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ACTA PROTOZOOLOGICA

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Electrical Properties of Cell Membrane in Protozoan, *Stentor coeruleus*. I. Modification of Resting Membrane Potential by Extracellular Ions

Received on 12 November 1979

Synopsis. The resting membrane potential measurements have been carried out in *Stentor coeruleus* by means of glass micropipette electrodes and standard electrophysiological recording technique. The potential was sensitive to the concentration change of extracellular potassium, sodium, hydrogen and divalent ions as well (Ca^{++}, Mg^{++}) but it did not vary in a manner which is readily explained by the Nernst's equation. The extracellular solution containing potassium chloride (or sulphate) induced the membrane potential depolarization with a slope of 34 mV per tenfold increase of potassium concentration, over the range of 0.5 mM to about 10 mM. At lower concentration of K^+ this concentration-potential dependency was less linear. The similar effect have been found with extracellular sodium, however, the slope was 22 mV/log unit. External calcium diminished the influence of both potassium and sodium in the medium. With rise of concentration of hydrogen, the internal potential was markedly shifted toward zero with the slope of 4 mV/pH unit. There was no detectible effect of the replacement of chloride by sulphate on the potential changes. The obtained data for the potential-concentration relationship in *Stentor* suggests that this membrane seems to behave at rest conditions as a multiionic electrode.

It is generally acknowledged that the membrane potential plays an important role in the organization of cell movement of various protozoan cells. The recent experimental findings show that the ciliary motor activity of the ciliated unicellular organisms is correlated with the membrane potential variation (e.g., Kinosita et al. 1964, Naitoh 1974, Byrne and Byrne 1978, Machemer and de Peyer 1977). Upon the depolarization of cell membrane the beat orientation of cilia reverses toward the cell anterior, and cyclic beating frequency increases. In contrast to that, the membrane hyperpolarization induces the cilia

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to beat more posteriorly in conjunction with increased frequency. Thus, the changes in the ionic environment of the cell might produce the shifts in the membrane potential, implicating changes in functioning of the ciliary mechanism, thereby modifying the locomotor behavior of cell. Therefore, in the context of this dependency between ciliary motor and bioelectric membrane activities, the ionic requirements for the generation of membrane potential seem to be important question to solve. The present investigations are concerning the significance of some external cations on the resting membrane potential changes in a protozoan, *Stentor coeruleus*.

Materials and Methods

(1) Animals and Culture Conditions

Stock cultures of *Stentor coeruleus* were grown in a medium of 1 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$ and 1 mM Tris/HCl (pH 7.3) under condition of semidarkness at room temperature. According to the method of DeTerra (1966), colpidium was used as a food source for *Stentor*.

(2) Measurement Procedure

Measurements of membrane potential were conducted in the designed plexiglass or teflon chamber, mounted on the stereoscopic microscope stage. Tested solution were exchanged with the aid of peristaltic pump continuously or by means of automatic micropipette.

Temperature of external medium was controlled by feedback peltier device with possibility of temperature settings in the range of 0°C to 20°C. Additionally, the bath temperature was monitored by a solid state thermistor, calibrated against a Hg-thermometer. Cells taken from the stock culture were washed in desired test solution and placed then in the recording chamber with the same medium. After several minutes of adaptation, the recording electrodes were inserted near the oral region (Wood 1970, Morgenhagen 1971), with the aid of micromanipulator. During experiment the temperature of tested solution in recording chamber was maintained at 14°C.

(3) Recording Equipment

Glass recording microelectrodes were drawn from pyrex tubes with vertical electric puller. Each electrode was filled with 0.5 M KCl (Gotow et al. 1977) contained some amount of neutral dye to better visualization of the electrode tip during insertion into the cell. In the beginning of the recordings, the filled electrode was placed in recording chamber and with the aid of micromanipulator the electrode tip by gentle touch to the chamber bottom was broken to obtain the tip diameter of 0.5 to 2 µm with a resistance between 30 to 50 Mohm. The resistance was usually checked before and after each cell penetration and the measured potential value was taken into account when no resistance change occurred. A liquid junction potential for the employed electrodes was less than 3 mV (Adrian 1956, Bingley 1966). The voltage electrode was connected to

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the recording system through nonpolarizable $Ag/AgCl_2$ bridge. The bath solution in the plexiglass chamber was coupled to the system via $Ag/AgCl_2$ half cell also.

(4) Solutions

For the potential measurements the following experimental solutions were used:

(a) solutions containing separately Na^+, K^+, Mg^{2+} and Ca^{2+} and buffered with 1 mM Tris-HCl or 1 mM Tris-H_2SO_4 at pH 7.3;

(b) solutions containing simultaneously sodium in constant concentration (2 mM and 5 mM) and K^+ ions at different concentration buffered with 1 mM Tris-HCl at pH 7.3;

(c) solutions of Ca^{2+} at constant concentration level (1 mM $CaCl_2$) and different concentration of potassium buffered with 1 mM Tris-HCl at pH 7.3;

(d) culture solution buffered with Tris-HCl, Tris-H_2SO_4 or Tris-H_3PO_4 at pH in the range between 3 and 8;

All the solutions were osmotically balanced and prepared on the tripple glass-distilled water. Chemical reagents were of analytical grade.

Results

(1) Effect of External K^+ and Na^+ on Resting Potential

The various cells show the high dependency of transmembrane potential magnitude on changes of external concentration of potassium in a manner suggesting that a passive diffusion of potassium to outside of the cell gives the rise to a membrane potential. For this reason, the internal potential was measured as a function of extracellular K^+ concentration. The data of such measurements are presented in Fig. 1 and show that in the absence of external cations other than potassium, the strong depolarization of the membrane occurs as K^+ increases. In essence, the same data were obtained with $(K^+)_o$ when external chloride are replaced by sulphate. The potential changes on passing from one solution with given potassium concentration to another were fully reversible, except when the cells were exposed for a longer time to higher concentration than 8 mM potassium. In the range from 0.5 mM to 8 mM the observed potential altering is approximately equal to 34 mV/log unit.

The concentration-potential relation for external potassium shows evidently that the potassium gradient across the protozoan membrane in tested range of concentrations is less negative than it might be expected from the Nernst's diffusion equation, assuming that membrane is permeable to potassium only. Over tested $(K^+)_o$-concentration range, the slope of the potential against potassium differs considerably from

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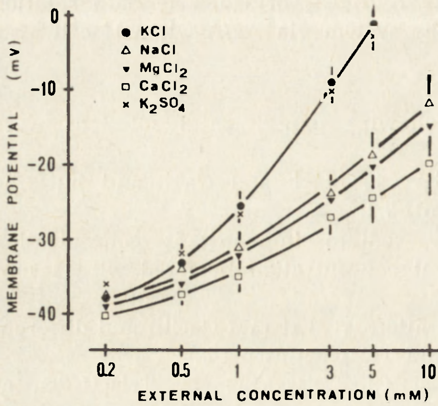

Fig. 1. Internal membrane potential vs. concentration of different cations at pH 7.3 and temperature 14 ± 0.5°C. Each point represents the mean value of 25–30 measurements. Vertical bars denote ± S.E.

the theoretical value of 58 mM/log unit (Hodgkin 1959). A slope smaller than that value indicates that the membrane is appreciably permeable to ions other than K^{+}.

In favour of this, it has been obtained direct evidence from the study of the modifying of potential by extracellular sodium. In absence of other ions than $(Na^{+})_{o}$, the magnitude of potential varied systematically with Na^{+} change and the slope was 22 mV per tenfold change of sodium over the concentration range 0.5–10 mM (Fig. 1).

Data of measurement of potential in the external medium, with constant concentration of $(Na^{+})_{o}$ as a function of potassium concentration, show the existence of an ionic interaction in the cell membrane, especially pronounced in the lower K^{+} concentration (Fig. 2).

Comparison of data in Fig. 2 suggests the sodium/potassium permeability ratio of about 0.2, because in circumstances of one extracellular cationic species the internal potential has the same value of −35 mV

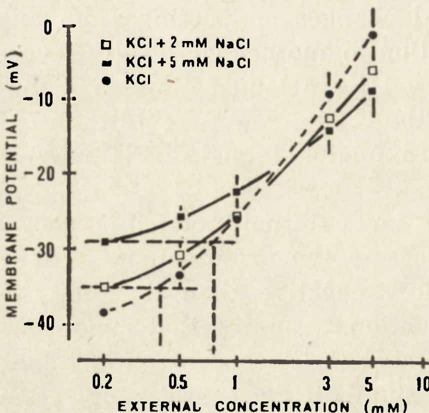

Fig. 2. Membrane potential in *Stentor* immersed in 0.2 to 8 mM KCl at different Na^{+} concentration (2 mM and 5 mM). The mean values for 25–30 sampled cells are given with ± S.E. (vertical bars)

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for 0.4 mM K^+ as for 2 mM Na^+ or −29 mV corresponds with concentration of 0.7 mM K^+ and 5 mM Na^+.

Similar results have been obtained for Na/K ratio in amoeba by Kokina and Doronin (1970). The total contribution of internal sodium to the membrane potential in *Stentor* can not be large, however. As it is estimated by Wood (1973), the internal Na^+ content is only one hundredth as large as internal potassium. Even assuming that the cell surface membrane in *Stentor* is equally permeable to the both mentioned cations, the expression of $\log(Na_i^+ + K_i^+)$ would be about 0.2% greater than the value of $\log K_i^+$ in the Nernst's equation. In fact, the cell membrane discriminates slightly against sodium ions and therefore the sodium contribution to the value of $\log(Na_i^+ + K_i^+)$ has to be less than 0.2%.

(2) Effect of Concentration Changes of External Ca^{2+} and Mg^{2+} on the Resting Potential

As it was confirmed in many cases, Ca^{2+} ion takes part in stabilization of cell membrane and is able to modify membrane permeability, particularly to monovalent cations (Osterhout and Hill 1938, Apter and Koketsu 1960, Tobias et al. 1962, Borle and Loweday 1968). As it can be seen in the Fig. 3, calcium seems to have the similar effect on protozoan membrane. Ca^{2+} added to the external medium changed the potential value, shifting towards more negative values at higher K^+ concentration. The transmembrane potential at presence of Ca^{2+} in the medium is rised by about 6 mV (Fig. 3).

Magnesium, the other divalent cation, demonstrates the influences on membrane potential as well but this effect is lower than that of calcium.

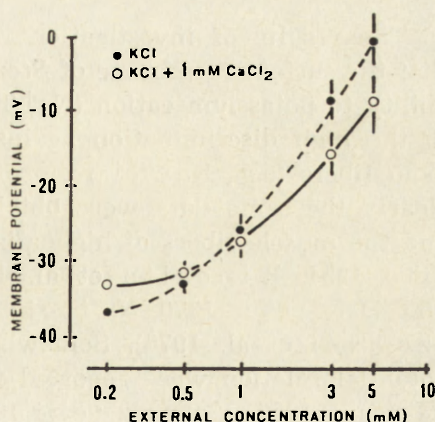

Fig. 3. Membrane potential value measured in external solution contained K^+ ions in the range of 0.2 to 8 mM and 1 mM $CaCl_2$ as well. Vertical bars denote ± S.E.

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(3) Effect of External pH on Membrane Potential

Membrane potential was measured in culture medium with different pH and different external anions (Cl^-, SO_4^{2-}, PO_4^{3-}) to ascertain the dependence of the membrane potential on external anions. Data from these measurements in pH between 3 and 8 are plotted in Fig. 4. Internal potential appeared to alter monotonically with the concentration variation of H^+ and the slope was about 4 mV/pH unit. This indicates

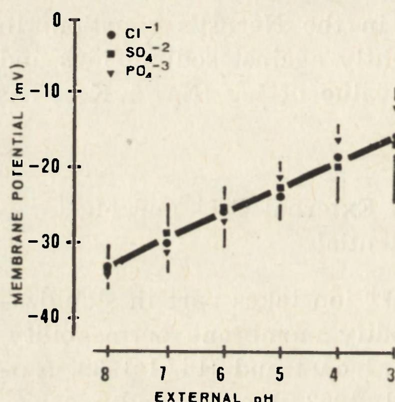

Fig. 4. Membrane potential vs. pH values of external medium in the presence of different anions (Cl^{-1}, SO_4^{-2}, PO_4^{-3})

a substantial influence of H^+ concentration change on the properties of cell membrane. It may be assumed that H^+ diffusion potential would make the contribution to the membrane potential per se or this cation is able to control indirectly the membrane permeability as in the case of calcium ion (Kitasato 1968, Rent et al. 1972).

Discussion

The results of investigation reported in this work clearly showed that the surface membrane of *Stentor* had relatively low specific permeability to potassium cation (Na^+/K^+ permeability ratio of 0.2), contrary to the high discrimination against other cations in muscle and nerve axon fibers (e.g., Na^+/K^+ ratio of about 0.01) (Hodgkin 1951, 1959). Nearly the same data were obtained for several other protozoan cells and the muscle fibers of higher insects as well (Hisada 1957, Kinosita 1954, Kinosita et al. 1964, Usherwood 1969, Kokina and Doronin 1970, Naitoh 1974, Josefson et. al. 1975, Janiszewski et al. 1976). Somewhat different results of concentration-potential relation were reported for *Paramecium* and eggs of the toad (Maeno 1959, Yamaguchi 1960). The membrane of this protozoan

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and egg indicated no discrimination against monovalent cations especially. However, it has been found later by Kinosita with his collaborators (1964) that the membrane of *Paramecium* possesses also the cationic selectivity which is very similar to that observed for *Stentor's* membrane regardless of use of different temperature during the experiments in the both studies. Recently was shown for the membrane of the bigest protozoan *Spirostomum* that internal membrane potential was sensitive to the changes in the external concentration of various cations but the authors were not able to obtain the exact potential-concentration relationship in consideration of high scattering of data (Doronin and Galkin 1974).

As it can be seen from previous section, the values obtained for the linear relation between internal potential and external concentration of $(K^+)_o$ considerably differed from those predicted by the Nernst's diffusion hypothesis under assumption that membrane of the cell had to behave as the potassium electrode. Such estimation suggests the quite low membrane selectivity for the membrane in *Stentor* like other protozoan cell membranes (Kinosita et al. 1964, Naitoh 1974, Josefson et al. 1975). Lack of influence of the replacement of the anions in the external medium on the value of potential indicated that the protozoan membrane has no detectabile permeability to the anionic molecules or the membrane permeability for different anions is similar.

The process in which H^+ ions are able to contribute to the resting membrane potential in the *Stentor's* membrane or as it was shown for membrane of *Paramecium* (Kinosita et al. 1964) remains unknown yet. Possibly that there is the same mechanism as for the plant cells where was shown the existing of the hydrogen diffusion toward the cell interior (Kitasato 1968, Rent et al. 1972).

When to the external medium calcium was added the internal potential became to be less sensitive to the alteration of the extracellular potassium concentration. There is interesting that the same external calcium action on the membrane of *Paramecium* was observed as well (Kinosita et al. 1964). The above-mentioned data might suggest that calcium is able to change indirectly the relative membrane permeability in compliance with the suggestion proposed by Apter and other authors for the muscle membrane (Apter and Koketsu 1960, Tobias et al. 1962).

At any rate, it may be concluded that the outer membrane in *Stentor* is more permeable to the external cations than anions and it shows the higher permeability to monovalent than to divalent cations. Thus, the membrane of *Stentor* and other protozoa as well differs in respect to their permeable selectivity from those in cells of higher organisms.

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Therefore, it seems that membrane with low cationic selectivity is a common feature which characterizes the protozoan cell membranes and, behaving like multiionic electrode, reflects the accomodation to the different living condition in comparison to that for nerve and muscle.

In order to proceed with the detailed explanation of the genesis of resting membrane potential in *Stentor* or for other protozoan membranes, in general, it is necessary to know both the mechanism by which ion pass through the cell membrane (e.g., energy metabolism-potential coupling) and ion distribution within the cell.

RÉSUMÉ

Le potentiel de répos mesuré à travers la membrane cellulaire de *Stentor coeruleus* est sensible aux changements des concentrations extracellulaires du potassium, du sodium, de l'hydrogène et des ions bivalents (Ca^{++}, Mg^{++}), mais il change de manière qui est difficile à interpréter par l'équation de Nernst. Les solutions contenantes de 0.5 mM à 10 mM de chlorure (ou de sulphate) de potassium dépolarisent le potentiel de la membrane de 34 mV quand la concentration du potassium augmente 10 fois. Dans les concentrations plus basses cette relation est moins linéaire. Les mêmes changements de la concentration du sodium apportent une dépolarisation de 22 mV. La calcium extracellulaire réduit les effets du potassium ainsi que ceux du sodium. Avec l'augmentation de la concentration de l'hydrogène, le potentiel tombe à la proportion de 4 mV par l'unité pH. Les résultats suggèrent que la membrane non-stimulée de *Stentor* se comporte comme une éléctrode polyionique.

REFERENCES

Adrian R. H. 1956: The effect of internal and external potassium concentration on the membrane potential of frog muscle. J. Physiol., 133, 631–653.

Apter J. T. and Koketsu K. 1960: Temperature studies implication calcium in regulation of muscle membrane potential. J. Cell Comp. Physiol., 56, 123–127.

Bingley M. S. 1966: Membrane potential in *Amoeba proteus*. J. Exp. Biol., 45, 251–267.

Borle A. B. and Loweday J. 1968: Effects of temperature, potassium and calcium on the external potential difference in HeLa cells. Cancer Res., 28, 2401–2405.

Byrne B. J. and Byrne B. C. 1978: Behavior and the excitable membrane in *Paramecium*. CRC Crit. Rev. Microbiol., 53–108.

Doronin Y. K. and Gałkin A. A. 1974: Bioelectrical phenomena in ciliates, *Spirostomum ambiguum*. Cytologia, 16, 362–369.

Gotow T., Ohba M. and Tomita T. 1977: Tip potential and resistance of microelectrodes filled with KCl solution by boiling and nonboiling methods. IEEE Trans. Biomed. Ingr., 24, 366–371.

Hisada M.: 1957: Membrane resting and action potential from a protozoan, *Noctiluca scintilana*. J. Cell Comp. Physiol., 50, 57–71.

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Hodgkin A. L. 1951: The basis of electrical activity in nerve and muscle. Biol. Rev., 26, 339–381.
Hodgkin A. L. 1959: The Cronian Lecture: Ionic movements and electrical activity in giant nerve fibres. Proc. R. Soc., B, 148, 1–37.
Janiszewski L., Olszewska E. and Borkowska M. J. 1976: The effect of some ions on the muscle fiber resting potential in the cricet *Acheta domesticus*. Acta Physiol. Pol., 27, 401–407.
Josefson J.-O., Holmer N. G. and Hannson S. E. 1975: Membrane potential and conductance during pinocytosis induced in *Amoeba proteus* with alkali ions. Acta Physiol. Scand., 94, 278–288.
Kinosita H. 1954: Electrical potentials and ciliary response in Opalina. J. Fac. Sci. Univ. Tokyo, 4, 1–12.
Kinosita H., Dryl S. and Naitoh Y. 1964: Relation between the magnitude of membrane potential and ciliary activity in *Paramecium*. J. Fac. Sci. Univ. Tokyo, Sec. IV, 10, 303–309.
Kitasato H. 1968: The influence of H^+ ions on the membrane potential and ion fluxes of *Nitella*. J. Gen. Physiol., 52, 60–86.
Kokina N. N. and Doronin Y. K. 1970: Electrical characteristics of the excitables structures of some unicellular organisms. Cytologia, 12, 804–809.
Machemer H. and de Peyer J. 1977: Swimming sensory cells: Electrical membrane parameters, receptor properties and motor control in ciliated *Protozoa*. Verh. Dtsch. Zool. Ges., 86–110.
Maeno T. 1959: Electrical characteristics and activation potential of Buffo eggs. J. Gen. Physiol., 43, 139–158.
Morgenhagen D. 1971: Membrane potential in *Stentor coeruleus*. Protoplasma, 72, 359–365.
Naitoh Y. 1974: Bioelectrical basis of electrical behavior in protozoa. Am. Zool., 14, 883–893.
Osterhout W. J. and Hill S. E. 1938: Calculation of bioelectric potential. IV. Some effect of calcium on potential in *Nitella*. J. Gen. Physiol., 22, 139–141.
Rent R. K. and Johnson R. A., Barr C. E. 1972: Net H^+ influx in *Nitella clavate*. J. Membr. Biol., 7, 231–244.
De Terra M. 1966: Culture of *Stentor coeruleus* on *Colpidium compylum*. J. Protozool., 13, 491–492.
Tobias A. M., Agin D. P. and Pawłowski R. 1962: The excitable system in the cell surface. Circulation, 26, 1145–1152.
Usherwood P. H. R. 1969: Electrochemistry in insect muscle. Adv. Insect Physiol., 6, 205–278.
Wood D. C. 1970: Electrophysiological studies of the protozoan, *Stentor coeruleus*. J. Neurobiol., 1, 367–377.
Wood D. C. 1973: Physiological correlates of habituation in *Stentor coeruleus*. In: Behavior of Microorganism, Mexico, 234–246.
Yamaguchi T. 1960: Studies on the modes of ionic behavior across the cytoplasmic membrane of *Paramecium*. I. Electric potential differences measured by the intracellular microelectrode. J. Fac. Sci. Univ. Tokyo, Sect. IV, 8, 573–591.

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Electrical Properties of Cell Membrane in Protozoan *Stentor coeruleus*. II. Effect of Temperature on Internal Membrane Potential

Received on 12 November 1979

Synopsis. The glass intracellular microelectrodes have been employed to investigate the influence of temperature chages of the environment on the resting and action potentials in the free-living ciliate *Stentor coeruleus.* In the temperature range of 5–18° C the membrane potential was depolarized by increase of temperature of external solution. Also the maximum rate of rising and falling phases of the spike were continuously increased with the rise of temperature. However, the maximum rate of rise of spike had a considerably higher temperature coefficient than that of fall, the Q_{10} values between 8 and 18° C being about 10 and 5 for the rise and decay of spike respectively. The action potential in amplitude is substantially decreased with rise of temperature of external medium. The measured input transmembrane resistance and capacitance have been found to rise when temperature was reduced. The obtained results suggest that both the resting and action potentials in the membrane of *Stentor* are generated by temperature-sensitive mechanism.

Although electrophysiological and behavioral studies carried out on the protozoan cells have been revealed a close correlation of the cell locomotor behavior with both bioelectrical phenomena within the cell surface membrane (Kinosita et al. 1964, Dryl and Grębecki 1966, Byrne and Byrne 1978, Machemer and de Peyer 1977, Naitoh 1974) and the temperature changes in the external solutions (Oliphant 1938, Dryl and Łukowicz 1974, Hildebrandt 1978), the direct effect of temperature on the various parameters of electrical activity of cell membrane in protozoan cells have not hitherto been reported. According to the well-known Nernst's ionic hypothesis, the membrane potential across the semipermeable cell membrane is directly related to absolute temperature. Attempts to demonstrate that the Nernst's equation describes precisely the behavior of intact membrane, apparently succeeded for muscle (e.g., Ling and Woodbury

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1949, Jenerick and Gerard 1953) but failed appreciable for some nerve axon (e.g., Hodgkin and Katz 1949, Senft 1967, Ayrapetyan 1969, Marchiafava 1970). Thus, to explain the divergences observed in different preparations it was suggested that temperature might differentially alter the permeability of the membrane to various ions, making the membrane potential a complex function of temperature (Goldmann 1943, Senft 1967, Hodgkin and Katz 1949, Apter and Koketsu 1960). The similar arguments have been proposed for the explanation the mentioned divergences in the behavior of resting membrane potential observed in the ciliate cell, *Stentor coeruleus.*

Materials and Methods

Fresh water ciliates, *Stentor coeruleus* were cultured in the manner described elsewhere (Wood 1973, de Terra 1966). The cells selected for the experiments were washed in the tested solutions, then were adapted to it in the recording chamber during several minutes. After that, two 0.5 M KCL — filled microelectrodes were inserted into the chosen cell in order of voltage recording and injection of the electrical current by means of conventional electrophysiological methods (Fabczak 1979). The bath temperature was maintained with a feedback peltier device in the range of 0°C to 20°C. Additionally, the current temperature was monitored continuously by a thermistor probe.

Results

(1) Steady-state Potential

Initially, the potential measurements were carried on the cells at 18°C only. Under this condition both negative and positive shifts in membrane potential from zero level were observed. Thus, in order to reduce the significant scatterings in potential values, the cells showing the negative potential were chosen for further experiments exclusively. The typical distribution curve of the 98 potential recordings for the individual cells is showed in Fig. 1. As it become obvious from the preliminary tests, the temperature parameter appeared to be an important variable, so the measurements were conducted at different temperatures in the range 5°C to 18°C. In the all tested range, the decrease of temperature caused the rising of potential (hyperpolarization) up to value of about −55 mV at 5°C (Fig. 2). In the circumstances when the cooling of environment did not exceed 5°C, in all cases the influence of temperature changes was reversible and the values given for the higher tem-

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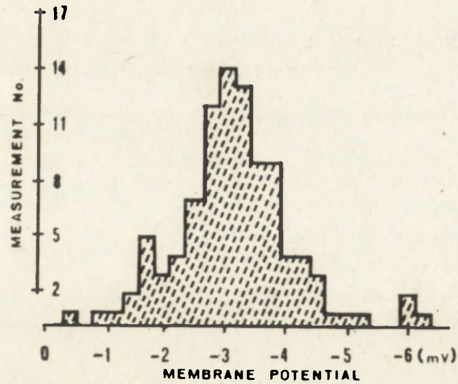

Fig. 1. Histogram of negative membrane potential in *Stentor* at 18°C in culture medium. The mean value for 98 measurements is -3.1 ± 0.2 mV

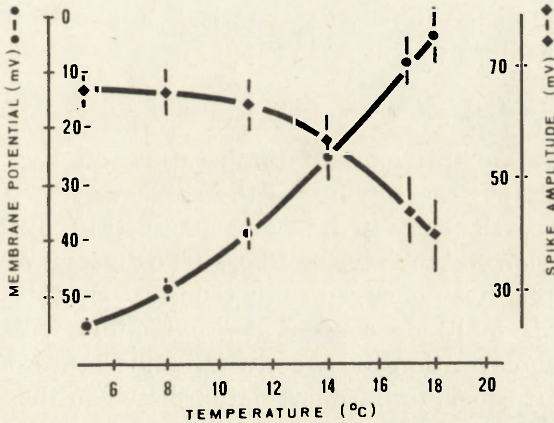

Fig. 2. Comparison of temperature effect on resting membrane potential and spike height. Bars are ± S.E.

perature in Fig. 2 are the mean from measurements made both at the beginning and the end of each experiments.

The determination of the membrane constants were made in accordance with the method employed for muscle fibers by Fatt and Katz (1951). The time constant, τ, was obtained by measuring of the time decay to 15% of potential value. The specific input resistance and capacitance were computed from the measurement of cell surface, assuming that contracted animal is spherical in the shape and cytoplasmic resistivity can be neglected. The plotted values of specific input resistance, R_m, are showed in the Table 1 and indicate that resistance at high temperature is only about half of that in low temperature. The average value of specific membrane resistivity obtained in the present work at 18°C is within the range of those previously reported for

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Table 1

Electric membrane constants in *Stentor* (mean values), derived from "square pulse analysis" and parameters of action potential (mean values) at different temperatures

Temperature (°C)	5	10	15	18
Resting potential (mV)	−55.3	−42.3	−22.8	−3.1
Spike height (mV)	66.5	63.2	52.8	38.9
Max. rate of rise (Vs^{-1})	—	0.15	2.25	6.13
Max. rate of fall (Vs^{-1})	—	0.10	1.15	3.12
Specific resistance ($Ohm \cdot cm^2$)	4193	3892	3127	2675
Specific capacitance ($\mu F \cdot cm^{-2}$)	7.69	6.82	6.75	6.21

Stentor (Wood 1970) or *Spirostomum* (Kokina and Doronin 1970) and muscle fibers as well (e.g., Castillo and Machne 1953). The specific membrane capacitance, C_m, has been found to increase slightly when external medium was cooled (Table 1).

(2) Action Potential

The most obvious influence of temperature on the time course of the action potential is the slowing of the spike wave with the lowering of temperature, particularly in its rising phase (Fig. 3), contrary to the opposite observation for the muscle fibers (Hodgkin and Katz 1949). The height of spike was changed with temperature as well. The reduction of the spike amplitude coursed approximately with the same rate as membrane depolarization did when the temperature of external solution was raised. It seems to be a correlation between those two variables (Fig. 2). At temperature below 5°C in the external medium the action potential for all tested cell behaved in graded manner and such measurement were excluded from the investigation.

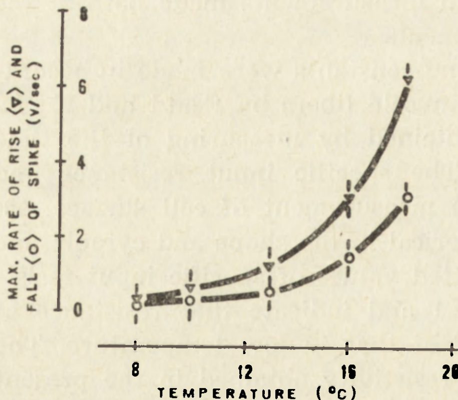

Fig. 3. Effect of temperature on the maximum rate of rise and fall phases of action potential. Bars denote ± S.E.

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The exact calculation of the values of the maximum rise and fall rates of the action potentials indicated that the rate of falling phase had the lower temperature coefficient than that of rise (Fig. 3 and Table 1). The obtained data lend some support to the suggestion that different cell mechanisms within the membrane of *Stentor* are responsible for the generation of those two phases of action potential.

Discussion

The present results showed explicitly that altering temperature of external medium appreciable changed the membrane potential in protozoan cells. The well-marked negative temperature-potential relationship is in apparent disagreement with those expected from classical ion hypothesis. The divergences from Nernst's equation have been obtained for muscle and nerve fibers (Apter and Koketsu 1960, Tobias 1950, Shaw et al. 1956, Stampfi 1959). Thus, the resting membrane potential of squid axon is little affected by temperature changes in the range of 2–20°C (Hodgkin and Katz 1949), whereas in both lobster axon and muscle fibers of frog, the resting potential was depolarized markedly with rising of temperature (Senft 1967). Another finding was reported by Apter and Koketsu (1960) and related to the temperature-potential relationship in sartorius frog muscle. The membrane potential in this preparation has been found to alter the potential value variously with temperature change. In Ca^{++}-rich external solution with usual K^{+} concentration the steady-state potential was independent of temperature, while this potential was significantly depolarized with warming of Ca^{++}-free external solution containing both K^{+} and Na^{+} ions. Such negative temperature-potential relation was entirely reversible in all media containing no Ca^{++} or low calcium concentration. The results obtained for mentioned relationship for sartorius frog muscle seem to be consistent with the data of the present investigation on protozoan cell.

From the foregoing evidence it is clear that the generation of resting potential in some muscle and nerve fibers or in protozoan cell, especially in the range from 2°C to 20°C, is rather difficult or even impossible to explain if someone accepts that the resting potential should change in direct proportion to absolute temperature change in compliance with the Nernst's equation. It has to be much less change in potential magnitude with temperature than it is practically. As it was pointed out previously by Hodgkin and Katz (1949) and later by other physiologists (e.g., Koketsu and Kimura 1960, Ling 1960, Apter and Koketsu

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1960, Senft 1967, Stephenson 1957), the divergences observed in membrane potential data might be explained by assuming that the relative ion permeability in membrane alters with the temperature changes. And indeed, the mechanism governing potential seems to involve the interaction of Ca^{++} ions, incorporated within membrane, with external calcium and potassium in order to change the permeability to the individual ions in intact membrane (Apter and Koketsu 1960). The close similarity in the temperature course of both membrane potential and specific resistance in muscle and protozoan cell allows to utilize the similar arguments as mentioned above for the better explanation of membrane behavior in *Stentor* with temperature variation than the Nernst's hypothesis did. The exact nature of the genesis of membrane potential in *Stentor* needs further experiments to be elucidated.

On the other hand, the recent data indicate that neither Nernst's nor other hypotheses (Goldman 1943) are sufficiently helpful in the interpretation of membrane potential phenomena. There was proved that the temperature changes might modify the potential in the membrane by variation of metabolic energy output responsible for active ion transport in membrane of metazoan cells (Senft 1967, Ayrepetyan 1969). In lobster axon the hyperpolarization of membrane potential was observed when temperature was increased. This polarization resulted from an active efflux of Na^{++}, which was considerably diminished in the presence of metabolic inhibitor, 2,4-dinitrophenol. There was ascertained that for the proper functioning of the ionic pump in the membrane, the higher concentration of sodium in the cell, sufficiently high temperature and the presence of potassium in the external medium is necessary (e.g., Kernan 1962, Mullins and Noda 1963, Mullins and Adaw 1964, Kerkut and Thomas 1965, Ayrapetyan 1969, Nakijama and Takahashi 1966). The possibility of existence of active ion transport in *Stentor* was not investigated yet.

