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Response of the Hyalospheres of *Amoeba proteus* to Direct Electric Current

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Synopsis. Reactions of the hyalospheres to direct electric current (DC) were examined. The stimulating field intensity was constant in all experiments and established at 10V/cm potential gradient. The dissociation of the consecutive actin layers from the plasma membrane appeared more frequent in the electric field than in its absence. The frequency of the cortical detachment was always higher at the cathodal side. This phenomenon was accompanied by translocation of the hyaloplasm toward the cathode and respective shift of the granuloplasmic core in the opposite direction, i.e., toward the anode. It is concluded that probably in the normal moving amoebae, like in the hyalospheres, the response to DC is also based on the successive separation of cortical sheets by the cathodal current.

It is generally known that in each frontal region of a moving *Amoeba proteus* the actin network is dissociated from the membrane and withdrawn to the hyalo-granuloplasm border (Grębecka and Hrebenda 1979, Wehland et al. 1979, Stockem et al. 1982, Hoffmann et al. 1984). These local discontinuities in the cortex structure explain endoplasmic flow in the frontal direction and, as a result, the amoeba's locomotion (Grębecki 1982, Grębecka 1988, Stockem and Kłopocka 1988, Dembo 1989). So far, however, all existing studies relating to the reconstruction of the events taking place in the front of moving amoebae were mostly based on the examination of fixed cells.

As we demonstrated in a previous paper (Grębecki and Kwiatkowska 1988) the spontaneous dissociation of the successive cortical sheets from the membrane takes place in amoebae pretreated with heat

shock (the hyalospheres). Therefore, we treat the hyalospheres as an excellent model of the frontal activity of amoeba making the dissociation of membrane-cortex contacts accessible for a direct study. Our preceding investigations of the light and shade effects on the hyalospheres (Kwiatkowska and Grębecki 1988) were the first approache made in vivo to the role of breaking membrane-cytoskeleton contacts in amoeba's reaction to the positive and negative external stimuli. Consequently, the dissociation of membrane-cortex contacts found in the hyalospheres has been now re-investigated under the influence of the electric current.

Material and Methods

Cultures of *Amoeba proteus* were grown on the standard Pringsheim medium and fed on *Tetrahymena pyriformis*. The hyalospheres were produced as reported previously (Grębecki and Kwiatkowska 1988) by heat shock (40°C for 20 min). They were examined in the direct electric field established along an experimental chamber with two silver coated electrodes. The distance between the electrodes was 3 cm and the stabilized potential difference of 30 V (from a DC generator) was applied to them. It produced 10 V/cm potential gradient in the test chamber.

All observations of the hyalospheres' reactions to DC were carried out in Pringsheim solution.

All experiments were followed in the differential interference optics and recorded on video tape, which enabled a later careful study of the produced responses.

Results

Without electric field, the intracellular movement and its dynamics in the hyalospheres were, in the present study, approximately the same as we observed and reported earlier (Grębecki and Kwiatkowska 1988).

The examination of hyalospheres exposed to direct electric fields has shown that most distinct responses occurred at the field gradient of about 10 V/cm (similar to the normal moving *Amoeba proteus* — Korohoda and Kurowska 1970, and fibroblasts — Onuma and Hui 1988). Generally, the dissociation of membrane-cortex complex in the hyalospheres was better expressed in DC presence, than in its absence. The optically dense sheets moving backwards through the hyaline layer appeared much more frequently on the cathodal side than on the opposite one. Sometimes, their detachment took place on the anodal side too, however it was a rather sporadic event.

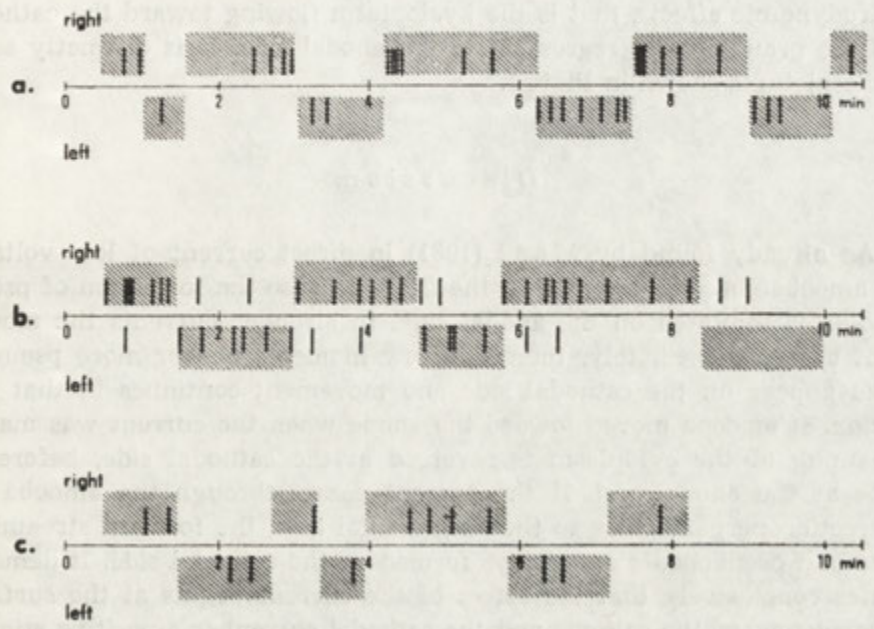

Fig. 1. Frequency of the detachment of cortical sheets in 3 hyalospheres (a, b and c) exposed to direct electric current (10 V/cm). The dashed segments represent the position of the cathode, which alternated between the left and right side relative to the cell

Alternating changes of polarization of the applied current, produced at regular or irregular intervals, provided similar effects, as shown on Plate I.

Experiments were continued for ten or more minutes. The frequency of the cortex dissociation during that time was measured and analyzed from the video tape. In Fig. 1 three typical examples of such experiments are presented. They show that in the DC field the detachments of cortical sheets is significantly reduced in frequency on the anodal side or even arrested, while on the cathodal side this phenomenon is incessantly produced. As a result the frequencies of detachment manifest very significant differencies on the two sides. In 15 experiments, which totalized over 110 minutes of observation, 231 sheets dissociated at the cathodal side and only 83 at the anodal one were recorded. Usually, in the case of periodical alternating the direction of electric current, the cortex disassembly is restored on the formerly anodal side, after it became exposed to cathode. Together with the inversion of the current direction the polarity of cell cortex response is also almost immediately reversed.

In most experiments the cortical detachment brings the expected

hydrodynamic effects, that is the hyaloplasm flowing toward the cathode and the granuloplasm regressing to the anodal side. It is distinctly seen in the pictures shown in Plate I.

Discussion

As already found by Mast (1931) in direct current of low voltage the amoebae become oriented in the field, because the formation of pseudopodia is inhibited on the anodal side. In stronger currents the movement ceases immediately, then in a few moments one or more pseudopodia appear on the cathodal side and movement continues in that direction. If amoeba moved toward the anode when the current was made, streaming of the cytoplasm is reversed at the cathodal side, before it stops at the anodal end. If the current passes through the amoeba in a direction perpendicular to the longitudinal axis, the forward streaming stops and pseudopodia are always formed on the cathodal side. It demonstrates conclusively that the effect of the current begins at the surface directed toward the cathode and the cathodal current is a positive stimulus which initiates and enhances the movement of fronts. That means that the detachment of cortical layers from the cathodal side in the hyalospheres, is very similar to the behaviour of advancing fronts of normal amoebae moving in the direct electric field. As a matter of fact, the identity of this phenomenon in the untreated locomoting amoebae during the current-induced and spontaneous migration has been recently demonstrated by one of us by video enhancement procedures (Grębecki, in press). Therefore, in our opinion, this cyclic dissociation of the actin submembrane network which was analyzed here in the hyalosphere model, is the fundamental function of normal migrating fronts.

The mechanism of this field-oriented membrane-cytoskeleton dissociation remains hypothetical. It may be postulated however, that the current-induced penetration of extracellular calcium through the voltage-gated Ca channels is limited to the cell surface facing the cathode (Nuccitelli 1983, Cooper and Schliwa 1985, Robinson 1985). The calcium entry may lead to weakening the cortical actin network and/or its disengagement from the membrane-attachment sites. That effect is attributed in the literature to the calcium movements provoked by local anaesthetics (Nicolson et al. 1977, Low et al. 1979, Nachmias et al. 1979) and electric field (Onuma and Hui 1988). This interpretation has already been put forward by us (Grębecka 1988, Grębecki and Kwiatkowska 1988) as explanation given to other cases of stimulating the frontal activity of amoebae.

ACKNOWLEGMENTS

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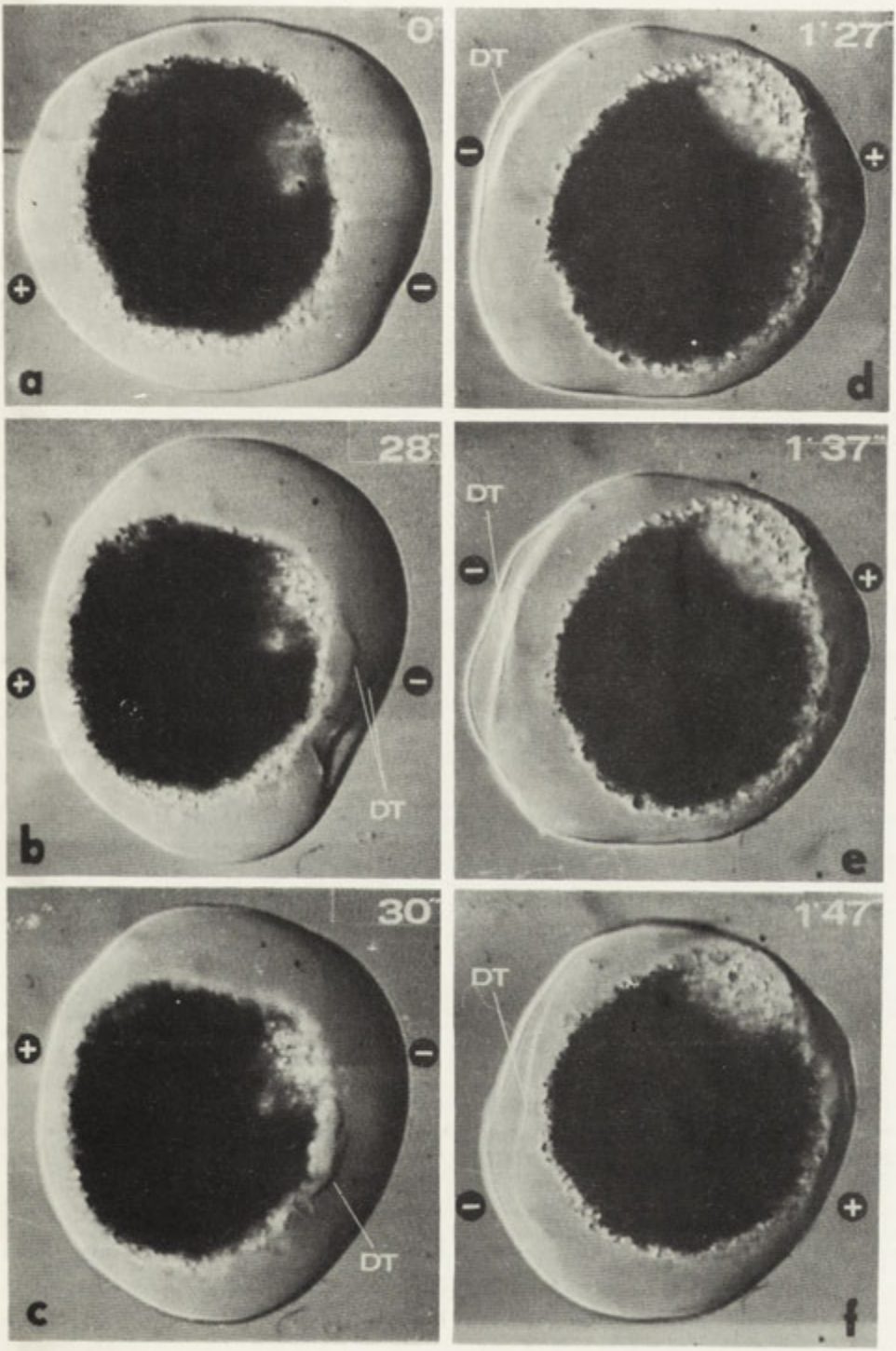
EXPLANATION OF PLATE I

The hyalosphere kept in direct electric current (10 V/cm). Note the detachment of submembrane contractile layer (DT in b-f). In a-c the cathode was applied at the right side of the cell, and in d-f at the left. Selected stages from a video record. Timing in seconds. (× 500)



E. M. Kwiatkowska and A. Grębecki auctores phot.

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Action of Calcium Channel Blockers on Potassium-Induced Ciliary Reversal in *Paramecium octaurelia* (Strain 299s)

A g n i e s z k a U C I E K L A K and S t a n i s ł a w D R Y L

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Synopsis. Both inorganic (Co^{2+}, Cd^{2+}, Mn^{2+}, La^{2+}) and organic (verapamil, D-600, nifedipine) calcium channel blockers caused shortening of potassium-induced ciliary reversal in *Paramecium octaurelia* (strain 299s). The observed effect of Ca channel blockers at their sublethal concentrations was more strongly pronounced in case of inorganic blockers than in organic ones. It is suggested that the above mentioned inhibition of potassium-induced ciliary reversal is associated with the lowering activity of voltage sensitive calcium channels located within ciliary plasma membrane.

