ACTA PROTOZOOLOGICA

NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY
WARSAW, POLAND

1991

VOLUME 30 NUMBER 2
ISSN 0065-1583

Polish Academy of Sciences
Nencki Institute of Experimental Biology

ACTA PROTOZOOLOGICA

International Journal on Protistology

ACTA PROTOZOOLOGICA appears quarterly.
Indexed in Current Contents in CABS and in Protozoological Abstracts.

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 63-71

Phototaxis, Gravitaxis and Vertical Migrations in the Marine Dinoflagellates, *Peridinium faeroense* and *Amphidinium caterea*

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Summary. Phototaxis and gravitaxis were studied in the marine dinoflagellates *Amphidinium caterea* and *Peridinium faeroense* at several culture ages. *P. faeroense* showed a positive gravitaxis and *A. caterea* a negative one, the precision of which varied in dependence of the time of the day. While no obvious phototaxis could be observed in *A. caterea*, *P. faeroense* showed a pronounced positive phototaxis at low and intermediate fluence rates and a diaphototaxis (perpendicular to the light beam) at high fluence rates. As a consequence of the orientation mechanisms *P. faeroense* exhibited vertical migrations in a 3-m water column and was found near the surface in the late morning hours and late afternoon and were more randomly distributed during the rest of the time. In contrast, *A. caterea* failed to show vertical migrations.

Key words. Marine dinoflagellates, phototaxis, gravitaxis.

INTRODUCTION

The marine phytoplankton represents the biggest ecosystem on the earth; it has been estimated to be responsible for more biomass production than all terrestrial ecosystems taken together (Häder et al. 1989). Consequently, these photosynthetic organisms are also responsible for the incorporation of about two thirds of the atmospheric carbon dioxid and production of the same amount of atmospheric oxygen (Round 1981, Ryther 1969).

Dinoflagellates are one of the most productive taxonomic groups both in freshwater and marine phytoplankton communities. Like many other motile microorganisms, these flagellates orient within the water column and move into and stay in zones of suitable light conditions. The orientation patterns (and possibly an endogenous component) result in pronounced daily vertical migrations in the water column (Yentsch et al. 1964, Taylor et al. 1966, Tyler and Seliger 1978, 1981). Several species have been observed to move to the surface during daytime and to lower layers at night (Estrada et al. 1987, Holmes et al. 1967).

Most species, however, are not adapted to the high irradiances of unfiltered sunlight close to the surface. When exposed to unattenuated solar radiation the pigments are photobleached in many photosynthetic microorganisms (Ekelund and Häder 1988, Häder et al. 1988) and photosynthesis is inhibited (Nultsch and Agel 1986). Therefore it is not amazing that they avoid the top layer of a few decimeters which attenuates the detrimetal high illuminances (Hasel 1950, Talling 1971). In addition to visible light, the ultraviolet component of solar radiation has been found to be inhibitory for many microorganisms (Häder 1986, 1987, 1988). The percentage of motile cells and their swimming velocity is affected by both solar and artificial ultraviolet radiation in a number of fresh-

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water and marine flagellates (Häder and Häder 1988a,b, 1989a,b,c). Furthermore, both phototaxis (orientation of the cell with respect to the light direction) and gravitaxis (orientation in the earth's gravity field) are affected by ultraviolet radiation (Häder and Liu 1990a,b). Action spectroscopy has shown that specifically ultraviolet radiation in the wavelength band between 280 nm and 320 nm (UV-B) is effective. It is this UV band, in which the highest increase in solar radiation is expected due to a partial destruction of the stratospheric ozone layer caused by manmade chlorinated fluorocarbons. Both the inhibition of motility and orientation of the cells might have far reaching effects on the survival of the populations of phytoplankton.

The aim of this paper is to study daily vertical migrations of the marine dinoflagellate, *Peridinium faeroense*, in a 3-m water column and to correlate this behavior with photo- and graviorientation of the organisms.

MATERIALS AND METHODS

Organisms and their culture

The dinoflagellates *Peridinium faeroense* (Paulsen) and *Amphidinium caterea* (Hulbert) were grown in 5 or 10 l glass containers without additional gassing in natural sea water pumped from the North Sea near Helgoland which was used after coarse and fine filtration and sterilization. A number of minerals and vitamins were added (Guillard and Ryther 1962). The organisms were cultivated at 18° C and at an irradiance of 1000 lx from fluorescence lamps in a light (6 a.m. to 10 p.m.)/dark cycle.

Determination of vertical movements

The vertical movements of the organisms were monitored in a Plexiglas column with an inner diameter of 70 mm and a total length of 3 m which had 18 evenly spaced outlets along the length of the column starting 20 mm above the bottom. Each outlet was connected with a 4 m long silicon hose (1 mm inner diameter) to a peristaltic pump (STA, Desaga, Heidelberg, FRG) which could handle 18 samples in parallel (1.3 ml each) drawn at regular time intervals (Häder and Griebenow 1988). The Plexiglas column was surrounded by an opaque outer column (diameter 400 mm) in which sea water circulated to minimize heating and the generation of a thermal gradient within the inner column during irradiation. If there were convection currents they were negligible compared to the velocity of the swimming cells. Each experiment was conducted for two days and repeated a few times.

The samples were stored in test tubes, and the cell densities were determined using the image analysis system described below using algorithms to calculate the number and areas of the organisms in the field of view (Häder and Griebenow 1987). Each measurement was repeated at least three times in order to increase the statistical significance. The column was irradiated from above using a 250 W slide projector (Prado, Leitz, Wetzlar, FRG) equipped with a 24 V quartz halogen bulb and an infrared cut-off filter (KG 2, Schott & Gen., Mainz, FRG) via a mirror. The light intensity distribution within the column was measured with a waterproof silicon diode (OSI-5K, Centronic Ltd, Croydon, U.K.). The oxygen concentration along the water column was determined with an oxygen electrode (EOT 196-4, Wiss. Techn. Werke, Weilheim, FRG), which also allowed to determine the temperature. Both values were monitored at the bottom of the column during the measurements, and the oxygen and temperature gradient was measured which had established within the column at the end of the experiment. A chi square test was used to detect deviations from a random distribution of the cells in the column.

Measurement of photo- and graviorientation

The movements of the organisms were tracked with an automatic image analysis system capable of handling up to 200 flagellates in real time (Häder and Vogel 1991). The cell suspension was filled into a sealed glass cuvette (40 x 40 x 0.17 mm^3 inner dimensions), located on the stage of a light microscope (Zeiss Standard, Oberkochen FRG). Objective magnification was 2.5 x for *Peridinium faeroense* and 6.3 x for *Amphidinium caterea*. Phototaxis was determined in a horizontal cuvette and gravitaxis was measured in a vertical cuvette (microscope 90° tilted). The image of the moving cells was recorded by a CCD b/w camera (LHD 0600, Philips, Netherlands) mounted on top of the microscope in dark field to improve the contrast. An infrared cut-off filter (RG 695, Schott & Gen., Mainz, FRG) was mounted in front of the light source to avoid disturbing the cells by the monitoring light. The CCD video image was digitized by a Matrox (PIP 1024, Quebec, Canada) card at a frequency of 50 half frames per second with a spatial resolution of 512 x 512 pixels at 256 possible grey levels. The digitizer card was plugged into an IBM AT compatible microcomputer (Tatung CS 8000, Taipei, Taiwan).

In order to follow the cells' movements the outline and position of each organism was determined in four consecutive images taken at 80 ms intervals each. The main program was written in the computer language C while fast input/output routines were developed in assembler. The chain code algorithm was employed to differentiate the organisms from the image background (Freeman 1961, 1974, 1980). The movement vectors of all tracked cells (deviation angles from the stimulus direction - light or gravity defined as 0°) were stored for subsequent mathematical and statistical analysis in a disk file. The velocity of the organisms was calculated from the distances the organisms had moved during the measurement interval. The precision of orientation was calculated using the Rayleigh test (Batschelet 1965, 1981, Mardia 1972) which yields a value between 0 (random orientation) and 1 (precise orientation of all cells in one direction). Circular histgrams were constructed by binning the angular deviations in 64 sectors. In addition, a quadrant summation was used for bimodal orientations, for which the Rayleigh test is not defined: the percentage of cells moving in the lateral quadrants is compared with those moving upwards or downwards. Phototaxis was induced by an actinic light beam produced from a slide projector described above. The intensity of the actinic light was determined with a light meter (Li-185A Licor, Lincoln, USA) which could be equipped with either an Li-210S or an Li-190S sensor head.

RESULTS

Gravitaxis measurements

Peridinium faeroense showed a precise positive gravitaxis (downward) when observed in vertical cuvette (Fig. 1a). Especially older cultures oriented with a high precision. In contrast, *Amphidinium caterea* showed a negative gravitaxis (moving upward) with slightly less precision (Fig. 1b). When samples were taken at 2-h intervals over a whole day the precision of orientation was found to reach a maximum between 4 p.m. and 10 p.m. in *Peridinium faeroense*. This behavior was obvious in all three age groups studied (Fig. 2). When, in contrast, one sample was confined to a closed cuvette and the gravitactic behavior was monitored over a prolonged period of time, the sense of orientation inversed within 1 h and the precision gradually increased with time (Fig. 3). The diurnal variation in the precision of negative gravitaxis is not so obvious in *Amphidinium caterea* (Fig. 4). Also the degree of gravitactic orientation did not alter remarkably, when

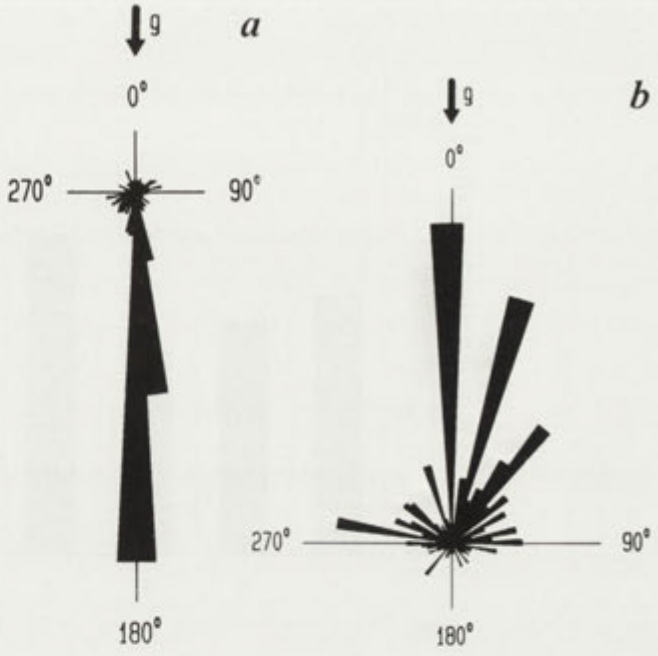


Fig. 1. Circular histograms of (a) positive gravitaxis in *Peridinium faeroense* and (b) negative gravitaxis in *Amphidinium caterea* measured in a vertical cuvette

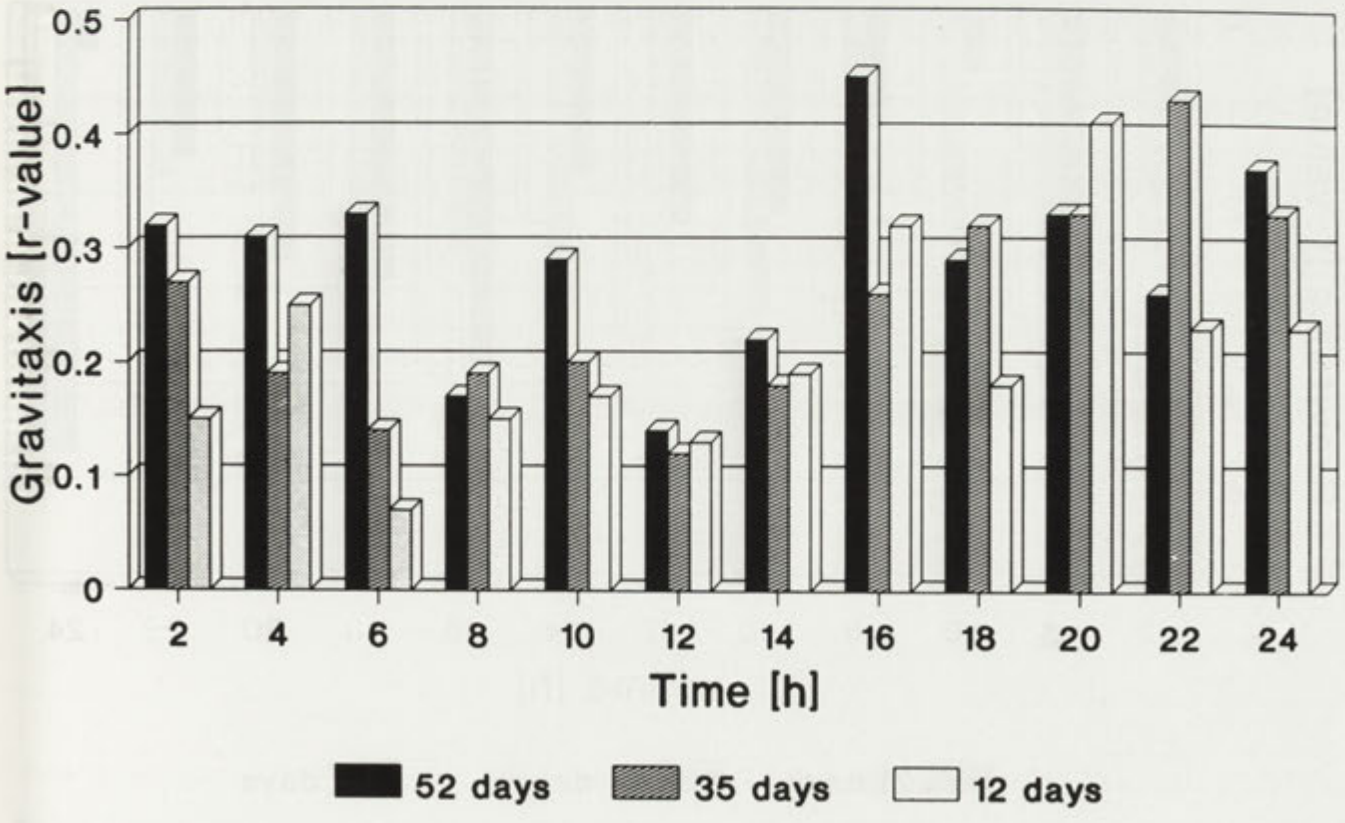


Fig. 2. Precision of positive gravitaxis in *Peridinium faeroense* in dependence of the time of day at three different culture ages

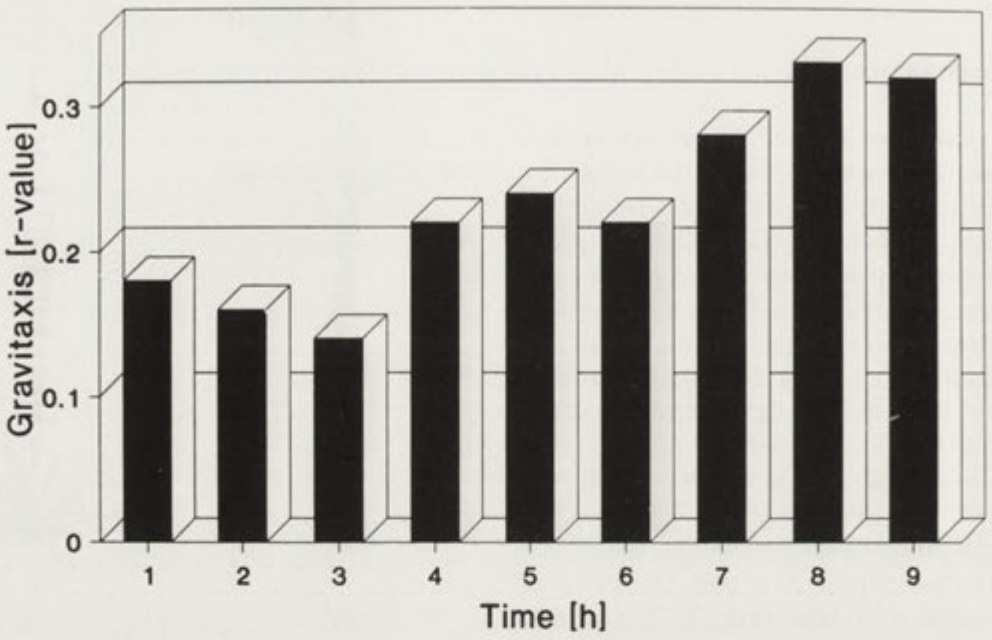


Fig. 3. Degree of positive gravitactic orientation in *Peridinium faeroense* in dependence of the time the organisms were confined to a closed cuvette

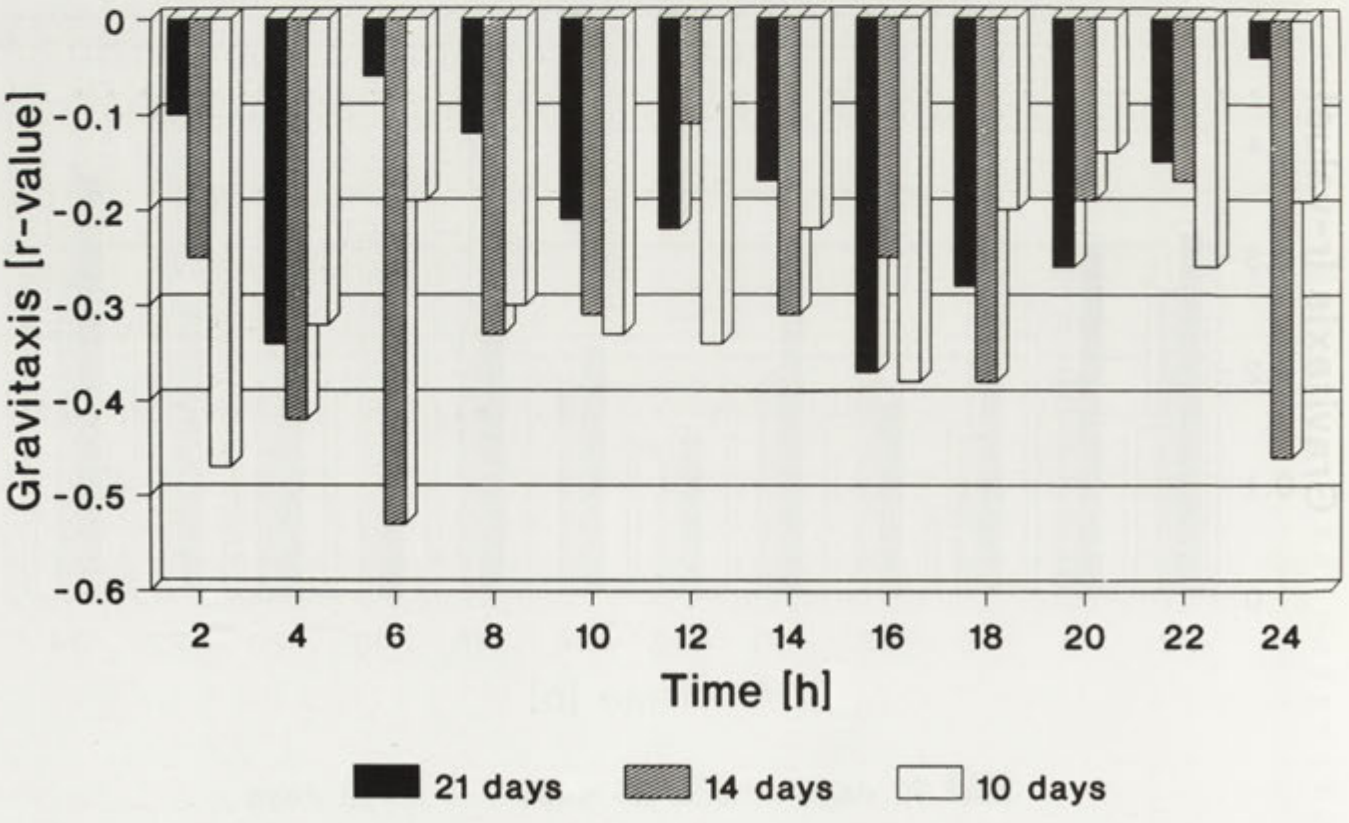
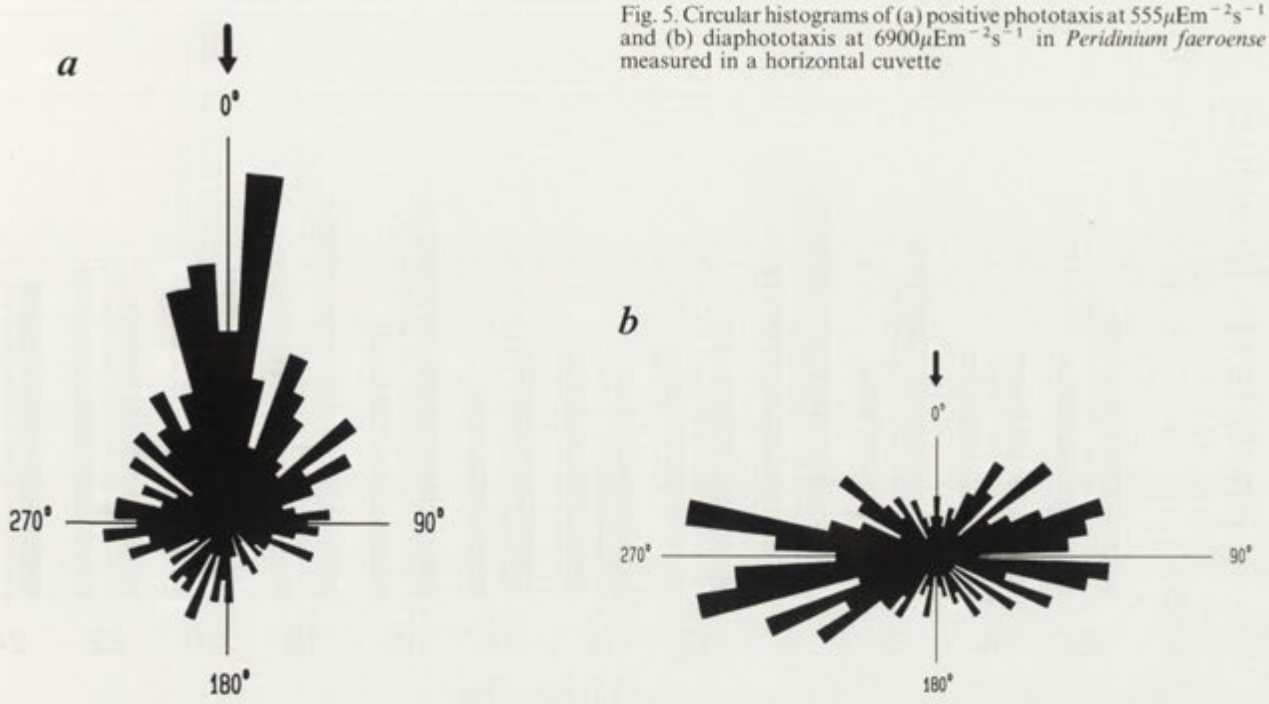


Fig. 4. Precision of positive gravitaxis in *Amphidinium caterea* in dependence of the time of day at three different culture ages

Fig. 5. Circular histograms of (a) positive phototaxis at 555μEm^{-2}s^{-1} and (b) diaphototaxis at 6900μEm^{-2}s^{-1} in *Peridinium faeroense* measured in a horizontal cuvette



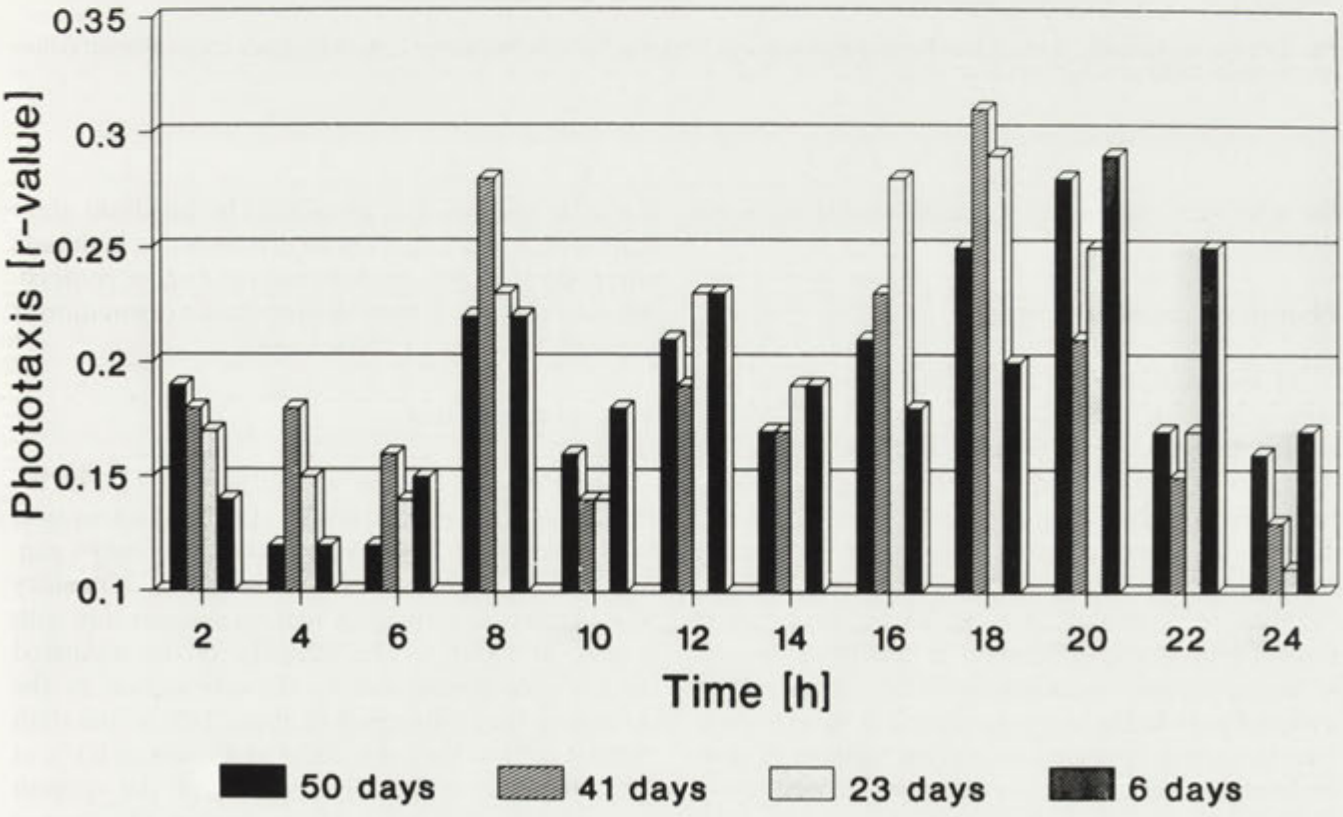


Fig. 6. Precision of positive phototaxis in *Peridinium faeroense* at 555 μE m^{-2}s^{-1} in dependence of the time of day at four different culture ages as quantified by the Rayleigh test

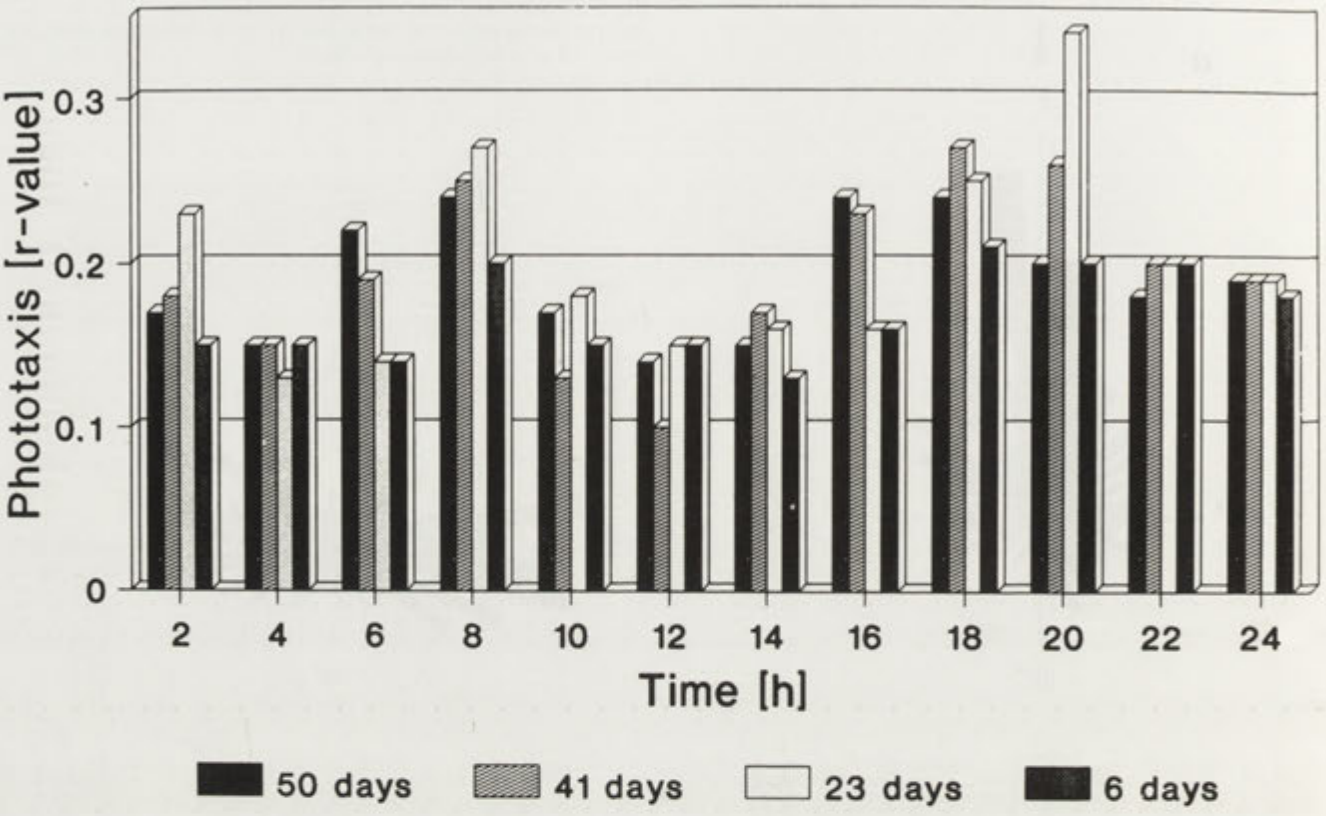


Fig. 7. Precision of positive phototaxis in *Peridinium faeroense* at 1750 $\mu Em^{-2}s^{-1}$ in dependence of the time of day at four different culture ages as quantified by the Rayleigh test

the cells were confined to a sealed cuvette (data not shown).