It was showed that, simultaneously with the high temperature dependence of internal potential in *Stentor*, a strong temperature influence on the spike parameters (height, rate of rise and fall) was consistently observed. The spike height was maximal at relatively low temperature (5–8°C) and it was diminished with temperature rising (Fig. 2). In approximation, the depolarization magnitude of the resting potential is similar to the magnitude of the spike height changes by the same temperature jump. This observation is consistent with the idea of a concentration potential possibility in *Stentor* like in other protozoan, *Paramecium* (Naitoh 1974, Byrne and Byrne 1978), an analoque of sodium concentration hypothesis in some metazoa (e.g., Hodgkin and Katz 1949 b). The maximum rate of spike rising and

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falling phases increased as the temperature was lowered to 5°C (Fig. 3). Over the tested temperature range the maximal rate of rising part of spike substantially exceeded the rate of falling one, thus showing an opposite behavior to that in giant axon of squid (Hodgkin and Katz 1949 a). The reversal of temperature effect on the spike rates in different phases is suggested to be due to a different ionic mechanisms underlying the generation of the action potentials in *Stentor* and membranes of higher organisms.

On the basis of data for the effect of temperature on the electrical parameters of membrane in *Stentor* it is possible to suppose that a number of protozoan membrane properties is analogous to muscle and nerve tissue properties in general, but show the differences in details as well.

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RÉSUMÉ

L'influence de la température ambiante sur le potentiel de repos et sur le potentiel d'action a été étudiée chez *Stentor coeruleus*. Entre 5 et 18° C le potentiel de membrane est dépolarisé avec l'augmentation de la température du milieu. Les vitesses maximales des phases ascendante et descendante du spike augmentent également de façon continue avec l'augmentation de la température. Cependant, le coefficient thermique est considérablement plus élevé pour l'ascension du spike que pour son delin (le Q_{10} s'élève approximativement à 10 dans le premier cas et à 5 dans le deuxième, centre 8 et 18° C). L'amplitude du potentiel d'action est clairement réduite avec l'augmentation de la température ambiante. La résistance et la capacité de membrane augmentent quand la température tombe. Les résultats suggèrent que le mécanisme de génération du potentiel de repos, ainsi que celui du potentiel d'action, sont chez le *Stentor* sensibles à la température.

REFERENCES

Apter J. T. and Koketsu A. 1960: Temperature studies implicating calcium in regulation of muscle membrane potential. J. Cell Comp. Physiol., 56, 123–127.

Ayrepatyan S. N. 1969: The effect of temperature on the membrane potential of giant neurons of snails. Biofizika, 14, 663–668.

Byrne B. J. and Byrne B. C. 1978: Behavior and the excitable membranes in *Paramecium*. CRC Crit. Rev. Microbiol., 58–108.

http://rcin.org.pl

Castillo J. and Machne X. 1953: Effect of temperature on the passive electrical properties of the muscle fibre membrane. J. Physiol., 120, 431–434.
Dryl S. and Gręebecki A. 1966: Progress in the study of excitation and response in ciliates. Protoplasma, 2–3, 255–284.
Dryl S. and Łukowicz M. 1974: In: *Paramecium,* a Current Survey (ed. Wagtendonk W. J.), Amsterdam (Lond.) N. Y., 165–218.
Fabczak S. 1979: Electrical properties of cell membrane in protozoan, *Stentor coeruleus.* I. Modification of resting potential by extracellular ions. Acta Protozool., (in press).
Fatt P. and Katz B. 1951: An analysis of the end-plate potential recorded with an intracellular electrode. J. Physiol., 115, 320–370.
Goldmann D. E. 1943: Potential, impedance, and rectification in membranes. J. Gen. Physiol., 27, 37–60.
Hildebrandt E. 1978: Ciliary reversal in *Paramecium*: Temperature dependence of K^{+}-induced excitability, decrease and recovery. J. Comp. Physiol., 127, 39–44.
Hodgkin A. L. and Katz B. 1949: Effect of temperature on electrical activity of giant axon of squid. J. Physiol., 109, 240–249.
Hodgkin A. L. and Katz B. 1949: The effect of sodium ions on the electrical activity of the giant axon of squid. J. Physiol., 108, 11–37.
Jenerick H. P. and Gerard R. W. 1953: Membrane potential and threshold of single muscle fibers. J. Cell Comp. Physiol., 42, 79–102.
Kerkut G. A. and Thomas R. C. 1965: An electrogenic sodium pump in snail nerve cells. Comp. Bioch. Physiol., 14, 167–183.
Kernan R. P. 1962: The role of lactate in the active excretion of sodium by frog muscle. J. Physiol., 162, 129–137.
Kinosita H., Dryl S. and Naitoh Y. 1964: Relation between the magnitude of membrane potential and ciliary activity in *Paramecium.* J. Fac. Sci. Univ. Tokyo, Sect. IV, Vol. 10, 303–309.
Koketsu K. Y. and Kimura Y. 1960: The resting potential and intracellular potassium of sceletal muscle in frogs. J. Cell Comp. Physiol., 55, 105–128.
Kokina N. N. and Doronin Y. K. 1970: Electrical characteristics of the excitables structures of some unicellular organisms. Cytologia, 12, 804–809.
Ling G. N. and Woodbury J. W. 1949: Effect of the temperature on the membrane potential of frog muscle fibers. J. Comp. Cell Physiol., 34, 407–412.
Ling G. N. 1960: The interpretation of selective ionic permeability and cellular potential in terms of the fixed charge-induction hypothesis. J. Gen. Physiol., 43, 149–174.
Machemer H. and J. de Peyer J. 1977: Swimming sensory cells: Electrical membrane parameters, receptor-properties and motor control in ciliated *Protozoa.* Verh. Dtsch. Zool. Ges., 86–110.
Marchiafava P. L. 1970: The effect of temperature change on membrane potential and conductance in *Aplysia* giant nerve cell. Comp. Biochem. Physiol., 34, 847–852.
Mullins L. J. and Noda K. J. 1963: The influence of sodium-free solutions on the membrane potential of frog muscle fibers. J. Gen. Physiol., 47, 117–132.
Mullins L. J. and Adaw M. W. 1964: The membrane potential and sodium efflux from sodium-loaded muscle fibers. Fed. Proc., 23, 212.
Naitoh Y. 1974: Bioelectric basis of behavior in protozoa. Am. Zool. 14, 873–893.
Nakijama S. and Takahashi K. J. 1966: Post-tetanic hyperpolarization and electrogenic Na-pump in stretch receptor neurone of crayfish. J. Physiol., 187, 105–127.
Oliphant J. F. 1938: The effect of chemicals and temperature on reversal in ciliary action in *Paramecium.* Physiol. Zool., 11, 19–30.
Senft J. P. 1967: Effects of some inhibitors on the temperature-component of resting potential in lobster axon. J. Gen. Physiol., 50, 1835–1848.
Shaw F. H., Simon S. E. and Johnstone B. M. 1956: The noncorrelation of bioelectric potentials with ionic gradients. J. Gen. Physiol., 40, 1–18.
Stampfi R. 1959: Is the resting potential of Ranvier nodes a potasium potential? Ann. N. Y. Acad. Sci., 81, 265–284.
Stephenson W. K. 1957: Membrane potential changes and ion movements in the frog sartorius muscle. J. Cell Comp. Physiol., 50, 105–128.

http://rcin.org.pl

De Terra N. 1966: Culture of *Stentor coeruleus* and *Colpidium compylum*. J. Protozool., 11, 491–492.
Tobias H. J. 1950: Injury and membrane potential in frog muscle after depleting potassium and producing other changes by soaking in potassium-free salt solution or distilled water. J. Cell Comp. Physiol., 36, 1–13.
Wood D. C. 1970: Electrophysiological studies of the protozoan, *Stentor coeruleus*. J. Neurobiol., 1, 363–367.
Wood D. C. 1963: Stimulus specific habituation in a protozoan. Physiol. Behav., 11, 349–354.

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Effects of Cytochalasin B and Colchicine on Cytoplasmic Streaming in *Paramecium bursaria*

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Synopsis. Immobilized *Paramecium bursaria* were exposed to cytochalasin B (CB), dimethyl sulfoxide (DMSO) — the solvent for CB, colchicine and lumicolchicine to distinguish between the effects of these agents on a rotational cytoplasmic streaming. CB-treated cells had higher percentage of specimens in a sample with streaming inhibited than cells exposed to the equivalent DMSO concentrations. Colchicine and lumicolchicine at high concentrations stopped as well cytoplasmic streaming, therefore this effect seems to be unspecific and not related to microtubule disassembly effect. These facts point out to the conclusion that structures and/or mechanism affected by CB and DMSO are involved in cytoplasmic streaming propulsion in *Paramecium* cell.

Streaming of cytoplasm among protists is promoted at the expense of endogenous cellular energy by means of two main systems. It is suggested that in amoebae the motile system depends mainly upon the activity of thin and thick filaments (Pollard and Weihing 1974, Taylor and Condeelis 1979) identified as an actin and myosin (see review Allen and Allen 1978) whereas in other protists like: heliozoa (Shigenaka 1976, Shigenaka et al. 1975, 1979, Toyohara et al. 1978), foraminifera (Edds 1975 a, 1975 b) and ciliates (Tucker 1972, 1978, Bardele 1974, Tucker and Mackie 1975, Hausman and Peck 1978) microtubules or microtubules with microfilaments (Hauser et al. 1980) are considered as a structures responsible for force generation.

Up to now there is a lack of knowledge about ultrastructures which might be involved in propulsion of cytoplasmic streaming in *Paramecium.* On account of that, microfilaments and/or microtubules are being suspected to act in this phenomenon, the effects of cytochalasin B (CB) and colchicine on cytoplasmic streaming have been investigated. CB and its solvent dimethyl sulfoxide (DMSO) are known as drugs which affect

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microfilaments and membranes (Wessels et al. 1971, Estensen et al. 1971, Sanger and Holtzer 1972, Bradly 1973). In *Paramecium* cell CB affects membranes (Tołłoczko 1977), occasionally infraciliary lattice, while cortical microfilaments remain unchanged (Sibley et al. 1977). *Paramecium's* filamentous systems were investigated so far mainly in the region close to the cortex of the cell (Pitelka 1969, Ehret and McArdle 1974) whereas the inner parts of the cell, where the rotational streaming of cytoplasm occurs, only "irregular fibrous elements near to the limits of resolution" have been reported (Jurand and Selman 1969). The colchicine is known as a specific compound which binds tubulin subunits and inhibits microtubule polymerization also in protists (Rosenbaum and Carlson 1969, Nelsen 1970) with some exceptions (Wunderlich and Peyk 1969, Flanagan and Warr 1978). Therefore physiological experiments as a preliminary test for identification of ultrastructures which might be involved in motile system of cytoplasmic streaming in *Paramecium bursaria* have been attempted.

Material and Methods

Paramecium bursaria grown at room temperature 19 ± 2° C on lettuce medium with unspecified bacteria was used. To decrease the number of symbiotic green algae in the cell the culture was breaded in darkness. The purpose of this study was to determine the influence of two drugs: cytochalasin B and colchicine on cytoplasmic streaming within *Paramecium bursaria* cells. To visualize the changes in cytoplasmic flow, the experimental culture was immobilized by nickel method (Sikora and Wasik 1978). All observations were done under bright light microscope at room temperature 19 ± 2° C.

Cytochalasin B. Pure cytochalasin B (Serva) was dissolved in DMSO (Reachim) to make a stock solution 1 μg/ml. Before experiments different concentrations of CB in DMSO and corresponding concentrations of pure DMSO as a control were made by dilution in the Tris-HCl buffer at pH 7.2 (Ionic strength = = 0.01) containing KCl and $CaCl_2$ both in final concentration 1 mM. *Paramecium* cells washed in Tris-HCl buffer were added to give the desired concentrations of CB in DMSO (20–75 μg/ml) and pure DMSO (2–7.5% v/v).

Colchicine. The pure colchicine (BDH Chemicals Ltd) was dissolved in the phosphate buffer at pH 6.8 (Ionic strength = 0.015) containing KCl and $CaCl_2$ both in final concentration 1 mM. The final concentrations of colchicine (0.02–10 mM) were made by adding washed *Paramecium* cells in phosphate buffer. Colchicine decomposes in Tris-HCl buffer (Margulis 1973) therefore the change of buffer was necessary. As a control of the effect of colchicine, the lumicolchicine in corresponding concentrations have been used. The lumicolchicine was obtained by using Wilson and Friedkin (1966) method. The conversion of colchicine into lumicolchicine was determined by change in the absorption spectrum.

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Results

The purpose of the study was to examine the influence of cytochalasin B and colchicine on rotational cytoplasmic streaming within immobilized *Paramecium bursaria* cell. Both drugs cause a distinct retardation and eventually cessation of the streaming. The cessation of the flow takes place uniformly in the whole route of the cytoplasmic streaming. All the agents used (CB + DMSO, DMSO, colchicine and lumicolchicine) did not cause any changes in *Paramecium bursaria* cell shape even at the highest concentrations used. Cells undergo body deformation only directly before death. The CB + DMSO and DMSO in concentrations tested did not produce higher lethality than found by Sibley et al. (1977).

Ciliates exposed to 2% DMSO do not show changes in streaming of cytoplasm whereas in 20 μg/ml of CB + DMSO about 30% of surviving Paramecium cells undergo cessation of rotational streaming. After 1 h of exposure the percentage of cells with arrested streaming increases in higher concentrations of CB + DMSO and DMSO, however, in all cases the combined effect of drug and its solvent was higher as compared to the effect of DMSO alone (Fig. 1). After 10 min exposure the number of cells with cytoplasmic streaming abolished was distinctly higher than after 1 or 2 h exposure to CB + DMSO or DMSO only.

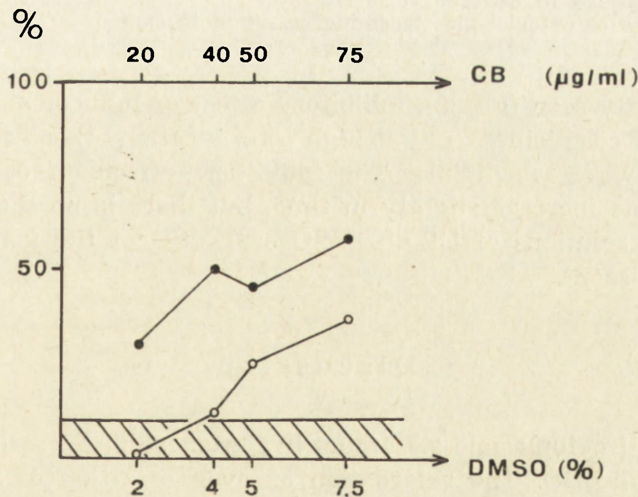

Fig. 1. Percentage of *Paramecium bursaria* cells with cytoplasmic streaming stopped after 1 h exposure to different concentrations of CB + DMSO (·) and DMSO (∘) Range of cytoplasmic streaming cessation in control cells is expressed as a lined area

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The reactivation of streaming take place in *Paramecium* cells remained in the solution containing drugs.

The colchicine, the second drug analysed, also inhibits cytoplasmic streaming but only at hight concentrations. Remarkable cessation was seen at 5 mM and higher concentrations (Fig. 2). As a control of possible nonspecific effect of colchicine, the isomer — lumicolchicine was used.

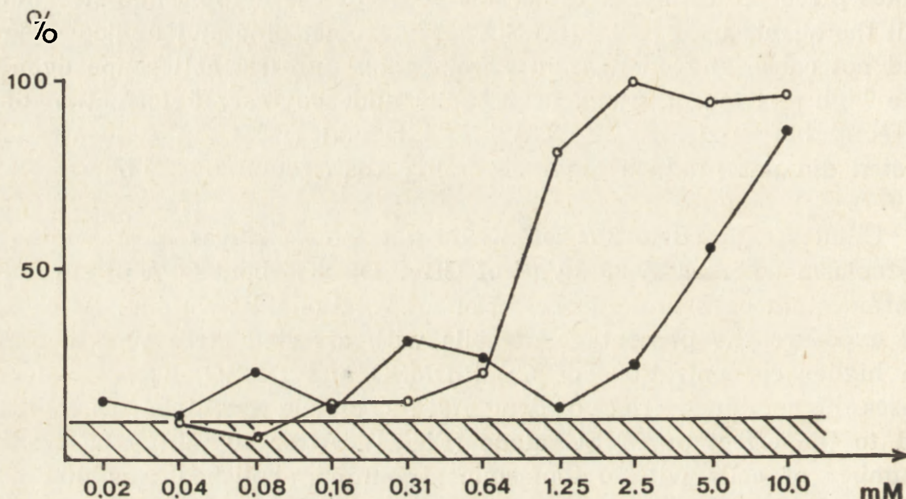

Fig. 2. Percentage of *Paramecium bursaria* cells with cytoplasmic streaming stopped after 1 h exposure to different concentrations of colchicine (•) and lumicolchicine (◦). Range of cytoplasmic streaming cessation in control cells is expressed as a lined area

As it is clearly seen (Fig. 2), inhibitory effect of lumicolchicine is much alike that of colchicine. Only at high concentrations there are some differences, however negligible. The inhibitory effects of colchicine and lumicolchicine increase slightly in time, but there is no shock effect as observed in solutions of CB + DMSO or DMSO at the very beginning of drug treatment.

Discussion

Rotational cytoplasmic streaming in *Paramecium bursaria* is affected by CB and DMSO. The retardation or even cessation of streaming is more frequent among *Paramecium* cells during the first 10 min of exposure to the drugs than later, after 1 or 2 h. Between 10 min and 1 h percentage of animals with cytoplasmic flow arrested decrease to the level shown on Fig. 1. Therefore it might be suggested that, espe-

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cially at the very beginning of experiments, dramatic increase of cell membrane permeability caused by DMSO (Szmant 1975) evoke osmotic disturbances which are responsible for retardation of cytoplasmic streaming (Yamada 1969).

The CB-treated samples show after 1 h a higher percentage of cells with cytoplasmic streaming arrested than the samples exposed to the equivalent concentrations of DMSO alone. Although numerous data show unspecific effects of CB (Estensen et al. 1971, Spooner et al. 1971, Sanger and Holtzer 1972, Tołłoczko 1977), it seems possible that mechanism and/or structures (possibly microfilaments — Pollack and Rifkin 1976) which are involved in causing the cytoplasmic streaming are affected by CB in an unknown way. This conclusion is reinforced by comparison the colchicine and lumicolchicine effects on cytoplasmic streaming in *Paramecium bursaria*.

Colchicine, the specific agent binding to the subunits of microtubules, is as effective as lumicolchicine which does not bind to tubulin (Wilson and Bryan 1974). This clearly shows that microtubules are not likely to be involved in propulsion of *Paramecium* cytoplasm. Cytoplasmic streaming and food vacuole transport in some other ciliates are promoted by mechanism which involves microtubules as being responsible for force generation (Kitching 1938, Tucker 1972, 1978, Allen 1974, 1975, Allen and Wolf 1974, Bardele 1974, Tucker and Mackie 1975, Hausman and Peck 1978, Hauser et al. 1980, Tołłoczko 1980 a, 1980 b), while in *Paramecium* it seems more probable that microtubules may provide a guiding system beyond the "channel" (Sikora et al. 1979 a) but microfilaments seem to be involved in guiding (Sikora et al. 1979 b) and propulsion of the cytoplasm inside the "channel". Unfortunately filamentous elements in the *Paramecium* cytoplasm showing ability to stream are not sufficiently known (Jurand and Selman 1969) to indicate what structures are involved in the mechanism of cytoplasmic motility. Bundles of microfilaments were found at the cortical region of *Paramecium* cell (Hufnagel 1969, Pitelka 1969, Allen 1971, Sibley et al. 1977), however) up to now there is no data proving their presence in the inner parts of the cell. Therefore the present data might suggest that mechanism promoting rotational cytoplasmic streaming in *Paramecium* is probably related to the activity of microfilaments, though they have not been visualized yet, while microtubules seem not to be involved.

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RÉSUMÉ

Les cellules immobilisées de *Paramecium bursaria* étaient exposées à la cytochalasine B(CB), au DMSO — le solvant pour la CB, à la colchicine et lumicolchicine, pour faire distinction entre les effets que ces substances exercent sur le courant rotatoire du cytoplasme. Les échantillons traités à la CB démontraient un pourcentage plus élevé des individus avec le courant supprimé que les échantillons exposés aux concentrations équivalentes du DMSO seul. La colchicine et la lumicolchicine à des concentrations élevées arrêtent également le courant cytoplasmique, mais leurs effets ne semblent pas être spécifiques et liés à une disjonction des microtubules. Ces résultats suggèrent que la propulsion du courant cytoplasmique dans la cellule de *Paramecium* dépend des structures et des mécanismes sensibles à la CB et au DMSO.

REFERENCES

Allen R. D. 1971: Fine structure of membranous and microfibrillar system in the cortex of *Paramecium caudatum*. J. Cell Biol., 49, 1–20.

Allen R. D. 1974: Food vacuole membrane growth with microtubule-associated membrane transport in *Paramecium*. J. Cell Biol., 63, 904–922.

Allen R. D. 1975: Evidence for firm linkages between microtubules and membrane-bounded vesicles. J. Cell Biol., 64, 497–503.

Allen R. D. and Wolf R. W. 1974: The cytoproct of *Paramecium caudatum*: structure and function, microtubules and fate of food vacuole membranes. J. Cell Sci., 14, 611–631.

Allen R. D. and Allen N. 1978: Cytoplasmic streaming in amoeboid movement. Annu. Rev. Biophys. Bioeng., 7, 469–495.

Bardele C. F. 1974: Transport of materials in the suctorian tentacle. Symp. Soc. Exp. Biol., 28, 191–208.

Bradly M. O. 1973: Microfilaments and cytoplasmic streaming: inhibition of streaming with cytochalsin. J. Cell Sci., 12, 327–343.

Edds K. T. 1975 a: Motility in *Echinosphaerium nucleofilum*. I. An analysis of particle motions in axopodia and a direct test of the involvement of the axoneme. J. Cell Biol., 66, 145–155.

Edds K. T. 1975 b: Motility in *Echinosphaerium nucleofilum*. II. Cytoplasmic contractility and its molecular basis. J. Cell Biol., 66, 156–164.

Ehret C. F. and McArdle E. W. 1974: The structure of *Paramecium* as viewed from its constituent levels of organization. In: *Paramecium*, A Current Survey (ed. W. J. wan Wagtendonk), Elsevier Sci. Publ. Comp., Amsterdam, New York, 263–338.

Estensen R. D., Rosenberg M. and Sheriden J. D. 1971: Cytochalasin B: microfilaments and "contractile" processes. Science, 173, 356–357.

Flanagan D. J. and Warr J. R. 1978: Colchicine — binding of cell extracts from colchicine resistant mutants of *Chlamydomonas reinhardi*. Exp. Cell Res., 116, 474–478.

Hauser M., Hausmann K. and Jockusch B. M. 1980: The microtubular--microfilament complex of the cytopharyngeal basket of the ciliate *Pseudomicrothorax dubius* contains tubulin, actin and α-actinin: an immunofluorescence microscopic study (in press).

Hausmann K. and Peck R. K. 1978: Microtubules and microfilaments as major components of a phagocytic apparatus: the cytopharyngeal basket of the ciliate *Pseudomicrothorax dubius*. Different., 11, 157–168.

Hufnagel L. A. 1969: Cortical structure of *Paramecium aurelia*. J. Cell Biol., 40, 779–801.

Jurand A. and Selman G. G. 1969: The Anatomy of *Paramecium aurelia*. Macmillan, St. Martin's Press, New York, 1–218.

http://rcin.org.pl

Kitching J. A. 1938: On the mechanism of movement of food vacuoles in peritrich Ciliates. Arch. Protistenkd., 91, 77–87.

Margulis L. 1973: Colchicine-sensitive microtubules. Int. Rev. Cytol., 34, 333–361.

Nelsen E. M. 1970: Division delays and abnormal oral development produced by colchicine in *Tetrahymena*. J. Exp. Zool., 175, 69–84.

Pitelka D. R. 1969: Fibrillar systems in protozoa. In: Research in Protozoology, (ed. T. T. Chen), 3, 279–388.

Pollack R. and Rifkin D. B. 1976: Modification of mammalian cell shape: Redistribution of intracellular action by SV 40 virus, proteases, cytochalsin B and dimethylsulfoxide In: Cell Motility (eds. R. Goldman, T. Pollard and J. Rosenbaum), Cold Spring Harbor Conferences on Cell Proliferation, Cold Spring Harbor Laboratories, 3, 389–401.

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

Rosenbaum J. L. and Carlson K. 1969: Cilia regeneration in *Tetrahymena* and its inhibition by colchicine. J. Cell Biol., 40, 415–425.

Sanger J. W. and Holtzer H. 1972: Cytochalasin B: effects on cell morphology, cell adhesion and mucopolysaccaride synthesis. Proc. Natl. Acad. Sci. U.S.A., 69, 253–257.

Shigenaka Y. 1976: Microtubules in protozoan cells. II. Heavy metal ion effects on degradation and stabilization of heliozoan microtubules. Annot. Zool. Jpn., 49, 164–176.

Shigenaka Y., Tadokoro I. and Kaneda M. 1975: Microtubules in protozoan cells. I. Effect of light metal ions on the heliozoan microtubules and their kinetic analysis. Annot. Zool. Jpn., 48, 227–241.

Shigenaka Y., Maruoka T., Toyohara A. and Suzaki T. 1979: Microtubules in protozoan cells. IV. Effects of sulfhydryl-blocking reagents on axopodial degradation and reformation in heliozoa. Cell Struct. Funct., 4, 23–34.

Sibley J. T., Paul M. D. and Hanson E. D. 1977: Subcellular effects of cytochalasin B and dimethylsulfoxide on *Paramecium aurelia*. J. Protozool., 24, 595–604.

Sikora J. and Wasik A. 1978: Cytoplasmic streaming within Ni^{2+} immobilized *Paramecium aurelia*. Acta Protozool., 17, 389–397.

Sikora J., Wasik A. and Baranowski Z. 1979 a: The estimation of velocity distribution profile of *Paramecium* cytoplasmic streaming. Europ. J. Cell Biol., 19, 184–188.

Sikora J., Wasik A. and Allen R. D. 1979 b: The role of single particle saltation in *Paramecium* cytoplasmic streaming. Acta Protozool., 18, 201.

Spooner B. S., Yamada K. M. and Wessels N. K. 1971: Microfilaments and cell locomotion. J. Cell Biol., 49, 595–613.

Szmant H. H. 1975: Physical properties of dimethyl sulfoxide and its function in biological systems. Ann. New York Acad. Sci.. 243. 20–23.

Taylor D. L. and Condeelis J. S. 1979: Cytoplasmic structure and contractility in amoeboid cells. Int. Rev. Cytol., 56, 57–144.

Tołłoczko B. 1977: Endocytosis in *Paramecium*. III. Effect of cytochalsine B and colchicine. Acta Protozool., 16, 185–193.

Tołłoczko B. 1980 a: Cytochalsine B induced inhibition of food ingestion in *Dileptus anser*. Acta Protozool., (in press).

Tołłoczko B. 1980 b: Effect of colchicine on food ingestion in *Dileptus anser*. Acta Protozool., (in press).

Toyohara A., Shigenaka Y. and Mohri H. 1978: Microtubules in protozoan cells. III. Ultrastructural changes during disintegration and reformation of heliozoan microtubules. J. Cell Sci., 32, 87–98.

Tucker J. B. 1972: Microtubule-arms and propulsion of food particles inside a large feeding organelle in the ciliate *Phascolodon vorticella*. J. Cell. Sci., 10, 883–903.

Tucker J. B. 1978: Endocytosis and streaming of highly gelated cytoplasm alongside rows of arm-bearing microtubules in the ciliate *Nassula*. J. Cell Sci., 29, 213–232.

Tucker J. B. and Mackie J. B. 1975: Configurational changes in helical

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microtubule frameworks in feeding tentacles of the suctorian ciliate *Tokophrya*. Tissue and Cell, 7, 601–612.

Wessels N. K., Spooner B. S., Ash J. F., Bradley M. O., Luduena M. A., Taylor E. L., Wrenn J. T. and Yamada K. M. 1971: Microfilaments in cellular and developmental processes. Science, 171, 135–143.

Wilson L. and Bryan J. 1974: Biochemical and pharmacological properties of microtubules. In: Advance in Cell and Molecular Biology (ed. E. J. Du Praw) Academic Press, N. Y., San Francisco, London, 3, 21–72.

Wilson L. and Friedkin M. 1966: The biochemical events of mitosis. I. Synthesis and properties of colchicine labeled with tritium in its acetyl moiety. Biochem., 5, 2463–2468.

Wunderlich F. and Peyk D. 1969: Antimitotic agents and macronuclear division of ciliates. II. Endogenous recovery from colchicine and colcemid — a new method of synchronization in *Tetrahymena pyriformis* GL. Exp. Cell Res., 57, 142–144.

Yamada K. 1969: A comparative study on cyclosis in *Paramecium* J. Sci. Hiroshima Univ. Ser. B, Div. I, 22, 127–153.

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Effect of Colchicine on Food Ingestion in *Dileptus anser*

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Synopsis. Colchicine causes the diminishing of food vacuoles formation rate. Its action is probably due to the disturbances caused in functioning of labile microtubules which take part in the transport of membranous material in the area of oral apparatus. Endocytosis is also inhibited when colchicine blocks the regeneration of nemadesmal basket.

Three main classes of ultrastructural elements are visible in the region of oral apparatus in carnivorous ciliate *Dileptus*: microtubules, microfilaments and membranes (Grain and Golińska 1969, Kink 1973, Golińska and Kink 1976, Golińska 1978). The first ones are extremely numerous forming nemadesmata of the oral basket, transverse fibers and perpendicular microtubules. It has been suggested that perpendicular microtubules separately lying in the area of so called "phagoplasm" are labile and can play a role in the transport of membranous material to the nascent food vacuole (Tołłoczko 1978). In order to establish the role of microtubular elements in the process of food ingestion in *Dileptus* the effect of antimicrotubular drug colchicine was studied.

Material and Methods

Dileptus anser O. F. M. cells were cultivated in Pringsheim solution. They were fed every other day with filtrated and condensed culture of *Colpidium colpods*. Colpidia were cultivated separately in Pringsheim fluid with addition of egg yolk.

For experiments cells before feeding were collected and washed with 1 mM phosphate buffer pH 7.1. They were kept in this buffer overnight. Three kinds of experiments were carried out.

(1) Colchicine (BDH Chemicals Ltd., Poole, England) in concentrations 1–10 mM was added to the samples containing about 20 cells each. After 5, 30, 60 min or

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24 h of colchicine treatment colpidia were added for 5 or 30 min. Samples were fixed with 10% formalin and the number of specimens containing and lacking of food vacuoles was counted in colchicine treated and in control cells treated with phosphate buffer. The experiment was repeated three times and the percentage of dileptuses with food vacuoles and without vacuoles was calculated.

(2) The observations of single living cells of *Dileptus* exposed to the prey after and without colchicine treatment were also carried out. The number of food vacuoles formed during 1–30 min exposure to Colpidia was counted in each single cell of *Dileptus*. 10–17 cells in each colchicine concentration were observed and the mean number of vacuoles was calculated.

(3) The effect of 5 mM colchicine added in different stages during regeneration of oral apparatus in posterior fragments of *Dileptus* was studied in order to elucidate the role of nemadesmal basket in the process of endocytosis.

For studies with electron microscope colchicine treated and control cells were prepared as described by Golińska and Kink (1977). Sections obtained with the LKB ultramicrotome were stained with uranyl acetate and lead citrate and observed in the JEM 100B electron microscope.

Results

The effects of colchicine appear to be time and concentration dependent (Table 1, 2). Five min treatment with 10 mM solution of the drug causes that almost 50% of *Dileptus* cells are not able to form food vacuoles during 5 min exposure to colpidia, whereas it was 60 min before 1 mM colchicine inhibited phagocytosis to the same extent. All specimens from the control group ingest the prey during 5 min exposure to it. It is significant that during longer exposure to colpidia (30 min) higher per cent of dileptuses form food vacuoles. It indicates, that probably the rate of endocytosis is slowed by colchicine rather then complete inhibition occurs. After 24 h of treatment with 10 mM and 5 mM colchicine the cells became spherical and obviously exhibit no feeding reaction. After 2.5 mM colchicine treatment dileptuses have

Table 1

Percentage of *Dileptus anser* cells with (+) and without (—) food vacuoles exposed to colpidia for 5 min after different times of colchicine treatment

Time of treatment	Concentration of colchicine (mM)									
	10		5		2.5		1		Control	
	+	—	+	—	+	—	+	—	+	—
5	46	54	86	4	97	3	94	6	100	0
30	32	68	58	42	79	21	87	13	100	0
60	0	100	0	100	54.5	45.5	60	40	100	0
24h	—	—	—	—	0	100	16	84	100	0

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Table 2

Percentage of *Dileptus anser* cells with (+) and without (−) food vacuoles exposed to colpidia for 30 min after different times of colchicine treatment

Time of treatment	Concentration of colchicine (mM)									
	10		5		2.5		1		Control	
	+	−	+	−	+	−	+	−	+	−
5	82	18	100	0	100	0	100	0	100	0
30	55	45	74	26	100	0	100	0	100	0
60	0	100	23	77	83	17	84	16	100	0
24h	—	—	—	—	0	100	30	70	100	0

shorter proboscises than control cells and do not form food vacuoles, either. Cells treated 24 h with 1 mM colchicine do not differ in their appearance from the control ones but their ability to endocytosis is much lower than in buffer treated cells.