During last decaded the ciliate protozoa proved to be model unicellular organisms for studies on excitability and motor response to external stimuli at the cellular level. Among ciliates *Paramecium* became one of the most useful tool for experimental studies under laboratory conditions. It shows typical motor reactions in response to various kinds of external stimuli in form of the longer lasting reversed beat of cilia or short lasting "avoiding reactions" (J e n n i n g s 1906). Ciliary reversal (CR) is triggered as a result of activation of calcium conductance of the ciliary membrane and the resulting influx of external calcium ions into the intraciliary space which activates the reversal mechanism (D r y l 1974, D o u t h y and D r y l 1980). So it is evident that CR induced by external stimuli is associated with the opening of voltage sensitive calcium channels within ciliary plasma membrane which encloses the axoneme. It should be added that calcium channels have been found in many excitable cells — from single cell organism to mammalian nerve, muscle

and secretory cells (Hagiwara and Byerly 1983). Ehrich et al. (1984) brought evidence that ciliary membrane of *Paramecium* contains ionic channels that are divalent cations selective. They are moderately selective (calcium and barium can be electric current carriers) and are strongly voltage dependent, being responsible for intracellular recording of calcium inward current.

It is known that calcium transmembrane currents can be blocked by certain polyvalent metal ions (La, Cd, Co, Ni, Mn) and by some organic substances (verapamil, D-600, nifedipine, nitrendipine, diltiazem etc.), which appear to be specific in blocking calcium currents of vertebrate heart and smooth muscles, but they are much less effective against activity of Ca channels of other membranes (Hagiwara and Byerly 1983).

The aim of the present study was to analyze the possible effect of some above mentioned Ca channel blockers on the excitability of *Paramecium octaurelia* (strain 299s). The indication of the effective blocking of calcium channels was decrease of induced CR duration caused by external potassium ions (Dryl and Totwen-Nowakowska 1985).

Material and Methods

Experiments were carried out on *Paramecium octaurelia* strain 299s, cultivated in axenic medium (Soldo et al. 1966, 1969), temperature 22-24°C. Before starting experiments the ciliates were collected by low speed centrifugation, washed twice in buffer solution: 1 mM $CaCl_2$ + 1 mM Tris/HCl (pH 7.2) and left for starvation during period of 20 h.

It should be pointed out that potassium chloride and calcium channel blocker substances were also prepared on the basis of above mentioned buffer solution.

After twenty hours of washing, paramecia were incubated for three minutes in LD_{50} concentrations (1 h exp.) of applied Ca channel blocker substances and then exposed to solutions of KCl (30 mM, 40 mM and 80 mM) in order to check the duration of K-induced CR. Potassium chloride solutions contained the same concentration of Ca channel blockers as incubation medium.

Observations were carried out on 50 paramecia in depression slides containing 0.5 ml of experimental medium. The motile behaviour of ciliates was checked under low power microscope and the criterion of duration of observed CR response was renormalization of movement observed in 25 ciliates (i.e. in 50 per cent of population). Calculation were done on basis of 20 repeated experiments.

Control experiments were performed in similar way on Paramecia exposed to KCl solution devoid of Ca channel blockers. Data concerning the duration of K-induced CR included in Table 1 (for inorganic) and Table 2 (for organic Ca channel blockers) have been recalculated as percentage in relation to control mean

values and were presented in diagram (Fig. 1) This way of presentation of achieved experimental data gave good opportunity to compare results from both groups of applied Ca channel blockers.

Results and Discussion

It was proved in the preliminary series of experiments that LD_{50} concentrations (1 h exp.) of Ca channel blockers were sufficient high to ascertain hundred per cent survival of paramecia during relatively short lasting experimental procedure (not exceeding 300 s). Only in case of $NiCl_2$ discoordination of ciliary movement rendered impossible observations of altered motor reactions of ciliates induced by KCl solutions.

Following LD_{50} concentrations (1 h exp.) of applied Ca channel blockers were established: 1.7×10^{-3} M $CoCl_2$, 3.8×10^{-3} M $MnCl_2$, 5×10^{-5} M $CdCl_2$, 3.3×10^{-4} M $LaCl_3$, 2.1×10^{-5} M verapamil, 2.5×10^{-5} M D-600, 2.5×10^{-4} M nifedipine.

Paramecia exposed for three minutes to above mentioned solutions of Ca channel blockers showed reduced rate of forward movement and in case of inorganic blockers short lasting intermittent CR responses which lasted not longer than 100 seconds.

The exposure to KCl solutions showed in comparison to control marked decrease in duration of K-induced CR as a consequence of three minutes lasting incubation in medium containing Ca channel blockers. Only lanthanium ($LaCl_3$) was ineffective at 30 mM KCl and showed rather negligible effect at 40 mM KCl.

Data included in Table 1 and 2 suggest that Ca channel blockers can be arranged in the following way in relation to their relative blocking strength:

Inorganic blockers: Co > Mn > Cd > La

Organic blockers: nifedipine > verapamil > D-600

Data presented in Fig. 1 show additionally that blocking effects of Co, Mn, Cd are more strongly expressed at 30 mM KCl than at higher KCl concentration, whereas — vice versa — blocking effects of organic Ca channel blockers were weaker at 30 mM KCl than at 40 mM and 80 mM KCl.

On general the achieved results on action of Ca channel blockers in paramecia are in good agreement with the analogous data reported for heart and smooth cells of vertebrate animals. Even effective concentrations of blockers proved to be similar, although little higher than in case of metazoan cells (F l e c k e n s t e i n 1983).

Lanthanium in relatively high concentration did not show any bloc-

Table 1

Inhibiting action of inorganic calcium channel blockers on potassium-induced ciliary reversal in *Paramecium octaurelia* (strain 299s)

Concentration of KCL (in Ca-Tris/HCL buffer solution)	Duration (s) of potassium-induced ciliary reversal			
	$CoCl_2$	$MnCl_2$	$CdCl_2$	$LaCl_3$
30 mM	24.7± 3.3	31.6± 5.2	31.3± 2.1	51.0± 3.6
	(59.5± 4.0)	(59.5± 4.0)	(45.5± 2.7)	(51.0± 3.3)
40 mM	35.2± 4.0	44.8± 4.3	55.0±4.6	53.0±11.0
	(74.7± 4.8)	(74.7± 4.8)	(69.8±2.8)	(57.0± 3.4)
80 mM	60.4± 7.0	69.3±18.5	51.3±5.0	no reversal
	(83.8±19.0)	(83.8±19.0)	(65.6±8.9)	(69.2± 2.2)

Data presented in columns correspond to duration (s) of potassium-induced ciliary reversal after exposure to LD_{50} (1 h exp.) concentrations of applied blockers. Data included in parantheses correspond to duration (s) of potassium-induced ciliary reversal in control medium, devoid of calcium channel blocker. Average values were calculated from twenty repeated measurements. Data concerning the LD_{50} concentrations of blockers are included in chapter "Material and Methods".

king effect on paramecia exposed to 30 mM KCl solution. This result seems rather surprising since for long time lanthanium was considered as one of the most effective inorganic blockers (Hagiwara and Byerly 1983). It should be pointed out in this respect that in general the inhibiting effects of Ca channel blockers (both inorganic and organic) proved to be more strongly expressed in case of hypotrich ciliate *Stylonychia mytilus* (Dryl and Totwen-Nowakowska 1955), where high concentrations of $CoCl_2$ and $MnCl_2$ caused complete inhibition of K-induced reversed beat of cirri. On the other hand, the effect of verapamil and D-600 on K-induced CR was much weaker and rather similar to ef-

Table 2

Inhibiting action of organic calcium channel blockers on potassium-induced ciliary reversa in *Paramecium octaurelia* (strain 299s)

Concentration of KCL (in Ca-Tris/HCL buffer solution)	Duration (s) of potassium-induced ciliary reversal		
	verapamil	D-600	nifedipine
30 mM	57.0± 6.5	55.0± 4.0	35.0±2.0
	(59.5± 4.0)	(59.5± 4.0)	(42.0±1.5)
40 mM	61.0± 8.0	66.3± 4.5	39.0±2.2
	(74.7± 4.8)	(74.7± 4.8)	(51.0±5.0)
80 mM	66.4± 4.6	67.0± 4.5	42.0±3.4
	(83.8±19.0)	(83.8±19.0)	(55.0±5.2)

For explanation cf. Table 1.

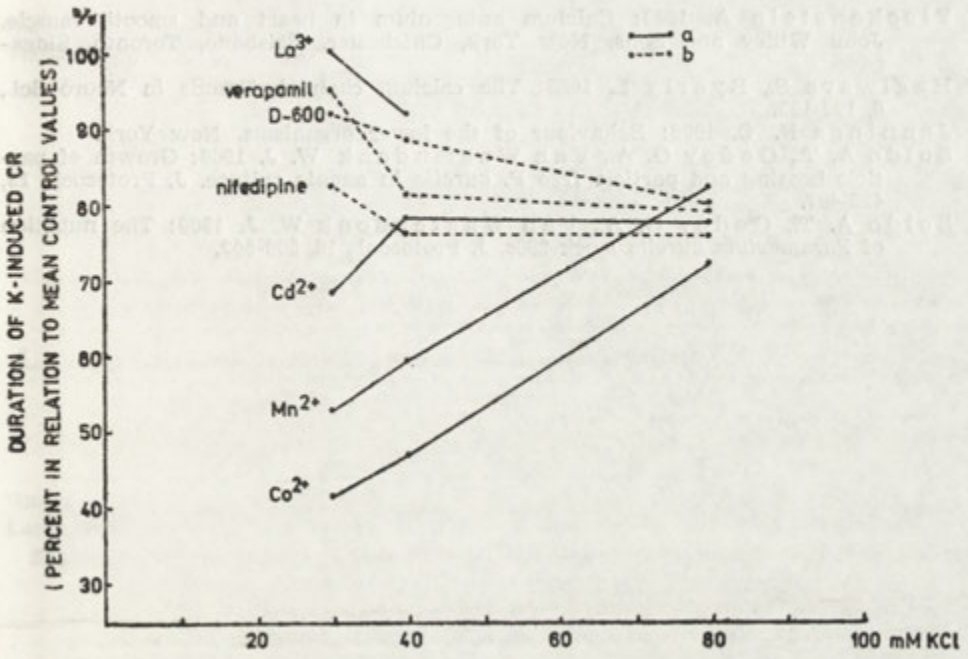

Fig. 1. Changes in duration (s) of potassium-induced ciliary reversal in *Paramecium octuarelia* (strain 299 s) by inorganic and organic blockers applied at LD_{50} (1 h exposure) concentration, a — inorganic Ca channel blockers, b — organic Ca channel blockers. The points represent data from Table 1 and 2 recalculated as percentage in relation to control

fects reported in the present paper. In marine ciliate *Fabrea salina* CR inhibiting effects of Ca channel blockers were much stronger pronounced (Dryl and Łopatowska — pers. comm.).