Phototaxis measurements

At low and intermediate fluence rates *Peridinium faeroense* showed a positive phototaxis (Fig. 5a) while at high fluence rates the cells showed a diaphototaxis: the cells oriented perpendicular to the impinging light beam (Fig 5b). The slight left/right asymmetry in this diagram is not statistically meaningful as seen by comparison with other data sets. When monitored at 555$\mu Em^{-2}s^{-1}$ over a 24 h period the precision of positive phototactic orientation in *Peridinium faeroense* had an obvious maximum in the late afternoon and a second peak in the early morning (Fig. 6) which was found in young, intermediate and old cultures. A similar behavior was seen at intermediate fluence rates of 1750$\mu Em^{-2}s^{-1}$ (Fig. 7). At high fluence rates (6900$\mu Em^{-2}s^{-1}$) the organisms showed an obvious diaphototaxis, so that the quantification using the Rayleigh test was not possible. The quadrant summation indicates a high degree of orientation in the late afternoon (Fig. 8). *Amphidinium caterea*, in contrast, did not show any detectable phototactic orientation at any light intensity or culture age.

Vertical migrations

The Plexiglas column was filled with 11 l of the *Peridinium faeroense* suspension at night at a density of 8100 cells per ml. It was kept in darkness until 4 a.m. when the first samples were drawn. The light intensity was adjusted to simulate a natural summer day with a peak at 13.30 h. The intensity of the unfiltered radiation decreased due to the attenuation in the column so that it dropped to about 10% at the sixth outlet, to 1% at the tenth outlet and to about 0.1% at the thirteenth outlet. The bottom of the column received less than 10^{-6} of the surface illuminance (50 klx = 1750$\mu Em^{-2}s^{-1}$). The temperature was constantly measured throughout the experiment and

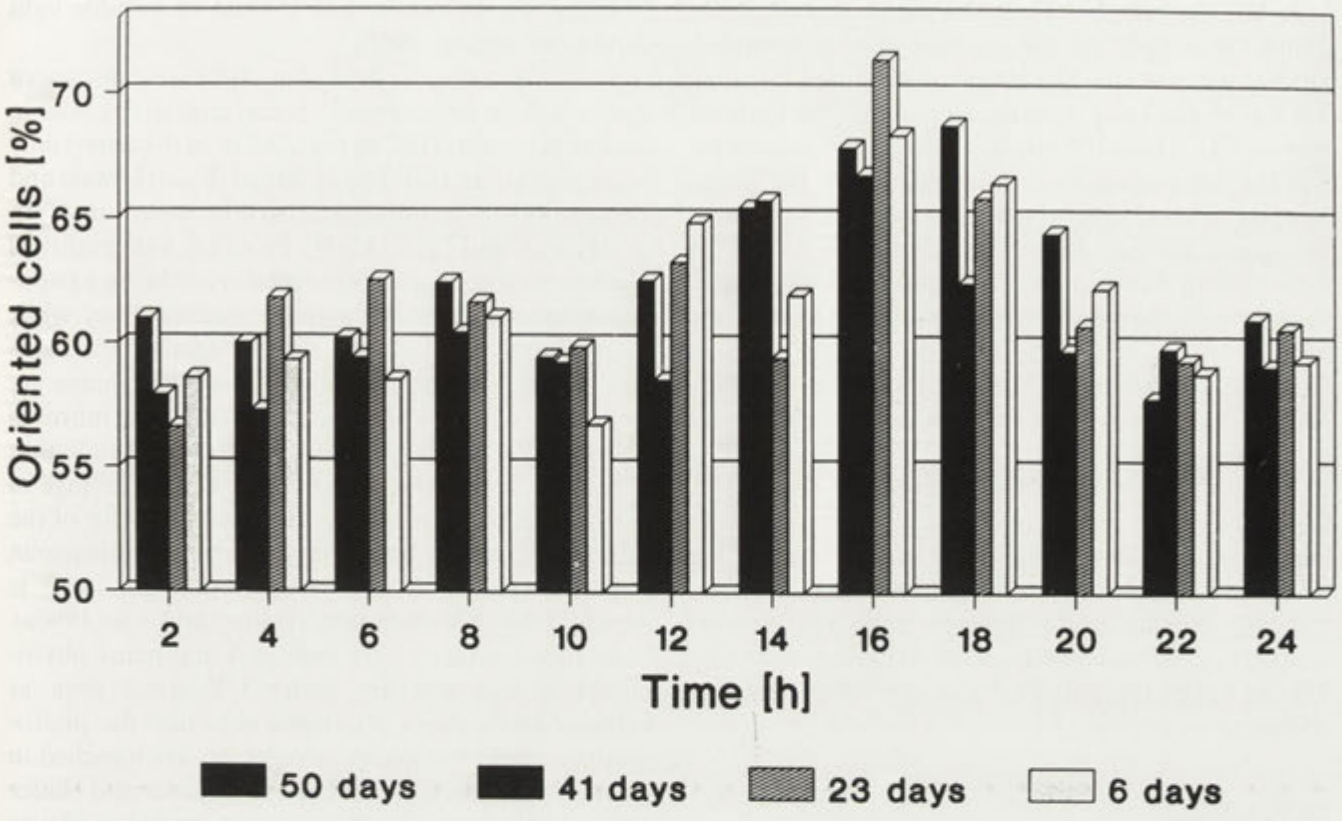


Fig. 8. Precision of diaphototaxis in *Peridinium faeroense* at 6900 $\mu Em^{-2}s^{-1}$ in dependence of the time of day at four different culture ages indicated by the percentage of cells moving in the lateral quadrants ($90° \pm 45°$ and $270° \pm 45°$) of the total population

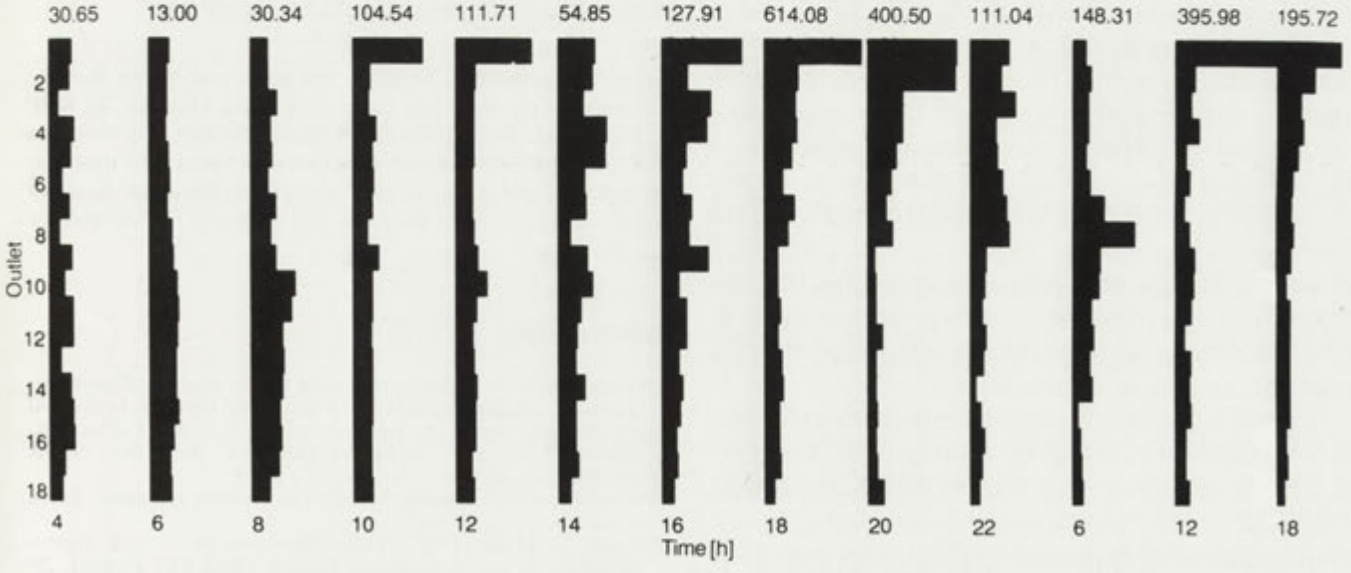


Fig. 9. Cell densities of *Peridinium faeroense* measured in samples taken from the 18 outlets of the vertical column between 4 a.m. and 10 p.m. on two consecutive days. The chi square values for each set of data are indicated above each density histogram

after the last sample was drawn the thermal gradient along the length of the column was determined. During the exposure the temperature varied less than 0.8°C over the 2 day monitoring and also the gradient was < 1°C. The cell densities for each outlet (number 1 is the top outlet) were determined with the image analysis system and plotted in the form of linear histograms for each time (Fig. 9). In order to test for non-random distributions a chi square test was performed for each histogram (numbers indicated over the histograms). A random distribution (using 17 degrees of freedom according to the 18 samples) on the 99% probability level is represented when the chi square value falls below 33.41.

While the cells are initially randomly distributed, they move to the surface in the late morning hours. In the early afternoon they move downward in the column and return to the surface in the later afternoon and early evening. In *Amphidinium caterea* no obvious vertical migrations could be detected (data not shown). The chi square test indicated a random distribution at all times.

DISCUSSION

Both positive and negative phototaxis have been found in several dinoflagellates (Forward 1976, Ekelund and Häder 1988, Liu et al. 1990). Furthermore, at high fluence rates *Peridinium gatunense* had been found to show a pronounced orientation perpendicular to the light beam (diaphototaxis) which was also found in *Peridinium faeroense* during this study. Taking into account only phototaxis the cells would be expected to start moving upward after sunrise. The diaphototaxis would tend to disperse the cells during exposure to high fluence rates and in the afternoon a second movement to the surface would be expected when the light intensity decreases, which has actually been observed. Thermal gradients had probably no effect on the vertical migrations because they were too small. In addition, preliminary experiments using larger thermal gradients caused no thermotaxis.

Similar vertical movements have been observed also in field studies in a number of dinoflagellates (Eppley et al. 1968, Levandowsky and Kaneta 1987, Kamykowski 1981). However, the downward movement in the evening cannot be explained by phototaxis, but rather by positive gravitaxis. In contrast, the green flagellate *Euglena gracilis* shows a negative gravitaxis which operates antagonistically with the negative phototaxis to bring the organisms into a band of suitable light conditions (Häder 1987).

It is interesting to note that *Amphidinium caterea* does not show pronounced vertical migrations. Also in tank experiments (10.7 m high, 3.7 m in diameter) only weak migrations could be observed (Kamykowski and Zentara 1977): the cells meandered between 1 m depth (at daytime) and 2 m (at night). Probably antagonistical orientation mechanisms are necessary to bring a population into a band of suitable fluence rate, while *Amphidinium caterea* only shows negative gravitaxis and no pronounced phototaxis. Since *Peridinium* accumulates close to the surface in the late morning hours, the cells are exposed to the ultraviolet radiation of the sun. In *Peridinium gatunense* the percentage of motile organisms as well as the linear velocity of the cells are affected by both solar and artificial ultraviolet radiation (Häder et al. 1990a,b). Also gravitaxis is impaired by UV radiation (Häder and Liu 1990a). Field measurements have indicated that many phytoplankton organims are under UV stress even at ambient levels. After prolonged exposure the photosynthetic and photoreceptor pigments are bleached in several flagellates (Häder et al. 1990, Häder and Häder 1989, 1990), and the fluorescence emission shows dramatic changes; also the photosynthetic oxygen production declines drastically (unpublished data). Thus, any substantial increase in the solar UV-B radiation due to a further decrease in the ozone layer caused by the emission of chlorinated fluorocarbons could have marked effects on the growth and survival of dinoflagellate phytoplankton organisms.

Acknowledgements. This work was supported by the Bundesminister für Forschung und Technologie under Contract No. KBF 57. The authors thank C. Bäz-Oberhäuser, J. Schäfer, U. Schleier and H. Vieten for skillful technical assistance. We owe specific thanks to the technical and scientific personnel of the Biological Research Station in Helgoland for their help and especially E. Hagmeier for valuable support.

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Received on 5th February, 1991

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 73-77

Influence of Light of Different Colors on Motile Behavior and Cytoplasmic Streaming in *Amoeba proteus*.

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Summary. Responses of the colorless protozoan *Amoeba proteus* to a sudden increase in light intensity of different wavelengths are described. Antagonistic action of blue and orange-red radiation on the cytoplasmic streaming and locomotion has been examined by a prolonged observation of amoebae stimulated with high photon fluence rate of $314x10^{18}$ photons $m^{-2}s^{-1}$ (intensity 109 W m^{-2}), one second orange light pulses and by a prolonged irradiation (orange light) with medium photon fluence rate of 63x1018 photons $m^{-2}s^{-1}$ (intensity 20-30 W m^{-2}). The presented results reveal: (1) a very weak sensitivity of *Amoeba proteus* to orange-red light, (2) the same mode of action of both red and blue light, which supports the involvement of only one photoresponsse mechanism, (3) the photophobic reaction of *A. proteus* is a two-stage reaction and consists of: (a) initial photophobic reaction - a transient solation and acceleration of cytoplasmic streaming, (b) specific photophobic reaction a transient cessation of both cytoplasmic streaming and cell motility.

Key words. *Amoeba proteus*, blue light, red light, photomechanical transduction.

INTRODUCTION

Amoeba proteus is a colorless, carnivorous amoeba, which responds to a sudden increase in light intensity with a transient cessation of both cytoplasmic streaming and locomotion (it is a step-up photophobic response) and to lateral light illumination with a negative phototactic reaction.

Harrington and Leaming (1900) were the first who did the initial investigations on the effect of different wavelengths upon amoeboid movement of *Amoeba proteus*. They found that a sudden increase in intensity causes an immediate cessation of cytoplasmic streaming either in white or blue light. Irradiation with green, yellow and red, after white or blue, accelerated the recovery of locomotor activity and caused an increase in the rate of streaming and movement. The authors also claim "that red light is the most powerful excitant to flow as is indicated by the shorter latent period after quiescence in white light". The results they presented were seriously questioned because of methodological deficiencies. They used broad, Bierstad-colored, celluloid plates with unknown spectral properties and they did not determine the intensity of the stimulating light.

The experiments were repeated by Mast (1910). She used spectroscopically tested, color filters which were, however, faintly transparent in the violet and blue ranges. The data she obtained showed that "The blue (430-490 nm) is nearly as efficient in causing reactions of *Amoeba proteus* as white light. Violet, green, yellow and red are only very slightly active". In the second set of experiments, Mast (1911) used a glass prism to obtain purer hues. It allowed to cut off the interming-

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ling of rays in distant parts of the spectrum. The results she obtained agreed in general with her previous conclusion. The blue region had a very marked effect on the rate of movement of *Amoeba proteus*, while the violet and green hues had only a slight effect. Contrary to the results presented by Harrington and Leaming (1900), that red light has a stimulating effect on the streaming and movement of amoebae, Mast (1911) found that orange and red had a rather slight effect on movement.

The latter observation was indirectly revised by Mast and Stahler (1937). The authors supported the observation made by Harrington and Leaming (1900). They found the gelating effect of short wavelengths on the cytoplasm of *Amoeba proteus*, which leads to a decrease in velocity of streaming and movement. On the other hand, they observed the solating effect of longer wavelengths which results in the acceleration of movement. These earlier results were re-evaluated by Hitchcock (1961) under conditions of constant radiant energy level of all wavelengths used. As a criterion of relative hue effectiveness, he used the duration of streaming suppression after light stimulation. The authors found, "The longest mean suppression was obtained under illumination of 515 nm, in contrast to the 450-490 nm range previously declared most effective". He also found the effective region of stimulating light ranged from 420 to 600 nm.

The discrepancy of the results presented above was initially tested by Łazowski and Kuźnicki (1984) and Łazowski (1987a). They found that the blue light in the range 440-482 nm has a very distinct effect on the streaming and gelation of plasmasol with maximum efficiency around 450 nm, while light in the 511-625 nm range has only a slight effect.

The studies presented in this paper were conducted in order to analyze the photoresponses of *A. proteus* induced by light of different wavelengths and to find out whether these photoresponses are mutually related.

MATERIALS AND METHODS

Amoeba proteus strain "C" grew in a discontinuous culture in 0.3 l glass Petri dishes containing 0.2 l of a medium (Łazowski and Kuźnicki 1985). The culture was kept at 21°C in a very dim light (5 lx) during daytime and in darkness during night. Well attached and actively motile cells (velocity of uroid propagation $> 2\ \mu m\ s^{-2}$) were harvested by pipette and transferred to an experimental chamber. Amoebae were observed under low power magnification (microscope Biolar, PZO or Standard 14, Zeiss). The monitoring beam was produced from the internal microscope illuminator, equipped with red R241 (Carl Zeiss, Jena) and CuSO4 aq. heat filters. In the monitoring light beam an additional blue light cut-off filter was inserted (4-Nitroso-N, N-dimethylanilin water solution). In both systems red, cut- off filters were mounted above the specimen. The filters attenuate and/or cut off the intensity of the stimulating light beam passing through the viewing system of the microscope. It allows to monitor the behavior of amoebae during and after stimulation.

Photophobic reactions were evoked by impulse (1 second) or continuous irradiation by means of the microbeam system (Łazowski and Kuźnicki 1984); only the fragments of a leading pseudopod were irradiated. The experiments were repeated using a double-beam microscope system (Łazowski 1987b), which allows irradiation of a whole cell. The actinic light was produced from a 100 W/12 V halogen quartz lamp and measured with a calibrated photodiode BPW 32 (Siemens). Monochromatic light was obtained by means of a metal or dielectric interference filter (Carl Zeiss, Jena) with half-band widths of about 10 nm additionally coupled with color glass filters of spectral properties suitable to cut off the range, residual transmission.

Photophobic responses were observed microscopically either in real time or on a video monitor. A response was considered as valid only when an active pseudopod (microbeam stimulation) or an amoeba (whole specimen stimulation by means of the double beam system) had stopped within 5 or 10 seconds respectively. The amoebae, stoppage or movement response, and the results were compared with controls (spontaneous stoppages).

RESULTS

Response of *Amoeba proteus* to different spectral light ranges

Irradiation of *Amoeba proteus* with short pulses of light of different colors induces different reactions (Table 1). Near UV and blue light (365-500 nm) induce a distinct cessation of cytoplasmic streaming and

Table 1

Reaction of *Amoeba proteus* to different light ranges. Observation time – first 10 seconds

Filter	Spectral range	Reaction
B 223	330–500 nm	cesation of cytoplasmic streaming and locomotion after 1 second
GG 11/2	475–700 nm	cessation of cytoplasmic streaming and locomotion during first 5 second
OG 2/2 RG 2/4	550–700 nm 600–750 nm	transient acceleration of cytoplasmic streaming

Table 2

Effect of light of different wavelengths on *Amoeba proteus* responses after 1 second stimulation (+) – indicates occurrence of the reaction.

Filter λ (nm)	$\frac{\lambda}{2}$	Photon fluence rate factor $\times 10^{18} m^{-2} s^{-1}$	Reactions of the cells: Transient acceleration of streaming	Inhibition of: pseudopods expansion	Inhibition of: streaming and motility
365	(15)		+	+	+
375	(13)		+	+	+
383	(17)		+	+	+
400	(12)	27	+	+	+
425	(9)	24	+	+	+
436	(10.5)	14	+	+	+
450	(11.5)	22	+	+	+
466	(9.5)	16	+	+	+
475	(9)	15	+	+	+
491	(7.5)	32	+	+	+
500	(10)	80	+	+	+
512	(5)	24:47.5	+	−?	−
528	(5.5)	44	+	−?	−
535	(4.5)	24:44.1	+	−	−
546	(5.5)	88:136	+	−	−
554	(4)	42:96.5	+	−	−
575	(7)	60	+	−	−
589	(5.5)	37	+	−	−
600	(5.5)	46	+	−	−
650	(5.5)	22	+	−	−

locomotion. The decrease of the streaming velocity and the stopping of cytoplasm is first seen in the peripheral parts of amoeba, except the uroid, and expands toward the central part of the cell until the whole amoeba becomes motionless. Longer wavelengths ($\lambda > 550$ nm) produce a visible acceleration of cytoplasmic streaming. The acceleration of the cytoplasmic streaming and locomotion is seen 5 to 10 seconds after stimulation and is transient (lasting for 1 or 2 s). In several cases a sudden contraction of the cell was seen.

Reactions of amoebae to irradiations of different wavelengths and similar photon fluence rates are presented in Table 2. Irradiation with 450 nm light produces 100° of reaction and leads to a total cessation of streaming throughout the cell. Stimulation with 500 nm induces reactions of 50° of the cells. Irradiations with 512 or 528 nm wavelengths lead to a transient decrease of the rate of streaming in advancing pseudopods while only a few amoebae stop their movement. In 535 nm and longer wavelengths the cessation of streaming in pseudopods and the inhibition of amoebae movement is on the same level as in the control specimens.

Comparison of the action of blue and red light

Comparison of amoebic responses to blue, orange or red light gave indications on antagonistic action of these light ranges (Table 1). On the other hand, results presented in Table 2 indicate the same initial mode of action of the blue light as at longer wavelengths. Thus the question arises, whether the differences in the motile behavior found in the presence of blue and longer wavelengths are due to the different action of both ranges, which might be related to different photoreceptors, or whether they are due to the effectiveness of both ranges related to the spectral properties of the same chromophore molecule.

To answer this question, experiments with the following irradiations have been done (Table 3): (1) high photon fluence rate, impulse, 575 nm irradiation, and

Table 3

Final behavioral reactions of *Amoeba proteus* induced by hight fluence rate, impulse or medium fluence rate, continuous light stimuli (575 nm). For comparision the effect of impulse, 450 nm, hight flence rate irradiation is shown.

Wavelength (nm)	Photon fluence rate $\times 10^{18} m^{-2} s^{-1}$	Stimulation time (s)	Final response
575	314	1	Active pseudopod stops after 10 seconds
450	186	1	Ceasing of cytoplasmic streaming and motility within 3 seconds after stimulation. Repolarization of functional polarity after recovery.
575	63	continuous	Ceasing of streaming and motility after 30 to 40 seconds. After recovery the amoebae might move in the same direction.

(2) medium photon fluence rate, continuous, 575 nm irradiation.

Observation of amoebae irradiated with a strong, 1 second of monochromatic light (λ 575 nm) reveals the stopping of the leading pseudopods after 10 seconds. The pseudopods became transiently frozen and were withdrawn after 3 to 8 seconds. A control irradiation with 450 nm induced a global cessation of streaming and pseudopods movement within 3 seconds. After the recovery of locomotion, a reversal of function polarity was observed. An active pseudopod develops in the previous tail part of the cell, while the previously active pseudopods form a new uroid.

Continuous irradiation of dark-adapted amoebae with 574 nm or red light (RG2/4 glass filter) induces the transient ceasing of cytoplasmic streaming, however, no change of functional polarity of the cell after recovery is seen. The reaction time for the irradiation is several times longer than for the blue light and reaches 40 seconds in comparison to 3-5 seconds for blue light. For both stimulation modes the initial acceleration of the protoplasmic streaming is seen within the first 10 seconds following stimulation.

DISCUSSION

We have presented several lines of evidence which show that the antagonistic effect of short wavelengths (blue) and long wavelengths (orange and red, Table 1) in *Amoeba proteus*, might be based on the same photomechanical transducing system. These include: (1) the time-course of the blue light reaction, (2) amoebic response to continuous irradiation with orange or red light (at medium photon fluence rates), and (3) retarded response to strong impulses of orange or red light.

A comparison of amoebic reactions to blue light irradiation with the reaction to orange and red high fluence rate pulses or continuous and medium fluence rate irradiation reveals the same sequence of events. In all cases the light produces the initial acceleration of the streaming followed by retarded cessation of the streaming and locomotion. Cytoplasmic streaming acceleration is clearly seen in amoebae irradiated with longer wavelengths (orange or red), while in amoebae irradiated with blue the effect is not so clearly marked. The observed difference in the induction of a transient acceleration between both light ranges is related to the differences in their effectiveness, which is expressed in the differences between the reaction times. For orange and red light irradiations the reaction times are several times longer (over 30 s) than for the blue (3-5 s) of a similar photon density, which allows to spread out the initial photoreaction (acceleration of streaming and partial solation of cytogel). For the blue light stimulus the initial photoreactions is sometimes occluded by a rapidly succeeding specific photophobic reaction (i.e., cessation of streaming and other motile activity) before it develops to a perceivable form and extent. The results presented above do not agree with the previous ones (Harrington and Leaming 1900, Mast and Stahler 1937) pointing out an antagonistic action of both light ranges (red vs. blue). The inconsistencies might result from the method of observation, too short observation times, as well as from the experimental set-ups. The previously used set-ups did not allow to separate the stimulating light beam and microscope monitoring beam, or amoebae were stimulated by a sudden increase in intensity of the monitoring beam. The stimulus beam in these systems temporarily blinded the observer. It hindered, during and shortly after stimulation, the complex time-table of the blue light reaction, in particular, the transient acceleration of the streaming velocity. However, Mast (1931) described a light- induced acceleration of streaming followed by its slowing in a small number of specimens with extremely slow streaming: she first suggested the tendency of light to produce an increase and then a decrease in the rate of streaming, but as she pointed out, this tendency is too small to be perceptible.

The parallels between the time table of photophobic reaction and the table of events triggered by intracellular injections of Ca^{2+} ions (Alsup 1942, Heilbrunn 1958, Kuroda et al. 1988, Taylor et al. 1973, Taylor and Fechheimer 1982) indicate that light might influence the Ca^{2+}–handling systems of *A. proteus*. The observation indirectly supports the solation-contraction coupling hypothesis (Condeelis and Taylor 1977, Taylor 1977).

This research was supported by Grant No CPBP-04.01.

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Received on 2nd January, 1991.

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 79-85

Effect of the Angle in Incidence of a Stimulating Light Beam on Phototactic Orientation of *Amoeba proteus*

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Summary. *Amoeba proteus* exhibits negative phototaxis to blue light. The time-course of orientation as well as amoeba cell shape and trajectories of movement are found to be dependent on the angele of incidence of stimulating light beam. Correlations between angle of incidence and the amoebae cell shape and their trajectories of movement suggest that the determinations of light propagation direction by the amoeba is based on the detection of the internally created gradient of light intensity. The difference in light absorption is then processed into the changes of a mechanorheological properties of cytoplasm.

Key words. *Amoeba proteus*, light stimulation, phototaxis

INTRODUCTION

The colorless Rhizopod *Amoeba protues* exposed to a horizontal light beam of direct sunlight migrates outward the source of light (Davenport 1897, Mast 1910, Muller 1965, Haberey and Stockem 1971, Łazowski 1986) exhibiting negative phototaxis. Unevenly illuminated amoebae extend pseudopods on the more shaded side and contract the pseudopods located on the illuminated side. They escape from the enlightened area and accumulate in darkness – a negative phototopotactic response (Mast 1910. 1911, 1926, 1931, Muller 1965, Grębecki 1980, 1981).

These both reactions allow the protozoan to find a suitable niche in a natural environment or at least to avoid unfavorable light conditions. Though *A. proteus* phototaxis and phototopotaxis has been known for more than a century, little is known about the mechanism underlying these photoresponses.

In the following paper we present the study of the effect of oblique light beams on movement, trajectory patterns and cell shape of *Amoeba protues*. On the basis of presented results, the mechanism underlying the phototactic orientation is proposed.

MATERIALS AND METHODS

The movement and the cell shape changes of amoebae were studied with a videomicroscopic system (Saba VCR 6038, VC 79) (Łazowski 1984a). The trajectories of the movement were recorded on photographic film NP55 using dark field techniques and prolonged exposure methods (up to 30 minutes) (Łazowski and Kuźnicki 1985). Standard 14 (Opton, Oberkochen) and Docuval (Zeiss, Jena) microscopes fitted with phase contrast condensers and low power objectives (2.5x, 6.3x and 3.2x, 6.3x respectively) were used. The monitoring beam was produced form an incandescent lamp equipped with a red light filter and warm filters: the $CuSO_4$ aq. and W 302 (Zeiss, Jena). Cells were stimulated with blue light (450 nm interference filter). The actinic beam was provided by a halogen quartz lamp

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100W/12V and impinged the sample chamber at different angles. Horizontal stimulation was produced in a special chamber connected with the light source by an optical fiber (Fig. 1). The intensity of

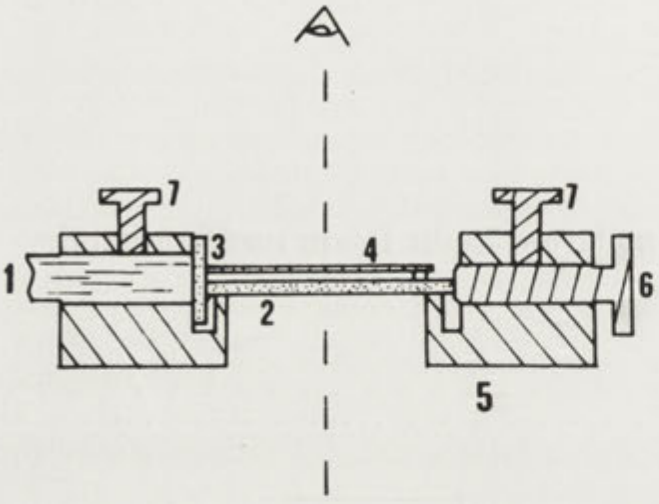

Fig. 1. A setup for horizontal stimulation, 1-opitc fiber, 2 and 3 and 4-sample compartment (made of glass), 5-main body, 6 and 7 screws to hold sample compartment and optic fiber

stimulus was measured with a BPW32 (Siemens) photometric diode placed, instead of a sample chamber, on a microscope stage. The diode was additionally covered with a cover glass to imitate the experimental conditions. The light intensity was adjusted to a constant, arbitrary chosen level for every series of experiments by inserting neutral density filters or suitably crossed polarizers.