Since dileptuses are able to divide food vacuoles already few minutes after the ingestion, it was impossible to count the number of vacuoles in each cell fixed after 5 or 30 min exposure to the prey. The observations of single, living cells allowed to measure the rate of food vacuole formation (Fig. 1). These data support the supposition that the rate of vacuole formation is much slower in colchicine treated cells. Even in the group where no differences between experimental and control cells are visible when the percentage of vacuole containing cells was calculated — the differences are very significant when we compare the number of vacuoles which are formed (for example during 30 min exposure to colpidia after 5 and 30 min treatment with 1 mM colchicine).

The observations of the living cells have revealed that both functions (i.e., food trapping and food ingestion) are slowed in similar manner, since the prey killing without subsequent mouth opening and vacuole formation was only very rarely observed. The electronmicrographs show that in colchicine treated cells neither nemadesmal basket nor transverse fibers on the cytostome area are disrupted or changed, but the number of labile microtubules and disc shape vesicles (which are very numerous in control cells) seems to be diminished (Pl. I 1–4).

In order to establish the role of nemadesmal basket in the process of food vacuoles formation 5 mM colchicine was added in different stages of regeneration of posterior fragments as it is shown on the diagram (Fig. 2). *Dileptus* cells were sectioned in halves and posterior fragments were isolated. Colchicine solution was added just after the operation (group I), 1 h after the operation (group II) and 2 h after the operation (group III). Control cells were treated with 1 mM phosphate buffer. After 3 h (when regeneration is complete in control cells) all groups

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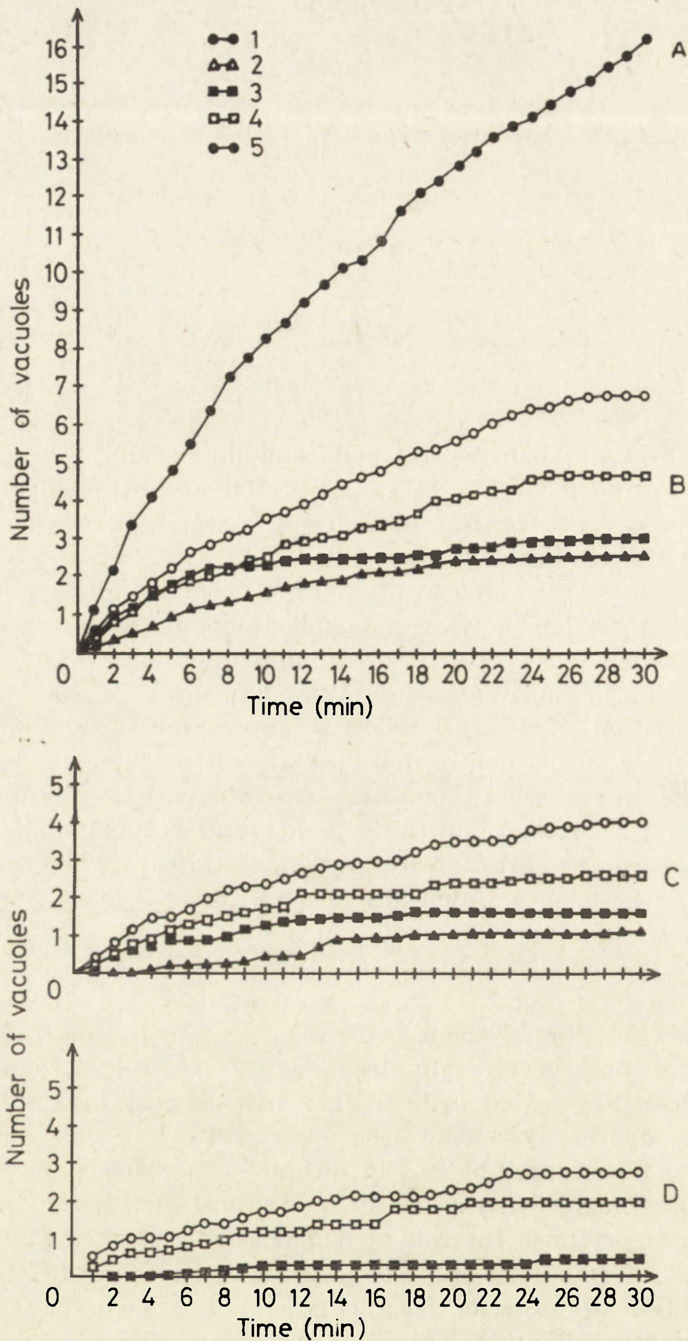

Fig. 1. The rate of food vacuole formation in *Dileptus anser* after different time of colchicine treatment. A — control cells, B — the rate of vacuole formation after 5 min of colchicine treatment, C — the rate of vacuole formation after 30 min of colchicine treatment, D — the rate of vacuole formation after 1 h of colchicine treatment. *1* — control cells, *2* — 10 mM colchicine, *3* — 5 mM colchicine, *4* — 2.5 mM colchicine, *5* — 1 mM colchicine

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were exposed to colpidia and their ability to capture the prey (C) and of ingestion (I) were observed. Since during such treatment both effects of colchicine: the influence on nemadesmal basket formation and its action during the exposure to colpidia can be observed it may be difficult to distinguish these two effects. Hence, other *Dileptus* fragments were treated with colchicine from 0 to 1st h after the operation

Table 3

Food capturing (C) and food ingestion (I) in posterior fragments of *Dileptus anser* treated with colchicine in different stages of regeneration

No. exp. group	No. of series 1		2		3		4		5	
	Function C	I	C	I	C	I	C	I	C	I
C	+	+	+	+	+	+	+	+	+	+
I	–	–	–	–	–	–	–	–	–	–
II	–	–	–	–	–	–	–	–	–	–
III	–	–	–	–	+	–	–	–	+	–
C′	+	+	+	+	+	+	+	+	+	+
I′	–	–	–	–	–	–	–	–	–	–
II′	–	–	–	–	–	–	–	–	–	–
III′	+	+	+	+	+	+	+	+	+	+

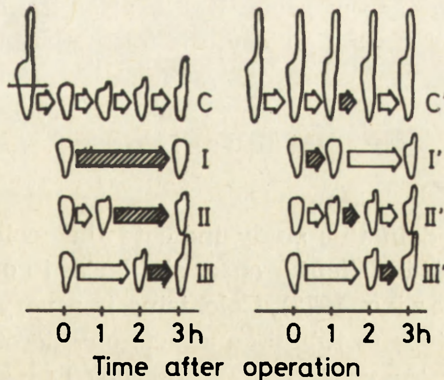

Fig. 2. The scheme of experiments with regeneration of posterior fragments of *Dileptus anser* treated with 5 mM colchicine (arrows) after different time after the operation. C — control group, I — cells treated with colchicine during the whole time of regeneration (0–3rd h), II — cells treated with colchicine from 2nd to 3rd of regeneration, III — cells treated with colchicine during the last h of regeneration (2nd to 3rd h), C — cells which were not operated but treated with colchicine during the same period of time as cells from group II', I' — cells treated with colchicine during the first h of regeneration II' — cells treated with colchicine from 1st to 2nd h of regeneration, III' — cells treated with colchicine during the last h of regeneration and washed with buffer before adding colpidia

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(group I′), from 1st to 2nd h after the operation (group II′) and from 2nd to 3rd h after the operation (group III′) and their ability to endocytosis was observed after washing them in phosphate buffer. As an additional control nonoperated dileptuses were treated with colchicine during one hour in the same time as group II′. The data obtained from these experiments indicate that neither prey capturing, nor food ingestion occur when colchicine is added before nemadesmal basket formation, which takes place (according to Bohatier and Kink 1977) 1–2 h after the operation (Table 3). Even cells treated with colchicine from 2nd h after the operation (group III) do not form food vacuoles and only some of them are able to kill colpidia, although their appearance resembles this of control cells. This effect may be caused by colchicine action on labile microtubules since 100% of nonoperated cells treated 1 h with 5 mM colchicine were not able to the food ingestion, either (Table 1, 2). The observation of the cells from group III′ in which normal endocytosis occurs, also support this interpretation. This conclusion is also supported by observations in EM which indicate that ciliates from group III have normally build nemadesmal basket, whereas the cells from group I have no nemadesma 3 h after the operation. In group I only dense microfibrillar material equipped with smooth vesicles and nonciliated kinetosomes can be observed and can be interpreted as the place where oral regeneration starts (Pl. II 1) Similar microfibrillar material can be also seen in the endoplasm (Pl. II 2). In dileptuses from group II 3 h after the operation no nemadesmal basket occurs and no endocytosis takes place, either.

Discussion

The data obtained in this study indicate that colchicine inhibits the process of endocytosis in *Dileptus anser* similar as in other ciliates (Nilsson 1973, Tolloczko 1977). Colchicine is a very well known agent which specifically binds to tubulin and acts on microtubules of different cells (Borisy and Taylor 1967 a, b, Shelansky and Taylor 1967, Olmsted and Borisy 1973, Wilson et al. 1974, Cheung et al. 1978). However, when it is added to preformed microtubules *in vitro* it is unable to induce their dissociation (Margolis and Wilson 1977). Neither it does disrupt microtubules of formed oral apparatus and cilia in *Tetrahymena* (Tamura et al. 1969, Nelson 1970, Wunderlich and Heuman 1974). It seems possible that in *Dileptus* its action can be explained as the effect of preventing the as-

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sembly of labile microtubules which are present in the area of the oral apparatus. These microtubules may play a role in the transport of phagoplasmic vesicles which are the membrane pool for food vacuoles similar as in *Paramecium* (Allen 1974), *Peritricha* (McKanna 1973 a, b) or *Tetrahymena* (Nilsson 1976). The disorganization of this transport causes probably that the mouth can not be opened and food vacuole is not formed. This assumption is supported by the observation that there are differences in the ultrastructure of phagoplasm between colchicine treated and control cells.

On the basis of different experiments (Taylor 1965, Rosenbaum et al. 1969, Nelson 1970, Schömharting et al. 1977) it can be assumed that the effect is not due to the influence of the drug on protein synthesis. However, it should be pointed out that while it is very likely that colchicine operates through microtubules nonetheless it can not be ruled out that there may be some other ways of its action, since colchicine inhibits also processes which obviously are not mediated by microtubules (Douglas and Sorimachi 1972, Gabbay and Tze 1972, Orr et al. 1972, Tritaro et al. 1972). A lot of data indicate that colchicine can act on plasma membrane (Feit and Barrondes 1972, Stadler and Franke 1972, Pitman et al. 1972, Berlin and Ukena 1972, Price 1974, Wunderlich and Heuman 1974, Byers 1974, Bhattacharyja and Wolff 1975, Chajek et al. 1975, Lagunoff and Chi 1976, Rembold and Langenbach 1978). On the other hand the persistence of apparently intact microtubules does not imply their unimpaired function, so it can not be excluded that both; function of labile microtubules and of microtubules of nemadesmata are disturbed during colchicine action.

Colchicine causes also the disturbance of the process of regeneration of *Dileptus*. The abnormal development of microtubules after colchicine treatment has been also observed in cytopharynx of *Nassula* (Tucker et al. 1975). In *Deleptus anser* stomatogenesis is normally achieved after 3 h (Bohatier and Kink 1977) whereas in colchicine treated cells only specimens from group III (i.e., treated with colchicine from 2nd to 3rd h after the operation) posses normal nemadesmal basket. These data are in agreement with studies of Bohatier and Kink (1977) who have observed basket formation in *D. anser* 1–2 h after the operation and Golińska and Grain (1969) who have obtained similar data for *D. cygnus*. In the cells treated with colchicine just after the operation or 1 h later the regeneration seems to be arrested. The complete inhibition of endocytosis in dileptuses which do not regenerate complet basket indicate that this structure plays a role not only in the stiffening the mouth or in guidance of form-

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ed vacuoles but plays an active role in the process of mouth opening. This hypothesis supports the investigations of Tucker (1978) and Hauser et al. (1979) who have postulated an active role of microtubules during food ingestion in other ciliata.

ZUSAMMENFASSUNG

Nach der Behandlung von *Dileptus anser* mit Kolchicine das Tempo von der Nahrungsvakuolen Ausbildung ist langsamer als in der Kontrole. Das ist wahrscheinlich veranlasst von der Störung von der Aktion der labilen Mikrotubulen. Die Endocytose ist auch aufgehaltet, wenn Kolchicine die Regeneration von Nemadesmalkorb blokiert.

REFERENCES

Allen R. D. 1974: Food vacuole membrane growth with microtubule associated membrane transport in *Paramecium*. J. Cell Biol., 63, 904–922.

Berlin R. D. and Ukena T. E. 1972: Agregation of polymorphonuclear leukocytes by concanavalin A is inhibited by colchicine and vinblastine which bind microtubular proteins. Nat. New Biol., 238, 525–534.

Bhattacharyja B. and Wolff J. 1975: Membrane bound tubulin in brain and thyroid tissue. J. Biol. Chem., 250, 7639–7646.

Bohatier J. and Kink J. 1977: Etude des synthèses protéiques au cours des processus morphogénetiques de division et de régenération chez *Dileptus anser*: Action de la cycloheximide. Protistologica, 13, 509–528.

Borisy G. C. and Taylor E. W. 1967 a: The mechanism of action of colchicine. Binding of colchicine 3H to cellular protein. J. Cell Biol., 34, 525–534.

Borisy G. C. and Taylor E. W. 1967 b: The mechanism of action of colchicine. Colchicine binding to the urchin eggs and the mitotic apparatus. J. Cell Biol., 34, 535–548.

Byers M. R. 1974: Structural correlates of rapid axonal transport: evidence that microtubules may not be directly involved. Brain Res., 75, 97–113.

Chajek T., Stein O. and Stein G. 1975: Colchicine induced inhibition of plasma lipoprotein lipase release in the intact rat. BBA 380, 127–131.

Cheung H. T., Centarow W. D. and Sundharados G. 1978: Colchicine and cytochalasin B (CB) effects on random movement, spreading and adhesion of mouse macrophages. Exp. Cell Res., 111, 95–103.

Douglas W. W. and Sorimachi M. 1972: Colchicine inhibits adrenal medollary secretion evoked by acetylcholine without affecting that evoked by potassium. J. Pharmacol., 43, 129–132.

Feit H. and Barrondes S. H. 1970: Colchicine activity in particulate fractions of mouse brain. J. Neurochem., 17, 1355–1364.

Gabbay K. H. and Tze W. J. 1972: Inhibition of glucose induced release of insulin by aldose reductase inhibitors. Proc. Natl. Acad. Sci. USA, 69, 1435–1439.

Golińska K. and Grain J. 1969: Observations sur les modifications ultrastructurales lors de la régénération chez *Dileptus cygnus* Clap et Lach 1859, Cilié, Holotriche, Gymnostome. Protistologica, 5, 447–463.

Golińska K. and Kink J. 1976: The regrowth of oral structures in *Dileptus cygnus* after partial excision. Acta Protozool., 15, 143–163.

Golińska K. and Kink J. 1977: Proportional regulation of body form and cortical organelle pattern in the ciliate *Dileptus*. J. Cell Sci., 24, 11–29.

http://rcin.org.pl

Golinska K. 1978: The course of *in situ* remodeling of injured mouth parts in *Dileptus* (*Ciliata, Gymnostomata*). Acta Protozool., 17, 47–67.

Grain J. and Golinska K. 1969: Structure et ultrastructure de *Dileptus cygnus* Claparede et Lachmann 1859, Cilié, Holotriche, Gymnostome. Protistologica, 5, 269–290.

Hauser M., Hausmann K. and Jokusch B. M. 1979: The microtubular-microfilament complex of the cytopharyngeal basket of the ciliate *Pseudomacrothorax dubius* contains tubulin, actin and α-actinin: An immunofluorescence microscopic study. J. Eur. Cytol., (in press).

Kink J. 1973: The organization of fibrillar structures in the trophic and encysted *Dileptus visscheri* (*Ciliata, Rhabdophorina*). Acta Protozool., 18, 173–194.

Lagunoff D. and Chi E. Y. 1976: Effect of colchicine on rat mast cells. J. Cell Biol. 71, 182–195.

Margolis R. L. and Wilson L. 1977: Addition of colchicine-tubulin complex to microtubule ends: The mechanism of substoichiometric colchicine poisoning (protein self-assembly during binding). Proc. Natl. Acad. Sci. USA, 74, 3466–3470.

McKanna J. A. 1973 a: Cyclic membrane flow in the ingestive–digestive system of peritrich protozoans. I. Vesicular fusion at the cytopharynx. J. Cell Sci., 13, 663–675.

McKanna J. A. 1973 b: Cyclic membrane flow in the ingestive–digestive system of peritrich protozoans. II. Cup-shaped coated vesicles. J. Cell Sci. 13, 677–686.

Nelson E. M. 1970: Division delays and abnormal oral development produced by colchicine in *Tetrahymena*. J. Exp. Zool., 174, 69–84.

Nilsson R. J. 1973: Certain factors influencing vacuole formation in *Tetrahymena*. Progress in Protozool., Abstr. Fourth Int. Congr. Protozool., Clermont-Ferrand 1973, 297.

Nilsson R. J. 1976: Physiological and structural studies on *Tetrahymena pyriformis* G. L. C. R. Trav. Lab. Carlsberg, 40, 215–355.

Olmsted J. B. and Borisy C. C. 1973: Microtubules. Ann. Rev. Biochem., 42, 507–539.

Orr T. S. C., Hall D. E. and Allison A. C. 1972: Role of contractile microfilaments in the release of histamine from mast cells. Nature, 236, 350–351.

Pitman R. H., Tweedle C. D. and Cohen H. J. 1972: Electrical responses of insect central neurons: Augmentation by nerve section and colchicine. Science, 178, 507–509.

Price M. T. 1974: The effects of colchicine and lumicolchicine on the rapid phase of axonal transport in the rabbit visual system. Brain Res., 77, 497–501.

Rembold H. and Langenbach T. 1978: Effect of colchicine on cell membrane and on biopterin transport in *Crithidia fasciculata*. J. Protozool.. 25, 404–408.

Rosenbaum J. L., Houlder J. E. and Ringo D. L. 1969: Flagellar elongation and shortening in *Chlamydomonas*. The use of cyclohexamide and colchicine to study the synthesis and assembly of flagellar proteins. J. Cell. Biol., 41, 600–619.

Schömharting M., Breer H., Rahmaun H., Siebert G. and Romer H. 1977: Colchicine, a novel inhibitor of fast axonal transport without tubulin binding properties. Cytobiologie, 16, 106–117.

Shelansky M. L. and Taylor E. W. 1967: Isolation of a protein subunit from microtubules. J. Cell Biol., 34, 549–554.

Stadler J. and Franke W. W. 1972: Colchicine binding in chromatin and membranes. Nat. New Biol., 237, 237–238.

Tamura S., Tsuruhara T. and Watanabe T. 1969: Function of nuclear microtubules in macronuclear division of *Tetrahymena pyriformis*. Exp. Cell Res., 55, 351–358.

Taylor E. W. 1965: The mechanism of colchicine inhibition of mitosis. I. Kinetics of inhibition and the binding of 3H colchicine. J. Cell Biol., 25, 145–160.

Tołłoczko B. 1977: Endocytosis in *Paramecium*. III. Effect of cytochalasin B and colchicine. Acta Protozool., 16, 185–193.

Tołłoczko B. 1978: Food ingestion in *Dileptus anser*. Acta Protozool., 18, 213–214.

http://rcin.org.pl

Tritaro J. M., Collier B., Lastowecka A. and Stern D. 1972: Inhibition by colchicine and by vinblastine of acetylcholinoinduced catecholamine release from the adrenal gland: anti-cholinergic action, not an effect on microtubules. Mol. Pharmacol., 8, 264–267.
Tucker J. B., Dunn M. and Pattison J. B. 1975: Control of microtubule pattern during the development of a large organelle in the ciliate Nassula. Dev. Biol., 47, 439–453.
Tucker J. B. 1978: Endocytosis and streaming of highly gelated cytoplasm alongside rows of arm-beasing microtubules in the ciliate *Nassula*. J. Cell Sci., 29, 213.
Wilson L., Bomburg J. R., Mizel S. B., Grisham L. M. and Creswell K. M. 1974: Interaction of drugs with microtubule proteins. Fed. Proc., 33, 158–166.
Wunderlich and Heuman 1974: *In vivo* reassembly of microtubules in presence of intracellular colchicine. Cytobiologie 10, 140–151.

EXPLANATIONS OF PLATES I–II

Plate I. The area of the oral apparatus of *Dileptus anser* in control (1) and colchicine treated cells (2–4)

1: Section through right part of nemadesmal basked in control cell. A lot of phagoplasmic vesicles (pv) and labile microtubules (arrows) can be seen between outer (n) and inner (n') basket and inside the basket. tf — transverse fibers, v — smooth vesicles. × 11 400
2: Similar section of the cell treated with 5 mM colchicine during 1 h. The number of phagoplasmic vesicles (pv) .is significantly diminished and labile microtubules can not be observed. Microtubules of nemadesmata of outer (n) and inner (n') basket and transverse fibers are intact. × 14 400
3: Higher magnification of the fragment of phot. 2 showing transversal fibers (tf). No labile microtubules and no phagoplasmic vesicles can be observed. × 45 000
4: Fragment of the cytoplasm between outer (n) and inner (n') nemadesmat basket. Very few phagoplasmic vesicles can be seen. × 27 000

Plate II. Posterior fragment of *Dileptus anser* treated with 5 mM colchicine during 3 h after the operation (group I)

1: Anterior part of the fragment. Nonciliated kinetosomes (nk) and microfibrillar material (m) equipped with smooth vesicles (v) can be observed. × 27 000
2: The cytoplasm inside the same fragment. Microfibrillar material (m) and smooth vesicles (v) can be also seen. × 18 000

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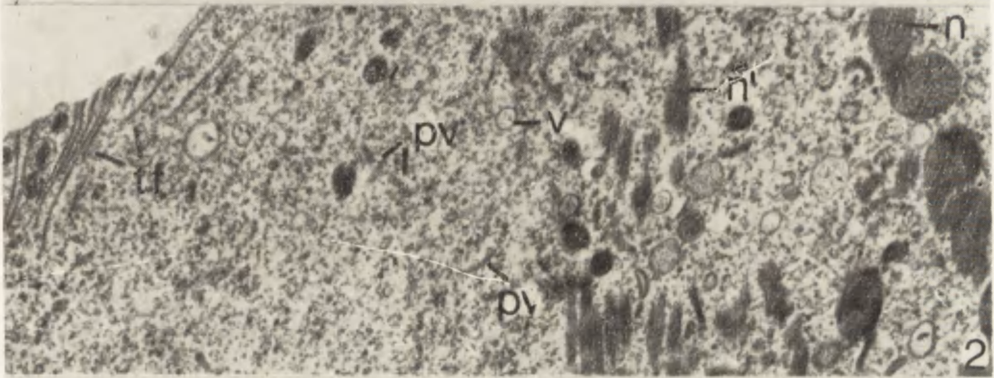

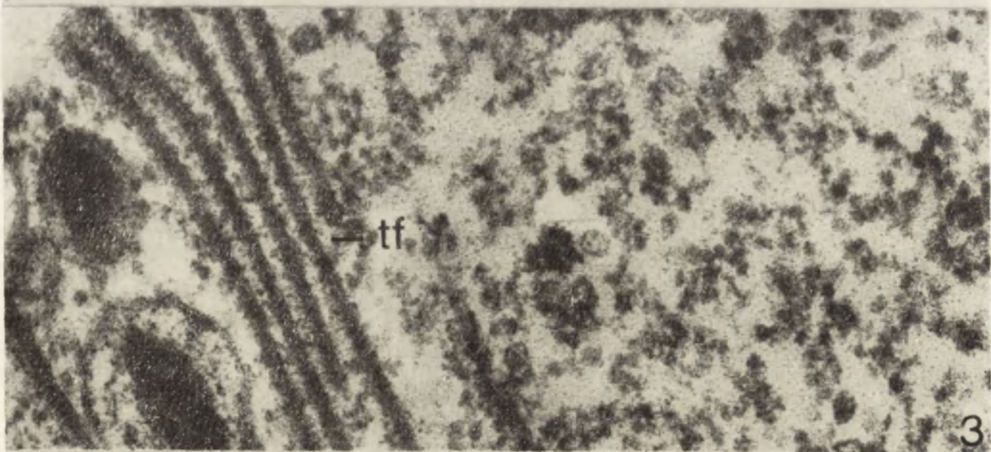

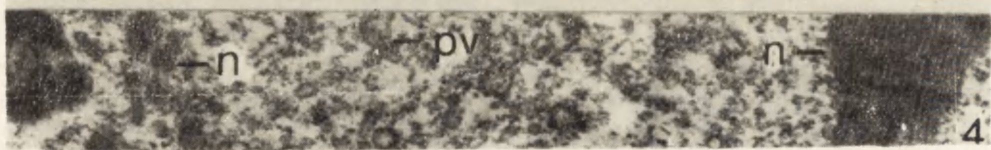

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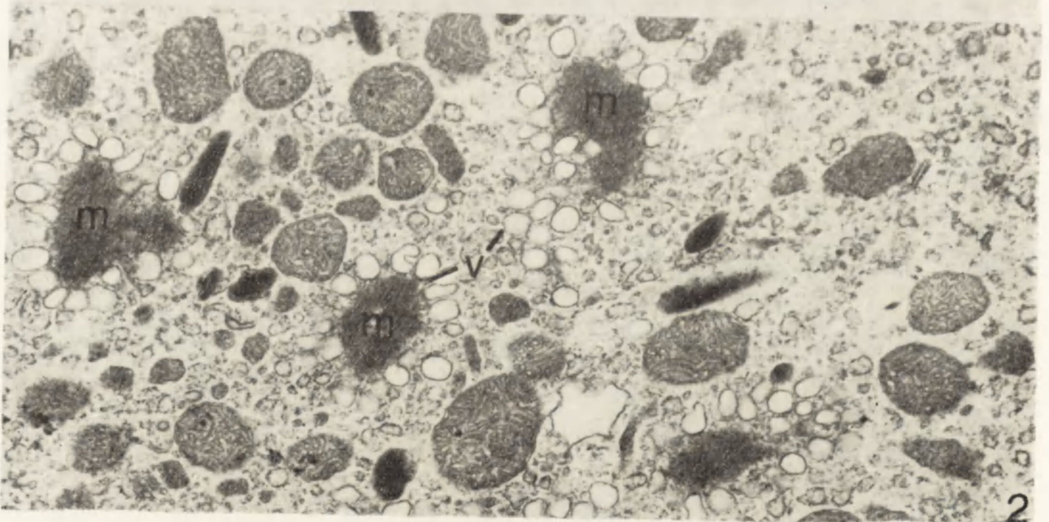

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Motile Behaviour of *Amoeba proteus* Under Various Ionic Conditions

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Synopsis. The normal and electrically stimulated movement of *A. proteus* in solutions with varying Ca^{2+}, K^{+} and Mg^{2+} concentrations is studied. The movement of the cell is described by a random walk model (Sayers et al. 1979). The non-directional rate of locomotion (u) is found to be the most sensitive parameter of this model to the changes in external ionic conditions. It appears to be more sensitive to changes in external K^{+} concentration than to Ca^{2+} or Mg^{2+} concentrations and has a maximum value of about 6 μm s^{-1} when the K^{+} concentration is very low, both in the normal and electrically stimulated movement. The orientation of the cells towards the cathode in a uniform electric field is also very high in solutions with low K^{+} concentration.

The purpose of this paper is to present results of a study of the normal and electrically stimulated movement of *Amoeba proteus* in various concentrations of Ca^{2+}, K^{+} and Mg^{2+} in the external medium. The effects of the calcium ionophore A23187 on the movement of the cell have also been studied. A random walk model (Sayers et al. 1979) is used to describe the movement of the cell and the response to changes in the external medium is measured in terms of the parameters of this model. The details and parameters of the random walk model are summarised in Fig. 1.

Materials and Methods

Amoeba proteus used in these experiments was cultured in a standard medium (Prescott and James 1955). Details of the culture and the experimental techniques are given elsewhere (Sayers et al. 1979).

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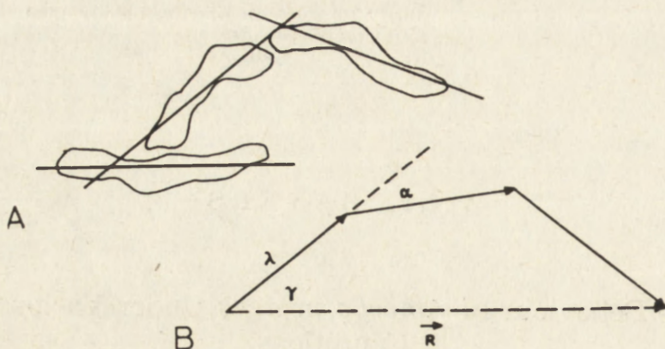

Fig. 1. A — Example of a part of the path of an *A. proteus*, B — Schematic representation of a part of the path of an *A. proteus*
Explanation: The random walk model of movement of *A. proteus*: λ — mean free path, τ — time taken to traverse λ, α: turning angle, γ — orientation of the cell, R — the position of the cell at a given time, u — non-directional rate of locomotion (λ/τ)

For a given population $\langle R^2 \rangle = 4Dt$, where

$$D = \frac{1}{4}\,\frac{\lambda^2}{\tau^2}\left(\frac{1+\cos\bar{\alpha}}{1-\cos\bar{\alpha}}\right)$$

is the diffusion coefficient.

The movements of 105 amoebae were recorded by time-lapse photography and a single frame analysis was carried out on the recordings.

The ionophore experiments were carried out using the calcium ionophore A23187 kindly supplied by Dr. B. Gompertz, University of London and by Dr. R. Hamill, Eli Lilly and Co., Indianapolis, Indiana U.S.A. In these experiments the final concentration of A23187 in the Prescott-James medium was about 10^{-7} M and that of ethanol and DMSO, used to dissolve the ionophore, was 0.5%. During these experiments amoebae were placed on a microscope slide with a cover slip over them. When the cells began normal movement a small amount of ionophore solution was applied across them, thus subjecting them to a rough gradient. Amoebae were observed under a Nomarsky interference microscope and the photographs were taken at regular intervals the illumination being provided by a flash light.

Results

Changes in the Unstimulated Movement

The compositions of the solutions used and the parameters of the random walk in these solutions are given in Tables 1–8. Solution I given in Table 1 is the control medium and the values of the parameters in this solution, given in the first column of Table 2, are the control values.

From Tables 2, 4, 6 and 8 it is seen that the non-directional rate of locomotion, u (Fig. 1), is the most sensitive parameter to changes in the

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external ionic conditions. It has a maximum value of 6.2 $\mu m\ s^{-1}$ (as compared to the control value of about 4.0 $\mu m\ s^{-1}$) in low external K^+ concentration and is not affected as much by the variations in the external Ca^{2+} and Mg^{2+} concentrations. Changes in the concentrations of Ca^{2+}, K^+ and Mg^{2+} simultaneously result in the reduction of u below its control value (about 2.0 $\mu m\ s^{-1}$).