The achieved results suggest that similarly as in metazoan cells, the Ca channels of ciliate protozoa show great diversity of functional properties.

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Heat-Shock Proteins in *Amoeba*. II. The Effect of Cooling on *Amoeba borokensis*

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Synopsis. It was found that in *Amoeba borokensis* exposed to low temperature (6°C) the pattern of synthesized proteins changes while survival of cells is unaffected during up to 51 h of incubation. The cells produce viable hyalospheres and the majority of cells restore their normal amoeboid shape and motility on rewarming to room temperature. The possible associations between enhancement of the synthesis of low molecular weight proteins of amoebae exposed to decreased temperature and the ability to withstand the seasonal changes of the surrounding temperature is discussed.

It became widely accepted that environmental stress has a distinct and dramatic effect on gene expression in living organisms (Alahiotis 1983, Snutch and Baillie 1983, Schlesinger 1986, Bond and Schlesinger 1987). Most animals respond to thermal stress by reducing the synthesis of normal cell proteins and initiating the synthesis of a set of proteins termed heat-shock proteins (hsps) or stress proteins (Craig 1985). The synthesis of heat-shock proteins depends on the limited number of low active or quiet genes in normal conditions. It was found that in stress the new mRNA transcriptional level is rapidly increased (Ritossa 1962, 1964, Schlesinger et al. 1982) while normal mRNAs are stored or even repression of the normal genes is noted (Ashburner 1970, Bonner and Pardue 1976, Findley and Pe-

derson 1981). Since a number of agents (not only supraoptimal temperatures) induce heat-shock proteins synthesis, it is understood that the hypothetical common inducing factor is rather elusive (Tanguay 1983). According to Craig (1985) the proteins found in *Drosophila melanogaster* after exposure to stress conditions can be classified into three families on the basis of structural homologies: the first — hsp 83 kD, second — hsps 70 and 68 kD and third — hsps 27, 26, 23 and 22 kD. More or less homologues to the above cited hsps have been identified in many species. In a number of species of Protista heat-shock proteins were noted during normal growth or in response to shock temperatures, as for instance in *Dictyostelium* (Loomis and Wheeler 1980), *Tetrahymena pyriformis* and *T. thermophila* (Fink and Zeuthen 1980, Guttman et al. 1980, Gorovsky et al. 1982, Amaral et al. 1988), *Amoeba proteus* and *Amoeba borokensis* (Kalinina et al. 1988).

The aim of the present report is the determination of the motility, behaviour and survival of free living *Amoeba borokensis* exposed to a temperature below optimal for its growth (6°C) and estimation of the heat-shock proteins synthesis pattern. We were searching for the positive correlation between the presence of hsps in cells and the thermotolerance of amoebae to cold shock.

Material and Methods

Fresh water free living *Amoeba borokensis* (Pl. I 1) (Kalinina et al. 1988) from the collection of the Institute of Cytology Academy of Sciences of the USSR were cultured in Petri dishes at 22 ± 1°C by the method of Prescott and James (1955). Cells were fed twice weekly with washed *Tetrahymena pyriformis* and starved 2-3 days before experimental treatment.

Samples (approximately 150-200 cells) of *A. borokensis* in Petri dishes (4 cm in diameter) were exposed to the reduced temperature (6°C) for 8, 11, 26, 31 and 51 h. The cytoplasmic streaming, ability to locomotion and viability of amoebae were determined 24 h after rewarming of the samples to 22°C. Cells were considered normal if cytoplasmic streaming, amoeboid morphology and motility were restored. Observations were made under a binocular microscope of low magnification. The heat-shock proteins assay in cold shocked amoebae was based on the previously reported procedure (Kalinina et al. 1988). Pulse labelling with ^{35}S-methionine (100 μCr ml^{-1}) was performed during the last 3 h of incubation at reduced temperature. The samples of concentrated amoebae (10^4 cells in 0.5 ml) were exposed to 6°C for 5, 9, 23, 28 and 48 h. Therefore the total duration of incubation at lowered temperature was: 8, 11, 26, 31 and 51 h. The appropriate time of labelling with radioactive methionine at 6°C was determined in preliminary experiments. Autoradiograms on disc polyacrylamide gels were analysed by means of the microdensitometer MD-1000 (Zeiss).

Results

Most of *Amoeba borokensis* cells exposed to decreased temperature form hyalospheres (Pl. II 4,5) (Seravin 1966, Grębecki and Kwiatkowska 1988) and on return to room temperature restore their amoeboid shape (Pl. II 2, 3), cytoplasmic streaming, and locomotive ability. Since the majority of cells restore their normal morphology and physiological functions it may suggest that the primary response of amoebae to the decreased temperature appeared rather in gene expression. The first signs of differences in the pattern of the newly synthesized proteins labelled in vivo with ^{35}S-methionine were noted in gel electrophorisis and visualized by autoradiography (Pl. III 6) in samples of amoebae incubated for 8 h. The label appeared in newly synthesized proteins of molecular weight 21, 30 and 31 kD. Prolongation of incubation to 11 h led to the appearance of the following bands: 24, 27 and 37 kD, while the density of 30, 31 and 52 kD (52 kD also present in the control) apparently increased. Low temperature exposure for 26 h evoked the 26 and 55 kD bands and subsequent increase in density of the 24, 30, 31, and 52 kD bands. Further maintenance at supraoptimal temperature did not cause synthesis of the new proteins. The synthesis of 18, 28, 29, 58, 65, 70 and 83 kD proteins remained fairly constant during the whole period of exposure to reduced temperature. Finally, it is worth mention that the synthesis of 42 kD actin decreased as the time of incubation at low temperature was prolonged.

Discussion

The enhanced synthesis of several heat-shock proteins as a result of stress conditions is the universal response of all sepcies of the organisms examined up till now (Craig 1985). The most probable function of heat-shock (or stress-shock) proteins is to increase the cellular resistance to the adverse conditions (Loomis and Wheeler 1980, Li and Werb 1982, Plesset et al. 1982, Ketola-Pirie and Atkinson 1983, Landry and Chretien 1983, Snutch and Baillie 1983, Tanguay 1983). *Amoeba borokensis* and *Amoeba proteus* (strain War), differing markedly in their thermoresistance when exposed to the elevated temperatures, synthesized heat-shock proteins of a similar pattern. It was therefore concluded that there is no sufficient evidence for a correlation between synthesis of polypeptides which might be regarded as

heat-shock proteins and the thermoresistance to temperatures elevated above optimum for growth (Kalinina et al. 1988). On the other hand, the *Dictyostelium discoideum* mutant lacking the ability to synthesize hsps loses its resistance to heat shock (Loomis and Wheeler 1982 a, b). Therefore, the determination in *Amoeba borokensis* of the correlation between enhancement of hsps synthesis during exposure to decreased temperature may give us a hint in respect of the function of hsps in free living-amoebae. In *Amoeba borokensis* cells exposed to low temperatures for 8 to 26 h synthesis of new proteins: 21, 24, 27, 30, 31, 37 and 55 kD was enhanced. Prolongation of this treatment leads only to the enhancement of synthesis of low molecular weight proteins. The results presented in this paper do not give unequivocal evidence that hsps are responsible for the increase of thermoresitance of *A. borokensis*, but point to an association between the enhancement of hsps synthesis and the successive appearance of hsps during prolonged cooling. Possibly, this might indicate the involvement of hsps in the process of adaptation to the adverse environmental conditions. The induction of new and/or enhanced gene expression in response to cold is known, however it is restricted to a few examples. For instance the new hsps of 20, 34, 45 and 83 kD appeared in the spectrum of *Tetrahymena* incubated at 10°C for 24 h (Fink and Zeuthen 1980). Enhanced synthesis of 65 kD polypeptide in epidermal cell cultures of *Rana catesbeiana* have been found after a short time exposure to elevated (32°C) or depressed (5°C) temperatures (Ketola-Pirie and Atkinson 1983). On the other hand, the kidney epithelial cell line of *Xenopus laevis* responds to heat shock but does not respond to cold shock (Ketola-Pirie and Atkinson 1983). So-called antifreeze proteins or lypoproteins of 10-32 kD molecular weight in many teleost fish species inhabiting polar waters are believed to be heat-shock proteins protecting the fish serum against freezing (Feeney and Yen 1978). It seems possible that hsps synthesized at decreased temperatures are involved in the process of "hardening" the organisms exposed in nature to the seasonal changes of the ambient temperature (Volger and Heber 1975, Hew and Yip 1976, Alahiotis 1983, Kishore and Upadhyaya 1988). Other mechanisms responsible for the changes of membrane fluidity, underlying longer-term changes in metabolism, cannot be excluded (Johnston and Dunn 1987, Thompson 1989). It is not clear therefore, whether the associations between enhance of synthesis of low molecular weight polypeptides in *Amoeba borokensis* exposed to decreased temperatures depend on the nonspecific interactions or are the expression of changed gene expression. However, the idea that the inducible proteins (hsps) are responsible for the increase in the resistance is of great importance in the explana-

tion of the mechanism leading to acquisition of thermoresistance to deceased temperatures in protozoans which do not have the ability to form cysts.

ACKNOWLEDGMENTS

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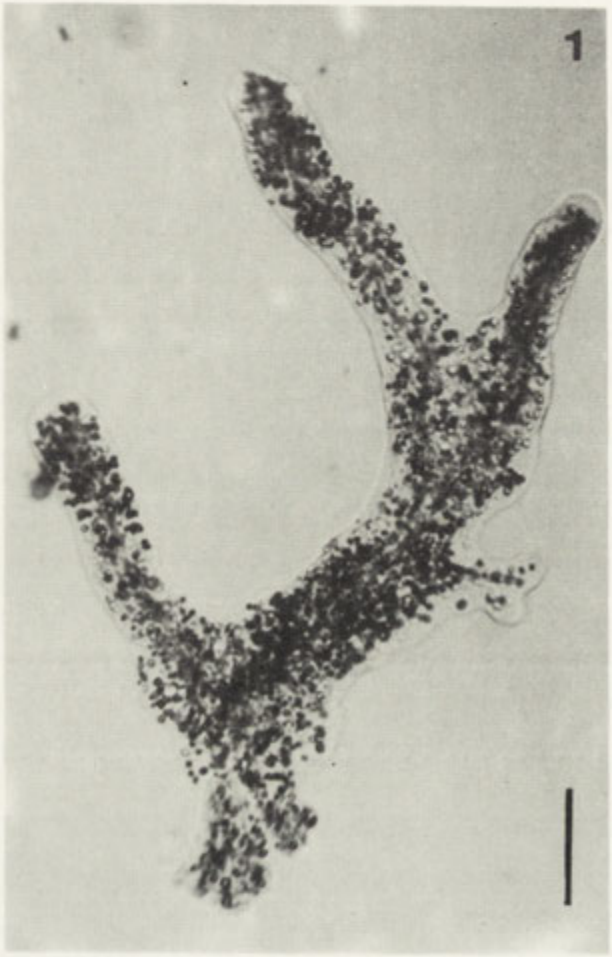
EXPLANATION OF PLATES I—III

1: Light microscopy of *Amoeba borokensis* the control. Scale bar — 100 μm
2—5: Light microscopy of *Amoeba borokensis* after exposure to low temperature (6°C), 2—3, locomotive forms after resumption of motility, and 4—5, hyalospheres. Scale bars — 100 μm
6: Autoradiograms of disc (8—15%) SDS polyacrylamide gels subjected to electrophoresis according to the procedure of Laemmli (1970) containing *Amoeba borokensis* samples maintained at room temperature 22 ± 1°C — control, and at 6°C during 8, 11, 26, 31 and 51 h. ^{35}S-Methionine labelling was introduced into the samples of concentrated cells for the last 3 h of incubation in lowered temperature (for details see Kalinina et al. 1988). Molecular weights determined by comparison with the LMW standards are indicated in kD × 10^3 at the right side of the figure