In the present study we examine the effect of different angles of incidence of the stimulating light beam on the phase of orientation and on the cell shape and trajectories of movement in the phase of oriented movement. Phototactic responses were measured at the following angles of incidence of light stimulus with respect to the plane of amoeba movement: 0° (horizontal stimulation) and 45°.

RESULTS

When *Amoeba proteus* is placed in the path of a stimulating light beam, a photophobic response induced by a sudden increase in light intensity is seen. Stimulated amoebae stopped their transient movement following cessation of protoplasmic streaming. After remaining motionless for a period of time (a quiescence period (Folger 1925)), the recovery of a cytoplasmic streaming and locomotion succeeds. The time-course of events following the recovery of streaming is related to mutual orientation of the direction of stimulating light bean propagation and the direction of movement of unstimulated amoebae.

There are three main configurations in the horizontal projection of the direction of propagated light: (1) parallel to the direction of amoeba movement (homodrome position), it means amoeba was moving before stimulation outward the light source; (2) antiparallel to the direction of amoeba movement (antidrome position), it means unstimulated amoeba was moving toward the light source; (2) antiparallel to the direction of amoeba movement (antidrome position), it means unstimulated amoeba was moving toward the light source; (3) perpendicular to the direction of amoeba movement.

Amoeba proteus response to a laterally impingent light beam consists of two phases: the phase of orientation in respect of the direction of light beam propagation, and the phase of oriented movement. The same two phases were found during chemotaxis of *Dictyostelium* to cAMP (Swanson and Taylor 1982).

Phase of orientation

Parallel configuration (Homodrome position)

In the case of horizontal stimulation, light beam impinges on the rear end of cell and illuminates the cell area perpendicular to the direction of light propagation. Some of the stimulated amoebae do not stop after onest of the light beam though the light beam of the same intensity impinging vertically induce 100 percent of photophobic reaction. In amoebae which stopped, the recovery of streaming is seen shortly after stimulation. The recovery of streaming is probably a common expression of *Amoeba proteus* avoiding reaction (Grębecki at al. 1987, Dołowy and Grębecki 1988). Numerous, small pseudopods are formed mainly in the anterior part of the amoeba. Pseudopods which have been extended laterally are quickly withdrawn or turn away from the light source. They very rarely undertake the role of a leading front. The active front is formed mainly by the pseudopods, which have protruded from the old front and, during their elongation, have been shaded partly or completely by the cell parts proximal to the light source. Reversal of cytoplasmic streaming was never observed in all tested light intensities.

In the case of a 45° light beam illuminating the upper side and partly rear end of amoeba cell, after recovery of streaming, pseudopods are extruded not only from the anterior part, but also from the middle region of the cell. A lateral pseudopod might undertake

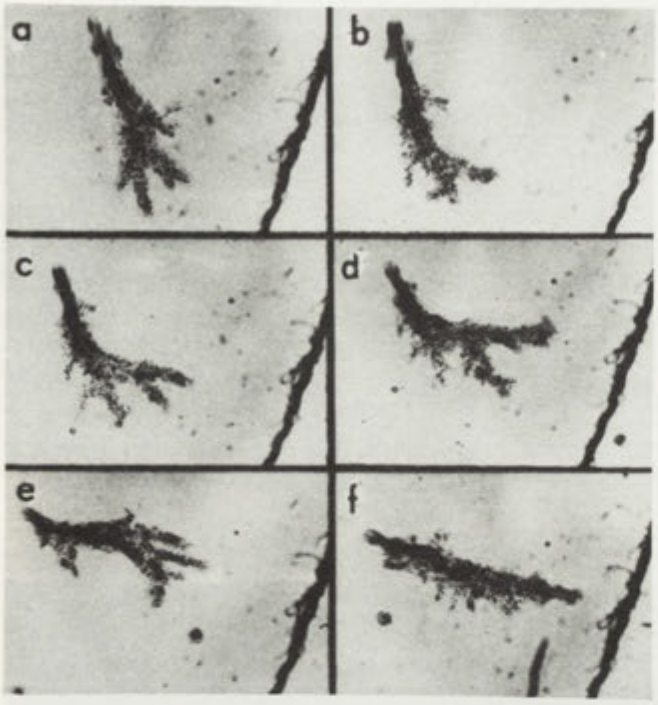

Fig. 2. a-f. Orientation of *Amoeba proteus* in horizontal light beam. Light beam travells perpendicularly to the black stripe marker on the right side of pictures

a role of a leading one and form a new front. However, during 30 sec. after extrusion, the pseudopod turns away from the light source or new pseudopods develop on its side distal to light source. The flowing endoplasm is directed toward the new ones and a gradual change of the streaming direction as well as the direction of the new front expansion is observed.

If the actively advancing pseudopod expands from the old frontal part the same events are seen, as in the case of the horizontal stimulation.

Transient reversal of the streaming direction following its recovery was seen in few specimens stimulated with the highest light intensity.

Antiparallel configuration

Horizontally impinging light beam illuminates the frontal part of the cell and ceasing of streaming as well as stoppage of locomotion occurs. After recovery of streaming, its reversal is observed and new pseudopods are formed in the previous amoeba tail part, and several pseudopods are elongated from the old uroid. Lateral small pseudopods are quickly withdrawn and they have never any influence on a cytoplasmic streaming direction.

During the 45 degree stimulation, the light beam impinges on the dorsal cell side and partly on the front. Ceasing of a cytoplasmic streaming and stoppage of locomotion is always seen as in the case of horizontal stimulation. In the majority of cases, almost immediate streaming reversal follows its recovery, and new pseudopods are protruded from the middle and previous posterior parts.

Small pseudopods are also elongated from the old anterior part, despite the 180 degree reversal of the main cytoplasmic streaming direction. This is due to the existence of short-lived local domains of a cytoplasm characterized by different directions of streaming, in particular directed oppositely to main streaming.

In some cases amoebae preserve the previous direction of a streaming. Pseudopods continually protrude from the anterior part. The pseudopods, elongated toward the light source, are more quickly eliminated than the pseudopods which are expanding laterally. New pseudopods are formed along the middle part or at the base of the lateral one, mainly on its side distal to the light source. During such events, a new front is created from some of the formerly lateral pseudopods and the amoeba gradually turns away from the light.

Perpendicular configuration

In the perpendicular configuration the duration of orientation of amoebae, stimulated horizontally and obliquely, is similar. After recovery of a cytoplasmic streaming numerous small, thin pseudopods are formed in the frontal and central parts of the cell. If the stimulating light beam is strong enough, reversal of cytoplasmic streaming occurs and pseudopods are formed in the previously posterior part. Pseudopods formed at the illuminated cell side are quickly withdrawn, while the pseudopods on the side distal extend in the shaded area. If the new dominating front develops from pseudopods extending from the previous frontal or tail part of the cell, the amoeba gradually turns, away from the light source (Fig. 2). In the sequence of events the amoeba preserve its functional polarity or its complete repolarization occurs, respectively. If the new front develops from lateral pseudopods, the previous anterior part of the cell is retracted and the flowing cytoplasm is directed to the active lateral pseudopod oriented parallel to the horizontal projection of the stimulating light beam. In the sequence of events the laterally extending pseudopods become a new anterior part.

The phase of oriented movement

The horizontally stimulated amoeba assumes a cylindric sharpe with one main leading pseudopod, which extends ahead of the moving cell (Figs 3, 4). During movement, a second pseudopod may be formed at the basal region of the old one. In this case both pseudopods progress in the same direction for a short time, but soon one of them slows down and stops. Small, thin lateral pseudopods are formed, but they never develop to dimensions which might disturb the cytoplasmic streaming and change the direction of movement. In the final phase of the reaction, amoebae move away of light source, minimizing the cell area exposed to light. The movement trajectories are fairly straight lines (Fig. 5). For comparison, the trajectories of amoebae moving in vertical light beam are shown (Fig. 6).

An obliquely stimulated amoeba (45 degree stimulation) also assumes a cylindric shape as in the case of horizontal stimulation, but often several competing pseudopods in the anterior part are formed (Fig. 7)

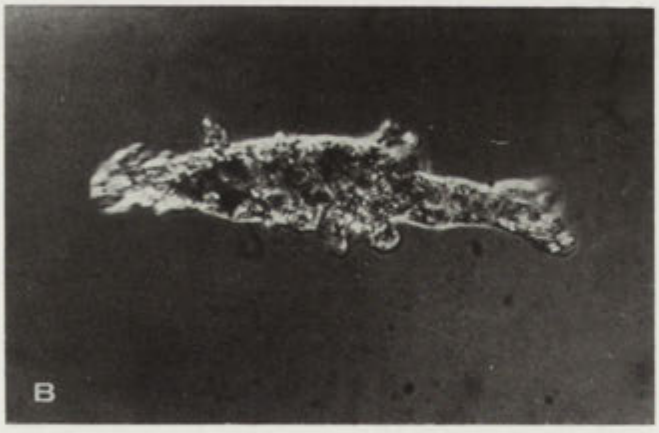

Fig. 3 A, B, C. Typical shapes of amebas moving in horizontal light beam. Photographs of the same specimen taken in 30 sec. intervals; A = 0 sec., B = 30 sec., C = 60 sec. (Upper specimen)

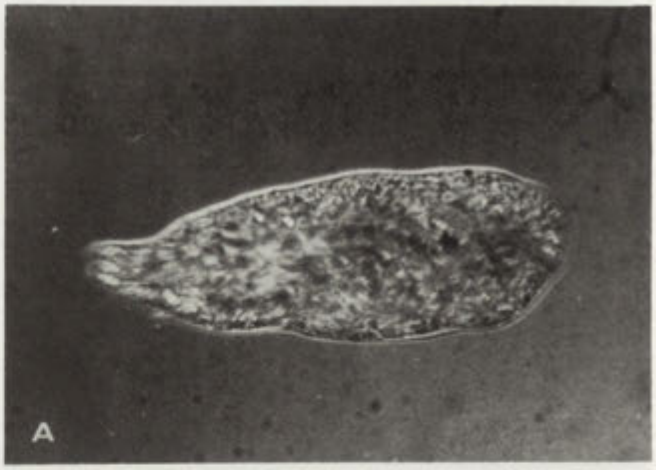

Fig. 4 A, B. Typical shapes of two young amebas (about 4 to 6 hours after division) in horizontal light beam

Fig. 5. Trajectories of amebas moving in horizontal light beam

measurement of the difference of external light intensity around the cell, as it is in the case of a local light-shade gradient stimulation – a phototopotactic response. In the case of horizontal stimulation, an amoeba assumes an orthotactic form (Grębecki and Grębecka 1978) with one leading pseudopod and the movement trajectories are in straight lines. The relative change of external light intensity along the cell is not greater then $10^{-5}m^{-1}$, while in the case of a local light-shade gradient stimulation the smallest relative difference was found to be between 1.1 and 1.3 (unpublished results). In the case of oblique stimulation the relative change of intensity of external light is of the same order as in the case of horizontal stimulation, but

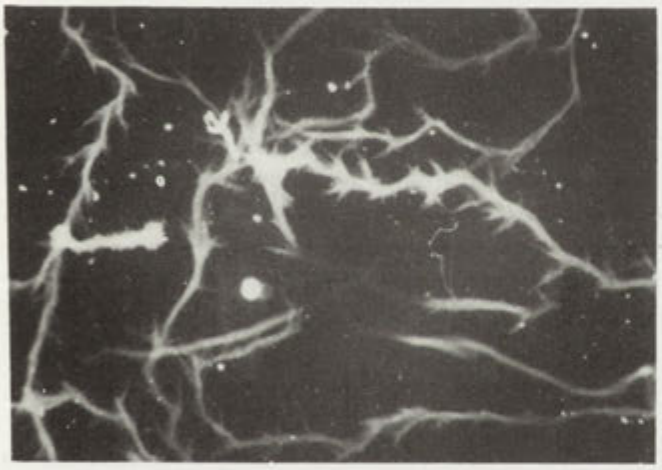

Fig. 6. Trajectories of amoebas in vertical light beam

Fig. 7. Typical shapes of amebas moving in obeique light beam

They extend laterally with respect to the direction of light propagation, but angles between the horizontal projection of light beam and the direction of pseudopod expansion are always smaller than 90 degres. This means that lateral pseudopods also move outward away from the light source. The laterally protruded pseudopods change their transient direction of movement. The trajectories are no longer straight lines, but exhibit an arrow-head pattern (Fig. 8).

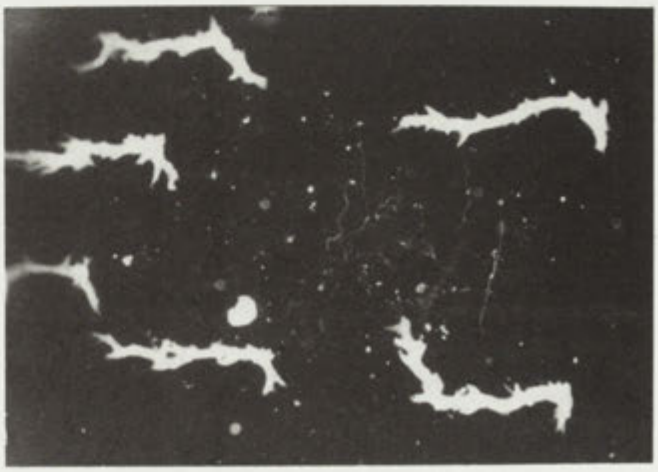

Fig. 8. Trajectories of amebas moving in oblique light beam

DISCUSSION

The observations presented in this study show that *Amoeba proteus* detects the direction of light propagation. The detecting procedure is not related to the

amoebae posses several laterally propagating pseudopods and movement trajectories are no longer straight lines but exhibit an arrow-head pattern.

The evidence clearly indicates that the determination of the direction of light propagation by the amoeba is based on the detection of the internally created gradient of light intensity. This internal light gradient is measured simultaneously by spatially separated detectors in different regions of the cell (Łazowski and Kuźnicki 1984). The difference in light absorption is then processed into the changes of a mechano-rheological properties of cytoplasm, by a still unknown mechanism which results in the observed cell shape (orthotactic form) and motile behavior. In the case of horizontal stimulation, the light gradient is established along the length of cell between its tail and frontal parts. The usual length of orthotactic amoeba is between 0.3 and 0.5 mm and is much greater than in the case of 45 degree stimulation, when a light gradient is produced on the thickness of the cell equal to about 50 x 10^{-6}m. This is about 0.1 of the cell length. Light intensity attenuation coefficient of *Amoeba proteus* cytoplasm is about $\mu = 0.004\mu m^{-1}$ (Łazowski 1987). For horizontal stimulation the rate of difference of light intensity – I_{front}/I_{tail}, between front -I_{front} and tail-I_{tail} of amoeba may reach the value of 7 ($I_{front}/I_{tail} = \exp(\mu x$ amoeba length) while for oblique stimulation the value is about 1.2. the internal translation of the information of the direction of light propagation into the difference of light intensity in different cell parts is confirmed by the duration of the phase of orientation.Several amoebae, which are horizontally stimulated in parallel configuration (homodrome position) never stop their movement, However, the light of the same intensity impinging vertically on them induces 100 percent of a photophobic reaction. This means that the internally attenuated intensity of the light beam penetrating the cell was to low to produce a total photophobic reaction and to stop the shaded, leading pseudopod. If the same experiment was repeated with obliquely impinging light, the photophobic reaction was always seen. In antiparallelly (antidrome) and horizontally stimulated amoebae, reversal of streaming is seen and the active front is produced instead of the previous tail part. In several amoebae, stimulated antiparallelly (in the antidrome position) and obliquely, the streaming direction is preserved and amoebae gradually turn away from light.

Considering that *Amoeba proteus* cytoplasm is a fairly random dispersion of different granules, vacuoles, crystals and small organelles which very effectively diffuse the light beam (Fig. 9), the multiscattering effect might be responsible for generation of the light gradient along unilaterally illuminated cell.

Fig. 9. Dark field picture of polytactic *Amoeba proteus* with numerous cristals and others structures dispersing light

This work was supported by Grant No CPBP-04.01.

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Received on 15th January, 1991

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 87-92

Surface Oscillations in *Physarum polycephalum* – Computer Simulation and Comparison With the Local Influence of the Respiratory Inhibitors

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Summary. Plasmodial strand of *Physarum polycephalum* partially submerged in solution containing respiratory inhibitors, potassium cyanide (KCN) and salicylhydroxamic acid (SHAM) preserves its contraction-relaxation cycle in the submerged fragment while the following total submersion evokes cessation of the force oscillations. The effect of the local application of KCN and SHAM was compared with a computer simulation based on the model of the contraction-relaxation cycle (Teplov and Romanovsky 1987). The influence of the respiratory blockade on the regulation of the contractile activity was studied. It is suggested that the respiratory blockade is not sufficient for suppressing the contractile activity of the plasmodium.

Key words. *Physarum polycephalum*, respiratory inhibitors, computer simulation, contractile activity.

INTRODUCTION

The plasmodium of *Physarum polycephalum* is a very convenient object to build and test the theoretical models because of its relatively low complexity and facility of experimental verification. One of such models, proposed by Teplov and Romanovsky (Teplov and Romanovsky 1987), tied in system of the differential equations: i) the deformation of a strand surface, ii) the active pressure and iii) the concentration of the calcium ions in plasmodium (see Appendix A; for detail analysis see also Latushkin et al. 1988). An advantage of that model was clearly described space dependence. It gave a new starting point for studying the spatial aspects of contractile activity in plasmodia.

The purpose of the present investigations was to study an influence of the local changes in the kinetic parameters on the behavior of simulated plasmodium in comparison with experimental data, i.e., with the influence of the respiratory inhibitors, KCN and SHAM, on the contractile activity of a real plasmodium (see also Baranowski 1985, Naib-Majani et al. 1988). In both cases the change in the calcium homeostasis is supposed. Such local impact was relatively easy to perform because of earlier underlined a very clear space dependence of the model.

The present results suggest that the blockade of the respiration is the necessary, but not the sufficient condition for the cessation of the contraction-relaxation cycle in plasmodia.

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MATERIALS AND METHODS

Simulation

The computations of the model were carried out with the aid of the author program (see Appendix B) that solved a given system of differential equations using the Gaussian elimination method (Potter 1973, Samarsky and Nikolayev 1988). This program was written in the computer language Turbo Pascal version 5.5 from Borland, Inc. on the IBM PC/AT – compatible computer. The values of the viscoelastic and geometric parameters of simulated plasmodial strand were taken from (Teplov and Romanovsky 1987).

To avoid a discontinuous dependence of the kinetic constants k_3 and k_4 (Appendix A, eq. 3) on horizontal coordinate z we assumed that these parameters change along z axis in a continuous manner. Furthermore, for more exact simulation of the real experimental situation of penetrating the inner plasmodial space by an active factor we assumed a diffusion-like time dependence of k_3 and k_4 (ibidem).

Experiment

Plasmodia of the myxomycete *Physarum polycephalum* were maintained as a surface culture and fed with the oatflakes. Prior to the experiment the plasmodium migrated on 2% water agar overnight and thus the formation of smooth strands was achieved. The fragments of the plasmodial strands about 2 cm long without any branches were excised from the plasmodium and used for the experiment.

The respiratory inhibitors KCN and SHAM (stock solution in DMSO) were dissolved in salt solution containing 1 mM $CaCl_2$, 2 mM NaCl, 1 mM KCl and 3 mM $MgCl_2$ to obtain 7 mM SHAM and 5 mM KCN final concentrations. Solutions were adjusted to pH 7.2 with the aid of NaOH. The final concentration of DMSO was 2% (vol.).

Tensiometric investigations were carried out under isometric conditions (Wohlfarth-Bottermann 1975). The isolated strand in a vertical position was attached in its middle part to the arm of a force transducer with the aid of a drop of 2% water agar. The lower end of the strand was mounted in a bottom of the test chamber. It enabled the registration of the force that was generated by the lower part of the strand only. The upper end was vertically tied to the arm of a force transducer to avoid its influence on the registration. This also allowed to achieve the gradual KCN/SHAM treatment by changing the solution level in the chamber.

All experiments were performed at 20°C.

RESULTS

Cessation of the oscillations

Figures 1(a) and 1(b) show the comparison between the simulation and the experimental data in case of the cessation of the oscillations. In part (a) (simulation) we put k_3 and k_4 equal to zero in a selected part of the simulated plasmodial strand after 1500 s (vertical

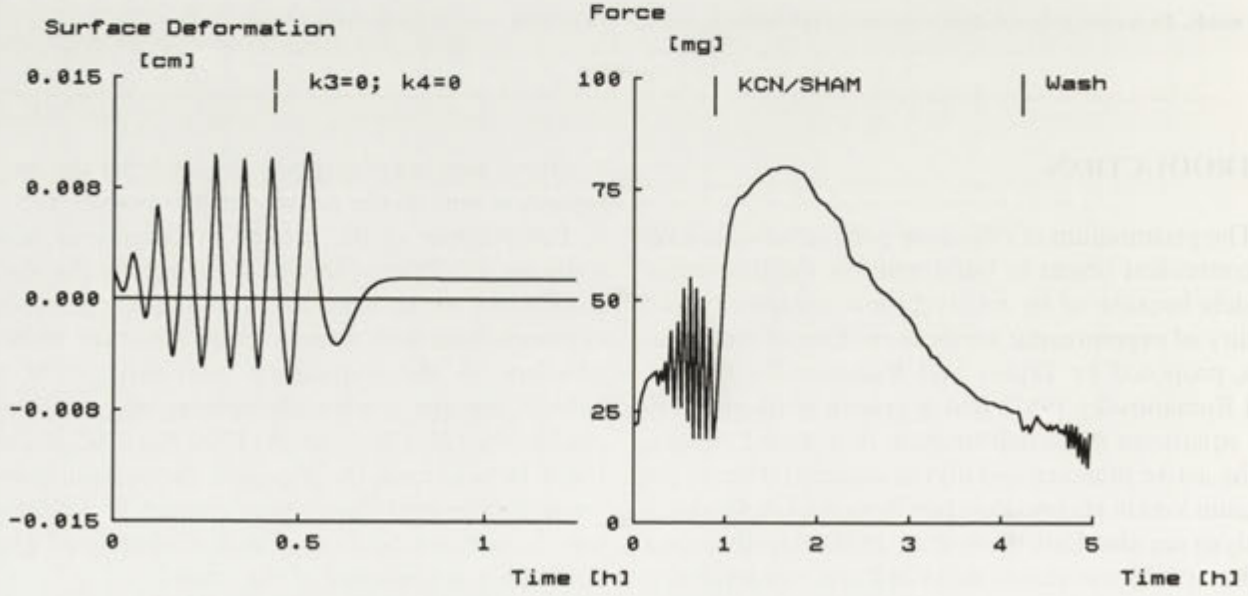

Fig. 1. The comparison between the simulation and the experiment in case of the cessation of the oscillations. A: The simulated changes in the surface deformation. The kinetic parameters k_3 and k_4 after 1500 s became a zero (vertical marker). B: The experimental data. The plasmodial strand after 1 hour was treated with the test KCN/SHAM solution (first vertical marker). After 4 hours (next vertical marker) the strand was washed with the aid of salt (control) solution.

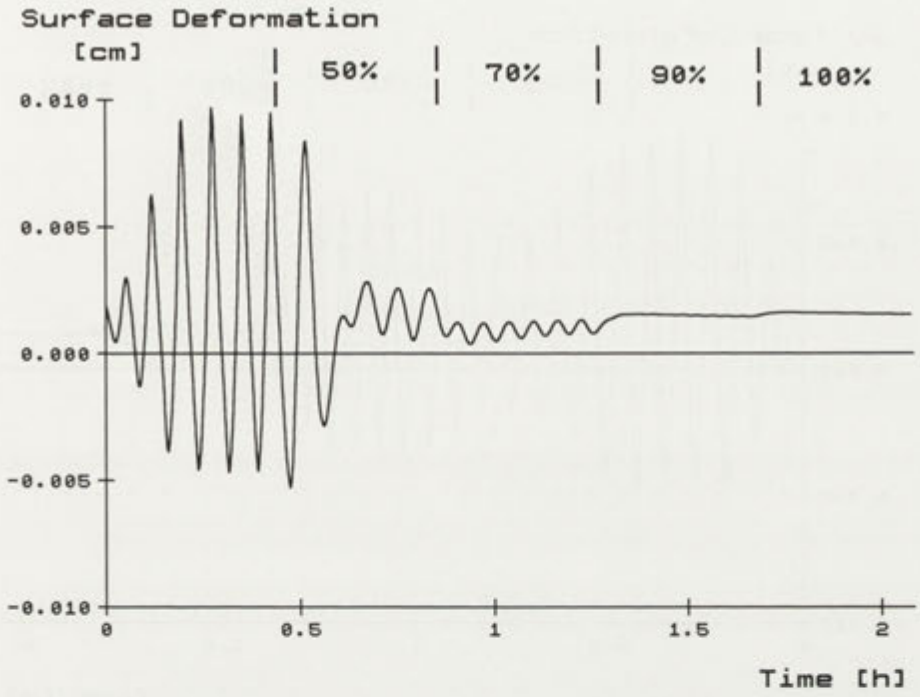

Fig. 2. The behavior of the "lower" end (for explanation see Results – Gradual treatment) of the simulated plasmodial strand. The kinetic parameters k_3 and k_4 became a zero (after 1500, 3000, 4500 and 6000 s – vertical time markers) in a given part of the plasmodial volume (50%, 70%, 90% and 100%, respectively).

marker). As a matter of a fact, we assumed this way that the concentration of the free calcium, which took part in a contraction-relaxation process, was constant. It is clearly seen that after a certain time (adequate to the constant of a diffusion time T – see Appendix A) oscillations totally disappeared. In part (b) (experiment) the plasmodial strand after about 1 hour was submerged in a test (KCN/SHAM) solution. After a certain time the oscillations of a longitudinal force disappeared in a similar, diffusion-like way. It should be underlined here that removing those active factors simply by washing with the salt solution (wash – second time marker in Fig. 1(b)) caused reappearing of the oscillations in the same manner as restoring the initial values of k_3 and k_4 might cause reappearance of the rhythmical activity.

Gradual treatment

Figures 2, 3 and 4 show the comparison between the simulation and the experimental data in case of gradual treatment. Figures 2 and 3 show the results of the simulation. In this part of investigation we put k_3 and k_4 equal to zero in a given volume of the simulated strand – 50%, 70%, 90% and 100% – after 1500, 3000, 4500 and 6000 s, respectively (vertical time markers). In Fig. 2 we show a behavior of this end of the simulated strand, at which k_3 and k_4 are always equal to zero ("submerged" or "lower" end), while Fig. 3 shows the changes in an oscillation pattern of the opposite ("upper") end of the strand. It means, at this end k_3 and k_4 differ from zero until the entire strand is treated.

In order to check, whether the predictions of the model are correct we performed the experimental verification. The results are shown in Fig. 4. Plasmodial strand was initially located in a salt solution (see Materials and Methods). About 30 minutes after the establishment of oscillations (first vertical time marker) circa 30% of strand was treated with the test KCN/SHAM solution. This procedure was reiterated for 50% and 70% of the strand. After 3.5 hours only the upper attach point, i.e., about 2 mm long piece of the plasmodial strand surrounded by the agar drop, was not in the solution of the inhibitors. Finally, after 4.5 hours the entire strand was poured over. As it is seen, oscillations of the longitudinal force were detectable

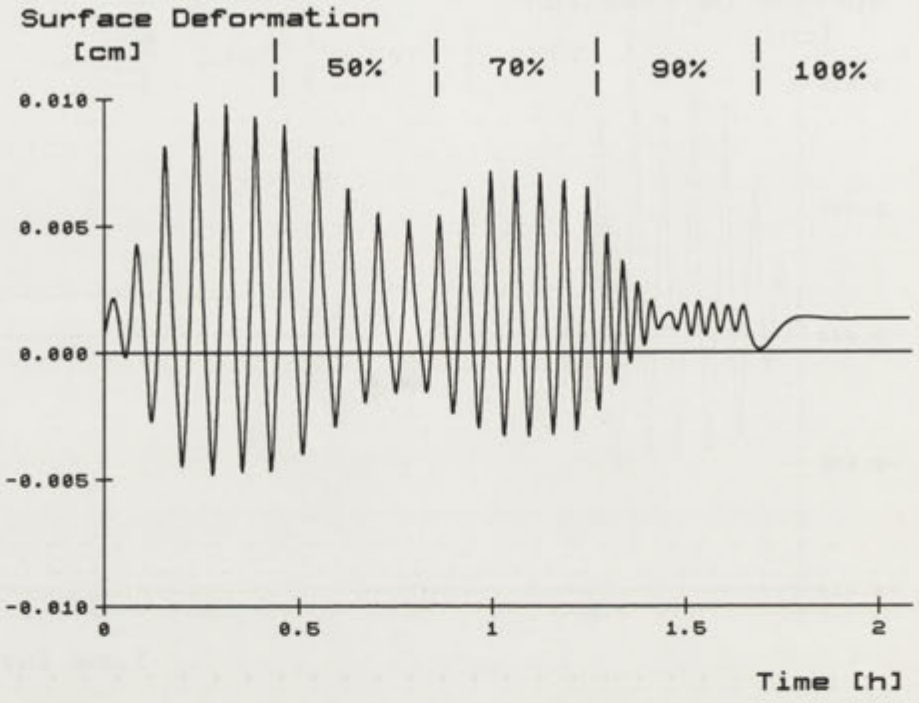

Fig. 3. The behavior of the "upper" end (for explanation see Results – Gradual treatment) of the simulated plasmodial strand. The kinetic parameters k_3 and k_4 became a zero (after 1500, 3000, 4500 and 6000 s – vertical time markers) in a given part of the plasmodial volume (50%, 70%, 90% and 100%, respectively).