In the experiments where the calcium ionophore A23187 was used the ionophore was dissolved in ethanol or DMSO and was expected to facilitate calcium ion entry into the cell (Arnold 1975). Plate I 1, 2, 3 and 4 show *A. proteus* in Prescott-James medium, 0.5% ethanol, 0.5%

Table 1

Compositions of solutions I, I a and I b used in the experiments in which the Ca^{2+} concentration was varied

Compound (mM)	Solution I	Solution I a	Solution I b
$CaCl_2$	0.030	0.150	0.600
KCl	0.022	0.022	0.022
K_2HPO_4	0.030	0.030	0.030
$MgSO_4 \times 7H_2O$	0.011	0.011	0.011

Table 2

Parameters of the random walk in solutions I, I a and I b (mean values with standard errors for 10, 7 and 5 amoebae, respectively)

Parameter	Solution I	Solution I a	Solution I b
$\bar{\lambda}$ (μm)	520 ± 30	530 ± 40	510 ± 60
τ (min)	2.5 ± 0.3	2.4 ± 0.4	2.4 ± 0.4
$\bar{\alpha}$ (deg)	35 ± 2	34 ± 3	23 ± 1
u ($\mu m \cdot s^{-1}$)	3.9 ± 0.6	4.0 ± 0.4	3.8 ± 0.4
D ($m^2 \cdot s^{-1}$)	$(4.7 \pm 2.0) \cdot 10^{-9}$	$(5.3 \pm 3.0) \cdot 10^{-9}$	$(12.0 \pm 6.0) \cdot 10^{-9}$

Table 3

Composition of solutions II a, II b and II c used in the experiments in which the K^+ concentration was varied

Compound (mM)	Solution II a	Solution II b	Solution II c
$CaCl_2$	0.030	0.030	0.030
KCl	0.000	0.164	3.230
K_2HPO_4	0.000	0.030	0.030
$MgSO_4 \times 7H_2O$	0.011	0.011	0.011

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Table 4

Parameters of the random walk in solutions II a, II b and II c (mean values with standard errors for 7, 5 and 4 amoebae, respectively)

Parameter	Solution II a	Solution II b	Solution II c
$\bar{\lambda}$ (μm)	540±40	550±40	540±40
$\bar{\tau}$ (min)	1.4±0.1	2.1±0.2	3.0±0.3
$\bar{\alpha}$ (deg)	25±3	25±2	33±1
$\bar{u}$ ($\mu m \cdot s^{-1}$)	6.2±0.5	4.3±0.2	3.2±0.1
D ($m^2 \cdot s^{-1}$)	$(19.0\pm9.0)\cdot10^{-9}$	$(13.0\pm5.0)\cdot10^{-9}$	$(4.7\pm1.5)\cdot10^{-9}$

Table 5

Compositions of solutions III a and III b used in the experiments in which the Mg^{2+} concentration was varied

Compound (mM)	Solution III a	Solution III b
$CaCl_2$	0.030	0.030
KCl	0.022	0.022
K_2HPO_4	0.030	0.030
$MgSO_4 \times 7H_2O$	0.055	0.220

Table 6

Parameters of the random walk in solutions III a and III b (mean values for 6 and 5 amoebae, respectively)

Parameter	Solution III a	Solution III b
$\bar{\lambda}$ (μm)	510±30	580±20
$\bar{\tau}$ (min)	2.0±0.1	3.0±0.3
$\bar{\alpha}$ (deg)	23±25	26±2
$\bar{u}$ ($\mu m \cdot s^{-1}$)	4.5±0.1	3.6±0.3
D ($m^2 \cdot s^{-1}$)	$(13.0\pm6.0)\cdot10^{-9}$	$(9.0\pm3.0)\cdot10^{-9}$

Table 7

Compositions of solutions IV a, IV b and IV c used in the experiments in which the Ca^{2+}, K^+ and Mg^{2+} concentrations were varied

Compound (mM)	Solution IV a	Solution IV b	Solution IV c
$CaCl_2$	0.150	0.600	0.600
KCl	0.110	3.230	3.230
K_2HPO_4	0.030	0.030	0.030
$MgSO_4 \times 7\ H_2O$	0.010	0.010	0.200

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Table 8

Parameters of the random walk in solutions IV a, IV b and IV c (mean values with standard errors for 10, 9 and 7 amoebae, respectively)

Parameter	Solution IV a	Solution IV b	Solution IV c
$\bar{\lambda}$ (μm)	440±20	430±20	410±20
τ (min)	2.9±0.2	2.7±0.2	10.7±5.4
$\bar{\alpha}$ (deg)	30±5	33±3	40±3
$\bar{u}$ (μm·s^{-1})	2.6±0.2	2.8±0.2	1.4±0.4
D (m^2·s^{-1})	(4.0±2.0)·10^{-9}	(3.3±1.0)·10^{-9}	(0.7±0.5)·10^{-9}

DMSO and in 10^{-7} M A23187 (with 0.5% ethanol) respectively. It was observed that the application of the ionophore from a given direction results in the reversal of the direction of cytoplasmic streaming such that the cell moves away from the ionophore source. Further application results in hyaline cap formation and appearance of small blob like pseudopodia at the advancing end. The advancing end of the cell broadens and tail of the cell becomes a persistent structure (Plate I 4). However, as can be seen from the control Plates in ethanol (Plate I 2) and in DMSO (Plate I 3) similar effects are also produced by these solutions without the ionophore A23187. Other investigators have also reported similar effects on ameboid movement by anaesthtics (Hulsmann et al. 1976) and by DMSO (Grebecka and Kalinina 1979). It is therefore difficult to distinguish between the effects of the ionophore and the effects of the solvent used.

Changes in the Electrically Stimulated Movement

In these experiments the concentrations of Ca^{2+} and K^{+} in the external medium were changed individually, in a uniform horizontal electric field of 200 V m^{-1}.

Table 9

Parameters of the random walk in solutions IV a, I a and I b at 200 V m^{-1}, where $\gamma = 0°$ (or 360°) is the cathode direction (mean values with standard errors for 12, 6 and 9 amoebae, respectively)

Parameter	Solution IV a	Solution I a	Solution I b
$\bar{\lambda}$ (μm)	470±10	600±50	510±20
τ (min)	2.5±0.1	3.0±0.5	3.3±0.4
$\bar{\alpha}$ (deg)	26±3	37±3	33±3
$\bar{u}$ (μm·s^{-1})	3.2±0.1	3.5±0.4	3.0±0.4
D (m^2·s^{-1})	(7+2)·10^{-9}	(5.0+2.5)·10^{-9}	(3.8+2.0)·10^{-9}
γ (deg)	25	299	320

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Table 10
Parameters of the random walk in solutions II a, II b and II c at 200 Vm^{-1}, where $\bar{\gamma} = 0°$ (or 360°) is the cathode direction (mean values for 6, 6 and 7 amoebae, respectively)

Parameter	Solution II a	Solution II b	Solution II c
$\bar{\lambda}$ (μm)	710±70	730±40	520±20
$\bar{\tau}$ (min)	2.8±0.3	3.2±0.3	3.0±0.4
$\bar{a}$ (deg)	19±1.0	24±3	35±4
$\bar{u}$ ($\mu m \cdot s^{-1}$)	4.3±0.2	4±0.4	3.4±0.3
D ($m^2 \cdot s^{-1}$)	$(28 \pm 10) \cdot 10^{-9}$	$(16 \pm 7) \cdot 10^{-9}$	$4.0 \pm 1.5) \cdot 10^{-9}$
γ (deg)	350	287	298

The solutions used are indicated in Tables 9 and 10 together with the parameters of the random walk in these solutions. From these tables it is seen that as in the unstimulated case, the movement is affected mostly by the changes in external K^+ concentration. The rate of orientation towards the cathode is significantly higher than in any other solution and the rate of locomotion is higher. Changes in the external Ca^{2+} and Mg^{2+} concentrations do not appear to affect the galvanotaxis strongly.

Discussion

The experiments reported in this paper indicate that both normal and electrically stimulated movement of *A. proteus* are strongly affected by the changes in the external K^+ concentration but not by the changes in $Ca^{2+}$and Mg^{2+} concentrations. This may be explained in terms of the mechanisms by which the internal concentrations of these ions are controlled. The cell is likely to possess active mechanisms for maintaining the internal Ca^{2+} concentration (Reinold and Stockem 1972, Coleman et al. 1973, Taylor et al. 1976) because of the involvement of this ion in contraction and in the determination of direction of movement (Nuccitelli et al. 1977). However, if the cell membrane is freely permeable to K^+ (Bruce and Marshall 1965), then the changes in the external K^+ concentration will readily affect the cell and its movement. In the experiments reported above it is likely that very low external K^+ concentration will cause a leakage of K^+ from the cell interior and thus hyperpolarise the cell membrane. This high membrane potential may in turn cause small increases in the local free Ca^{2+} concentration or affect the Ca^{2+} transport across the membrane thus promoting contractions and resulting in a high rate of locomotion.

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Braatz-Schade and Haberey (1975) have also reported high rates of locomotion from amoebae with high membrane potentials.

Nuccitelli et al. (1977) have measured electrical currents associated with both stationary and moving *A. proteus* in the normal culture medium. The steady current they measured had a density of 1.0–2.0 $mA \cdot m^{-2}$ and entered the cell at the tail and left the cell at the front end. Ion substitution experiments suggested that the steady current was carried into the cell mainly by Ca^{2+}. We have reported earlier that in a uniform horizontal electric field (about $270\ V \cdot m^{-1}$) the cathodal migration of *A. proteus* is a weak long term response following the strong initial response and that although a current of about 150 $mA \cdot m^{-2}$ is forced through the cell membrane in these fields the direction of movement is not altered strongly (Sayers et al. 1979).

The experiments reported in this paper indicate that the current imposed on the cell membrane by the externally applied electric field is probably carried by K^{+} in the medium and does not necessarily interfere with the natural direction determining Ca^{2+} current of the cell. However, when there is no K^{+} available in the medium the current due to the imposed electric field also has to be carried by Ca^{2+} and this interferes with the natural current of the cell. Since the current flow is towards the cathode, the cell continues to move towards the cathode after the initial response which had already affected it strongly and made it turn towards the cathode.

The accounts of the motile behaviour of *A. proteus* given in this paper and an earlier one (Sayers et al. 1979) have been qualitative. During the course of this study several attempts were made to understand the problem quantitatively but the calculations could never go far due to the of data on the ionic fluxes and the ions involved in the fluxes across *A. proteus* membrane. Although difficult this is a very important area for further investigations.

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RÉSUMÉ

Le mouvement normal de l'*Amoeba proteus* et sa migration dans le champ électrique étaient étudiées dans les solutions contenant les différentes concentrations de Ca^{2+}, K^{+} et Mg^{2+}. Le mouvement est décrit en termes du modèle de

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mouvement fortuit (Sayers et al. 1979). La vitesse de locomotion non-directionelle s'est avérée comme paramètre du modèle le plus sensible aux changements des conditions ioniques ambiantes. Ce paramètre paraît plus sensible aux variations de la concentration extérieure de K^+ qu'à celle de Ca^{2+} ou Mg^{2+} et il atteint sa valeur maximale d'environ 6 $\mu m \cdot s^{-1}$ quand la concentration de K^+ est très basse, aussi bien pour le mouvement normal qu'au cas de la stimulation électrique. L'orientation des cellules vers la cathode dans un champ électrique uniforme est également très élevée dans des basses concentrations de K^+.

REFERENCES

Arnold J. M. 1975: An effect of calcium cytokinesis as demonstrated with ionophore A23187. Cytobiologie, 11, 1–9.

Bruce D. L. and Marshall Jr. J. M. 1965: Some ionic and bioelectric properties of the amoeba *Chaos Chaos*. J. Gen. Physiol., 49, 151–178.

Coleman J. R., Nilsson J. R., Warner R. R. and Batt P. 1973: Electron probe analysis of refractive bodies in *Amoeba proteus*. Exp. Cell Res., 76, 31–40.

Grebecka L. and Kalinina V. 1979: Effects of DMSO on the motory behaviour of *Amoeba proteus*. Acta Protozool., 18, 327–332.

Hulsmann N., Korohoda W. and Stockem W. 1976: Reversible disorganisation of motile activities in *Amoeba proteus* induced by general anaesthetics. Acta Protozool., 15, 187–194.

Nuccitelli R., Poo M. M. and Jaffe L. 1977: Relations between ameboid movement and membrane controlled electrical currents. J. Gen. Physiol., 69, 743–763.

Prescott D. M. and James T. W. 1955: Culturing of *Amoeba proteus* on *Tetrahymena*. Exp. Cell Res., 8, 256–258.

Reinold M. and Stockem W. 1972: Darstellung eines ATP-sensitiven membransystems mit Ca^{2+} transportieren der funktion bei amoben. Cytobiologie, 6, 182–194.

Sayers Z., Roberts A. M. and Bannister L. H. 1979: Random walk analysis of movement and galvanotaxis of *Amoeba proteus*. Acta Protozool., 18, 313–325.

Taylor D. L., Moore P. L., Condeelis J. S. and Allen R. D. 1976: The mechanochemical basis of ameboid movement: I. Ionic requirements for maintaining viscoelasticity and contractility of amoeba cytoplasm. Exp. Cell Res., 101, 127–133.

EXPLANATION OF PLATE I

1: *A. proteus* in Prescott-James medium
2: *A. proteus* in 0.5% ethanol
3: *A. proteus* in 0.5% DMSO
4: *A. proteus* in 10^{-7} M A23187

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Motory Interdependence of Pseudopodia in Freely Moving *Amoeba proteus*

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Synopsis. Pseudopodia of polytactic amoebae, which assume for a long time the leading role, are usually polycyclic with phases of acceleration and of retardation of movement. The retraction of the uroid is always, in an unstimulated cell, a steady process without fluctuations of velocity. Therefore, such events as the formation of new pseudopodia, the changing rate of their frontal advancing, and the initiation of their contraction, cannot be explained by any oscillations of activity of the contracting posterior body regions. But a distinct correlation in time and in space is found between the moments of initial formation of each pseudopodium and the beginning of inhibition or of contraction in some others. Velocity fluctuations of sister pseudopodia are often antagonistic. This indicates that probably the pressure exerted by the cortical contraction in amoeba is steady but its distribution across the frontal body regions is variable in time and in space due to the local control.

In early behavioural studies of amoeboid movement the idea was sometimes expressed that the motory phenomena manifested at different regions of the moving cell may be co-ordinated by impulses transmitted to all parts of the body (cf. Jennings 1904, McClendon 1909, Verworn 1913, Edwards 1923). This concept was dismissed by the experiments of Mast (1932) in which amoebae were exposed to localized photic stimulation. Mast emphasizes that "amoeba acts as an organized unit...", but he concludes that "the action at a distance from the region stimulated is... merely the result of transmission of pressure".

This point of view is intrinsically linked to two basic elements of the classical theory of amoeboid movement (Mast 1926): (1) that the endoplasmic stream follows the intracellular pressure gradients, and (2) that amoeba is a turgid system, i.e., its peripheral cell layers are under tension.

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The idea that amoeba, similar to each other cell and organism, "acts as an organized unit" is now contested by the frontal zone contraction theory implying "the independence of streaming in sister pseudopodia" (Allen 1973), and even stating explicit that "each stream of endoplasm with its hyaline cap behaves as an independent functional unit" (Allen and Allen 1978). Similar earlier statements of the same author (Allen 1968) that "pseudopods of normal polypodial specimens form initially and continue to move independently of one another" and that "...spurts of streaming in different pseudopodia are not synchronized", were presented as "observations" but not supported by any documented evidence.

It is certainly true that such a discoordination of motory events in a polytactic amoeba is a postulate logically coherent with the theory which localizes the motive force in the tips of all advancing pseudopodia and rejects the integrating role of intracellular pressure. This conviction lead us to analyze the cinematographic records of moving polytactic amoebae in the hope to learn whether such critical events in the cycles of different pseudopodia as their initial formation, temporary inhibition, and beginning of retraction, are mutually interrelated in time and in space or completely independent of one another.

Material and Methods

Amoebae were cultured in Pringsheim medium and fed twice a week on *Tetrahymena pyriformis*. Samples containing amoebae with the original culture medium, taken not earlier than two days after feeding, were transferred to a standard slide. A few glass beads were added to the preparation to keep the cover glass at the distance of 0.5 mm from the slide. Polytactic specimens were usually selected, except a few experiments in which orthotactic or monotactic forms were used too (terminology of Grębecki and Grębecka 1978). They were filmed 10–15 min after the transfer from the stock culture, what allowed them to recover the normal motory behaviour.

The microscope was equipped with the PZO variable phase contrast device which served to produce the dark field type of illumination. Cinematographic pictures were taken with 10× standard lens, without eye-piece. It allowed to reproduce on each frame of the 16 mm film a field measuring 0.7×1 mm. Spontaneous locomotion of unstimulated amoebae was filmed during the full length of time needed by an individual to cross that field of view. Pictures were taken at the frequency of 4 fr./s with a camera controlled by Bolex frame-by-frame device. All the records were taken in a semi-dark room, at 18 ± 2°C.

The produced films were either analyzed frame-by-frame or served to plot photokimographic records of the pseudopodial activity. In the first case they were projected on the tracing paper, and contours of amoebae were redrawn from each 10th frame which produced pictures of superposed motion stages

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succeeding one another at the intervals of 2.5 s. Photokimographic records were obtained by the same procedures which were earlier introduced by Grębecki and Moczoń (1978) and Cieślawska and Grębecki (1978) to analyze the contraction-expansion phenomena in the slime moulds plasmodia. The films were projected with the LW photo-optical data analyzer on the screen in which a narrow vertical slit was cut out and covered with a semi-transparent material. The image rotating device mounted on the projector served to adjust the position of moving amoeba in such a way that the analyzed pseudopodium, during all its history, was intersected by the slit along its longitudinal axis (cf. Pl. I 1 a, b). A 70 mm recording camera with continuous film run was situated behind the screen. The image of the slit was perpendicular to the running recording film. As a result, the progressive shading of the slit during projection by an advancing pseudopodium was recorded in the form of a curve. The pictures which were taken at 4 fr./s were projected at 24 fr./s, and the recording film moved with the speed of 0.5 cm/s. It resulted in reproducing 12 s of the real time on 1 cm of the record.

This method permits to plot the activity curves of all pseudopodia of a moving specimen in the course of many successive projections of the same film, with the different positions of the slit. The photokimographic recording in each case was started from the same film frame chosen to represent t_0 and, therefore, all the obtained curves were synchronized and permitted to confront the timing of events manifested simultaneously at different cell regions.

Results

It is generally known that the progressive movement of the frontal part of polytactic amoeba has no constant rate because of the continuous substitution of older advancing pseudopodia by the new ones. This phenomenon is graphically demonstrated in the upper part of Fig. 1 A, in which the full contour of amoeba is shown at the stage t_0 and the increasing area covered by the advancing body regions is indicated every 2.5 s up t_{148s}. The picture suggests however that the velocity of forward movement may also fluctuate during the life of the same advancing pseudopodium.

This phenomenon is more clearly demonstrated by the photokimographic records. The shape of moving amoeba and the positions of slit applied to its projected image are shown at the initial and at the final stage of recording by the Pl. I 1 a and b. The activity curves of two advancing pseudopodia P_1 and P_2, corresponding to these two positions of the slit, are presented in the Pl. I 1 c and d. Both pseudopodia are characterized by fluctuating velocity of their progressive motion. Similar fluctuations are seen in the activity curves of pseudopodia drawn in Fig. 5. There is no regular periodicity in such velocity changes of advancing pseudopodia. Generally, the phases of slowing down or cessation of movement and those of its acceleration succeed at the intervals of 0.5–2 min.

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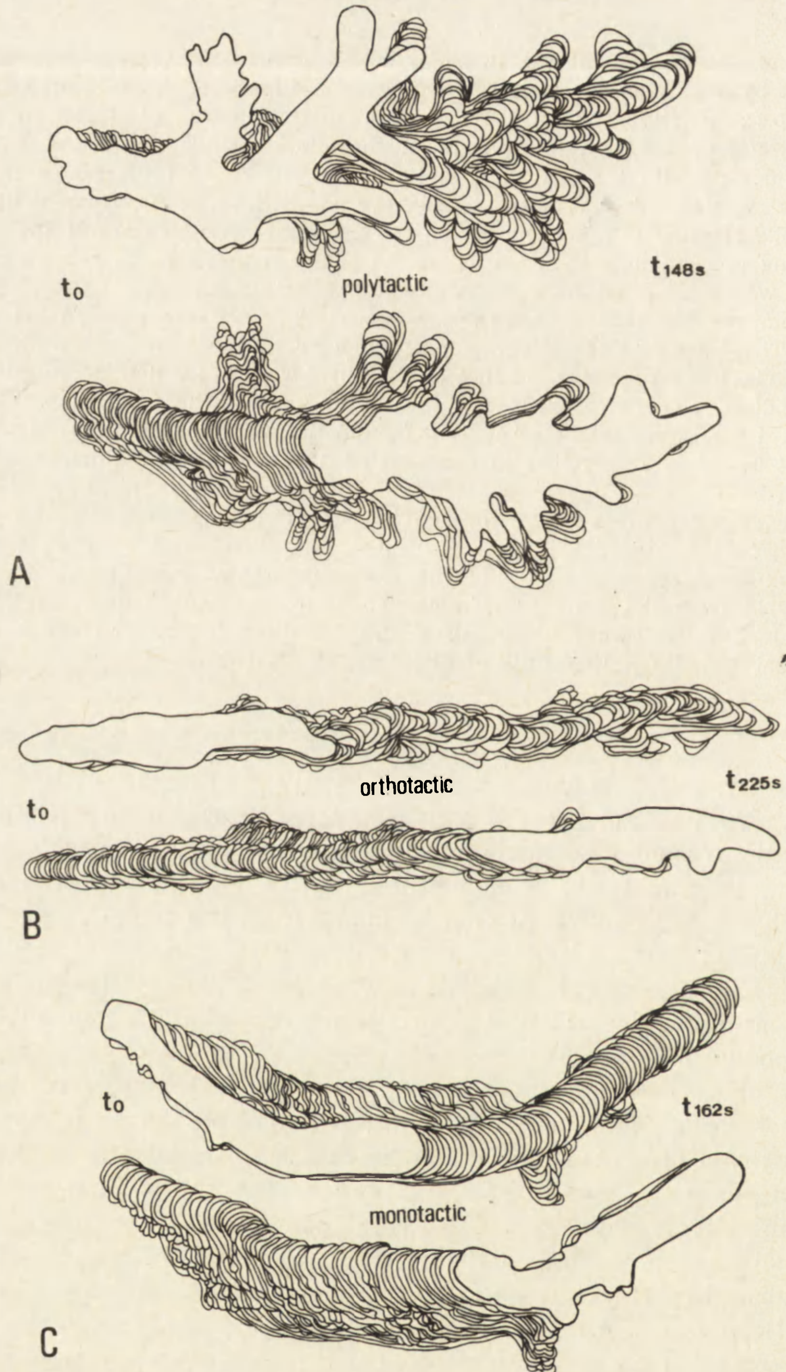

Fig. 1. Sequence of contours of moving polytactic (A), orthotactic (B) and monotactic amoeba (C) redrawn at the intervals of 2.5 s, in the manner to show the character of their frontal progression (the upper element of each drawing) and of their posterior withdrawal (lower elements). Note the steady character of retraction of the uroid

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It was probable that such critical changes in the motory behaviour of the frontal part of amoeba as formation of new pseudopodia, reversals of motion in older ones, and temporary inhibition of some others, might be related to some changes of the motory force exerted by the contracting posterior body regions. This possibility has been checked by photokimographic records of motion of the uroid, obtained through the slit applied at the position indicated as U in the example shown in the Pl I 1 a and b. The records proved however that the retraction of uroid is absolutely steady (P. 1 1 e) and there is no means to relate it to the velocity changes of advancing pseudopodia (Pl. I 1 c and d). The invariable velocity of retraction of the uroid may be also read out from the graph shown in the lower part of Fig.1 A.

The steady character of uroid retraction was also demonstrated in orthotactic amoebae (Pl. I 2 and Fig. 1 B)[1] and in monotactic ones

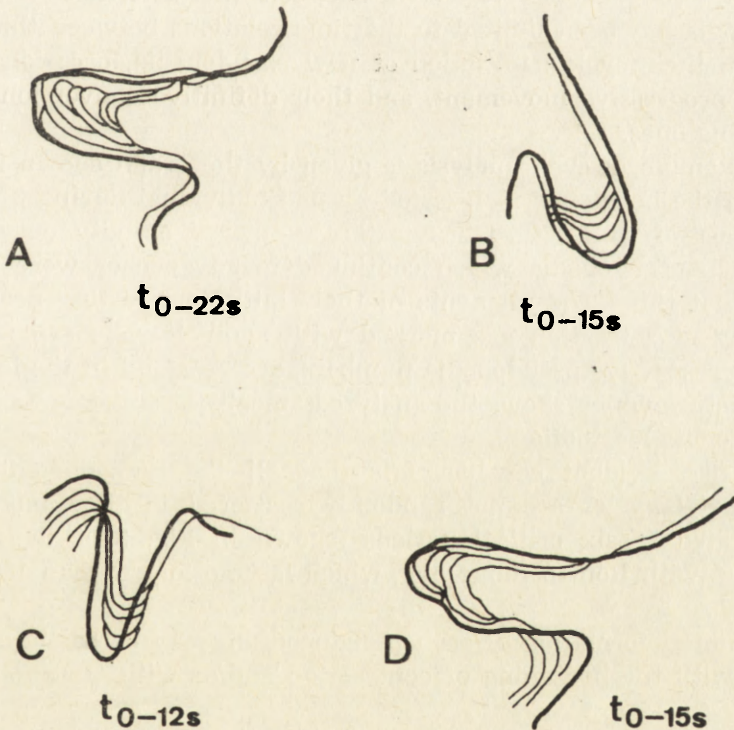

Fig. 2. Contour drawings demonstrating different modes of withdrawal of contracting pseudopodia: with shortening perceptible only at the retracted tips (A and B) or with the simultaneous shifts of the pseudopodial basis (C and D)

[1] Orthotactic amoebae were filmed in the lateral illumination produced by a halogen lamp, which served to induce their unidirectional locomotion.

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(Pl. I 3 and Fig. 1 C). In both these forms of amoeba the speed of frontal progression is also rather uniform. But in polytactic cells the absolutely steady rate of the uroid retraction creates a difficulty how to explain the existing fluctuations in the frontal velocities. They need another explanation, probably in the changing activity of other body regions, in particular in the interdependence of different pseudopodia of the same cell.

The methods used in this study do not permit to obtain reliable activity curves of contracting pseudopodia, because (as indicated in Fig. 2) the retraction of their tips gives no full information on the rate of their shortening, which depends in some extent also on the shifts of the pseudopodial basis. On the other hand, the curves of extension of advancing pseudopodia provide only very rough estimates of the intensity of endoplasm transport. For these reasons, the analysis of the interdependence expected between the activities of different pseudopodia has been limited to the time relations between three major events in their cycles: initiation of new pseudopodia, occasional pauses of their progressive movement, and their definitive conversion into the contracting ones.

An example of such analysis is given by the graph shown in Fig. 3, based on the behaviour manifested by one individual during 3 min. The upper part of the picture brings the numbered activity curves of all the fifteen pseudopodia which continued to advance or were produced during that time. The moments of their initiation, resting periods, and beginning of retraction are marked with symbols. The same symbols, with the respective pseudopodia numbers, are repeated in the lower part of the picture which shows the analyzed amoeba at six stages apparently critical for its locomotion.

The first evident conclusion is that all the major events in the pseudopodial cycles are not randomly scattered in time but strongly concentrated at six critical periods (shown in Fig. 3 as dashed areas numbered with Roman numerals), which last no longer than 10% of the total time.

The initial formation of each pseudopodium may be easily correlated in time with the beginning of contraction and/or with temporary cessa-

Fig. 3. Graphic analysis of the pseudopodial activity of one single polytactic specimen during 3 min. In the upper part of the picture the numbered activity curves of all extended pseudopodia are shown. Dashed columns numbered with Roman numerals correspond to six periods at which critical changes in pseudopodial activity were observed. The shape of amoeba at these six periods is presented at the bottom of the diagram. In both parts of the drawing the respective pseudopodia are labelled with the same numbers, and the same symbols are used to indicate their initial formation (empty arrows), temporary inhibition (plain circles), and beginning of retraction (plain arrows)

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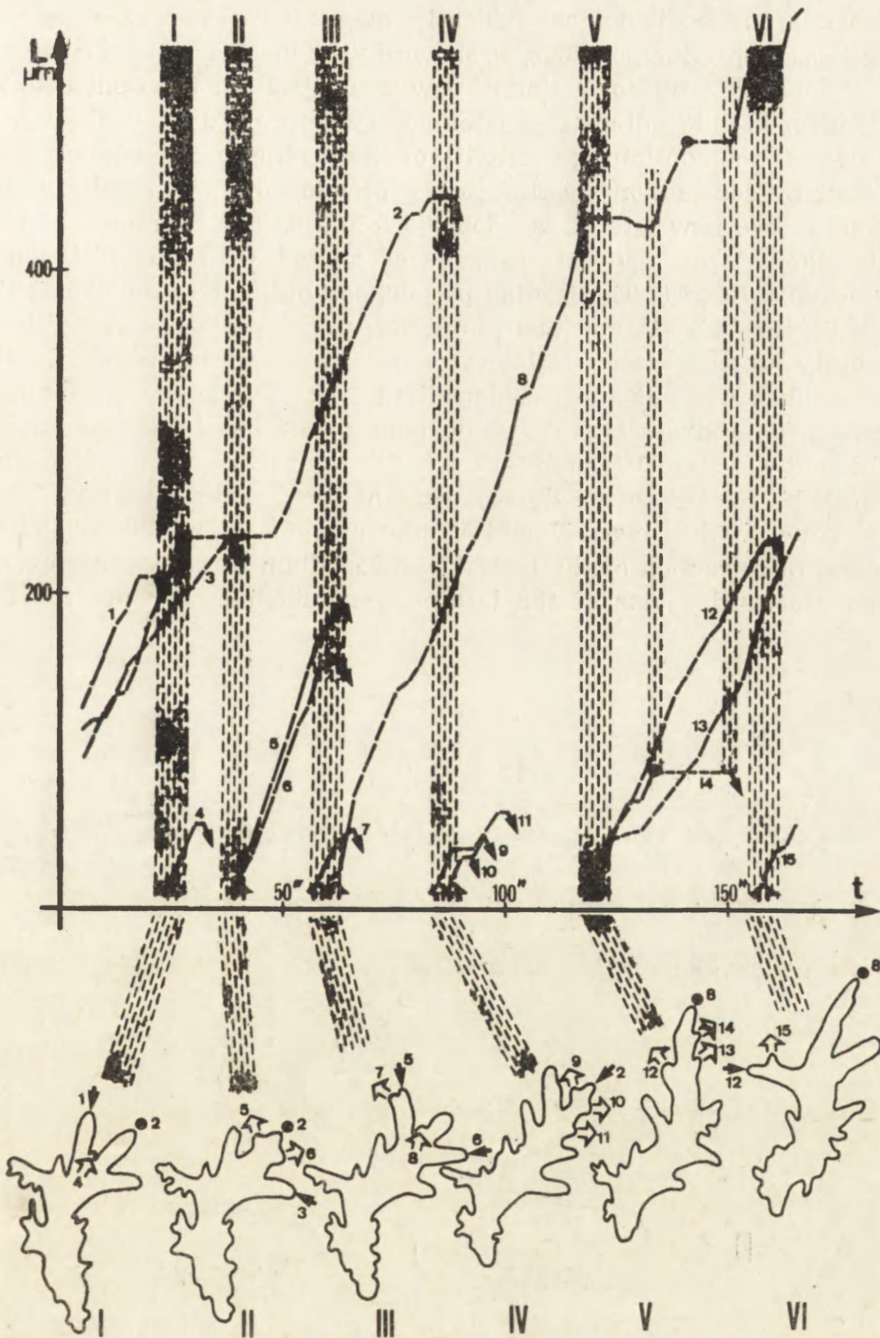

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tion of movement in some others. Moreover, this correlation is seen between pseudopodia which evidently may easily interact because of their close respective positions, as shown in the lower part of the picture. So, at the stage I the formation of new pseudopodium 4 is related to the reversal of pseudopodium 1 and temporary stop of pseudopodium 2. At the stage II the continuing inactivity of the leading pseudopodium 2, reinforced by contraction starting in the pseudopodium 3, results in formation of two new lateral pseudopodia 5 and 6. The beginning of their contraction at the stage III may be correlated with the initiation of pseudopodia 7 and 8. The leading pseudopodium 2 enters the contraction phase at the stage IV, and then three new pseudopodia (9, 10, 11) simultaneously protrude from its lateral walls. In the meantime the leading role is taken over by the pseudopodium 8. Its temporary inhibition at the stage V produces lateral pseudopodia 12, 13 and 14. Its another inhibition at the stage VI, simultaneous with the reversal of pseudopodium 12, may be related to the development of the pseudopodium 15. Moreover, between the stages V and VI the moment of inhibition and the moment of retraction of the lateral pseudopodium 14 correspond exactly to two forward spurts of the leading pseudopodium 8. Only the con-

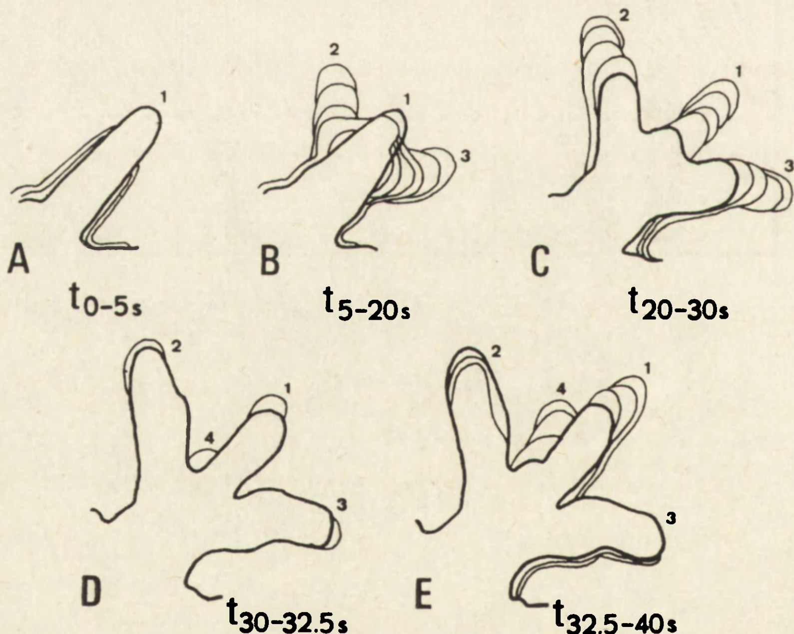

Fig. 4. Example of interrelation between the activity of central advancing pseudopodium (1) and the behaviour of its lateral branches (2, 3 and 4). The successive motion stages (A–E) are described in the text

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tractions of a few smallest short-living pseudopodia (4, 7, 9, 10, 11) seem unable to produce clear motory effects in other areas. In general, all the critical moments of all the pseudopodial cycles are distinctly interrelated in time and in space.

