2+}-adenosine triphosphatase activity. J. Biol. Chem., 248, 4691–4697.

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

Potter J. D. and Gergely J. 1975: The calcium and magnesium sites on troponin and their role in the regulation of myofibrillar adenosine triphosphatase. J. Biol. Chem., 250, 4628–4633.

Small J. V. and Sobieszek A. 1977: Ca-regulation of mammalian smooth muscle actomyosin via a kinase–phosphatase-dependent phosphorylation and dephosphorylation of the 20.000-M light chain of myosin. Eur. J. Biochem., 76, 521–530.

Stevens F. C., Walsh M., Ho H. C., Teo T. S. and Wang J. H. 1976: Comparison of Calcium-binding Proteins. Bovine heart and brain protein activators of cyclic nucleotide phosphodiesterase and rabbit skeletal muscle troponin C. J. Biol. Chem., 251, 4495–4500.

Szent-Gyorgyi A. G., Szentkiralyi E. M. and Kendrick-Jones J. 1973: The light chains of Scallop Myosin as regulatory subunits. J. Mol. Biol., 74, 179–203.

Waisman D., Stevens F. C. and Wang J. H. 1975: The distribution of the Ca^{2+}-dependent protein activator of cyclic nucleotide phosphodiesterase in invertebrates. Biochem. Biophys. Res. Commun., 65, 975–982.

Walsh M., Stevens F. C., Kuznicki J. and Drabikowski W. 1977: Characterization of tryptic fragments obtained from bovine brain modulator protein of cyclic nucleotide phosphodiesterase. J. Biol. Chem., 252, 7440–7443.

Watterson D. M., Harrelson W. G., Keller P. M., Sharief F. and Vanaman T. C. 1976: Structural similarities between the Ca^{2+}-dependent regulatory proteins of 3' : 5'-cyclic nucleotide phosphodiesterase and actomyosin ATPase. J. Biol. Chem., 251, 4501–4513.

Weihing R. R. and Korn E. D. 1971: *Acanthamoeba* actin. Isolation and properties. Biochemistry, 10, 590–600.

Weihing R. R. and Korn E. D. 1972: *Acanthamoeba* actin. Composition of the peptide that contains 3-methylhistidine and a peptide that contains N-methyllysine. Biochemistry, 11, 1538–1543.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 109–110
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Thymus Actin

K. AJTAI, S. DANKO, V. HARSÁNYI and E. N. A. BIRÓ. Department of Biochemistry, Eotvos Lorand University, Budapest, Hungary

By combination of the C-γ-alumina adsorption procedure of Lindberg and Skoog (1970) with the traditional actin purification procedure by polymerization–depolymerization cycle a simple procedure for the preparation of actin from (fresh or acetone dried) thymus tissue was obtained.

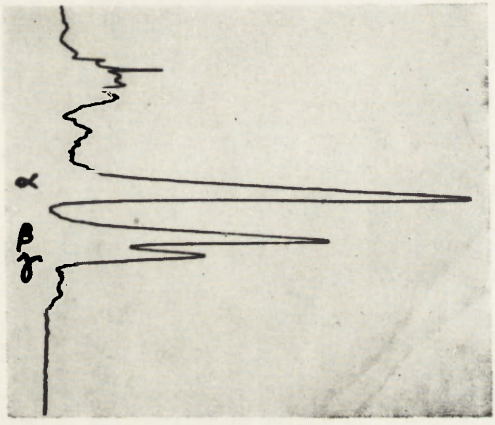
Actin obtained by this procedure from thymus was homogeneous and comigrated with skeletal actin in SDS gel electrophoresis (Pl. I A). In isoelectric focusing according to O'Farrel (1975) it was shown to contain β and γ actin (without a trace of α; Pl. I B). The proportion of β to γ was 2 to 1.

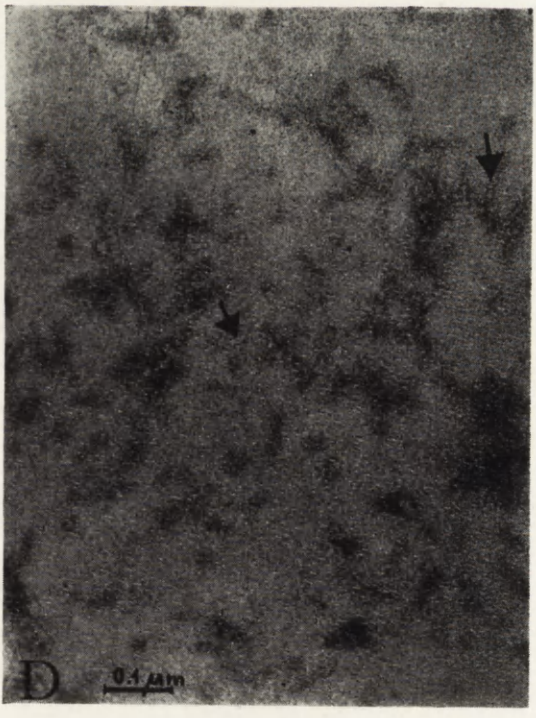
This actin, similarly to several other nonmuscle actins (Korn 1978) polymerized poorly. Up to a concentration of 1 mg/ml there was no polymerization as judged by viscosity measurement. It was native, however, as judged by its DN-ase I inhibitor activity which equalled this of skeletal actin. It also activated ATP-ase activity of myosin but to a lesser extent as compared to skeletal actin.

Studied by electron microscopy under polymerizing conditions only few and very short filaments were seen (Pl. I C). On addition of HMM to G actin decorated filaments formed abundantly but these were also rather short (Pl. I D).

REFERENCES: Korn E. D. 1978: Proc. natn. Acad. Sci., USA, 75, 588–599. Lindberg M. U. and Skoog L. 1970: Eur. J. Biochem., 13, 326–335. Lazarides E. and Lindberg M. U. 1974: Proc. natn. Acad. Sci. USA, 71, 4742–4746. O'Farrel P. H. 1975: Biol. Chem., 250, 4005–4021.

Plate I A — Coelectrophoresis of skeletal and thymus actin in SDS gel: Gel 1: thymus actin, Gel 2: 1 : 1 mixture of skeletal and thymus actin; I B — Isoelectric focusing and densitometric tracings of skeletal and thymus actin, Gel 1: thymus actin, Gel 2: skeletal actin, Gel 3: 1 : 1 mixture of skeletal and thymus actin; I C — Thymus actin; I D — Thymus actin filaments decorated by HMM





K. Ajtai et al.

auctores phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 111–112
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Mechanical Responses of Hypertrophied Denervated Eye Muscles of the Rabbit to Direct Stimulation

G. ASMUSSEN and U. GAUNITZ. Carl-Ludwig-Institute of Physiology University of Leipzig, GDR

After deprivation of its motor innervation the inferior oblique muscle (IO) of the rabbit undergoes a long-lasting hypertrophy (Asmussen and Kiessling 1976). This study describes the changes of the contractile properties of IO during denervation hypertrophy. In 32 rabbits the oculomotor nerve branch to the right IO were transected in the orbita. After 2 to 76 days isometric contractions of these muscles in response to direct massive stimulation were recorded *in vitro* at temperatures of 35°C. The IO of the contralateral (left) eye of the same animal were used as controls (see Gaunitz and Asmussen, this symposium).

Figure 1 demonstrates that the time parameters of a single twitch increase considerably following denervation: the contraction time grows from 6–7 ms to 20–30 ms, the half-relaxation time from 6.5–7.5 ms to 40–60 ms and the latency period from 1.5 ms to 2.3 ms. The rate of rise and the rate of fall in tetanic tension development decrease. The tension developed in a single twitch and the maximum tetanic tension drop progressively to only 5–15% in the first two weeks of those of the control muscles. The largest part of the fall occurs within the first 5 days. After the 2nd week the single twitch tension and the maximum tetanic tension increases slowly to about 50% of those of the controls. Following denervation the fusion frequency decreases from 300 pps to 120 pps. Denervated muscles exhibit a higher fatigue resistance than normal control muscles. The prolongation of the time characteristics and the decrease of the developed tension in the denervated IO of the rabbit are comparable with changes in denervated skeletal muscles of the cat which exhibit a considerable atrophy (Lewis 1972). However, the improvement of the tension development observed in later states of denervation of IO is probably caused by the hypertrophy of special muscle fibres (Asmussen and Kiessling 1976).

REFERENCES: Asmusssen G., Kiessling A. 1976: Acta anat., 96, 386-403, Lewis D. M. 1972: J. Physiol., 222, 51–75.

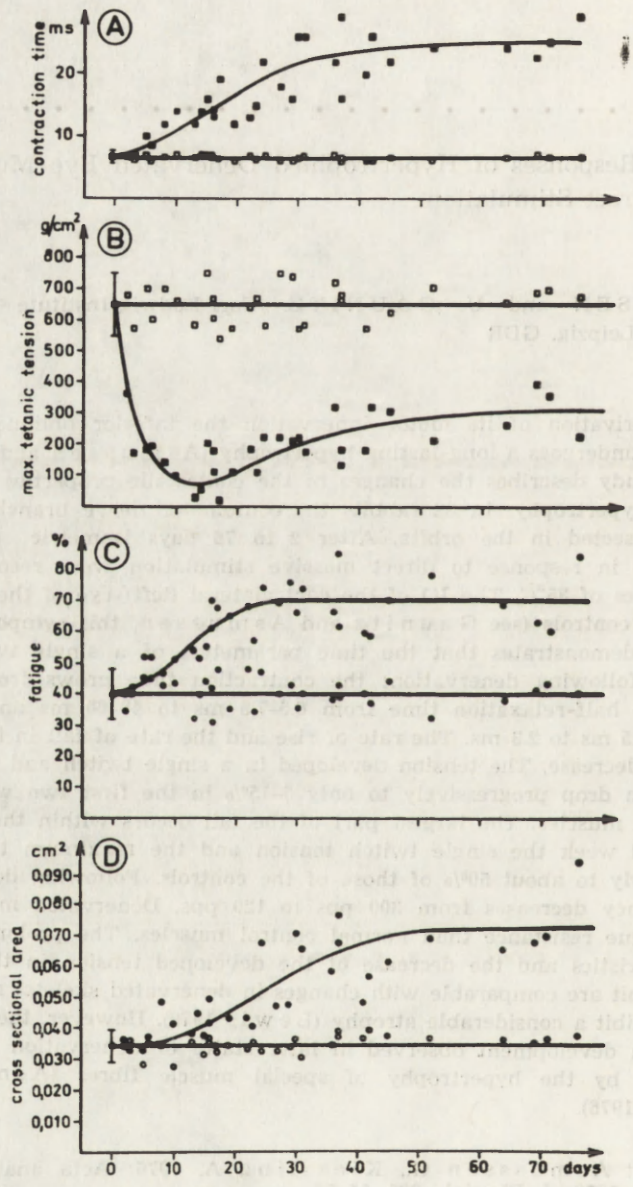


Fig. 1. Graph of some properties of the inferior oblique muscle plotted against period of denervation (open symbols = normal control muscles, filled symbols = denervated muscles). A — contraction time, B — maximum tetanic tension per unit cross sectional area, C — fatigue resistance (residual tension expressed as a percentage of the initial tension after a stimulation of 5 s duration at rates of fusion frequency) D — cross sectional area

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 1113–114
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Thickness Changes and Flow of Endoplasm in Frontal Zones of *Physarum polycephalum* Plasmodia

Z. BARANOWSKI. Department of Cell Biology, M. Nencki Institute of Experimental Biology, 02-093 Warszawa, Poland

Frontal zones of freely migrating plasmodia of *Physarum polycephalum* which covered about 1 mm² of non-nutritive agar gel surface were observed with the aid of holographic microscope. The observed interferograms were recorded on cine film with frequency of 6 frames/s, which allowed simultaneous recording of changes of both endoplasmic flow direction and thickness of the plasmodium. The latter was seen as an appearance and disappearance of fringes or fringe displacements in a given area of the plasmodium. The method used was more detaily described in the previous paper (Baranowski 1976 a).

The observations concerned: 1 — time and space pattern of thickness changes of frontal zones of the plasmodium, and 2 — relations between thickness changes and endoplasmic flow in the plasmodium.

Two main patterns of space order of thickness changes may be distinguished: a — "flickering", i. e., thickness changes disordered in space, and b — quasi synchronic contraction activity, i. e., with small fluctuactions of phase shift existing between contractions of separate areas of the plasmodium. When the activity of the front could be measured (the method of observation in principle is applicable to thin protoplasmic layers), quasi synchronic contraction activity was connected with wave-like changes of thickness in the narrow zone of migrating plasmodium. The analysis revealed wave propagation with the speed about 5–8 μm/s in the direction of plasmodium migration. The transition, with the passage of time, from "flickering" to quasi synchronic contraction activity was observed.

The microinterferometric observations revealed the lack of time and space correlation between flow of endoplasm and changes of plasmodium thickness in "flickering" pattern of thickness changes. On the contrary, in the quasi synchronic contraction activity a good correlation between these two processes is observed, i. e., during contraction endoplasm flows in the direction of plasmodium edge.

In the previous papers (Baranowski 1976 b, 1978) the propagation of wave-like changes of thickness with the speed of 35 μm/s in about 1 cm² size plasmodium were shown. In about 1 mm² size plasmodium the speed of wave propagation is reduced to 5–8 μm/s. It seems to be indicative of the dependence of wave parameters (length and speed of propagation) upon a size of the organism. During active migration the zone following the frontal one contracts and expands more or less synchronously. It was also shown by Grębecki and Cieślawska

(1978) for the zone of separate channels. In the quasi synchronic pattern of contraction activity, the contraction (as it was mentioned above) is connected with the flow of endoplasm in the direction of plasmodium migration and expansion of plasmodium edge (Grębecki and Cieślawska 1978), so the movement of the organism, which is caused by the polarity of endoplasm transport seems to be determined by an unknown factor which leads to the synchronous activity of contractile apparatus in large areas of the plasmodium. The waving thickness of frontal zone of the plasmodium is probably determined by varying elasto-viscous properties of protoplasm and dynamics of translocation and organization of contractile apparatus.

REFERENCES: Baranowski Z. 1976 a: Cytobiologie. 13, 118–131, Baranowski Z. 1976 b: In: Niemyshetchnyie formy podviznosti (ed. G. M. Frank), Acad. Nauk USSR, Pushchino 47–50, Baranowski Z. 1978: Acta Protozool., 17, 377–388, Grębecki A. and Cieślawska M. 1978: Cytobiologie, (in press).

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 115
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Photobehavior of *Euglena gracilis:* Effects of Drugs on Sensory Transduction Chain

C. BARGHIGIANI, G. COLOMBETTI and F. LENCI. Laboratorio Studio Proprieta Fisiche Biomolecole e Cellule, C.N.R., Pisa; Italy

The capability of several microorganisms to react to variations in illumination conditions with an alteration of their motile properties offers an interesting model system for the study of sensory transduction in aneural organisms. The receptor–effector chain is in many cases still largely unknown, some information being only available on the nature of the receptor pigments. One possible approach to the investigation of the metabolic pathways that may be involved in the sensory transduction chain is the use of metabolic drugs that block the cellular biochemistry at specific sites. We have studied the photobehavior of *Euglena gracilis* in the presence of three metabolic inhibitors: sodium azide, 2,4-dinitrophenol and DCMU. The behavior was followed under the microscope with the help of a TV recording system, whereas the effects of the drugs on motility were monitored using a laser light scattering technique. The results show no specific effect of these substances on *Euglena* photobehavior. This could indicate a possible relevance in the photomotile response of membrane potentials due to ions as e. g., Ca^{++}, Mg^{++}, K^{+} or Na^{+}.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 117–119
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Free Space of *Physarum polycephalum* on the Regional Differences in Ion Concentration in Migrating Plasmodia

S. I. BEYLINA, D. B. LAYRAND, N. B. MATVEEVA, V. A. TEPLOV.
Institute of Biological Physics, Acad. Sci. USSR. Pushchino, Moscow Region, USSR

Solution of the problem of myxomycetes motion regulation depends, to a large extent, on the knowledge of the fundamental principles of membrane system function of these cells. Since the electrophysiological investigations and the data on ion exchange of myxomycetes are neither numerous nor systematized, we were interested in works permitting to develop a methodical basis of such investigations. In the present work, carried out on plasmodium *Physarum polycephalum* using flame photometric technique, an attempt is made to apply the approaches developed in the studies of ion exchange in plants, to ameboid cell.

The semilogarithmic plot of cation absorption from solutions of potassium and sodium chlorides has a two-component character (Fig. 1). The first step of absorption, with the time constant about 15 min, is nonselective, easily reversible and

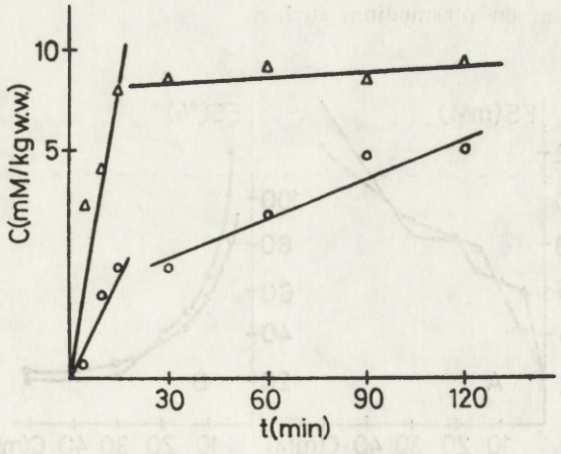


Fig. 1. Double semilogarithmic plots of cation absorption by plasmodium. Absorption of K^+ from 30 mM KCl solution (triangles); absorption of Na^+ from 15 mM NaCl solution (circles); both solutions contain 0.5 mM $CaCl_2$

temperature independent within the limits of the experiment accuracy. This complex of features is characteristic for the absorption into extracellular, the so called "free" space. Reversibility of absorption enables to determine the value of this cell compartment from the ion output into the medium of a given volume (Fig. 2).

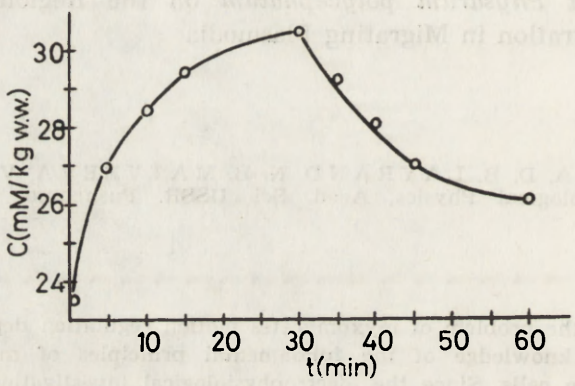


Fig. 2. Reversibility of the initial fraction of K^+ absorption by plasmodium myxomycete. Absorption from 15 mM KCl solution — 0.30 min; K^+ output from the cell into the medium (0.5 mM $CaCl_2$, averaged values of 15 experiments)

The dependence of absorption on equilibrant solution concentration, which permits to define the structure of space, suggests the presence of adsorptive component of absorption whose contribution is substantial in the concentration range between 1 and 30 mM (Fig. 3). At concentrations of cation higher than 30 mM water part becomes the main in calculating the free space. In a given case it is 20% of cell weight. A considerable part of water space may be determined by invagination system on plasmodium surface.

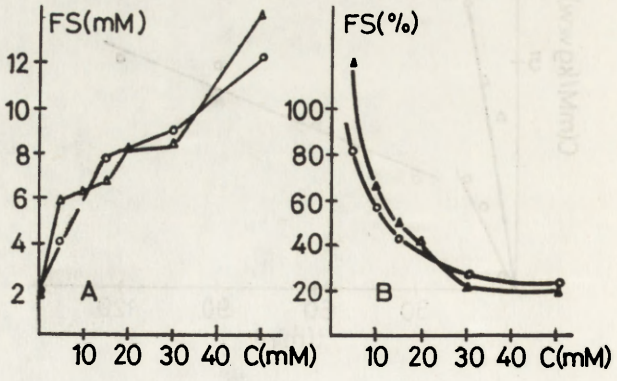


Fig. 3. Dependence of the absorptive space value on the concentration of the equilibrant medium: A — concentration of cations in exodiffusional solution; B — space value in per cent of cell volume. K^+ and Na^+ are designated as in Fig. 1

The value of the water space depends on the external factors, in particular, on pH (Fig. 4). It is shown that absorption as well as reabsorption of cation (sodium) has the maximum at pH 6.0. The effect is not metabolically dependent, nor depends it on the buffer system. The ion output into the exodiffusional medium is the same at both low and normal temperatures. The increase of the free space is not related to the disturbance of membrane semipermeability, since the output of K+ (1 mM/g w.w) is practically pH-independent. The most likely explanation for these effects might be a closing of invagination connected with the pH stimulated change in the state of cortical gel.

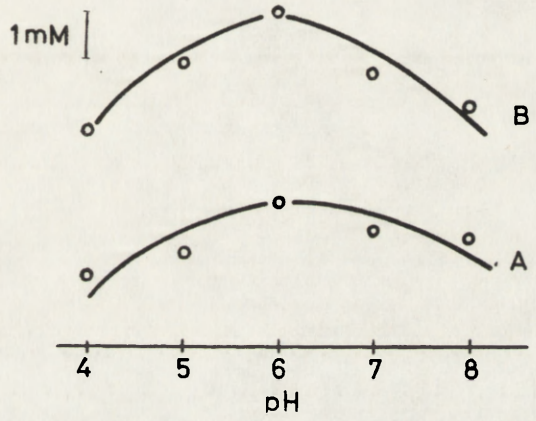
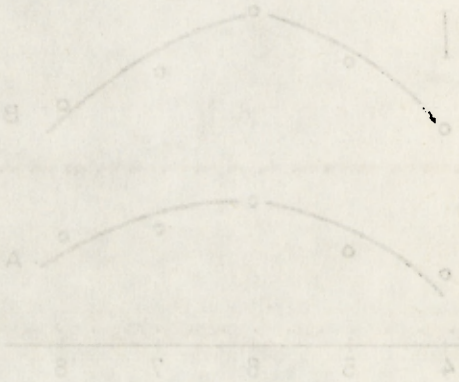


Fig. 4. Effect of pH medium on the absorption and reabsorption of Na^+. A — dependence of Na^+ uptake on pH of the incubation medium (0.5 mM $CaCl_2$, 15 mM NaCl); B — dependence of Na^+ output on pH of exodiffusional medium (0.5 mM $CaCl_2$ incubation in the medium 0.5 mM $CaCl_2$, 15 mM NaCl, pH 7.0)

Considerable value and lability of free space of plasmodium permits to account for the difference in cation concentration between the advancing front and channel region of migrating plasmodium observed by Anderson. The concentration of K^+ is higher in the front advancing region and that of Na^+ — in the channel region. Anderson has also demonstrated that Na^+ may be almost completely removed by flushing water over the organism. It would be natural to assume that the easily washed fraction is connected with extracellular space. If the distribution of Na^+ reflects the regional difference in space value, then for intracellular cation (K^+) the concentration in the region of advancing front should be higher and closer to the true value in cytoplasm.

All the experiments performed demonstrate that in studies of ion exchange of ameboid cells, one should take into account the considerable extracellular space and the possibility that experimental factors and chemicals might affect its value and structure.



ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 121
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Properties of ATPase of *Tetrahymena pyriformis* Cilia and of *Strigomonas (Crithidia) oncopelti* Flagella

S. A. BURNASHEVA, Bach Institute of Biochemistry, Acad. Sci. USSR, Moscow, USSR

ATPases similar in some respects to myosin has been extracted from cilia of *Tetrahymena pyriformis* and from flagella of *Strigomonas (Crithidia) oncopelti* by saline solution used for extraction of myosin from muscles.

The activity of ATPases of cilia and flagella, like myosin, is stimulated by Ca++, is high specific for ATP with two optima of pH (6.3 and 8.3). The K_m for ATP calculated according to Lineweavor–Burk method is 6.6×10^{-4} M for flagellar ATPase and 1.6×10^{-4} M for ciliary ATPase. The characteristic feature for this ATPases is inhibition of enzymatic activity by excess of substrate (substrate inhibition). Molecular weight of ciliary ATPase is 480 000 and flagellar ATPase is 400 000 dalton.

Cytochemical studies have demonstrated ATPase activities in cilia and basal bodies of *T. pyriformis*. In the cilium reaction product was associated with membrane of cilia, the arms, the periphery of the outer doublet microtubules. The strong distribution of the lead phosphate precipitate was found along the length of the part of outer microtubules, running into the basal bodies and within the basal body.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 123–124
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Preliminary Morphological Study on the Ehrlich Ascites Tumour Cells Induced to Spread on Glass at the Presence of Dexstran T 500

Jolanta CIEŚLAK and Włodzimierz KOROHODA. The Institute of Molecular Biology of the Jagellonian University, Kraków, Poland

It has been recently reported that high molecular polyglycans, Dextran T 500 and Dextran T 2000, when present in serum free medium, induce rapid and reversible attachment and spreading in Ehrlich ascites tumour (EAT) cells (1). This suggests that the cell surface properties can control the cell adhesiveness and the capacity of cells to spread on solid substrata. However, in the literature of the subject it was reported that the differences between the cell surface complex in normal and cancer cells concern not only the cell surface itself but also the cell membrane permeability and the arrangement of cyctoplasmic filaments. In particular, a numerous suggestions have been put forward which postulate a central role of the amount and spatial organization of cytoplasmic contractile elements for regulation of cell morphology, attachment to and spreading on a solid substratum and contact inhibition of movement (2, 3, 4, 5). The question therefore arises which of the observed differences in the cell surface complex plays a decisive role in control of the cell behaviour and whether it could be possible that only one of the differences has a primary character and all others are its direct or indirect consequences. It has been also observed that cell spreading and an induction of cell motile activity cause great changes in metabolism of cells and in plasma membrane permeability (6, 7, 8, 9).

Since in normal cells it was found that cell attachment and spreading on glass are sufficient to induce a formation of bundles of "fibrils" of parallely oriented filaments (10) the electron microscopic study were started on experimentally spread EAT cells at a presence of Dextran T 500 in Eagle's minimal essential medium.

In the preliminary experiments it was found that:

(1) The spreading of EAT cells on glass at the presence of Dextran T 500 is not sufficient per se to induce the parallel orientation of filaments and formation of their bundles in the cells.

(2) The filaments are localized mainly in the region of smooth endoplasmic reticulum where they attach to smooth membranes and in the extending lamellipodia, where they attach to plasma membranes.

(3) In the spreading cells a great number of long polyribosomes is present

what seems to suggest a stimulation of protein (contractile proteins?) biosynthesis by the processes of cell attachment and spreading. In the same cells a great activity of nuclear envelope and "budding" of its external membrane which can lead to the development of endoplasmic reticulum can be observed.

The further morphological studies will concern the questions: (1) Whether induction of contraction and/or locomotion in the spread EAT cells can cause the parallel orientation of filaments in the retracting lamellipodia? (2) What are the differences in spatial organization of the filaments in extending and retracting cell surface processes?

REFERENCES: (1) Cieślak J. and Korohoda W. 1978: Cytobiologie, 16, 381–392, (2) Wang E. and Goldberg A. L. 1976: Proc. natn. Acad. Sci., USA, 73, 4065-4069, (3) Wickus G., Gruenstein E., Robbins P. W. and Rich A. 1975: Proc. natn. Acad. Sci. USA, 72, 746–749, (4) Pollack R., Osborn M. and Weber K. 1975: Proc. natn. Acad. Sci. USA, 72, 994–998, (5) Vollet J. J., Brugge J. S., Noonan C. A. and Butel J. S. 1977: Expl. Cell Res., 105, 119–126, (6) Clarke G. D. Stoker M. G. P., Ludlow A. and Thornton M. 1970: Nature, 227, 798–801, (7) Otsuka H. and Moskowitz M. 1975: J. Cell Physiol., 86, 379–388, (8) Korohoda W. 1971: Folia Biol., (Kraków), 19, 41–51, (9) Bissell M. J., Hatie C. and Rubin H. 1972: J. natn. Cancer Inst., 49. 555–565, (10) Lloyd C. W., Smith C. G., Woods A. and Rees D. A. 1977: Exp. Cell Res., 110, 427–437.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 125
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Synchrony of Contractions in Freely Migrating Plasmodia of *Physarum polycephalum*

M. CIEŚLAWSKA and A. GRĘBECKI. Department of Cell Biology, M. Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3, Poland.

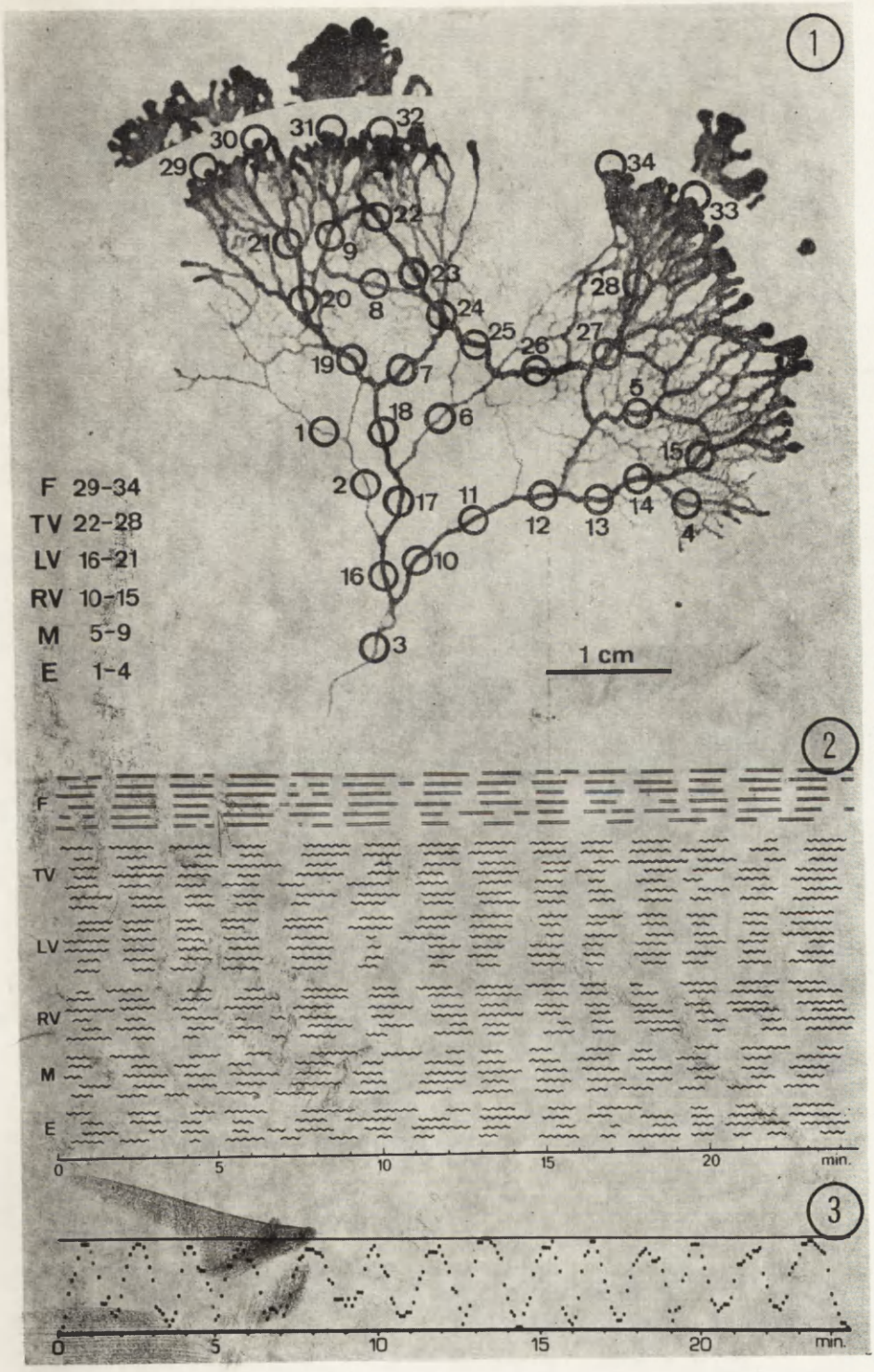
There are no models of the contractile activity of plasmodium integrating the actual data collected simultaneously across the entire system. Past models were indirectly derived from observations of the streaming pattern or from investigating contraction rhythms in minute areas *in situ*. It has been thus postulated that generation of contraction is restricted to specific active centres and/or that the contraction is propagated in peristaltic way. In this study, pulsation patterns are examined simultaneously at numerous sites scattered over the whole surface of plasmodium to obtain a comprehensive picture of the time-space distribution of contraction-relaxation cycles.

Macrocinematographic pictures of plasmodia freely migrating on non-nutritive agar gel were taken in dim light. Pulsation rhythms of veins and of the frontal edge were recorded photometrically at the screen plane, at many points of plasmodium (Fig. 1) in the course of repeated projections of the film. The general periodicity diagrams (Fig. 2) were constructed basing on the photocurrent curves obtained at all the tested sites. Each row of wavy lines, parallel to the time scale, represents the contraction periods of one of the sites situated in the network of veins (the rows are grouped as in Fig. 1). The double straight lines correspond to the periods of intense expansion recorded at the sites disposed along the frontal margin.

It is evident that the wavy lines in the diagram shown form 14 distinct vertical columns. That means that the whole network of veins of this plasmodium performed 14 synchronous contractions. The frontal margin also performed, at the same time, 14 steps forward. They clearly coincided in time with the contraction phases of the network of veins. The revealed synchrony is not always exactly precise, but deviations fail to present any stable pattern necessary to produce the peristaltic propagation of contraction, and any desynchronized site quickly returns to the common rhythm. The high degree of general synchrony of contractions in the whole network of veins is demonstrated by the diagram (Fig. 3) in which the number of tested sites undergoing contraction was marked, every 2 s, by a point over the time scale. The distribution of points follows a fairly regular sinusoid.

It became evident that contractions (and relaxations) coincide in time in the whole network of veins. The front expands and steps forward synchronously with

each such contraction. It indicates that simultaneous general contractions of all the system of veins squeeze the protoplasm out, toward the peripheral regions. The morphology of the frontal periphery predestinates it to accept the major part of the squeezed protoplasm. As a result, the common rhythm of pulsation of all the veins produces the pulsation of the frontal margin, characterized by the same period but opposite in phase. In general, plasmodium may be considered as an imperfectly synchronized monorhythmic contractile system.



M. Cieślawska et A. Grębecki auctores phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 127–128
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Polar Effects of DC on the Ciliary Response of Wild Type (Stock 51 S) and Pawn Mutant (Stock d4-94) of *Paramecium tetraurelia*

Stanisław DRYL and Jacek KURDYBACHA, Department of Cell Biology, Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3; Poland

In recent study the authors (Dryl and Kurdybacha 1977) attributed the increased threshold for galvanotaxis at low levels of external calcium (within range of pCa 6.0–9.0) to possible release of calcium ions from hypothetical intracellular stores in the cathodal region of cell. However, Kung (1971) reported the occurrence of cathodal orientation in the behavioral mutant stock of *Paramecium aurelia* "Pawn", which does not respond with ciliary reversal to external stimuli, taking all these facts in account, it appeared necessary to make a careful analysis of the cathodal and anodal effects of DC on ciliary response in "Pawn" behavioral mutant and wild strain stock *Paramecium tetraurelia* at high low levels of external calcium.

To render possible a direct observation of ciliary response the animals were immobilized by 1 h lasting homologous antiserum (1 : 100 diluted antiserum A) treatment. After being washed out from antiserum protozoa were placed into 1 mM TRIS/HCl solution (pH 7.1) with various Ca^{2+}_{ex} levels. At pCa 3.0 paramecia

Table 1

Polar effects of DC on the ciliary beat of *Paramecium tetraurelia* in wild type (stock 51 S) and behavioral mutant „Pawn" (stock d4-94) at high (pCa 3.0) and low (pCa 7.0) level of external calcium

Strain of *Paramecium tetraurelia* studies	pCa 3.0		pCa 7.0	
	C.R.	A.R.	C.R.	A.R.
51.8 S (wild type)	+(10)	+(10)	−(10)	+(10)
Behavioral mutant "Pawn" d4-94	−(10)	+(10)	−(6)	+(6)

C. R. = Cathodal Response i.e., ciliary reversal at cathodal end
A. R. = Anodal Response i.e., increased frequency of ciliary beat at the anodal end
+ means that reaction was present
− means lack of reaction
Value in brackets (e.g., 6 or 10) represents number of observations

were stimulated with DC of voltage gradient 2.0 V cm^{-1}, while at pCa 7.0 potential gradient of 12 V cm^{-1} was applied. DC exposure time was 15 s.

The activity of cilia was visualized by motion of suspended PLP latex beads (diameter 0.794 μm).

In separate series of experiments the swimming rate of galvanotactically stimulated paramecia was estimated by measuring the photo-recorded paths (Dryl 1958) of animals according to method by Fritsch (1975).

The data from the Table indicate that there is no cathodal ciliary reversal in "Pawn" at pCa 3.0 and 7.0 as well as in wild types at pCa 7.0. However, the anodal response was preserved in all cases. This explains why cathodal orientation is observed in behavioral mutant "Pawn" and also in wild type of *Paramecium aurelia* at extremely low levels of external calcium. This conclusion seems to be supported by evident increase of swimming rate of "Pawn" exposed to DC, when compared with behavior of wild strain of *Paramecium aurelia* (Fig. 1). The increased

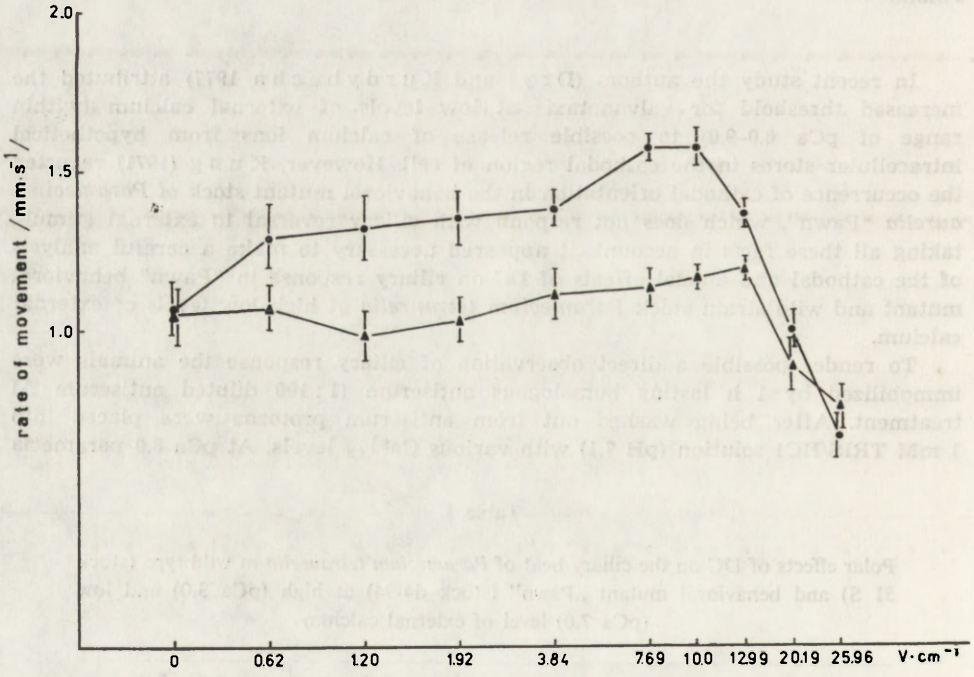


Fig. 1. Rate of forward movement in *P. aurelia* 51 S (wild type) and in behavioral mutant "Pawn" d4-94 at various voltage gradient. Each point of cummulative diagram represents mean value (and S.D.) from 25 measurements. Behavioral mutant "Pawn" d4-94-circle, *P. aurelia* 51 S (wild type) -triangle.

rate of forward swimming of "Pawn" was noticed at wide range of applied DC stimulation. This can be explained by the lack of the inhibitory action of ciliary reversal on the cathodal side of animal.

REFERENCES: Dryl S. 1958: Bull. Acad. Pol., Sci. Ser. Sci. Biol. Cl. II 6, 429–430, Dryl S. and Kurdybacha J. 1977: Progress in Protozoology. Vth Intern. Congress Protozool. New York (ed. Hunter). Abstr. 167, Fritsch P. S. 1975: Nova Acta Leopoldina 217, 41, 205–234, Kung Ch. 1971: Z. vergl. Physiol., 71, 142–164.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 129
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Ca^{2+} in the Protein Structure. Recording of Binding by IR-Spectrum

O. V. FEDOROV. Institute of Protein Research, Academy of Sciences of the USRR, 142292 Puschino, Moscow Region, USSR

The ligands of calcium ions in calcium-binding centers of proteins are mainly the carboxylic groups of dicarboxylic amino acid residues. In the infrared spectrum the ionized forms of these residues have absorption bands of about 1584 and 1567 cm^{-1} (asparagine and glutamine acids, respectively). A quantitative analysis of the spectral parameters of the absorption bands of these residues was done three years ago (Chirgadze et al. 1975). A strict reciprocal orientation of carboxylic groups occurring in the calcium-binding center in the presence of Ca^{2+}, may change the spectral appearance of these groups. The IR-spectra of carp parvalbumin and troponin C from rabbit skeletal muscle have shown a decrease of absorption at the described frequencies and the appearance of an additional band of about 1557 cm^{-1}. When Ca^{2+} is removed from the protein structure with EDTA or EGTA, the observed spectrum is characteristic for the given Asp and Glu content in the protein molecule. This is a reversible process. The observed spectral effect can be used for analytical purposes.

REFERENCE: Yu. N. Chirgadze, Fedorov O. V. and Trushina N. P. 1975: Biopolymers, 14, 679–694.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 131-132
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

The Effect of Temperature and CO_2 Contraction on Light-induced Chloroplast Movements in *Tradescantia* Leaves

H. GABRYŚ and T. WALCZAK. Department of Plant Physiology, Institute of Molecular Biology, Jagiellonian University, Grodzka 52, 31-001 Kraków, and Department of Nuclear Spectroscopy, Institute of Nuclear Physics, Radzikowskiego 152, 31-342 Kraków, Poland

The double-beam photometer was used for the study of the effect of temperature and CO_2 concentration on light-induced chloroplast displacements in *Tradescantia* leaves. The investigations were performed at temperatures of 10°, 20°, 30°C and at light intensities of 0.05, 2, 10, 20, 100 Wm^{-2}. The CO_2 concentrations were 0.003, 0.03, 0.3 and 3%.

A series of experiments dealing with temperature effects was performed starting from the dark chloroplast arrangement. For the light intensities which saturated the strong light reactions, the amplitude of the transmission changes was constant. Their velocities changed as Q_{10} ranging from 2 to 3. For lower light intensities (10, 20 Wm^{-2}) two phases of the reaction could usually be detected (Fig. 1). The slope of the curve for the first phase was temperature dependent according to the Q_{10} of about 2. The direction and amplitude of the second phase was dependent on the temperature in the following way: At a temperature of 10°C

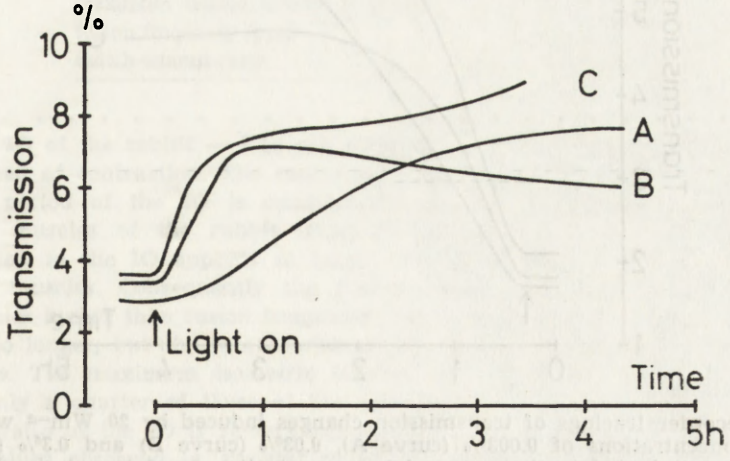


Fig. 1. Recorder tracings of transmission changes induced by 10 Wm^{-2} white light at different temperatures: 10°C (curve A), 20°C (curve B) and 30°C (curve C). The leaf was adapted to each temperature for 2 h

The IO of the rabbit is composed of several types of muscle fibres. Their distinction is possible by morphological methods (see Asmussen and Kiessling 1971) and it can be assumed that they also differ in their physiological properties. However, such differences are not detectable in stimulation experiments of the whole muscle as in this study (see also Close and Luff 1974).

REFERENCES: Asmussen G. and Kiessling A. 1971: Acta anat., 79, 526–545, Bass A. et al. 1973: Physiol. Bohemoslov., 22, 477–486, Close R. and Luff A. R. 1974: J. Physiol., 236, 259–270, Lennerstrand G. 1974: Acta physiol. scand., 91, 458–474.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 135
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Structure and Physiology of a "6 + 0" Flagellum

S. F. GOLDSTEIN, C. BESSE et J. SCHREVEL. Laboratoire de Zoologie et Biologie Cellulaire et S.G.M.E.A.B. 40, Avenue du Recteur Pineau, 86022 Poitiers Cedex, France

An actively beating flagellum with a "6 + 0" ultrastructure is found in the male gamete of the parasitic marine protozoan, *Lecudina tuzetae*. No central microtubules or sheath are exhibited in electron micrographs. The 6 doublets appear to have the same structure as those of "9 + 2" flagella, and bear dynein-like arms. The flagella are about 15 μm in length. The axoneme is only about 1000 Å in diameter, compared to at least 1600 Å for "9 + 2" flagella.

Gametes beating freely in sea water exhibit a wave form in which circular bends travel along an essentially helical path. The bends move from base to tip. In contrast to most protozoa — but similarly to most spermatozoa — they beat continuously, without starting and stopping. Beating is very slow, with a period of about 2 s. They can withstand high viscosities, and show relatively little change in waveform and frequency between sea water and 500 cP.

These flagella demonstrate that the central microtubules are not necessary for flagellar beating, and provide a good system for the study of the peripheral doublets.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 137
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

On the Relations between Different Forms of Non-Muscle Motility

V. A. GOLICHENKOV. Laboratory of Experimental and Evolutionary Embryology, Biological Faculty, Moscow State University, Moscow, USSR

There are various forms of non-muscle motility: cell movements, changes of cell morphology, streaming of cytoplasm, movements of intracellular components, mitotic movements.