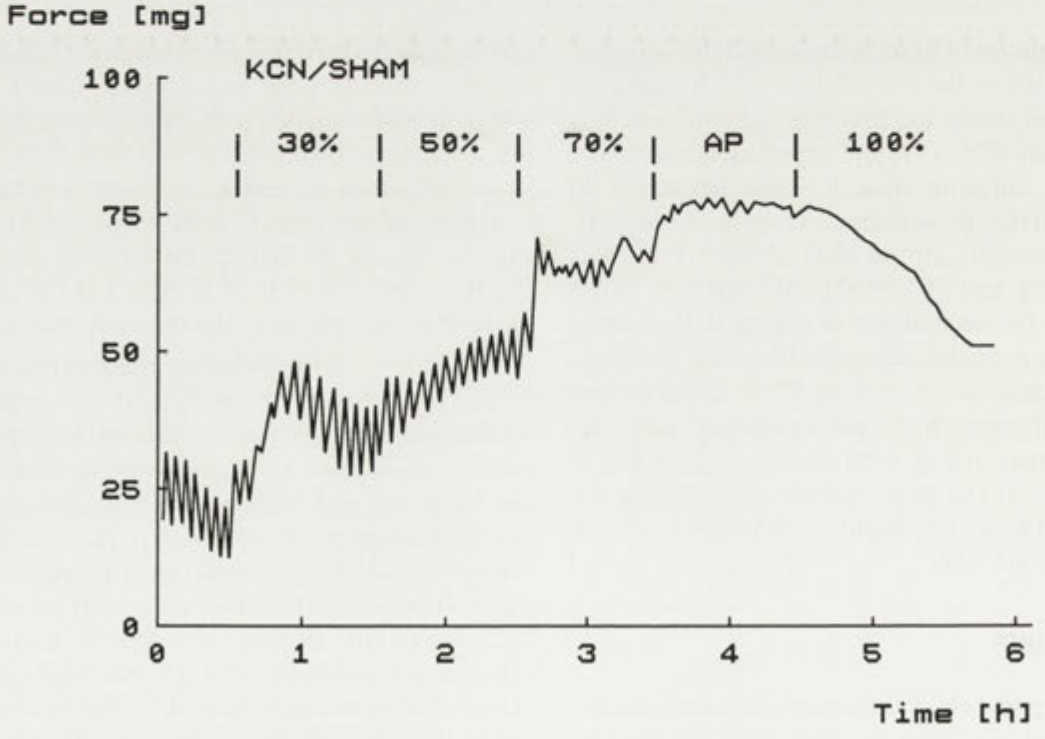

Fig. 4. The gradual treatment of the plasmodial strand with the aid of KCN/SHAM solution. Markers indicate the successive steps of experiment – 30%, 50%, 70%, the entire strand except the attach point (AP) and 100% of the length of the plasmodial strand submerged in the test solution.

until the entire strand was under the influence of inhibitors, just like in the simulated situation.

Beside this similarity we should underline that in both theoretical and experimental results the resemblances in changes of amplitude also occur. This effect is clearly seen in Fig. 3 in comparison with experimental data presented in Fig. 4.

DISCUSSION

The presented results show the satisfactory similarities between the model and the experiment as far as the radial and longitudinal contractile activities are comparable. Of course, in the present shape this model does not predestinate to describe the mechanism of the generation of the longitudinal force, however, basing on the quantitative equivalence between the tensiometric measurements and the method of the infrared reflection (Samans et al., 1978), we still may try to draw out some conclusions about the contractile activity in general.

It is well known that KCN and SHAM cut off both cytochromal and alternate respiratory pathway in *Physarum polycephalum* (Baranowski 1985, Beylina et al. 1988). Then, because 10% of the plasmodial volume (see Fig. 3.) is much enough to preserve the contractile activity, so the respiration blockade is the necessary, but not the sufficient condition for cessation of the contractile activity in plasmodium. It seems to be evident because it is hard to expect that in spite of the presence of endoplasmic streaming under the partial KCN/SHAM treatment (unpublished results) the respiration occurs in this 10% of the strand. We suggest that the respiratory inhibitors also may have an influence on the plasma membrane processes in a certain way. If so, we could say that either: i) in non-treated part of the plasmodial strand the concentration of KCN and SHAM is sufficient to evoke the blockade of the respiration but not the earlier suggested membrane process (self-defense effect) or: ii) the putative membrane process has a vector character, i.e., it is disturbed when the inhibitors act on the outer side of the membrane only. The occurrence of such a process was already observed for the caffeine and ethanol influence on the plasmodium (Ueda et al. 1978).

In this work we assumed that treated part was fully passive because the free calcium concentration and, thus, the active pressure were constant (see Appendix A). In case when the k_3 and k_4 are decreased, but not equal to zero, the treated part can react in an active way on the trigger impulse from the rest of the strand (unpublished results).

APPENDIX A

In the Teplov-Romanovsky model it is assumed, that the surface deformation (x), active pressure (Pa) and the level of the calcium ions (c) are tied in the system of differential equations. This system is closed and resolvable.

$$\frac{16 \cdot u}{R^3} \cdot \frac{\partial x}{\partial t} = \frac{Eh}{R^2} \cdot \frac{\partial^2 x}{\partial z^2} + \frac{nh}{R^2} \cdot \frac{\partial^3 x}{\partial z^2 \partial t} + \frac{\partial^2 Pa}{\partial z^2} \qquad (1)$$

$$\frac{\partial Pa}{\partial t} = k_1 \cdot f(c) \cdot (P_o - Pa) - k_2 Pa \qquad (2)$$

$$\frac{\partial c}{\partial t} = k_3 x - k_4 c \qquad (3)$$

where : k_1, k_2, k_3, k_4 – kinetic constants;
u – endoplasm viscosity;
n – ectoplasm viscosity coefficient for radial deformations;
E – Young's modulus for radial deformation;
P_o – maximum possible active pressure;
f(c) – dimensionless activation function (Teplov and Romanovsky 1987; see also Yoshimoto and Kamiya 1984), here assumed as follows:

$$f(c) = A * \arctan (B * c) \qquad (4)$$

where A and B – constants.

In order to simulate a diffusion of the active factor from an outer space that can possibly change the values of kinetic parameters we assumed the following time-dependence:

$$k_i = k_{i0} * \{1 - d(z) * (1 - \exp [- (t/T)])\}; \qquad (5)$$

where: i = 3, 4;
k_{i0} – the initial values of k_i;
T – penetration time (here 200 s);
d(z) – special factor that describes changes of parameters along with the horizontal z axis, here given as:

$$d(z) = E * \arctan (G * z + H); \qquad (6)$$

where E, G and H – constants.

A constant written as G has a special meaning for simulation results. It describes the gradient of the changes of k_i along with the horizontal axis. When it is relatively large (10 [cm^{-1}] here) the changes in parameters in non-treated part of the simulated plasmodial strand are practically not detectable.

In this way (equation 6) we assumed that at one end (for values of the z coordinate close to zero) the kinetic parameters should always be equal to zero while on the opposite end (values of the z coordinate close to the length of the strand) they differ from zero until the entire strand is treated.

APPENDIX B

In order to solve equations (1)-(4) using computer program we used the procedure described in (Tchernavsky et al. 1975). In this procedure the continuous axis z was divided into N equal pieces and the z-derivatives were changed into difference quotients (this is especially important for the first equation). After such operation we got the system of 3N-equation as follows:

$$\frac{dx_i}{dt} = g\,(x_{i-i},\ x_i,\ x_{i+i},\ c_i,\ Pa_i;t) \tag{7}$$

$$\frac{dPa_i}{dt} = h\,(x_{i-i},\ x_i,\ x_{i+i},\ c_i,\ Pa_i;t) \tag{8}$$

$$\frac{dc_i}{dt} = l\,(x_{i-i},\ x_i,\ x_{i+i},\ c_i,\ Pa_i;t) \tag{9}$$

where g, h and l – continuous functions of x, Pa, c and t.

The next step was to change the time derivatives into difference quotients. This way we got the system of the linear equations as follows:

$$A * x = w \tag{10}$$

where A – the three-diagonal matrix of connections between three succeeding points x_{i-1}, x_i, x_{i-1};

x – vector of the x_i;

w – vector of constant coefficients.

At last, using the Gaussian elimination method (Samarsky and Nikolayev 1988, Potter 1973) we found inverse matrix A^{-1} in order to solve the following equation:

$$x = A^{-1} * w \tag{11}$$

This work was supported by Polish Academy of Sciences – CPBP-04- .01

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Received on 26th October, 1990.

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 93-98

Cell-cell Recognition in *Dileptus*. The Dynamics of Homo- and Heterotypic Pair Formation During Conjugation

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Summary. The number of homo- and heterotypic pairs marked with chinese ink was registered during conjugation of complementary clones of *Dileptus margaritifer*. For each mating type of *Dileptus* both homo- and heterotypic pairs were studied. At the early stage of the process, 2 h after the mixing of clones all three types of pairs (two homotypic and one heterotypic) were present in the mixture, their ratio being approximately 1:2:1. Five-six h after beginning the experiment heterotypic pairs predominated in the mixtures. The homotypic pairs were formed in a great number in mixtures of clones where one of the mating types was represented by only a few cells, but they disintegrated later. It is suggested that the formation of pairs is due to the expression of mating-type nonspecific adhesive molecules on cell surface. The complementary cells in pairs are assumed to continue to stimulate the expression of the adhesines with their mating pheromones. The fact that heterotypic pairs appear to be more stable than homotypic ones is ascribed to this hypothetical mechanism. The stimulation of expression of the hypothetical mating-type nonspecific adhesines with heterotypic mating pheromones may be regarded as a mechanism which prevent inbreeding in ciliates.

Key words. *Dileptus margaritifer*, cell-cell recognition, conjugation.

INTRODUCTION

In ciliates, the intraspecific exchange of genetic information is realized in the form of conjugation. In this group of unicellular organisms, the process does not involve the multiplication of cells. The lack of free gametes in ciliates is usually considered as a consequence of their cortex complexity. This cell structure can be relatively independent of genes which encode its structural elements when it is reproduced during vegetative growth of cells (Ossipov 1979). As a rule, cells which can conjugate are morphologically identical. Nevertheless, it is easy to reveal that in many thoroughly investigated species some mechanisms exist which prevent inbreeding (intraclonal conjugation, or selfing). The clones which form a species can be classified into mating types.

Usually, with exception of the selfing phenomenon, only cells of different, or complementary mating types can conjugate. The mating type can be changed after conjugation and nuclear reorganization. As a rule, it remains constant during vegetative growth of a clone. The exceptions to this general rule involve the alteration of mating types in a clone of some ciliate species (Barnett 1966, Sonneborn and Barnett 1958), as well as the unstable clones whose mating types can

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be reversibly changed depending on their multiplication rate (Hiwatashi 1960), and irreversibly, in the process of a clone aging (Myohara and Hiwatashi 1975, Myohara 1980).

The significance of mating types consists of a certain level of outbreeding which can be achieved by means of the veto in intraclonal conjugation. The strength of the veto may be different in various species of ciliates. Spontaneous selfing without any change of mating types was registered in *Euplotes patella* (Kimball 1939, 1942), *E. octocarinatus* (Heckmann and Kuhlmann 1986), *Oxytricha bifaria* (Siegel 1956) and in many other ciliates (Katashima 1959, Takahashi 1973, Kosaka 1982 a, b, Jerka-Dziadosz and Dubielecka 1985, Valbonesi et al. 1987). Nevertheless, all these species have mating types and selfing in them may be considered as an infringement of the veto. The homotypic pairs may be induced artificially. Thus, in mating pheromone-excreting species the pair formation within a clone is usually induced by treating cells with heterotypic culture fluids (Kimball 1942, Miceli et al. 1983, Akada 1985 a, b). In that case the early steps of conjugation were imitated experimentally because homotypic pairs were also formed in the mixture of two complementary clones. But their percentage was usually small enough and pairs in the mixture were mostly heterotypic.

The ratio and dynamics of pair formation in a cell mixture may be an important source of information about the mechanisms of cell-cell recognition during early steps of conjugation (Kimball 1942, Luporini and Miceli 1984, Heckmann and Kuhlmann 1986).

Present work describes the dynamics of homo- and heterotypic pair formation during conjugation of a ciliate *Dileptus margaritifer*. This species is very convenient for investigation of cell-cell recognition in ciliates. It has only three mating types (Tavrovskaja 1979, Yudin and Afon'kin 1987), each of which excretes its own mating pheromone into the culture medium (Afon'kin and Yudin 1987, Parfenova et al. 1989).

MATERIALS AND METHODS

Dileptus margaritifer, formerly called *Dileptus anser* (see revision by Wirnsberger et al. 1984) was used for the study. Stock cultures were kept at +25°C and fed with *Tetrahymena pyriformis* GL (Nikolaeva 1968). Three clones nos. 52, 83 and 84 belonging to three complementary mating types I, II and III, respectively, were used. The clones were isolated from a natural population near Leningrad in 1984-1988. In preliminary experiments, no antagonistic cannibalistic interrelationships between them were detected, although sometimes cannibalistic clones of *Dileptus* can be picked up from nature (Tavrovskaja 1984). The chemoattraction activity of their mating pheromones was confirmed for clones 52, 83 and 84 (Afon'kin and Yudin 1987). In each experiment two complementary clones were mixed together and all three possible combinations of clones were studied. To discriminate homo- and heterotypic pairs, one of the mixed clones was marked in the following way. Cells were fed with tetrahymenas preliminarily incubated with Chinese ink suspension. Black food vacuoles of tetrahymenas were distinctly visible within food vacuoles of *Dileptus* during the whole time of the experiment (Fig.1). To be sure that the physiological state of the cells in the experiment was similar, the ciliates of the complementary clone were fed with unmarked tetrahymenas. Before the start of each experiment tetrahymenas were washed off and dilepti were kept in fresh culture medium for 2 h.

Cells were mixed in depressions of plastic plates for immunological research (Afon'kin and Yudin 1985). The volume of a depression was 20 μl. In the first series of experiments six cells of each clone were placed into each depression. The total number of depressions was 20. The number of homo- and heterotypic pairs was registered every 30 min for 5-6 h. The theoretically possible number of pairs (120) was assumed as 100% in each experiment.

In the second series of experiments the unbalanced mixtures of cells were made up. Ten cells of one clone and only 2 cells of the other one were placed in each of 10 depression. The number of pairs was registered as in the first series of experiments.

Fig. 1.The heterotypic pair of *Dileptus margaritifer*. One cell is marked with Chinese ink granules

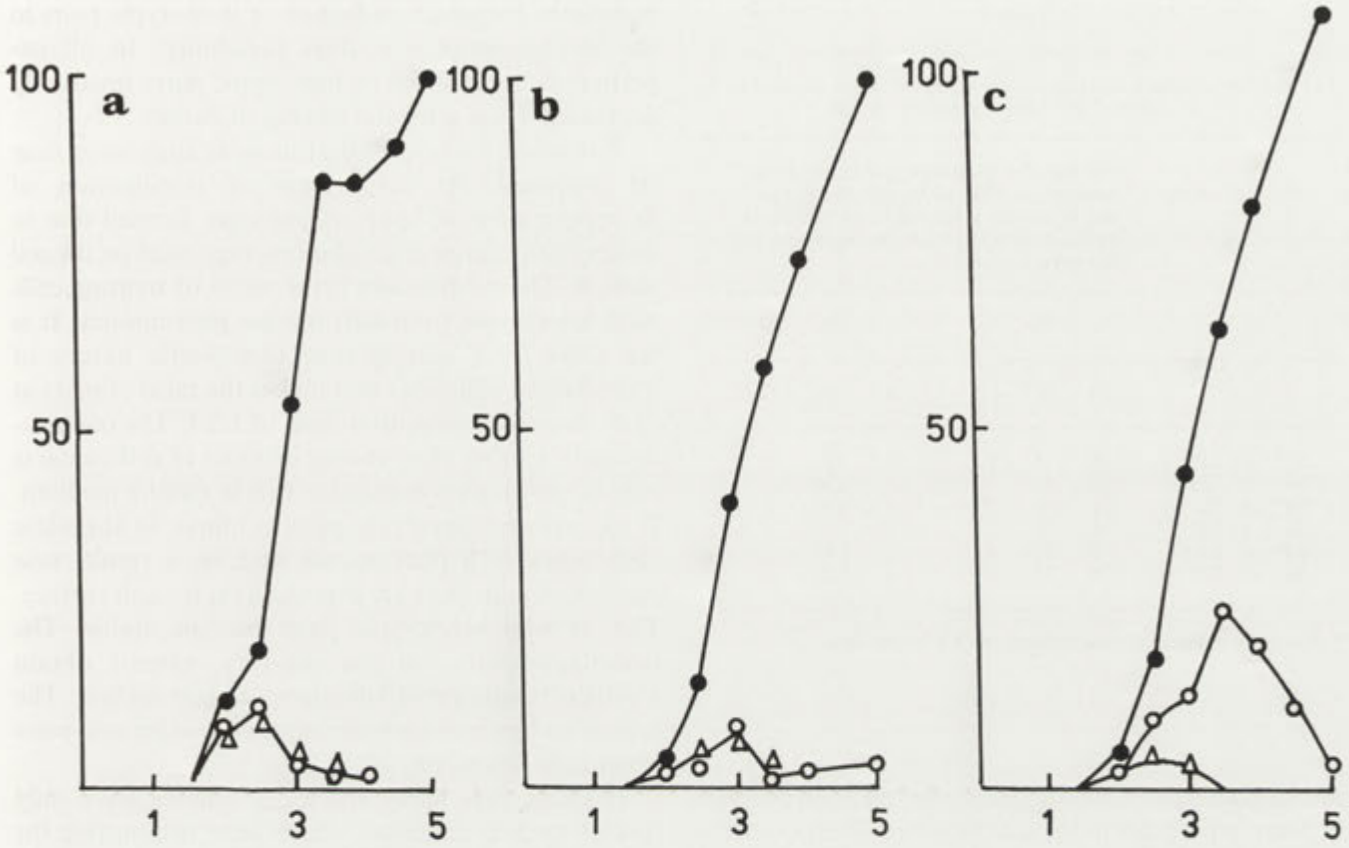

Fig. 2.The number of homo- and heterotypic pairs in equal mixtures of cells. a – clone no. 52 + clone no. 83; b – no. 52 + no. 84; c – no. 83 + no. 84. Abscissa, hours from the begining of the experiment; ordinate, the number of pairs; open circiles and two types of triangles, homotypic pairs

RESULTS AND DISCUSSION

All three possible combinations of complementary clones were used in the experiments. Each experiment was twice replicated. One of the two clones in the first replica and the other clone in the second one was marked with Chinese ink. Comparison of the two replicas shows that the ink granules per se do not affect the conjugation process in *Dileptus*.

In *D. margaritifer* the preliminary feeding did not prevent conjugation. The first cell-cell contacts which could be confidently defined as homo- and heterotypic ones were recorded 1.5-2 h after mixing complementary cells. At this time all three possible types of pairs were found in the mixtures. The cell pairs were unstable and could dissociate and reassociate. Later on the portion of heterotypic pairs rapidly increased (Fig.2). During conjugation some of the cells divide and new dilepti started to conjugate in their turn. As a result the number of heterotypic pairs in mixtures might exceed 100%. The number of homotypic pairs 3-4 h after the beginning of the experiment might increase to 10-25% (Fig.2c), but later it decreased in all cases to several percents or to zero at the end of the experiment.

Two hours after the cell mixing the ratio of the three kinds of pairs approximated 1:2:1 (Table 1). Only clone no. 84 showed lower ability to form homotypic pairs in the mixture with clone no. 83 (Table 1).

It may be suggested that heterotypic pairs are more stable in mixtures than homotypic ones. At early steps of conjugation heterotypic pairs disintegrate with lesser probability than homotypic pairs. As a result, the number of heterotypic pairs increases and the number of homotypic pairs decreases with time. If this is true, the number of homotypic pairs may be expected to increase in unbalanced mixtures where one clone is in excess. Indeed, in most cases the number of homotypic pairs in unabalanced mixtures was greater than that in the first series of experiment and they

Table 1

The number of homo- and heterotypic pairs formed in mixture of the *D. margaritifer* complementary clones

Pairs of clones	The number of homotypic marked (A), heterotypic (B) and homotypic unmarked (C) pairs observed in cell mixtures at different times after the beginning of the experiment					
	2h			5h		
	A	B	C	A	B	C
83* + 52	5	16	6	4	110	3
83 + 52*	7	16	7	6	120	0
84* + 52	2	5	2	1	120	7
84 + 52*	1	2	2	0	75	6
84* + 83	2	22	9	1	145	2
84 + 83*	8	11	2	1	120	1

* Asterisks indicate clones marked with Chinese ink

disintegrated more slowly. However, the total number of homotypic pairs in the second series of experiments varied for different cases and for different combinations of complementary clones. Figure 3 presents most dissimilar situations. As in the first series of experiments, the common feature of homotypic pairs in the second series was their instability. In all experiments the number of homotypic pairs invariably decreased 5-6 h after the mixing of clones.

To explain the results, the following suggestion may be proposed. At early steps of conjugation of *D. margaritifer* all kinds of pairs are formed due to mating type nonspecific adhesines expressed on the cell surface. Their expression is the result of treating cells with heterotypic (non-self) mating pheromones. It is the clone- and mating type nonspecific nature of hypothetical adhesines that makes the ratio of pairs at early steps of conjugation close to 1:2:1. The concentration of mating pheromones in zones of cell contacts may be very high compared to that in culture medium. If so, cells in heterotypic pairs continue to stimulate each other with pheromones and, as a result, new portion of adhesines are expressed on the cell surface. That is why heterotypic pairs become stable. The homotypic pairs, on the contrary, cannot obtain a sufficient amount of adhesines on their surface. The cells in such pairs do not stimulate each other and pairs eventually dissociate.

It seems that many species of ciliates have only species-specific adhesines which were responsible for the pair formation during conjugation. The only exception is the *Paramecium* group where conjugation begins with mass agglutination of cells and where the

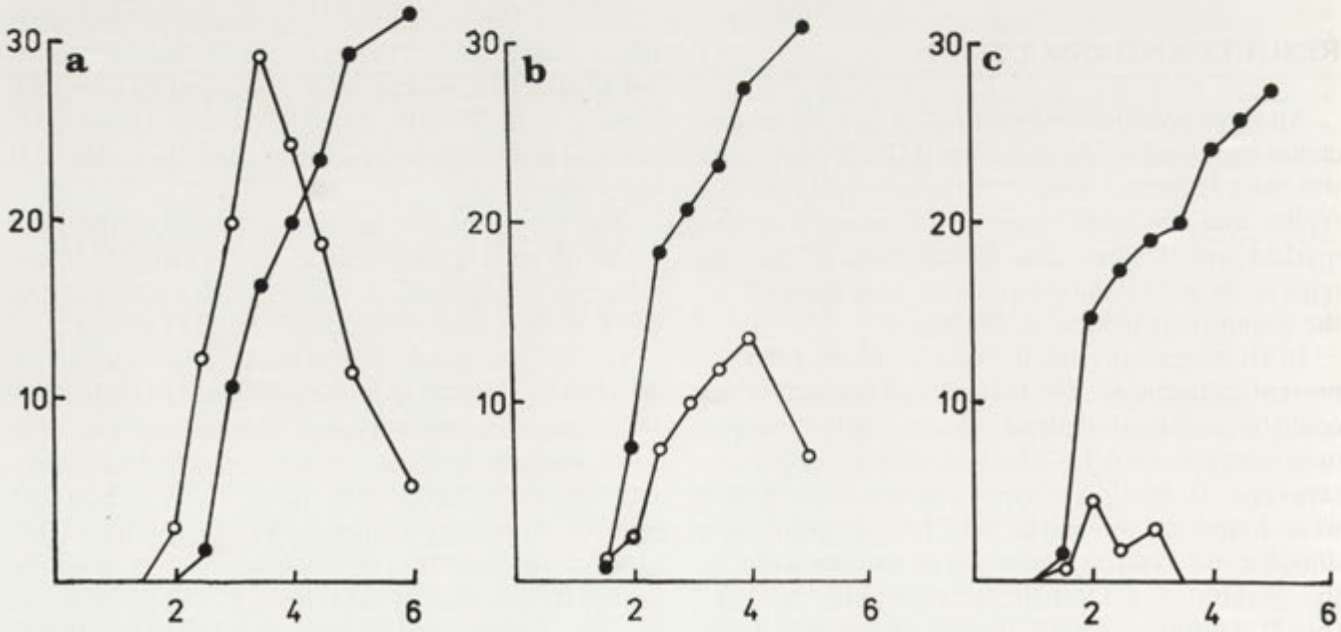

Fig. 3.The number of homo – and heterotypic pairs in unbalanced cell mixtures; a – clone no. 83 in excess, no. 84 few in number; b – no. 84 in excess, no. 83 few in number. Abscissa, hours from the begining of the experiments; ordinate, the number of pairs. Other designation as in Fig. 2

adhesines must be mating type-specific (the mating type substances). The limited portion of homotypic pairs is found during conjugation of *Euplotes patella* (Akada 1985 a, b), *E. raikovi* (Miceli et al. 1981), *E. octocarinatus* (Heckmann and Kuhlmann 1986), *E. charon* (Valbonesi et al. 1987). The hypothesis of mating type - nonspecific nature of the adhesive molecules seems to contradict the fact that only heterotypic pairs are formed during conjugation of *E. minuta* (Nobili 1966, Dini and Luporini 1985), *Glaucoma scintillans* (Nakata 1969) and *Tetrahymena thermophila* (Nanney 1977). More detailed analysis of the data shows, however, that in *Tetrahymena* homotypic pairs do appear at the early steps of conjugation, but they are unstable (Kitamura et al. 1986). It is probable that in other cases homotypic pairs were not registered due to their instability in time.

Another mechanism of pair formation is supposed from the study of homo- and heterotypic pair formation in *Euplotes patella* syngen 2 (Akada 1985 b). The author assumes that the different mechanisms are involved in the homo- and heterotypic pair formation. The suggestion is based on differential influence of $[Ca^{2+}]$ on the proportion of the two kinds of pairs observed in the mixtures of complementary clones. However, the author noted that the increased Ca^{2+} concentration in the medium (0.4 mM) induces selfing in one of the clones (Akada 1985 b). Taking this fact into consideration, it seems logical to suggest that reception or transduction of the mating pheromone signal is under the influence of Ca ions. The former induces the expression of hypothetical species-specific adhesines.

It may be supposed that the evolution of "information molecules" was involved in the evolution of mating types in some ciliates. These molecules are represented either by mating pheromones (Luporini and Miceli 1986) or by cell surface-bound molecules. In these two cases the adhesines retain only their species- specificity. In *Euplotes crassus* which does not excrete mating pheromones, substances of the "first generation" were initiated by the former ones and they may be the candidates for mating type nonspecific adhesines (Dini and Miyake 1977).

In *D. margaritifer* the whole situation seems to be similar. The "information molecules" are represented in this species by excreted proteins with Mw 3000-4500 Da (Afon'kin et al. 1988, Parfenova et al. 1989). No information is at present available on adhesines molecules. It is only known that their expression is independent of the kind of heterotypic pheromones in the medium (Afon'kin and Skovorodkin 1987). The same result was obtained in *Oxytricha bifaria* (Esposito et al. 1976).

In nature, clones of dilepti with a high level of pheromone induced-adhesive expression may be found. This is the probable reason for successful selfing in clones nos. 11 and 20 induced by heterotypic pheromones (Yudin and Afon'kin 1987). In these clones, the intraclonal conjugation results in viable exconjugant clones. It may be suggested that the level of adhesive expression in these clones is sufficient for the formation of tight pairs and subsequent nuclear reorganization. On the contrary, the pheromone-induced expression of adhesines in the clone no.84 seems to be low. Just this feature may cause low number of homotypic pairs in unbalanced mixtures with this clone. The number of homotypic pairs in unbalanced mixtures or the percentage of cells which successfully complete selfing under the influence of heterotypic mating pheromones may be used as an index of adhesive expression intensity in various clones of dilepti.

Acknowledgements. The author is indebted to Dr. A.L. Yudin for advice during critical reading of the manuscript.

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Received on 26th October, 1989.

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 99-102

Histochemical Investigation of the Synthetase of Prostaglandin in Pathogenic and Non-pathogenic Strains of *Acanthamoeba castellanii* and Infected Host Tissue

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Summary. The objective of the study was a histochemical analysis of the activity of prostaglandin synthetase in the trophozoits of the amoebae cultivatet in vitro as well as in vivo in the tissues of infected animals. No important differences were found between the pathogenic and non-pathogenic strains as far as the activity and localization of synthetase in the amoebae cultivated in vitro are concerned. The higher activity of sythetase in the amoebae in relation to the activity of the host's tissue, found in vivo, may point to the participation of prostaglandins in the amoebae's pathogenicity.

Key words. Prostaglandin, *Acanthamoeba castellanii*.

INTRODUCTION

With particular respect to arachidonic acid, unsaturated fatty acids of the length of the chains C-18 to C-24 play a vital role in organisms. Freed from phospholipid membrane, these acids – helped by phospholipase A – lead to the creation of prostaglandins (PGs), which are compounds bearing characteristics of tissue hormones. The synthesis of prostaglandins is begun by a multi-enzymatic microsomal complex, that is, by the synthetase of prostaglandins (E.C. 1.14.99.1). In the synthesis a crucial role is played by cyclical peroxides of prostaglandins G and H (PGG and PGH) which are direct precursors of prostaglandins. The synthesis of prostaglandins in cells may be provoked by such factors as: neuro-hormonal stimulation, hormones, mechanical and chemical stimulations as well as by the activity of pathogens. The retardation of the biosynthesis of prostaglandins follows the application of the agents of acetylosalicylic acid and its derivatives, and also of steridic hormones. Synthetized prostaglandins are immediately freed without accumulation and their period of semi-duration is very short. The synthesis of prostaglandins is localized in the cells and tissues of many organs as well as in the cells of some micro-organisms. The fundamental importance of prostaglandins and the broad sphere of their activity led us to investigate the presumable mechanisms of the impact of the prostaglandins in parasites on the host's organism (Hadaś 1988a).

The immediate target of the present investigation was to carry out a comparison in vitro of the activity of prostaglandin synthetase in the cells of the non-pathogenic (Neff) and pathogenic (309) strains of

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Acanthamoeba castellanii and to apply histochemical techniques in examining the lungs of the infected mice in vivo.

MATERIALS AND METHODS

Strains: The following strains of *Acanthamoeba castellanii* were examined: strain 309, pathogenic for mice, isolated from its natural environment (Kasprzak and Mazur 1982); non-pathogenic strain Neff, isolated from its natural environment (Neff 1957).

Culture: The amoebae were cultivated at a temperature of 24°C in 100 ml bulbs containing 25 ml medium (Cerva 1966, 1969) composed of 2% cassein hydrolizate Bacto-Casaitone (Difco) and 10% horse's serum. In cytochemical examinations microscopic slides grown with amoeba were put into the bulbs of the cultures.

Infecting the animals: Mice (strain Swiss) weighing 10-15 g were infected intranasally by administering a suspension of 10.000-20.000 pathogenic amoebae of strain 309 of *Acanthamoeba castellanii* from a liquid 4-day-old culture.