The example of dependence between the inhibition of an advancing pseudopodium and the development of its lateral branches is shown in more detailed manner in Fig. 4. The temporary cessation of movement of the leading pseudopodium 1 resulted in its inflation during the first 5 s, and in formation of two lateral pseudopodia (2 and 3) during next 15 s. Then, during 10 s all the three pseudopodia advanced. The initiation of fourth pseudopodium (30–32.5 s) inhibited the further development of pseudopodia 2 and 3 (32.5–40 s).

The interdependence of different pseudopodia is often manifested in the form of alternation of their phases of acceleration and slowing down. Such antagonism is very distinct in the photokimographic activity

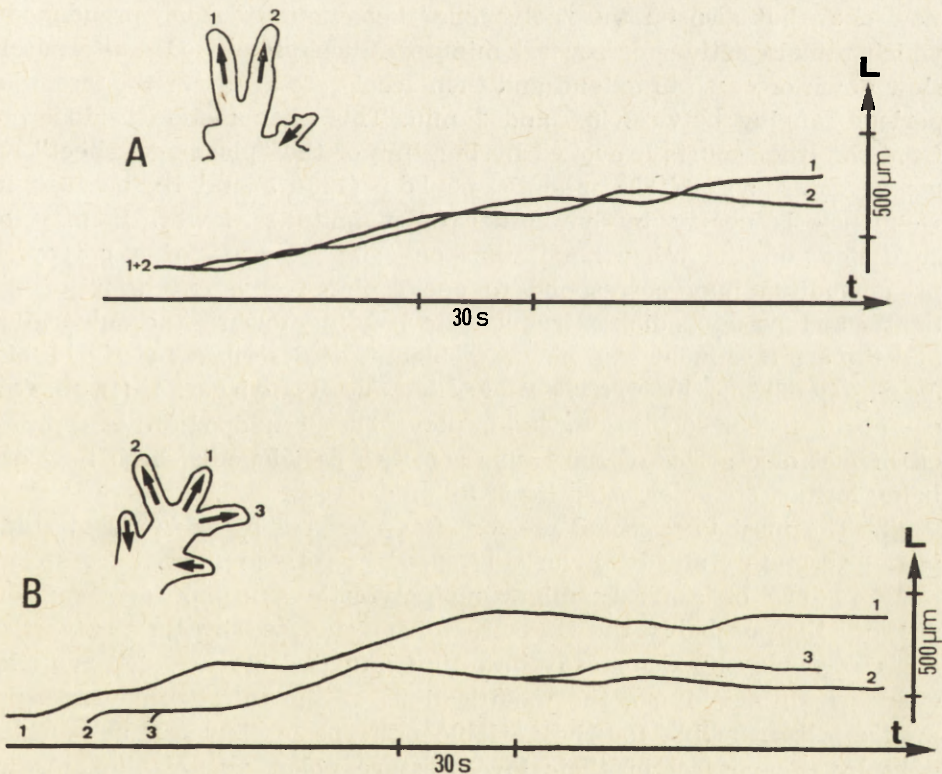

Fig. 5. Activity curves of frontal pseudopodia redrawn from photokinographic records. A — antagonist behaviour of two sister pseudopodia 1 and 2, B — antagonist behaviour of central pseudopodium 1 in respect to its lateral branches 2 and 3

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curves of pseudopodia P_1 and P_2 shown in the Pl. I 1 c and d. Some other examples of this phenomenon are presented by the activity curves redrawn in Fig. 5. The fluctuating velocities of two nearly equivalent sister pseudopodia (Fig. 5 A) seem to be opposed in phase. The progress of the central pseudopodium 1 shown in Fig. 5 B seems to be antagonistic in respect to the concurrent advancing of both lateral pseudopodia 2 and 3. This type of behaviour of pseudopodia suggests that they compete for the material transported from the posterior cell regions.

Discussion

The material exposed above permits to establish three groups of facts:

(1) The unsteady character of frontal progression of polytactic amoebae depends not only on the substitution of older pseudopodia by the new ones, but also on the "polycyclic" behaviour of some pseudopodia which remain active for several minutes. Such pseudopodia alternately slow down or cease to extend and then accelerate again, at the irregular periods ranging between 0.5 and 2 min. This phenomenon is different from the brief spurts provoked by bursting of the "plasmagel sheet" reported by Mast (1926) and Rinaldi (1964 a and b), because its frequency is nearly by one order of magnitudes lower. It may be suggested, on the other hand, that one single period of a polycyclic pseudopodium may correspond to one cycle of free extension of the unattached pseudopodial extremity, its bending toward the substratum and the establishment of a new attachment, as described by Bell and Jeon (1963) and by Nowakowska and Grębecki (1978). One can also consider such a long-living polycyclic pseudopodium as composed in fact of a series of successive separate pseudopodia, each new one being formed at the arrested tip of its predecessor.

(2) The most unexpected phenomenon revealed by the present study is the absolutely steady, invariable speed of retraction of the posterior body regions of a freely migrating polytactic amoeba. It permits to suppose that probably the pressure force generated by the contracting cell cortex may be also steady in an unstimulated individual kept under stable conditions. If so, the modifications of the intracellular pressure gradients responsible for the variable patterns of flow can be effected only by the control of their low pressure poles, i.e., at the anterior extremities of the moving cell.

(3) As a matter of fact, the activity cycles of different pseudopodia of a freely moving polytactic amoeba proved to be interdependent. In

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particular, the initial formation of each new pseudopodium can be clearly correlated in time with the cessation of movement or with the beginning of contraction of one or two other pseudopodia. Moreover, such interdependence is always detected between pseudopodia which seem to be predestinated to interact by their position in respect to one another and in respect to the main motory axis of amoeba. The same interdependence may be also manifested by the antagonistic alternation of acceleration and retardation phases in the equivalent sister pseudopodia, or in a central pseudopodium and its lateral branches. The interdependence of different pseudopodia based on their antagonism has already been demonstrated earlier in the specific case of pseudopodia headed by natural vesicular frontal caps or by artificial oil caps (Grębecka 1977 and 1978 a).

One important question arises which can not be unequivocally answered on the basis of present material: are the new pseudopodia formed because some older ones stop and begin to contract, or on the contrary — is the formation of a new pseudopodium the primary event responsible for the reversal of streaming in some others? The succession of both related phenomena in time, if it was enough exactly measured by the present methods, suggests that both situations are possible and they do happen more or less commonly. The distant effects of the experimental induction or inhibition of single pseudopodia by localized stimuli are now under study in the hope to solve this question.

In general, the present results contradict the pretended independence of pseudopodia stressed by Allen (1968, 1973, 1978) as an argument in favour of the frontal zone contraction theory. They confirm that "amoeba acts as an organized unit" (Mast 1932), and are interpretable by the concept that its different body parts may interact by the transmission of pressure. On the other hand, the steady rate of withdrawal of the posterior cell regions suggests that the intracellular pressure created by the peripheral contraction of the cell cortex provides the motive force for the endoplasm flow but the control of the rate and of the direction of streaming is probably located at the pseudopodial tips. It satisfies the objection made by Allen (1968), which was justified when applied to the classical pressure theories, that the movement should not be controlled in the tail, but "...in the front, where the behavioral events occur..". The mechanism of this control depends on local disruption of the cell cortex or its dissociation from the cell membrane, and on its reconstitution, as recently indicated by Grębecka (1978 a and b), Hrebenda and Grębecka (1978), Grębecka and Hrebenda (1979), Grębecki (1979), and Wehland et al. (1979). But the motory activity of the whole moving cell is

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integrated because changes taking place at the tip of one advancing pseudopodium exert their influence upon other pseudopodia by the transmission of pressure, as in the circuit of any hydraulic or pneumatic transducer.

RÉSUMÉ

Chez les amibes polytactiques les pseudopodes principaux qui gardent longtemps leur activité sont souvent polycycliques — avec des phases du mouvement accéléré et du mouvement ralenti ou suspendu. Chez les cellules qui ne sont pas soumises à la stimulation le retrait de l'uroïde est toujours uniforme, sans fluctuations de vitesse. Par conséquent, des événements tels que la formation des pseudopodes nouveaux, les changements de vitesse de leur progression frontale et le début de leur contraction, ne trouvent pas d'explication dans les oscillations de l'activité contractile des régions postérieures du corps. Cependant, on trouve des corrélations bien distinctes dans le temps et dans l'espace entre le moment initial de formation de chaque nouveau pseudopode et de début de l'inhibition ou de la contraction de certains pseudopodes plus anciens. Les fluctuations de vitesse chez les pseudopodes équivalents sont souvent antagonistes. Ces résultats suggèrent que la pression exercée chez l'amibe par la contraction corticale est uniforme, mais la distribution des gradients de pression dans les régions frontales du corps varie dans le temps et dans l'espace sous les effets d'un contrôle local.

REFERENCES

Allen R. D. 1968: Differences of a fundamental nature among several types of amoeboid movement. Symp. Soc. Exp. Biol., 22, 151–168.

Allen R. D. 1973: Biophysical aspects of pseudopodium formation and retraction. In: The Biology of *Amoeba* (ed. K. W. Jeon), Academic Press, 201–247.

Allen R. D. and Allen N. S. 1978: Cytoplasmic streaming in amoeboid movement. Ann. Rev. Biophys. Bioeng., 7, 469–495.

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

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

Edwards J. G. 1923: The effect of chemicals on locomotion in *Amoeba*. J. Exp. Zool., 38, 1–43.

Grębecka L. 1977: 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 Hrebenda B. 1979: Topography of cortical layer in *Amoeba proteus* as related to the dynamic morphology of moving cell. Acta Protozool., 18, 493–502.

Grębecki A. 1979: Organization of motory functions in amoebae and in slime moulds plasmodia. Acta Protozool., 18, 43–58.

Grębecki A. and Grębecka L. 1978: Morphodynamic types of *Amoeba proteus*: a terminological proposal. Protistologica, 14, 349–358.

http://rcin.org.pl

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.
Hrebenda B. and Grębecka L. 1978: Ultrastructure of the frontal cap of monotactic forms of *Amoeba proteus*. Cytobiologie, 17, 62–72.
Jennings H. S. 1904: Contributions to the study of the behavior of lower organisms. Pub. Carnegie Inst. Wash., 1–257.
Mast S. O. 1926: Structure, movement, locomotion and stimulation in *Amoeba*. J. Morphol., 41, 347–425.
Mast S. O. 1932: Localized stimulation, transmission of impulses, and the nature of response in amoeba. Physiol. Zool., 5, 1–15.
McClendon J. F. 1909: Protozoan studies. J. Exp. Zool., 6, 265–287.
Nowakowska G. and Grębecki A. 1978: Attachment of *Amoeba proteus* to the substrate during upside-down crawling. Acta Protozool., 17, 353–358.
Rinaldi R. A. 1964 a: Pictographs and flow analysis of the hyaline cap in *Chaos chaos*. Protoplasma, 58, 603–620.
Rinaldi R. A. 1964 b: The plasmagel sheet of *Amoeba proteus*. Protoplasma, 59, 480–484.
Verworn M. 1913: Irritability. New Haven, 1–264.
Wehland J., Weber K., Gawlitta W. and Stockem W. 1979: Effects of the actin binding protein DNAase I on cytoplasmic streaming and ultrastructure of *Amoeba proteus*. An attempt to explain amoeboid movement. Cell Tiss. Res., 199, 353–372.

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EXPLANATION OF PLATE I

1: Photokimographic records of the extension of advancing pseudopodia and of the retraction of the uroid in a polytactic amoeba. The initial and the final stage of migration recorded cinematographically are shown respectively in 1 a and 1 b, with the positions of the slit when it was applied to two pseudopodia (P_1 and P_2) and to the uroid (U). The obtained activity curve of the pseudopodium P_1 is given in 1 c, that of the pseudopodium P_2 in 1 d, and that of the uroid in 1 e
2: Photokimographic records of the frontal advancing (F in 2 a) and of the uroid retraction (U in 2 b) of an orthotactic amoeba
3: Photokimographic records of the frontal advancing (F in 3 a) and of the uroid retraction (U in 3 b) of a monotactic amoeba.

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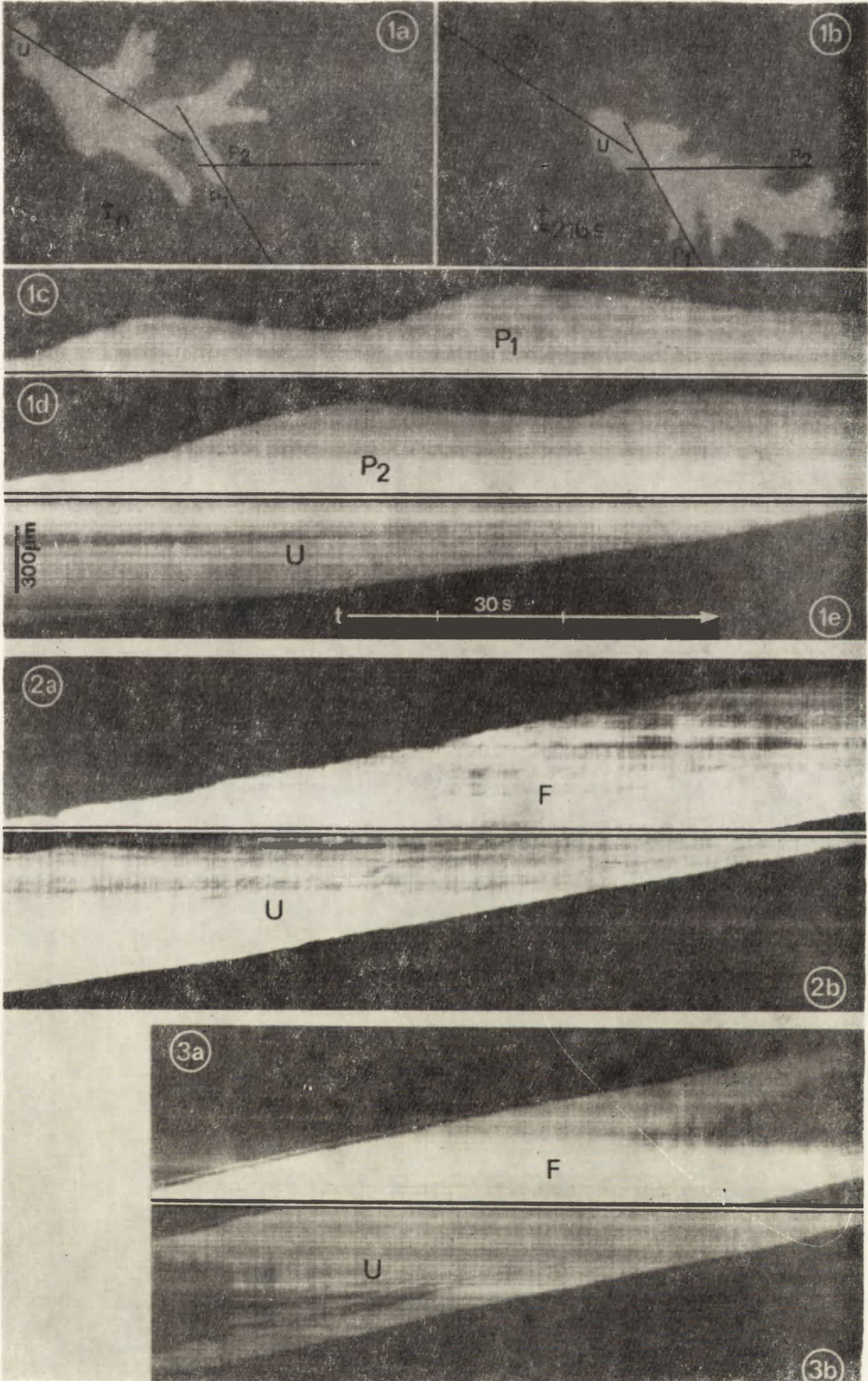

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Dynamics of the Ending Veins in Plasmodia of *Physarum polycephalum*

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Synopsis. The ending veins of plasmodia of *Physarum polycephalum* were observed *in situ*. Macro- and microcinematographic time-lapse pictures were taken. The pulsation of retracting ends was compared with that of the main veins. Additionally, the protoplasmic inflow and outflow were kept under control in the main veins and in the terminal branching strands. Those data served to complete the image of the mechanisms leading to the progressive movement of the organism.

The investigations initiated recently to describe the time and space distribution of contractions and relaxations in plasmodia of *Physarum* revealed the existence of a common rhythm of pulsation along single strands isolated from plasmodium (Krüger and Wohlfarth-Bottermann 1978, Yoshimoto and Kamiya 1978 a) and in the artificial system of interconnected strands (Takeuchi and Yoneda 1977). The common rhythm was then revealed in the whole intact plasmodia by Grębecki and Cieślawska (1978) and Cieślawska and Grębecki (1979) who qualified this organism as "an imperfectly synchronised monorhythmic contractile system". The schematic sequence of events leading to the progressive movement of plasmodium was thus described as being similar to a rhythmically operating syringe. All the main veins of plasmodial network squeeze simultaneously the protoplasm towards the peripheral areas and "as the frontal periphery may accept the greatest volume of the endoplasmic material the most efficient outflow goes in this direction". As a result the advancing edge makes in this time one step forward (Grębecki and Cieślawska 1978). But this statement does not fully inform what happens at the rear parts of plasmodium. Obviously, it is perfectly known that contractions and relaxations can be observed in each vein, no matter which part of the network it belongs to. But only suggestions

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and speculations could be made about how the rhythmicity in ending veins is related to that in the main ones.

The present investigation was carried out to compare the pulsation rhythmicity of the plasmodial ending veins with that of their mother main strands and thus to make one step more in completing the image of mechanisms leading to the locomotion of plasmodium of *Physarum*.

Material and Methods

Cultivation of plasmodia of *Physarum polycephalum* followed the technique described by Camp (1936). The small pieces of protoplasmic mass were transferred from the stock cultures on the non-nutritive 2% agar gel and investigated at least 24 h later.

Observations of radial pulsation and the way of withdrawing of the ending veins were made under low power through the PZO MPI 3 microscope. The time lapse pictures were taken every second by means of 16 mm Bolex camera with Variotimer. The analysis was made by redrawing the contours of the ending veins from each 10th frame of the film. The numeral data obtained after measuring appropriate parts of the drawings served to construct the graphs.

To compare the pulsation rhythmicity of the main veins with that of branching ending strands the macrocinematographic pictures were taken with the speed of 1 fr./s and then the pulsation at different places of the image was recorded photometrically from the screen during several projections of the same film. The filming procedure and the way of analysis followed the technique described in detail elsewhere (Grębecki and Cieslawska 1978).

The retracting ending veins were filmed as well in the withdrawing rear parts of the plasmodial network as in the region lying behind the front where many thin interconnecting strands are disrupted and retract before being resorbed by the main veins.

Results

The observation of microcinematographic pictures of the withdrawing veins reveals that there are two ways in which their regressive movement is achieved: (a) thicker endings demonstrate radial oscillations and their effective backward motion is also a pulsating one (Fig. 1 A), (b) thinner terminal veins perform indistinct radial oscillations and they withdraw more or less continuously (Fig. 1 B, C). It is worth to notice that in the thicker withdrawing vein (Fig. 1 A) the periods of diminishing the vein's diameter occur simultaneously with the outflow of protoplasm and with the highest speed of shortening of the vein. On the graphs B and C this coincidence is not so evident.

The pulsation rhythmicity of the ending branching veins was compared with that of their mother strands. Plate I 1, 2 and 3 shows three

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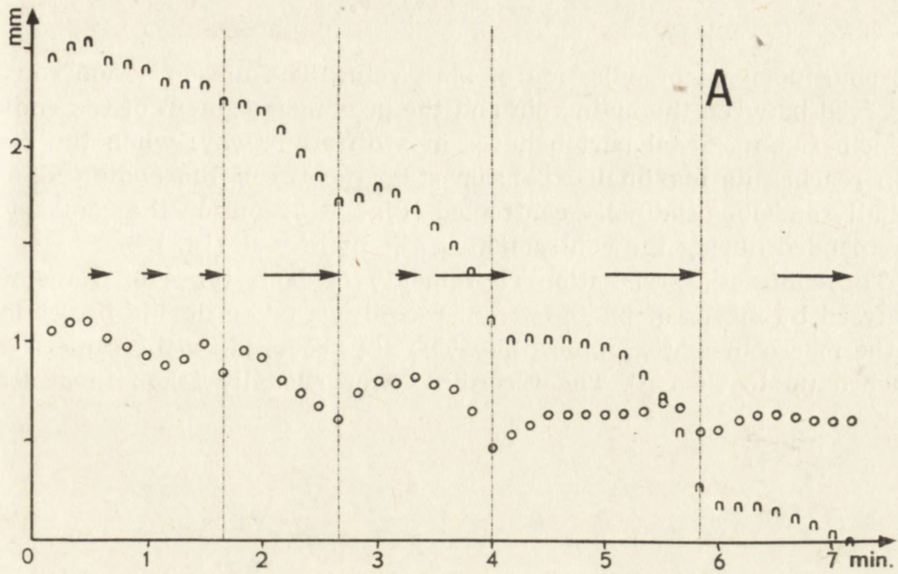

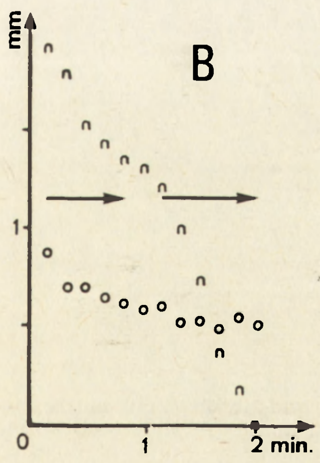

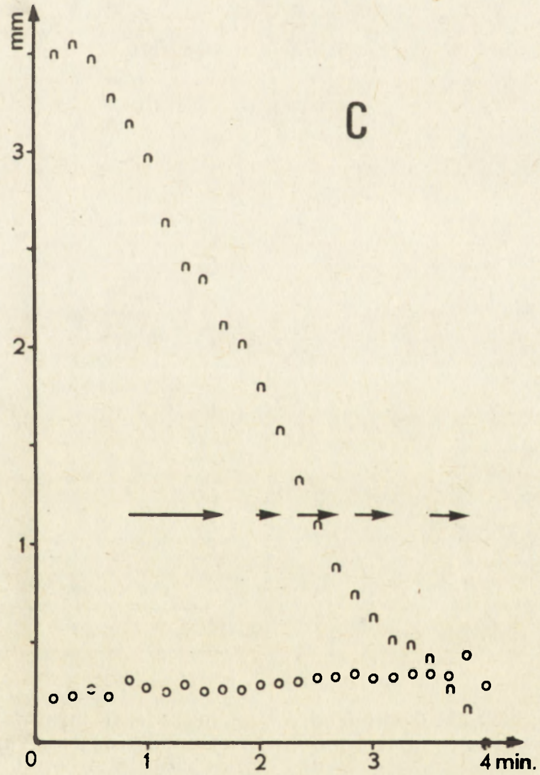

Fig. 1. Graphs of the length and terminal diameter changing in the ending strands, marked against the time scale. The horizontal arrows cover the periods of protoplasm outflow from the ending vein towards the main strand. A — thicker ending: oscillating changes of its terminal diameter are correlated with its pulsating backward movement and with the alterations of protoplasm flowing. B and C — thinner endings: their withdrawal is rather continuous. The changes of the vein's terminal diameters are indistinct.

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extreme moments of pulsation of both veins. The unison action can be observed between the main vein and the proximal segment of the ending branch. But its distal part behaves in a different way: when the main vein reaches its maximal expansion state the tip of the ending vein is radially and longitudinally contracted (Pl. I 1, 3) and on the contrary it is expanded during the contraction of the main vein (Pl. I 2).

The contraction-relaxation rhythmicity of both types of veins was analysed by means of photo-current recordings taken during projections of the macrocinematographic films. The area covered by the camera eye reached up to 4.5 cm². The recordings were usually taken in at least

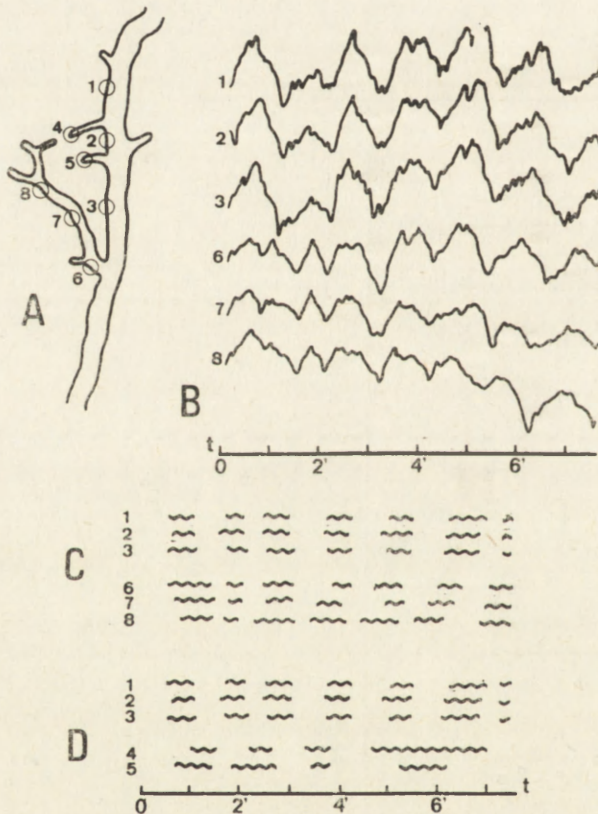

Fig. 2. A — Contour drawing of several terminal strands with their mother vein in which the pulsation was put under control. The points of the photo-cell application are marked with circles and numbered. B—Curves of the photo-current oscillations representing the pulsation of the veins. The numbers correspond to those on the Fig. 1 A. C and D — Diagrams demonstrating the contraction periods recorded at the marked sites of the veins. The descending parts of the curves of the photo-current changes are substituted by the wavy lines of corresponding length. Note that the contractions of the tips of the ending veins (lines *4* and *5*) occur during the relaxation periods of the major vein (lines *1*, *2* and *3*)

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two spots chosen on the main vein and in three others along the terminal vein, including or not its very tip.

Figure 2A is a contour drawing of a main vein giving branches to several terminals. The points of the photo-cell application are marked with circles and numbered. The curves of photo-current oscillations representing the rhythmicity and relative intensity of pulsation in the chosen points are presented on the Fig. 2 B. It can be noticed that corresponding maxima and minima of the curves *1, 2* and *3* recorded at the main vein, and those of the curve *6* recorded at the basal part of the longest terminal branch show almost no phase shift indicating that contractions and expansions in the points under control occurred in the same times. The curves *7* and *8* representing the median segments of the same terminal strand slightly differ in their rhythmicity: they display a marked tendency to the phase shifts leading to some overlaping in time of the contraction and expansion phases manifested respectively by the main vein and the ending branch. This is more clearly visualized on the diagram (Fig. 2 C) in which the descending parts of the curves (equivalent to the contraction periods) are substituted by the wavy lines of corresponding length.

The pulsation rhythmicity of the very tips of short terminal veins was measured as well. In those cases the position of photo-cell was gradually shifted to follow the withdrawing end of the vein, as in the method used to check the pulsation of the frontal edges (Grębecki and Cieślawska 1978). The periods of vein's shortening are shown by the lines *4* and *5* (Fig. 2 D). They are compared with contractions in three points at the main vein (lines *1*, *2* and *3*). It can be seen that the tips of the terminal veins reveal a different pattern of rhythmicity, their contractions covering more or less exactly the periods of the main veine expansions. This is also seen on the Fig. 3 where the retraction of the terminal vein's tip was followed by the photo-cell (line *5*) and compared with the pulsation in two points of the main vein (*1* and *2*) and in more proximal segments of its branching (*3* and *4*).

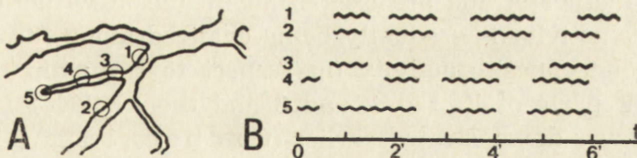

Fig. 3. Diagram of the contraction periods (B) recorded at the sites marked on the contours of the veins (A). The main vein and the proximal part of the ending branch oscillate in unison. The distal segment of the terminal strand acts almost exactly in opposite phase.

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On the Fig. 4 despite a certain irregularity in the contraction-relaxation pattern revealed by the main vein (lines *1* and *2*) again the contractions of the main strand are synchronous with the contraction periods of the terminal vein in its proximal region (line *3*) but opposite in phase at the withdrawing distal end (line *5*).

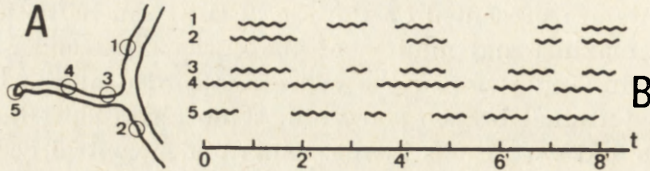

Fig. 4. Diagram of the contraction periods (B) recorded at the sites marked on the schematic drawing (A). Despite a certain irregularity in the oscillation pattern of the main vein its contractions are synchronous with those at the proximal part of the ending strand, opposite in phase in respect to the pulsation of the ending vein's tip.

Figure 5 presents the rhythmicity of pulsation measured along the main vein and relatively thin terminal one. Here, the whole ending strand, including even its part quite close to the main vein shrinks during the expansion periods of the main vein. However, it should be stressed that this situation is rather exceptional and in most cases the

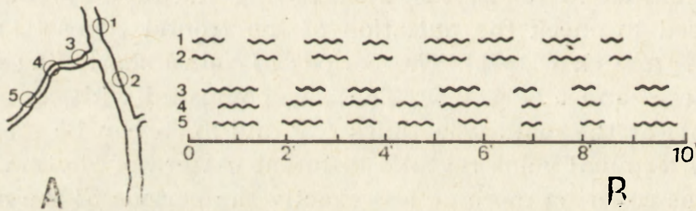

Fig. 5. Diagram of contractions (E) recorded along the major vein and along a relatively thin terminal branch (A). Here, the whole ending strand, including even its part quite close to the main vein, shrinks during the expansion periods of the main vein.

parts of the branching veins remaining in the closest neighbourhood with their mother strand are oscillating in unison with the latter. This synchronous activity is gradually being changed along the terminal vein leading to the opposite activity (in respect to the main strand) at the most distant parts of it (Fig. 2 and 3 and the Pl. I 1–3). The working antagonism was demonstrated earlier (Grębecki and Cieślawska 1978) between the network of major veins and the advancing frontal margin. It allows to expect the synchronous activity of the frontal edge and of the terminal veins' endings.