We suppose that these forms of motility have the same mechanism and apparatus. Moreover, we suggest that competitive relations exist between the various ways of using of motility apparatus. This type of relationships may be determined for example by the limited amount of the proper intracellular material (tubulin-like, actin-like and other substances). It may explain the fact that a cell can fully exhibit only one form of motility at any moment but not two forms.

We offer to use amphibian melanophores as a model for studying of relations between various forms of non-muscle motility. These cells provide an accessible and convenient system for *in vivo* and *in vitro* study of cell movement, division and intracellular movement of melanosomes.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 139
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Pigment Migrations in the Retinal Epithelial Cells of the Amphibian Eyes

V. A. GOLICHENKOV and O. V. BURLAKOVA. Laboratory of Experimental and Evolutionary Embriology, Chair of Darwinism of Biological Faculty, Moscow State University, Moscow, USSR

The subject of our investigation was the development of retinal epithelial cell reactions during the change of the light conditions in the ontogenesis of *Rana temporaria* L. It was shown that the adaptive reactions of these cells are preceded by a certain phase (18–21th stages of normal development by Kopsh) in which the pigment is dispersed independently on light conditions. The first reactions of retinal epithelium are detected at the 22nd stage of the development and they result in a pigment aggregation during the tadpole adaptation to the darkness and in it's dispersion — at light. Beginning with the same stage of development in constant darkness we have revealed certain circadian fluctuations of pigment dispersion with the minimum at night hours and with the period — 26–28 h. Being exposed to constant light the pigment is always fully dispersed. A decrease in illumination intensity (up to 70 lx in our experiments) produces temporary pigment aggregation, and in this case the rate of aggregation as well as its duration do not depend on the drop in the illumination intensity.

It is expected that both dispersion and aggregation processes in the retinal epithelial cells follow different regulatory ways in the organism by virtue of the fact that: (1) they have different time parameters (the aggregation is going more slowly than the dispersion); (2) the aggregation is preceded by latent period, during dispersion the latent period is not revealed; (3) they exhibit different temperature dependence; (4) the aggregation reaction undergoes certain changes in the ontogenesis; the process of dispersion is the same at all investigated stages of development.

It is shown that the general regularities characterizing the pigment migrations in the retinal epithelial cells are similar to those characterizing the reactions of another type of melanin-containing cells of the organism, i.e., the dermal melanophores.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 141–142
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Cornea Pigmentation after the Extirpation of the Eye in *Anura* Larvae as a Model for Studying Cells' Mobility

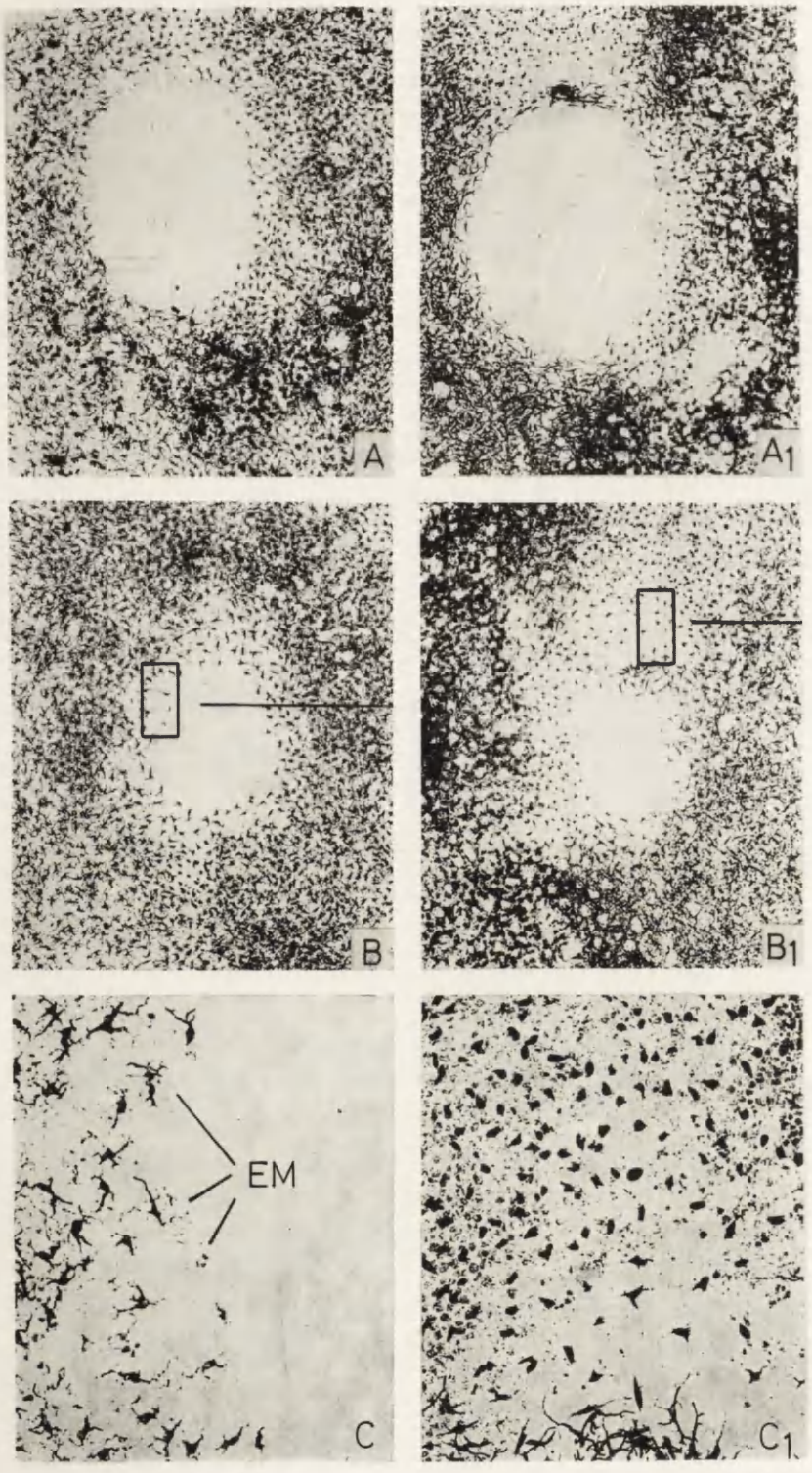
V. A. GOLICHENKOV, E. N. KALISTRATOVA, D. V. POPOW, Z. A. SOKOLOVA and S. M. STARODUBOV. Laboratory of Experimental and Evolutionary Embryology, Biological Faculty, Moscow State University, USSR

Amphibian larvae need their eyes not only in the process of cornea induction but also to upkeep its normal structure. The extirpation of the eye in *Anura* larvae results in the conversion of cornea into skin with the structures which are typical of it. In this case the epidermal melanophoric cells migrate filling up the space of the transparent cornea (Lewis 1905, Dürken 1913). Their migration might be determined with the help of the endogenic factors secreted by certain pigmentary cells, and these factors cause the melanophores to hold the minimum spacings between them. As a consequence of an increase in the population density of cells, the originating population pressure is directed towards the space free of melanophores, and in our case it is oriented in the direction of the cornea (Twitty and Niu 1954, Niu 1954). As well as Bytinski-Salz (1961, 1964) we believe that cornea pigmentation after the extirpation of the eye is a good model for studying the migration of epidermal melanophores.

We studied the cornea pigmentation after the extirpation of the eye in tadpoles (*Rana temporaria* L.) when they pass the 22nd stage in their development (Kopsch 1952). It has been found out that penetration of epidermal melanophores into the cornea epithelium occurs on the very first days after the operation and by the 7th day it can be well marked in the total preparations. As this takes place, the bulk of melanophores penetrate into the cornea from the dorsal and ventral sides. By the beginning of the metamorphosis (30 days after the operation) the process of cornea pigmentation is completed in the majority of animals. After the extirpation of the eye the cornea pigmentation process can well be expressed by means of the rectilinear dependence. The correlation of the data of the present investigation with the data obtained earlier (Popov and Starodubov 1975, 1977) shows that cornea pigmentation is proceeding 1.8 times slower than depigmentation during the process of induction.

We have noted two points which are of particular importance: (1) Since the 9 th day one can easily trace free migration of epidermal melanophores among the cells of the cornea epithelium separated from the cells of epidermis (Fig. 1 B, C); (2) Since the 26th day after the operation in the nasal area of the pigmenting cornea of all animals there appears a zone of epidermal melanophores in the state of contraction (Fig. 1 B_1, C_1). As the size of these cells is different it may be suggested that they are in the state of differentiation from melanoblasts.

Plate I: The total cornea preparation in the midst of the surrounding skin after the operation (9–26 days). The distribution of the photographs corresponds to the position of the areas on the surface of the larva. A–A_1 — the left test eye; B–B_1 — the eye is extracted. A, B, C — 9 days after the operation; A_1, B_1, C_1 — 26 days after the operation (20 ×). C — the area on which one can well trace the displacement of the epidermal melanophores (E. M.) among the cells of the cornea epithelium; C_1 — differentiation of melanophores from melanoblasts (140 ×).



V. A. Golichenkov et. al. auctores phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 143–144
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Dynamics of the Cortical Layer in Moving *Amoeba proteus*

Lucyna GRĘBECKA and Barbara HREBENDA. Department of Cell Biology, M. Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3, Poland

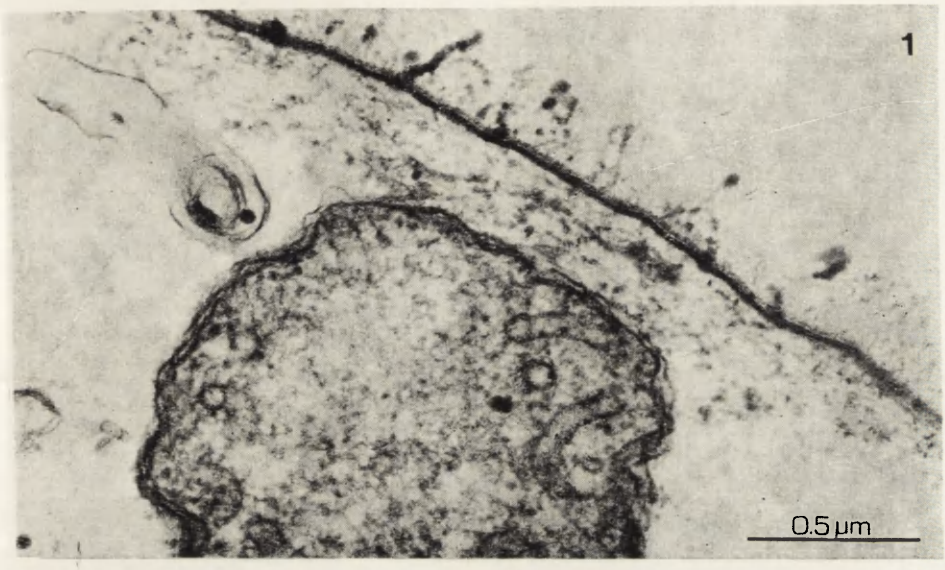
The cortex of *Amoeba proteus*, composed of the cell membrane and of the subjacent 1–2 μm thick cytoplasm layer, is considered by the majority of recent authors as the site of generation of motory forces responsible for cell locomotion. Localization of the contractile activity in this stratum is justified by the abundance of filaments, either anchored to the inner side of the cell membrane or crossing the underlying zone. It was traditionally described as the "hyaline layer". The general definition of the cortex of amoeba quoted above, and its existing descriptions, permit to conclude that it is regarded as a continuous structure, or at least that its possible discontinuity and differentiation along the cell axis has not been raised in the literature.

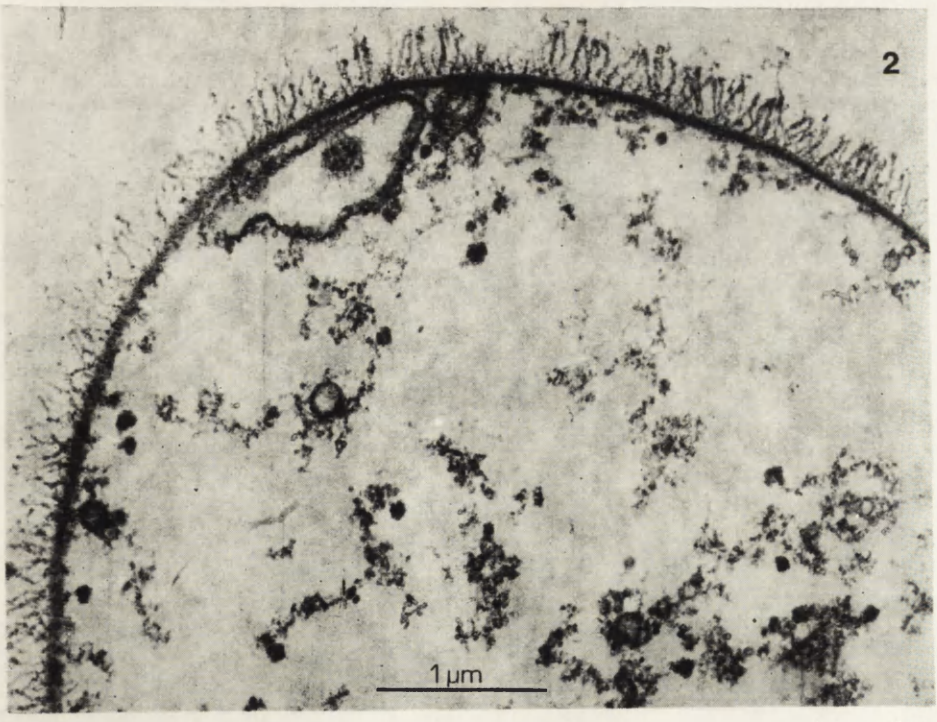
The basic method employed in this study consisted in dissecting polytactic amoebae, after fixation, to isolate the principal functional body parts and to investigate them separately in the electron microscope. The following differences in the fine structure were found between the retracting tail region, the walls of main body trunk, and the fronts of advancing pseudopodia: (1) In the tail region of moving amoeba the cortical cytoplasm is electron dense and, inside it, bundles of filaments parallel or oblique to the cell surface, are clearly seen. (2) Under the membrane of the side walls of the main body part the cortex is very well developed and characterized by the abundance of filaments. The great majority of them run in the longitudinal direction (Fig. 1). In many other sites of the lateral surface of amoeba the filaments form distinct bundles. (3) No contractile cortex could be found beneath the frontal membrane of advancing pseudopodia. In many cases the corresponding cytoplasm layer appears to be electron empty. The fronts of advancing pseudopodia often contain, under the cell membrane, the vacuoles of different size and some granules, but no organized filamentous material (Fig. 2).

The present results show that: (1) The filamentous layer, being *ex definitio* the essential constituent of the cortex, is not continuous and it fails to envelop tightly the whole body of amoeba. (2) The topography of this layer is clearly related to the dynamic morphology of the moving cell.

It means that the hyaline layer of light microscopists is not a homogenous stratum with evenly distributed filamentous structures, capable of contraction in any place. It presents a mosaic system in which, besides the regions predestinated by their structure to contract, other areas exist where the contraction is impossible

because the necessary structured material is absent. The present research demonstrated that the areas lacking of contractile cortex are predominantly distributed at the advancing front of migrating polytactic amoebae. That is consistent with the absence of cortex at the front of monotactic amoebae, as described elsewhere by the present authors. It allows to conclude that, in general, a local discontinuity of the cell cortex is the prerequisite of initiation of a pseudopodium and the necessary condition of its further advancing.





L. Grębecka et B. Hrebenda auctores phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 145
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Short-Term Reactions in Phytochrome-Regulation Chloroplast Orientations

Wolfgang HAUPT and Günter REIF. University of Erlangen-Nürnberg, Schlossgarten 4, D-8520 Erlangen, Fed. Rep. Germany

The chloroplast of the algae *Mougeotia* and *Mesotaenium* turns in the cell as a response to the light direction. This light effect is triggered by the active form of phytochrome P_{fr}. There is, however, one important difference between the two algae: In *Mougeotia*, a single red pulse photoconverting P_r to P_{fr} is sufficient to trigger the response during the following 20–40 min in the dark. In contrast, *Mesotaenium* requires either continuous red light during the whole response or repetitive pulses with dark intervals not exceeding a few minutes. Obviously, the intracellular P_{fr} gradient looses its effectivity very fast in *Mesotaenium*. The physiology of this phenomenon is the topic of this report.

Theoretically, there are mainly three possibilities how the effectivity of P_{fr} is lost: (1) Dark reversion of P_{fr} to P_r. (2) Dark destruction of P_{fr} to an unknown component. Both these dark reactions are well-known in higher plants, but in most cases they require much more time than a few minutes. (3) Change in properties of P_{fr} in such a way that its physiological activity is lost, but that photochemically the substance still is P_{fr}. The possibility that "newly formed" P_{fr} is more active than "old" P_{fr} is being discussed recently in some systems.

By proper combinations of repetitive pulses of red and far-red, the third possibility has been proven for *Mesotaenium*. The difference between *Mougeotia* and *Mesotaenium*, then, is quantitative rather than qualitative: In both these systems, newly formed P_{fr} is more effective than old P_{fr}. Whereas, however, in *Mougeotia* old P_{fr} has still some effectivity in triggering the movement, even after more than an hour, in *Mesotaenium* the effectivity is completely lost within a few minutes.

Obviously, continuous action of P_{fr} is required for sustaining the chloroplast movement. This is strong evidence for assuming that in the reaction chain of phytochrome-controlled chloroplast movement P_{fr} acts very closely to the motor apparatus.

A detailed publication with the proper references is being prepared.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 147
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Further Evidences for a Ca^{2+}-Mediated Coupling between Excitation and Inactivation (Adaptation) in *Paramecium*

E. HILDEBRAND. Institute of Neurobiology, KFA Juelich, Fed. Rep. Germany

It is well known that *Paramecium* exhibits a continuous ciliary reversal (CCR) and hence backward swimming if its cell membrane is steadily depolarized by high external K^+ concentration. The ciliary reversal is brought about by an influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels (Eckert 1972, Science, 176, 473), in the ciliary membrane (Dunlap 1977, J. Physiol., 271, 119). The K^+-induced CCR lasts several s or min depending on the external conc. of K^+ and of Ca^{2+}. Its duration is constant at a certain concentration range of these cations if the relation $[K^+]_o/[Ca^{2+}]_o$ is kept constant, indicating that the amount of membrane-bound calcium determines in some way the excitability (Grębecki 1964, Acta Protozool., 2, 69; Naitoh 1968, J. gen. Physiol., 51, 85).

After passing through CCR *Paramecium* becomes inexcitable. The excitability remains reduced even after the cells are washed for some min in a medium devoid of K^+. The half time of recovery is about 8 min. Besides the transient decrease of excitability an increase of the swimming rate can be measured. If the membrane is depolarized by K^+ at extremely low external Ca^{2+}-concentrations so as to abolish the inward-directed electrochemical Ca^{2+} gradient, the organism fails to show both the excitability decrease (Dryl and Hildebrand, in prep.) and the enhanced swimming. These results led to the hypothesis that the observed inactivation is coupled to the degree of preceding excitation and that the coupling is mediated by the Ca^{2+} influx. The recovery of excitability is thus thought to be caused by Ca^{2+} extrusion.

The K^+-induced inactivation shows a temperature coefficient of 1.3, consistant with a passive ion diffusion. The time course of the recovery has a Q_{10} of 2–3, indicating an active process. This is in line with the hypothesis stated above. Further support comes from the observation that a gradually reduced Ca^{2+} influx (caused by a decreased Ca^{2+} conc. gradient) which is measurable as a shortened CCR causes a decrease of inactivation.

The recovery of excitation after the cells have been washed free of K^+ is not exactly paralleled by the renormalization of the swimming rate, indicating that the mechanisms for both phenomena are not identical. The inactivation (adaptation) is thought to be caused by calcium which is bound to the cytoplasmic

face of the membrane thereby blocking the Ca^{2+} gates (Hildebrand and Dryl 1976, Bioelectrochem. Bioenerg. 3, 543). The increased swimming rate, on the other hand, may be either due to an increased K^+ permeability which would hyperpolarize the membrane and in some way reduce the Ca^{2+} leakage or to an enhanced active Ca^{2+} extrusion. Further experiments are presently performed to answer this question.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 149–150
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Localization of Filamentous Structures in Small Fragments of *Amoeba proteus*

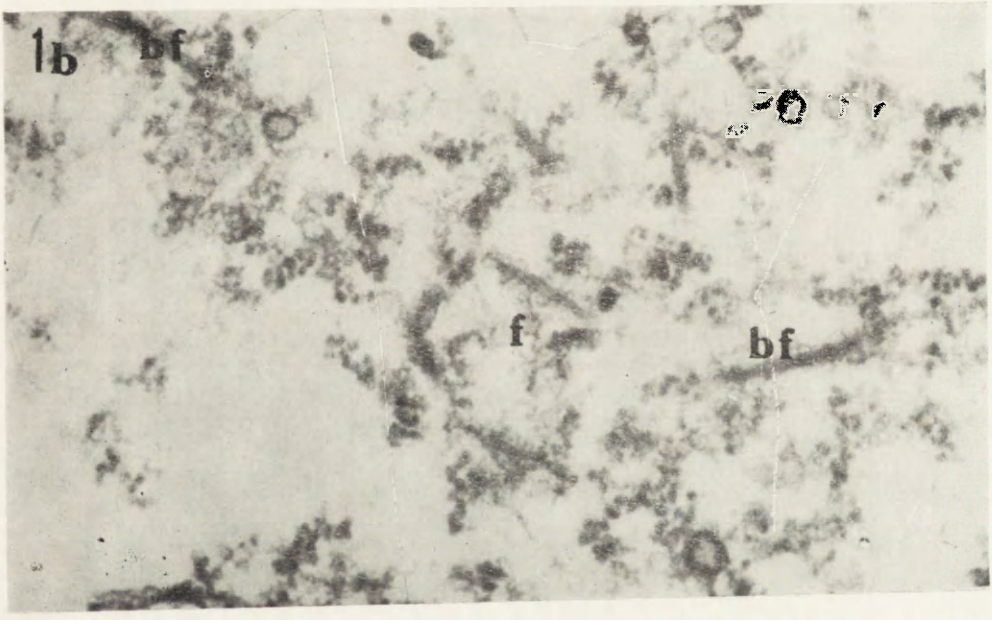
Barbara HREBENDA. Department of Cell Biology, Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3, Poland

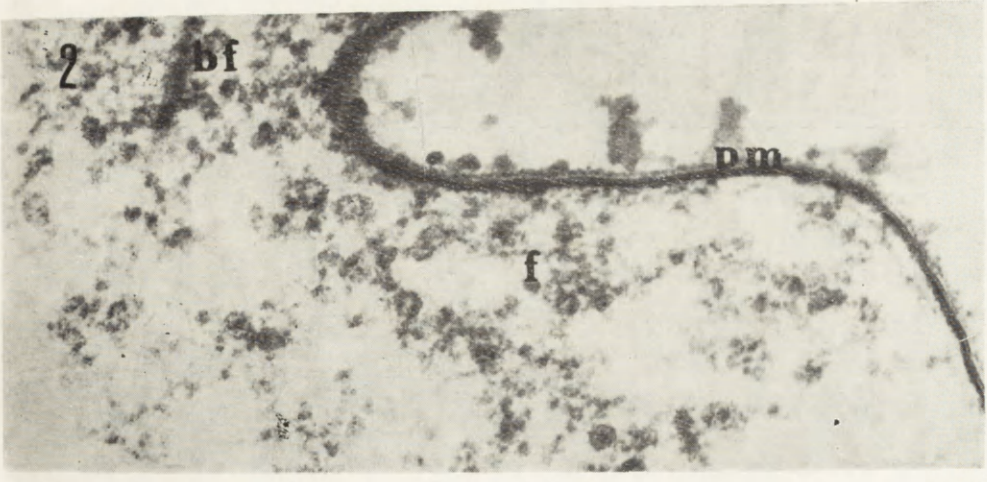
Investigations carried out during the last years establish in several primitive cells the presence of proteins similar to the contractile proteins in skeletal muscle. Such proteins has been also found in free living amebae using EM and different biochemical techniques (Nachmias 1964, Morgan et al. 1967) Nachmias 1968, Morgan 1971, Pollard and Korn 1971, Comly 1973). In naked cytoplasm of *Amoeba proteus* organization of filaments during ATP-stimulated moving can be observed (Pollard and Ito 1970). In more detail studies on extracts of slime molds cytoplasm the transformation of actin from non-filamentous to filamentous state has been observed. This phenomenon is interpretated as basic process in contraction–relaxation cycle in *Physarum* (Isenberg and Wohlfarth-Botterman 1976). The filamentous material in normally moving *Amoeba proteus* can exist as: (a) more or less arranged layer at the periphery of the cell (Fig. 1 a), (b) disordered filaments and their bundles in streaming cytoplasm (Fig. 1 b).