Histological investigations: Lungs obtained from dead mice on the 9-11th day post infection were frozen in a mixture of dry ice, ethanol and isopentan in -70°C. Sections of 5 μm thick were cut in cryostat at -25°C and fixed in ethanol-acetic acid mixture (97.2 – 2.5 v/v).

Localization of prostaglandin synthetase in cells: The amoebae growing on slides were fixed for 20 minutes and then rinsed several times with the cacodylic buffer (Karnovsky 1965). Histochemical reaction on the prostaglandin synthetase was performed by incubating the cells in a staining mixture containing 100 μM arachidonic acid, 1 mM 3,3'-diaminobenzidine, 2 mM potassium cyanide in 100 mM Tris-HCl buffer pH 8.2 (Ledwozyw et al. 1986). The reaction was stopped by rinsing the cells with water and then with cacodylic buffer. The samples were embedded in 2% gelatin in 50% glycerin. The controls were stained by a mixture without arachidonic acid or mixture with lmM acetylosalicylic acid as synthetase inhibitor.

Localization of the amoeba and prostaglandin synthetase in tissue: Both the localization of the amoebae and the activity of prostaglandin synthetase were determined in lungs from infected mice according to the above mentioned method. To accurately localize the amoebae, the lungs were stained with hematoxylin and eosin (H+E) as and also after Gomori's method (Burck 1975).

RESULTS

The cytochemical examination of prostaglandin synthetase activity in a pathogenic (309) strain of *Acanthamoeba castellanii* is presented in Fig. 1. In the cytoplasma of the amoebae numerous, tiny, brown granular products existing on the site of the enzyme's activity were discovered. No spots of particularly high activity of the enzyme in the cytoplasm of the amoebae were detected; neither were differences in the localization and activity of synthetase between pathogenic (309) and non-pathogenic (Neff) strains of *Acanthamoeba castellanii* observed. In the lungs stained with hematoxylin and eosin or trichrome we found that pulmonary vesicles were enlarged and that they contained numerous infiltrating foci of single-nucleus cells and individual leukocytes. The amoebae were located mainly on the margin of the abscesses. We also discovered some amoebae and single leukocytes in the lumen of bronchi (Fig. 2). In the lungs stained for activity of prostaglandin synthetase, a strong reaction in the amoebae and weak reaction in single leukocytes were found (Fig. 3). In the walls of the bronchi a mean diffusive reaction to the presence of prostaglandins syntethase was found (Fig. 4). No extensive activity of

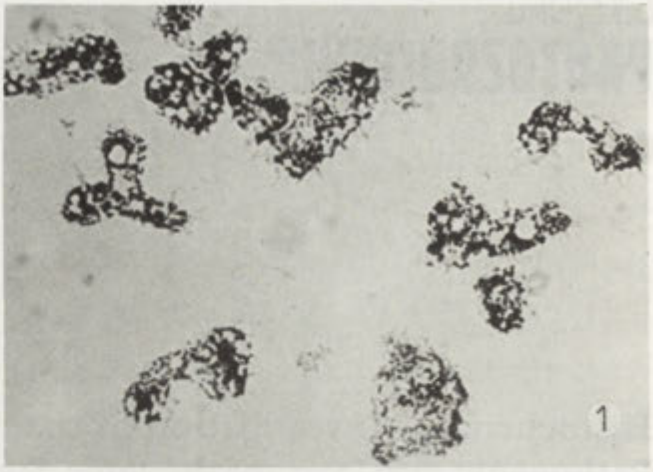

Fig. 1 Cytochemical examination of prostaglandin synthetase activity in a pathogenic strain (309) of *Acanthamoeba castellani*; × 400

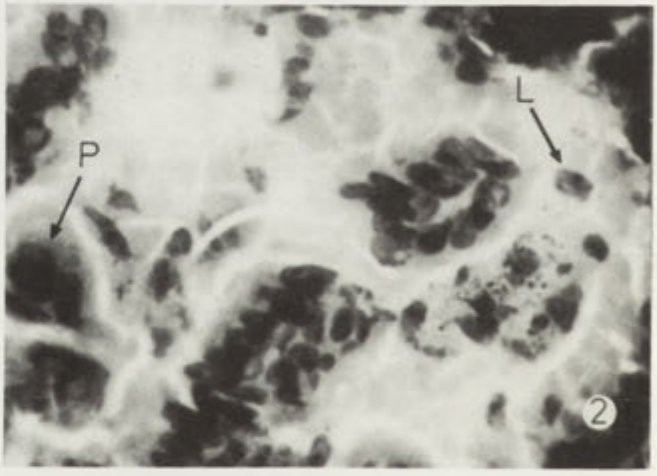

Fig. 2 Lungs stained by the Gomori method; × 150 P – amoebae, L – leukocytes

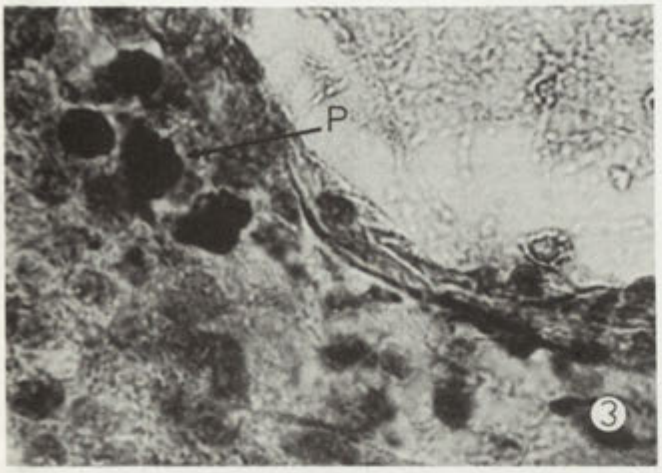

Fig. 3 Histochemical reaction of prostaglandin synthetase activity in the lungs of an infected mouse; × 150 P – amoebae

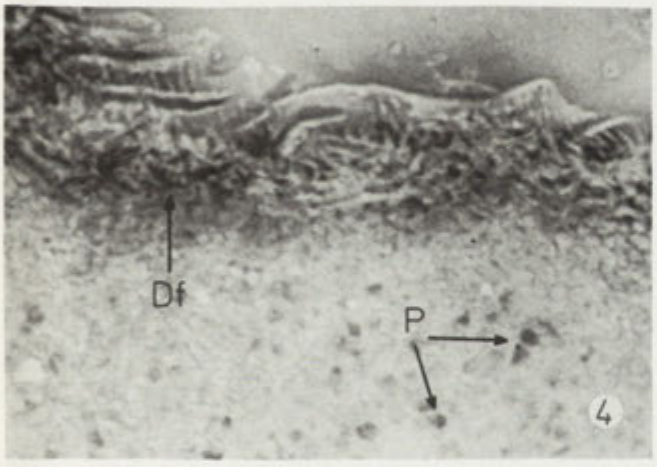

Fig. 4 positive diffused reaction of prostaglandin synthetase activity localzed around bronchi and pulmonary vesicles; × 50
Df – diffusive reaction, P – amoebae

the synthetase of prostaglandins was found in the lungs of non-infected mice. Control staining with acteylsalicylic acid for the activity of the synthetase of prostaglandins was negative.

DISCUSSION

The amoebae of *Acanthamoeba* species may cause granulomatosis inflammation in men and animals. Inflammation, which belongs to the sphere of the oldest medical notions, was only until a short while ago investigated almost entirely in morphological terms. Only recently locally acting mediators were discovered and that led to the explanation of the mechanisms of the symptoms of inflammation. Prostaglandins (PGs) are, among others, such mediators of inflammation and prostaglandins E and F play the main role in this process. In comparison with other known compounds, prostaglandins as mediators of inflammation and activators or inhibitors of immunological reactions are characterized by their ability to provoke a biological effect by an extremely small dose (Horrobin 1978). Determination of the factors that facilitate infiltration and breeding of the free-living amoebae in the tissues of the host and which condition the period of the amoebae's resting in the tissues poses a vital problem in parastiological research.

It is well known that the synthetase of prostaglandins is an enzymatic complex that catalyzes 3 reactions: lipoxygenation, cyclooxygenation and peroxidation (Robak and Kasperczyk 1979). The method used in the present study for localizing the activity of synthetase is based on histochemical determination of the reaction of peroxidation of endoperoxides PGs (Janszen and Nugteren 1971, Ledwozyw et al. 1986). In contrast to biochemical methods that necessitate the use of abundant material, this method is extremely sensitive and enables detection of the enzyme even in single cells.

The results of cytochemical investigations proved that there are no essential differences in vitro as far as the activity and localization of the synthetase of prostaglandins between the pathogenic (309) and non-pathogenic (Neff) strains of *Acanthamoeba castellanii* are concerned. The same was proved by earlier biochemical studies (Hadaś 1988b).

All these findings lead us to presume that the amoebae's ability to penetrate the host's tissues may depend on the potential capability for biosynthesis of proper prostaglandins rather than on the quantity of prostaglandins produced (Hadaś 1988c). Proved in vivo, the higher activity of the synthesis of prostaglandins in the amoebae in relation to the surrounding tissue and the amoebae's ability to move quickly in the host's tissue may enable the amoebae to avoid the host's defensive reactions and to stay in its tissue. The inflammatory reactions in the host's tissue may, on the one hand, be the result of the biochemical processes that take place when the host's tissue is damaged by invasion of the amoebae, and on the other, they may be the symptom of the host's immunological defence (Callahan et al. 1988).

The above description of the histochemical method

of investigating the localization of the synthetase of prostaglandins offers a handy tool for detecting the presence of the enzyme in cells and for observing the changes that take place in the host's tissue invaded by parasites.

As none of the previously published studies seem to have utilised the above method, the present work is perhaps the first to have used this histochemical technique to establish the activity of the synthetase of prostaglandins in parasitological protozoa and to examine the reactions present in host-parasite relationship.

Acknowledgements. The authors wishes to thank Dr. L. Gustowska from Department of Clinical Pathomorphology of Academy of Medicine in Poznań for her cooperation in this study.

This study was supported by grant CPBP 04.07-II.6 from the Stefański Institute of Parasitology, Polish Academy of Sciences, Warszawa.

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Received on 17th April, 1990.

ACTA
PROTOZOOLOGICA

Acta Prozoologica (1991) 30: 103-106

Anti-oxidant Enzymes of *Acanthamoeba castellanii* Strains

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Summary. The aim of this study was to compare in vitro and in vivo activities of some enzymes of anti-oxidant specificity in various strains of *Acanthamoeba castellanii*. The survey has shown that peroxidases can act as an agent protecting the amoeba from being eliminated from the host and that it may be a manifestation of the amoebae's pathogenicity. It has also shown that catalase acts as an element protecting the amoebae from the destructive activity of oxygen in their natural environment.

Key words. *Acanthamoeba castellanii*, enzymes.

INTRODUCTION

The ability of amphizoic amoebae to invade a host is connected with many agents. Apart from temperature barrier, they have to overcome a number of obstacles whose role is to eliminate them. One of the agents is the process of phagocytosis during which free radicals and peroxides are produced. The amoebae can be protected from the products of phagocytosis by such enzymes of anti-oxidant and poison counter-acting properties as peroxidase, catalase and superoxide dismutase.

This survey has to compared the activity of catalase and peroxidase to determined the isoenzyme composition of peroxidase in various strains of *Acanthamoeba castellanii* as well as examined the localisation of the activity of peroxidase in the amoebae cultivated in vitro and in tissue of an invaded host. The examination also attempted to defined the amoebae's ability to eliminate the oxidants produced by the host during the invasion and to establish the degree of participation of the examined enzymes the pathogenicity of the amoebae.

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MATERIALS AND METHODS

The following strains of *Acanthamoeba castellanii* were examined: strain 309, pathogenic for mice, isolated from natural environment (Kasprzak and Mazur 1972); pathogenic strains 2094 and 2119, freshly isolated from the environment; non-pathogenic strain Neff, isolated from natural environment (Neff 1957). The amoebae were cultivated at a temperature of 24°C in 100 ml flasks containing 25 ml medium (Cerva 1966, 1969) composed of 2% casein hydrolyzate Bacto-Casitone (Difco) and 10° normal horse serum. In cytochemical examinations microscopic slides grown with amoeba were put into the cultures flasks. Amoebae from 4-day-old cultures were centrifuged at 900 x g and then rinsed with distilled water. The precipitat was suspended in the extraction buffer (0.1 M potassium-phosphate with pH 7.0) at the ratio of 1:4 and homogenized in 4°C. The homogenates were centrifuged for 30 minutes at 15,000 x g. The supernatant was used for enzymatic and electrophoretic examinations after having been stored in ice up to the moment of determination. The content of protein in the supernatants from the amoebae was determined according to the colorimetric method of Lowry et al. (1951).

The enzymatic activity of peroxidase and catalase was determined in the supernatants from the amoebae by using spectrophotometric methods (Putter 1974, Aebi 1974).

The electrophoretic mobility and isoenzyme composition of peroxidase were determined by using specific staining after the electrophoretic separation of the protein from the supernatants. Disc electrophoresis of proteins was carried out according to Davis and Ornstein method (1959). It was accomplished under the influence of the substrate in presence of benzidine or 3'3-diaminobenzidine (DAB) in the spot in which the enzyme occurred (Maurer 1971). The staining was conducted for 10-90 minutes at room temperature.

For histochemical investigations mice (strain Swiss) weighing 10-15 g were infected intranasally by administering a suspension of 10,000-20,000 amoebae (*Acanthamoeba castellanii* - strain 309) from a liquid 4-day-old culture. Lungs obtained from dead mice on the 9th-11th day post infection were frozen in a mixture of dry ice, ethanol and isopentane at -70°C and cut into 5 μm scraps in cryostat at a temperature of -25°C. Some scraps were fixed in the mixture of ethanol (97.2 ml) and acetic acid (2.5 ml), and some were left not fixed.

The activity of peroxidase was localized in the cells of the amoebae growing on microscopic slides and in the lungs of dead mice. The amoebae growing on slides were fixed for 20 minutes and rinsed several times with the cacodylic buffer (Karnovsky 1965). Histochemical reaction on peroxidase was performed by incubating the slides and scraps in a staining mixture containing 1 mM DAB, 0.05% hydrogen peroxide in 0.2 M Tris-HCl buffer pH 7.6. The reaction was stopped by rinsing the slides with water and then with cacodylic buffer. The specimens were embedded in 2° gelatin in 50° glycerol. The control specimens were stained by a mixture without hydrogen peroxide. For accurate localization of the amoebae, the scraps were stained with hematoxylin and eosin (H + E) as well as according to Gomori's method (Burck 1975).

RESULTS

The results of the specific activity of the peroxidase and catalase activity in the examined strains of *Acanthamoeba castellanii* are presented in Table 1. It was found that the average specific activity in the non-pathogenic strain was 0.117 IU/mg of protein, whereas in the pathogenic strains it ranged between 0.153 to 0.204 IU/mg of protein. The average specific activity of catalase in the examined strains ranged between 79.73 to 99.56 IU/mg of protein. Statistical analysis showed significant difference in the activity of peroxidase between the pathogenic and non-pathogenic strains of *Acanthamoeba castellanii*, but showed no significant difference in the activity of catalase.

Table 1

Specific activity of peroxidase and catalase in different strains of *Acanthamoeba castellanii*. The activity is expressed in IU/mg of protein (mean ± SD, $n^{max}7$)

Strain	Peroxidase	Catalase
Neft	0.117 ± 0.021	93.70 ± 17.29
309	0.153 ± 0.025	79.73 ± 18.99
2094	0.167 ± 0.026	99.56 ± 18.20
2219	0.204 ± 0.030	93.84 ± 16.40

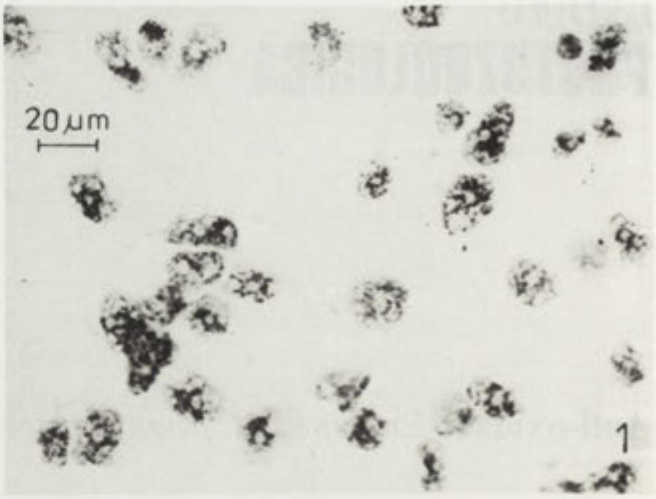

Fig. 1. The activity of peroxidase in the cells of pathogenic strain (309) *Acanthamoeba castellanii*

Determining the isoenzyme composition of peroxidase showed one enzymatic fraction of identical electrophoretic mobility Rf = 0.27 in the strains of *Acanthamoeba castellanii*.

No difference in the localization of peroxidases in the cells of the amoebae were found. Peroxidase activity in pathogenic (309) strain of *Acanthamoeba castellanii* is presented in Fig. 1. In the cytoplasma of the amoebae numerous, tiny, brown granules of reactions products were found.

In the specimens of lungs we found that pulmonary vesicles were enlarged and that they contained numerous infiltrating foci of single-nuclear cells and individual leukocytes. We also discovered some amoebae and single leukocytes in the lumen and on the rim of bronchi. In the examined specimens of lungs we found a strong activity of peroxidase in the amoebae and weak activity in single leukocytes (Fig. 2). We also found a positive diffusive reaction in the walls of the bronchi (Fig. 3).

DISCUSSION

Catalase and superoxide dismutase are widespread in animals, however, peroxidases are not; they are

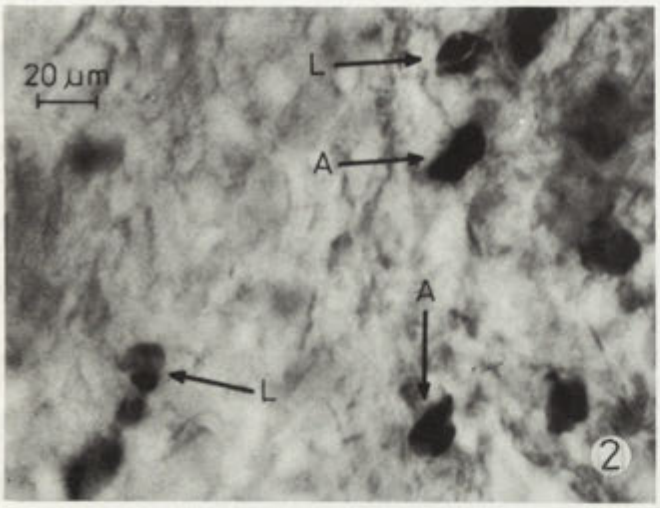

Fig. 2. The activity of peroxidase in the tissue of mouse lung; A – amoebae, L – leukocytes

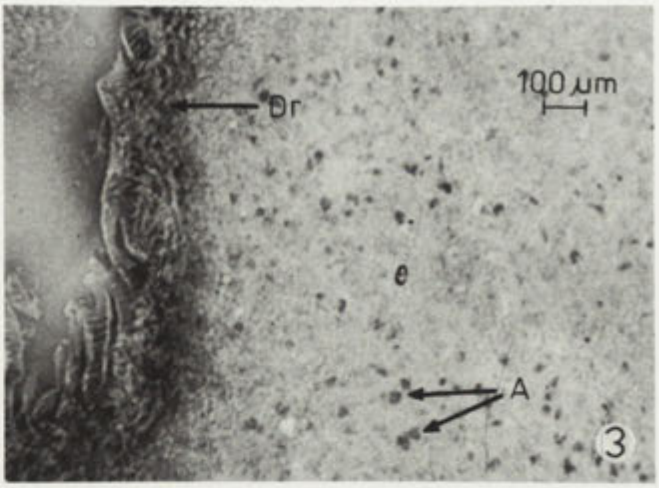

Fig. 3. The activity of peroxidase in the tissue of mouse lung; Dr – diffusive reaction, A – amoebae

much more prevalent in plants. Callahan et al. (1988) proposed that all helmints and parasitic protozoa might contain at least one of the main anti-oxidant enzymes such as: superoxide dismutase, catalase or peroxidase. Catalase and peroxidase in protozoa has been found only in *Leishmania tropica, Leishmania donovani, Toxoplasma gondi* and *Plasmodium berghei* (Callahan et al. 1988). Superoxide dismutase, however is present in many species, including *Leishmania tropica* and *Leishmania donovani* (Murray 1981), *Toxoplasma gondii* (Sibley et al. 1986), *Trichomonas foetus* (Kitchner et al. 1984) *Trypanosoma cruzi* (Boveris et al. 1980), *Entamoeba histolytica* (Mehlotra and Shukla 1988) and in the amoeba of *Naegleria* species (Pernin et al. 1985), among others. These enzymes play an important role in protecting the parasites against the destructive influence of free radicals and peroxides which: (1) are produced by the host during normal metabolism; (2) are induced by some anti-parasitic drugs; (3) are excreted during immunological and biochemical reactions in response to the presence of a parasite.

Phagocytosis is one of the reactions that results in the production of peroxides. During this process hydrogen peroxide is created via reactions catalysed by xantine, glucose or polyamine oxidase. Later on, hydrogen peroxide enters into the Haber-Weiss reaction producing singlet oxygen ($^1O_2$) as well as hydroxyl radicals (-OH) that damage or destroy the parasites. Damaging can also occur with the help of other radicals which are produced not exclusively in immunological reactions, but not via the Haber-Weiss reaction. Biosynthesis of prostaglandins and leukotrienes is the process during which oxidants are created (Zaorska 1986, Hadaś 1988). Leukotrienes destroy the cells of the parasites whereas prostaglandins act indirectly, activating the host's immunological system, phagocytosis included. Damaging activity of the products of the host metabolism or phagocytosis can be neutralized by peroxidation, among other factors. It is presumed that it constitutes the main mechanism in defending the parasites against being eliminated from the host organism.

The scrutiny permit a supposition that a higher activity of peroxidase (30-100° higher) in the pathogenic strains of *Acanthamoeba castellanii* in comparison with the non-pathogenic strain, as well as higher activity of peroxidases in the amoebae in proportion to the invaded tissue and phagocytic cells, protect the amoebae against being eliminated from the host by neutralizing the activity of free radicals and peroxides. They also render possible the survival of the amoeba and their proliferation in the host's tissues. In addition, electrophoretic survey testifies to the fact that the activity of peroxidase in an indicator of the pathogenicity of the amoebae and not the result of the differences in its isoenzymatic composition. On the other hand the almost identical activity of catalase in the examined strains indicates that it plays a role in protecting the amoebae in their natural environment against the damaging work of oxygen. However, the above presented results of our investigations do not

exclude the possibility of other anti-oxidant enzymes or a set of enzymes participating in defending the amoebae against the damaging effects of oxidants in the host's organisms. Thus, investigating anti-oxidant enzymes we call get closer to understanding of the mechanisms in the parasite-host relationship.

Acknowledgements. The author wishes to thank Dr. L. Gustowska from Department of Clinical Pathomorphology Academy of Medicine in Poznań for her cooperation in this study.

This study was supported by grants CPBP 04.07-II.6 and CPBP 04.07-III.10 from the Stefański Institute of Parasitology, Polish Academy of Sciences, Warszawa.

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Received on 21st August, 1990

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 107-113

Impact of Hormonal Imprinting on the Hydrolysis Time of the Feulgen Reaction in *Tetrahymena*. Do Nuclear Level Changes Appear after Imprinting?

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Summary. The macronucleus of normal and insulin pretreated (imprinted) *Tetrahymena pyriformis* and *Tetrahymena malaccensis* has been studied under the effect of insulin treatment by using Feulgen reaction. The intensity of the reaction was different in the normal and imprinted cells. The duration of hydrolysis disparately influenced the Feulgen reaction in the normal and insulin imprinted cells. The experiments render probable the nuclear effect of hormonal imprinting.

Key words. *Tetrahymena pyriformis*, hormonal imprinting, macronucleus.

INTRODUCTION

Hormonal imprinting occurs at primary interaction of a hormone with its target cell, and takes place at both ontogenetic and phylogenetic level (Csaba 1980, 1981). Hormonal imprinting alters considerably several life functions of the ciliated unicellular *Tetrahymena*. For example, imprinting with insulin changes the number of receptors (18) and the level and activity of certain second messenger systems (Ca^{2+}; calmodulin; guanylate cyclase; cAMP) (Csaba and Nagy 1976, Kovács and Csaba 1987, Kovács et al. 1989), and the phagocytic activity, growth rate and PAS-positive material content of the unicellular as well (Csaba and Kovács 1979). The effect of hormonal imprinting persists long, over several hundred generations of *Tetrahymena* (Csaba et al. 1982). Since hereditary transmission of this kind is presumably associated with a nuclear-level mechanism, the present studies were performed to obtain more information on the problem.

The mechanism of action of the laboratory diagnostic test known as the Feulgen reaction (Feulgen and Rosenbeck 1924) was originally described as follows: The weak hydrolytic activity of hydrochloric acid splits first the phosphodiester bonds of DNA, and secondly the glycosidic bonds between sugar and purine bases. Deprived of purine bases, the deoxyribose residues (so-called apurinic acid) transform to furanose, whose free aldehyde groups interact with the Schiff reagent. Further studies have demonstrated that two changes take place in the course of acid hydrolysis, in that first the purine bases are split from the deoxyribose molecule, then the histones and apurinic acid leave the DNA molecule. It follows that the intensity of the associated colour reaction (which is suitable for cytophotometric analysis) depends on the duration and the temperature of hydrolysis (Pearse 1968). Moreover, there is reason to postulate that the intensity of the colour reaction also depends on the quality and quantity of the

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histones involved (Bohm and Sandritter 1966) demonstrated that intact cell lines differed from tumor cells in sensitivity to acid hydrolysis.

The macronucleus of *Tetrahymena* contains histones (Gorovsky 1970), and its nucleosomal structure resembles that of the eukaryotic cell components of multicellular organisms (Gorovsky 1970, Gorovsky et al.1978). We speculated that certain environmental effects may probably induce eu- or heterochromatinisation-like changes in the nuclear chromatin of *Tetrahymena*, and these changes might probably be indicated by the colour intensity of the Feulgen reaction.

Variations in the intensity of the Feulgen reaction could also indicate changes in the ploidity of *Tetrahymena* macronucleus. It is known that ciliates possess a polyploidic macronucleus, and the degree of ploidity (the DNA content) may change among others with the ageing of the cultures (Klass and Smith-Sonneborn 1976), or with alterations in body dimension or conditions of nutrition (Dupy-Blanc and Metenier 1975).

In the present study we investigated the sensitivity of *Tetrahymena pyriformis* GL (devoid of micronucleus) and *Tetrahymena malaccensis* macronuclei to acid hydrolysis in normal conditions and after insulin imprinting, with special regard to the impact of insulin on the intensity of the Feulgen reaction. Since the examined two *Tetrahymena* taxa responded differently to insulin imprinting in earlier studies (Kovács and Csaba 1987, 1989) it seemed worthwhile to compare their behaviour also in this respect.

MATERIALS AND METHODS

Tetrahymena pyriformis GL and *T.malaccensis* MP 75 strains, cultured in 0.1 per cent yeast extract containing 1.0 per cent Bacto tryptone medium (Difco, Michigan, U.S.A.) at 28°C, were used in the logarithmic phase of growth.

Hormonal imprinting was induced with 10^{-6}M insulin (Insulin Semilente MC; Novo, Copenhagen, Denmark) for 1 h. After treatment the cells were washed in plain medium and were returned to plain medium for 24 h. The control cultures were similarly treated, except for insulin preeposure.

Examination of the impact of the duration of hydrolysis

Part of the cells was not treated, to serve as control (a), part was treated with 10^{-6}M insulin for 1 h (b). Subsequently the cells were fixed in 4 per cent formalin solution (in PH 7.2 PBS), washed in two changes of PBS, hydrolysed with normal HCl for 5, 10, 20, 30 or 60 min at 60°C, and treated with the Schiff reagent.

Examination for effect of insulin treatment

The control and the imprinted cultures were divided into two subgroups, of which one was not treated, and the other was treated with 10^{-6}M insulin. The cultures of both subgroups were sampled after 10, 20, 30 or 60 min. The samples were fixed in formalin, hydrolysed with 1.0 N HCl for 20 min, and treated with the Schiff reagent.

Construction of histogrammes

The control and the insulin-treated cultures were assigned to two subgroups, of which one was not treated, and the other was treated with 10^{-6}M insulin for 60 min. This was followed by washing in PBS, fixation in formalin, hydrolysis in 1.0 N HCl for 20 min, and performance of the Schiff reaction. The values of 100 cells were recorded in each group.

Examination of *T. malaccensis* micronuclei

The micronuclei were counted in 1000 cells in each group treated as described in previous paragraph and the proportions of the cells containing 0, 1, 2, 3 or more micronuclei were expressed in percentual terms.

Assessment of the growth rate

The control and the insulin-imprinted cells were returned to plain growth medium after imprinting, and 24 h later each culture was assigned to two subgroups of which one was treated with 10^{-6}M insulin for 20 h, and the other was not treated. The cell counts were determined in a Fuchs-Rosenthal counting chamber after culturing for 20 h.

Evaluation and statistical analysis

The intensity of the Feulgen reaction was assessed with a Zeiss Amplival cytophotometer at 546 nm. The analogous signals of the cytophotometer were transformed to digital signals and were recorded in a Hewlett Packard HP 41 CX calculator for determination of mean values, standard deviation and significance of inter-group variations (by Student's t-test). Twenty cells (for histograms 100 cells) were evaluated in each group and each assay was done in three replicates. Determination of colour intensity was limited to the macronuclei of interphase cells (Fig. 1).