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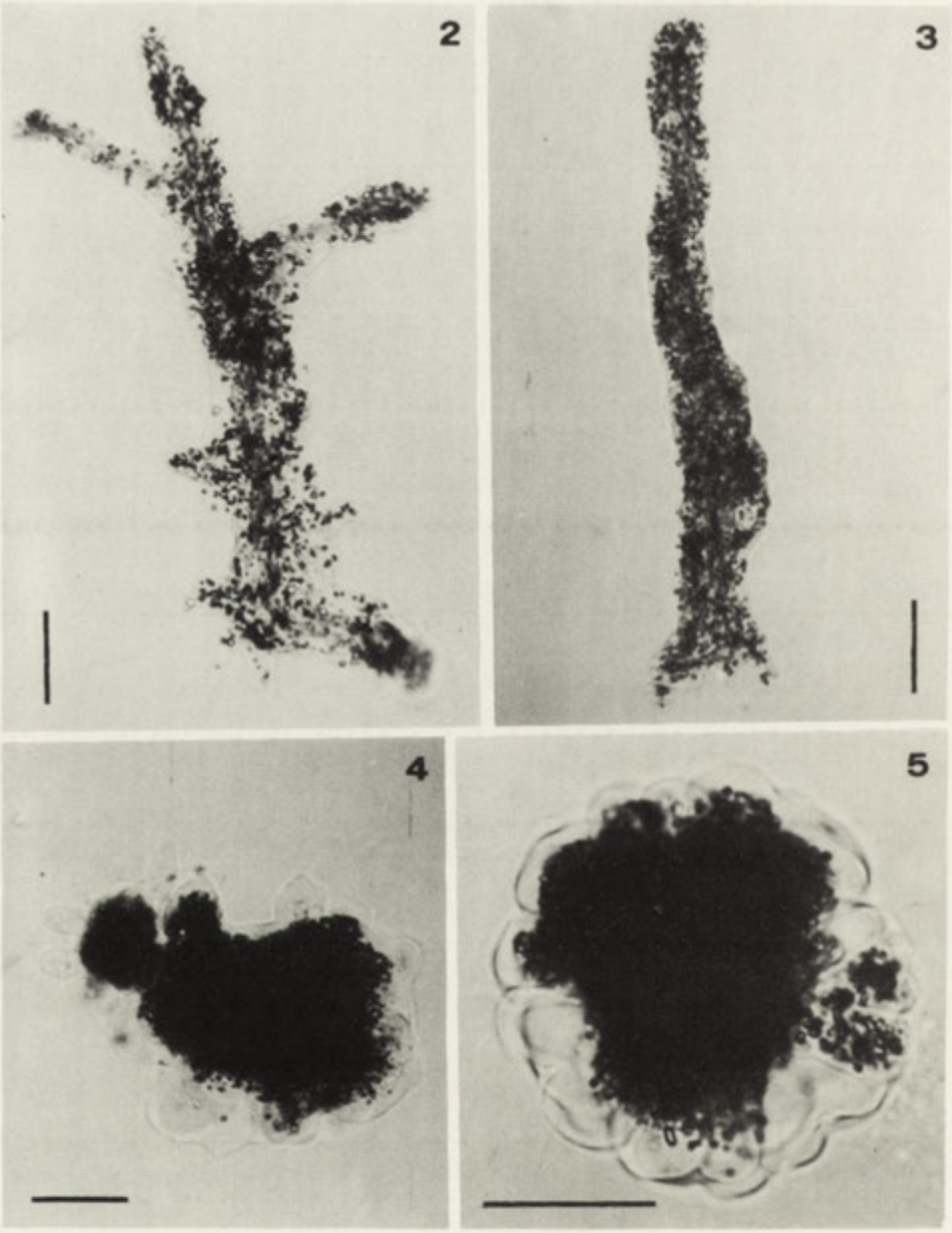

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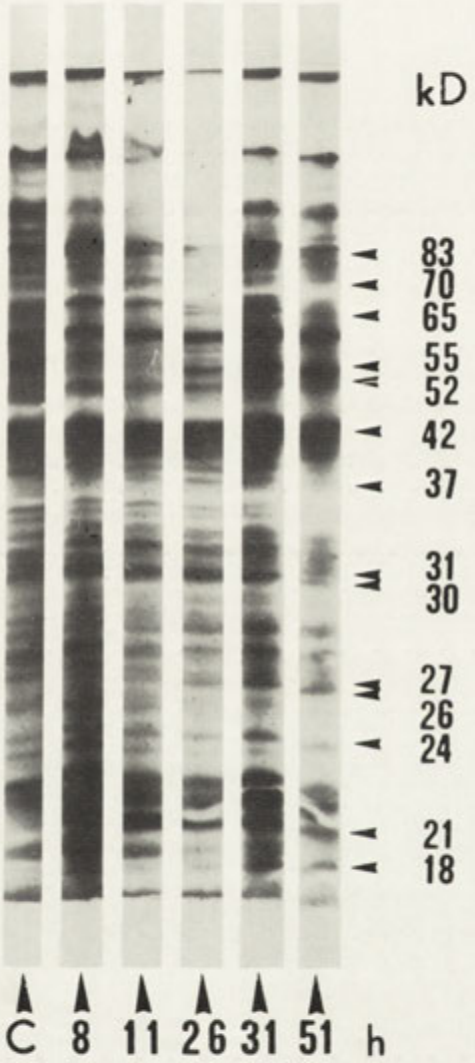

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Effect of Insulin on *Blepharisma undulans* (Stein) at Primary Exposure and Reexposure

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Synopsis. Blepharisma undulans (Stein) retired from insulin i.e. showed a negative chemotactic behaviour, after primary interaction with that hormone. This negative trend tended to decrease considerably for 7 days after the second interaction and reversion to a positive chemoattractant behaviour occured after 13 days. After a second interaction with insulin, the positive chemoattractant action was readily obvious already within 4 days. After one hour hydrolysis the Feulgen reaction was significantly reduced in insulin treated cells. The Fast-green stain alsoi was diminished in the nuclei and abolished in mitochondria. The ciliary and nuclear membrane bound FITC-or isotopically-labeled insulin at the first meeting and this was not observable at the second one. It is shown that the insulin treatment changes the further reaction of *Blepharisma* to the hormones.

The ciliated protozoa respond to environmental influences and to the changes induced by these among others by active movement, whose direction depends on the location of the source of the stimulus (e.g. photo-, geo, rheo- or chemo- taxis). The degree and trend of movement also depends considerably on the actual state (age, nutritional condition) of the unicellular organism, but primarily on the beneficial or adverse quality of the stimulus (Hellung-Larsen et al. 1986, Leick and Hellung-Larsen, 1985). The protozoon possesses receptor-like surface structures, which enable it to differentiate even related molecules from one another and to store the "memory" of the primary interaction with these (Csaba 1980, 1981).

Above all the signal molecules (hormones) are capable of inducing

that kind of "memory" in unicellular organisms (Csaba 1986). Hormonal imprinting, which occurs at the primary interaction of *Tetrahymena* with a hormone, results not only in the establishment of a "memory", but also in an altered — increased or decreased — response to the ligand by the progeny generations (Csaba 1986, Csaba et al. 1982). Although certain details of the intracellular events associated with the mechanism of imprinting are still obscure, the fact remains that a precisely reproducible imprinting takes place at interaction with all active molecules, which act at the receptor level.

At the unicellular level the occurrence of imprinting can be substantiated by determination of several parameters, such as changes in the number of binding sites for the hormone in question, as well as in phagocytotic activity, growth rate, membrane potential, intracellular Ca^{2+} level, etc. (Csaba 1981, Köhidai et al. 1987). In earlier studies the growth rate of populations of the heterotrich ciliated *Blepharisma undulans* (Stein) had been considerably suppressed by insulin treatment, but the second treatment had no such effect, in fact it had no effect at all, inasmuch as the protist seemed to become indifferent to hormonal influence (Kovács and Csaba 1988). Since the suppressive effect on the growth rate can also be attributed to toxicity, whereas the indifference observed at the second exposure to desensitization, we investigated the insulin effect by using cytochemical methods for studying the nucleus and by using a physiological model for the observation of chemoattractant action on *Blepharisma undulans* at primary exposure and reexposure. At last radioauthography and fluorescence label was used to clear the binding of the hormone to the cells.

Material and Methods

Chemoattraction

Thirty *Blepharisma* each were placed in 10 ml mineral water (Theodora springs, Hungary) of the following composition: K^+ 23 mg; Na^+ 45mg; NH^+_4 0.28 mg; Ca^{2+} 57.6 mg; Fe^{2+} 6.1 mg; Mg^{2+} 0.71 mg; Sr^{2+} 3.8 mg; Cl^- 11 mg; F^- 0.84 mg; Br^- 0.1 mg; SO^{2}_4 40 mg; HCO^+_3 1.7 mg. One group was not treated to serve as control, the other was treated with 10^{-6} M insulin (Insulin Semilente MC, Novo, Copenhagen) for 1 h. After treatment the cells were washed in 4 changes of mineral water, and were returned to 10 ml plain mineral water in which a single boiled rice-grain was placed. Chemotaxis was assessed 1, 4, 7 and 13 days after primary insulin treatment in the presence of 10^{-6} M insulin. In a further group the insulin-treated cells were reexposed to insulin for 1 h again 7 days after the first treatment. After reexposure they were washed, returned to plain mineral water, and examined for chemoattractant effect 4 days later.

For studying the chemoattractant effect a modified construction of the equipment developed by Taneda (1987) for geotaxis determination (Fig. 1.) was used. The working principle of the equipment was the following: A Zeiss K 200 compensation recorder was connected with a bidirectionally movable stage, who-

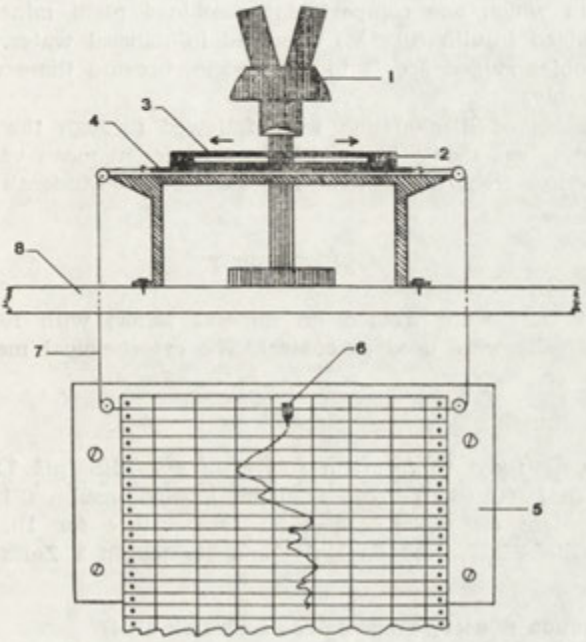

Fig. 1 a. Schematic presentation of the test system. 1 — stereomicroscope, 2 — chemotaxis-chamber with capillary tube, 3 — capillary tube, 4 — movable part, 5 — recorded, 6 — recorded head, 7 — cord, 8 — table

se movement caused the pencil to function like a chymograph. The movements were magnified twice on the record (thus Fig. 2 shows the doubled values). On the stage was mounted the system holding the *Blepharisma* containing capillary tube (Fig. 1 b). The movements of *Blepharisma* could be conveniently followed up through an appropriately placed stereomicroscope. The stage was so moved as to keep *Blepharisma* always in the centre of the reticule.

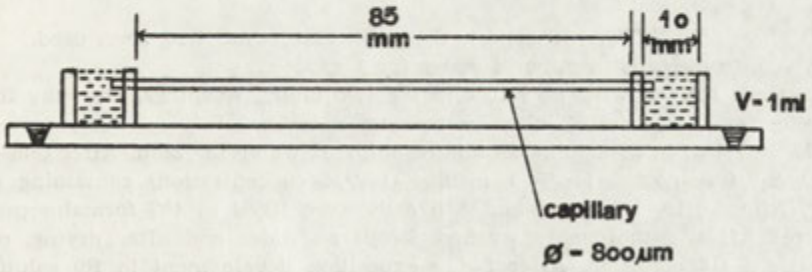

Fig. 1 b. Separated picture of the chemotaxis-chamber

The *Blepharisma* cells were placed in plain mineral water in the central part of a 10 cm long glass capillary, 800 μm in inner diameter. The capillary tube was sealed at both ends with a 1 mm thick 1% agar layer (in mineral water), which prevented effluescence of the medium, but did not interfere with diffusion. The capillary tube so prepared was fixed in the centre of a two-compartment system, in which one compartment contained plain mineral water, while the other contained insulin (10^{-6} M) dissolved in mineral water. Care was taken that no air bubbles gained access to the system, because these would inhibit diffusion considerably.