In fragments of *Amoeba proteus* obtained by centrifugation different kind of motility dependent on size, presence of nucleus and granular cytoplasm were noted (Kalisz 1977). In order to establish the role of filamentous arrangement in ameboid movement different kind of fragments manifesting different kind of movement should be investigated in EM. The first data concern filaments organization in the simplest, anuclear fragments. These fragments show only the pulsative movement. On micrographs the distribution of filamentous structures resembles the type "b" of arrangement in normal amoeba i. e., only disordered filaments and bundles (Fig. 2). The same kind of filaments distribution can be observed in amoeba cells treated with 2% ethanol or preheated (45°C). In these cells locomotorically ineffective pulsation has been observed too in spite of presence of nucleus (Korohoda and Stockem 1975). Thus, is seems possible that the presence of arranged layer of filaments in cortex is one of the basic conditions for normal locomotion of amoebae.

REFERENCES: Comly L. 1973: J. Cell Biol., 58, 230–237, Isenberg G. and Wohlfarth-Botterman K. E. 1976: Cell Tiss. Res., 173, 495–528, Kalisz B. 1977: Ph. D. Thesis, Korohoda W. and Stockem W. 1975: Microscop. Acta, 77, 129–141, Morgan J. 1971: Exp. Cell Res., 65, 7–16, Morgan J., Fyfe D. and Wolpert L. 1967: J. Cell Biol., 46, 267–289, Nachmias V. T. 1964: J. Cell Biol., 23, 183–188, Nachmias V. T. 1968: J. Cell Biol., 38, 40–50, Pollard Th. D. and Korn E. D. 1971: J. Cell Biol., 48, 216–219, Pollard Th. D. and Ito S. 1970: J. Cell Biol., 46, 267–289.

Plate: Fig. 1: Pattern of filamentous arrangement in intact amoeba, 1 a: filamentous layer parallel to plasma membrane, 54 000 × 1 b: filaments and their bundles inside cytoplasm, 54 000 ×, Fig. 2: Filaments distribution inside small fragments Abbreviations: filaments — f, bundles of filaments — bf, plasma membrane — pm





B. Hrebenda auctor phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 151
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Film: Rotating Filaments in an Eucaryotic Flagellum (*Synura sphagnicola*)

Robert JAROSCH Bot. Inst., Univ. of Salzburg, Austria

The film demonstrates by phase-contrast microscopy the genuine rotation of slender stiff filaments occassionally projecting out of the flagellum-tip (see Protoplasma, 85, 1975, 285–290).

Film: Filament-Dynamics in the Cytoplasm of *Nitella*

Robert JAROSCH. Bot. Inst., Univ. of Salzburg, Austria

"Active sliding force", transversal waves and pedulum-motion of filament-bundles are shown in cytoplasmic drops of *Nitella* (*Characeae*) by dark-field and negative phase-contrast microscopy. The branching-dynamics of very slender filaments are demonstrated. These dynamical networks move with the protoplasmic streaming in a rhizoid cell.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 153–156
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

How and Why Filaments Rotate

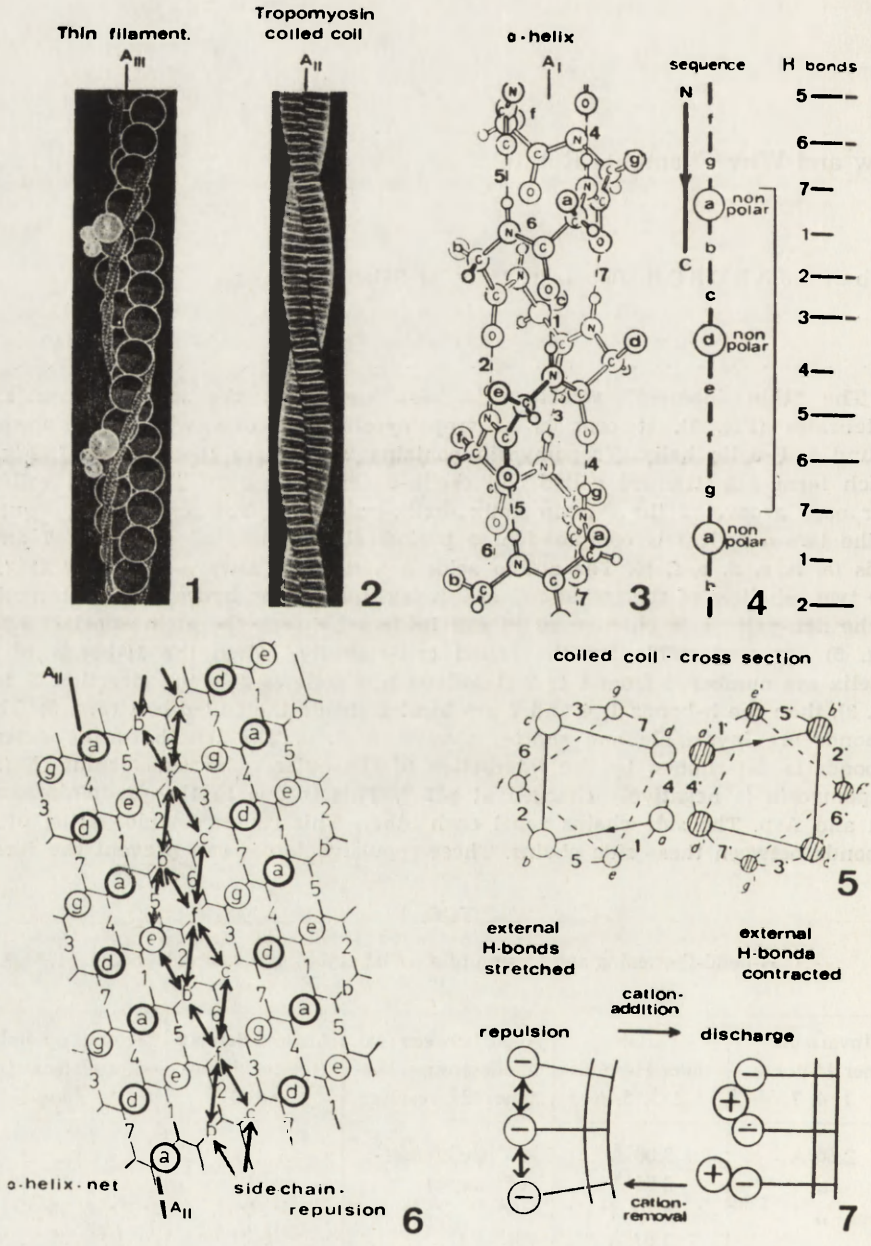
Robert JAROSCH. Bot. Inst., Univ. of Salzburg, Austria

The "thin filament" structure is best known in the striated muscle of vertebrates (Fig. 1). It consists of tropomyosin molecules which are wrapped around a F-actin helix. Tropomyosin contains 2 α-helices (helix-order I, Fig. 3) which form a 2-stranded coiled coil (helix-order II, Fig. 2). The coiled coil lies near each groove of the F-actin helix (helix-order III). The amino acid sequence of the two α-helices is composed of a periodical repetition of groups of 7 amino acids (a, b, c, d, e, f, g). The amino acids a and d are always non-polar (Fig. 4). The two α-helices of the coiled coil are in register. Strong hydrophobic interactions of the non-polar side chains and 18 salt bridges between the amino acids e and g (Fig. 5) are responsible for the coiled coil-stability. When the H-bonds of the α-helix are numbered from 1 to 7 (1 follows a, 7 follows g in the direction N to C, Fig. 3), than the h-bonds 1, 4 and 7 are located inside the coiled coil (Fig. 5). These H-bonds are invariable and shorter than 2, 3, 5, 6. The length of the external H-bonds is determined by the interaction of the outer polar side chains (Fig. 6). Tropomyosin is negatively charged at pH 7. This is due to the predominance of Glu and Asp. The side chains repel each other. This causes an elongation of the H-bonds between these side chains. These repulsive forces can prevent the forma-

Table 1

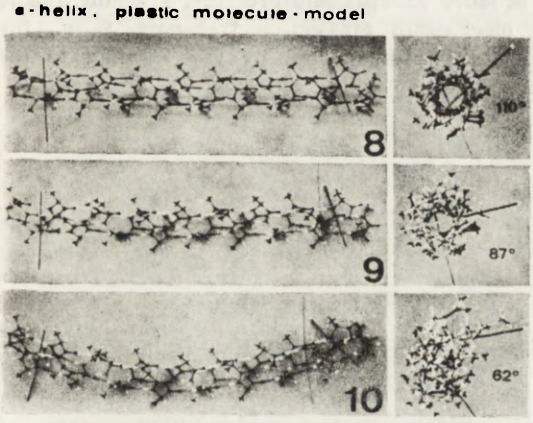
< H-bond-shortening and torsion of a coiled coil α-helix molecule-model

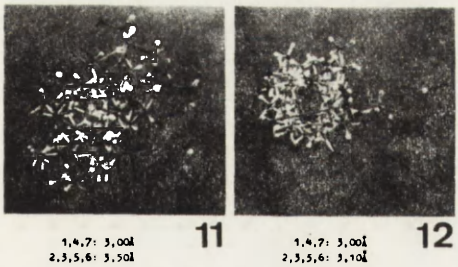
Invariable inner H-bonds 1, 4, 7	Variable outer H-bonds 2, 3, 5, 6	Angle between two amide-groups, distance 27 residues	Angle-difference (torsional motion)	Rotation-number of a filament 1 μm long
2.60 Å	3.00 Å	63° (reference-		
,,	2.85 Å	85° angle)	22°	15
,,	2.70 Å	99°	36°	25
,,	2.67 Å	101°	38°	27
,,	2.60 Å	110° (Fig. 8)	47°	33
2.67 Å	3.00 Å	62° (Fig. 10)	25°	17
,,	2.71 Å	87° (Fig. 9)		
3.00 Å	3.50 Å	−1° (Fig. 11)	45°	33
,,	3.10 Å	44° (Fig. 12)		

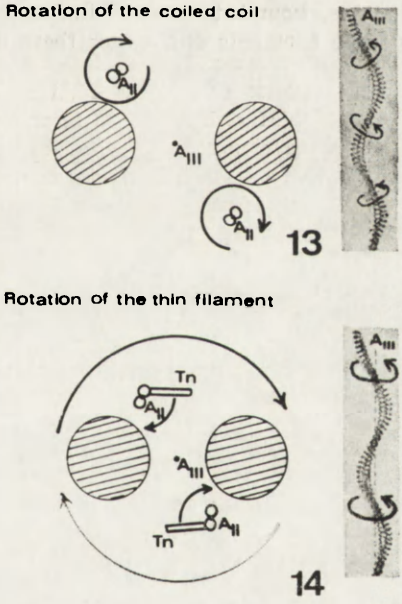


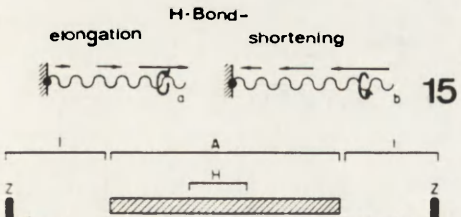
tion of H-bonds e. g., in polyglutamic acid. The addition of cations or a decrease in the pH value causes discharge and shortening of the H-bonds (Fig. 7). The positevely charged sites of Lys and Arg are more peripherally situated. Their side chains show a higher degree of rotatory freedom. Their attractive forces to Glu and Asp act therefore more vertically to the H-bonds and do not counteract

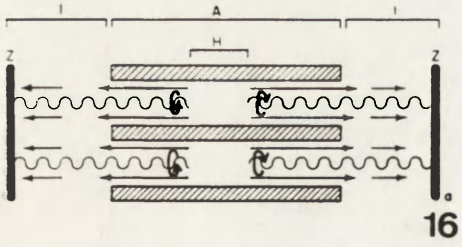
the shortening of the H-bonds. For steric reasons is each H-bond-shortening responsible for torsional movements of the coiled coil. This torsion can be demonstrated and measured on a molecule model (Figs. 8 to 12). Table 1 shows the torsion angles which arise after shortening of the H-bonds 2, 3, 5, and 6, and the calculated numbers of revolution being performed by the free end of a filament 1 μm long (cumulative effect). The action of the H-bonds seems to be cooperative in real α-helices. The length of the tropomyosin molecule does not change since the H-bonds along the axis A_{II} (Fig. 2) are invariant. There is therefore no change in the length of the unit cell-diagonal of Bailey-crystals although the other parameters are variable. The tropomyosin-rotation need not to be transmitted to the actin helix. It may take place around the axis A_{II} of the coiled coil (Fig. 13). This rotation would be transmitted in any case when

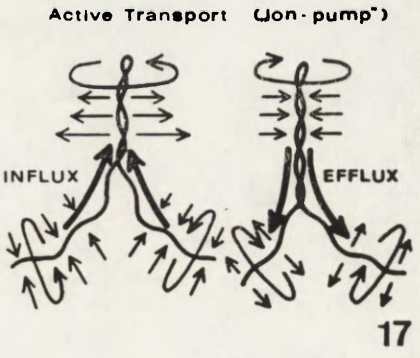












a binding linkage existed. This could be the mechanical function of troponin (Fig. 14).

Rotating helical filaments show either helical waves (arrows in Figs. 15 and 16) or the filaments drill into the opposite direction to these waves. In vertebrate striated muscle, the thin filaments may drill into the space between the myosin filaments and their cross bridges (Fig. 16) causing isotonic contraction. The assumption of a cyclic "power stroke" of these cross bridges is not necessary. There are supposedly other α-helical proteins besides tropomyosin which may generate torsion in a filament. Rotating thin filaments can be found together in a bundle where submicroscopic or microscopic waves occur ("active sliding force", active protoplasmic streaming, flagellar motion). The thin filaments can also branch into a netlike arrangement by winding and unwinding. This motion takes place without waves and may produce endoplasmic pressure. I would like to propose that this could also be a mechanism for active transport. E. g., the cations, bound to single filaments could be transported by the winding to the double filaments and under these new conditions displaced (Fig. 17).

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 157
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Twitch, Tetanus and Contracture of Frog Muscle Fibres Exposed to Na-octanoate

F. KÖSSLER. Central Institute Occupational Medicine, 1134 Berlin, GDR

Mechanical responses of thin bundles of frog muscles (*m. semitendinosus, m. iliofibularis,* about 10 fibres) were induced by square pulses (twitch), trains of pulses (tetanus) and by chemical agents such as potassium ions or caffeine. The induced tension was recorded isometrically by means of an RCA 5734 mechano-electric transducer and a direct print-out galvanometer oscillograph. Na-octanoate was added to the ringer solution flowing through a chamber which contains the bundle of fibres.

Provided that the electrical stimulation is supramaximal (length of muscles and pulse voltages were adjusted to give a maximum contractile response), the twitch: tetanus ratio in ringer solution takes a value of 0.5 ± 0.1 ($n = 100$). This relation is changed by hyperosmotic solutions and, for instance, attains a value of 0.1 in solution of threefold tonicity. An exposure of muscle fibres in 2.5 to 10 mM octanoate for 10 min decreases the amplitudes of twitch and tetanus nearly in the same degree, but does not effect the proportion of twitch and tetanus significantly.

Further, the effect of fatty acids on electrical and chemical induced tension was compared. The potassium induced contractures in ringer solution show a high variability which may be related to the physiological state of muscle cells. Usually, the contracture amplitude is higher than the twitch tension but always lower than the caffeine induced contracture. The contracture induced by 190 mM potassium is decreased or abolished if the fibres are bathed 10 min in 5 mM or 10 mM octanoate, respectively.

With 10 mM or more caffeine a contracture could be induced which is not lower than the tetanus. This mechanical response seems to be the most sensitive process to fatty acids. In muscle fibres, bathed 10 min in 2.0 mM octanoate before the addition of 10 mM caffeine, the increase of tension is delayed but at last nearly the same amplitude as in controls is recorded. Therefore it is to suppose that fatty acids influence the process of calcium release by caffeine but do not alterate the contractile proteins. This suggestion could be confirmed by investigations with glyzerinated muscle fibres. Such fibres develop with ATP tensions, which are not changed by pretreatment in 10 mM octanoate. Especially if the caffeine solution also contains fatty acid a very strong protraction and a dissociation between the initial quick increase of tension and a second slow one could be seen. In preliminary investigations of the motion of *Paramecium* and of the contractility of *Vorticella* a depressive effect of fatty acids could be observed, too.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 159
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Changes of Intracellular Potential in *Stylonychia mytilus* at Low Level of External Calcium

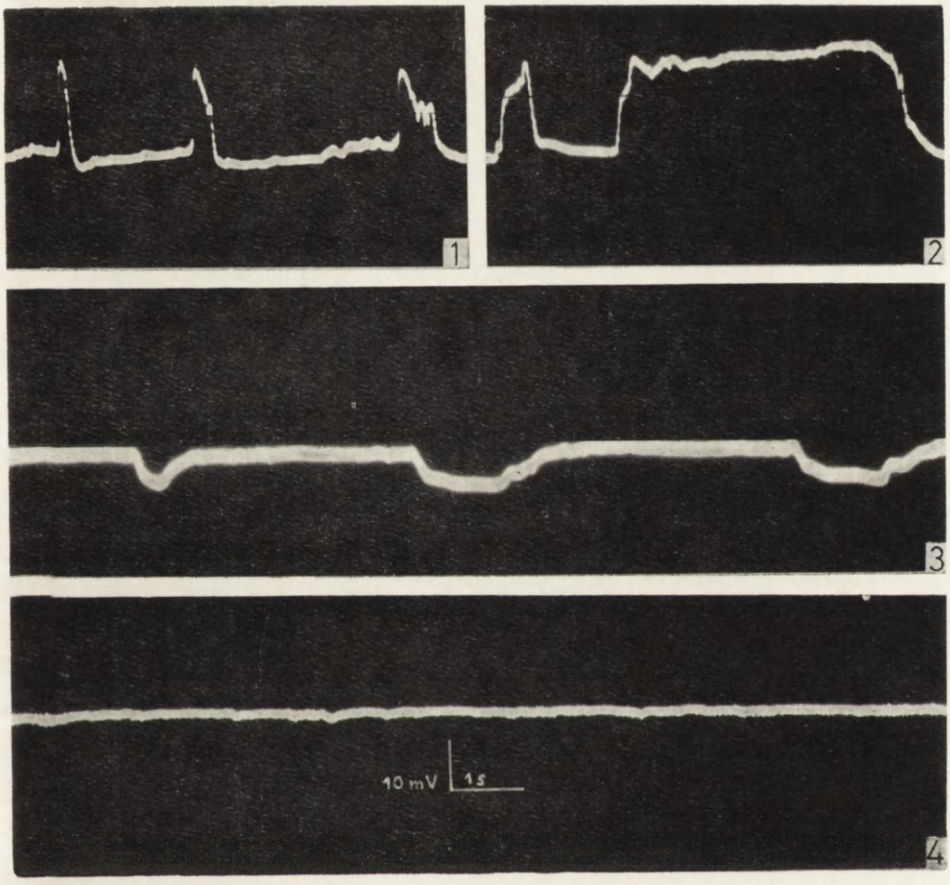
Andrzej KUBALSKI, Stanisław DRYL and Anna ŁOPATOWSKA, Department of Cell Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, 02-093 Warszawa, Poland

Under normal conditions *Stylonychia mytilus* moves forwards along arcs or is swimming along spiraling line with frequent spontaneous, short-lasting backward turnings due to reversed beat of AZM or somatic cirri. Depolarizing spikes (usually with characteristic prolonged plateau) are accompanying the above mentioned motor responses (Fig. 1 and 2).

In solution 1 mM $CaCl_2$ + 1 mM Tris/HCl (pH 7.1) *Stylonychia* shows resting potential (RP) of average value — 19–20 m[1]. Marked depolarization of cell membrane to average values of RP — 11–12 m[1] was noticed in all ciliate cells transfered to EGTA/Ca buffer solutions of pCa 5.5 or 6.0. Spontaneous "pacemaker" activity of AZM and cirri was strongly decreased at pCa 5.0 (Fig. 3) and completely abolished at pCa 6.0 (Fig. 4). This is apparently in good agreement with recent behavioral studies by Dryl et al. (1977) who noticed loss of reactivity in *Stylonychia mytilus* exposed to mechanical or chemical (KCl) stimulation at low level (pCa 5.0–6.0) of external calcium.

REFERENCES: Dryl S., Kurdybacha J. and Totwen-Nowakowska I. 1977: Abstr. Fifth int. Congr. Protozool., New York 1977 (ed. S. H. Hutner) (abstr. 304)

[1] Based on measurements of intracellular potential from ten ciliates.



A. Kubalski et al. auctores phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 161
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Identification of Myosin Thiol Groups

P. A. KUNZ, J. T. WALSER, J. G. WATTERSON and M. C. SCHAUB.
Department of Pharmacology, University of Zürich, Gloriastrasse 32, CH-8006 Zürich, Switzerland.

The myosin molecule consisting of 2 heavy chains (200 000) and 4 light chains contains 2 active centers. Limited proteolysis with chymotrypsin produces active isolated heads, each containing a single heavy chain (now 90 000) but only one light chain, since during the digestion 2 light chains (both DTNB-chains) become separated from the heads. Associated with each active center are 2 essential thiol groups which can be alkylated with radioactive reagents in either intact myosin or isolated heads. In the presence of the hydrolytic product Mg-ADP, these 2 groups can be selectively labeled since the binding of this ligand renders them the most reactive of all thiol groups. After treatment of such labeled preparations with CNBr, a single peptide (10 000) was isolated from the heavy chain of both myosin and heads containing the 2 essential groups. Tryptic digestion followed by 2-dimensional high voltage electrophoresis at pH 6.5 and 1.9 resolved the radioactivity into 2 distinct peptides. Amino acid analysis identified them to be the known tryptic peptides of thiol-1 and thiol-2.