To compare the movement of the retracting parts with the behaviour

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of the frontal edge the photo-current changes were recorded at the points chosen on the one and the another type of plasmodium periphery. The results are presented by the example shown on the Fig. 6. The cur-

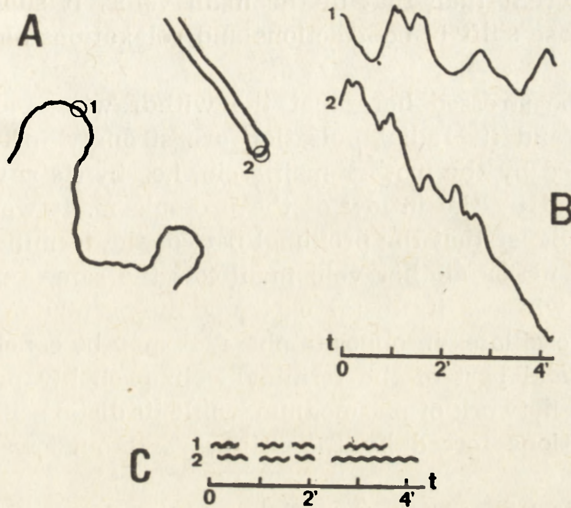

Fig. 6. Curves (B) and diagram (C) demonstrating the pulsation rhythmicity of the frontal edge and of one ending strand of the same plasmodium (A). Regressing of the frontal edge and retracting of the terminal vein coincide in time.

ves and the diagram demonstrate that the rhythmicity of pulsation of both peripheral areas is the same. Retracting of the terminal vein and regressing of the frontal edge coincide in time.

Retraction of the veins is also easily observable in this part of plasmodial network of veins which lies just behind the continuous protoplasmic layer. There, the anastomosing connections between the veins are loosing their continuity, retract after disruption and in the consequence are being incorporated into the main veins. The sequence of events leading to the retraction of two parts of the disrupted vein is shown on the Plate I 4–9. It is very characteristic of this process that immediately after the rupture the remaining two segments of the vein perform an extremely quick retracting step (Pl. I 6, 7). On the cine-film this movement resembles shortening of a rubber stripe which was formerly expanded.

Discussion

The previous studies of the ending veins of intact plasmodia carried in the polarized light revealed that the shape of the upper surface of those veins is similar to that of the main ones, and that the pulsation

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can be easily observed at their ends (Cieślawska and Grębecki 1978). The dynamics of the bright stripes appearing and disappearing at both sides of the ending veins indicated however that their pulsation is somewhat different than that of the main veins. It suggested the existence of a phase shift in contractions and relaxations along such a terminal strand.

It should be stressed here that the withdrawing movement of the terminal vein and its radial pulsation are strongly influenced by two factors produced by the mother main vein, i.e., by its rhythmic contraction pattern and by the inflow of the protoplasm. It was demonstrated in the present paper that the proximal part of the terminal strand which is close to the major mother vein manifests the same pulsation pattern as the latter, whereas its distal part, and in particular the tip of the ending strand oscillates in opposite phase. It may be concluded therefore that the proximal part of the terminal vein probably pulsates actively with the whole network of plasmodium, while its distal segment undergoes passive oscillations forced by the inflows and outflows of the protoplasm.

The pulsating character of withdrawal is almost indistinct in the thin terminal veins as the amount of the protoplasm entering them is limited by the narrowness of their channels. The retractions of such terminals is more or less continuous process. Also the disrupting interconnections between the veins of the network initially perform a very quick steady retracting movement. A similar type of motion was already described by Haberey (1971) and by Nowakowska and Grębecki (1977) in the small adhesive pseudopodia of the uroid region of *Amoeba proteus* when they detach from the substratum. The speculation can be made whether the quick and uniform retracting movement of the ending veins of plasmodium is also produced by the release of their adhesion to the surface. If so, it would be a case of the elastic retraction and it could confirm the suggestion of Yoshimoto and Kamiya (1978b) that the veins of plasmodium adhering to the substratum may be under longitudinal tension. However, further assumption of these authors that the veins *in situ* fail to manifest longitudinal oscillations is inconsistent with the findings of Hülsmann and Wohlfarth-Bottermann (1978) and Moczoń and Grębecki (1978).

The data presented here, together with those obtained in the previous studies, describe the following facts involved in the mechanism of plasmodial activity:

(1) The main veins of the plasmodial network perform simultaneous oscillations over the whole their length (Grębecki and Cieślawska 1978, and Cieślawska and Grębecki 1979).

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(2) The veins and the frontal edge manifest the same pulsation rhythmicity but opposite in phase (*ibidem*).

(3) Tip regions of the terminal veins and the main strands oscillate in opposite phases (Pl. I 1, 2, 3 Fig. 2, 3, 4, 5).

(4) In the thick ending veins the radial pulsation and the longitudinal dynamics of the vein are dependent on the protoplasm inflow and outflow (Fig. 1 A).

(5) In the thin ending veins this correlation is indistinct. Those veins follow a rather continuous way of withdrawing (Fig. 1 B, C).

(6) The anastomosing veins of the network after they disrupt begin to function as a part of plasmodial periphery.

Thus the dynamics of the frontal and of the rear peripheries of plasmodium seems to be in a similar way dependent on the movements of the protoplasm squeezed by active contractions from the central parts of the network.

RÉSUMÉ

Les extrémités des veines terminales, retirées par le plasmodium de *Physarum polycephalum*, étaient étudiées *in situ* par la macro- et microcinématographie à cadence ralentie. Leur pulsation était comparée à celle des veines principales. En plus, les mouvements du protoplasme étaient tenus sous contrôle dans les veines principales et dans leur branches terminales. Ces données ont servi à compléter l'image des mécanismes dont dépend le mouvement progressif de cet organisme.

REFERENCES

Camp W. G. 1936: A method of cultivating myxomycete plasmodia. Bull. Torrey Bot. Club, 63, 205–210.

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

Cieślawska M. and Grębecki A. 1979: Synchrony of contractions in freely migrating plasmodia of *Physarum polycephalum*. Acta Protozool., 18, 125–126.

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

Haberey M. 1971: Bewegunsverhalten und Untergrundkontakt von *Amoeba proteus*. Mikroskopie, 27, 226–234.

Hülsmann N. und Wohlfarth-Bottermann K. E. 1978: Räumliche und zeitliche Analyse von Kontraktionsabhängigen Oberflächenbewegungen bei *Physarum polycephalum*. Cytobiologie, 17, 23–41.

Krüger J. and Wohlfarth-Bottermann K. E. 1978: Oscillating contractions in protoplasmic strands of *Physarum*: Stretch-induced phase shifts and their synchronization. J. Interdiscipl. Cycle. Res., 9, 61–71.

Moczoń M. and Grębecki A. 1978: Time relationships between the lon-

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gitudinal and radial contraction in plasmodial veins of *Physarum polycephalum*. Acta Protozool., 17, 543–550.
Nowakowska G. and Grębecki A. 1978: Attachment of *Amoeba proteus* to the substrate during upside-down crawling. Acta Protozool., 17, 353–358.
Takeuchi Y. and Yoneda M. 1977: Synchrony in the rhythm of the contraction-relaxation cycle in two plasmodium strands of *Physarum polycephalum*. J. Cell Sci., 26, 151–160.
Yoshimoto Y. and Kamiya N. 1978 a: Studies on contraction rhythm of the plasmodial strand. I. Synchronization of local rhythms. Protoplasma, 95, 89–99.
Yoshimoto Y. and Kamiya N. 1978 b: Studies on contraction rhythm of the plasmodial strand. IV. Site of active oscillation in an advancing plasmodium. Protoplasma, 95, 123–133.

EXPLANATION OF PLATE I

1–3: Pulsation of the major vein and its ending branch shown in its extrema. Note the unison action between the main strand and the proximal segment of the ending branch. On the contrary to this the tip of the terminal vein acts in opposition to the main vein; 160 ×
4–9: Sequence of the film frames demonstrating the succesive stages of the vein's rupture. Immediately after the rupture (between the stages 6 and 7) the vein produces a very quick retracting step; 125 ×

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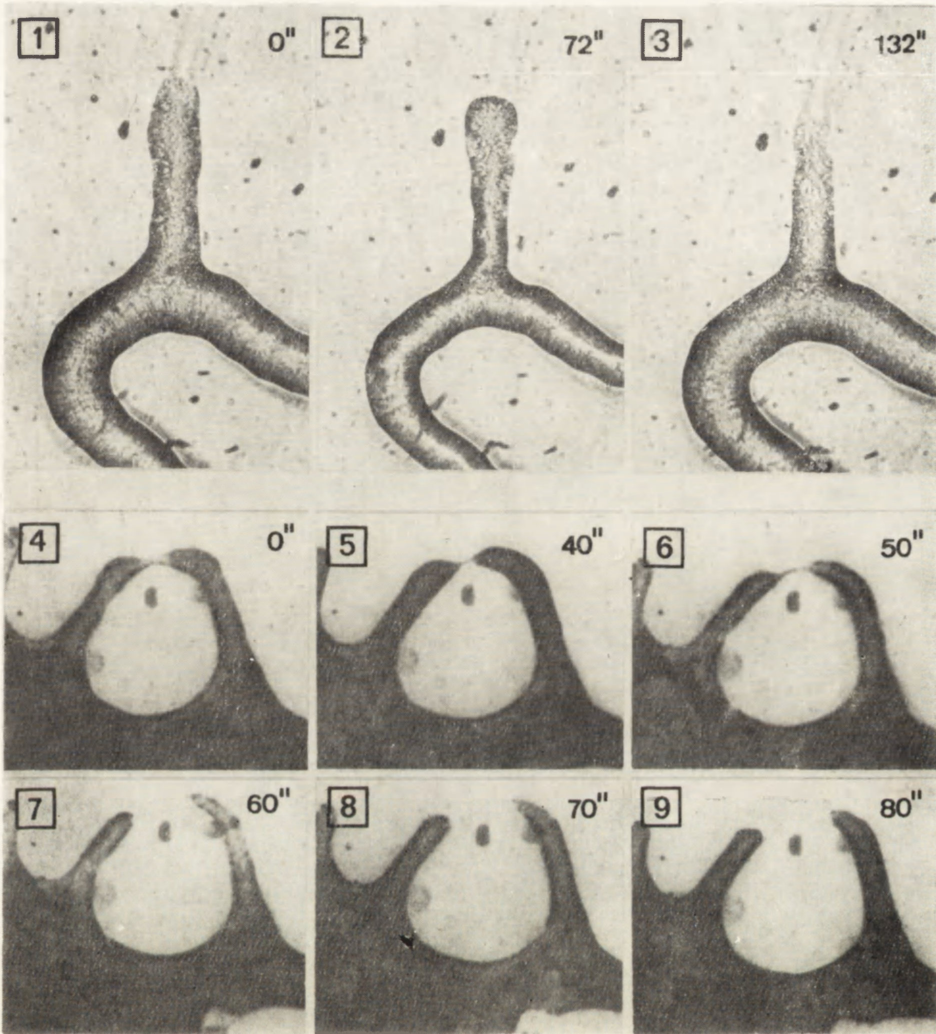

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Dynamics of the Frontal Margin in Plasmodia of *Physarum polycephalum*

Received on 25 October 1979

Synopsis. The forward extension of frontal margin of *Physarum* plasmodia was studied in the plane of migration and in the side-view by cinematographic techniques. During the phase of rapid progression the frontal ridge does not spread flatly on the substratum, but its expansion is initially directed obliquely upwards. Then, the expanded frontal margin falls down and establishes new contact with the agar gel surface. During the recessive phase the frontal ridge flattens but usually does not lose the territory which has already been occupied. Some digitated fronts may progress in uniform manner without distinct oscillations of velocity.

The question of space and time ogranization of the contractile activity in plasmodia of *Physarum polycephalum* became recently the subject of many studies. Hülsmann and Wohlfarth-Bottermann (1978 a and b) and Moczoń and Grębecki (1978) found that radial and longitudinal components of contraction of veins follow the same rhythm. The synchronization of contraction periods was demonstrated in isolated veins by Krüger and Wohlfarth-Bottermann (1978) and by Yoshimoto and Kamiya (1978), in a more complicated artificial system of interconnected veins by Takeuchi and Yoneda (1977), and *in situ* — in intact migrating plasmodia by Grębecki and Cieślawska (1978). The synchronous pattern of contraction-expansion cycles manifested in the whole network of veins becomes more complicated at the peripheries of plasmodium. In the narrow, 3–4 mm large zone behind the frontal edge, the thickness changes of plasmodium assume a wave-like character which was recently studied by Baranowski (1976, 1978), and the advancing edge itself oscillates in phase opposition in respect to the veins (Grębecki and Cieślawska 1978). As well the functionally posterior peri-

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pheries, i.e., the extremities of terminal retracting veins pulsate in opposition to the main network of plasmodium (Cieślawska 1980).

The objective of the present study was to complete the image of dynamics of different regions of migrating plasmodium by a more detailed investigation of behaviour of its frontal margin during the successive contraction-expansion cycles.

Material and Methods

The fragments of plasmodia cultivated on wet filter paper and fed on oat flakes (Camp 1936) were transferred on the non-nutritive agar gel substratum, and their motory behaviour was recorded on the first or on the second day after the transfer. Macrocinematographic pictures of the frontal zones of migrating plasmodia were taken with a 16 mm camera at the frequency of 1 frame per second. Plasmodia were filmed in the uniform and very weak white light illumination (80 lux) which does not disturb their mode of locomotion. Pictures were taken either in the plane of migration (Pl. I 1) or in the side-view (Pl. I 2). In the first case, plasmodia moved on the surface of agar gel covering the bottom of a Petri dish, and the camera was vertically mounted. In the second case, plasmodia migrated along the upper edge of an agar gel block, and the camera was mounted horizontally. The air humidity was assured in both situations.

The film records were used either for frame-by-frame analysis or served to produce photokimographic curves of activity. By the first procedure the sequencies of successive motion stages were obtained. The contours of the fronts of plasmodia were redrawn on the tracing paper, and then superposed to one another, at the regular intervals of 10 frames (= 10 s.) or at the selected critical moments of motion. The technique of producing the photokimographic curves of activity (the slit technique) was the same as described by Cieślawska and Grębecki (1978) and Grębecki and Moczoń (1978).

Results

Under the conditions of present experiments plasmodia transferred to the non-nutritive agar gel developed the fan-like frontal zones (Pl. I 1 a), and sometimes they produced digitated fronts (Pl. I 1 b). Fan-like fronts characterize the exploratory behaviour of starving plasmodia, whereas the conditions favouring the development of digitated ones are not defined. The fan-like fronts observed in the present study behaved in more regular manner, manifesting the propagation of pulsation waves in the narrow zone behind the advancing edge as described by Baranowski (1976, 1978), and the synchronization of expansion phases along their circumference as described by Grębecki and Cieślawska (1978). In the digitated fronts the waves and the co-or-

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dination of different branches were not distinct. Several cases of transition from the fan-like form to the digitated one were also observed.

The velocity oscillations of progression of the frontal margin were investigated by the slit technique in plasmodia which were filmed in the plane of their migration. Several examples of the activity curves obtained by this method are presented in the Pl. II 4. Contrary to the expectations, it proved to be rather difficult to find the fronts which regularly manifest clear regressive phases between two successive steps forward. Such an example is shown in the Pl. II 4 a. Most commonly the frontal margins were nearly stationary between the progressive phases, instead of being withdrawn (Pl. II 4 b–c). In some others the velocity was always positive, with oscillations expressed only by rhythmic accelerations and retardations of forward motion (Pl. II 4 d–e). In several more rare cases the progressive movement was so steady that any oscillations could be hardly detected along the activity curves (Pl. II 4 f).

The same differences appear in the contour drawings of fronts recorded in the side-view. Not all the fronts withdraw between their

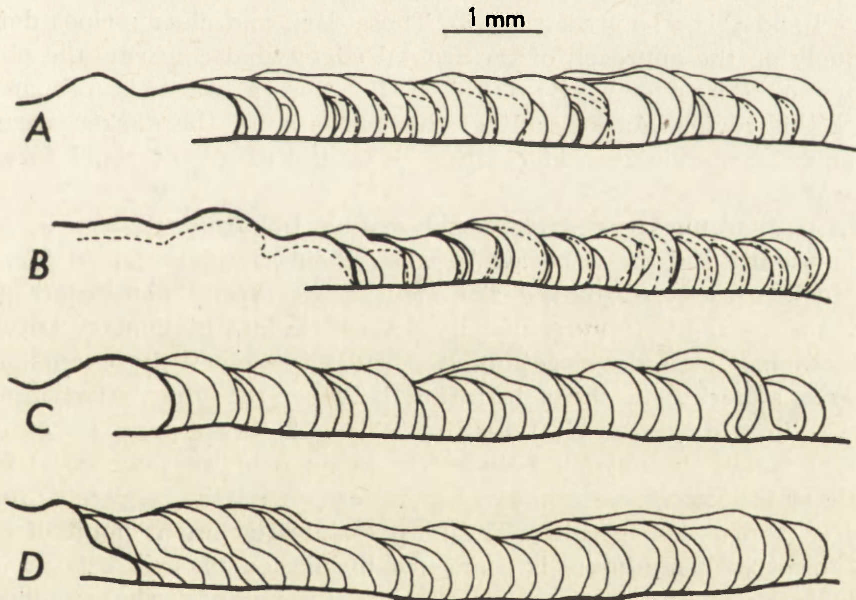

Fig. 1. Superposed contours of frontal profiles, spaced at 10 s intervals, redrawn from cinematographic pictures taken in side-view. First position of the front is marked with heavy line, its further extension with thin lines, and withdrawals of its contour with dashed lines. Note that the front may be sometimes periodically withdrawn (A and B), but it may also only periodically slow down its progression (C) or even progress in a more or less continuous way (D)

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successive forward steps, as in the Fig. 1 A and B. The Fig. 1 C shows an example of front which only retarded periodically its progression, and the Fig. 1 D a front which advanced in almost uniform manner.

It should be pointed out that there is a full gradation of possible transitions between the situations shown in the Pl. II 4 and in the Fig. 1. The character of frontal progression may also smoothly change during the migration of the same plasmodium. For example, it may happen when a fan-like front becomes digitated. In general, the steady progression with no distinct oscillations in velocity is exceptional in the fan-like fronts, and common only in the digitated ones. It should be noted that the film records demonstrate that plasmodia with digitated fronts which may advance in almost continuous manner manifest, however, the normal pulsation pattern in their network of veins.

In many activity curves of fan-like fronts their rhythmic changes of thickness were also recorded in form of alternation of dark and clear areas behind the advancing edge (Pl. I 3). In more posterior zones the darker areas coincide in time with slowing down or resting periods of the frontal edge, according to the opposition of pulsation phases of the whole plasmodium and of its advancing margin, demonstrated by Grębecki and Cieślawska (1978). These dark and clear stripes deflect obliquely on the approach of the frontal edge, what confirms the observations of Baranowski (1976, 1978) that a wave is propagated across this region. As a result of this phase shift, the darker areas at the anterior margin coincide in time with the phases of rapid forward movement.

The rather unexpected phenomenon that the frontal edges of plasmodia usually fail to withdraw between their successive steps forward was further analysed in the side-view. Such profile pictures (Fig. 2) show the position of most distally located points of contact between plasmodium and the substratum. It permits to survey the extension of the area adhering to the substratum between the alternating minima and maxima of front pulsation. In the Fig. 2 the upper part of each drawing shows the changing shape and position of the plasmodial front profile at the successive minima. In the lower part the successive maxima are presented in parallel. The most distal attachment point of plasmodium at each minimum is connected by dashed line with its position at the next maximum. The dotted lines interconnect the attachment points between maxima and their subsequent minima. It may be seen that almost all dashed lines are obliquely inclined forwards what reflects the previsible fact that the front spreads to a new territory between each minimum and the next maximum of its pulsation. It is more interesting to note the course of the dotted lines. They are very

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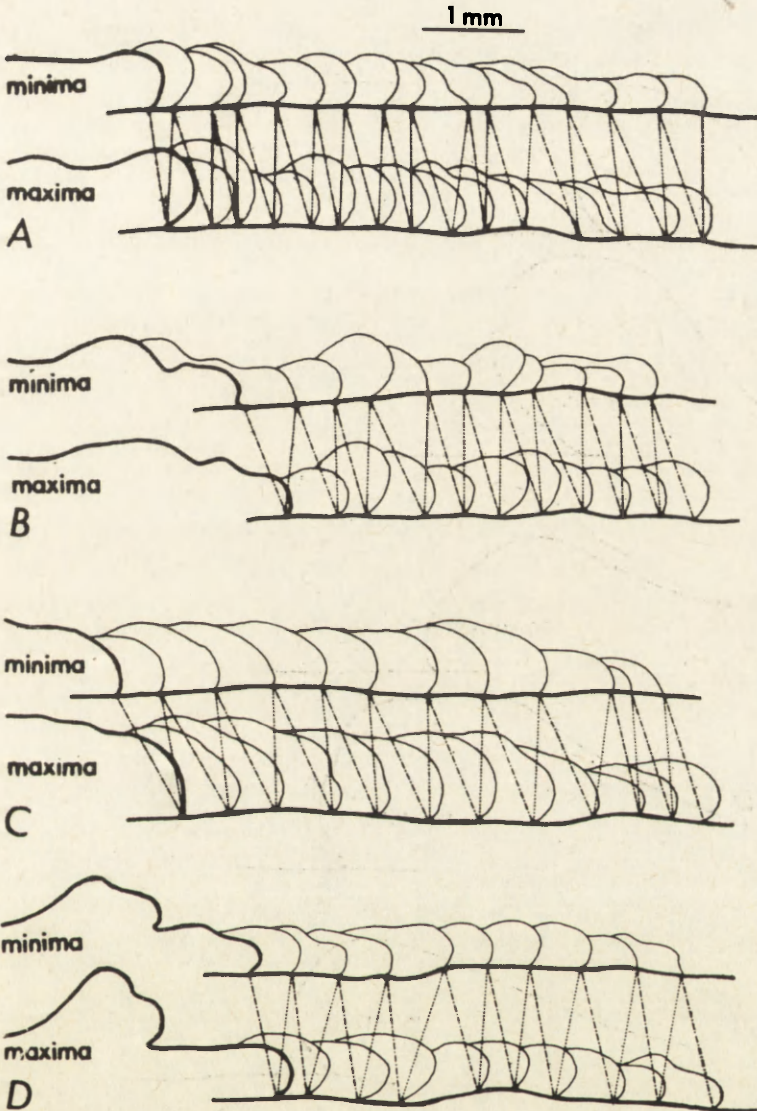

Fig. 2. Parallel presentation of the shape and position of frontal profiles of four different plasmodia (A, B, C, D), at the successive minima and maxima of their pulsation. Note that the area adhering to the substratum usually extends forward between a minimum and its subsequent maximum (dashed connecting lines), and it remains either unchanged or continues to extend between a maximum and the next minimum (dotted connecting lines)

rarely inclined backwards, what means that during the recessive phase (from a maximum to the next minimum) the frontal margin only exceptionally detaches from the substratum and effectively withdraws. Usually the dotted lines are approximately vertical, i.e., the attach-

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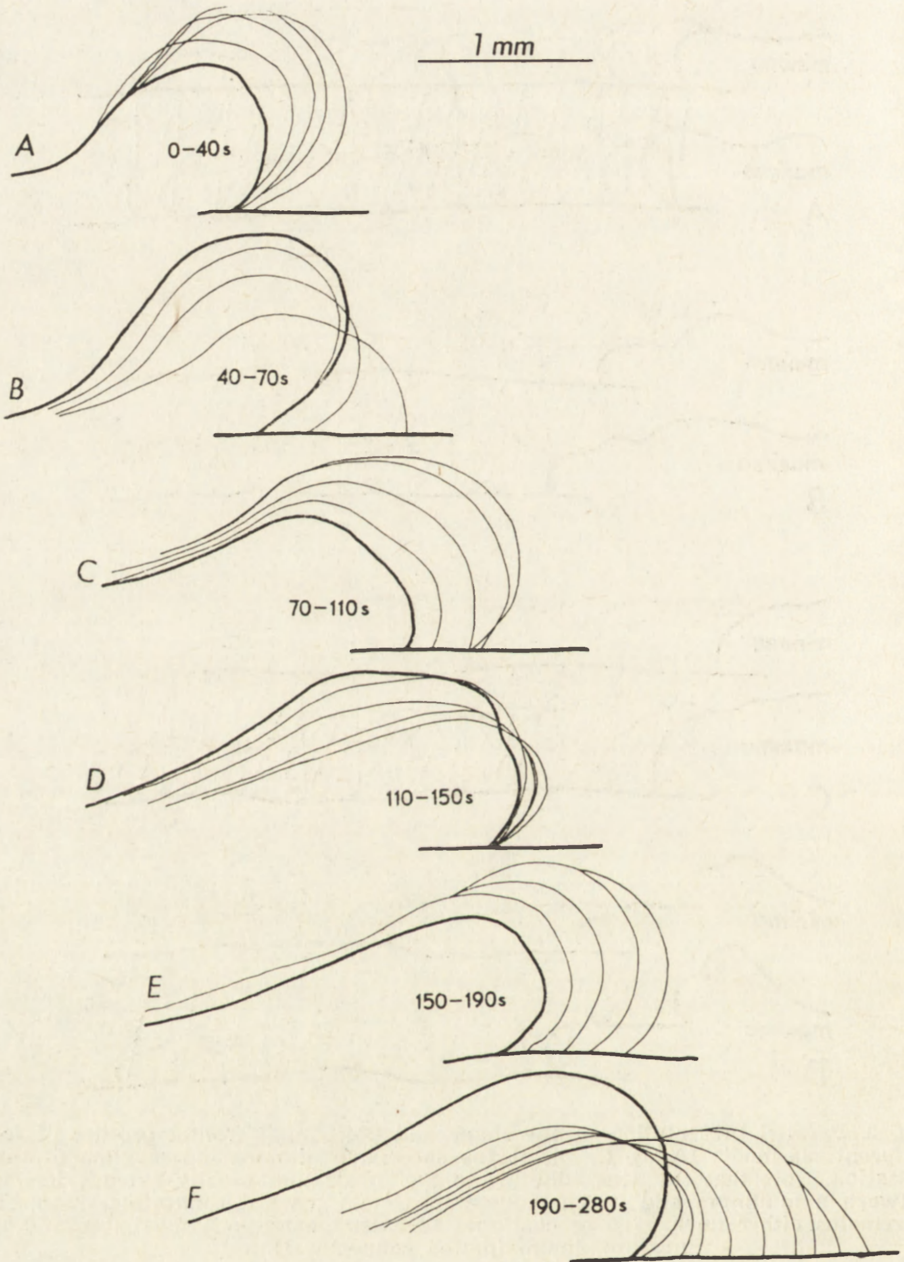

Fig. 3. Sequence of profile pictures of the frontal ridge of plasmodium demonstrating the phases of expansion directed obliquely upwards (A, C, E) and the phases of flattening and falling down (B, D, F). Contours are marked every 10 s

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ment established during front extension remains almost stable during the recessive phase. In some cases the attachment may be even further extended forwards at this phase of pulsation (dotted lines inclined forwards).

The side-view pictures of fronts (Pl. I 2) and their profiles redrawn from the films taken in vertical plane (Fig. 3) show that the advancing margin is much thicker than the zone lying behind it, and it forms an inflated ridge. The sequence of the motion stages in Fig. 3 demonstrates that the frontal ridge does not simply spread horizontally forward upon the substratum, but it manifests distinct phases of growing obliquely upward (Fig. 3 A, C, and E), and the phases of flattening (Fig. 3 B, D, and F) which are often accompanied by further progression forwards and establishment of new contact with the substratum.

The time relations between the growing up and the forward extension of the frontal ridge are also illustrated in the Fig. 4. The contours of plasmodial fronts at their successive motion stages present here the bundles of lines crossing one another, what demonstrates the existence of a phase shift between both these processes contributing to the frontal advance of plasmodium.

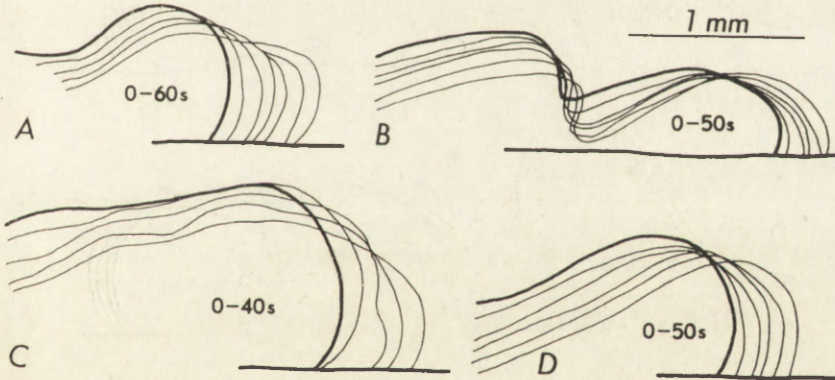

Fig. 4. Four examples (A, B, C, D) of frontal profiles in which the phase shift between the processes of growing up and of extending forwards is clearly seen in form of intercrossed contour lines

The convex shape of the anterior ridge and its tendency to grow obliquely upward during expansion creates periodically situations, seen in the Fig. 3, when the distal part of the front hangs over the substratum. In some plasmodia this tendency to expand upwards may be so extremely pronounced that it may cover much more than one pulsation cycle, as in the example presented by Fig. 5. During the first $2^1/_2$

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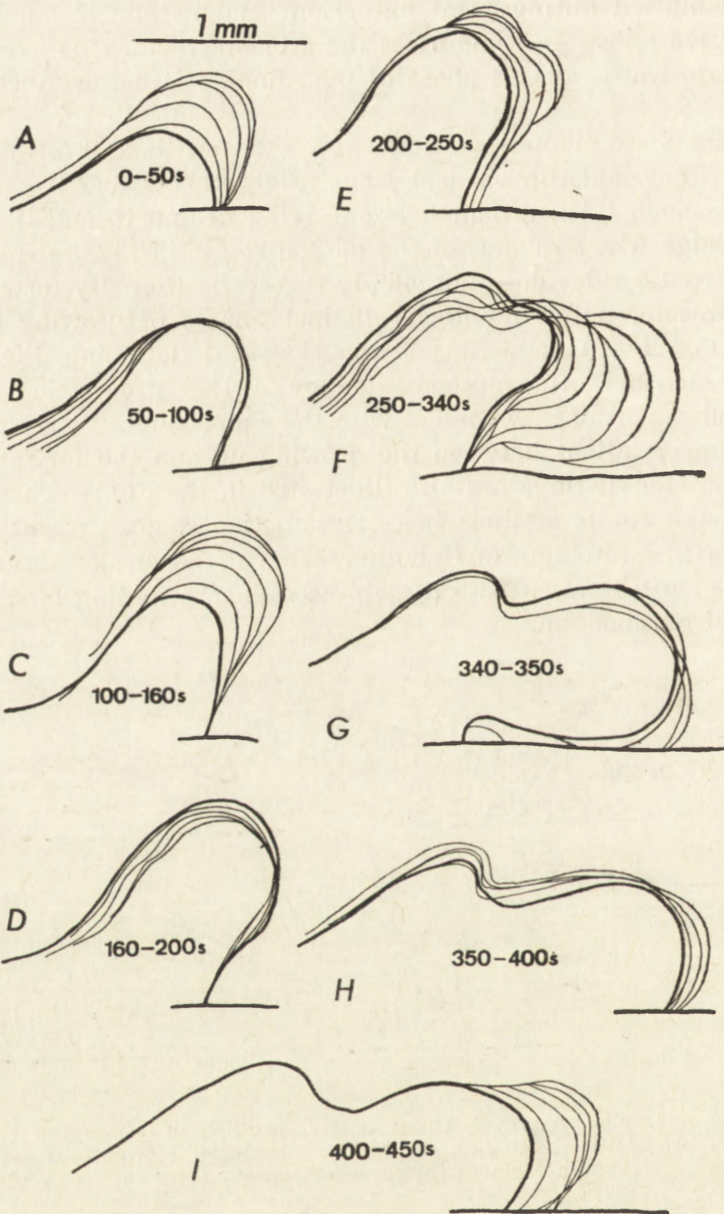

Fig. 5. Example of a frontal ridge with extremely well pronounced tendency to growing upwards. Note that the ridge continued to extend obliquely up during first 250 s, i.e., during $2^1/_2$ pulsation cycles (A–E). Its falling down took next 100 s (F-G). Later on, the front recovered the usual way of advancing (H-I). The contour lines are marked at 10 s intervals (except of G)

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cycles (Fig. 5 A–E) the investigated front of plasmodium did not spread over a new territory but it continued the oscillating extension upward. During the third cycle it already accumulated so much material in its obliquely inclined anterior extremity, that at the next 100 s period (Fig. 5 F–G) the ridge was progressively falling down toward the substratum. At the next stages (Fig. 5 H–I) plasmodium returned to the more usual mode of locomotion.

Discussion

The slit records of activity of fronts filmed in the horizontal plane covered the zone extending up to 3–6 mm behind the anterior edge of plasmodium. This distance was already sufficient to reveal the time relations between the thickness changes occurring inside the frontal region and the dynamics of the frontal margin itself. The results confirm the finding of Grębecki and Cieślawska (1978) that the advancing margin and more posteriorly located regions pulsate in phase opposition. The slit records presented here confirmed also that the transition between these two antagonistic activities is manifested, in the 3–4 mm large zone behind the anterior edge, in form of propagation of thickness waves, according to the description of Baranowski (1976, 1978).