RESULTS

The control cells of both taxa showed peak intensity of the Feulgen reaction after acid hydrolysis for 30 min (Figs. 2A and B), and a considerable reduction in colour intesity (increase in permeability to light) after hydrolysis for 60 min. The insulin-imprinted cells behaved differently, inasmuch as colour intensity was

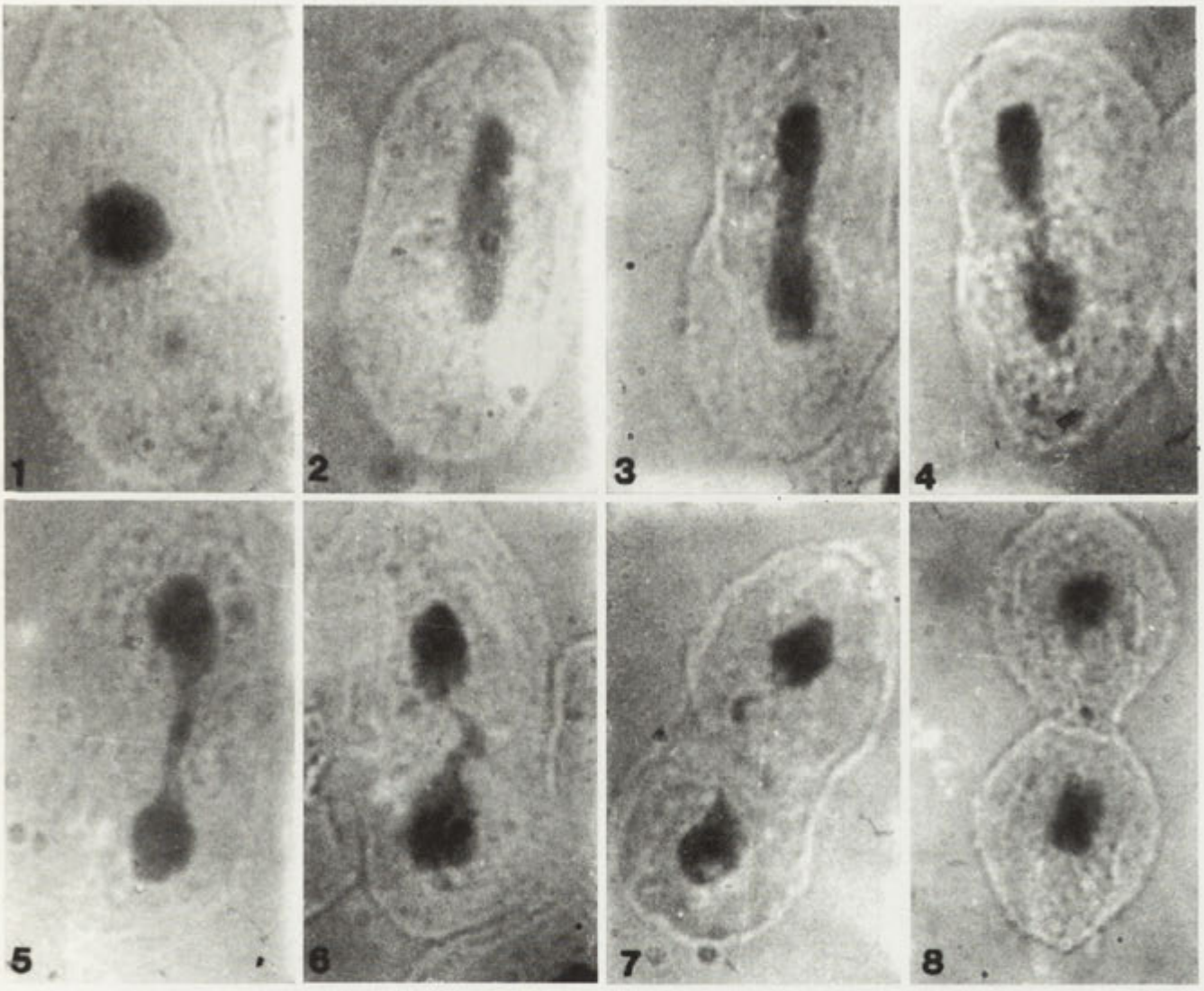

Fig. 1. Feulgen reaction of *Tetrahymena pyriformis* cells. Different stages of the cell cycle. The measurements were made on phase 1 cells. × 900

at peak after 20 min and 30 min hydrolysis, respectively, in the *T. pyriformis* and *T. malaccensis* cultures. The Feulgen reaction of the imprinted *T. pyriformis* cells differed significantly ($p < 0.01$) from the control after 20 and 30 min of hydrolysis, but did not appreciably differ from it after 60 min. The *T. malaccensis* cells still showed a significant ($p < 0.01$) difference from the control after 60 min; that taxon obviously yielded less readily to "overhydrolysis". Interesting to note that both taxa showed after hydrolysis for 20 min a significantly more intensive macronuclear reaction than the control cells.

The colour intensity of the Feulgen reaction changed differently in the control and imprinted cell populations after identical times (20 min) of hydrolysis (Figs. 3A and B). Treatment of the control cells with insulin accounted for a significant ($p < 0.01$) decrease in the intensity of the colour reaction after 30 and 60 min in the case of *T. pyriformis* and already after 10 min in the case of *T. malaccensis*. With *T. pyriformis*, the difference was greatest at 60 min, whereas with *T. malaccensis*, no appreciable change followed after 10 min.

The macronuclei of the imprinted *T. pyriformis* cells showed a significant ($p < 0.01$) increase in the intensity of the colour reaction already after 10 min reexposure

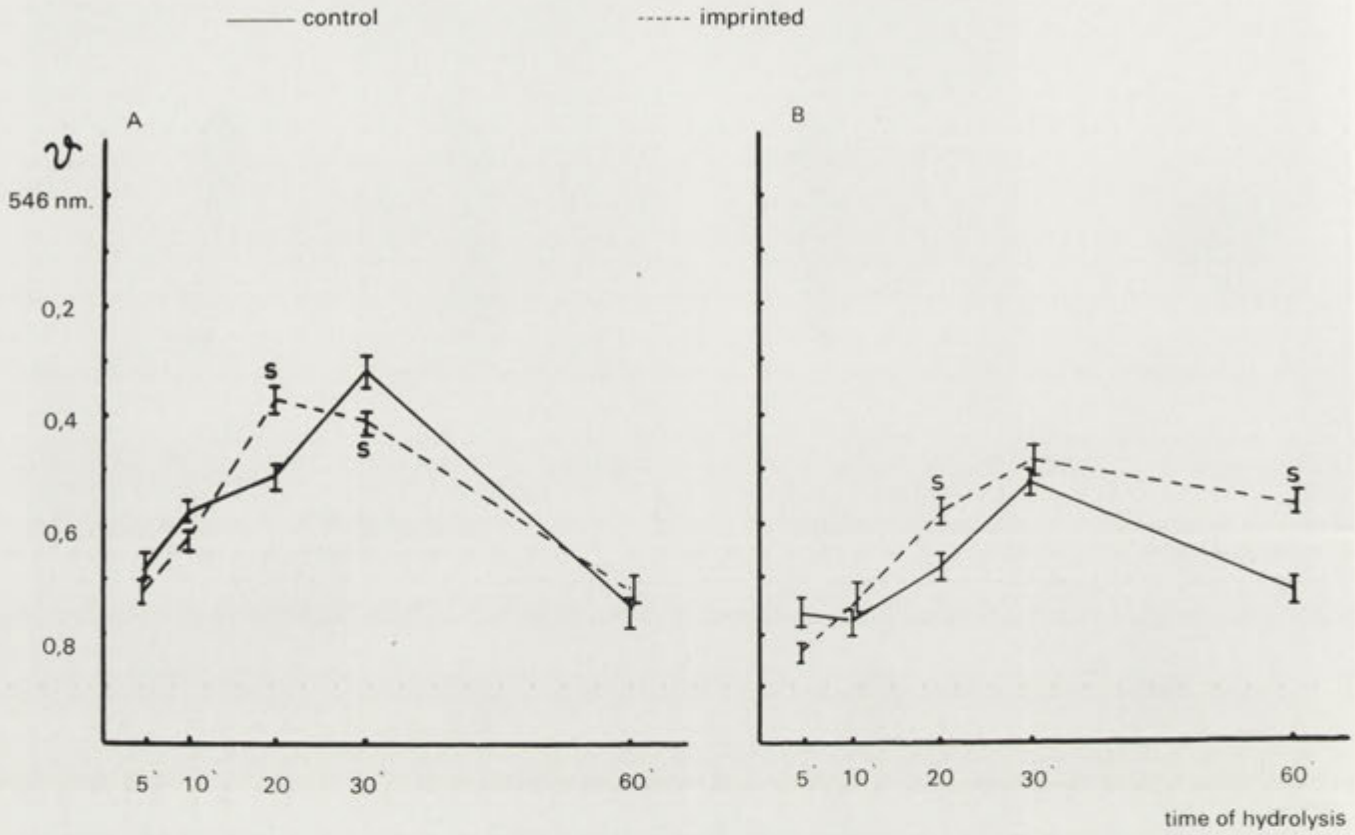

Fig. 2. Impact of the time of hydrolysis on the Feulgen reaction in *T. pyriformis* macronucleus (A) and *T. malaccensis* macronucleus (B). s = significant difference ($p < 0.01$) relative to the control

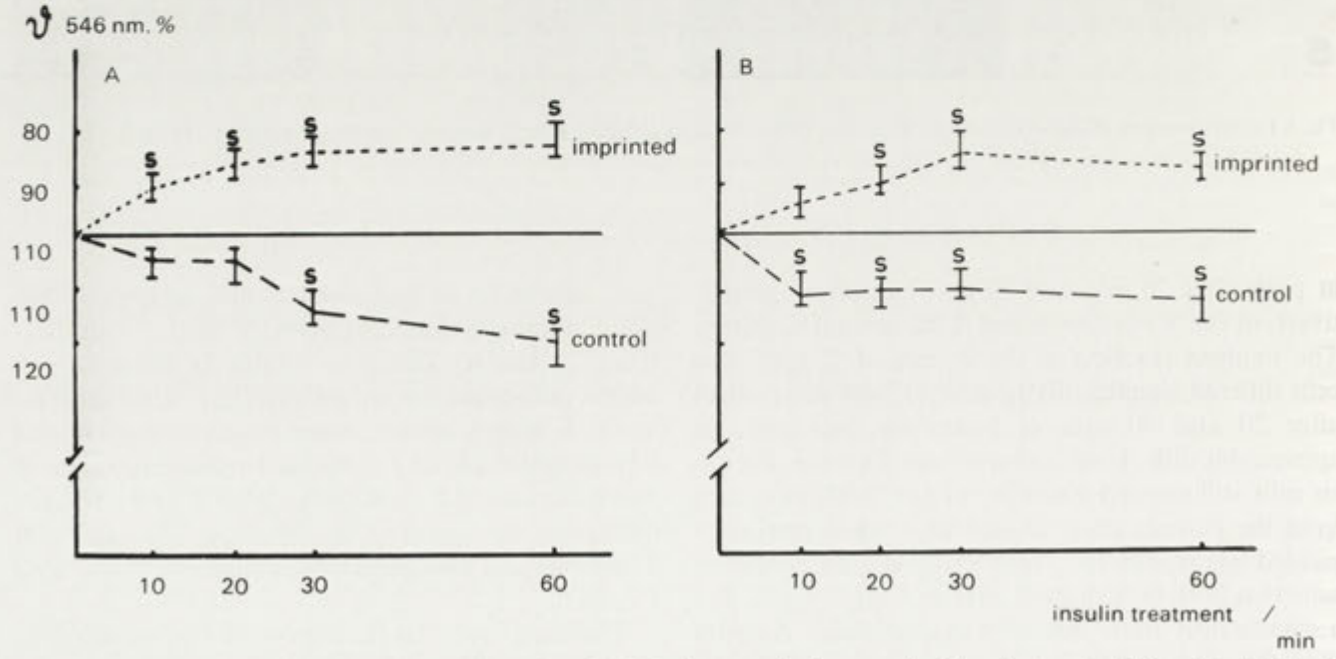

Fig. 3. Impact of the time of insulin exposure on intensity of the Feulgen reaction in control and insulin-imprinted *T. pyriformis* (A) and *T. malaccensis* (B) cells. 100% = cells not treated with insulin. s = significant difference ($p < 0.01$) from cells not exposed to insulin

Table 1

Percentual proportions of different micronucleus counts in control and insulin-imprinted *T. malaccensis* cells

	Micronucleus count/cell (%)				
	0	1	2	3	4
Control	5.01	61.01	27.69	5.8	0.34
Control + insulin	13.49	77.77*	8.73*	–	
Insulin – imprinted	–	67.24	31.89	0.86	–
Imprinted + insulin	3.03	80.00	16.96	–	–

* – $p < 0{,}01$ relative to cells not treated with insulin.

to insulin, whereas the imprinted *T. malaccensis* cells showed it after reexposure for 20 min.

This difference became expressed in the histograms, too. The slopes of the curves indicated that not so much the degree of ploidity as the sensitivity of DNA to hydrolysis was changed by reexposure to insulin for 60 min. (Figs. 4 and 5A and B).

Treatment with insulin for 60 min altered considerably the proportions of *T.malaccensis* cells with dissimilar micronucleus counts as shown in the Table 1.

Insulin treatment accounted for a decrease in micronucleus count in both the imprinted and not imprinted cell population, although in the latter to a lesser

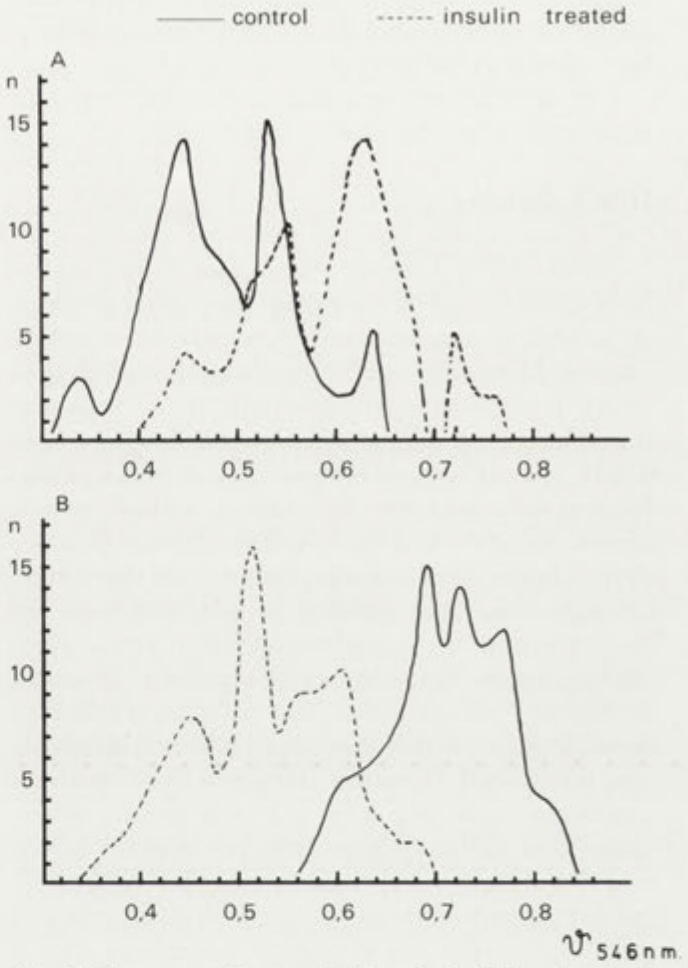

Fig. 4. Histogram of control (A) and insulin-imprinted (B) *T. pyriformis* cultures for intensity of the macronuclear Feulgen reaction in populations exposed to insulin for 0 min (control) and 60 min

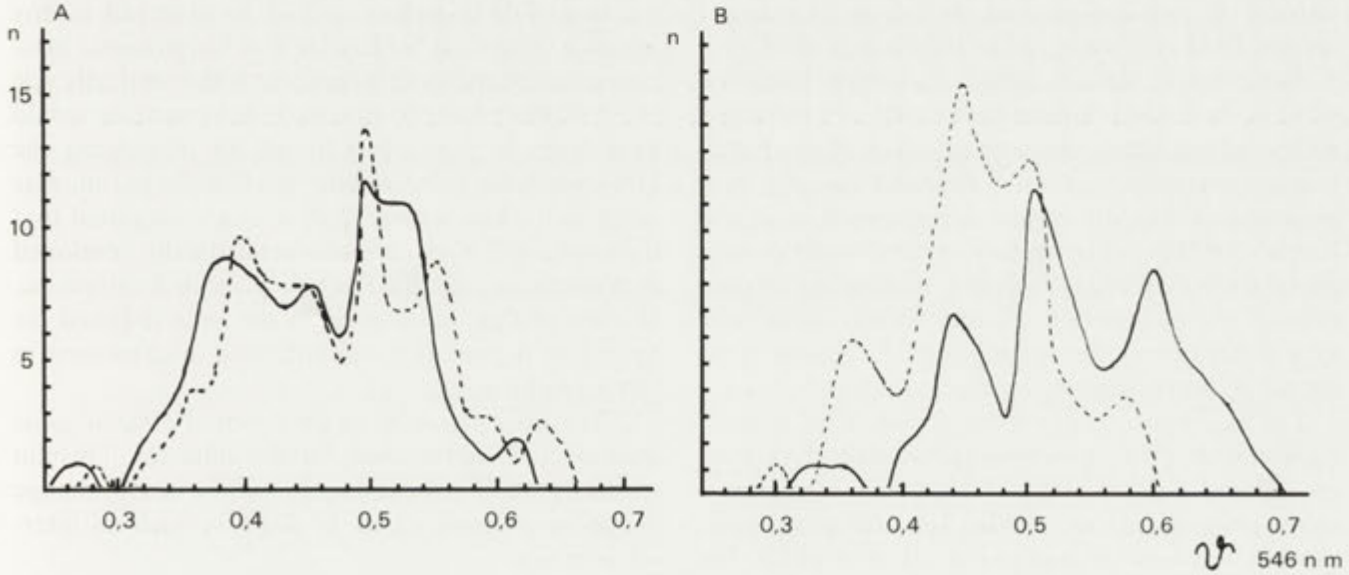

Fig. 5. Histogram of control (A) and insulin-imprinted (B) *T. malaccensis* cultures for intensity of the macronuclear Feuglen reaction in populations exposed to insulin fot 0 min (control) and 60 (symbols used same as in Fig.5)

degree. The percentage of cells with zero micronucleus count was considerably decreased by insulin imprinting relative to the control.

Cell division was not appreciably influenced by either one or two treatments with insulin.

DISCUSSION

Although the existence of hormonal imprinting has been substantiated by multiple experimental evidence, certain details of its mechanism and persistence are still obscure. Demonstration of the effect of imprinting in scores of offspring generations indicates a continuous transmission of the information to daughter cells (Csaba et al. 1982). Transmission can in principle take place at different levels, for example without genetic control, by analogy of the so-celled cytotaxis (Nanney 1985). The theory of clonal heredity implies that certain changes, which are induced in the membrane by environmental effects, are transmitted to the daughter cells during mitosis, and the new patterns so arisen induce the formation of similar patterns, which may persist long unless they represent a selection disadvantage for the cell. However, this attractive hypothesis cannot be reconciled with our experimental observation that although imprinting presupposes intactness of the membrane, second messenger system and cellular protein synthesis ((Kovács 1986), no injury to these mechanisms can extinguish imprinting after it has become established (Nozawa et al. 1985). In this light there is reason to postulate that the information associated with hormonal imprinting is, after mediation by the membrane and the second messenger system, fixed and preserved at the nuclear level.

Information transfer could also take place via secretion by the imprinted cells of a mediator substance which endows that nonimprinted cells with the potentials conveyed by imprinting. Evidence has also been presented in support of this hypothesis (Csaba and Kovács 1987), but this mechanism, too, would presuppose the involvement of nuclear control in the production of the information transfer factor. Thus both hypotheses permit the assumption of a nuclear level impact of hormonal imprinting.

The macronucleus of ciliates, the so-celled somatic nucleus, controls the processes taking place during cell growth and division. The macronucleus of ciliates is either polyploidic, or contains isolated gene copies capable of a selective duplication (Raikov 1982). The ploidity of the macronucleus, which is as a rule assessed relative to the DNA content of the diploidic micronucleus, varies greatly according to the literary data, and its value is 35 for cells of the *T. pyriformis* HSM strain (Raikov 1982). The macro and micronucleus play a different role in conjugation, in which the meiotic division of the latter plays the leading part,while the former becomes degraded (Allen and Gibson 1973).

We demonstrated earlier that the development and quality of imprinting differ between *Tetrahymena* taxa possessing (*T. thermophila, T. malaccensis*) and not possessing a micronucleus (*T. pyriformis*) (Kovács and Csaba 1987,1989). This could account for the differences between the behaviour of the *T. pyriformis* and *T. malaccensis* cells. It is known that certain environmental influences alter the DNA content of the macronucleus (Dupy-Blanc and Metenier 1975, Klass and Smith-Sonneborn 1976), but have no such effect on the micronucleus. The fact that conjugation involves transfer of the genetic information associated with the micronucleus to the daughter cells could explain the superior "stability" of the sexually reproducing micronucleated species. However, the possibility of a so-called negative imprinting in micronucleated taxa suggests that a change in the quantity of information could occur also in these.

The present experimental observations indicate that insulin treatment changed macronuclear sensitivity to acid hydrolysis in both the control and the imprinted cells. The histograms did not indicate any change in macronuclear ploidity after insulin treatment for 60 min, but since insulin is known to stimulate DNA synthesis (and accordingly, mitosis) in mammalian cells, we also studied cell growth in the different conditions of experiment. No appreciable indication of increased DNA synthesis could be observed in any instance. Since the S phase of the *Tetrahymena* macronucleus covers about 50 per cent of the total cell cycle (3-3.5 h) (Holz 1960) 60-min hormone treatment would have been, in principle,sufficient for influencing the DNA synthesis. However, the fact that the mitotic rate failed to increase within 20 h, strongly suggested that the insulin effect which is characteristically developed in mammalian cells, did not take place in *Tetrahymena*. In view of this, the change in the time required for hydrolytic degradation was unrelated to an increase in DNA synthesis.

The impact of insulin on the count of *T. malaccensis* micronuclei could account for the influence of insulin on the duration of the cell cycle, but the lack of change in the mitotic rate seems to disprove such an interrelationship.

It follows from the foregoing considerations that imprinting, i.e. primary treatment with isnulin, gave

rise to marked changes in the state of the nucleus, as indicated by the alteration of nuclear sensitivity to hydrolysis and by the structural alterations demonstrated earlier by electron microscopic studies. RNA and proteins mask the DNA, and influence thereby the result of the Feulgen reaction. The changes observed in the present experiments may have been due to this circumstance. From this emerges additional proof that hormone treatment, i.e. the imprinting induced by it, gives rise to nuclear-level changes.

The histograms indicated shift to the right of the curve, i.e. facilitation of hydrolysis on primary exposure to insulin. This phenomenon may have been related to the de-masking of DNA under hormonal influence. This effect of insulin was durable, and even tended to increase after 24 h, owing in all probability to the establishment of imprinting. In contrast, second exposure shifted the histogram to the left, to approximately the same place which it had occupied after primary exposure. Shift to the right after primary interaction and shift to the left after secondary interaction indicated the impact of insulin imprinting, and suppor- ted the implication that the insulin-induced functions require a given state of the proteins which are masking the DNA, and this state can be assumed by the chromatin complement from either direction. Similar "bidirectional" changes were already observed in earlier studies along this line (Kovács and Csaba 1989, 1990).

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Received on 7th June, 1990

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 115-120

Factors Influencing the Growth of Rumen Ciliates *Eudiplodinium maggii* in vitro

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Summary. The rumen ciliates *Eudiplodinium maggii* were cultured in vitro in different salt solutions and with different compositions of food. The ciliates distinctly preferred the salt solution based on phosphates. The protozoa were unable to survive for a longer time when food consisted of hay only and survived for a long time when food consisted of hay and wheat gluten. When protein was supplied in the ratio of 0.25 mg/ml/day the protozoa counts varied from 400 to 800 cells/ml in relation to bacteria number. Cellulose support the growth of population of *Eudipodinium maggii* while starch had a slightly positive effect only when its proportion in the food was 3.5%. Xylose, glucose, galactose, fructose, sucrose, rafinose and pectins restricted the growth of protozoa or eliminated these organisms from the cultures and this effect depended on the dose of the supplement. The protozoa did not survive more than 20 days when soluble sugars were supplied to the medium in the ratio of 0.25 mg/ml/day. The death of protozoa was not accompanied by a decrease in pH.

Key words. Rumen ciliates, cultivation, nutrition.

INTRODUCTION

The ciliates from the family *Ophryoscolecidae* occupy the forestomachs of ruminants and *Eudiplodinium maggii* is a common species of rumen microfauna of large animals (Eadie 1962). These protozoa belong to large ophryoscolecids (Dogiel 1927) and are equipped with many different enzymes hydrolyzing different components of the ruminants food (Williams et al. 1986). Due to this they should contribute to considerable part of protozoal biomass and of protozoal activity in the rumen in spite of their low concentration. On the other hand, the large ciliates are preferentially retained in the rumen (Michałowski et al. 1986a). This suggests that importance of these ciliates should be considered in terms of their involvment in the food conversion processes inside the rumen, rather than as the source of protein for the host. Taking this as a starting point we have recently examined the ability of some species of diplodinia to utilize different substances for their growth in vitro (Michałowski et al. 1986b, 1989). The present paper is concerned to such ability of *Eudiplodinum maggii*.

MATERIALS AND METHODS

The protozoa originated from the rumen of cattle fed hay-concentrate diet. The single species population of these organisms was obtained by picking of the ciliates from a mixed population also containing entodinia, different diplodinia and *Epidinium ecaudatum*.

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Fifty to hundred cells were picked up and introduced to the small volume (2-5 ml) of "caudatum type" salt solution (Coleman et al. 1972). The initial cultures were diluted with a fresh portion (1-2 ml) of salt solution every day until reaching 20 ml of volume and small portion of food (1-5 mg) was also added. The ciliates were then transferred to the Erlenmeyer flasks (volume 50 ml), diluted with "caudatum type" salt solution up to 40 ml and cultured by a routine method as described by Michałowski et al. (1985, 1986b, 1989). The ciliates were transferred to the continuous culture system (Michałowski 1979) when their concentration reached about 1000 cells/ml and cultured continuously for over 2 years. The food was given once daily in the ratio of about 2 mg/ml. The food consisted of hay (60%) cellulose powder (25%) and wheat gluten (15%). The samples of protozoa from the continuous culture system were used as inocula in experiments presented in the paper.

Table 1

The chemical composition of salt solutions used for cultivation of *Eudiplodinium maggii* (g/l)

Ingredient	A	B	C	D
K_2HPO_4	6.3	4.9	0.0	0.0
KH_2PO_4	5.0	3.8	1.0	0.0
$Na_2HPO_4 \times 12H_20$	0.0	0.0	0.0	9.3
$NaHCO_3$	0.0	6.6	5.0	9.8
KCl	0.0	0.0	0.0	0.56
NaCl	0.65	0.49	6.0	0.47
$CaCl_2 \times 7H_2O$	0.09	0.07	0.2	0.08
$MgCl_2$	0.0	0.0	0.0	0.06
$MgSO_4 \times 7H_2O$	0.09	0.07	0.2	0.0
CH_3COONa	0.75	0.0	0.0	0.0

A – „caudatum type" salt solution, B – „simplex type" salt solution (both according to Coleman et al., 1972), C – „Hungate type" salt solution (according to Hungate, 1942). D – artificial saliva (after McDougall 1948).

Four different salt solutions were tested as a liquid part of the culture medium (Table 1). The other components used were: powdered hay, barley flour, powdered beet pulp, cellulose powder (Koch Light), pure barley starch, pectins, xylose, glucose, fructose, galactose, sucrose and rafinose (11 soluble sugars were supplied by Polskie Odczynniki Chemiczne, Gliwice), casein (BDH) and wheat gluten. The substances not available commercially were prepared in the laboratory. Pure barley starch was obtained accoring to Whelan (1955) and wheat gluten according to Klein (1933) and Pace (1955). Insoluble casein was prepared from the Light White Soluble Casein (BDH). It was precipitated with 10% TCA, then with saturated TCA and dried at 50°C. Finally the precipitate was washed several times to remove remains of soluble protein.

All experimental cultures were initiated by introducing the samples of protozoa (20 ml) to Erlenmeyer flasks with 20 ml of culture salt solution and mixture of appropriate food. Three cultures were run simultaneously in relation to any factor studied. The ciliates were fed every day and the content of flask was gassed with CO2 according to Michałowski (1975). Every fourth day a half of the culture volume was transferred, after thorough mixing, into another flask containing fresh medium.

Samples for ciliates and bacteria count were taken every fourth day from the material remaining after ciliate transfer to the fresh medium. They were fixed with 4% formaldehyde solution. Occasionally the sample of ciliates from the continuous culture system were also fixed and counted. The ciliate number was estimated by counting all the cells present in 0.1 ml of the fixed sample and bacteria number in a Thoma counting chamber. Each sample was counted three times using the light microscope. The statistical analysis of the data was made according to Ruszczyc (1970).

RESULTS

From four types of the salt solution teste only the "caudatum type" salt solution and "Hungate type" salt solution supported growth of *Eudiplodinium maggii* in vitro. The concentration, however, of protozoa was higher when "caudatum" salt solution was used (Fig. 1).

Ciliates survived in continuous culture system for over 2 years and the "caudatum" salt solution was then always used there. The food (about 2 mg/ml/day) consisted of hay (60%), cellulose powder (25%) and wheat gluten (15%). The ciliate concentration exceeded there 1000 cells/ml and the maximum density

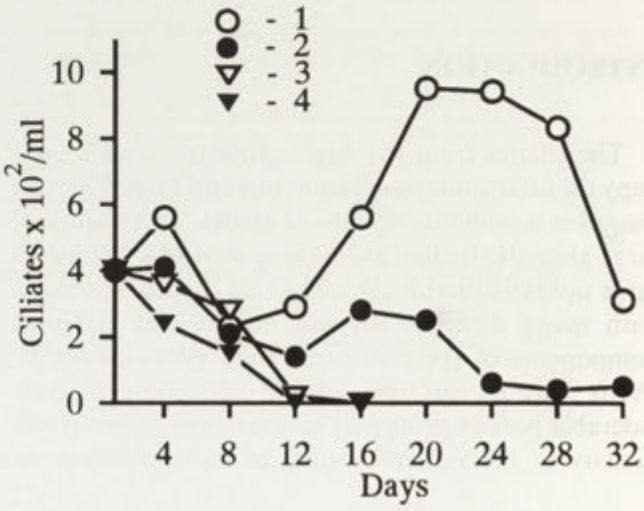

Fig. 1. The concentration and survival of *Eudiplodinium maggii* cultured in „caudatum type" salt solution (1), „Hungate type" salt solution (2), „simplex type" salt solution (3) and artificial saliva (4). The food given consisted of hay (0.835 mg/ml/d) and wheat gluten (0.165 mg/ml/d)

Table 2

The concentration of *Eudiplodinium maggii* ($\times 10^2$/ml) and bacteria ($\times 10^7$/ml) in the cultures supplemented with different doses of wheat gluten (mg/ml/day). (All the cultures were given powdered hay in the ration of 0.8 mg/ml/day). Mean values ± SE

Wheat gluten	Ciliates	Bacteria
0.040	2.5 ± 0.58	10.3 ± 1.42
0.083	3.6 ± 0.34	10.5 ± 1.67
0.165	4.8 ± 0.41	11.7 ± 2.61
0.206	5.0 ± 0.55	12.8 ± 2.01
0.248	4.7 ± 0.67	11.6 ± 1.30
0.289	4.8 ± 0.67	10.7 ± 1.93
0.330	4.8 ± 0.79	10.8 ± 3.01

was about 4000 cells/ml. Turnover rate of the contents of the culture flask was about 0.5-07/day.