The movements of *Blepharisma* were followed through the stereomicroscope for 12 min. The values shown in the Figure 2 represent mean values. The significance of differences from the control was assessed by Student's *t*-test.

Cytochemistry

Blepharisma cells were treated (in mineral water) with 10^{-6} M insulin for 24 h. Untreated cells were used as control. The cytochemical methods were applied after this time.

FITC-insulin binding

The cells were fixed in formalin solved in 4% PBS (pH 7.2) washed twice, and incubated in FITC (BDH-Poole England) labeled insulin (FITC-protein ratio: 0.37, protein content 0.2 mg/ml), in room temperature for 1h. The cells were washed trice (with PBS) dried on slides and studied in a Zeiss Fluoval microscope.

Feulgen-reaction (Feulgen and Rosenbeck 1924)

The hydrolysis was done (with nHCl) at 60°C for 20 or 60 min. Before the reaction the cells were fixed in ethanol (5 min) for dissolving zoopurpurin.

Color intensity was registered by a Zeiss Amplival cytophotometer connected with a HP 41CX minicomputer. 10 nuclei per group were measured at 546 nm wavelength. The Figure 2 demonstrates the means fo trice repeated experiments.

Fast-green FCF-reaction (Alfert and Geschwind 1953)

The cells were fixed in formalin. Evaluation of color intensity at 625 nm wavelength. The Figure 4 demonstrates the means of trice repeated experiments.

Autoradiography

^{125}I insulin (IZINTA, Budapest, spec. act. 33 kBc/ml) had been used.

(a) Control — cells in mineral water for 1 day

(b) Cells treated with 10^{-6} insulin for two hours, washings, and stay in mineral water for 22 h.

(c) Stay in 10^{-6}M insulin containing mineral water for 24 h. After one day the cells were transported for ^{125}I insulin (10^{-9}M concentration) containing mineral water. After 5, 10, 30 min, 1 and 3 h cells were fixed in 4% formalin (solved in PBS, pH 7.2) and thoroughly washed. Drops on slides and after drying, covering with Ilford G5 emulsion. After 5-day exposition development in R9 solution and staining with diluted Giemsa stain.

Results

Chemoattraction

The cells not treated with insulin on any occasion (absolute control) made random movements in the centre of the tube to right and to left alike. The control cells showed a distinct negative chemotaxis, in that they moved almost continuously away from the insulin compartment,

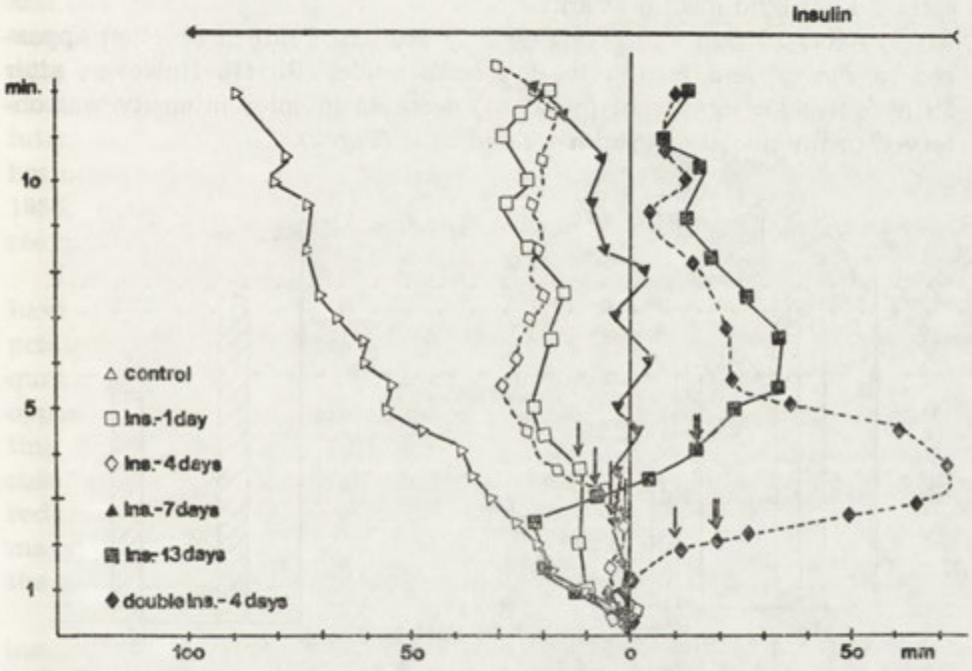

Fig. 2. Recorded movements of *Blepharisma* (means). Ordinate time after starting experiment. Abscina distance from starting point. On left control compartment, on right insulin compartment. The single arrows indicate the places in the line from which there is significant difference from the control and the double arrow indicates similarly the significant difference from the results read at 4 days

i.e. towards the compartment filled with pure mineral water (Fig. 2). The insulin-pretreated (imprinted) cells showed a less distinct negative chemotactic behaviour than the control 1 day after reexposure to the hormone. At 4 days they performed random movements similar to the absolute control for 8 min, and began to show a negative chemotactic behaviour only thereafter. At 7 days random movements could be seen for the initial 3 min, after which the signs of negative chemotaxis appeared again at a reduced, but not significantly less intensity than at 4

days. At 13 days an intesive negative chemotactic behaviour was followed after 3 min by a positive trend of movement.

On reexposure to insulin followed after 2-3 min an extraordinarily distinct positive chemotactic response appeared, after wich the cells returned to the region of their original localization, where they performed random movements further on.

Cytochemistry

(1) The control cells bound FITC-insulin. This was observed on cilia of the oral apparatus and the nuclear mambrane (Pl. I). Insulin treated cells did not bind insulin at all.

(2) After 20 min hydrolysis equally positive Feulgen reaction appeared in control and insulin treated cell's nuclei (Pl. II). However, after 1h hydrolysis a significant ($p < 0.01$) decrease in color intensity was observed in the nuclei of insulin treated cells (Fig. 3).

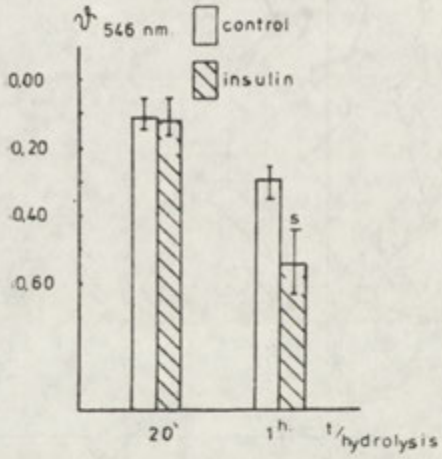

Fig. 3. Cytoplasmic analysis of Feulegen-reactions, s — $p < 0.01$ related to the control

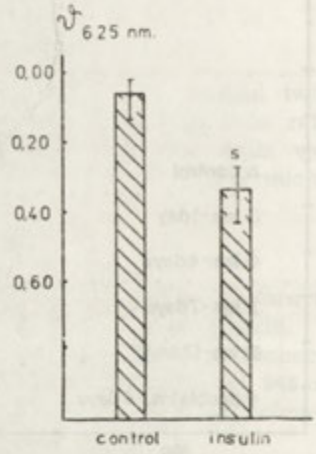

Fig. 4. Cytophotometric analysis of Fast-green FCF-reaction, s — $p < 0.01$ related to the control

(3) A strong reaction of Fast-green stained the nuclei and mitochondria in control cells in contrast to treated cells (Pl. III) where the reaction (Fig. 4) was significantly ($p < 0.01$) diminished (in nuclei) or completely negative (in mitochondria).

Radioautography

Only over the control cells were grains, 90% of these over the food vacuoles. After 5 min the insulin containing vacuoles were in the whole length of the cell, after 10 and 30 min many of them were localized in the area of cytopyge (Pl. IV). After 1 and 3 hours most of the grains was

over the bottom of the cells. In that time grains were over the borderline of the cells, too. There were no specific grains over the insulin treated cells.

Discussion

The unicellular organisms contain hormone-like materials characteristic of higher organisms (Blum 1967, Le Roith et al. 1980, 1983) and are able to respond to vertebrate hormones. Their membrane structures are capable of binding these hormones, and the specificity of binding can be, in certain cases, substantiated by binding studies (Legros et al. 1975, Le Roith et al. 1987, McKenzie et al. 1988). The unicellulars acquire a "memory" of the primary interaction with a vertebrate hormone, which causes them to change their binding capacity (Csaba 1986, Csaba et al. 1982) for, and behaviour towards, the hormone at reexposure (s).

The primary interaction with the hormone (hormonal imprinting) may have a positive or a negative effect. *Tetrahymena pyriformis* cells imprinted with insulin show an increased binding capacity for it at subsequent interactions, while *Tetrahymena thermophila* shows exactly the opposite behaviour (Csaba and Kovács 1987). At all events, imprinting takes place in both cases, and its trend is characteristic of the species. In earlier studies (Kovács and Csaba 1988) insulin treatment reduced the gowth rate of *Blepharisma*, and this effect lasted over as many as 45 generations. This supports the hypothetical conclusion that the effect of insulin on *Blepharisma* is long-lasting.

The present experiments support the earlier observations, that the first insulin effect on *Blepharisma* provokes a change in the reaction of the cell which is resulted in a disparate response in the case of the second encounter. However, as clear it is in the *Tetrahymena*, that a receptorial effect appears after the first hormonal treatment (imprinting), as dubious this is in *Blepharisma*.

There were cytochemically demonstrated important changes in the nucleus of the cell. The disparate Feulgen reaction to hydrolysis and the parallel decrease of Fast-green staining justifies the change in protein-nucleic acid interaction (Böhm and Sandritter 1966) under the effect of insulin. Since these proteins have a regulatory role in the manifestation of genetic information, this may explain the different reaction of cells to insulin in the case of the second encounter This nuclear change can also explain the inhibition of cell division — in earlier (Kovács and Csaba 1988) experiments — as well, as the "switch off" of mito-

chondrial cytochrome C (which is is responsible for the binding of Fast-green) and the decrease of ATP-synthesis by it.

The chemoattractant studies have also shown the difference in the responses between the first and second hormone treatment. Primary exposure to insulin caused the cells to retire continuosly from the insulin-containing compartment, whereas reexposure to insulin had a much less pronounced effect after 14 and 7 days, and accounted for a distinct chemoattractant effect after 13 days. Further exposure to 10^{-6} M insulin had a still greater positive effect: 4 days later (in the test system) caused the cells to move towards the region of the highest insulin concentration. Thus the results of the growth and chemoattraction experiments were essentially similar, in that the negative effect observed after the primary interaction was arrested, and even took a positive turn, after reexposures. The reversion of the tendency of chemoattractant behaviour can be accelerated by repeated hormone exposure.

From these experiments we can conclude that insulin influences the *Blepharisma* in the first occassion of contact to change its reaction in the case of the next one. However we can not conclude unanimously to the level of the change. *Blepharisma can bind* insulin when meet it at first, and can internalize it. The hormone appears on the nuclear membrane and — may be as a consequence of this — changes the protein-nucleic acid relations. Later — at the second occasion — the cells do not bind insulin at all and give positive movement — response to insulin. If insulin provoked a positive change in receptor development — and this follows from the positive chemoattraction — why does not bind the cell insulin at all at the second time? If there is no binding in this case, how can sense the cell the presence of insulin what is a preperequisite of the positive chemoattraction? Presently we can not answer the questions. The fact nevertheless remains that the *Blepharisma undulans* does respond to insulin, and its primary interaction with the latter does influence its behaviour on subsequent interactions with that vertebrate hormone.