When intact myosin is labeled during hydrolysis of Mg-ATP at 25°C a third type of thiol group not essential for the enzymic activity is the first to be blocked. However, it is not possible to label this group in isolated heads where the essential thiol groups are the most reactive also under this condition. In fact, heads prepared from such labeled myosin lose the radioactivity. During the proteolysis with chymotrypsin a soluble fragment (11 000) which is simultaneously produced along with the heads, was found to carry this label. Electrophoretic mapping after tryptic digestion of intact myosin labeled in this way, identified a third class of thiol group distinct from thiol-1 and thiol-2. Using the same technique this class was also found to be distinct from the 2 thiols of the DTNB-chain. Thus there is a class of non-essential thiol-3 groups located in the myosin heavy chain and separable from the active center of isolated heads.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 163
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Anodal Ectoplasmic Contraction in *Paramecium caudatum* at Various Levels of External Calcium

Jacek KURDYBACHA and Stanisław DRYL. Department of Cell Biology, Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3, Poland

It was suggested by the authors elsewhere (Dryl and Kurdybacha, Acta Protozool., this volume), that the cathodal orientation of *Paramecium* in medium of low concentration of external calcium (pCa 6.0) is due to the anodal response of cilia. The ectoplasmic contraction is another well known phenomenon which can be induced by the DC stimulation. Preliminary observations of the authors (Kurdybacha and Dryl, unpublished) revealed that the voltage gradient of approximate value 7.1 V cm^{-1} proved to be sufficiently high to induce strong contraction of cell body without marked injury of the cell membrane.

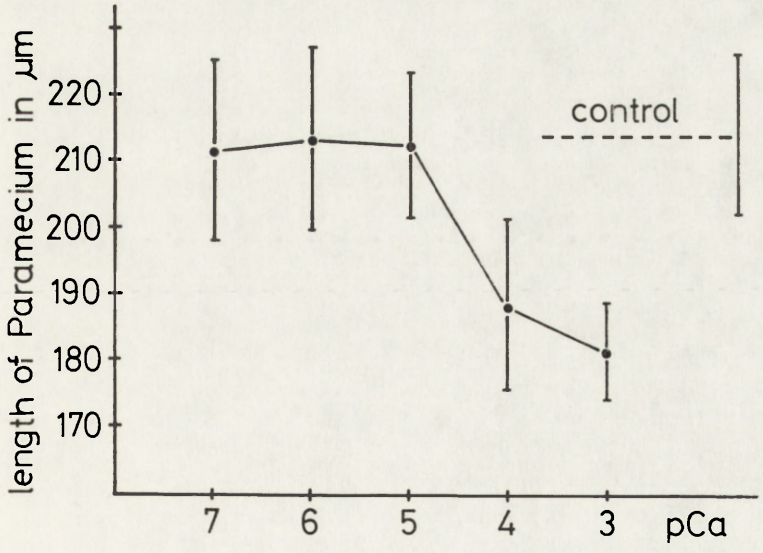


Fig. 1 The dependence of DC induced ectoplasmic contraction in *Paramecium caudatum* on the level of calcium ions in external medium. The applied voltage gradient was 7.1 V cm^{-1} in all experiments. Each point of the cummulative diagram represents the mean value (and S. D.) from 10 measurements

Changes of the length of *Paramecium* was the measure of the degree of contraction. The results of experiments from Fig. 1 show no contraction of cell body at pCa 5.0, while graded increase of contraction was noticed in the range of pCa 5.0. The achieved data suggest that external calcium plays essential role in induction of contractile phenomena within cytoplasm of *Paramecium* during DC stimulation. This suggests possibility that in some way the external calcium may control the transmission of information from outside to the cell interior, contributing to eventual release of calcium from cellular internal stores. The mechanism of this information transfer depends probably on conformational changes within cell membrane induced by external stimuli under control of external calcium ions.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 165
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Computer Image Processing in the Analysis of Leukemia Cell Locomotion

Krystyna LEWANDOWSKA, Jan DOROSZEWSKI, Gisela HAEMMERLI and Peter STRÄULI. Department of Biophysics and Biomathematics, Medical Center of Postgraduate Education, Warsaw, Poland, Division of Cancer Research, Institute of Pathology, University of Zürich, Zürich, Switzerland

A computer image processing system CPO-2/K-202 has been used in the study of locomotion of leukemia cells performed in collaboration with the Institute of Biocybernetics and Biomedical Engineering, Warsaw. The system consists of a TV camera, a digital converter and a memory and is connected to a minicomputer. It comprises also two TV monitors and a joy-stick marker.

The cells used in this study were L 5222 rat leukemia cells suspended in MEM with 20% FCS. They display locomotive activity on a glass surface and adopt a polarized shape with one major cytoplasmic extension. Changes of shape occurring during dislocation of the cell were recorded by means of time-laps cinematography.

The cells to be analyzed were filmed with an oil-phase contrast objective on 16 mm film at intervals of 2 s. A phase contrast photograph was first converted into the digital picture consisting of 512 × 512 points in 16 grey levels. A program has been developed that transforms the digitized picture into binary cell image, on which various measurements could be performed.

The following parameters were analyzed: "center of mass" for the cell body and the whole cell, direction of the main axis of the whole cell, its area and perimeter, directional characteristics of largest extension and cell shape factors (rectangular approximation of shape, six auxiliary diameters and global shape factors). For each cell a sequence of time-lapse film frames were chosen and the above parameters were computed.

The usefulness of different quantitative factors was investigated in respect to the description of the shape and position changes of cells during their active movement. This approach provides a more detailed and precise information as can be obtained by graphic evaluation alone. The possible applications of the computer cell locomotion analysis are discussed as well as the limitation of this method.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 167
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Chemotactic Stimulation in *Dictyostelium disoideum:* Mechanism of Sensory Transduction

Jose M. MATO and Theo M. KONIJN. Cell Biology and Morphogenesis Unit, Zoological Laboratory, University at Leiden, the Netherlands

The present interest of chemotaxis is concentrated on the following three problems: (i) what are the chemotactic signals and how are they detected; (ii) what regulatory molecules change their level during chemotactic stimulation; and (iii) how modulate these molecules the motile response. Amoebae of *D. discoideum* show many advantages to solve these problems. Adenosine 3' : 5'-cyclic monophosphate (cAMP) is the chemotactic molecule of aggregative cells of *D. discoideum* (1). A model for the interaction cAMP-chemoreceptor, based on the chemotactic activity of more than 50 different cAMP derivatives, will be discussed. Guanosine 3' : 5'cyclic monophosphate (cGMP) mediates chemosensory transduction in *D. discoideum* (2–5). Evidence indicating that cAMP activates guanylate cyclase in *D. discoideum* will be given. cGMP action is mediated by an intracellular cGMP receptor(s) (6). The properties and possible function of this receptor during chemotaxis will also be discussed.

REFERENCES: (1) Konijn T. M., van de Meene J. G. C., Bonner J. T. and Barkley D. S. 1967: Proc. natn. Acad. Sci. USA, 58, 1152–1154, (2) Mato J. M., Krens F. A., van Haastert P. J. M. and Konijn T. M. 1977: Proc. natn. Acad Sci. USA, 74, 2348–2351, (3) Wurster B., Schubiger K., Wick U. and Gerisch G. 1977: FEBS Lett., 76, 141–144, (4) Mato J. M., van Haastert P. J. M., Krens F. A., Rhijnsburger E., Dobbe F. and Konijn T. M. 1977: FEBS Lett., 79, 331–336, (5) Mato J. M., Krens F. A., van Haastert P. J. M. and Konijn T. M. 1977: Biochim. Biophys. Res. Commun., 77, 399-402, (6) Mato J. M., Woelders H., van Haastert P. J. M. and Konijn T. M. 1978: FEBS Lett., (in press).

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 169–171
Int. Symposium on Cell Motility, June 26–28, Warszawa, Poland

Active Proton Transport Across the Surface Membrane of the Slime Mold *Physarum polycephalum*

N. B. MATVEEVA, S. I. BEYLINA, V. A. TEPLOV and D. B. LAYRAND. Institute of Biological Physics, USSR Academy of Sciences, Pushchino, Moscow Region, USSR

In order to understand the mechanism of cell locomotion, it is essential to know what is the connecting link between reception of the external stimulus and the regulating system of the contractile apparatus.

In approaching this problem, investigation of all membrane processes, in particular the study of the active proton transport across the plasmodium membrane presented here is of great interest.

The nature of the revealed phenomenon of acidification of the medium by plasmodium *Physarum polycephalum* has been studied. The slime mold was cultivated by the method of Camp. The experiments were carried out on plasmodium films. The methods of pH-measurement, H^+-titration and inhibitor analysis were used.

The plasmodium acidifies intensively nonbuffered media. The acidification is a metabolically dependent process. The titration curve in the acid range shows a peak of buffering capacity at pK of lactic acid. The acidification and the lactate peak increase under anaerobic conditions. In the presence of glucose and cyanide and decrease after addition of iodoacetate. The production of lactate in normal conditions suggests the occurrence of aerobic glycolysis in plasmodium.

However, the below presented results and arguments suggest a proton transport which, along with the lactate release, contributes to the acidification. In the presence of potassium salts (10^{-4} to 10^{-2} M) the acidification rate increases distinctly. This effect is characteristic of K^+ and Rb^+ and is not observed in the presence of Na^+ (Fig. 1). The rise of acidification rate is accompanied neither by increased production of acids (Fig. 3) nor by increased CO_2 concentration in the medium (Fig. 2). This enables one to relate this process to the proton transport across the plasmodium membrane.

Intracellular pH determined by redistribution of protons between cell and medium upon disturbance of the membrane selectivity is found to be about 7.5 (Fig. 4). The temperature dependence of the K^+-effect as well as the data of experiments with inhibitors have shown the proton efflux to be metabolically dependent. This rules out the possibility of a passive exchange. The proton efflux occurs against the gradient of the H^+-electrochemical potential and requires energy supply, the direct one or via an exchange process.

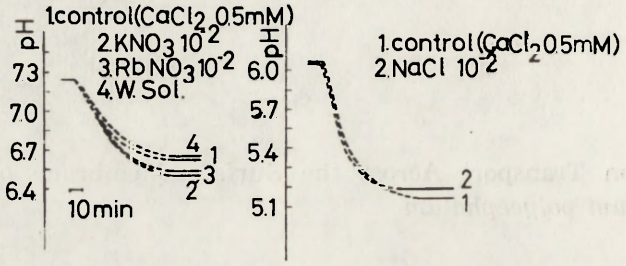


Fig. 1. Effects of monovalent cations on the rate of plasmodium-induced acidification. Measurements were carried out in perfusion chamber. Proton output in the presence of potassium

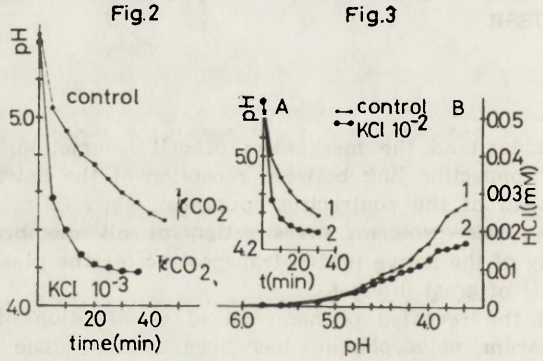


Fig. 2. Absence of potassium activation of CO_2 evolution in plasmodium. The bars indicate the pH-shifts produced by the complete release of CO_2 from medium. Both pH-shifts are equal in H^+-concentration units

Fig. 3. Effect of potassium on the acidification and buffering capacity of the medium. A — increase of intensity of the medium acidifaction in response to KCl 10^{-2} M, B — corresponding change of the medium buffering capacity; 1 — control, 2 — KCl 10^{-2} M. Prevention of the medium against the trace amount of CO_2 was made in the course of titration

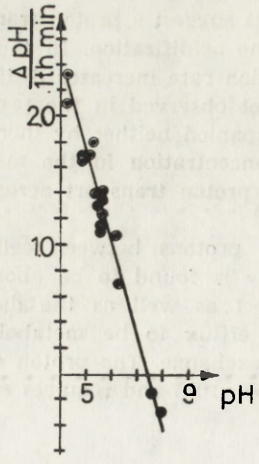


Fig. 4. pH-relationship of the initial rate of the medium pH-changes upon damage of the plasmodium membrane. The linear character of this plot is indicative of the absence of processes other than diffusion. The cross point with the abscissa corresponds to the intracellular pH

In the course of valinomycin-induced alterations in the passive potassium flux, changes in the medium pH were observed. The changes were dependent upon external concentration of the protons and potassium (Fig. 5). This enables us to conclude that the plasmodial membrane is permeable to proton.

With constant potassium concentration, the intensity of proton transport determined by the difference between the acidification rate in the K+-medium and in the control increase (Fig. 6).

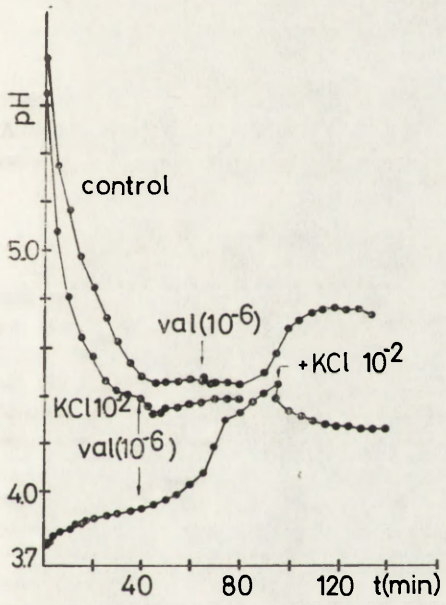


Fig. 5. Passive K+–H+ exchange in the presence of valinomycin

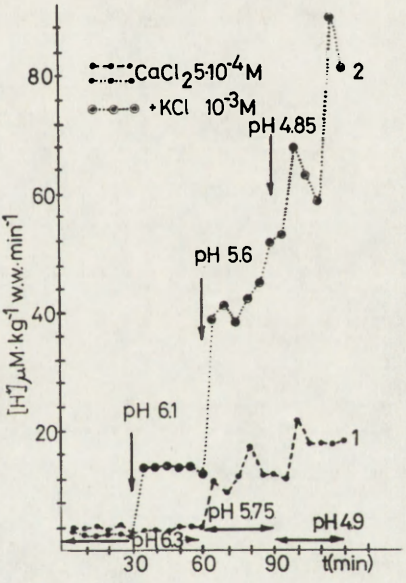


Fig. 6. Effect of external pH on the rate of medium acidification 1 — control, 2 — in the presence of potassium

These results enables the assumption that on the plasmodium membrane there exists a K+-activated system of active proton transport.

External calcium is shown to affect the proton transport. The physiological functions of the active proton transport system might be electrogenesis, transport of ions and nonelectrolytes as well as the membrane control of pH changes in the plasmodium cortex. These changes are able to affect the state of contractile proteins both directly and indirectly, for example, via changes in the level of free Ca++ induced by mitochondria and/or the calcium-pumping system in vacuoles.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 173–176
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Chemotactic and Proton Responses of the Slime Mold *Physarum polycephalum* to Non-Metabolizable Glucose Analogues

N. B. MATVEEVA, S. I. BEYLINA, V. A. TEPLOV and D. B. LAYRAND. Institute of Biological Physics, Academy of Sciences USSR, Pushchino, Moscow Region, USSR

An assumption is made that the motile response of the plasmodium during taxic might be due to pH-changes in the cortical gel layer under the membrane. The basis for this assumption is the presence of K^+–H^+-activated system of proton transport in the membrane of myxomycete *Physarum polycephalum* as well as the evidence for the decrease of intracellular pH in response to glucose, an attractant for plasmodium, and the increase of pH by repellents (KCl and monoiodacetate).

In view of this, the effects of structural glucose analogues 2-deoxy-D-glucose (2-DOG) and 3-O-methyl-D-glucose (3-MeG) on both the motile reactions and the proton response of plasmodium have been studied.

Local treatment by the analogues caused a reorientation of the migrating plasmodia. The motive force of such a movement could be registered as locomotive pressure occurring in the direction of the applied stimulus (Fig. 1). Upon total treatment, the plasmodium stopped to migrate, it lost its specific migrating form and looked like flattened sheet protoplasm with a smooth surface. The durations of the stop and the morphological reaction were the same as in the case with glucose.

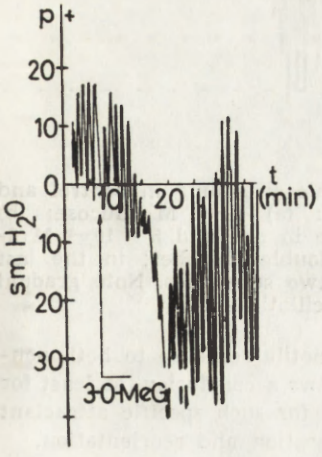


Fig. 1. Polarization of the motive force of protoplasmic streaming (locomotive pressure) in direction to the applied stimulus. Plasmodium was treated in one of the compartments of double chamber with 10^{-2} M 3-O-Methyl-D-glucose. Control solution 0.5 mM $CaCl_2$

Both analogues caused a well-known reaction of isometric tension decrease (Ueda et al. 1975) and a pronounced drop of elasticity, which could be judged from strand reaction to stretch (Fig. 3). The spreading as well as the decrease of the motive force in response to treatment of the whole plasmodium in the double-chamber by both glucose and its analogues seems to be connected with this drop of elasticity. As shown in Fig. 2, a slow restoration of the motive force oscillations occurred. The time of that restoration corresponded to that required for the appearance of chaannels in the smooth film. The time of elasticity restoration depended on the concentration of the attractant. We did not make a comparson between the thresholds of glucose and its analogues but at the same concentration (5×10^{-3} M in Fig. 2 c), no preference for the substratum was observed.

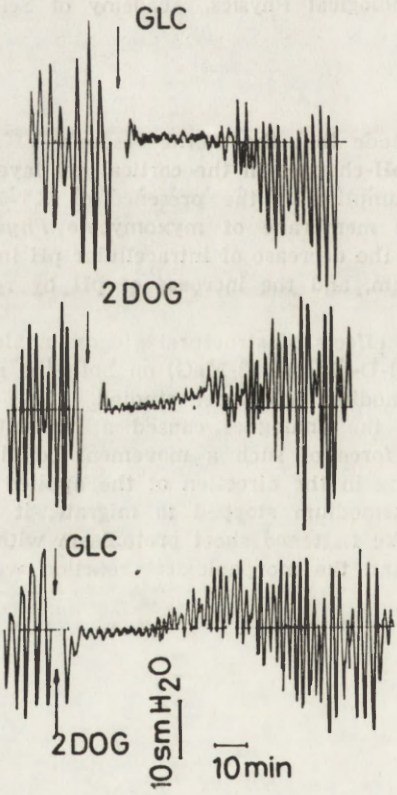


Fig. 2. Time course of the motive force of protoplasmic streaming in control and under simultaneous treatment of plasmodium with: (a) 10^{-2} M glucose; (b) 5×10^{-3} M 2-deoxy-D-glucose; (c) 5×10^{-3} M glucose in one and 5×10^{-3} M 2-deoxy-D-glucose in the other compartment of the double chamber; in the last case plasmodium does not distinguish between these two substrates. Note gradual restoration of the motive force oscillations

Thus *Physarum polycephalum* exhibits the same motile response to both non-metabolizable glucose analogues and glucose, which allows a conclusion, at least for carbohydrates, that metabolic activity is not necessary for such specific attractant reactions as elasticity decrease, spreading, stop of migration and reorientation.

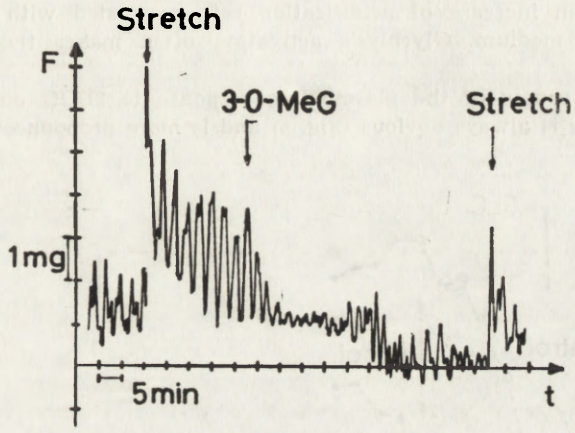


Fig. 3. Effect of 3-O-Methyl-D-glucose 10^{-2} M on the isometric tension of the plasmodial strand. The difference in the height of the spikes in response to the stretch (20% in control and in the presence of the carbohydrate is due to the drop of elasticity)

The evidence for the reversibility of elasticity decrease seems to be important in view of the nature of chemotactic motile response since this mode of action correlates with general principles of chemotactic reactions. The chemotactic reactions to the structural analogues and the absence of changes in oscillation patterns in the plasmodium strands whose membrane is damaged by detergent (Fig. 4) support a conclusion on the presence of a receptor system on the plasmodium membrane (U e d a et al. 1975).

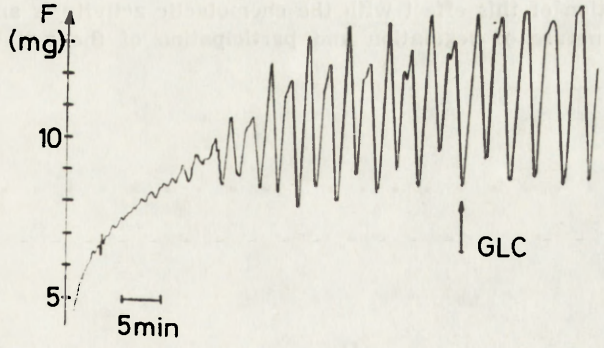


Fig. 4. The absence of changes in the pattern of force oscillations in the detergent-treated plasmodium strand in response to glucose/10^{-2} M

The changes of the plasmodium intracellular pH produced by the non-metabolizable analogues were compared with those produced by glucose and were about 0.2–0.4 pH units. The nature of this phenomenon may be related to the mode of carbohydrate transport.

The time course of the medium pH changes in the presence of glucose is, in some cases, of double-phase nature (Fig. 5). The first short phase, alkalization,

is followed by an increase of acidification rate associated with the lactic acid release into the medium. Glycolysis activation often makes the starting phase obscure.

In the time course of the plasmodium response to 2DOG and 3-O-MeG the alkalization phase is always obvious (Fig. 5) and is more pronounced and prolonged.

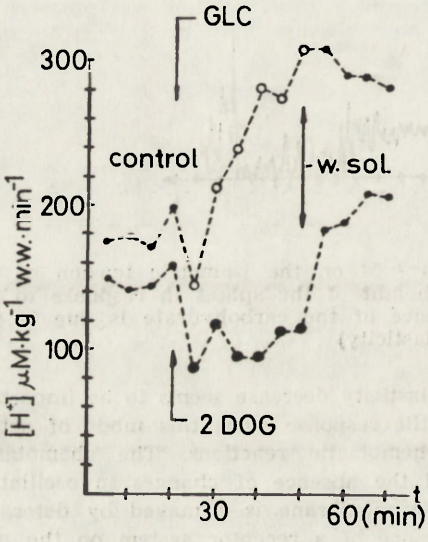


Fig. 5. The time course of the rate of plasmodium-induced changes in pH of the medium in the presence of glucose and 2-deoxy-D-glucose. The alkalization in the presence of 2-deoxy-glucose is more pronounced and prolonged

This suggests a possibility of intracellular pH changes connected with carbohydrate co-transport with protons.