It should be pointed out that the side-view records presented here in form of contour drawings were limited to the zone of 1–2 mm only, and therefore they could not reflect the relations discussed in the precedent paragraph. They allowed to follow the dynamics of the frontal ridge alone.

The following main conclusions may be drawn as to the behaviour of this most distal area of a migrating plasmodium:

(1) During the recessive phase of streaming the advancing front usually does not lose the territory which has been occupied before, and its decrease in volume depends merely on its flattening.

(2) During the progressive phase of streaming the volume increase determinates the frontal ridge expansion which is, at the first stage, not strictly directed along the agar gel surface but is deflected obliquely upwards.

(3) The falling down of the expanded frontal ridge and the establishment of contact between its lower surface and the substratum is produced with some delay and it may overlap in time with the phase of front flattening.

In general, this sequence of events seems to be, at least phenomeno-

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logically, similar to the cycles of extension and attachment of pseudopodia in polytactic forms of *Amoeba proteus*. According to the descriptions given by Bell and Jeon (1963) and by Nowakowska and Grębecki (1978), the first stage is characterized by a free extension of the pseudopodial extremity, then it bends and falls down, and eventually establishes a new attachment point. Its bending toward the substratum is an active movement, not depending on gravitation, and it was observed by Nowakowska and Grębecki (1978) even in amoebae walking in the upside-down position. In the case of plasmodium it remains unknown whether the falling down phase represents only a passive movement due to increase in weight of the expanded frontal ridge, or an active component of locomotion.

The observation that the front during its flattening phase usually fails to withdraw, suggests that probably in the regularly advancing fronts the areas which once adhered to the substratum are no more detached. It seems prematured to speculate why the periodicity is sometimes expressed only by cyclic retardations of frontal progression, and why some fronts may exceptionally progress without distinct velocity oscillations.

RÉSUMÉ

La progression de l'extrémité frontale de plasmodium de *Physarum polycephalum* était étudiée par les méthodes cinématographiques. Les prises de vue se faisaient sur le plan horizontal ainsi que sur le vertical (vues de profil). Pendant la phase de progression rapide la crête frontale ne suit pas étroitement la surface de l'agar, mais son expansion est d'abord dirigée obliquement vers le haut. Après cela, l'extrémité frontale retombe et elle établie un nouveau contact avec l'agar. Pendant la phase recessive la crête frontale devient aplatie, mais d'habitude elle ne quitte plus le territoire occupé précédemment. Des certains fronts digitiformes peuvent avancer de manière continue sans oscillations distinctes de leur vitesse.

REFERENCES

Baranowski Z. 1976: Svjaz volnovyh javlenij s migracjej plazmodija miksomiceta. In: Nemyšečnyje formy podvižnosti, Akademija Nauk SSSR, Puščino, 47–50.

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.

Camp W. G. 1936: A method of cultivating myxomycete plasmodia. Bull. Torrey Bot. Club, 63, 205–210.

http://rcin.org.pl

Cieślawska M. 1980: Dynamics of the ending veins in plasmodia of *Physarum polycephalum*. Acta Protozool., 19, 143–152.
Cieślawska M. and Grębecki A. 1978: Contraction-expansion rhythms simultaneously observed in two sites of *Physarum polycephalum* plasmodium. Acta Protozool., 17, 533–541.
Grębecki A. and Cieślawska M. 1978: Plasmodium of *Physarum polycephalum* as a synchronous contractile system. Cytobiologie, 17, 335–342.
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.
Hülsmann N. und Wohlfarth-Bottermann K. E. 1978 a: Räumliche und zeitliche Analyse von kontraktionsabhängigen Oberflächenbewegungen bei *Physarum polycephalum*. Cytobiologie, 17, 23–41.
Hülsmann N. and Wohlfarth-Bottermann K. E. 1978 b: Spatio--temporal relationships between protoplasmic streaming and contraction activities in plasmodial veins of *Physarum polycephalum*. Cytobiologie, 17, 317–334.
Krüger J. and Wohlfarth-Bottermann K. E. 1978: Oscillating contractions in protoplasmic strands of *Physarum*: Stretch induced phase shifts and their synchronization. J. Interdiscipl. Cycle Res., 9, 61–71.
Moczoń M. and Grębecki A. 1978: Time relationships between the longitudinal and radial contraction in plasmodial veins of *Physarum polycephalum*. Acta Protozool., 17, 543–550.
Nowakowska G. and Grębecki A. 1978: Attachment of *Amoeba proteus* to the substrate during upside-down crawling. Acta Protozool., 17, 353-358.
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.
Yoshimoto Y. and Kamiya N. 1978: Studies on contraction rhythm of the plasmodial strand. I. Synchronization of local rhythms. Protoplasma, 95, 89–99.

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EXPLANATION OF PLATES I–II

1: Selected frames from 16 mm cinematographic film taken in horizontal plane, showing the difference between the fan-like front (a), and the digitated one (b). The magnification bar applies to both pictures

2: Frame from 16 mm film taken in vertical plane, showing the profile of an advancing front as it appears in side-view

3: Photokimographic records of the frontal activity obtained with the slit applied across the frontal zone, perpendicular to the advancing edge. Note the oscillating velocity of frontal progression and the pattern of dark and clear stripes which reflect the thickness changes behind the anterior margin. The time scale and the magnification bar apply to all the pictures (a–d)

4: Selected photokimographic records of the frontal activity showing differences in behaviour of the advancing margin at the recessive oscillation phases. At this stage some few fronts retract (a), most often they are stationary (b–c), sometimes only slow down (d–e), and sometimes may progress without distinct oscillations of velocity (f). The time scale and the magnification bar apply to all the pictures (a–f)

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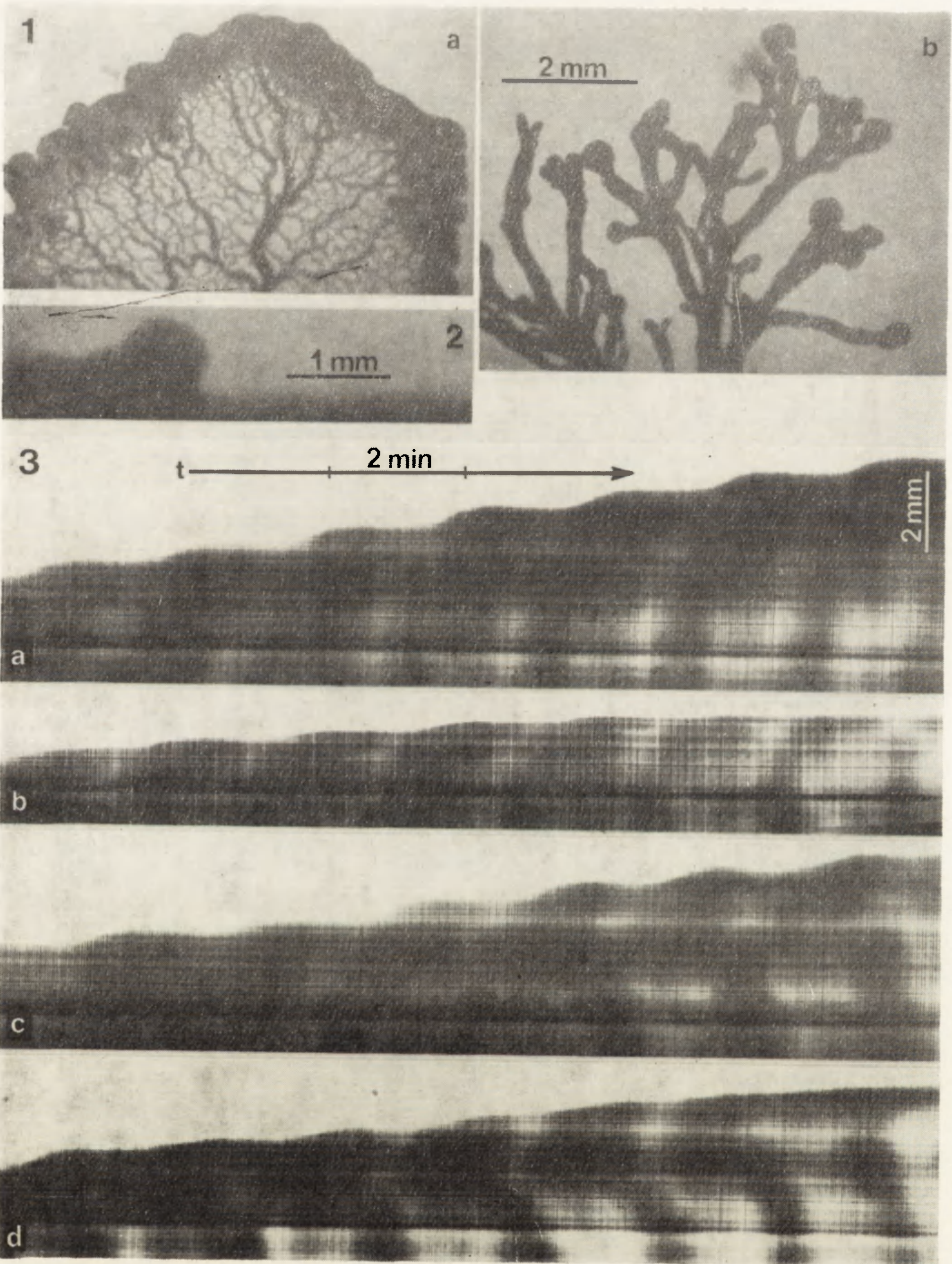

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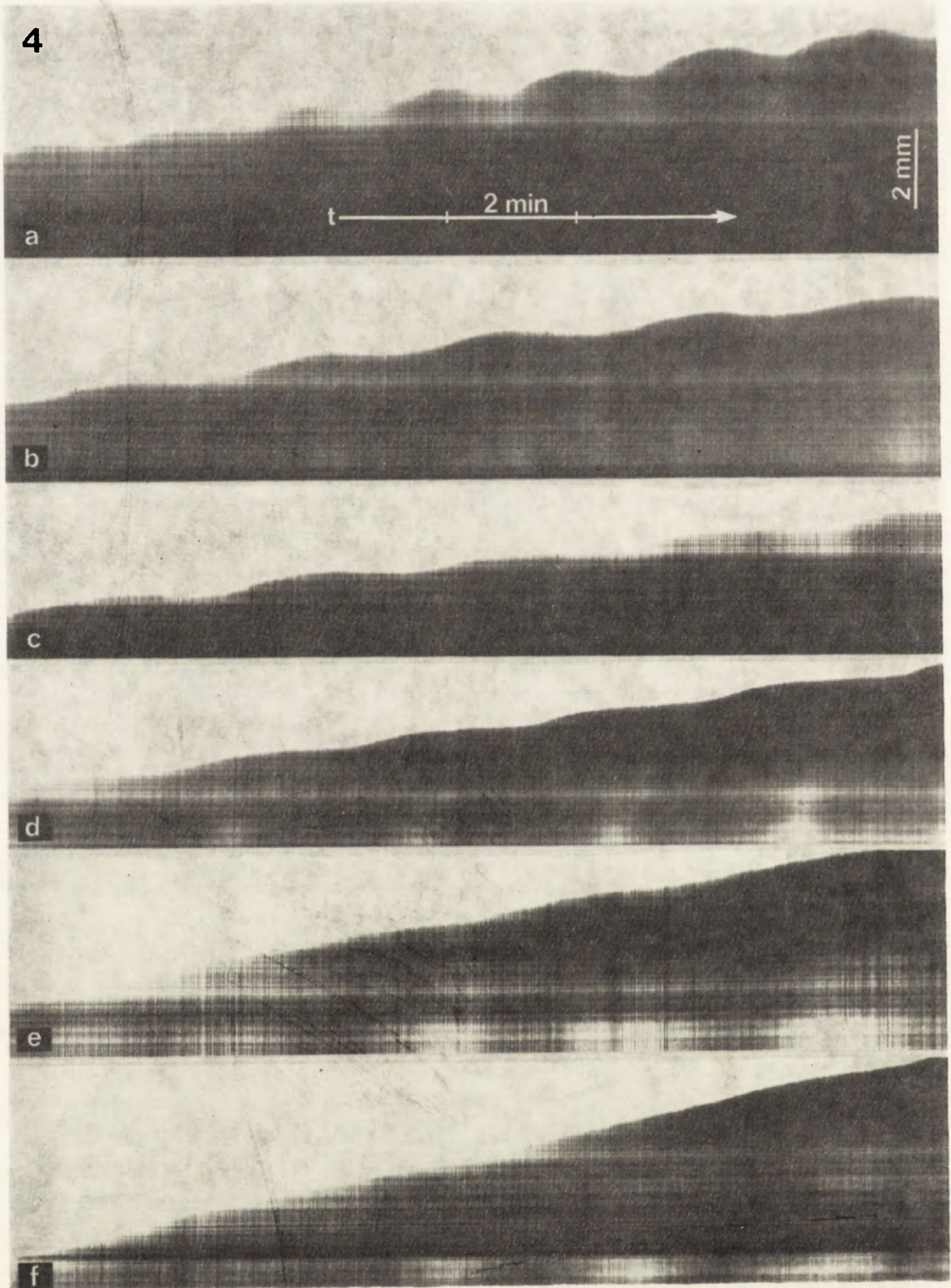

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Reproducibility of *Paramecium tetraurelia* strain 51 S and *Paramecium octaurelia* strain 299 S in Mass Cultures

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Synopsis. Paramecium tetraurelia and *P. octaurelia* populations grown in rigorous conditions are characterized by a similarity of their development. The proportions among the cells, being in different cell or life cycle stages, are at the given time of population development stable and reproducible, as ilustrated by the growth curves and the curves of percentual distribution of macronuclear stages of autogamy. Comparison of these curves allows to establish the best conditions for the growth and the optimal time when the population should be harvested for definite experimental purposes.

Mass cultures of *Paramecium* can be easily cultivated in laboratory conditions. However, little is known on the possibility of synchronization of their cell and life cycle. Only the first few generations may divide synchronously when the daughter cells are transfered into a fresh medium immediately after division, under rigorous culture conditions. This fact eliminates *Paramecium* grown in mass cultures from such studies in which cells deriving from a definite cell or life cycle stage should be harvested in large quantities. In some cases, however, for biochemical purposes it will suffice if the cultures containing different stages of the cell or life cycle are collected so that the proportions of different cell stages are comparable from experiment to experiment, from population to population. It is necessary, therefore, to find easily observable features characterizing mass cultures development, which would allow to fix the best routine ways of mass culture cultivation and to collect material homogeneous and reproducible as far as possible. The aim of these studies was to characterize and compare cell growth and the development of the nuclear events of autogamy in populations of *P. tetraurelia* grown in different axenic and monobacterized media and to find some common criterion for appreciation reproducibility of mass cultures.

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Materials and Methods

Organisms and Culture Media. *Paramecium tetraurelia* strain 51S (collection of T. M. Sonneborn, Department of Zoology, Indiana University, Bloomington, USA) and *P. octaurelia* strain 299S (collection of dr A. T. Soldo, Veterans Administration Hospital, Miami USA) were cultured on: (1) TEM-4T axenic medium described by Soldo et al. (1966), (2) SL axenic medium described by Skoczylas and Wagtendonk (1975), (3) lettuce monobacterized medium described by T. M. Sonneborn (1950). The source of lipids in the TEM-4T medium were diacetyl tartaric esters of tallow monoglycerides, in SL medium — an ethyl-ether extract of linseed, in the lettuce medium — *Enterobacter aerogenes*. Fresh medium (250 ml) in 1-litre Roux bottles were inoculated with 5 ml, of 7-day subcultures. The volume-to-surface ratio was 0.7. Cultures were grown at 27° C in the dark. pH of medium was 6.8.

Cell Counting. The population density was estimated by counting the number of living animals in a measured sample of the culture. The final result of counting was expressed as a number of cells per ml of cultures. Each time two samples, containing not less than 50 cells, were counted. Every experiment was replicated three times in two parallel bottles. The results were statistically analysed and the mean standard error was calculated. The number of cell divisions was calculated by the equation, $x = \log_2 a$, where a denotes the number of cells.

Autogamy Examination. Samples (5 ml) of culture were centrifuged 1 min at 250 x g then, 10 µl of cell suspension were stained with 5 µl of 0.5% w/v methyl green (National Anilin Division, Allied Chemical Day Corp.). In each of two parallel samples over 100 cells were examined and the percental distribution of full vegetative macronuclei, macronuclear fragments and anlagens was established.

Source of Material. Proteoso-peptone was purchased from Difco Co., Trypticase was a product of Baltimore Biochemical Laboratories and nucleic acids of BDH Biochemicals. Stigmasterol was obtained from Merck Co., vitamins from Nutritional Biochemical Co. TEM-4T was a kind gift of dr Soldo. SL extract was prepared and lettuce grown (without pesticides) in our laboratory. Enterobacter aerogenes was purchased from the bank of bacteria, Warsaw, Institute of Hygiene.

Results

(1) Characteristic of growth of the two *Paramecium aurelia* strains (299 S and strain 51 S) in mass cultures in the TEM-4T axenic medium.

(a) Features Common to both Strains

The growth of population is quick, mean division rate during the first five days — more than one division per day. In the standard inoculation

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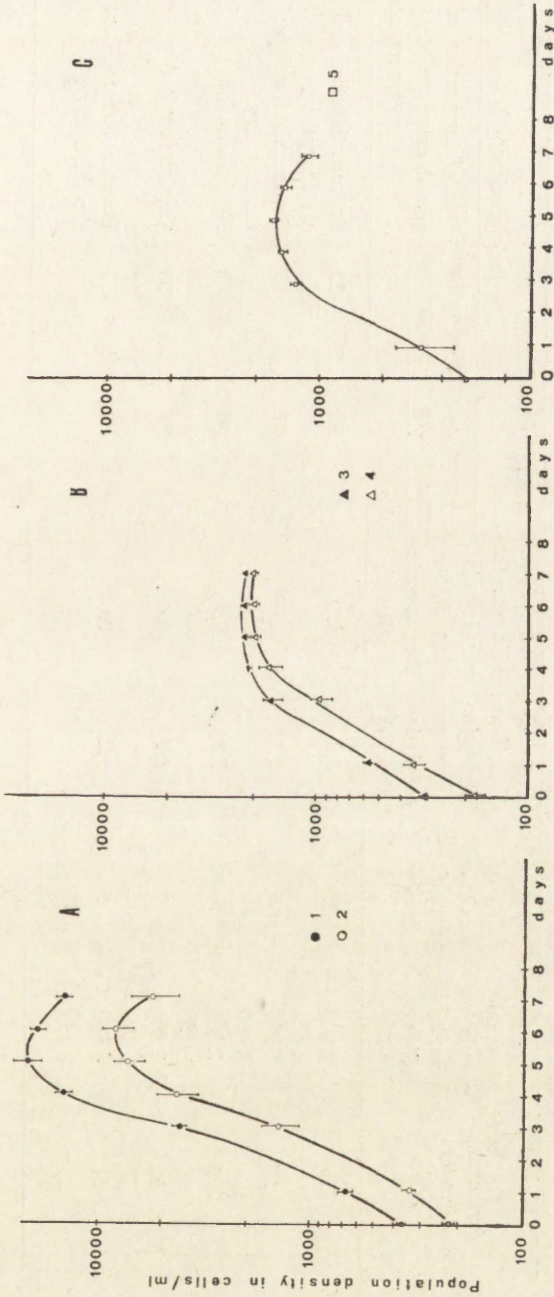

Fig. 1. *Paramecium aurelia* growth curves in: A — TEM-4T axenic medium, B — SL axenic medium, C — lettuce monobacterized medium. *1* — TEM-4T axenic medium, strain 299, 2 — TEM-4T axenic medium strain 51 S, 3 — SL axenic medium strain 299, 4 — SL axenic medium strain 51 S, 5 — lettuce monobacterized medium strain 51 S

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Table 1

Data characterizing growth and autogamy development of *Paramecium tetraurelia* and *P. octaurelia* strains in axenic and monobacterized media

Growth medium		*Paramecium aurelia* strain	Maximum population density (cells/ml)	Day of maximum population density	Δ of divisions (from inoculation to maximum population density)	Mean divisions number per day (during 5 days after inoculation)	Macronuclear autogamous stages on the day when they reach their maximum				
							Vegetative macronuclei		Fragmented macronuclei		Day of intersection of population growth curve and vegetative nuclei development curve
							day	%	day	%	
Axenice	TEM	299	21 600 ± 890	5	5.9	1.18	3	97	5	22	Between the third and a half, and the fourth day
Axenice	TEM	51	8 200 ±1 400	6	5.2	1.00	3–4	97	6	17	Between the fourth and a half, and the fifth day
Axenice	SL	299	2 200 ± 50	5–6	2.8	0.56	3	100		0	No intersection
Axenice	SL	51	2 000 ± 120	7	3.5	0.68	1–3	95	6	20	On the fourth day
Lettuce monobacterized		51	1 700 ± 100	5	3.2	0.64	1–3	82	4	17	Between the second and a half, and the third day

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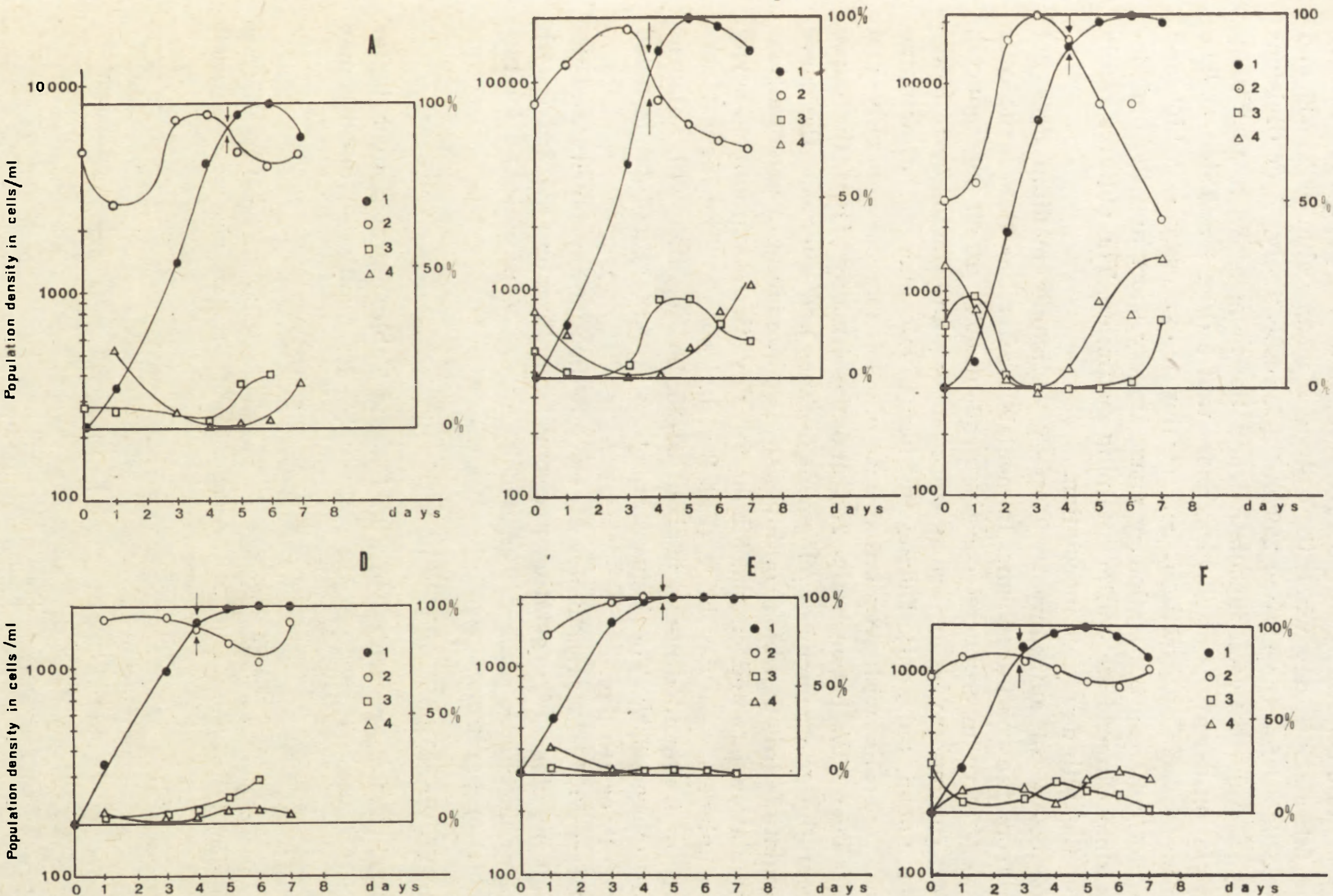

Fig. 2. Growth and autogamy development of the strain: A — 51 in TEM-4T axenic medium, B — 299 in TEM-4T axenic medium, C — 299 in TEM-4T axenic medium (series of experiments in Miami), D — 51 in SL axenic medium, E — 299 in SL axenic medium, F — 51 in lettuce monobacterized medium, *1* — population growth curve, *2* — vegetative macronuclei (%), *3* — fragmented macronuclei (%), *4* — anlagens (%), *5* — arrows intersections of population growth curves with curve of vegetative macronuclei

and cultivation conditions cultures reach maximum of population density on the 5th and 6th day when they attain a mean value of 21 600 and 14 000 cells/ml for the strains 299 and 51, respectively. Δ of divisions during the time between inoculation and the moment when maximum of population density is attained, is more that 5 (Fig. 1 a, Table 1). The logarithmic phase of growth, of both strains, is expressed as a high and steep plot, whereas the stationary phase is very short and the plot declines almost immediately after attaining maximum. The cultures rarely survive the 10th day after inoculation.

The course of autogamy in TEM-4T — axenic medium does not differ from the routine scheme. Immediately after inoculation the cells with vegetative macronuclei constitute about 80% of all macronuclei, the rest continue to be in various stages of the autogamous cycle (fragmented nuclei, anlagens). During the logarithmic phase of growth the number of cells with vegetative macronuclei increases quickly and reaches over 98% between the 2nd and the 4th day. Then, the curve of vegetative macronuclei development declines and intersects the curve of population growth which continues to rise. Maximum of macronuclear fragmentation is noted on the 5th or 6th day, the maximum of newly grown anlagens — a day later, (Fig. 2 a, b, Table 1). The highest percentage of fragmented nuclei is about 20–25, but together with anlagens it may exceed 35%. The highest anlagens percentage (15–20) is noted on the 6th or 7th day.

Data in Table 2 present mean values of population density and the mean percentage of vegetative macronuclei noticed on the 3rd or 4th day after inoculation, the day in which cultures were collected for biochemical preparations.

(b) Features Distinguishing both Strains

Strain 299 as presented in Fig. 1 a and Table 1 grows much better in TEM-4T-axenic medium than strain 51. Its population density may

Table 2

Mean values of population density and the mean percent of vegetative macronuclei attained from examination of several P. *octaurelia* 299 cultures (5 l each) harvested during the third or fourth day after inoculation

Day of harvesting	No. of collected cultures	Population density		Vegetative marconuclei (%)
		(cells/ml)	Mean st. error	
3	11	4290	457	99
4	14	14750	1764	87

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reach sometimes more than 22 000 cells/ml, corresponding to a division rate of 1.2 per day. The population growth is very stable and reproducible as may be judged from the mean standard error value. The same concerns autogamy development. Almost identical shapes of curves were obtained in another group of experiments carried out some years ago in Miami in dr Soldo's laboratory. The only difference distinguishing the present results and those from Miami was a higher percentage (sometimes up to 40 per cent) of cells with fragmented macronuclei in the Miami group of experiments (Fig. 1 a, 2 b, c).

Strain 51 grows in TEM-4T-axenic medium a little slower and never reaches more than 12 000 cells/ml, this being accompanied by a lag in initiation of the start of a new autogamous cycle. The growth of this strain is, however, very unstable, the mean standard error values are very high.

(2) Characteristic of growth of the two *Paramecium aurelia* strains (299 S and 51 S) in mass cultures in the SL-axenic medium.

(a) Features Common to both Strains

In this medium cells multiply very slowly. The development of the both strains is similar, even strain 51, seems to grow a little better. Mean division rate is 0.5–0.7 per day. Maximum of population density is reached between the 5th and 7th day after incoulation and its mean value amounts to 2200 and 2000 cells/ml in the strains 299 and 51, respectively. The mean standard error values are low (Fig. 1 b and Table 1).

(b) Features Distinguishing both Strains

A striking difference in the behaviour of the two strains is the course of the autogamous cycle. Strain 299 does not start a new autogamous cycle before the 14th day after inoculation and maintains 100% of vegetative nuclei, sometimes from the 2nd day after inoculation. The curve of vegetative nuclei development reaches the population growth curve in the 4th day and then runs parallely to it up to the 14th day after inoculation when, the first fragmented macronuclei appear. Single fragmented nuclei can be noticed earlier, but never anlagens (Fig. 2 d, e). Δ of divisions 5 days after inoculation for the strain 299 is below 3 whereas in the same time strain 51 passes 3.5 divisions (Table 1). In the latter vegetative macronuclei never reach more than 95%, but autogamy is also very poor, although the cells with fragmented macronuclei are present during the whole logarithmic and stationary phase of growth. The maximum of fragmentation is reached on the 6th day and does not exceed 20%. Anlagens level is always very low.

(3) Development of strain 51 in monobacterized lettuce medium.

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Division rate and population density are similar, but a little lower than in case of growth of the same strain in the axenic SL-medium. However, the shape of the growth curve indicates that the stationary phase starts to decline after the 5th or 6th day (Fig. 1 c, Table 1). The vegetative nuclei number never reaches more than 80%, and there is no distinct interval between two autogamous cycles. Thus, fragmented macronucli accompany the whole phase of growth in a higher percentage than in the case of axenic SL-medium. The new autogamous cycle starts its development very early, (Fig. 2 f).

Duscussion

The shape of the population growth curve, supplying information concerning the division rate, generation time and population density in the given time of the logarithmic and stationary phase of growth, allows to conclude about the course of series of cell cycles during population development, from cell inoculation into the fresh medium to culture harvesting (van Wagtendonk 1975). The shape of the population growth curves should be repeatable as long as all the growth conditions, all which can be foreseen (such as: temperature, pH, light, volume to surface ratio, inoculation conditions and medium composition), are rigorously followed. Moreover, when *Paramecium* mass cultures growth is examined, additional information about the population growth can be supplied by analysis of their life cycle, that is on autogamy development, a sexual process accompanying the development of each population of *Paramecium* (Diller 1936). An easily readable autogamy marker is the appearance in the cultures of cells in which macronuclei assume irregular shapes, or disintegrate into small fragments. Later new macronuclei, anlagens — small, bright stained, regular round shaped nuclei may appear. The percentual distribution of these different morphological forms of vegetative and autogamous macronuclei plotted against the time of culture growth is a good illustration of autogamy development in the culture. The shape of such curves can be also considered as a sensitive indicator of growth conditions.

Both these parameters can be compared as long as uniform criteria of plotting them together on one diagram will be established. In the presented diagrams (Fig. 2 a–f) the zero of each of the macronuclear autogamous stages (full vegetative macronuclei, fragmented macronuclei or anlangens) is marked on the ordinate at the point corresponding to the population density immediately after inoculation and 100% at the

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point corresponding to the maximum of population density attained by this culture.

In mass cultures autogamy develops cyclically. It starts to increase in the second part of the logarithmic phase of growth. Maximal fragmentation of macronuclei is observed simultaneously with the attaining by the culture of maximum population density or a day later when the population starts to decline. Anlagen development progresses a day later. Few of the fragmented nuclei and some anlagens are always noted at the beginning of the logarithmic phase of growth, but they are requisites of the earlier autogamous cycle transferred with inoculum into the new culture. These forms disappear during the first one or two days. Most interesting is the course of the curve presenting development of cells with vegetative macronuclei. Information on this is especially important, when for experimental purposes cells containing vegetative nuclei exclusively should be collected. The vegetative nuclei curve reaches its maximum in one to three days after inoculation, depending on the kind of medium used. Then, it slopes down, intersecting the population curve which still rises. The crossing point of both curves is a stable value in time, in the given culture condition. When cells with exclusively vegetative macronuclei are required, mass cultures should be harvested a day before the curve intersection, and just after it when a high quantity of fragmented nuclei is to be harvested.

Analysis of both these curves allows the choice of the best medium for cultivation of the given strain and the most useful for the given experiment.

Both studied strains reach a range of higher population density (around 10^7 cells/ml) when cultured in the TEM-4T medium, what allows to collect 10 times more packed cells from the same volume of culture medium. Strain 299, grows in this medium much better than strain 51 and its growth is very well reproducible (Fig. 1 a).

Culturing both strains, 299 and 51 in the TEM-4T-medium, one can harvest cells with 98–100% of vegetative nuclei on the 3rd and 4th day, respectively, when population density reaches about 400 cells/ml (Fig. 2 a, b, Table 2).