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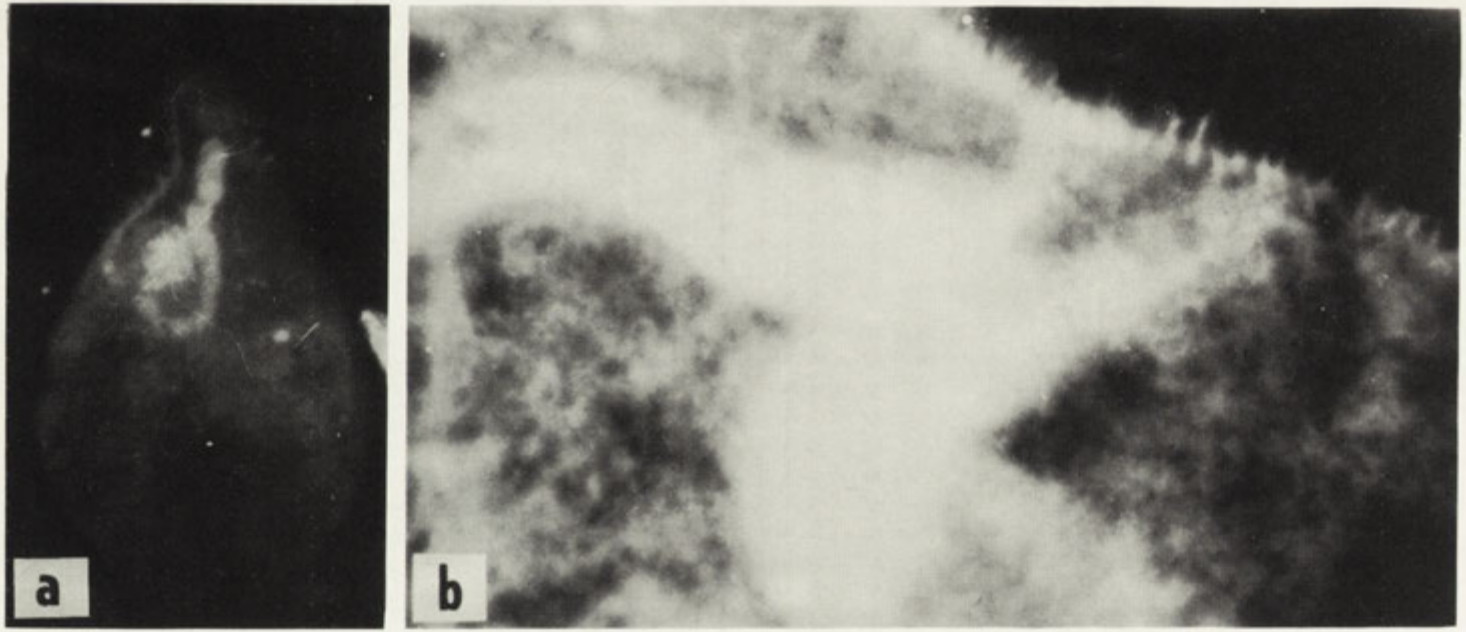
EXPLANATION OF PLATES I—IV

1: Binding of FITC-insulin to untreated *Blepharisma*, a — ×150, b — ×1200
2: Feulgen reaction in *Blepharisma*, a — control, 20 min. hydrolysis, b — insulin treated, 20 min. hydrolysis, c — control 1 h hydrolysis, d — insulin-treated, 1 h hydrolysis (×1200)
3: Fast-green FCF reaction, a — control, b — insulin treated cells (×1200)
4: Radioautograms of control cells, after a — 5 min (×1500), b — 30 min (×900), c — 1 h (×900) exposure in the ^{125}I-containing medium



P. Kovács and G. Csaba

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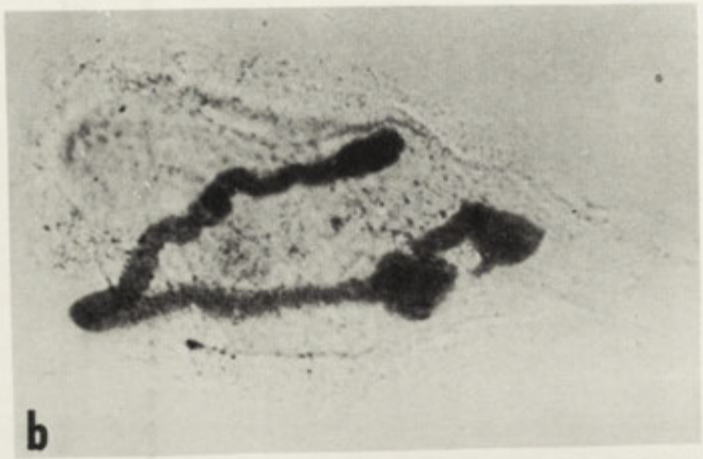

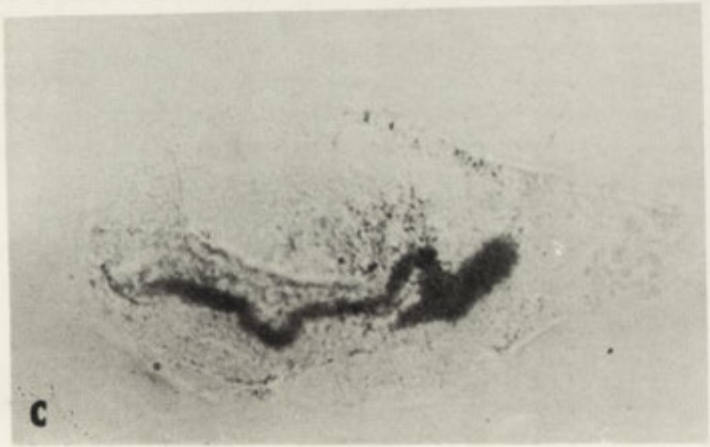

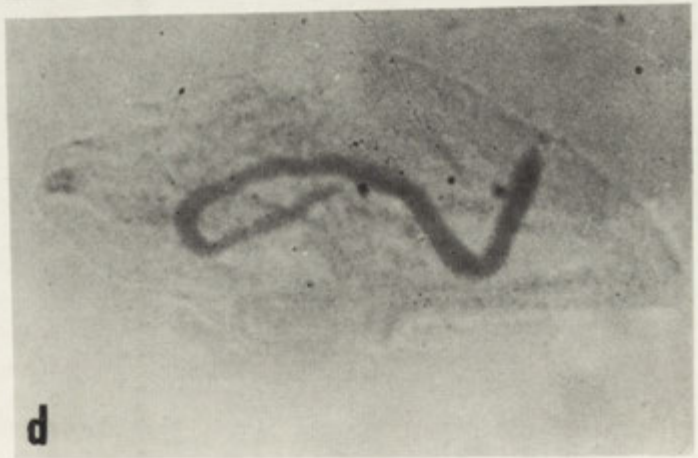

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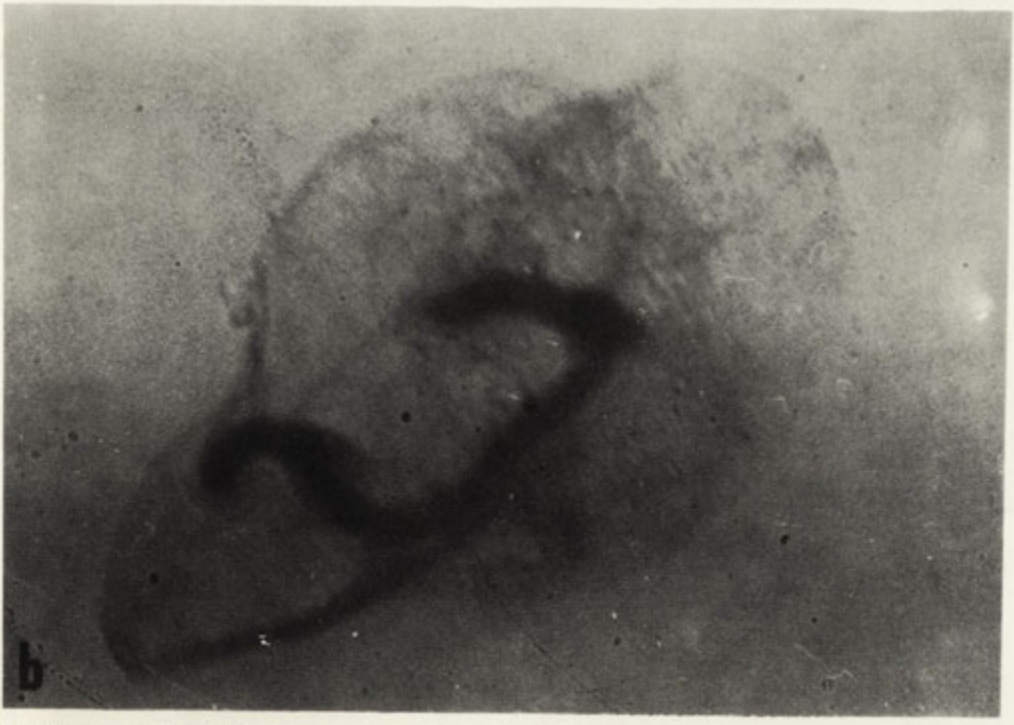

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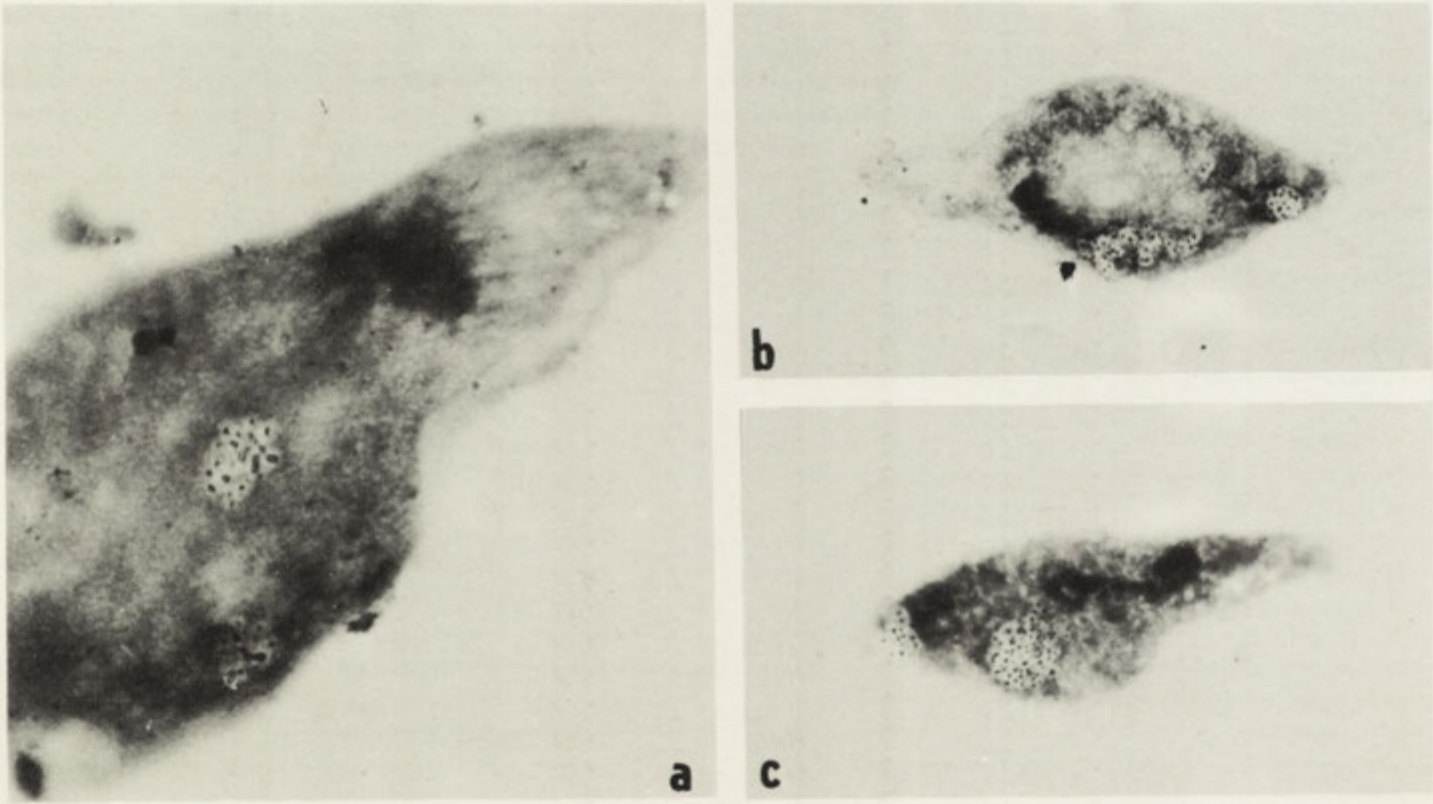

P. Kovács and G. Csaba auctores phot.

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Studies on Lectin-Induced Agglutination of *Acanthamoeba*

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Synopsis. Studies were carried out on lectin agglutination of different strains of the genus *Acanthamoeba*, using an "in vitro" agglutination test and affinity chromatography. The results show that this latter method may be useful in the isolation of several strains.