The correlation of this effect with the chemotactic activity is an argument for the membrane nature of regulation and participation of the active proton transport in it.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 177
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Short Range Co-ordination of Motory Functions in the Plasmodial Veins of *Physarum polycephalum*

Mariola MOCZOŃ and Andrzej GRĘBECKI. Department of Cell Biology, M. Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3, Poland

The widely accepted assumption that plasmodia are polyrhythmic systems, was tested to learn how the polyrhythmicity is expressed in short segments of veins: are the rhythm differences manifested either when two different parameters of contraction are confronted at the same site, when the same parameter is recorded at two sites, or when it is compared in two anastomosing veins.

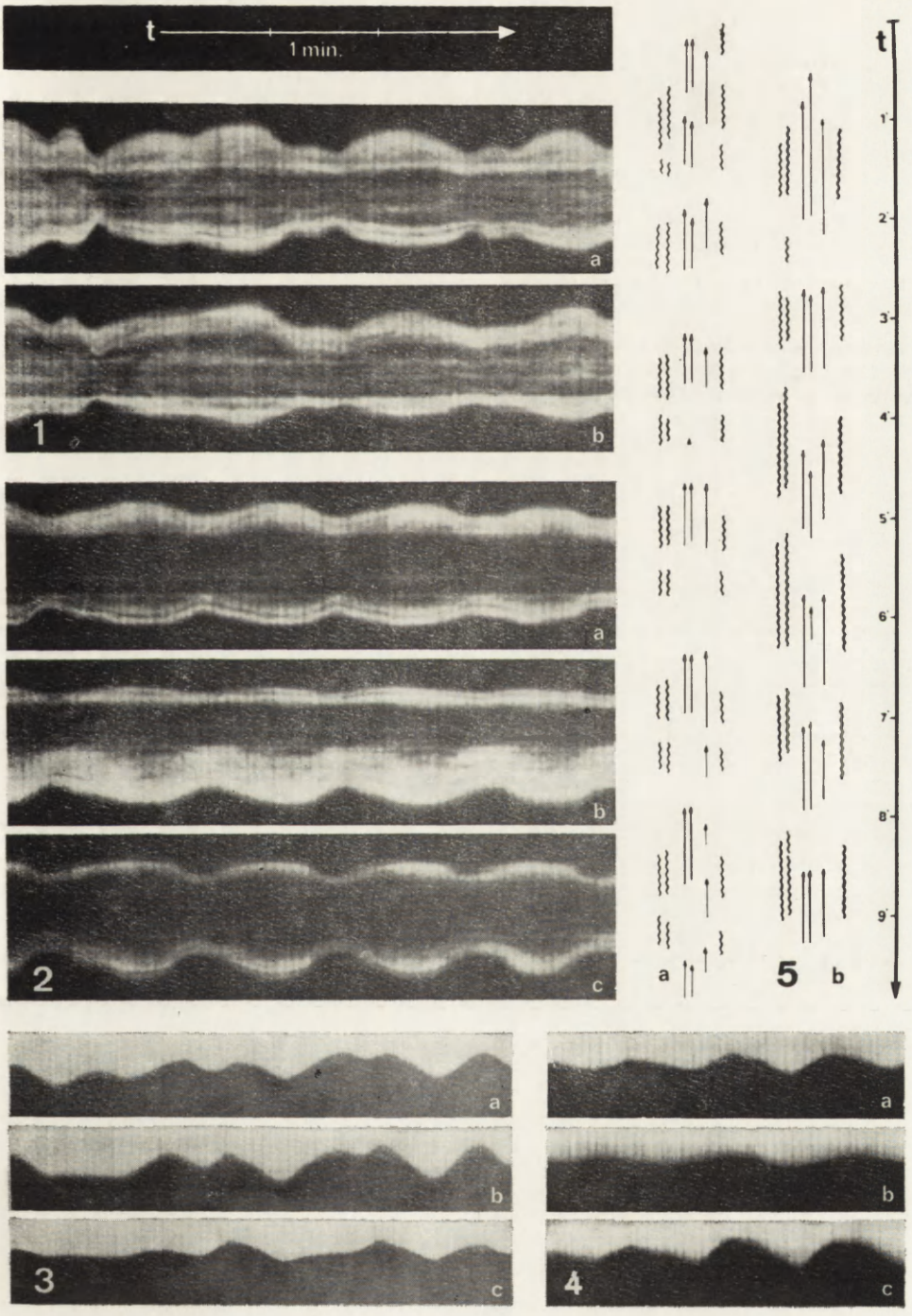
Time lapse films of plasmodial veins were projected on the screen with vertical slit covered with semi-transparent material. The 70 mm camera with horizontal continuous film run was focused upon the slit from the other side of the screen. The curves obtained on the 70 mm film strip express the rhythmical changes of vein's linear dimensions at the point of application of the slit.

The non-branched segments of veins *in situ* do not manifest any propagation of pulsation. Their contraction–relaxation rhythms recorded at two sites (Fig. 1 a and b), ca. 1 mm distant from one another appear almost exactly synchronous. The general synchrony of radial contraction is also evident when estimated in the vertical dimension. Only some slight and irregular shifts of phase may be found between up and down oscillations of the vein's ridge, recorded at three sites (Fig. 3 a, b, c) separated by the distance of ca. 0.5 mm from each other. Isolated fragments of veins were winded round cylindrical agar gel rods. When the microscope is focused upon the rod edge, the vein is seen in its optical cross section. The pulsation curves (Fig. 4) recorded at the top of vein (b) and at both its lateral slopes (a and c) follow the same general contraction-expansion rhythm, but large amplitude differences and slight phase differences are seen between the ridge and the slopes, leading form the more concave profile in the contracted state to the more convex shape under the expanded condition.

The periodicity of pulsation was investigated *in situ* in branching veins (Fig. 2): in a major vein before junction (a) and behind it (b), and in the lateral branch (c). Again, the three sites pulsate synchronously, indicating that the junction of veins has no influence on the rhythm of contractions. Periodicity diagrams of pulsation and of streaming alternations, at the junctions of veins, were constructed (Fig. 5). Two wavy lines on the left show the contraction periods of the main vein before and behind junction, and the double arrows indicate the periods of forward streaming at the same two sites. The single arrows and the single wavy lines

show the forward streaming and the contractions in the branch-vein. The pulsation periods are strongly correlated in the major vein before and behind junction, and in the lateral vein. Correlation of the flow directions is also high at both sites in the major vein, but insignificant between this vein and its branch. A significant but weak correlation is found between the contraction and the forward direction of flow.

It should be concluded in general that in short segments of veins, either investigated *in situ* or as isolated fragments, the different parameters of contraction–relaxation cycles manifest a common rhythm. It allows to expect that all the parameters of contraction are probably synchronized in the whole plasmodium.



M. Moczoń et A. Grębecki auctores phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 179
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Stability of a Resting Muscle Mechanism of Muscular Contraction and a Possible Role of the Two Heads of Myosin

Jean E. MOREL and Marcel P. GINGOLD. Département de Biologie. Service de Biophysique. Centre d'Etudes Nucléaires de Saclay. BP N° 2 91190-Gif-Sur-Yvette, France

We have recently proposed a new approach of the mechanism of muscle contraction and cytoplasmic streaming (Morel et al., 1976, J. Theor. Biol., 62, 17). The main feature of this model is that attached cross-bridges exert tractions on the thin filaments at rest. These tractions disappear during contraction and this leads to a lateral swelling of the myofibrillar lattice and therefore to contraction in the direction of the filaments. Electron micrographs show that MgATP acts on both the myosin-myosin and myosin-actin affinities. The MgATP sites of fixation on myosin being most likely entirely localized on the myosin heads, the variations in the myosin-myosin affinity vs. the concentration of MgATP is due to a variation in the head-to-head interactions. We therefore propose that head-to-head interactions exist in the myosin filaments and that one head of myosin interact with the head of another myosin inside the backbone of the thick filament. The other head of myosin lies outside the backbone and can interact with actin. With such a structure, the existence of restoring forces exerted by the cross-bridges attached at rest is easily explained. Under the action of MgATP at rest, the myosin head lying inside the backbone is submitted to a strong attraction in the direction of the centre of the backbone and the myosin molecule, attached to actin by the intermediary of its other head, is submitted to a restoring force directed from the thin towards the thick filament. The stability of the myofibrillar lattice at rest results thus from a balance between the electrostatic repulsive forces and the Van der Waals-London attractive forces, reinforced by the restoring forces exerted by the attached cross-bridges. During contraction, the chemical energy released from ATP splitting induces a conformational change in the myosin heads, which become incapable of fixing MgATP. MgATP being at the origin of the restoring forces, its release from the myosin heads results in the disappearance of the restoring forces. The electrostatic repulsive forces become therefore automatically predominant and contraction occurs by lateral swelling of the myofilament lattice. A similar reasoning may be proposed in the case of non-muscle contractile cells.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 181
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Cholinergic Control of Calcium Transport: Regulation of Sperm Motility

Leonard NELSON, Department of Physiology, Medical College of Ohio, Toledo, Ohio 43699, USA

The spermatozoa of *Arbacia punctulata* respond biphasically to some cholinergic agents, by increasing motility at low concentrations and decreasing movement at higher concentrations. Other agents, e.g., those that block acetylcholine synthesis or acetylcholine binding to cholinergic receptors (e.g., α-bungarotoxin) cause only a depression in progressive motility at all effective concentrations. Calcium ions and substances which alter calcium ion uptake or binding by the sperm cells also adversely affect the swim rate and pattern of motility. Since quaternary ammonium compounds penetrate the sperm cell only slowly or with difficulty except when facilitated by dimethyl sulfoxide, a hypothetical model has been formulated which depicts a cholinergic receptor extending through both the external and the cytoplasmic surfaces of the plasma membrane. It has been proposed that this receptor complex serves as a calcium ionophore which undergoes a conformational change in response to acetylcholine which is cyclically synthesized by intracellular choline acetyltransferase. Hydrolysis of the receptor-bound acetylcholine by the closely associated (spatially) acetylcholinesterase permits reversal of the conformationally changed calcium channel and simultaneous extrusion of the excess free calcium ion.

Normally the intracellular free calcium is considered to be maintained at a low level by sequestration at storage sites within the cell. Calcium, entering from the environment in response to cholinergic stimuli, is thought to trigger the release and transport of calcium ions to mediate the spermatozoan equivalent of excitation-contraction coupling of the flagellar contractile protein system.

Recent electron microscopic studies of calcium (and $LaCl_3$) distribution in isolated bull sperm plasma membrane vesicles clearly demonstrate that the cytoplasmic surface of the plasma membrane provides the most likely site for the sequestration and release of the intracellular calcium.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 183
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Electrophysiological Consequence of Deciliation in *Paramecium*

Akihiko OGURA[1] and Hans MACHEMER. Arbeitsgruppe Rezeptoren, Lehrstuhl Allgemeine Zoologie, Ruhr-Universität Bochum, D-4630 Bochum, Fed. Rep. Germany

Violent shaking of *Paramecium caudatum* (Ciliata) in 5% ethanol induces the loss of the somatic cilia, which regrow within 4 h in an ethanol-free medium (Ogura 1977). Electrophysiological experiments of the ethanol-deciliated paramecia in solutions of 1 mM $CaCl_2$ + 1 mM KCl gave the following results: (1) The cells fail to produce regenerative membrane depolarizations either to injected outward current pulses or to mechanical stimuli applied to the anterior region of the cell. These responses reappear after reciliation. On the other hand, the deciliated cells retain the hyperpolarizing membrane response to mechanostimulation of the posterior region of the cell. These results agree with those obtained in chloral hydrate-deciliated paramecia (Ogura and Takahashi 1976). (2) The resting membrane potential (V_r) is not affected by deciliation. V_r in deciliated cells is determined both by potassium (24.9 mV/10-fold change of $[K]_o$) and by calcium (20.8 mV/10-fold change of $[Ca]_o$) which corresponds to the K- and Ca-dependence of V_r in ciliated cells (Naitoh and Eckert 1968). Instantaneous I/V plots of voltage-clamped deciliated cells lack the negative resistance property characteristic of ciliated cells. The steady-state resting input resistance is slightly increased in deciliated cells (8.4×10^7 ohms vs. 6.2×10^7 ohms of untreated cells). This resistance increase is largely due to pharmacological effects of ethanol, since unshaken — thus still ciliated — cells show the resistance increase as well (8.6×10^7 ohms). (3) Mechanical stimulation of the anterior region of a deciliated cell elicits a non-regenerative depolarization (receptor potential). The most sensitive area for this depolarization is the ventral deciliated surface 25–50 μm posterior of the front end. With V_r under voltage-clamp, anterior mechanical stimulation of the deciliated cell generates a brief inward current in the order of 10^{-10} A. In the ciliated cell this receptor current is of the same magnitude. Posterior mechanical stimulation of the clamped cell, ciliated or deciliated, leads to comparatively large outward current of the order of 10^{-9} A.

It is concluded that the voltage-sensitive Ca-channels are restricted to the ciliary membrane of *Paramecium* (Ogura and Takahashi 1976, Dunlap 1976, 1977). Extraciliary membranes carry the voltage-insensitive Ca-channels (mechanoreceptor plus leakage channels) and mechanoreceptor as well as voltage-sensitive K-channels. This work is supported by the Deutsche Forschungsgemeinschaft (SFB 114, TP A5).

---

[1] On leave of absence from Zoological Institute, University of Tokyo, Tokyo 113, Japan

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 185
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Cell Surface-Substratum Interactions in Amoeboid Locomotion

Michał OPAS. M. Nencki Institute of Experimental Biology, Warsaw, Poland

The cell surface complex in amoebae which is constituted of thick filamentous glycocalyx, plasmalemma and cortical proteins provides a possibility of a motive force transmission on a substratum. It seems reasonable to assume that a probability of adhesion is determined by physicochemical character of substratum and amoeba surfaces, but whether the contact will be realized or not, and what will be its form — it is, at least partly determined by the cell cortex contractile activity.

Taking into account the recent data concerning the involvement of contractile structures in adhesion of tissue cells (Lloyd et al. 1977, Rees et al. 1977, Heath and Dunn 1978), it may be inferred from experiments on amoebae (Opas 1976, 1978, in preparation) that the role of the cell cortex in transmission of the motive force generated within an amoeba on a substratum consists in controlling the geometry of the cell surface being in contact with this substratum by means of contractile activity.

Little is known, however, about the very interaction of amoeba surface with that of a substratum. The useful tool in evaluating the closeness of cell-substratum approach — the interference reflection microscopy (Curtis 1964) — can not be successfully used as long the refractive indices of glycocalyx and cell cortex in amoebae are not known, since as it was shown by Izzard and Lochner (1976), in a microscope working at low illuminating numerical aperture the interference reflection image is formed partly by the cell interior. As the microscope I have used (Opas 1978) worked at extremely low illuminating numerical aperture, it was not possible to estimate the distance of separation between the amoeba and glass surfaces. On the other hand, my preliminary experiments employing the microscope working at high illuminating numerical aperture (supposed to collect reflections from amoeba and glass very surfaces only) suggest that changes of higher order interference colours generated by the amoeba interior in white light at low illuminating numerical aperture are usually not correlated with the changes of residual interference colours generated at the amoeba surface in white light at high illuminating numerical aperture. This indicates the involvement of more than two surfaces in the interference reflection image generation in amoebae.

Therefore, though we are gaining more data on involvement of contractile proteins in adhesion, the nature of contact of amoebic surface with a surface of substratum and the involvement of glycocalyx in adhesion of amoebae remains to be elucidated.

REFERENCES: Curtis A. S. G. 1964: J. Cell Biol., 20, 199–215, Heath J. P. and Dunn G. A. 1978: J. Cell Sci., 29, 197–212, Izzard C. S. and Lochner L. R. 1976: J. Cell Sci., 21, 121–159, Lloyd C. W. et al. 1977: Exp. Cell Res., 110, 427–437, Opas M. 1976: In: Microcinematography as a Research Method in Cytology. Hradec Kralove, 70–72, Opas M. 1978: J. Microsc., 112, 215–221, Rees D. A. 1977: Nature, 267, 124–128.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 187
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Possible Inducers of Actin Polymerization in the Cell

Christine ORIOL-AUDIT. Biochimie cellulaire — Collège de France — 75231 Paris Cedex 05, France

In the cell, the formation of arrays of actin microfilaments accompanies certain events such as cytokinesis or motility. In dividing cells, Schroeder (1972) has shown that the furrowing process is related to the appearance of a uniform ring of aligned actin microfilaments called the contractile ring. The biological factors which induce this alignement of microfilaments are unknown.

These arrangements of microfilaments have a short life span and the mechanism by which they are induced may have no counterpart in the muscular machinery. Even, it could be supposed that this transient polymerization is induced by non-protein factors.

As numerous investigations have shown an increased polyamine content in the rapidly growing cells, we have investigated the action of biological polyamines on cytoplasmic actin. Actin was prepared from pig kidney and it was observed that spermidine and spermine could induce the reversible polymerization of actin. The addition of 1 mM spermidine or spermine to the kidney G-actin dissolved in its depolymerization buffer induces its polymerization with a better yield (82–88%) than the addition of 1 mM $MgCl_2$ in the presence of 0.1 M KCL (70%).

In a previous study, we had demonstrated that biological polyamines (1,3-diaminopropane, putrescine, cadaverine, spermidine, spermine) can also induce the reversible polymerization of muscle actin *in vitro* (Oriol-Audit 1978).

The polyamine-induced F-actin has the well-known properties of the salt-induced F-actin (strong activation of the Mg-ATPase myosin, specific viscosity enhanced, blue shifts of the dichroic bands in the near UV). The actin filaments induced by spermidine or by spermine show different features by electron microscopy. Spermidine-induced F-actin displays single homogeneous filaments whereas spermine-induced F-actin shows parallel arrangements of filaments. The addition of spermidine to a salt-induced F-actin promotes a higher degree of polymerization.

In the cell, actin exists in the form of G-actin monomers and solitary microfilaments. If the polymerization process demonstrated *in vitro* also takes place *in vivo*, the precursors of aligned microfilaments could be either G-actin monomers polymerized by the action of spermine or single microfilaments aligned by spermidine.

Equal concentrations of spermidine and spermine are distributed in nuclear and extra-nuclear fractions. Therefore, after the disappearance of the nuclear membrane during mitosis, the concentration of polyamine in the cytoplasm may

increase and this changes in concentration could be an inducer of actin polymerization for the furrowing process.

As polyamines can form complexes with phospholipides, they may also play a role in the localization of contractile ring microfilaments by causing some binding between the actin filaments and the membrane or other phosphate-containing material.

REFERENCES: Schroeder T. E. 1972: J. Cell Biol., 53, 419–434, Oriol-Audit C. 1978: Eur. J. Biochem., (in the press).

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 189
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Electric and Motor Responses to Mechanical Stimulation in *Stylonychia*

Jacques E. de PEYER and Hans MACHEMER. Arbeitsgruppe Rezeptoren, Lehrstuhl für Allgemeine Zoologie, Ruhr-Universität Bochum, D-4630 Bochum, Fed. Rep. Germany

Mechanical stimulation of the anterior end of *Stylonychia mytilus* (*Ciliata*) produces a predominantly Ca-dependent depolarization, stimulation of the posterior end K-dependent hyperpolarization of the membrane. At resting potential the marginal cirri (compound cilia) are kept largely motionless with small angles of declination from a position perpendicular to the cell surface. High frequency cine analysis of the ciliary responses to mechanical stimuli reveals that upon dopolarizing stimulation the cirri turn in a reversed position and beat at high frequency. Upon hyperpolarizing stimulation the beating occurs in the posterior direction with the frequency raised.

Anterior mechanical stimulation, with the resting potential voltage-clamped, evokes an inward current of maximally 10^{-8} A which decays with a time constant of approximately 3 ms, significantly below the time constant of the depolarizing receptor potential. Similar stimulation of the posterior end induces an outward current of similar magnitude and decay time. No motor responses of ciliary organelles occur due to these inward or outward receptor currents.

Ohmic or electrogenic inward currents of magnitudes similar to inward receptor current were produced under voltage clamp by hyperpolarizing and depolarizing steps of membrane potential. 30 mV depolarizing steps of 10 ms lead to a reversal response of the cirri. 50 mV hyperpolarizing steps of 20 ms produced normal beating at increased frequency.

We conclude: (1) Inward or outward membrane currents do not, *sui generis*, activate the cilia. (2) Ca-receptor pores occur outside the ciliary membranes so that Ca receptor current cannot modify the Ca environment of the axoneme for ciliary activation. (3) Steps in the senso-motory coupling involve two species of membrane pores, (a) receptor K- or Ca-pores for non-regenerative changes in membrane potential; (b) voltage-sensitive Ca-pores on the ciliary membranes regulating, together with the Ca pump, the intraciliary Ca concentration.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 191
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

The Structure and Physico-Chemical Properties of Bacterial Flagella

M. N. POGLAZOVA, V. I. BIRJUZOVA, A. L. METLINA, T. I. MISHINA, B. F. POGLAZOV. Institute of Microbiology, Academy of Sciences, Institute of Biochemistry, Academy of Sciences, Moscow State University, Moscow, USSR

Highly purified preparation of flagella from *Bacillus brevis* and from a highly mobile mutant of *Escherichia coli* have been isolated. During the structural studies attention was focused on the mode attachment of flagella to bacterial cell.

Significant differences in the composition of the basal body in the studied bacterial strains were demonstrated. Sedimentation analyses and electrophoresis made it possible to establish that the molecular weights of *Bac. brevigs* and *E. coli* flagellines vere 39 000 and 45 000 dalton, respectively. The use of electron microscopy and viscosimetry allowed elucidation of certain physico-chemical properties of flagellines associated with their aggregation.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 193
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Some Aspects of *Labyrinthula* Movement

Heinz SCHMOLLER. Institute of Pharmacology and Toxicology, W. Pieck-University of Rostock. Rostock, Leninallee 70, GDR

Single "spindle-bodies" of *Labyrinthula* can be isolated from their net-plasmodium. They have no flagella or cilia and show no axial stream of protoplasm as it is common in ameboid movement. Though they possess a cell membrane (with some interruptions, called bothrosomes) and all structural characteristics of a cell, the spindle-bodies alone are immotile but move after formation of a membrane-bounded thread system within which they show a special type of sliding motility.