Both strains cultured in SL-medium never reach more than $2.3 \cdot 10^6$ cells/ml. Their stationary phase is very long, what makes easy their maintenance, (Skoczylas and Van Wagtendonk 1975). During all the time of population growth the development of autogamy is very poor and even in the case of strain 299 a new autogamous cycle does not start before the 14th day after inoculation. This fact may be a consequence of sexual immaturity of the *Paramecium* nuclear apparatus. According to Sonneborn (1954), both the strains need at least 15 successive

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divisions after the last autogamy to attain sexual maturity. These observations were made on strains grown in lettuce monobacterized medium in single cells isolations (Sonneborn 1970). Cells grown in SL axenic medium in mass cultures passed less than 3 divisions in the period between inoculation into fresh medium and attaining of maximum of population density. Likewise, cells transferred with inoculum into a fresh medium were probably far from fulfilling the criterion of maturity established by Sonneborn. However, recent studies suggest that autogamy and macronuclear regeneration, in contrast to conjugation, do not require the attaining of sexual maturity for initiation of the process of autogamy (Butzel 1974). Besides, Miyake (1978) have reported that autogamy initiation may be dependent on the chemical factors applied. The data reported in the present paper indicate that, with almost the same number of divisions passed after inoculation by the cells of strain 51, cultivated in two different media — lettuce monobacterized and SL axenic medium, in the first case autogamy is initiated in one or two days after inoculation and in the second one no autogamy could be detected during first two weeks (Fig. 2 d, f, Table 1). This fact indicates the significance of external enviroment for autogamy initiation and development.

Lettuce extract itself represents a medium poor in nutritional components and, consequently, is not easily subjected to any additional infection, thus making all handling of the cultures rather simple in comparison with the axenic cultures. However, in this medium inoculated with *Klebsiella aerogenes* such monobacterized cultures are biologically unhomogeneous. The stability of the culture growth depends in these conditions on the balance between the protozoan and bacterial cell growth. This deficiency of biological homogeneity excludes application of the lettuce infusion as a culture medium for collection of material for the majority of biochemical experiments. However, it has an advantage over the axenic medium in these experiments, where interaction with environment on the growing cell should be similar to natural conditions. It is generally konwn, for example, that in cells cultured in axenic medium it is difficult to maintain their surface antigen properties.

Examination of the slopes presented in Figs. 1 b, c and 2 d, f indicates that the yield of lettuce medium is similar to that of SL-axenic medium ($2 \cdot 10^6$ cells/ml), but autogamy starts to develop in this medium much earlier. Thus, the intersection of the growth curve with the curve of vegetative macronuclei development takes place between the 2nd and the 3rd day after inoculation and consequently the cells with vegetative macronuclei never reach 100%.

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This fact makes impossible collection from this medium of cells containing more than 80% of vegetative macronuclei, at the any growth stage.

Neither the presented ways of mass culture in the axenic media nor in the lettuce medium allows to collect cells containing more than 30–40% of fragmented macronuclei. This indicates that in the conditions described above neither autogamy nor cell divisions are ever fully synchronous.

ACKNOWLEDGMENTS

We thank Prof. Aleksandra Przełęcka for helpful suggestions during preparation of the manuscript. We also are much indebted to Dr. Anthony Soldo for instructing one of the authors (Dr. B. Skoczylas) in culturing *Paramecium* in axenic medium, during her stay in his Laboratory in Veterans Administration Hospital in Miami.

RÉSUMÉ

Les populations de *Paramecium tetraurelia* et *P. octaurelia*, cultivées sous les conditions rigoureusement standardisées, se caractérisent par la similitude de leur développement. Les proportions du nombre des individus qui ont atteint les différents stades de leur cycles cellulaires ou de leurs cycles de développement sont stabiles et reproduisibles pour tous les moments donnés de l'histoire de la population, comme le démontrent les courbes de croissance et les courbes des pourcentages des stades macronucléaires de l'autogamie. L'étude de ces courbes permet d'établir les meilleures conditions de croissance, ainsi que le temps optimal quand la population doit être récoltée pour satisfaire un besoin expérimental défini.

REFERENCES

Butzel H. M., Jr. 1974: Mating type determination and development in *Paramecium aurelia*. In: *Paramecium* a Current Survey (ed. W. J. van Wagtendonk), Elsevier Scientific Publishing Company, The Netherlands, 91–95.

Diller W. F. 1936: Nuclear reorganization processes in *Paramecium aurelia*, with descriptions of autogamy and "hemixis". J. Morphol., 59, 11–67.

Miyake A. 1978: Cell communication, cell union, and initiation of meiosis in ciliate conjugation. Current Topics in Developmental Biology, 12 37–82.

Skoczylas B. and van Wagtendonk W. J. 1975: A maintenance medium for the axenic culture of *Paramecium aurelia*. Acta Protozool., 14, 379–383.

Soldo A. T., Godoy G. A. and van Wagtendonk W. J. 1966: Growth of particle-bearing and particle-free *Paramecium aurelia* in axenic culture. J. Protozool., 13, 492–497.

Sonneborn T. M. 1950: Methods in the general biology and genetics of *Paramecium aurelia*. J. Exp. Zool., 113, 87–143.

Sonneborn T. M. 1954: The relation of autogamy to senescence and rejuvenescence in *Paramecium aurelia*. J. Protozool., 1, 38–53.

http://rcin.org.pl

S o n n e b o r n T. M. 1970: Methods in *Paramecium* research: In: Methods in Cell Physiology, (ed. D. M. P r e s c o t t), Academic Press, New York London, 241–339.
van W a g t e n d o n k W. J. 1974: Nutrition of *Paramecium*. In: *Paramecium* a Current Survey (ed. W. J. van W a g t e n d o n k), Elsevier Scientific Publishing Company, The Netherlands, 373–376.

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ACTA PROTOZOOLOGICA

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Eimeria garzettae sp. n. *(Coccidia: Eimeriidae)* in the Little Egret (*Egretta garzetta L.*) from *Bulgaria*

Received on 15 October 1979

Synopsis. A new species of coccidia, *Eimeria garzettae* sp. n. is described. The parasite was found in contents and scrapped epithelium of the small intestine of a Little Egret (*Egretta garzetta* L.) from Bulgaria.

Two specimens of Little Egrets (*Egretta garzetta* L.) collected in the vicinity of Sofia were studied in May 1979. Unsporolated oocysts of coccidia were found in the small intestine of one of the egrets. The tracing of the sporolation revealed that they belonged to a species which had not been described in the parasitological literature. In order to trace the sporolation of the oocysts a fraction of the content of the small intestine with a large number of oocysts was stored at room temperature ($t_0 = 22°C \pm 1°C$) in Petri dishes. The intestine content was analysed for oocysts after Fülleborn.

Eimeria garzettae sp. n. Fig. 1 a, b, Pl. I 1, 2, 3, 4, 5

Description: Oocysts (Fig. 1 a, b, Pl. I 1, 2, 3, 4) were ovoid or subspherical. Micropyle distinct 3–3.5 μm in diameter at the tapering front end. Oocyst wall doubled. Exocyst rather polymorphic: in some cases smooth and colourless (Fig. 1 a Pl. I 1, 3) and in most cases sculptured to different extent and with pale brown colour (Fig. 1 b, Pl. 2, 4). Sometimes exogenous elements are attached to the sculptured exocyst with make it semitransparent. Endocyst smooth and looking lake a more dense membrane. The total thickness of the oocyst wall is 1.2–2 μm.

Distinct micropyle at the front end of the oocysts where exocyst and endocyst are narrower. In some cases the unsporolatted oocysts resemble the oocysts of *Eimeria anatis* Scholtyseck from ducks (*Anas platyrhynchos* L.) in the structure of the micropyle. Often a groove or plug-like

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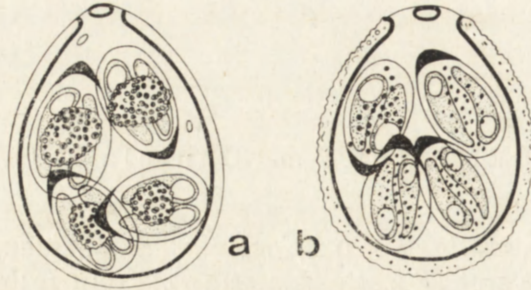

Fig. 1. Sporulated oocysts of *Eimeria garzettae* sp. n., a — oocyst with a smooth outer layer. × 2000, b — oocyst with a sculptured outer layer. × 2000

body about 3 μm in diameter looking like an air bubble is present in the center of the micropyle (PI. I 1, 3, 4, 5).

The dimensions of the oocysts vary between 17.6–21.2 × 14.4–16.2.

The duration of the sporulation of the oocysts at room temperature is 96–120 h. Oocyst residuum in sporulated oocysts absent, 1–2 polar granules present.

Sporocysts pear-shaped, with dimensions 9–11 × 6.5–8. Stieda body absent. Distinct hyaline body at the pointed front end. Sporocyst residuum compact shortly after sporolation and dispersed among the sporozoites in older oocysts. The elongated sporozoites are located longitudinally in the sporocysts and each has a refractile globule located at one of the poles.

Host: Little Egret (*Egretta garzetta* L., Aves: *Ardeidae*).
Location: Oocysts found in contents and scrapped epithelium of the small intestine.
Locality: Marsches near Dolni Bogrov, Sofia, Bulgaria, May, 6, 1979.
Prevalence: Oocysts were found in one of two adult Little Egrets.

Discussion: *E. garzettae* sp. n. is the only coccidian species found in the birds of the genus *Egretta* so far. It is remarkable that the birds of the entire order *Ciconiformes* are very slightly invased with coccidia and according to Pellerdy (1974) only in the Black Ibis (*Pseudibis papilosa*) *Eimeria bazi* Chauhan et Bhatia has been found. *E. garzettae* sp. n. resembles *E. anatis* Scholtyseck found in ducks in the oocyst shape. (Scholtyseck 1955). It differs from the latter by the dimensions of the oocysts, the type of the oocyst wall and the structure of the micropyle. Having in mind the strict species differentiation of the coccidia belonging to the genus *Eimeria* established so far, we conclude that in this case we have a new species parasitizing in the Little Egrets.

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RÉSUMÉ

Un nouveau espèce — *Eimeria garzettae* sp. n. (*Coccidia*: *Eimeriidae*) est décrite. Le parasite a été trouvé dans le contenue intestinal et l'épithelium de l'intestin grêle d'une Aigrette garzette de Bulgarie.

REFERENCES

Pellerdy L. 1974: Coccidia and Coccidiosis. Budapest, Akad. Kiado, 959 p.

Scholtyseck E. 1955: *Eimeria anatis* n. sp., ein neues Coccid aus der Stockende (*Anas platyrhynchos*). Arch. Protistenk., 100, 431–434.

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EXPLANATION OF PLATE I

1, 2: Unsporulated oocysts of *Eimeria garzettae* sp. n. × 1900
3: Sporulated oocyst of *E. garzettae* sp. n. with smooth outer layer. × 2500
4: Sporulated oocyst of *E. garzettae* sp. n. with sculptured outer layer. × 1900
5: View of the micropyle of *E. garzettae* sp. n. × 4000

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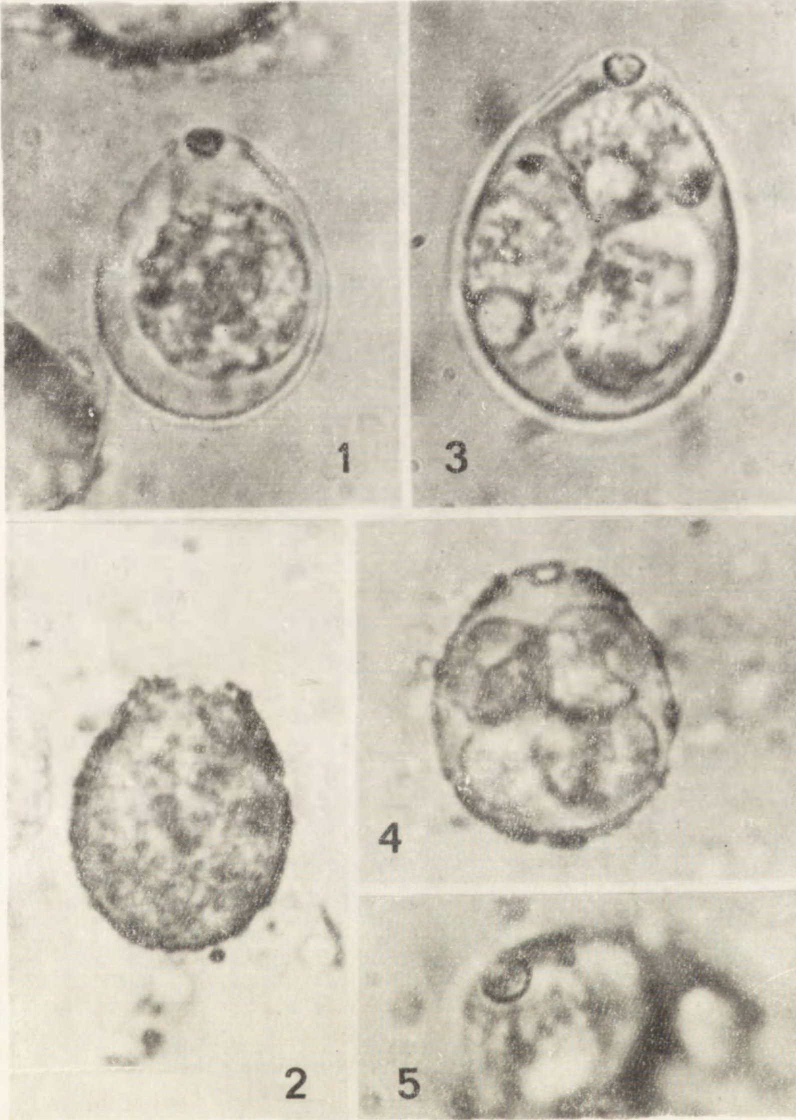

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ACTA PROTOZOOLOGICA

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Observations on *Eimeria glossogobii sp. n.* (*Sporozoa* : *Eimerridae*) from a Fresh Water Teleost Fish

Received on 3 October 1979

Synopsis. The communication records a new species of Coccidia (*Sporozoa: Eimeriidae*), ***Eimeria glossogobii***, from the fresh water teleost fish, ***Glossogobius giuris*** (Hamilton). It has a thin and delicate oocystic wall and the sporulation is completed inside the host gut, characters which necessitate its inclusion under the subgenus *Goussia* Labbé.

Fishes are known to harbour mainly the eimeriid coccidia in their intestines. According to Lom (1971), approximately one hundred coccidian species have been described from piscine hosts. A perusal into the literature reveals that only four out of these have been reported from Indian fishes. They exclusively belong to the genus *Eimeria* Schneider, 1881. It was Halwani who first described *E. southwelli* from the liver and gut of *Aetobatis narinari* in 1930. Later, Setna and Bana (1935) reported *E. harpodoni*, Chakravarty and Kar (1944) noted *E. notopteri* and Mandal and Chakravarty (1965) described *E. zygaenae*, all new species, from *Harpodon nehereus*, *Notopterus notopterus* and *Zygaena blochii* respectively. Mandal (1976) obtained *E. southewelli* from the small intestine of *Scoliodon sorrakowah* and *E. notopteri* from *Notopterus chitala* also.

In course of our studies on the protozoan parasites from edible fishes of this locality we have obtained a coccidium from *Glossogobius giuris* (Hamilton). Its mature oocysts have four sporocysts and inside each of them are two sporozoites, characterizing its inclusion under the genus *Eimeria* Schneider. It is described here as *Eimeria glossogobii* new species on the basis of its structural differences from the other fish coccidia.

Lom (1971) stressed the need for the preservation of the subgenus *Goussia* Labbé for those Coccidia having a delicate and thin oocystic

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membrane whose sporulation are completed inside the intestine of the host fish. Since *Eimeria glossogobii* has features similar to those of the subgenus *Goussia*, we propose to include it under the latter.

Material and Methods

The fishes were collected from the local fish market at Kalyani during our survey for the fish protozoans. They were brought to the laboratory for routine examination. Branchiae, kidney, brain, liver, blood, spleen, gall bladder, urinary bladder and alimentary canal were thoroughly examined for the parasites. Sporulated oocysts of *Eimeria* Schneider were found abundantly in the smears of intestinal contents. Smears of these sporulated oocysts were made on slides and a few drops of normal saline were put on it and they were covered with No "0" cover slips and sealed with wax. Examinations were made at different magnifications of a Carl Zeiss microscope fitted with achromatic objectives. Some oocysts were also preserved in 2.5% solution of pottasium dichromate for future examination. Oocysts were measured with an ocular micrometer and drawings were made with the aid of a camera lucida.

Observations

Eimeria glossogobii sp. n. (Fig. 1 *1–4*)

The oocysts obtained in the intestinal contents are all sporulated. They are faintly yellowish in tinge, spherical in outline with a very thin and delicate smooth oocystic membrane (Fig. 1 *1, 2*). Each oocyst contains four pyriform to oval sporocysts (Fig. 1 *3*), all of which contain two sporozoites. The sporozoites are colourless, elongated and sickle-shaped. A spherical refractile body appears at the broad end. The position of the nucleus cannot be ascertained definitely (Fig. 1 *4*). A distinct knob-like steida body is present at the pointed end of each sporocyst. Neither oocystic residuum nor sporocystic residuum are present.

The endogenous stages of the parasite are being studied in the laboratory and will be described in a future communication.

Diagnosis of *Eimeria glossogobii* sp. n.
Type host: *Glossogobius giuris* (Hamilton)
Locality: Kalyani, West Bengal, India
Site of infection: Alimentary canal
Oocyst: All mature; oocystic membrane very thin and delicate; four pyriform to oval sporocysts each containing two sickle-shaped sporozoites; stieda body present; oocystic and sporocystic residua absent
Measurements (in μm): Figures within parenthesis indicate the average of 20 specimens

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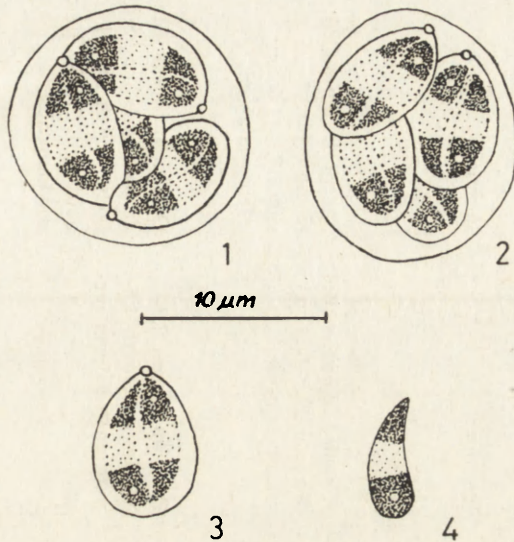

Fig. 1. Camera lucida drawings of *Eimeria glossogobii* sp. n. *1* and *2* — Two mature oocysts, *3* — A sporocyst, *4* — A single sporozoite liberated by mechanical damage of the oocyst and sporocyst

	Length	Breadth
Oocyst	7.1–12.2 (10.0)	7.1–11.0 (10.2)
Sporocyst	3.0–9.1 (7.2)	2.0–5.1 (3.9)

Pathogenicity: Any externally visible pathogenic symptoms are not seen, however, several necrotic patches are observed in the upper part of the small intestine where the oocysts are found in abundance.

Type Material: Holotype and paratypes (collection No. Coc/Bele/78) have been deposited in the Department of Zoology, University of Kalyani, Kalyani, India.

Systematic Position and Discussion

The coccidium from *Glossogobius giuris* undoubtedly belongs to the genus *Eimeria* Schneider because it possesses four sporocysts each with two sporozoites and to the subgenus *Goussia* Labbé since the walls of its oocysts are very thin and its sporulation is completed within the host's alimentary canal. It differs from those described from Indian fishes in the characters of the oocystic wall and the nature of sporulation. In all the species the wall of the oocyst is thick and sporulation is completed outside the hosts' gut. There are variations in the measurements as well as shape index. As such, it has been given a new specific status and is named *Eimeria glossogobii* sp. n. after the name of the

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Table 1

Comparative characters of the five species of Coccidia from Indian fishes

	Eimeria southwelli Halwani	*Eimeria harpodoni* Setna and Bana	*Eimeria notopteri* Chakravarty and Kar	*Eimeria zygaenae* Mandal and Chakravarty	*Eimeria glossogobii* sp. n.
Host/s	*Aetobatis narinari* Muhl and Henle *Scoloidon sorrakowah* Cuvier	*Harpodon nehereus* (Ham. Buch.)	*Notopterus notopterus* (Pallas) *N. chitala* Günther	*Zygaena blochii* Cuvier	*Glossogobius giuris* (Ham.)
Location	Gut, liver and spiral valve of intra-uterine embryo of *A. narinari*; intestine of *S. sorrakowah*	Intestine	Intestine	Intestine	Intestine
Oocyst	Cylindrical or sausage-shaped, colourless; double-walled; length 25.0–53.0 μm and width 10.5–15.4 μm; micropyle absent	Spherical, double-layered wall; transparent, colourless; diameter 12.0–17.3 μm; micropyle absent	Irregular in shape, double-walled; length 23.5–25.2 μm and width 21.4–22.5 μm; no micropyle	Rounded, double-walled; diameter 12.1–14.3 μm; micropyle absent	Spherical, thin-walled; length 7.1–12.2 μm and width 7.1–11.0 μm; micropyle absent
Sporocyst	Oval; length 10.0–12.0 μm and width 6.5 μm; sporocystic residuum absent	Ellipsoidal, protuberance at one end with a broad, inverted V-shaped appendage; length 8.5–10.8 μm and width 3.6–5.7 μm; sporocystic residuum present	Oval, both ends bluntly pointed; length 10.5–11.5 μm and width 5.5–7.3 μm; refractile knob and residual mass absent	Pyriform; length 7.7–8.9 μm and width 4.5–6.6 μm; residuum present	Oval or pyriform with a distinct knob-like "Stieda body" length 3.0–9.1 μm and width 2.0–5.1 μm; residuum absent
Sporozoite	Sausage-shaped with a pointed end; 10.1 μm long; arranged irregulary	Slightly curved with a pointed end; 7.3 μm long	Elongated with a pointed anterior end; 5.5 μm long	Elongated with a pointed anterior end; 6.6 μm long	Elongated, sickle-shaped; 6.6 μm long
Sporulation	48 h	24 to 36 h	48 to 50 h	72 to 80 h	Inside host gut
References	Halwani (1930) Mandal (1976)	Setna and Bana (1935) Mandal (1976)	Chakravarty and Kar (1944) Mandal (1976)	Mandal and Chakravarty (1965) Mandal (1976)	Present study

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host. A comparison of characters of the different piscine coccidian species from India has been made in Table 1.

It is interesting to note that most of the fish Coccidia reported from outside India have delicate oocystic membrane. The sporulation in these cases is completed inside the hosts' alimentary canal too. This is in great contrast to the eimeriids from warm blooded vertebrates. The placement of these piscine Coccidia in the subgenus *Goussia* Labbé would isolate them to some extent from the eimeriids from homoiothermous vertebrates as suggested by Lom (1971). However, the idea of Lom (1971) that thin wall in fish Coccidia is consequent to aquatic habitat where there are least possibilities against mechanical injuries or dessication has been questioned by Davies (1978), since there are marine fish Coccidia with thick walled oocysts and Coccidia of terrestrial vertebrates with thin oocystic wall (Péllerdy 1974). From the evolutionary point of view it may be suggested that the thin walled oocyst in fish coccidians indicates its primitiveness to that of thick walled oocyst of warm blooded vertebrates.

RÉSUMÉ

Une nouvelle espèce de coccidie (*Sporozoa*: *Eimeriidae*), *Eimeria glossogobii,* a été trouvé chez le poisson d'eau douce *Glossogobius giuris* (Hamilton). Elle se distingue par le paroi d'oocyte mince et délicat, et par la sporulation qui se fait entièrement à l'intérieur de l'intestin de l'hôte. Ce caractère la font inclure au sous-genre *Goussia* Labbé.

REFERENCES

Chakravarty M. M. and Kar A. B. 1944: A new coccidian from the intestine of the fish, *Notopterus notopterus* (Pallas). Curr. Sci., 13, 51.

Davies A. J. 1978: Coccidian parasites of intertidal fishes from Wales: Systematics, Development and Cytochemistry. J. Protozool., 25, 15–21.

Halwani A. 1930: On new species of *Eimeria* (*E. southwelli*) from *Aetobatis narinari*. Ann. Trop. Med. Parasit., 24, 1–3.

Lom J. 1971: Remarks on the spore envelopes in fish Coccidia. Folia Parasitol., 18, 289–293.

Mandal A. K. 1976: Coccidia of Indian vertebrates. Rec. Zool. Surv. India, 70, 39–120.

Mandal A. K. and Chakravarty M. M. 1965: A new coccidium, *Eimeria zygaenae* from hamm-headed shark, *Zygaena blochii*. Sci. Cult., 31, 381–382.

Pellerdy L. P. 1974: Coccidia and Coccidiosis. Second Edition. Akademiai Kiado, Budapest.

Setna S. B. and Bana R. H. 1935: *Eimeria harpodoni* sp. n. new coccidium from *Harpodon nehereus* (Ham. and Buch.). J. R Microsc. Soc., 55, 165–169.

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Быстрый метод изготовления постоянных препаратов инфузорий в экспедиционных условиях

A Quick Method of Making Permanent Preparations of Ciliates in Field Conditions

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Синопсис. Быстрый метод изготовления постоянных препаратов основывается на первичной адгезии объекта трет-бутиловым спиртом с одновременным его просветлением и разгонкой фиксатора. Для прокрашивания инфузории покрываются нитроцеллюлозной пленкой и окончательно заключаются в глицерин-желатину. Метод может использоваться для повышения качества видового определения инфузорий в полевых условиях.

В настоящее время предпринимаются все более интенсивные гидробиологические исследования инфузорий различных типов водоемов. К сожалению, во многих случаях видовой состав инфузорий при учетах их численности определяется исключительно на живом материале, что часто является причиной ошибок.

Еще Dragesco (1962) отметил необходимость дополнения прижизненных наблюдений над инфузориями цитологическими данными. В настоящее время существует ряд методов, позволяющих детально изучать цитологические структуры инфузорий. Это методы „сухого" и „мокрого" серебрения (Klein 1926, 1927, Chatton and Lwoff 1930, Corliss 1953, Foissner 1967 et al.) и окраски протарголом (Dragesco 1962, Tuffrau 1964, Wilbert 1976 et al.). Но оба эти метода громоздки и могут быть использованы при обработке проб в лабораторных условиях. Существующие методы адгезии нитроцеллюлозой (Chen 1944, Янковский 1975) предназначены для работы с несколькими инфузориями. Это же относится и к методу, предложенному Nissenbaum (1953). Слишком сложен для полевых условий и метод протаминовой пленки (Marsot and Conillard 1973).

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Сущность метода

Прижизненное изучение инфузорий проводится в висячей капле (Pennak 1953, Ковальчук и Бошко 1979). Для фиксации желательно отловить новые особи, так как метил- или оксипропилцеллюлоза, используемые в растворах для затормаживания простейших, плохо поддаются разгонке. Если исследуемые формы относятся к редким, то следует заменить тормозной раствор в капле, отсасывая его микропипеткой, на воду и только после этого приступить к фиксации инфузорий

Фиксация. Инфузории фиксируются по Шампи или 2–5 мин. в смеси следующего состава: к 5–10 ml 2% осмиевой кислоты прибавляется несколько капель 12–13% хромовой кислоты. В отличие от жидкости Шампи эта смесь может храниться длительное время не разлагаясь. Для более крупных инфузорий пригодны и другие фиксаторы (e. g.. Буэна). Фиксация проводится при увеличениях в 32–56 раз под стереомикроскопом. Фиксатор добавляется стеклянной палочкой, причем мелкие формы в процессе обработки желательно постоянно держать в поле зрения.

Первичная адгезия. Осторожно отсасываем фиксатор микропипеткой или фильтровальной бумагой, оставляя слой, превышающий толщину инфузории не более чем в 1,5 раза. Приближая к объекту стеклянную палочку, предварительно опущенную в смесь трет-бутилового спирта с ледяной уксусной кислотой (10:4), осторожно разгоняем фиксатор, после чего каплю смеси капаем непосредственно на инфузорию, которая приклеивается и обесцвечивается.

Заливка в нитроцеллюлозу. Не допуская подсыхания препарата, опускаем непосредственно на приклеенную инфузорию небольшую каплю 0,5% раствора нитроцеллюлозы в абсолютном спирте с эфиром (1:1). Через несколько секунд подсушиваем покровное стекло с образовавшейся пленкой в 70% спирту.

Окраска. Наиболее удобными для работы в полевых условиях являются красители на основе железного гематоксилина. Препараты следует несколько перекрашивать, так как при последующем заключении в глицерин-желатину они несколько просветляются. Можно пользоваться и любыми другими методами окраски.

Промывка. На протяжении 5–10 мин. желательно сменить несколько порций воды.

Заключение в глицерин-желатину. Проводится непосредственно после промывки. Если есть возможность прогревать глицерин-желатину, в которую следует добавить бактерицидное вещество, то можно пользоваться пропис-

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ной пропорцией приготовления. Если такой возможности нет, то следует развести среду до жидкого состояния водой и после заключения инфузории оставить препарат для подсыхания на сутки. После подсыхания покровное стекло стабилизируется по краям жидким канадским бальзамом и эпоксидной смолой.

Эта методика была разработана и использована нами в 1978–79 гг. при изучении бентосных инфузорий Киевского и Кременчугского водохранилищ. С успехом применялась она и нашими коллегами из институтов Зоологии и Гидробиологии АН УССР при изучении эктокомменсальных, паразитических и свободноживущих инфузорий.

SUMMARY

The proposed method consists of the following operations:

(1) Fixation. In Champy's fluid or in 2% osmic acid with a few drops of 12–13% chromic acid.

(2) The initial adhesion. After the fixator being sucked out with a micropipette put a drop of the third-buthyl alcohol with an ice acetic acid (10 : 4) on the ciliate.

(3) Putting into nitrocellulose. Immediately a drop of 0.5 solution of nitrocellulose in absolute alcohol with ether (1 : 1) should be put on the object. After a few seconds put the cover glass into 70% alcohol where it should be within 2–3 min.

(4) Staining. Any method. The simplest are on the base of iron hematoxyline.

(5) Washing. During 5–10 min 2–3 portions of water are changed.

(6) Final mounting in glycerine-gelatin.

A quick method of fixation and preparation of whole mounts of ciliates is proposed. Due to its simplicity the method is recommended to be used in field conditions.

ЛИТЕРАТУРА

Chatton E. et Lwoff A. 1930: Impregnation par diffusion argentique de l'infraciliature des Cilies marins et d'eau douce, apres fixation cytologique et sans dessication. C. r. Seances. Soc. Biol., Paris, 104, 834–836.

Chen T. T. 1944: Staining nuclei and chromosomes in *Protozoa*. Stain Technol., 19, 83–90.

Corliss J. O. 1953: Silver impregnation of ciliated *Protozoa* by the Chatton-Lwoff technic. Stain Technol., 28, 2, 97–100.

Dragesco J. 1962: L'orientation actuelle de la systematique des cilies et la technique d'impregnation au proteinate d'argent. Bull. microsc. appl., 12, 2, 49–58.

Foissner W. 1967: Wimpertiere im Silberpräparat. Ein verbessertes "trockenes" Verfahren zur Darstellung des Silberliniensystems. Mikrokosmos, 56, 122–126.

Янковский А. В. 1975: Новый метод приготовления глицериновых препаратов. Вест. зоол., 3, 80—81.

Klein B. 1926: Ergebnisse mit einer Silbermethode bei Ciliaten. Arch. Protistenk., 56, 243—279.

Klein B. 1927: Die Silberliniensysteme der Ciliaten. Ihr Verhalten während Teilung und Conjugation, neue Silberbilder, Nachtrage. Arch. Protistenkd., 58, 55–142.

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Ковальчук А. А. и Бошко Е. Г. 1979: Об использовании оксипропилцеллюлозы для затормаживания движения простейших. Вест. зоол., 2, 62.
Marsot P. and Conillard P. 1973: The use of protamine coated slides for immobilising protozoa. J. Protozool., 20, 1, 105–106.
Nissenbaum G. 1953: A combine method for the rapid fixation and adhesion of ciliates and flagellates. Science, 118, 3053, 31—32.
Pennak R. W. 1953: Fresh-water invertebrates of the United States. New-York.
Tuffrau M. 1964: Quelques variantes techniques de l'impregnation des Cilies par le proteinate d'argent. Arch. Zool. Exp. Gen., 104, 2, 186—190.
Wilbert N. 1976: A Standardized Method for Identifying and Counting the Vagile and Sessile Periphyton. Oecologia, 24, 343—347.

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