Lectins have been employed in the study of amoebae as a useful mean of separating different species (Josephson et al. 1977), and strains (Zubiaur and Alonso 1985), as well as for distinguishing virulent strains (Stevens and Kaufman 1974).

Two methods were employed for evaluating agglutination in seven amoebic strains of the genus *Acathamoeba*, with eight different animal and plant lectins. The affinity chromatography method was found to efficiently isolate and identify strains on the basis of their lectin agglutination affinity.

Material and Methods

Amoebae

Acanthamoeba culbertsoni A2, *A. lugdunensis* DPGr, *A. lugdunensis* SH565, *A. griffini* S7, *A. lenticulata* PD2, *A. castellanii* DPGr and *A. sp.* Gr-1, were used in this investigation. The amoebae were cultured axenically in CGV medium (Willaert 1976) supplemented with 10% (v/v) foetal calf serum (Gibco), in Leighton flasks at 37°C.

Cultures of amoebae were harvested by centrifugation at 600 g for 5 min.

Counts of amoebae were carried out using a haemocytometer chamber, and the organisms were then resuspended in PBS at a final concentration of 10^4 amoebae/ml.

Lectins

The lectins and their specific sugars for this test were: Concanavalin A (glucose/mannose), wheat germ lectin (WGA)-(N-acetyl-glucosamine), soybean lectin (SBA)-(N-acetyl-galactosamine), *Helix pomatia* lectin (N-acetyl-galactosamine), *Lens culinaris* lectin (glucose/mannose), peanut lectin (β-D-galactose (1, 3)-D-galactose-N-acetyl-glucosamine), *Ulex europeus* lectin (α-L-fucose) and *Ricinus comunis* lectin (β-D-galactose/α-D-galactose). All lectins were obtained from Boehringer and all specific sugars from Sigma.

Agglutination test

The lectins were used at final concentrations of 0.5, 1, 10, 20, 30, 40, 50, 100, 250 and 500 μg/ml. Fifty Microliter aliquots of amoeba suspension mixed with an equal volume of each lectin solution were plated on microtiter plates (Flow Lab). After 30 min, 1 h and 24 h, incubation at room temperature, agglutination was estimated on a subjective scale ranging from (+) to (+++), and (±) for trace agglutination, according to Zubiaur and Alonso (1985).

Affinity chromatography

The adsorber used was CNBr activated Sepharose 6MB (Pharmacia), coupled with lectins and was followed the metodology described in "Affinity chromatography: principles and methods" (Pharmacia Fine Chemicals A. B. Box 175), passing every strain of *Acanthamoeba* through each column linked to the different lectins. The amoebae were harvested from the culture, then resuspended in PBS at room temperature. The sample was placed at the top of the column and left for 10—20 min. Subsequently, non-retained amoebae were eliminated by washing the column with PBS (20 times the volume of the column) at a flow rate of 2—10 ml/min. Retained amoebae were obtained by passing 2 ml of a 0.1 M solution of the specific sugar of the lectin in destilled water through the column. The number of amoebae released by the addition of sugar was then counted in a Neuebauer chamber. The viability of the amoebae after chromatography was tested both by using Tripan blue and by growing them in 2% Bacto-Agar-Difco with heat-killed *E. coli*. The columns were regenerated after use and stored at 4°C with sodium azide.

Results

In general, the degree of agglutination does not increase with incubation time. The results obtained in the agglutination tests are shown in Table 1, and refer to the minimum concentration of lectins that lead to the maximum agglutination observed in each case. The results obtained by affinity chromatography are shown in Table 2, expressed as the approximate percentage of amoebas retained in the column in each case.

Table 1

Minimum lectin concentration (μg/ml) leading to maximum agglutination with each amoebae strain

Amoebae	Lectins							
	ConA	WGA	SBA	Helix	Lens	Peanut	Ulex	Ricinus
A. culbertsoni A2	100(+++)	500(+)	0.5(+++)	10(+++)	10(+++)	10(+)	0.5(+++)	0.5(+++)
A. lugdunensis DPGr	500(+++)	1(+++)	05.(+++)	40(+++)	–	20(+)	0.5(+++)	0.5(+++)
A. lugdunensis SH565	500(++)	–	30(++)	500(++)	100(+++)	–	40(±)	–
A. griffini S7	500(+++)	50(±)	500(++)	40(+++)	100(+++)	50(±)	–	0.5(+++)
A. lenticulata PD2	500(+++)	500(+)	0.5(+++)	–	20(++)	20(+)	–	1(++)
A. castellanii DPGr	250(+++)	10(++)	0.5(+++)	30(+)	–	–	250(+++)	0.5(++)
A. sp. Gr-1	500(+)	–	40(++)	30(+++)	100(+++)	–	–	20(++)

- — no agglutination, + — slight agglutination, ++ — moderate agglutination, +++ — marked aglutination.

Table 2

Affinity chromatography method: approximate percentage of amoeba retained in the column in each case

Amoebae	Lectins							
	Con A	WGA	SBA	Helix	Lens	Peanut	Ulex	Ricinus
A. culbertsoni	30	—	100	80	50	5	100	90
A. lugdunensis DPGr	5	70	90	45	—	5	90	90
A. lugdunensis SH565	1	—	10	10	20	—	5	—
A. griffini S7	1	—	5	60	30	5	—	60
A. lenticulata PD2	1	—	100	—	20	—	5	—
A. castellanii DPGr	5	50	90	10	—	—	20	5
A. sp. Gr-1	1	—	10	60	30	—	—	20

Discussion

According to Zubiaur and Alonso (1985), the sensitivity of some amoebic species to certain lectins is specific (Tab. 1). The results obtained in the different "in vitro" tests reveal notable differences in the agglutination patterns of the lectins tested. Maximum agglutination is obtained after incubation for a minimum time with a specific concentration of lectin, or else longer incubation periods. For instance with *A. castellanii* and the lectin *Ricinus* test, greatest agglutination (++) was obtained at a concentration of 0.5 μg/ml. Despite increasing the concentration to 500 μg/ml, agglutination failed to rise further.

The SBA and *Ricinus* lectins showed the greatest agglutination capacity, suggesting an abundance of N-acetyl-galactosamine and β-D-galactose/α-D-galactose on *Acanthamoeba spp.* cell surfaces.

Affinity chromatography is useful for the quantification of agglutination by lectins, and could well be applied in taxonomic studies, given at least for the strains tested here, the agglutination patterns of each strain obtained with the different lectins are clearly distinct.

ACKNOWLEDGEMENTS

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Thecamoebiens (*Rhizopoda, Testacea*) des millieux aniso-oligohydriques mousses et lichens

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Synopsis. Dans ce travail, nous décrivons *Centropyxis obscurus* sp. nov. présente dans les Mousses saxicoles, avec quelques commentaires sur les espèces habituellement fréquentes dans ces milieux peu humides.

Dans la classification des biotopes (Thomas 1961), le milieu aniso-oligohydrique, est représenté par des niches écologiques recevant l'eau uniquement par les pluies, sujettes a l'évaporation et souvent à une totale dessication.

Dans ce type de biotope, se forme avec le temps un néosol constitué de fines particules minérales diverses, d'origine éolienne très peu humifère, ce qui constitue les conditions les plus extrèmes pour l'établissement des Themacoebiens.

Dans cette note, nous avons recencé les espèces capables de vivrent dans ces milieux, ce sont généralement des espèces ubiquistes et cosmopolites, capable de former des kystes de résistance de longues durées.

Dans ce but, nous avons uniquement étudié des niches écologiques consituées par des Mousses saxicoles et des Lichens épiphytiques.

Matériel et méthode

Les Mousses

Les Mousses saxicoles des Genres: *Pleurosium, Bryum, Grimmia, Andreacea* et *Tortula*, croissant sur les murs, les pierres et les murets béton bordant les

sentiers de jardin, sont détachées ou coupées. Elles sont ensuite placées dans des boites de Petri et humectées d'eau distillée.

Après 24 heures à la température ambiante, l'eau est aspirée à l'aide de pipettes Pasteur et étalée sur lames. Les espèces sont ensuite déterminées et isolées à la micropipette pour étude.

Après ce court séjour dans leur milieu humidifié, la majorité des espèces ont repris leurs activités.

Les Lichens

Peu d'études, ont été faites spécialement sur ces végétaux particuliers (Thomas 1961, Decloitre 1962, 1970, 1973, Chardez 1975, Bonnet 1967), hélas, très souvent, les espèces trouvées proviennent du milieu environant par contamination, en réalité, les espèces qui peuvent vivrent et s'y développer sont assez rares.

Tous les Lichens étudiés sont des *Ascolichens*.

Cinquante six échantillons ont été inventoriés, 38 contenaient les espèces reprises dans cette note. Ces Lichens appartiennent aux Genres: *Usnea*, *Anaptychia*, *Evernia*, *Umbilica* et *Cladonia*. Ils ont été détachés des troncs d'arbes et placés dans des enveloppes, au laboratoire, la microséparation est effectuée après fragmentation par barbotage énergique, d'air dans 100 cc d'eau distillée.

La flottation est prélevée à la pipette et déposée sur lames dans une goutte d'une solution colorante de Ponceau de Xylidine à 1% possage dans le colorant permet de séparer les thèques vides des espèces actives ou enkystées.

Liste et répartition des espèces

	Mousses saxicoles	Lichens épiphitiques
Assulina muscorum Greff	+	+
Trinema lineare Penard	+	+
Trinema mineare f. minuscula Chardez	+	+
Arcella arenaria Greeff	+	
Heleopera sylvatica Penard	+	
Euglypha laevis Perty	+	
Euglypha megastoma Decloitre		+
Euglypha strigosa (Ehrenberg) Leidy	+	+
Euglypha strigosa v. *glabra* Wailes		+
Corythion dubium Taranek		+
Corythion dubium v. *orbicularia* Penard		+
Corythion dubium v. *aerophila* Decloitre		+
Centropyxis aerophila Deflandre	+	+
Centropyxis obscurus sp. nov.	+	

Notes sur les espèces

Assulina muscorum Greeff

Les spécimens observés, correspondent morphologiquement au type, toutefois, la majorité des espèces est de taille plus réduite et souvent

parfaitement transparente. La longueur de la thèque oscille entre 30 et 50 µm.

Euglypha megastoma Decloitre

La thèque est fortement comprimée avec un pseudostome large, elliptique presqu'en fente. La revêtement de la thèque est formé d'écailles étroites régulières assez petites, 14 à 16 écailles buccales denticulées rangées en ligne droite bordent le pseudostome, ce caractère est typique de l'espèce.

Decloitre (1969) décrit cette espèce d'une station bryomadicole sur rocher. Nous l'avons observée en quelques exemplaires parmi les Lichens du genre *Umbilicaria*. Un seul individu y était enkysté. Le kyste ovale, comprimé entre les deux parois de la thèque, assez foncé à membrane bien délimitée.

Dimensions relevées: L — 38-40 µm, l — 20-26 µm, epaisseur — 7-9 µm, pseudostome — 20-25 µm.

Euglypha strigosa (Ehrenberg) Leidy

Chez les Euglyphes de cette espèce, le pseudostome est très étroit avec tendence à former un col, le nombre des épines est assez rare. La forme glabra Wailes est plus fréquente que le type.

Corythion dubium v. *aerophila* Decloitre

Quelques exemplaires de cette variétés ont été signalées par Decloitre (1956) dans des Mousses et Lichens terricoles provenant de l'Eqe (Groenland).

Cette variété caractérisée par l'étroitesse de son pseudostome est assez fréquente parmi les Lichens du genre *Evernia*. L — 33-45 µm, l — 21-20 µm, pseudostome — 4-5 µm.

Corythion dubium v. *orbicularis* Penard

Cette variété représente environ 80% des populations observées sur l'ensemble de nos prélèvements.