Under cultural conditions on agar plates with streaks of food organisms they develop a wall-like aggregation of numerous spindle bodies within a common thread substance as the result of growth. This netplasmodium moves forward on the streaks. Locomotion includes both, the common thread substance and the spindle bodies. More in detail it can be observed that the two differ in respect to their velocity. The ectoplasmic thread substance limits the locomotion of the whole plasmodium with its locomotion rate of about 1.5 cm a day and reacts to adequate external stimuli which lead to a certain direction of its movement. Spindle bodies slide more quickly up to 230 μm per minute and aggregate therefore on the front of the netplasmodium. The thread substance of ectoplasmic nature moves forward in direction of the locomotion, where it forms ramifications or elongates the threads. Locomotion and changing of the ramifications are consequently initially performed by the ectoplasmic thread substance. But it can be negociated, that the spindle bodies are moved passively only by contraction of these ways, for they pass ramifications while the branches do not change their position. The tip of the spindle bodies plays an important role for its movement. In parallel orientation to the thread substance as the surrounding ectoplasm it acts as a center for the movement of the whole spindle body and is able to form a long extension with subsequent approach of the spindle body. If the tip is bent at right angles, movement of the whole spindle body ceases immediately. But spindle bodies possess no morphological polarity. If one of them turns the back to the front movement is continued in the former direction.

What *Labyrinthula* demonstrates is the existence of two different rates of movement in the locomotion of the plasmodium. It results only from co-operation of the two constituents, the nucleus bearing spindle bodies and the ectoplasmic threads. This seems to indicate the necessity of a plasmatic polarity for the action of contractile elements in both parts of this biological system.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 195
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Light-Oriented Chloroplast Movement in *Mougeotia* sp.

E. SCHÖNBOHM and W. HAUPT. Universities of Marburg and of Erlangen-Nürnberg, in cooperation with Institut für den wissenschaftlichen Film, Göttingen, Fed. Rep. Germany

A scientific film will be presented. In that film, the movement of the flat chloroplast in the cell of the filamentous green alga *Mougeotia* is shown. The chloroplast orients to the light by turning around the axis of the cylindrical cell. In strong white and blue light, the chloroplast exposes its profile to the light source, but in red or in low intensity white light, it exposes its face to the light. Both these types of movements are demonstrated in the film. It is shown, also, that the chloroplast not always responds as a unit to the light, but that the response is restricted to the illuminated part of the cell.

The red light effect can act as an "induction": i.e., a light pulse can initiate and orient a response which occurs in a subsequent dark period. This induction by a red pulse can be reversed by far-red following red. Hence phytochrome is the photoreceptor pigment for that orientation in *Mougeotia*. It becomes obvious from the film that far-red stops the movement immediately at any time; this points to a close connection between P_{fr} as the internal signal and the motor apparatus.

Finally, the action dichroism is shown: In polarized red light, the response strongly depends on the orientation of the electric vector of the light in relation to the cell axis. Again, in can be seen in the film that changes in the inducing light conditions immediately result in changes in the response.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 197
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Light Dependent Control of Cytoplasmic Streaming and Chloroplast Movement

Konrad SEITZ. Institut für Botanik der Universität Erlangen-Nürnberg, Fed. Rep. Germany

In plant cells light causes an increase in the rate of cytoplasmic streaming and induces cyclosis as well as phototactic orientation movement of chloroplasts. The movement of chloroplasts can be induced either by an activation of the motive mechanism of streaming or by a change in the mobility of chloroplasts in the cytoplasm which depends mainly upon their adhesion to the ectoplasmic layer (Seitz 1972, Zurzycki 1962).

The effect of the light on the mobility of chloroplasts has been analyzed in *Vallisneria* by centrifugation experiments. High intensity of light causes via a flavin as photoreceptor an increase in the centrifugability of chloroplasts. Low intensity in contrast causes via chlorophylls as receptor a decrease in centrifugability. Experiments with metabolic inhibitors revealed differences in the inhibition between the low and high intensity range and showed that the increase in centrifugability is dependent upon ATP from oxydative phosphorylation whilst the decrease in centrifugability is dependent upon the photosynthetic electron flow (Seitz 1978 a).

These results support a hypothetic model of the action mechanism of light suggested earlier (Seitz 1971, 1972). According to this model light induces in dependence upon the irradiance changes in the availability of ATP by an interaction of oxydative phosphorylation and photosynthetic reactions. Such light induced changes in ATP can control the mechanism of cytoplasmic streaming and may be important for other blue light dependent motion responses as well (Seitz 1974).

In the motive mechanism of cytoplasmic streaming the parallel-shifting force in the interface between endo- and ectoplasm probably is caused by an interaction of actin filaments anchored to the ectoplasm with myosin molecules in the endoplasm (Hepler and Palevitz 1974, Williamson 1975). In this molecular mechanism reversible crossbridges of myosin and actin would be formed between endo- and ectoplasm, involving attachment, motion and detachment as in the actomyosin system of muscle. ATP could have a dual function in this system. On the one side ATP as substrate of the myosin-ATPase would supply the energy for the motive force and would control the reaction velocity of this enzyme and thus the speed of cytoplasmic streaming. On the other side ATP would dissociate the crossbridges between actin and myosin and could thus modify the

linkage between ecto- and endoplasm. In cansequence in presence of ATP the endoplasm and chloroplasts could be moved more easily relative to the ectoplasm. Without ATP in contrast many crossbridges would be formed as in the rigor state of muscle and the endoplasm with chloroplasts could be moved less easily. Light induced changes in the availability of ATP can according to this mechanism control the rate of cytoplasmic streaming as well as the adhesion of chloroplasts to the ectoplasmic layer (Seitz 1978 b).

REFERENCES: Hepler P. K. and Palevitz B. A. 1974: A. Rev. Pl. Physiol., 25, 309-362, Seitz K. 1971: Zeitschr. Pflanzenphysiol., 64, 241–256, Seitz K. 1972: Acta Protozool., 11, 225–235, Seitz K. 1974: Ber. Deutsch. Bot. Ges., 87, 195–206, Seitz K. 1978 a: Z. Pflanzenphysiol., in preparation, Seitz K. 1978 b: Encyclopedia of Plant Physiology, (in preparation), Williamson R. E. A. 1975: J. Cell Sci., 17, 655–668, Zurzycki J. 1962: Acta Soc. Bot. Pol., 31, 489–538.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 199
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Structure and Function of Canine Cardiac Myosin during Pressure-Overload Cardiac Hypertrophy

R. F. SIEMANKOWSKI[1] and P. DREIZEN. Department of Medicine and Program in Biophysics, State University of New York, Downstate Medical Center, USA

Studies were conducted of myosin from left and right ventricles of normal canine hearts and hypertrophic canine hearts at 5 weeks and 13 weeks after aortic banding. On SDS-polyacrylamide gel electrophoresis, cardiac myosin shows degraded heavy chains (70 000–180 000 mol wt), the proportion of which is greater in myosin from hypertrophic hearts than normal hearts. Interestingly, comparable degradation occurs in preparations from left and right ventricles of banded hearts, although only the left ventricle was subjected to hemodynamic stress. Heavy chain fragmentation in the presence of dodecyl sulfate appears to have a complex origin, involving a non-enzymatic stochastic process and also proteolysis due to contaminant proteases. In addition, the susceptibility of heavy chains to protease-dependent proteolysis differs in myosin from normal and hypertrophic hearts. With precautions to minimize proteolytic artifacts, myosin preparations from left and right ventricles of normal and hypertrophic hearts exhibit comparable subunit composition, with molar ratios of heavy chains (200 000 d), light chain L1 (27 000 d), and light chain L2 (18 000 d). In studies of the microheterogeneity of myosin light chains, charge electrophoresis and a newly developed two-dimensional SDS-isoelectric focusing method reveal the same subunits in preparations from normal and hypertrophic hearts.

There is also evidence that ATPase activity is altered in purified myosin from hypertrophic hearts. At 5 weeks after aortic banding, there is diminished K^+/EDTA-ATPase, and no significant change in Ca^{++}-ATPase, in myosin from hypertrophic hearts as compared with normal hearts. Similar activities are obtained for left and right ventricle myosin. A wide wariation of K^+/EDTA-ATPase among individual preparations of myosin may be related to the extent of cardiac hypertrophy, in that activity is normal or slightly diminished in mild hypertrophy, and diminished approximately 50% in moderate hypertrophy. Insofar as there is no evidence for different myosin isozymes in normal and hypertrophic canine hearts, the observed changes in myosin ATPase during pressure-overload cardiac hypertrophy may reflect denaturation or other modification of myosin *in vivo*.

Supported in part by research grants from the National Institutes of Health (AM-06165 and HL/AM-16596), the New York Heart Association, and the New York State Health Research Council.

[1] Maynard-Crawford Fellow of the New York Heart Association, 1972–1975.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 201
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

The Role of Single Particle Saltations in *Paramecium* Cytoplasmic Streaming

Jerzy SIKORA, Anna WASIK and Robert D. ALLEN. Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland and Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire, USA

Cytoplasmic streaming is probably caused by some kind of force producer (Sikora et al. this issue) polarized by unknown ultrastructural elements that serve as a guidance system along the route of cytoplasmic rotation.

Streaming is polarized with reference to the outer surface and influenced by the geometry of some cell organelles. Almost all particles flow along with the main stream, but from time to time some particles show noticeable movement across or backwards with respect to the main stream, often in vicinity of trichocysts. Such behavior of particles has been found at different optical sections of the same cell. In most cases only single particles exhibit such movement at a given time. The behavior of particles resembles the saltatory motions of uninserted trichocysts and mitochondria described by Aufderheide (1977). However, mean values of saltating particles velocity are 3.55 ± 0.54 μm/s which is almost 3.5 fold higher than values cited. Granules saltations in the direction opposite to the bulk of cytoplasmic streaming is probably due to occasional local errors in the polarity of the guidance system.

The saltation of the particles against or across an established stream is hydrodynamically impossible unless the particles are acted upon throughout their entire course by a guidance system (e. g., a bundle of microfilaments). Furthermore, granules flowing along rotational route show a mean velocity of 2.83 ± 0.40 μm/s similar to velocities of particle saltation. Thus the observed saltations suggest that perhaps cytoplasmic streaming may be the result of well coordinated saltatory movements in association with a guidance system that occasionally shows polarity errors resulting in counter-current saltations (see abstract of Strömgren-Allen for description of similar phenomenon in *Nitella*, this issue).

REFERENCE: Aufderheide K. J. 1977: Science, 198, 299–300.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 203–204
Int. Symposium on Cell Motility, June 26–28, Warszawa, Poland

Velocity Profile of Cytoplasmic Streaming in *Paramecium*

Jerzy SIKORA, Anna WASIK and Zbigniew BARANOWSKI.
Department of Cell Biology, Nencki Institute of Experimental Biology. Warsaw, Poland

Mechanism of cytoplasmic streaming in *Paramecium* cell have been suggested to be comparable with that promoting cytoplasm in *Nitella* (Koenuma 1963, Jahn and Bovee 1964, 1967, 1969). This view was based on the Koenuma's (1963) observation of the velocity distribution of particles flowing within cytoplasm of *Paramecium caudatum*. The highest velocities near the outer surface of the streaming and gradually decreasing inward, had support the idea of an active-shearing propulsion mechanism (Kamiya and Kuroda 1956, Jahn and Bovee 1964, 1967, 1969, Donaldson 1972 a, b).

Investigation by means of other techniques (Kuźnicki and Sikora 1971, Sikora and Kuźnicki 1975, Sikora 1976) has shown that above mentioned view seems to be doubtful. The particle movement velocities recorded on the motion picture film and careful analysis by means of Kamiya's (1950) modified method enabled to state, that within whole cytoplasmic streaming route in *Paramecium* the velocity distribution profile is almost paraboidal (Fig. 1). The obtained data clearly show that flow of cytoplasm has a hydraulic character.

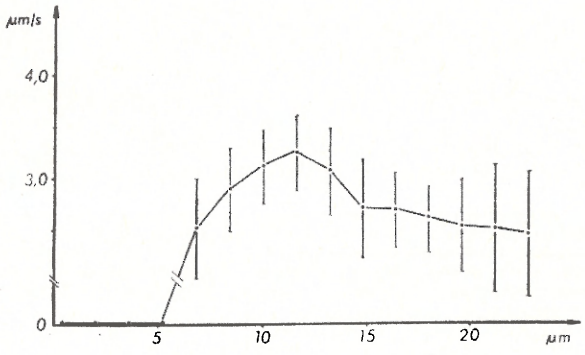


Fig. 1. Distance from the outer surface of the cell — *abscissa*, mean velocity of the particles — *ordinate*

This approach shows evidently, that active-shearing could not be responsble for propulsion of cytoplasm in *Paramecium*, because it assumes the force to be delivered at the boundary layer of the outer surface of flowing cytoplasm.

Up to now, there is no evidence showing distinguished tubular or fibrilar structure existence in *Paramecium* cell, which might be responsible for propulsion of cytoplasm (Hufnagel 1969, Jurand and Selman 1969, Allen 1971, Ehret and McArdle 1974).

The probable mechanism is based on rapid and extensive changes in cytoplasmic consistency. Theoretical consideration of Perkins and Babb (1977), with an explanation of mechanical implication of sol-to-gel transformation, provided as a basis for the new hypothesis of a sol-to "sol more organized" transformation as a source of motive force involved in propulsion of cytoplasm in *Paramecium*.

REFERENCES: Allen R. D. 1971: J. Cell Biol., 49, 1–20, Donaldson I. G. 1972 a: J. theor. Biol., 37, 75–91, Donaldson I. G. 1972 b: Protoplasma, 74, 329–344, Ehret C. F. and McArdle E. W. 1974: In: Paramecium a Current Survey, (ed. W. J. Wagtendonk), Elsevier Sc. Publ. Comp., Amsterdam, London, New York, pp. 263–336, Hufnagel L. A. 1969: J. Cell Biol., 40, 779–101, Jahn T. L. and Bovee E. C. 1964: In: Physiology and Biochemistry of *Protozoa*. (ed. S. H. Hutner), New York, Academic Press 3, pp. 62–129, Jahn T. L. and Bovee E. C. 1967: In: Research in Protozoology, (ed. T. T. Chen), New York — Pergamon Press, 1, pp. 41–200, Jahn T. L. and Bovee E. C. 1969: Physiol. Rev., 49, 793–862, Jurand A. and Selman G. G. 1969: The Anatomy of *Paramecum aurelia*. Macmillan, St. Martin's Press, New York, pp. 1–218, Kamiya N. 1950: Cytologia, 15, 194–204, Kamiya N. and Kuroda K. 1956: Botan. Mag., 69, 544–554, Koenuma A. 1963: Zool. Jap., 36, 66–71, Kuźnicki L. and Sikora J. 1971: Acta Protozool., 8, 439–445, Perkins D. L. and Babb S. E. 1977: J. theor. Biol., 69, 293–299, Sikora J. 1976: Int. Congr. Cell Biol., Boston, J. Cell Biol., 70, 399 a, Sikora J. and Kuźnicki L. 1976: Acta Protozool., 15, 173–178.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 205
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

The Movement of Melanosomes and the Division of Dermal Amphibian Melanophores

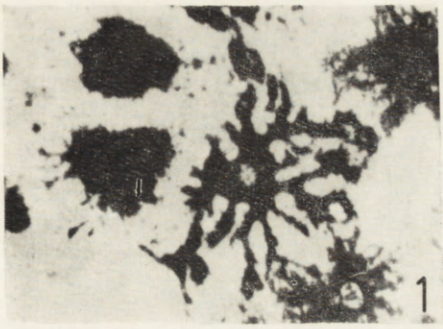
S. M. STARODUBOV and V. A. GOLICHENKOV. Laboratory of Experimental and Evolutionary Embryology, Biological Faculty, Moscow State University, Moscow, USSR

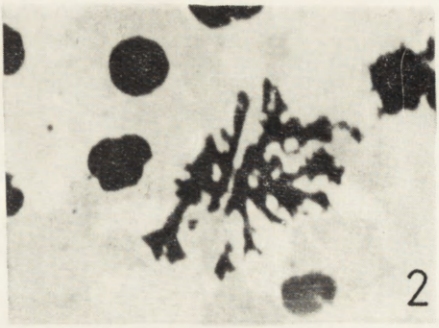
Tadpoles of *Rana temporaria*, stage 19–20 of Kopsch, were used in our experiments. Pieces of skin were dissected from cheek region and placed in transparent chamber with medium No. 199 diluted with distilled water (1 : 0.5). Light microscopic observations of dermal melanophores were performed at the magnification of 200 × or 400 ×.

We have found that dividing melanophores may be detected due to the characteristic appearance about an hour before the end of cytokinesis (Pl. I 1–2). Melanophore — stimulating hormone (MSH) or melatonin as well as changes in illumination have no notable effects on melanosome movement within these pigment cells.

After the ending of division the daughter cells become capable to melanosome translocations in response to various stimuli. It is suggested that incapability of dividing melanophores to exhibit melanosome displacement in response to light and certain hormones may be associated with the presence of a single substratum which is possibly need for cellular and intracellular movements as well as mitosis (Golichenkov 1977).

Plate I 1–2: Melatonin effect (10^{-6} g/l) on dermal melanophores in the tadpoles of *Rana temporaria*. The dividing melanophores is in the center





S. M. Starodubov et V. A. Golichenkov auctores phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 207
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

The Motile Cytoskeleton of *Nitella*

Nina STRÖMGREN ALLEN and George C. RUBEN. Dartmouth College, Hanover, NH 03755, USA

The large internodal cells of *Nitella* and other characean cells exhibit the fastest rational streaming in green plants (40–100 μm s^{-1}). The current investigations into the fine structure of these cells were undertaken after multitudes of longitudinally-oriented filaments had been discovered in the endoplasm of *Nitella* cells (Allen 1974).

The classical theory of streaming in these cells (Kamiya and Kuroda 1956, Kamiya 1977) postulated an active shearing force developed at the border between the cortex and the endoplasm. Subcortical fibrils (SF) were discovered by Kamitsubo (1966) at that location, and Kersey et al. (1976), showed that these fibrils were bundles of actin with HMM arrow polarity opposite to the direction of streaming.

The SF's are only part of a motile cytoskeleton, as they have branches of endoplasmic filaments (EF) extending from the SF's, Allen (1974, 1976). Both SF's and EF's can be observed to transport many sizes of particles including nuclei and detached chloroplasts at up to terminal velocities. The EF's, although often below Airy disc diameter can be clearly seen in optical sections of the entire cytoplasm (treated with 1 mM NEM) with a high extinction D I C microscope. NEM causes streaming to cease, but does not seen to cause damage EF's.

Ultrastructural methods are beginning to reveal further details regarding the organization of the motile cytoskeleton of *Nitella*. SEM preparations may be most prone to artifact, but with careful fixation one can at least see the general nature and extent of the beds of EF's with attachment to SF's. Depending on fixatives used no network or shows a well-preserved network with many particles (0.8 μm) still attached can be observed. HVEM enables one to see more detailed structure of the SF's. The branching of EF's from SF's have also been recorded. So far, conventional electron micrographs of endoplasm with EF's have not been productive due to destruction of endoplasm during fixation. Freeze-fracture and freeze-etching of rapidly frozen cells appears to cause the least disturbance of the organization of *Nitella*'s cytoskeleton. A network of filaments with attached particles is regularly seen in the cytoplasm.

Cine analysis of streaming endoplasm (recorded with the Zeiss Axiomat system) has revealed some new observations (see N. S. Allen, film). We have discovered Countercurrent Saltations, i. e., files of particles (ca. 0.8 μm) streaming counter to the direction of the mainstream. Particles have been recorded "spi-

ralling" around and along filaments; jumping onto or off filaments; and at times coming to a complete standstill, while other particles continue on at the same rate. Recorded rates of streaming very considerably within short distances in undisturbed endoplasm. Variation in streaming rates in *Nitella* endoplasm have also been recorded by Mustacich and Ware (1977) using laser-doppler spectroscopy.

Thus new concepts of streaming are evolving that take into account the interaction of particles with an extensive actin-containing motile cytoskeleton of SF's and EF's throughout the cytoplasm of *Nitella*, not only at the border between the cortex and endoplasm. The reactive surface is in effect much larger than was imagined untill recently.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 209–210
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Contractile Properties of Plasmodium Strand Models

V. A. TEPLOV, S. I. BEYLINA, D. B. LAYRAND and N. B. MATVEEVA. Institute of Biological Physics, USSR Academy of Sciences, Pushchino, Moscow Region, USSR

Contractile models allow investigation of a number of actual problems which are difficult or impossible to solve both on biochemical and physiological levels. Glycerinated plasmodium strands able to respond to ATP by volume contraction and torsion oscillations were obtained by Kamiya in the 1960's. In the present work the capacity of glycerol-extracted and detergent-treated strands of slime mold to generate a detectable tension was investigated.

Physarum polycephalum was cultivated by the Camp method. Segments of protoplasmic strands, usually 3 to 6 mm in length and 0.2 to 0.5 mm in diameter, were used in the experiments. A sensitive tension transducer was applied for the detection of small force generation. The experimental error was about 0.2 mg. The external potential difference was measured in a double-chamber, the isometric tension of the sample in one of the compartments was simultaneously recorded. The experimental chamber was equipped with a cooling device.

The glycerol-extracted model made by the Kamiya procedure (1968) responded to ATP by volume contraction but did not generate a tension which could be detected with the tension transducer (Fig. 1). All the experimental attempts such as shortening the extraction time to 20 min, decreasing glycerol concentration to 20% modifying the salt solutions, protecting the contractile proteins against oxidation by removal of oxygen from the solutions were uniformly unsuccessful.

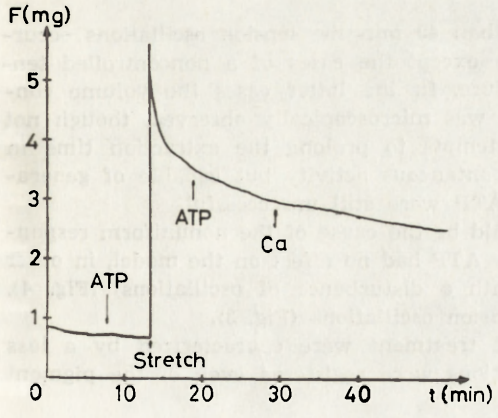


Fig. 1. Isometric tension record of the plasmodium strand glycerinated by Kamiya procedure. Temperature, 20°C. 5 mM ATP and 2 mM $CaCl_2$ do not affect tension. Usual relaxation is observed after 50% stretch

We used also detergent Tvin-20 to obtain the plasmodium model. The extraction was carried out in 1% Tvin-20, 30 mM KCL, 10 mM Tris-HCl buffer, pH 7.2. The same salt solution often with 2 mM $MgCl_2$ was used as a working and washing solution. The durations of extraction and washing were equal. Both procedures were carried out at 2°C, and isometric tension of the sample was simultaneously measured.

At cooling of strands the tension oscillations disappeared and montonous contraction was registered. The effect of cold was reversible. An additional contraction occurred in the presence of Tvin-20. After washing and warming-up a partial relaxation took place followed by spontaneous tension oscillations (Fig. 2).