The protozoa were unable to survive in a medium composed of culture salt solution and hay given at the ratio of 0.8 mg/ml/day. Such cultures were initiated many times, but the ciliates always died within 12-20 days of cultivation. Enrichment of such medium with wheat gluten provided appropriate conditions for the long-term culture of *Eudiplodinium maggii* when the protein was supplied in the ratio of 0.04 mg/ml/day or more. On the other hand there were no differences between protozoal concentration when the wheat gluten supplement dose varied between 0.165 and 0.33 mg/ml/day (Table 2). No significant differences in bacteria number were found ($p > 0.05$).

Table 3

The concentration of *Eudiplodinium maggii* ($\times 10^2$/ml) and bacteria ($\times 10^7$/ml) in the cultures supplemented with casein of different solubility in the culture salt solution (%). Mean values ± SE

Protein solubility	Protozoa	Bacteria
1	8.3 ± 1.05	11.4 ± 1.92
15	7.7 ± 1.08	10.5 ± 1.66
25	8.0 ± 1.07	10.9 ± 1.28
50	7.2 ± 0.74	10.1 ± 1.38
75	6.8 ± 0.22	10.4 ± 1.25
100	7.3 ± 0.65	11.3 ± 1.47

Table 4

The concentration of *Eudiplodinium maggii* ($\times 10^2$/ml) and bacteria ($\times 10^7$/ml) in the cultures supplemented with casein of different solubility in the culture salt solution (%). Mean values ± SE

Protein solubility	Protozoa	Bacteria
1	3.2 ± 0.16	20.3 ± 2.65
15	6.6 ± 1.27	12.2 ± 0.40
25	4.3 ± 0.84	18.8 ± 1.46
50	3.4 ± 0.38	18.1 ± 2.05
75	4.4 ± 0.27	16.7 ± 1.11
100	5.9 ± 0.51	14.1 ± 0.62

Table 5

The probability of correlation between ciliate number and protein solubility or bacteria counts in two cultivation experiments (see Table 3 and 4)

Correlated parameters	Correlation coefficient	Degrees of freedom	The level of statistical significance
Table 3			
Protozoa number vs. protein solubility	−0.805	4	0.1
Protozoa number vs. bacteria counts	0.229	„	n.s.
Table 4			
Protozoa number vs. protein solubility	0.261	„	n.s.
Protozoa number vs. bacteria counts	−0.995	„	0.01

Table 6

The concentration of *Eudiplodinium maggii* ($\times 10^2$/ml) and bacteria ($\times 10^7$/ml) in the medium composed of salt solution, hay and wheat gluten (A) or supplemented with cellulose (B), starch (C) and pectins (D). Mean values ± SE

Organisms	A	B	C	D
Ciliates	4.4 ± 0.96	9.9 ± 0.79	6.5 ± 1.18	2.1 ± 0.51
Bacteria	8.7 ± 0.33	9.5 ±0.21	9.9 ± 0.31	10.7 ± 0.38

Two experiments were made to examine the development of ciliates in relation to solubility of supplied protein. The number of protozoa varied there from 3.2 x 10^2 to 8.3 x 10^2/ml (Table 3 and 4). It showed a tendency to negative correlation with protein solubility in first experiment when the number of bacteria was similar in all cultures or was negatively correlated with the number of accompanying bacteria when it varied between 12.2 x 10^7 and 20.5 x 10^7/ml (Table 5).

The concentration of *Eudiploidinium maggii* in the cultures received hay (0.65 mg/ml/day) and wheat gluten (0.1 mg/ml/day) was 4.4 x 10^2/ml. Cellulose supplement (0.25 mg/ml/day) caused an increase in ciliate density by over 100% ($p < 0.001$). Some increase was also observed when starch was supplied in the same ratio while pectins supplement resulted in distinct decrease in population density (Table 6). No such effect was observed when powdered beet pulp was supplied as a source of pectins (Table 7). The differences in bacteria number were not significant ($p > 0.05$).

Table 7

The concentration of *Eudiplodinium maggii* ($\times 10^2$/ml) and bacteria ($\times 10^7$/ml) in the medium composed of salt solution, hay and wheat gluten (A) or supplemented with cellulose (B) and beet pulp (C). Mean values ± SE

Organisms	A	B	C
Ciliates	5.4 ± 0.21	10.4 ±0.43	8.2 ± 0.44
Bacteria	8.1 ± 0.25	8.5 ± 0.24	9.1 ± 0.32

Particular components were given in the ratio (mg/ml/day) of 0.65 (hay), 0.1 (wheat gluten) and 0.25 (cellulose and beet pulp).

Table 8

The concentration of *Eudiplodinium maggii* ($\times 10^2$/ml) in the control cultures (A) fed with basal ration consisting hay (0.65 mg/ml/day) and wheat gluten (0.1 mg/ml/day) or basal ration suplemented with cellulose, starch and beet pulp given in the ratio of 0.05 (B), 0.125 (C) and 0.25 mg/ml/day (D). Mean values ± SE

Supplement	A	B	C	D
Cellulose	8.2 ± 0.30	10.9 ± 0.40	14.7 ±0.70	19.9 ± 0.60
Starch	„	10.9 ± 0.65	11.2 ±0.85	8.3 ± 0.58
Beet pulp	„	10.1 ± 0.59	11.7 ± 0.72	9.8 ± 0.37

When the cellulose supplement rised from 0.05 mg/ml/day to 0.25 mg/ml/day an increase in protozoa count was observed. However, no increase was found when the same doses of starch or beet pulp powder were used for the supplementation of food

(Table 8). Bacteria number then varied there between 8.7 x 10^7 and 10.1 x 10^7/ml and pH was 6.3-6.6 ($p > 0.05$). It was measured 24 h after feeding.

The ciliates died when starch contributed to more than 23.5% of the ration consisting of hay, starch and wheat gluten. A positive effect of starch was observed only when it consisted 3.5% of the food mixture (Table 9). The highest concentration of *Eudiplodinium maggii* was faund when cellulose consisted 23.5% and hay 60% of the food dose. However, a stimulating effect was also observed when proportion of cellulose was 43.5% and that of hay 40% (Table 9).

Soluble sugars i. e. xylose, glucose, galactose, sucrose and raffinose caused an elimination of ciliates from the cultures within 10-24 days when supplied in the ratio of 0.25 mg/ml/day. Protozoa survived for over 30 days when glucose and fructose were supplied in the rate of 0.05 and 0.125 mg/ml/day, but the population density was lower than that of the control cultures receiving only hay and wheat gluten (Fig. 2). However, the protozoa died when sugar supplement was 0.25 mg/ml/day. The pH exceeded 6.0 in all types of the cultures.

Table 9

The concentration of *Eudiplodinium maggii* in the cultures receiving wheat gluten, hay and cellulose or starch. The food was supplied in the ratio of 1 mg/ml/day. Wheat gluten contributed to 16.5% of the ration. Proportion of hay and polysaccharides varied from 0 to 83.5% of the ration. Mean values ± SE

Polysaccharide/hay percentage	Protozoa concentration	
	Cellulose	Starch
0.0/83.5	4.0 ± 0.34	7.6 ± 0.43
3.5/80.0	4.4 ± 0.47	9.2 ± 0.45
23.5/60.0	9.6 ± 0.80	6.0 ± 1.19
43.5/40.0	6.3 ± 0.86	not survived
63.5/20.0	3.6 ± 0.64	"
83.5/0.00	not survived	"

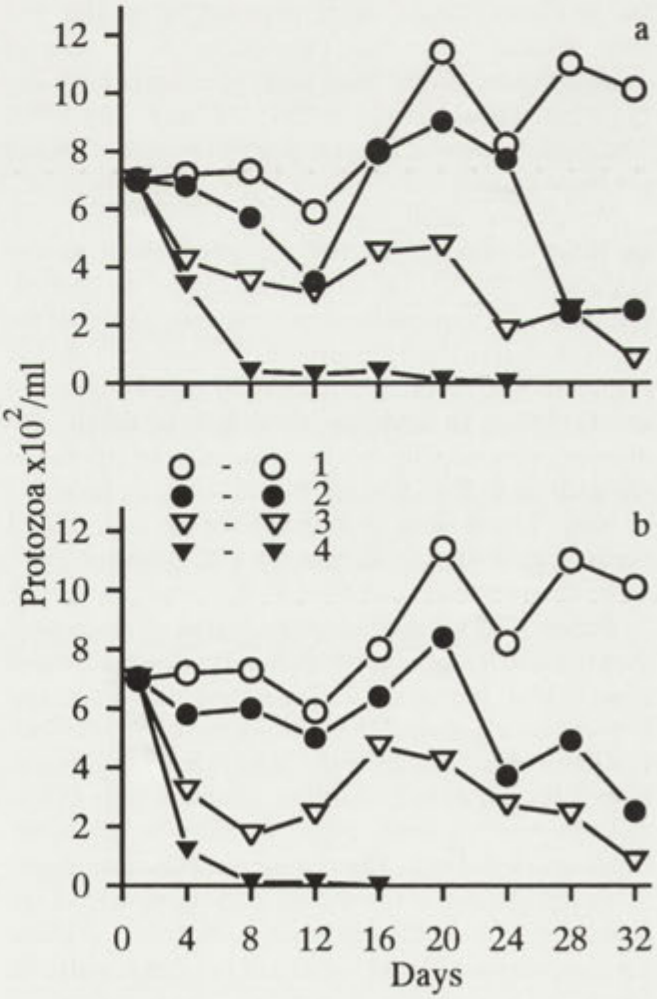

Fig. 2. The changes in concentration of *Eudiplodinium maggii* in the cultures fed hay (0.65 mg/ml/day) and wheat gluten (0.1 mg/ml/day) supplemented with glucose (a) or fructose (b) given at the ratio of 0.05 (2), 0.125 (3) and 0.25 mg/ml/day (4). 1 – control cultures

DISCUSSION

The presented results characterize the requirements of *Eudiplodinium maggii* in vitro. Some observations concerning the cultivation of *Eudiplodinium maggii* were previously made by Coleman et al. (1976). He found the initial addition of the fresh or autoclaved rumen fluid to be necessary. We never used any kind of rumen fluid as a component of the culture medium and the ciliates remained alive. That shows that rumen fluid can be omitted from the culture medium both during the isolation as well as the cultivation stage.

The importance of protein and carbohydrates was tested. *Eudiplodinium maggii* was unable to survive when powdered hay was the only food component in spite of the fact that bacteria exceeded 10^7 cells/ml. Thus these protozoa differ from *Anoplodinium denticulatum* which were cultured under such nutritional conditions for a long time (Michałowski et al. 1989).

The concentration of ciliates increased markedly when protein supplement increased from 0.04 to 0.165 mg/ml/day (Table 2) but further increase in the protein dose did not affect the growth of *Eudiplodinium maggii*. Thus the reaction of these protozoa on the level of protein supplementation was similar to that of other

species cultured in the laboratory (Michałowski 1989) and showed that the supplement of protein not lesser than 10% of the food dose is necessary to support intensive development of population of *Eudiplodinium maggii* in vitro.

In the cultures on casein bacteria influenced more effectively the development of *Eudiplodinium maggii* than protein solubility (Table 3 and 4). It was, perhaps, the case why the relationship between the last and the population density of ciliates was observed only during the first repetition of cultivation when the concentrations of bacteria in all cultures were similar (Table 3). The number of ciliates tended to increase there with the decrease in protein solubility. Similar relationship was found in the case of *Entodinium caudatum* and *Diplo-plastron affine* (Michałowski 1989).

Among the tested polysacharides cellulose effected positively the population density of *Eudiplodinium maggii*. Other informations (unpublished) showed that the cellulose particles were engulfed by ciliates and then gradually digested. The cellulolytic activity of *Eudiplodinium maggii* have been previously been demonstrated (Coleman et al. 1976, Michałowski 1990). Thus cellulose could be an important source of energy for these ciliates.

Starch was readilly engulfed by *Eudiplodinium maggii* (Michałowski 1990), but a positive effect on the population density was found only at low supplementation, while supplementation exceeding 23.5% of the food ration restricted the growth of protozoa (Table 8). A similar effect of starch was also observed by Coleman et al. (1976). In contrast to cellulose, starch was digested very rapidly by the protozoa (unpublished observations). Thus it is possible that certain products of starch metabolism liberated to the medium caused negative environmental changes. This problem needs more detailed research.

Pectins and soluble sugars restricted or eliminated the protozoa from the cultures. Similar effects were also observed in the case of *Entodinium caudatum* and *Entodinium exiguum* (Michałowski et al. 1985, 1986c). It is known that ciliates of the family *Ophryoscolecidae* have a limited ability to utilize soluble sugars (Abou Akkada and Howard 1960, Williams et al. 1961, Coleman 1969, 1972). Thus an excess of these nutrients in the culture medium could alter its properties which negatively affected the growth of protozoa. The changes in a compositions of bacterial flora can not also be omitted. It was often observed that soluble sugars and pectins supported a development of thread-like colonies of bacteria which in the majority of cases were accompanied by a decrease in protozoa number.

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Received on 28th November, 1990

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 121-127

Leptomonas rigidus sp. n. *(Trypanosomatidae)* – a Parasite of *Salda littoralis* L. *(Hemiptera: Heteroptera)*

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Summary. *Leptomonas rigidus* sp. n. from *Salda littoralis* (*Hemiptera*) is the first trypanosomatid found in bugs of the family *Saldidae* and is described below. Cultures in liquid and solid media were obtained. In the culture cells retained promastigote organization. There were many cells in the late stages of cytokinesis in the culture. Colonies were small, monomorphous and of regular shape. Ultrastructure of the cells show some peculiarities: the distance between subpellicular microtubules varies even within a single specimen and the mitochondrial part of the kinetoplast bears several ring– shaped cristae. Kinetoplast DNA is of a specific structure – both mini– and maxicircles are of small size (1.35 kbp and 2.8 kbp respectively). Peculiarities of *L. ridigus* separate it from the other lower trypanosomatids, which were studied earlier.

Key words. *Leptomonas rigidus* sp. n., Trypanosomatidae, parasite, *Salda littoralis*, Heteroptera.

INTRODUCTION

The fauna of the lower trypanosomatids is not still satisfactory described – considerable difference exists between the number of host insect and plant species and that of known trypanosomatid species from these hosts (Wallace 1966, Wallace et al. 1983). *Hemiptera* and *Diptera*, from this point of view, are studied better than other insect orders; however even among the *Hemiptera* there are some families where lower trypanosomatids still have not been found. Recently some species of the genus *Leptomonas* from *Nabidae* bugs were described (Podlipaev 1985), as well as species of the genus *Blastocrithidia* from *Miridae* bugs (Podlipaev and Frolov 1987). It appears that lower trypanosomatids have not been mentioned in the family Saldidae.

In the Soviet Union special studies of the fauna of the lower trypanosomatids have not been carried out, these parasite having been mentioned in only a few works (Ioff 1927, Hodukin 1927, Shakhov 1928, Petrisheva 1932, Muratov and Cheissin 1959, Arifdzhanov and Nikitina 1961). In recent years, in the European and Asian parts of the USSR, representatives of the genera *Leptomonas*, *Crithidia*, *Blastocrithidia* and *Phytomonas* have been found (Podlipaev 1982, 1985, 1986, 1988 a, b, Podlipaev and Frolov 1987).

Little is known about the variability of lower trypanosomatids, their dependence upon their hosts, or their geographical distribution; the degree of specificity of these parasites is obscure. The taxonomy of the lower trypanosomatids seems to be a complicated

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problem. The number of morphological characteristics is very low, those available being significantly variable and greatly influenced by environmental conditions. When looking for taxonomic criteria the main problem is to determine the value of the differences distinguished. It is difficult to differentiate criteria, connected with geographic variability and specificity from systematic criteria. It is also difficult to determine the value of morphometric data because of new knowledge about variability of these organisms.

The use of cultures of the lower trypanosomatids enlarges the possibilities of researching this group. Each new culture is a new model for studying the trypanosomatids themselves and also the phenomenon of parasitism.

Fig. 1. Colonies of *Leptomonas rigidus* sp. n. on solid medium

MATERIALS AND METHODS

Methods of culture isolation, cultivating and staining of preparations were described earlier (Haetsky 1982, Podlipaev 1982, 1985). To avoid fungoid infection, which often develops in culture isolated from the insect intestine, two quite opposite methods are applied. The first deals with the cloning of cultivated organisms. However, the procedure of cloning inevitably results in restriction of polymorphism of the population, the decrease of genetic variability and in selection of random genotypes in the isolated culture. Another method of purification is based on the segregation of the cells of various motility – mobile flagellates are separated from the immobile fungi. We have applied the last method, using special device (Podlipaev and Frolov 1987).

One hundred cells were measured on each slide.

For electron microscopy, 10-day cultures in liquid medium were used. After centrifugation (3000 rpm, 10 min) the cells were fixed with 1.5% glutaraldehyde in 0.1 M cacodylate buffer (75 min, 0°C). After washing with 0.1 M cacodylate buffer, containing 5% sucrose, postfixation was carried out with 2% OsO_4 (100 min, 0°C). Then the flagellates were embedded in epon– araldite mixture. Sections, stained 3 h with saturated aqueous uranyl acetate and 5 min with lead citrate were examined in a JEM–100 C electron microscope.

The purification of kDNA for the restriction cleavage analysis was done as reported earlier (Maslov et al. 1982).

RESULTS

Leptomonas rigidus Podlipaev, Malysheva et Kolesnikov, sp. n.

Host: *Salda littoralis* L. (*Hemiptera*: *Saldidae*) in one of three examined specimens.

Site: Gut.

Locality: White Sea, Tshupa–Bay, cape Kartesh, supralittoral under fucoids, 28.08.1987.

Types: holotype, xenotype* and the type culture (1b) are stored in the Zoological Institute, Leningrad. In a previous publication (Kolesnikov et al. 1990) *L. rigidus* was designated as "isolate 1b".

Diagnosis. Promastigotes. The body of cells in culture is often twisted once. Promastigote organization is retained in culture. On solid medium only regular semispherical colonies form, small ones predominate (Fig. 1). Symbionts are absent (Figs. 2, 3–9). Kinetoplast is situated close to the nucleus, the kinetoplast index reaches 4. Measurements are presented in Table 1. Results of restriction cleavage of kDNA are presented in Table 2.

The peculiar property of *L. rigidus* is maintenance of promastigotes in culture. One both solid and liquid media parasites may surely be determined as representatives of genus *Leptomonas* due to their morphology.

The kinetoplast index (KI) is extremely high because of the short distance between the kinetoplast and nucleus in the cells isolated from the insect and also in the cells in the culture.

The culture of *L. rigidus* achieves its maximum in 7–9 days (10^7–10^8 cells/ml) (Fig. 11). Hemin is necessary for normal development. Culture may grow under a layer of vaseline oil. During cultivation cells stretch, and many of them bear an attenuated posterior end.

On solid medium *L. rigidus* develop transparent colourless colonies of regular semispherical shape. The distribution of the colonies according to size is extremely asymmetrical – the small size groups dominate

* Xenotype – host specimen from which the parasite or commensal was collected and described (Jankowsky 1981).

(Fig. 12). Mean size of 20–days colony is 0.14 ± –0.01 mm.

The diameter of subpellicular microtubules is 25 nm. The distance between microtubules varies even in one specimen from 45 to 120 nm (Figs. 4,8,9) whereas in the other trypanosomatids this parameter is rather constant within every stage of the development (Anderson and Ellis 1965).

The mitochondrion is slightly ramified, 1–2 fragments of the mitochondrion occupy the posterior part of the cell and branches derived from the mitochondrion surround the reservoir (Fig. 4). Kinetoplast is of original structure – there are 1 or 2 ring–shaped cristae (Figs. 3, 4). Cristae in the posterior part of a cell are not numerous and disposed chaotically (Figs. 6, 7). Few peroxisome–like structures occur (Fig. 3). Dividing cells at various stages are common.

These cells are often connected by thin cytoplasmic bridges, containing microtubules (Figs. 8, 9, 10). Such appearances may be explained by the prolonged time of cytokinesis.

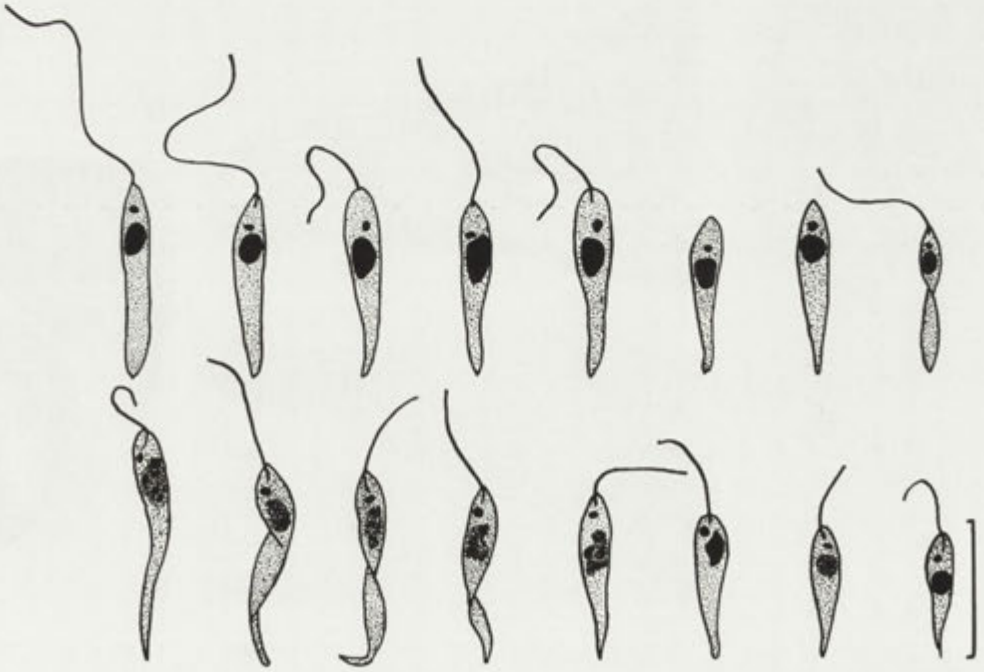
The molecular structure of kinetoplast DNA is original – both mini– and maxicircles are of small size – 1.35 kilobase pairs (kbp) and 25 kbp respectively.

Table 1

Body sizes of *Leptomonas rigidus* (μm, the mean value ± standart deviation is also indicated, in bracktets – maximal and minimal)

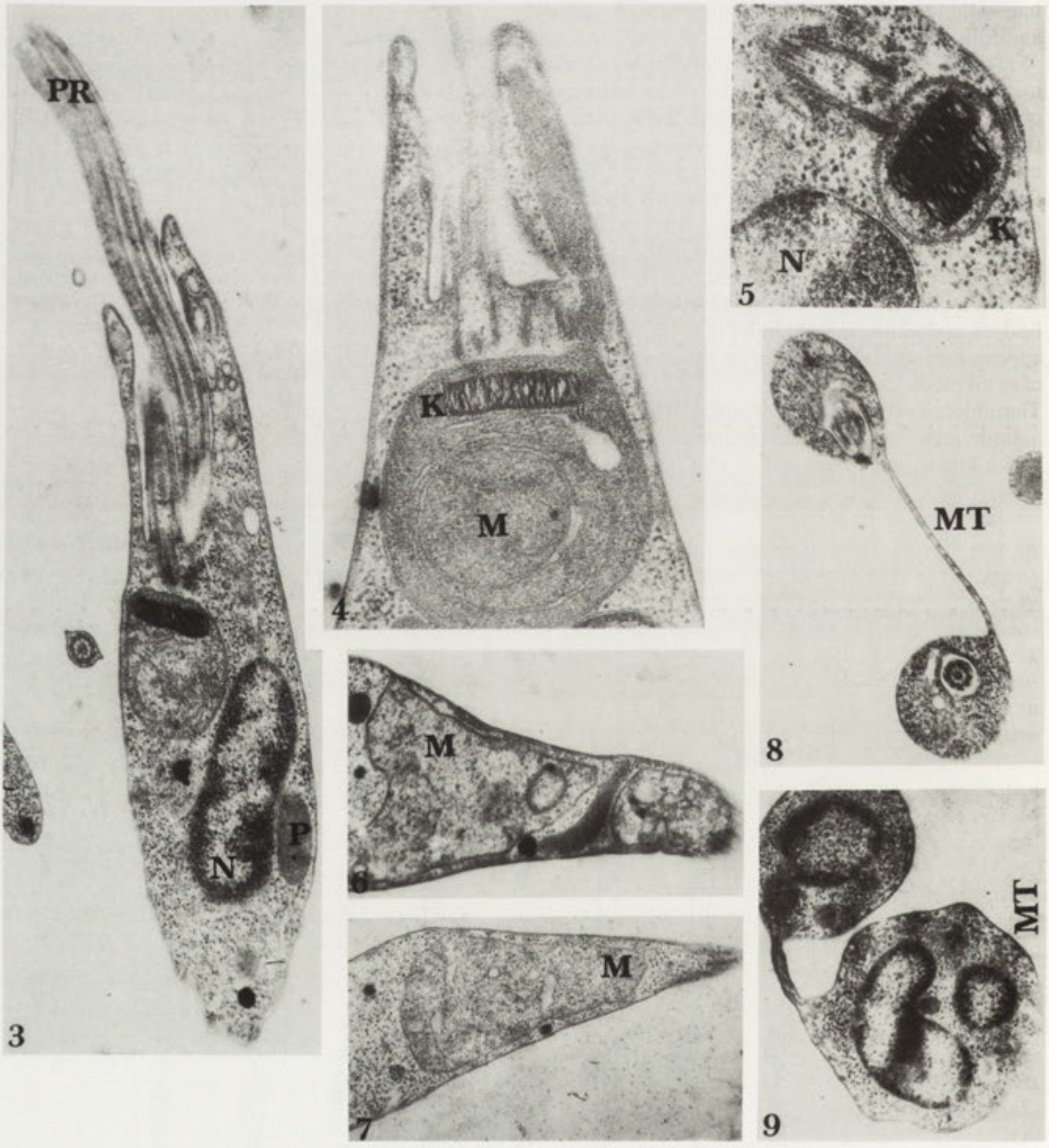
Measurements	From *Salda littoralis*	From culture (liquid medium)
Body length	12.33 ±0.43 (17.94 – 9.11)	10.39 ± 0.53 (14.23 – 6.93)
Body width	1.74 ± 0.43 (2.37 – 1.23)	1.71 ± 0.05 (2.09 – 1.52)
Nucleus	1.95 ± 0.22 (2.56 – 1.33)	1.66 ± 0.09 (2.37 – 1.14)
Kinetoplast	0.66 ± 0.33 (0.95 – 0.38)	0.51 ± 0.02 (0.66 – 0.30)
From anterior end of the body to nucleus (A-N)	2.44 ± 0.10 (3.80 – 1.61)	2.71 ± 0.07 (3.23 – 2.09)
From posterior end of the body to nucleus (P-N)	7.81 ± 0.42 (12.43 – 4.93)	5.32 ± 0.37 (7.88 – 2.66)
From anterior end of a body to kinetoplast (A-K)	1.65 ± 0.10 (2.75 – 0.85)	1.92 ± 0.06 (2.28 – 1.52)
From kinetoplast to nucleus (K-N)	0.83 ± 0.07 (1.61 – 0.38)	0.83 ± 0.05 (1.4 – 0)
Nuclear index – NI (A-N/P-N)	0.31 ± 0.01 (0.42 – 0.23)	0.55 ± 0.05 (0.93 – 0.32)
Kinetoplast index – KI (A-K/K-N)	2.33 ± 0.20 (4.24 – 1.44)	2.42 ±0.18 (4.0 – 1.35)

Fig. 2. Cells of *Leptomonas rigidus* sp. n. Above – in the insect gut, below – culture liquid medium, scale 10 μm



DISCUSSION

In studying naturally invaded and isolating laboratory cultures of lower trypanosomatids one should bear in mind probability of nonspecific invasion. This probability is especially high for predators, the last may be invaded while feeding on infected prey. The question arises whether laboratory culture corre-



Figs. 3-9. Ultrastructure of cultural forms of *Leptomonas rigidus* sp. n., cells in liquid medium 3 – 20000 x, 4 – 32000 x, 5 – 45000 x, 6-7 – 16000 x, 8 – 20000 x, 9 – 25000 x
Abbreviations: K – kinetoplast; M – mitochondrion, MT – microtubules, N – nucleus, P – peroxisomes, PR – paraxial rod.

Table 2

The cleavage of kinetoplast maxicircular DNA of *Leptomonas rigidus*

Restriction endonucleases	Pvu II	Hind III	Msp I	Cfr 131	Eco 471	Mva I
Recognizable sequence	CAGCTG	AAGCTT	CCGG	GGNCC	GG$\frac{A}{T}$CC	CC$\frac{A}{T}$GG
Fragment size (kbp)	25	25	12	8.2	8.3	15
			10	6.3	5.9	5.6
			3.7	6.0	5.7	4.6
				4.3	3.8	
Sum of fragment sizes (kbp)	25	25	25.7	24.8	23.7	25.2

Fig. 10. Alive cells of *Leptomonas rigidus* sp. n. in liquid medium

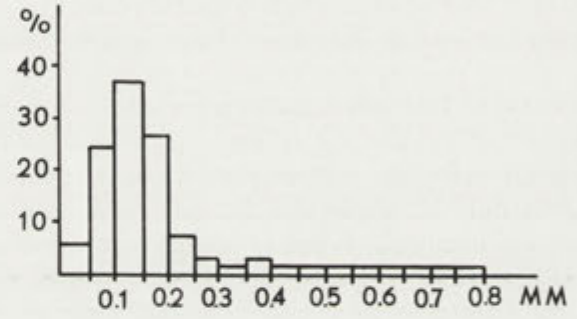


Fig. 12. Size of 10 day-colonies of *Leptomonas rigidus* on solid medium, in mm

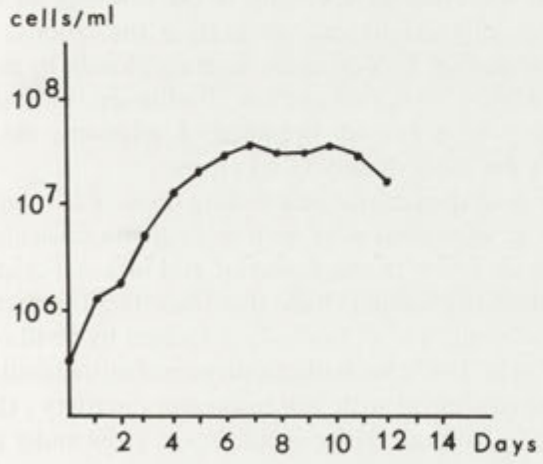


Fig. 11. Growth of the culture of *Leptomonas rigidus* in liquid medium

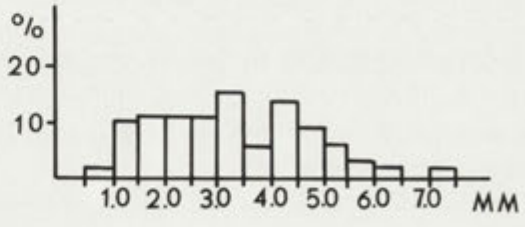


Fig. 13. Size of 10-day-colonies of *Blastocrithidia miridarum* on solid medium, in mm

sponds to the natural invasion? It is impossible, a priori, to exclude the selective affect of the medium or of the isolation procedure (Podlipaev 1985).

Salda littoralis was collected in the supralittoral zone of the White Sea under a thick layer of stranded fucoids. This is a unique biotope, situated on the border of the tidal zone. This particular bug was not found in another biotopes and, in this particular biotope, other

Hemiptera species were absent. The probability of specific invasions increases when ecologically isolated hosts are examined.

The most infected bugs in the region researched — *Nabicula flavomarginata* (fam. *Nabidae*), inhabiting forrests and meadows and *Gerris lacustris* (fam. *Gerridae*) inhabiting freshwater reservoirs in the woods. In *N. flavomarginata*, *Leptomonas* and *Crithidia* species were found and in *G. lacustris* – *Blastocrithidia* spp. *L. rigidus* differs greatly from the lower trypanosomatids, parasitizing these insect hosts both by size and cell morphology.

The proximity of kinetoplast and nucleus in the cells of *L. rigidus* (kinetoplast index reaches 4.2) distinguishes this species among the representatives of genus *Leptomonas* from *N. flavomarginata*, this index being usually no more than 2.6 (Podlipaev 1985). When cultivated the cells of many *Leptomonas* and *Blastocrithidia* species change their shape: become shorter and more round (Podlipaev 1985, Podlipaev and Frolov 1987). In our collection of the cultures of lower trypanosomatids, which are referred to these genera (more, than 20 isolates), only *L. rigidus* remains as typical promastigote cells in culture. When compared with the other researched *Leptomonas* species, *L. rigidus* has two peculiar features of its colonies on solid medium: (1) small size groups prevail; (2) the colonies are monomorphous in their size and shape (Fig. 1). The majority of species examined earlier were characterized by the presence of both small and large colonies of regular semispherical shape. In *Leptomonas* species from *N. flavomarginata* – *Leptomonas* sp., *L. occidentalis*, *L. nabiculae* and *L. peterhoffi* the size of the colonies varies in the range of several millimeters. In *L. peterhoffi* 2 classes of semispherical colonies are distinguished – "large" (1–2 mm) and "small" (0.1 – 0.3 mm). Representatives of genus *Blastocrithidia* – *B. gerricola* and *B. miridarum* have colonies of regular shape several millimeters in size (Podlipaev 1985, Podlipaev and Frolov 1987). *L. rigidus* differs from all species mentioned above by the absence of large colonies of regular shape.

It may be that the size of the colony is of some taxonomic value: in any case the colonies of *B. miridarum* are considerably larger, than these of *L. rigidus* (Fig. 13). The possible taxonomic value of the characteristics of the colonies had been already considered (Keppel and Janovy 1977; Podlipaev 1985).

The species described here differs also from the *Leptomonas* species from *N. flavomarginata* by the absence of polymorphism in the colony's shape. The variety of colony shapes of *Leptomonas* from *Nabicula* may be summarized as follows: (1) regular semispherical large; (2) regular semispherical small; (3) amoeba–like with salient or rough centre; (4) amoeba–like without a salient centre and (5) branched (Podlipaev 1985). Species of this group are characterized by the definite set of phenotypes of the colonies: *L. occidentalis* – 1, 2, 3, 4; *L. peterhoffi* – 1, 2, 4; *L. nabiculae* – 1, 3, 4, 5; *Leptomonas* sp. – 4 and also the unique colonies of regular shape with concave centre – never found in species of this group.

For *B. gerricola* and *B. miridarum* two classes of colonies are distinguished: (1) regular round and (2) irregular with projections; the last species forms the colonies with the salient centre (Podlipaev 1985, Podlipaev and Frolov 1987). Classification of colonies, given above is a preliminary one. Some phenotypes are inherited. For example – regular or irregular shape of the colonies of *L. peterhoffi*. It is doubtful that one a single characteristic itself of a colony would be of diagnostic value. More useful would be to systematize colony polymorphism within genus of family. Within the parameters of this variability, separate species (genera) would be characterized by expression of some specific forms.

Colonies of *L. rigidus* are not structured. Earlier were mentioned structural elements in the colonies of *Herpetomonas megaseliae* (Keppel and Janovy 1977) and in *Leptomonas* sp. (Podlipaev 1985) and *B. miridarum* (Podlipaev and Frolov 1987). It is possible that the definite structure of a colony is the evidence of the presence of mechanisms, integrating the colony. In *Leptomonas* sp. two colonies, coming closely to each other while growing do not fuse (Podlipaev 1985); this fact also supports the presence of relations, maintaining the individuality of a colony.

Ring–shaped cristae, resembling those of *L. rigidus*, but more numerous were seen in *Crithidia fasciculata* (Hill et al. 1968). In the posterior end of a cell cristae often bear an irregular shape that resembles the cristae of mitochondria of *C. fasciculata*, treated by acriflavin (Kusel et al. 1967). Such ultrastructure of mitochondria may be connected with low respiratory activity , that correlates with the ability of cultures to grow under the layer of the oil. Similar concentric structures in the mitochondria were described in one of the isolates of *Phytomonas* sp. (Attias et al. 1988).

Two parallel structures of the kinetoplast form the DNA–containing part of the kinetoplast in some cells of *L. rigidus*. Similar structures were mentioned in *Phytomonas* sp. (Attias et al. 1988).

The molecular structure of kinetoplast DNA in *L. rigidus* has some peculiarities. Among lower trypa-

nosomatids of Northwest of the USSR, which were examined in terms of kDNA structure (Kolesnikov et al. 1990), *L. rigidus* forms a separate group, which is characterized by the small size of minicircles – 1.35 kbp. The size of minicirles in *Leptomonas* from *N. flavomarginata* is 1.75–1.55 kbp. The size of maxicircles of kDNA in *L. rigidus* is 2.5 kbp whereas in the majority of lower trypanosomatids, including these isolated in the same region where *L. rigidus* was found, the size of maxicircles exceeds 3.0 kbp.

The design of maxicircle cleavage undoubtly separates *L. rigidus* from the representatives of genus *Leptomonas* from *Nabicula* and also from the other species examined.

Therefore, *L. rigidus* occupies an isolated position among lower trypanosomatids of the *Hempitera* of Northwest of the USSR. One should not exclude the possibility of raising the taxonomic status of *L. rigidus*.

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Received on 4th April, 1990

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1991) 30: 129-133

Orthoamphisiella stramenticola nov. gen., nov. spec., a New Hypotrichous Ciliate (*Ciliophora: Hypotrichida*) Occurring in Walnut Leaf Litter

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Summary. A new hypotrichous ciliate, *Orthoamphisiella stramenticola* nov. spec., was discovered in fresh walnut leaf litter in Austria. The description is based on live observations, protargol impregnation and morphometric techniques. *Orthoamphisiella stramenticola* is 80-140 × 30-45 μm in size and has 4 macronuclear segments, 20 adoral membranelles, 5 buccal cirri and 10 fronto-ventral cirri in 2-3 short rows on average. Transverse and caudal cirri are lacking. This and the short rows of fronto-ventral cirri left to the amphisiellid long ventral row are considered to be diagnostic characters of a new genus, *Orthoamphisiella* nov. gen., which is placed in the family *Amphisiellidae* Jankowski, 1979.

Key words. *Orthoamphisiella, Hypotrichida*, systematics.

INTRODUCTION

Hypotrichous ciliates are very common in soil. Many new genera and species have been discovered during the last decade (Foissner 1987a). The new species described in this paper was found during the investigation of ciliates inhabiting fresh leaf litter.

MATERIALS AND METHODS

Orthoamphisiella stramenticola occurred on fallen leaves of a walnut tree (*Juglans*) in the village of Schrötten, Styria, Austria next to an old farmhouse with the number 22. The meadow under the tree is mowed twice a year and is rich in flora.

The top leaf of at least 3 layers of dry walnut leaves was taken. Several such leaves were put in a petri dish and a raw culture according to Foissner (1987a) was set up. A clone was established in Volvic-yeast medium and maintained at 14-22°C. Protargol silver impregnation was used to reveal the infraciliature (Foissner 1982). Methyl green-pyronin was applied to differentiate certain cell organelles (Foissner 1979). Body shapes of living specimens were drawn from slides without cover glasses. Details were studied on slightly to heavily squeezed individuals using the oil immersion objective, bright field and phase contrast microscopy. Drawings were made with the help of a camera lucida. All counts and measurements were undertaken at a magnification of 945 x (1 unit of ocular micrometer = 1.25 μm).

All statistical procedures follow methods described in Sokal and Rohlf (1981).

RESULTS AND DISCUSSION

Orthoamphisiella nov. gen.

D i a g n o s i s: *Amphisiellidae* with 1 row of buccal cirri and 2-3 short rows of fronto-ventral cirri left of the long ventral row.

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Type species: *Orthoamphisiella stramenticola* nov. spec.

Derivatio nominis: Composite of "Orthos" (Greek.; straight; because of the straight rows of cirri in the frontal field) and "Amphisiella". Feminine gender.

Comparison with related genera: The conspicuous buccal and fronto-ventral rows and the lack of transverse and caudal cirri separate *Orthoamphisiella* from *Amphisiella* Gourret et Roeser, 1888 (*Amphisiellidae* with more than 1 cirrus left of ventral row in frontal field. Transverse cirri present; Fig. 2); from *Amphisiellides* Foissner, 1988 (*Amphisiellidae* with more than 1 cirrus left of ventral row in frontal field. Transverse cirri and caudal cirri present; Fig. 3); from *Paramphisiella* Foissner, 1988 (*Amphisiellidae* with 1 cirrus left of ventral row in frontal field. Caudal cirri present; Fig. 4); and from *Hemiam-*

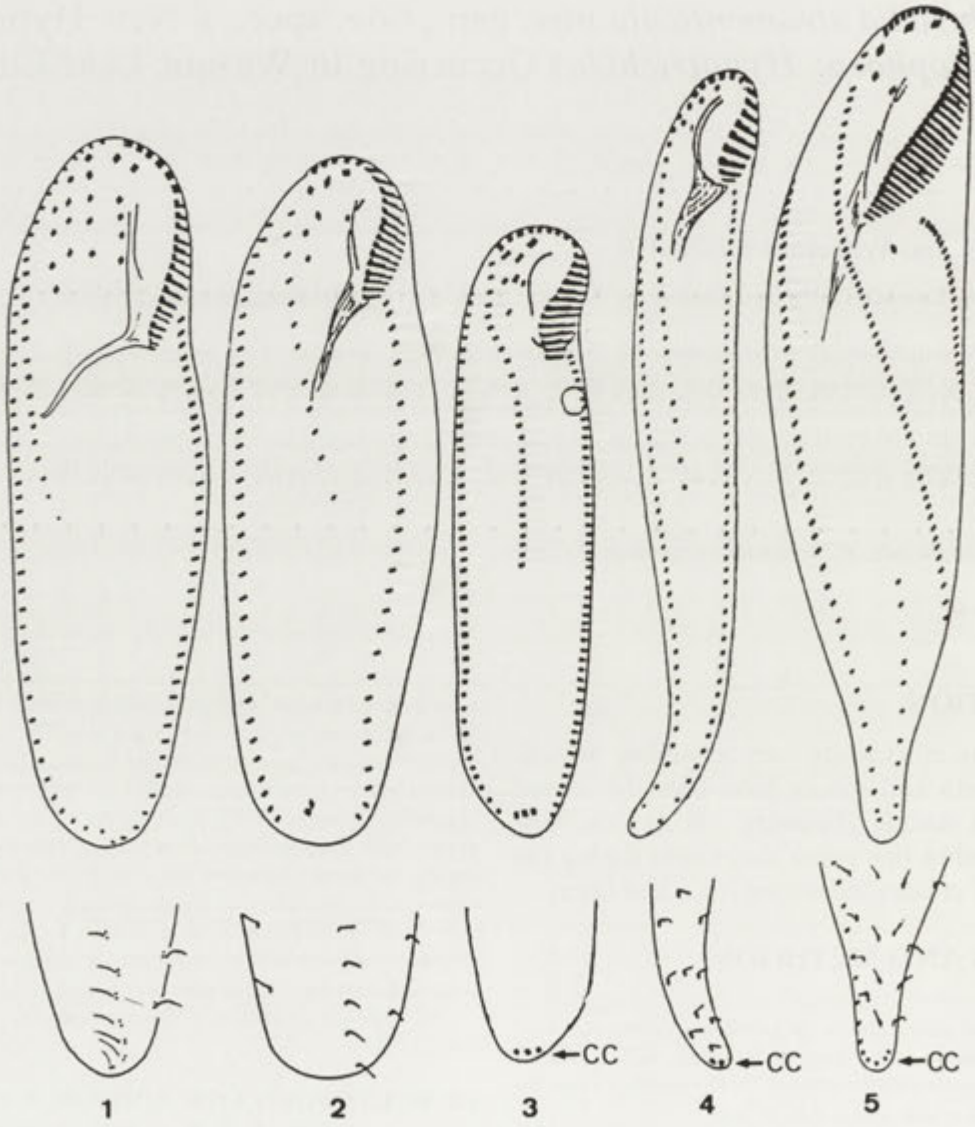

Fig. 1–5. Comparison of the 5 genera of the family Amphisiellidae. The upper series of figures shows the ventral infraciliature, the lower series shows the dorsal infraciliature in the posterior part. 1 – *Orthoamphisiella* (original). 2 – *Amphisiella* (from Foissner 1987b). 3 – *Amphisiellides* (from Hemberger 1985). 4 – *Paramphisiella* (from Foissner 1982). 5 – *Hemiamphisiella* (from Foissner 1988). CC – caudal cirri.

Table 1

Morphometric characterization of *Orthoamphisiella stramenticola*

Character[1]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, lenght	93.8	92.5	6.5	1.2	6.9	78.7	105.0	29
Body, width	28.8	27.5	6.6	1.2	22.9	18.7	50.0	29
AZM, lenght	27.5	27.5	4.6	0.8	16.7	22.5	37.5	29
VR, lenght	46.2	48.7	8.9	1.7	19.2	41.2	83.7	27
Ma, lenght[2]	10.9	11.3	1.7	0.4	15.2	6.8	13.8	22
Ma, width[2]	5.9	6.3	0.9	0.2	15.2	3.8	7.5	22
Ma, lenght[3]	11.9	12.5	3.0	0.6	25.2	5.0	17.5	28
Ma, width[3]	5.3	5.0	0.9	0.2	16.9	3.7	7.5	29
Ma, No.	4.3	4.0	0.6	0.1	13.9	4.0	6.0	30
Mi, No.	5.1	5.0	1.3	0.2	25.4	3.0	7.0	28
AM, No.	20.4	20.0	2.7	0.6	13.2	18.0	30.0	23
RMR, No. cirri	40.7	41.0	2.1	0.5	5.1	36.0	44.0	19
LMR, No. cirri	33.5	34.0	2.3	0.5	6.9	30.0	38.0	21
BC, No. cirri	4.7	5.0	1.0	0.2	21.2	4.0	7.0	22
FVR 1 No. cirri	3.7	4.0	0.7	0.1	18.9	3.0	5.0	22
FVR 2 No. cirri	4.0	4.0	0.6	0.1	15.0	3.0	5.0	23
FVR 3 No. cirri	2.8	3.0	1.0	0.2	35.7	1.0	5.0	15
VR, No. cirri	22.8	23.0	2.4	0.5	10.5	19.0	30.0	20

[1] All data are based on protargol impregnated specimens from a raw culture. All measurements in μm. AM – adoral membranelles; AZM – adoral zone of membranelles; BC – buccal row; LMR – left marginal row; Ma – macronuclear segment; Mi – micronuclei; RMR – right marginal row; VR – ventral row; FVR 1,2,3 – 1st, 2nd, 3rd fronto-ventral row; $\bar{x}$ – arithmetic mean; M – median; SD – standard deviation; SE – standard error of arithmetic mean; CV – coefficient of variation in %; Min – minimum value; Max – maximum value; n – sample size. [2] anterior segment. [3] second anterior segment.

phisiella Foissner, 1988 (*Amphisiellidae* with 1 cirrus left of ventral row in the frontal field. Underneath oral apparatus 1 isolated cirrus between ventral row and left marginal row. Caudal cirri present. Usually a second much shorter ventral row in posterior half of body; Fig. 5).

Orthoamphisiella stramenticola nov. spec. (Tab. 1, Fig. 6-13)

Diagnosis: Size in vivo 80-140 × 30-45 μm. Body shape almost rectangular. 4 macronuclear segments, 20 adoral membranelles, 5 buccal cirri and 10 fronto-ventral cirri in 2-3 rows on average.

Derivatio nominis: "Stramenticola" (lat.) due to its living in litter.

Type location: Walnut leaf litter in village Schrötten, Styria, Austria (Long. 15°49', Lat. 46°47', Alt. 320 m).

Type specimens: A holotype and a paratype of *O. stramenticola* as 2 slides of protargol impregnated cells have been deposited in the collection of microscope slides of the Oberösterreichischen Landesmuseums in Linz.

Description: Shape rectangular, right body margin straight to slightly convex, left slightly indented beneath adoral zone of membranelles. Both ends broadly rounded. Sometimes anterior end narrowed head-like, posterior pointed (Fig. 9) . Dorso-ventrally flattened about 2:1. Under coverslip pressure slightly contractile. Usually 4 macronuclear segments, three of them ellipsoid, lie slightly left of median, anteriormost segment almost spherical, located slightly right of median at level of cytopharynx. Chromatin bodies spherical. Usually 5 spherical micronuclei near macronuclear segments. Contractile vacuole on left border in mid-body, without collecting canals. Some colourless subpellicular granules irregularly and lo-

osely arranged, do not stain with methyl green-pyronin (Fig. 9). Cytoplasm filled with crystals and greasily shining globules. Food vacuoles 3-7 μm in diameter, containing colpodid ciliates and possibly bacteria. Cytopyge at posterior end of body. Movement fast, changes direction frequently.

Adoral zone of membranelles 30% of body length. Cilia of adoral zone of membranelles in vivo 13-17 μm. All cirri 8-12 μm long. Right marginal row commences at level of 3rd-4th cirrus of ventral row. Ventral row commences next to distal end of adoral zone of membranelles, usually terminates near centre of ventral surface. Marginal rows almost closed posteriorly. Bases of the 3 frontal cirri slightly enlarged. Undulating membranes almost straight, lie side by side. Buccal row in line with middle (second) frontal cirrus. First buccal cirrus lies at a level with 2nd cirrus of 1st fronto-ventral row. First fronto-ventral row in line with right frontal cirrus. Last cirri of fronto-ventral rows sometimes out of line. A 3rd fronto-ventral row exists in 64% of cells (including cells which have only 1 cirrus for the 3rd fronto-ventral row). Two dorsal kineties commence at anterior end of body, right kinety courses along right margin of body and ends slightly subterminally. Left kinety courses almost straight to posterior end of body, its last 3 cilia curved to left. Caudal and transverse cirri absent.

Prior to encystment in a clone 2 cells unite along their oral surfaces and the posterior body half attenuates. Pairs stop swimming and round up, still

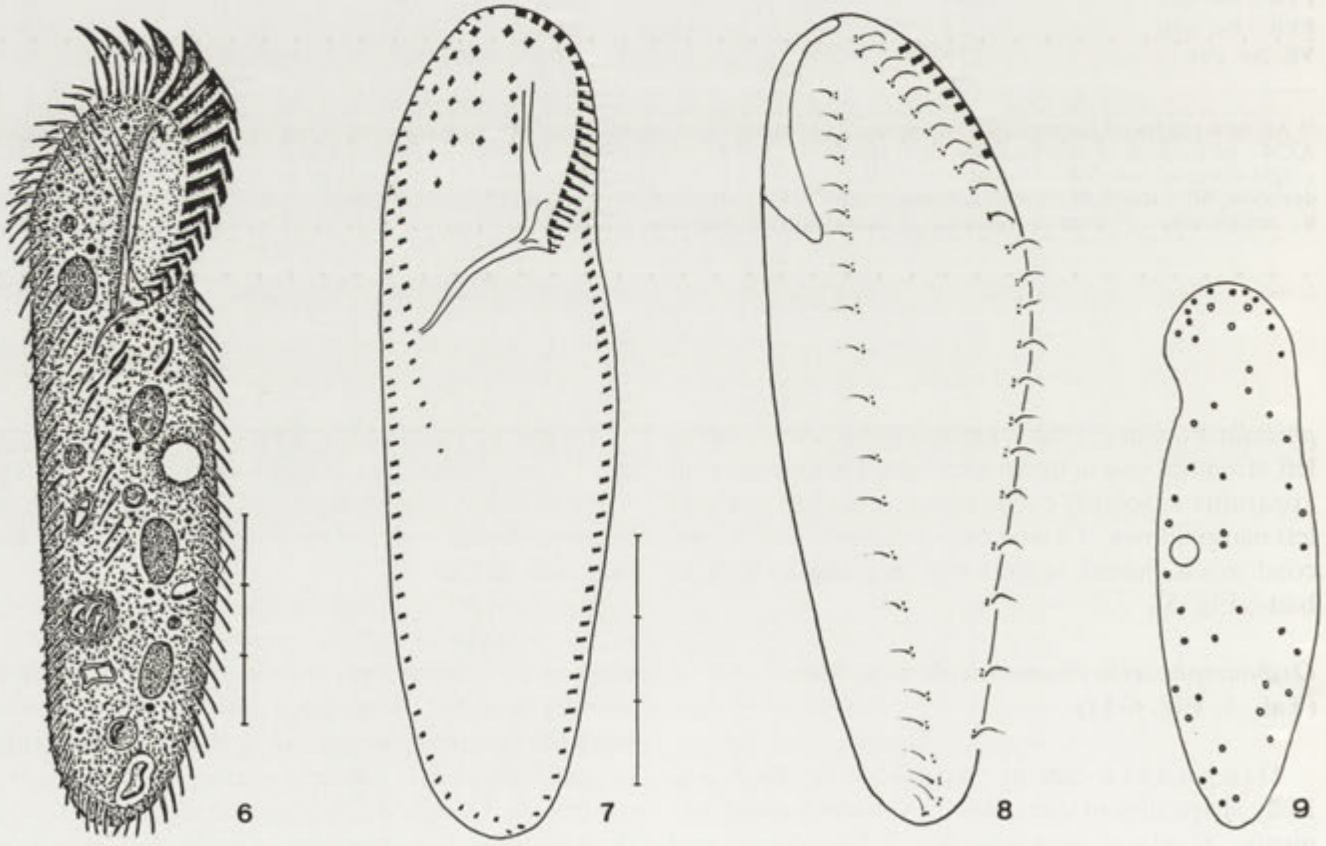

Fig. 6–9. *Orthoamphisiella stramenticola* from life (Fig. 6) and after protargol impregnation (Fig. 7, 8). 6, 7 – Ventral views. 8 – Dorsal view, 9 – Dorsal view of shape variant. Note loosely arranged subpellicular granules. Scale bar division = 10 μm.

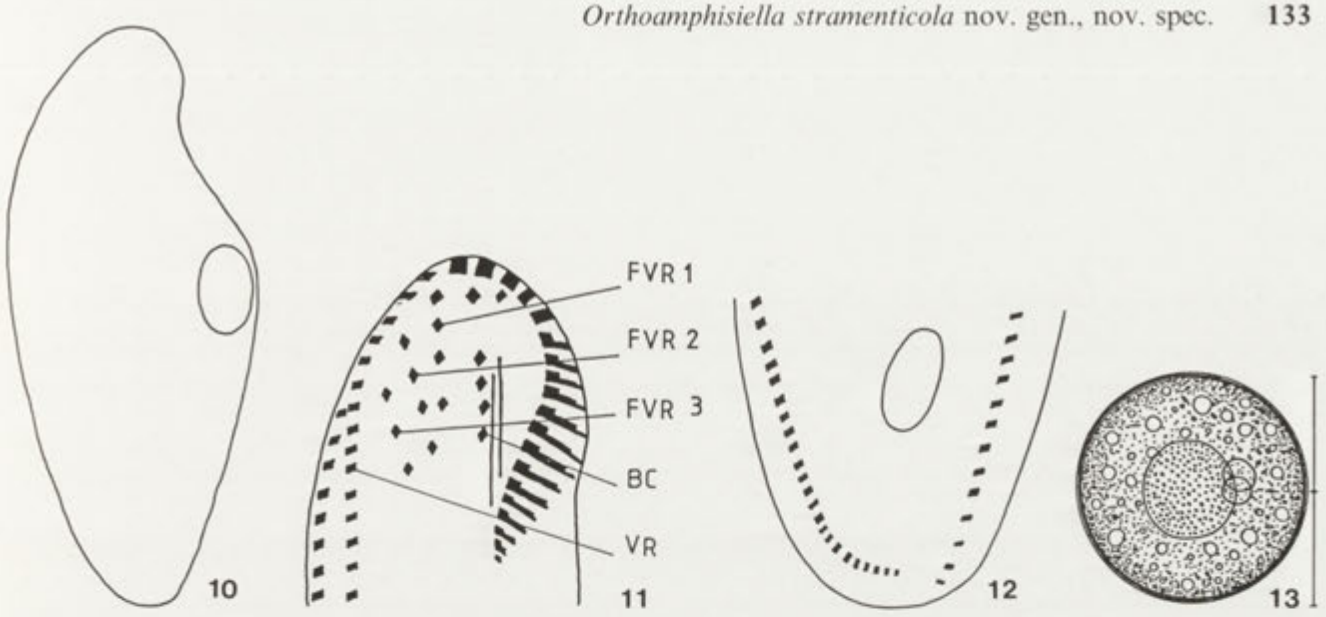

Fig. 10–13. *Orthoamphisiella stramenticola* from life (Fig. 10, 13) and after protargol impregnation (Fig. 11, 12). 10 – Body shape of a middle morphogenetic stage. 11, 12 – Anterior and posterior portion of ventral side higher magnified. 13 – Resting cyst. BC – buccal row; FVR 1, 2, 3, – 1st, 2nd, 3rd fronto-ventral row; VR – ventral row. Scale bare divisions = 10 μm.

united along their oral surfaces. They then separate by slow movement of their cirri which eventually disappear. Resting cysts usually lie by pairs, filled with greasily shining globules, 15-42 μm ($\bar{x}$ = 38 μm, n = 30) in diameter. Shape spherical to slightly ellipsoid, wall c. 1 μm thick (Fig. 13).

Comparison with related species: No other species is known to us with the characters of *Orthoamphisiella stramenticola*. Some, however, appear rather similar when superficially observed, e. g., *Hemiamphisiella quadrinucleata* (Foissner, 1984) Foissner, 1988 (caudal cirri present, 4 dorsal kineties, no fronto-ventral rows), *Amphisiella quadrinucleata* Berge et Foissner, 1989 (transverse cirri present, only 1 fronto-ventral row left of ventral row), *Amphisiella vitiphila* (Foissner, 1987) Foissner, 1988 (transverse cirri present, 3 dorsal kineties, usually only 3 ventral cirri behind the 3 frontal cirri).

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Received on 30th July, 1990

IN PREPARATION:

E. Hadaś and **T. Mazur**: Studies on biochemical indicators of virulence in the amoebae of the *Acanthamoeba* sp. – **J.J. Lipa** and **H.M. Hokkanen**: A haplosporidan *Haplosporidium meligethi* sp. n. and a microsporidian *Nosema meligethi* I. and R., two protozoan parasites from *Meligethes aeneus* L. (*Coleoptera: Nitidulidae*) – **L. Szablewski** and **E. Hadaś**: Prostaglandins of *Tetrahymena pyriformis* GL-C and *T. rostrata* – **V. Golemansky** and **J.J. Lipa**: Gregarines (*Apicomplexa: Eugregarinida*) from cave and terrestrial Arthropods in Bulgaria – **A. Ucieklak** and **S. Dryl**: Effect of organic calcium channel blockers on ciliary reversal in *Paramecium octaurelia* (strain 299 s) – **D.-P. Häder**: Advanced methods in photobiology of Protists – **P.K. Prasdan** and **K.P. Janardanan**: *Stenophora tamulini* sp. n. (*Apicomplexa: Cephalina*) from the centipede *Tamulinus ceylanicus* (Attems) of Kerala, India – **A.I. Darwish** and **V.G. Golemansky**: Coccidian parasites (*Coccidia: Eimeriidae*) of domestic rabbits (*Oryctolagus cuniculus domesticus*, L.) in Syria.

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