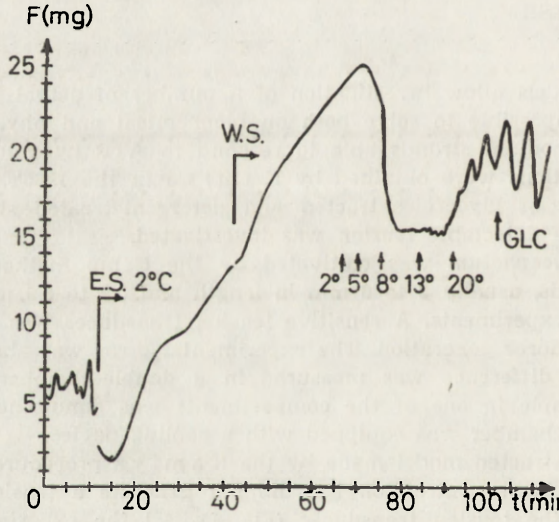


Fig. 2. The time course of isometric tension of the sample in the making of the model. The numbers and arrows indicate the temperature at a given moment. Note the absence of decrease in the tension oscillation amplitude in response to 10 mM glucose

With extraction times of no more than 40 min the tension oscillations occurred almost always (often with a delay) except the cases of a noncontrolled tension drop during the treatment procedure. In the latter cases the volume contraction of models in response to ATP was microscopically observed, though not detectable with the transducer. The attempts to prolong the extraction time in order to obtain a model deprived of spontaneous activity but capable of generating detectable tension in response to ATP were still unseccessful.

Incompleteness of the extraction could be the cause of the nonuniform responses of the models to ATP. In some cases ATP had no effect on the model, in other cases it caused a contraction (often with a disturbance of oscillations) (Fig. 4). Sometimes it stimulated spontaneous tension oscillations (Fig. 3).

The samples obtained by detergent treatment were characterized by a loss of pigment. Spontaneous tension oscillations were registered even of the pigment was completely lost.

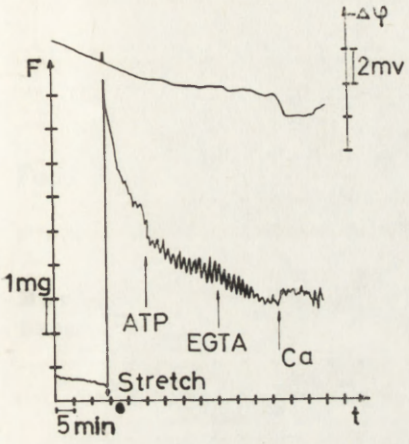


Fig. 3. Simultaneous registration of external potential and isometric tension of the plasmodium strand extracted for 60 min in solutions without oxygen. Chemicals used are 5 mM ATP, 1 mM EGTA, 5 mM $CaCl_2$. Temperature, 20°C. Note that the 30% stretch induces a very small depolarization

Small changes of potential difference were registered but neither the stretch nor the puncture of the sample produced the normal depolarization (Fig. 3). The standard reaction for glucose was always absent in these models (Fig. 2, 4). The detergent-treated plasmodium was unable to migrate. There was no protoplasmic streaming in it. Alteration of ground cytoplasm consistence and organelles disruption were observed.

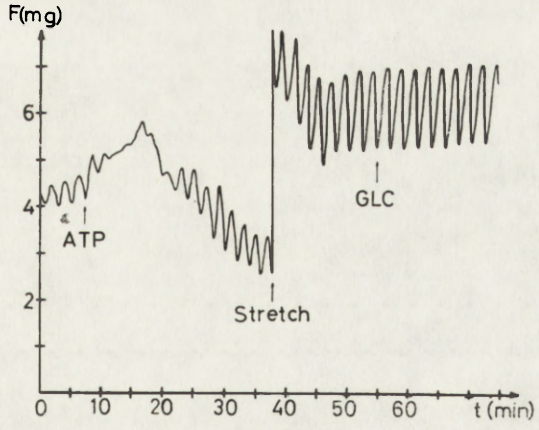


Fig. 4. Persistence of stretch activation in the plasmodium strand extracted for 30 min. The stretch is 25% of the sample length. 5 mM ATP has produced transient contraction. There is no response to 10 mM glucose

It is interesting to note that the effect of stretch activation persisted in spite of all these disturbances (Fig. 4). This enables us to hope that this model can be useful to study the structural and functional relations of the contractile apparatus and its regulation.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 213–214
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Food Ingestion in *Dileptus anser*

Barbara TOŁŁOCZKO. Department of Cell Biology, Nencki Institute of Experimental Biology, 02-093 Warszawa, Pasteura 3, Poland

Numerous studies have given evidence that the generation of oriented movements in eukaryotic, nonmuscle cells is associated with two basic classes of fibrous elements: microtubules and filaments. These structures are also present in oral apparatuses of different ciliates (Miller and Stone 1963, Grain and Golińska 1969, Kink 1973, Allen 1974). In *Dileptus* 3 main stages of food vacuole formation can be distinguished: (1) mouth opening, (2) food ingestion and vacuole formation, (3) food vacuole separation. Each of these stages must involve special kind of movement. In order to study the structural basis of these events the observations of the eating cells in EM were carried out. The influence of antimicrotubule agent colchicine and of cytochalasin B (which disturbes many processes involving filaments) was studied for the purpose of elucidating the role of fibrous elements in the process of food ingestion.

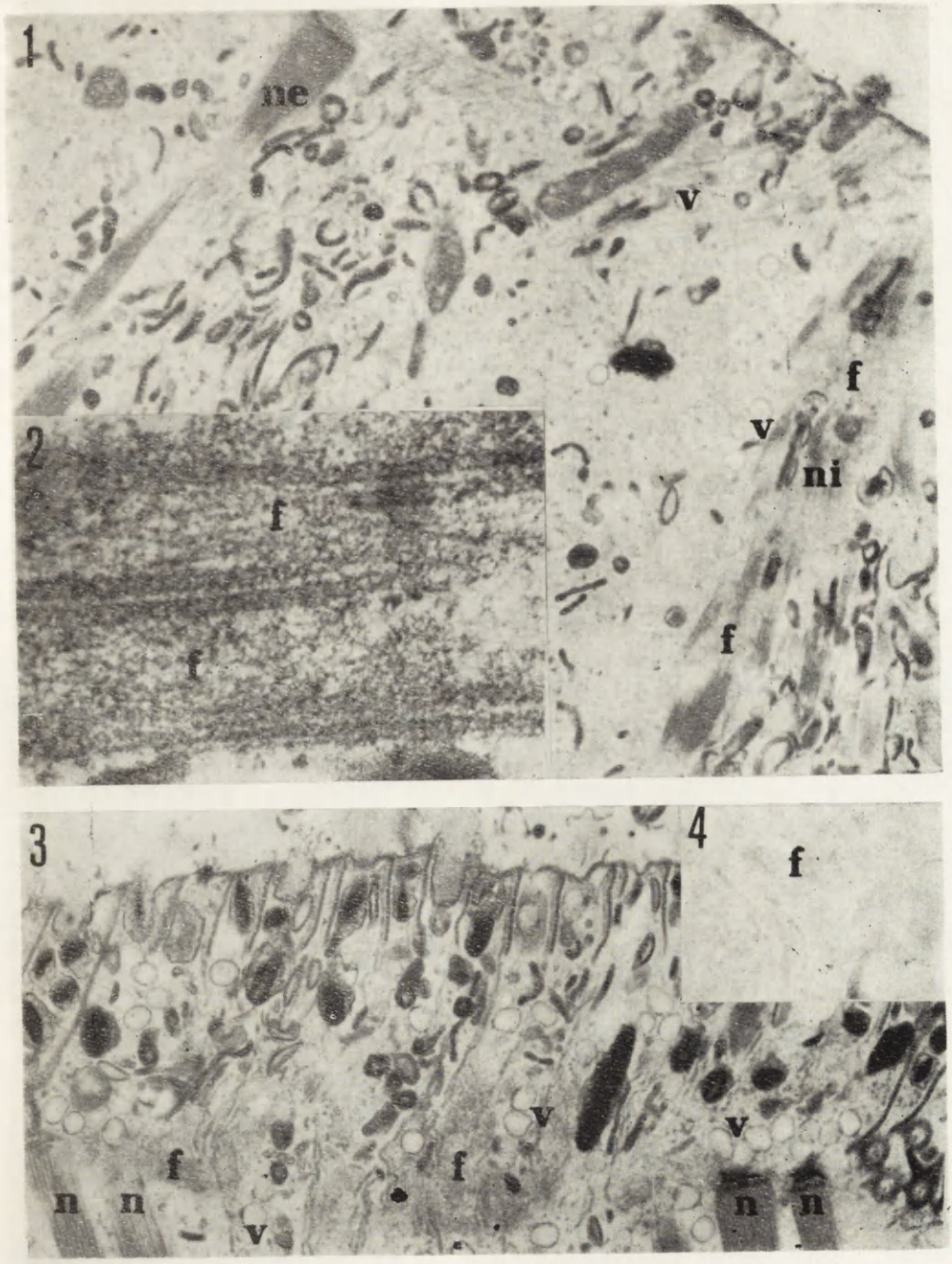
In the eating *Dileptus* the change of nemadesmata position must occur. In the latter the filaments connected with the inner basket (Pl. I 1, 2) can play significant role. The appearance of new, filamentous material during food ingestion was also observed. It is situated on the inner margin of the nemadesmal basket and equipped with smooth, spherical vesicles (Pl. I 3, 4). The same kind of structures were observed by Golińska and Kink (1977) during oral apparatus reorganization in *Dileptus anser*. These authors suggest that the microfibrils together with their vesicular elements are responsible for shape regulation in *Dileptus* and may be analogous to the contractile unit in *Spirostomum*.

The treatment of normal cells with colchicine (5 mg/ml) caused the decrease of vacuole formation rate similar as in *Paramecium* (Tołłoczko 1977). This effect might be due to the action of the drug on the cell membrane or labile microtubules. Whereas the addition of colchicine during nemadesmal basket formation inhibits completely the feeding reactions in regenerated *Dileptus*.

CB in concentration 10 μg/ml caused almost complet inhibition of food ingestion. The observations of the living cells revealed that the mouth opening was influenced, probably due to the disturbances in filaments action.

REFERENCES: Allen R. D. 1974: J. Cell Biol., 63, 904–922, Golińska K. and Kink J. 1977: J. Cell. Sci., 24, 11–29, Grain J. and Golińska K. 1969: Protistologica, 5, 269–291, Kink J. 1973: Acta Protozool., 12, 173–194, Miller O. L. and Stone G. E. 1963: J. Protozool., 10, 280–288, Tołłoczko B. 1977: Acta Protozool., 16, 185–193.

Plate I 1: Oblique section of the oral basket in *Dileptus anser*. ne — nemadesma of the external basket, ni — nemadesma of the inner basket, v — vesicle, f — filamentous material (× 14 000)
2: Fragment of filaments from 1 (× 90 000)
3: Longitudinal section of nemadesmal basket in the eating *Dileptus anser*. n — nemadesma, f — filamentous material, v — vesicle (× 14 400)
4: Fragment of filaments from 3 (× 27 000)



B. Tołłoczko auctor phot.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), p. 215
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Metabolic Dependency of Membrane Potential Oscillation Induced by Veratrine on Skeletal Muscle

E. VARGA, M. DANKÓ and Julia CSERI. Department of Physiology, Univ. Med. School, Debrecen, Hungary

Studying the mechanism of the depolarizing effect of veratrine on skeletal muscles, a long lasting rhythmic membrane potential oscillation was observed (Varga et al. 1972: Acta physiol. Acad. Sci. hung., 41, 81–99). The membrane potential oscillation is so regular and long lasting that it may be a suitable model for studying the rhythmic activity. Therefore, the mechanism of membrane potential oscillation has been investigated. Veratrine induced membrane potential oscillation is still apparent after the muscle has been treated with 1 mM KCN, 1 mM NaN_3, 0.2 mM DNP resp., to inhibit oxidative metabolism. However, the latency period in the NaN_3 — and DNP — pretreated muscle was shorter than in the control muscle. On the other hand the membrane potential oscillation ceased sooner in the treated muscle. These changes may be explained by our observation that NaN_3 and DNP depolarize the muscle membrane in the employed concentration. Repolarizing the membrane by electrotonus the membrane potential oscillation appeared again. This observation proves that aerobic metabolism is not involved in the mechanism of membrane potential oscillation. On the other hand it shows also the membrane potential dependency of the phenomenon. In this respect it seems important to consider that KCN which does not depolarize the muscle membrane has not any influence on the oscillation as DNP and NaN_3.

In view of the above experiments, glycolytic oscillation, as only known rhythmic metabolic process in the muscle which has the same range of frequency as the membrane potential oscillation, seemed to be worth investigating. On the effect of 1 mM phlorrhizin the latency period become prolonged and the frequency of oscillation decreased markedly. The frequency decreasing effect of veratrine is reversible. Furthermore, the inhibitory effect of phlorrhizin may be partially reversed by glucose despite the further presence of phlorrhizin. Finally the frequency decreasing effect of phlorrhizin is markedly observable even in muscles equilibrated in Li-Ringer.

Based on these results it seems probable that the frequency decreasing effect of phlorrhizin is more likely the result of the inhibition of glucolysis than some interference with Na-ion transport.

Veratrine causes no membrane potential oscillation but only depolarization in muscles pretreated with 0.5 mM iodoacetic acid, eventually a few abortive waves can be observed.

On the basis of the presented data it is suggested that rhythmic changes of anaerobic metabolism may be related to the oscillation of membrane potential.

ACTA PROTOZOOLOGICA, VOL. 18 (No. 1), pp. 217–218
Int. Symposium on Cell Motility, June 26–28, 1978, Warszawa, Poland

Application of Two Independent Light Beams for Measurements of Transmission Changes Corresponding to Chloroplast Movements in Leaves

Tadeusz WALCZAK. Department of Nuclear Spectroscopy, Institute of Nuclear Physics, Radzikowskiego 152, Kraków 31–342, Poland

A recording double-beam photometer was developed for measuring the reaction amplitude and the kinetics of light-induced chloroplast displacement in leaves. This apparatus enables the registering of light transmission changes independent of the intensity and spectral distribution of the actinic light.

The measuring beam is the monochromatic 658 nm light having an intensity of 50 mW m^{-2}, modulated with a frequency of 800 Hz. The modulated light transmitted through the leaf is detected by a sillicone photodiode. The AC-signal of 800 Hz is amplified with a linear amplifier and then rectified with a phase sensitive detector. The DC-signal from the detector is recorded. The range of linearity of the detecting diode is such that unmodulated actinic light does not influence the AC-component.

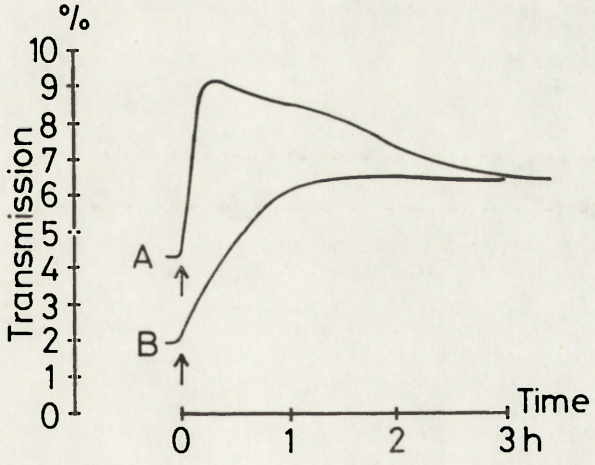


Fig. 1. Recorder tracing of transmission changes induced by 10 Wm^{-2} white light. Curve *A*: reaction starting from the dark chloroplast arrangement. Curve *B*: reaction starting from the low intensity chloroplast arrangement. Arrows indicate the beginning of the irradiation

The light transmission changes for *Tradescantia albiflora* leaves were recorded. The actinic light source (100 W, 12 V quartz-iodide lamp) was used with the output restricted to PhAR region. Power supply was stabilized with accuracy of ±0.2%. The light intensities ranged from 0.05 to 200 Wm^{-2}. Various types of chloroplast movement reactions were studied using different light regimes. Transmission curves for the reactions starting from the dark chloroplasts arrangement show two-phase kinetics with a pronounced over-shoot in the range of medium light intensities (Fig. 1, curve *A*). Curves starting from the low intensity chloroplasts arrangement exhibit no overshoot and are less steep (Fig. 1, curve *B*).

Similar results were obtained with blue actinic light 400–500 nm.

The complete dose-response curve for the white light is presented.

SHORT COMMUNICATIONS

CONTENTS

NOTICE TO AUTHORS

ACTA PROTOZOOLOGICA is intended as a journal serving for the publication of original papers embodying the results of experiment or theoretical research in all fields of protozoology with the exception of faunistic notices of the local character and purely clinical reports. The papers should be concise and will not be accepted if they have been previously published elsewhere. After acceptance by the Editors papers will be printed in the possibly shortest time.

Papers are accepted in English, French, German and Russian. Every paper should begin with the name and postal address of the laboratory, name and the surname of the author and title in the language of the text. The titles in German and French should be translated into English according to the demands of Current Contents. The paper should be accompanied by synopsis in the language of the text not exceeding 100 words and by short summary in one of 4 languages accepted in the Journal. In the Russian text also the name and the postal address of the laboratory, legends of tables, plates and text illustrations must be translated, the translation of the summary may be somewhat more extensive, and the name of the author should be given additionally also in the Latin characters.

Manuscripts should be doublespaced typescript (30 lines on one side of a sheet) with a normal margin. No elements of the text should be fully typed in capitals nor in spaced set (only underlining with pencil is admittable). In decimal fractions points not commas should be used. The generally accepted abbreviations and symbols are recomended. Nomenclature must agree with the International Code of Zoological Nomenclature, London 1961. The original and one carbon copy of the whole text material should be supplied. References must be cited in the text indicating only the author and year thus:

"Kinosita (1954) found that, etc." Only all references cited in the text should be listed. The list must be arranged as follows: Ehret C. F. and Powers E. L. 1959: The cell surface of Paramecium. Int. Rev. Cytol., 8, 97–133.

Gelei J. von 1939: Das aussere Stutzgerustsystem des Parameciumkorpers. Arch. Protistenk., 92, 245–272.

Titles of references are given in their original language (not translated). In papers written in English, French or German, the Cyrillic type of the Russian references is translitterated according to the international system (ISO Recommendation R 9 September). This regulation is not applied to names if there exists in their traditional spelling. Also author may freely choose in transliteration of his own name. In Russian papers, the Russian references are cited in Cyrillic, the others in the Latin characters, but they must be listed all together in the Latin alphabetical order.

The following material should be supplied on separate sheets 1. the running title for the page headlines, 2. tables, 3. legends for text figures, 4. legends for plates. Line-drawings will be published in the text, photographs and raster figures on separate plates. No coloured photographs can be published presently. Lettering on photographs and drawings should be marked in pencil. With no regard to the language of the text, only the Latin lettering arabic numerals or generaly accepted symbols are admittable for marking on illustrations. Numbering of text-figures, plates and tables must be also marked in pencil, as well in the legends as in the text.

Galley proofs are sent to the authors. Authors receive 75 reprints without covers.

First Announcement

Sixth International Congress of Protozoology is organized by the M. Nencki Institute of Experimental Biology in Warsaw, the Committee on Cell Biology of Polish Academy of Sciences (National Group of ECBO: a Federation of European Societies for Cell Biology) and the Protozoological Section of the Polish Zoological Society. It will be held in Warsaw on 5–11 July, 1981.

The Congress is open for Protozoologists from all countries and is not limited to members of organized protozoological societies.

The Programme will include: Invited Lectures from main fields of protozoology, Symposia, Contributed Paper Sessions and Poster Sessions.

The Symposia and Contributed Paper Sessions will be grouped into six sections:

Systematics and phylogeny of Protozoa
Genetics and morphogenesis
Development and life cycles
Physiology, biochemistry and immunology
Motility and behaviour
Ecology of free-living and parasitic Protozoa

Scientists interested in participating in the Congress should write, before 1 March 1980, to:

Dr. Stanisław L. Kazubski
Secretary General
VI International Congress of Protozoology
M. Nencki Institute of Experimental Biology
3. Pasteur str., 02-093 Warsaw, Poland

They will then receive the Second Announcement, registration forms, details about housing, as well as information about social activities, entertainment and tours (including possibilities of post-Congress trips).

In preparation:

M. Wolska: *Circodinium minimum* (Gassovsky, 1918), Electron-microscope Investigations — W. Krawczyńska: Ultrastructure of Isolated *Paramecium aurelia* Macronuclei — T. Einszporn-Orecka: Flagellates *Spironucleus anguillae* sp. n. Parasites of Eel (*Anguilla anguilla* L) — V. E. Madre: Two Species of Flagellates of the Genus *Chilomastix* Alexeieff, 1912 (*Mastigophora*: *Retortamonadida*) from Amphibians and Reptiles in India — R. Krishnamurthy and V. E. Madre: Studies on Two Flagellates of the Genus *Monocercomonoides* Travis. 1932 (*Mastigophora*: *Polymastigina*) from Amphibians and Reptiles in India — B. V. S. S. R. Subbarao, C. Kalavati and C. C. Narasimhamurti: A New Aseptate Gregarine, *Monocystis pontodrili* sp. n. from the Littoral Oligochaete, *Pentodrillus bermudensis* Beddard — В. Г. Ковалева и В. Г. Големански: Псаммофильные инфузории Болгарского побережья Черного моря — V. Golemansky: *Thécamoebiéns* psammobiontes du supralittoral vietnamien de la Mer Chinoise et description de *Cryptodifflugia brevicolla* sp. n. (*Rhizopoda*: *Arcellinida*) — M. Sudzuki: Psammobiont Rhizopoda and Actinopoda from Marine Beaches of Japan — S. F. Ng: The Precise Site of Origin of the Contractile Vacuole Pore in *Tetrahymena* and its Morphogenetic Implications — Z. Sayers, A. M. Roberts and L. H. Bannister: Random Walk Analysis of Amoeboid Movement and Galvanotaxis of *Amoeba proteus* — L. Grębecka and L. V. Kalinina: Effects of DMSO on the Motors Behaviour of *Amoeba proteus* — Cz. Balcerzak: The Radiation Changes of Activation Energy of Phosphate Ions Transport across the *Spirostomum ambiguum* Membrane and Accumulation of these Ions in Cells — R. L. Hoover and S. M. Gittleson: The Bicarbonate Ion as a Factor Influencing Cell Growth in *Polytomella agilis*.

Warunki prenumeraty

Cena prenumeraty krajowej: rocznie zł 200,—, półrocznie zł 100,—

Prenumeratę przyjmują Oddziały RSW „Prasa-Książka-Ruch" oraz urzędy pocztowe i doręczyciele w terminach:
— do dnia 25 listopada na styczeń, I kwartał, I półrocze i cały rok następny
— do dnia 10 każdego miesiąca (z wyjątkiem grudnia) poprzedzającego okres prenumeraty.
Jednostki gospodarki uspołecznionej, instytucje i organizacje społeczno-polityczne oraz wszelkiego rodzaju inne zakłady pracy, składają zamówienia w miejscowych Oddziałach RSW „Prasa-Książka-Ruch".
Zakłady pracy w miejscowościach, w których nie ma Oddziałów RSW, oraz prenumeratorzy indywidualni zamawiają prenumeratę w urzędach pocztowych lub u doręczycieli.
Prenumeratę ze zleceniem wysyłki za granicę, która jest o 50% droższa od prenumeraty krajowej, przyjmuje Biuro Kolportażu Wydawnictw Zagranicznych RSW „Prasa-Książka-Ruch", ul. Wronia 23, 00-958 Warszawa.

Bieżące i archiwalne numery można nabyć lub zamówić we Wzorcowni Wydawnictw Naukowych PAN-Ossolineum-PWN, Pałac Kultury i Nauki (wysoki parter), 00-901 Warszawa, oraz w księgarniach naukowych „Domu Książki".

Cena zł 50.—

CONTENTS

Index 35133