Tous les individus sont de petite talle et de teinte hyaline.

Dimensions relevées: L — 36-40 µm, l — 34-38 µm.

Centropyxis aerophila Deflandre

Cette espèce est assez fréquente parmi les Lichens du genre *Evernia* et *Anaptychia*, particulièrement riches en micromorpholithes; une distinction structurale de la thèque existe entre les grands individus et les petits, en effet, chez les petites formes le revêtement est moins pierreux,

principalement constituté d'un vernis organique de teinte foncée, incrustant quelques rares plaquettes minérales.

Trinema lineare Penard et la forme *minuscula* Chardez, ainsi que *Euglypha loevis* Perty étaient présents dans les Lichens uniquement sous forme de thèques vides.

Arcella arenaria Greeff et *Heleopera sylvatica* Penard, nombreux uniquement dans les mouses.

Corythion dubium Taranek, a été observé enkysté dans les Lichens.

Centropyxis obscurus sp. nov. (Pl. I)

Diagnose: Thèque circulaire, fortement comprimée dorso-ventralement, face ventrale plane, face dorsale bombée, légèrement plus haute dans la partie opposée au pseudostome. Structure constituée d'une matrice organique assez opaque généralement de teinte verte, incrustant de très fines particules formant une sorte de ponctuation régulière et quelques petites plaquettes poymorphes.

La surface de la thèque est quelquefois garnie de particules pierreuses plus abondantes dans la région postérieure. Cette thèque est peu rigide, facilement déformable.

Le pseudostome, petit est peu excentré, plus ou moins circulaire, peu invaginé, sans brides internes, mais toujours bordé d'un épaississement plus foncé.

Mensurations extrèmes: diamètre — 72-79 μm, hauteur — 20-29 μm, pseudostome — 12-16 μm (n — 15).

Les pseudopodes de type labosa, sont rares et timides, très hyalins ils se résument souvent à une simple lame cytoplasmique, dépassant rarement le contour de la thèque (Pl. I 5).

Discussion

Centropyxis obscurus sp. nov., se distingue de *C. ecornis* Leidy et de ses variétés par ses dimension plus réduites et surtout par la forme et les dimensions du pseudostome, elle se distingue également de *C. guyanensis* Coûteaux et Chardez par l'absence totale de brides internes.

A première vue, le pseudostome si peu excentré fait penser au genre *Cyclopyxis*, seule, la vue de profil montre nettement la compression de la partie antérieure, caractérisant le genre *Centropyxis* (Deflandre 1929).

Cette nouvelle espèce, n'est certainement pas rare, mais celle passe facilement inapercue, vivant dans des biotopes aériens et subaériens, elle se confond facilement avec diverses particules présentes dans ces Mousses.

Locus typicus: Mousses saxicoles et muricoles.

Preparation type: n° V2/7 et paratypes n° X1 et X2 (Dep. Fac. Sc. A. E. Gembloux).

Conclusion

Certains genres de Lichens, retiennent une assez grande quantité de micromorpholithes et de particules diverses d'origine éolienne, qui constituent un néosol plus ou moins minéralisé favorisant le développement de quelques espèces (Lambinon 1961).

La majorité des espèces de Thecamoebiens qui peuvent par leur nombre être considérées comme typiques des Lichens, ont un caractère commun, c'est la compression latérale de la thèque. L'effet de la dessication en est la cause principale. Cette compression extrême s'observe parfaitement sur les thèques vides, en particulier dans le genre *Corythion* dont certaines thèque apparaissent même concaves. Nous avons déjà signalé ce phénomène (Chardez 1965) chez plusieurs espèces des Mousses aerienne souvent sujettes à des périodes de sécheresse totale.

Par le nombre d'espèces en activité ou enkystées et le grand nombre de thèques vides observées, les neuf espèces citées peuvent être considérées comme consituant la faune véritable des Lichens.

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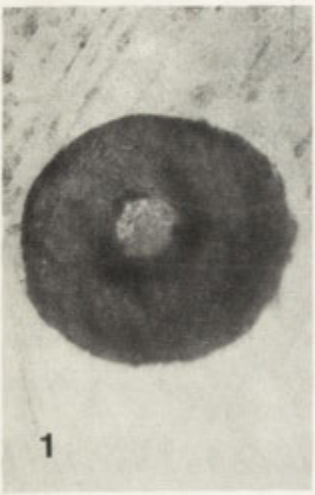
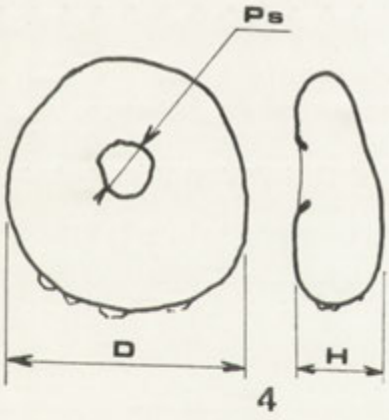
SUMMARY

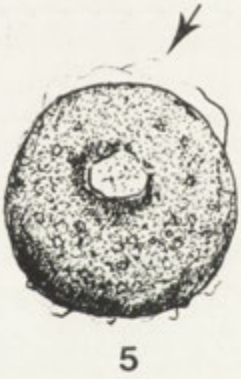
In this work we describe a new species present into the saxicoles Mosses: *Centropyxis obscurus* sp. nov. At the same time as we show off a few commentaries about the usual species from the not very humid medium.

EXPLICATION DE PLANCHE I

1—5: *Centropyxis obscurus* sp. nov. (les fleches indiquent les emissions pseudopodiques)







D. Chardez auctor phot.

ACTA PROTOZOOLOGICA
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Contribution a la connaissance des Thecamoebiens aquatiques du Tyrol allemand (*Rhizopoda, Testacea*)

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Synopsis. Des prélèvements du bord d'un lac (Tyrol allemand) nous a révéle une population de Thecamoebiens Lobosa, dont deux Taxons nouveaux.

Au cours des recherches sur la faune thecamoebienne du lac de Füssen dans le Tyrol allemand, situé a une altitude de 1800 m, une population principalement constituée d'espèces de la classe des Lobosa a été inventoriée parmi lesquelles deux taxons nouveaux: *Centropyxis declivistoma* sp. nov. et *Lagenodifflugia sphaeroideus* (Tarnogradsky) comb. nov.

Matériel et méthode

Les echantillons ont été prélevés dans les régions de la beine et de la déclivité, il s'agit principalement de sédiments peu sapropéliques, mélangés a un léger feutrage organique.

Le triage des espèces a été fait dans un premier temps au microscope inversé. Les espèces prélevées à la micropipette ont été ensuite placées en boites de Petri dans de l'eau d'origine filtrée, additionée de quelques grains de "Proténum neutre". Dans ces conditions, à la température ambiante, les Thecamoebiens se gardent en vie pendant quelques jours.

Les espèces

Centropyxis aerophila Deflandre
Centropyxis aerophila v. *sphagnicola* Deflandre
Centropyxis aculeata (Ehrenberg) Stein
Centropyxis aculeata v. *minima* van Oye
Centropyxis cassis Wallich
Centropyxis constricta (Ehrenberg) Defflandre
Centropyxis declivistoma sp. nov.
Centropyxis ecornis Leidy

Le genre *Centropyxis*, constitute environ 50% de l'ensemble de la population.

Cyclopyxis eurystoma Deflandre

Quelques exemplaires sous forme de thèques vide D — 50-55 µm.

Difflugia lobostoma Leidy

Nombreux exemplaires de 80 à 150 µm, dont le pseudostome est trilobé ou quadrilobé.

Schwabia regularis Jung

Assez nombreux, le thèque est ovale, régulière, lisse, formée de trés petits éléments minéraux plants polymorphes.

Le pseudostome apical est petit et circulaire à bord lisse, il représente une simple perforation. Hauteur de la thèque — 43-47 µm, diametre — 33-35 µm, diametre du pseudostome — 13-19 µm.

Toutes ces espèces, sont dans l'ensemble conformes aux différents types, chez les *Centropyxis corniculées*, le nombre de cornes est comme habituellement fort variable.

Centropyxis declivistoma sp. nov.

Diagnose: Thèque oviforme, légèrement comprimée dorso-ventralement, la compression s'accentuant sensiblement vers la partie anterieure, la thèque est constituée d'un abondant vernis organique, incrustant des plaquettes minérales polymorphes, l'ensemble formant une paroi assez épaisse, teintée de brun-rouge.

Le pseudostome situé a la partie étroite de la thèque, est formé par la paroi dorsale qui se prolonge en une courte visière. La paroi ventrale sensiblement plus courte, ménage une ouverture ovale, très allongée et très étroites, en vue de profil, ce pseudostome forme un angle de quelques degrès avec l'axe longitudinal de la thèque.

Observé en vue apicale, ce pseudostome se présente sous forme d'une simple fente.

Le cytoplasme n'a put être observé en raison de l'opacité de la thèque. Le pseudopode, généralement unique est long et lobé, très clair.

C. declivistoma, rampe sur la face ventrale, le pseudostome appliqué au substrat, l'arrière de la thèque légèrement surélevé. La reptation est extrêmement lente.

Dimensions: longueur — 150-154 µm, larguer — 98-100 µm, épaisseur — 70-76 µm, pseudostome — 19-20 × 6,5-9,9 µm (n — 11).

Préparation type: n° V3/5a, déposé dans les collections de la Faculté des Sciences Agronomiques de l'Etat à Gembloux.

Discussion. La position systématique de cette nouvelle espèce, est difficile à trancher. La structure et l'aspect très particulier de la thèque en raison de sa teinte, fait penser à *Heleopera petricola* v. *amethystea* Penard, mais le pseudostome, bien qu'en fente étroite, n'est pas placé comme chez les *Heleopera*, mais fait partie de la face qui doit être considérée comme ventrale.

Centropyxis declicistoma, entre donc dans le type morphologique des plagiostomes simples (PLS) comme l'a défini Bonnet (1975).

Lagenodifflugia sphaeroideus (Tarnogradsky) comb. nov.

Synonymie: *Pontigulasia sphaeroideus* Tarnogradsky, 1961

Diagnose: Thèque pyriforme, section transversale circulaire, la partie antérieure est prolongée en un col bien marqué.

La thèque est entièrement formée de particules minérales anguleuses soudées par un vernis organique.

La base du col est entourée de fragments quartzeux plus gros. Ces éléments pierreux débordent vers l'intérieur formant un étranglement faisant office de diaphragme.

Observé par l'intérieur du col en vue apicale, ces gros éléments pierreux, ménagent une ouverture irrégulière plus ou moins circulaire et plus ou moins centrale, assez petite, séparant de la sorte la thèque en deux parties distinctes, la panse plus ou moins ovale et le col aboutissant au pseudostome circulaire.

Si l'on couvre de Xylol une prépation sèche, la présense de bulles d'air à l'interieur des thèques, révèlent parfaitement leur bipartition interne et l'ouverture étroite au niveau du rétrécissement.

Dimensions: hauteur totale — 99-112 µm, diamètre — 50-60 µm, hauteur du col — 33-35 µm, diamètre du pseudostome — 18-30 µm (n + 14).

Répartition: Caucase, dépression a Sphaigne (Tarnograd-

sky 1961), Roumanie, Lac de la Porte de Fer (Godeanu 1971), Tyrol, Lac de Füssen (Chardez 1985).

Préparation type: n° X3/5b. Déposé dans les collections de la Faculté des Sciences Agronomiques de l'Etat à Gembloux.

Discussion: Tarnogradsky (1961), a décrit cette espèce sous le nom de *Pontigulasia sphaeroideus*, or, depuis le changement de status taxonomiques du groupe des Pontigulasies à la suite des travaux de Ogden (1963, 1987) et de Medioli et Scott (1983), ce groupe se divise en trois genres: *Pontigulasia* Rhumbler, 1896, *Zivkovicia* Ogden, 1983 et *Lagenodifflugia* Medioli et Scott, 1983.

La systématique de chacun de ces genres est actuellement basée sur la structure interne en forme de brides, de diaphragme ou de simple étranglement à la base du col séparant la thèque en deux parties.

Ces structures différentes, ne peuvent être révélées que par l'observation en vue apicale par l'ouverture du pseudostome.

Pour ces raisons de structures internes, il concient de placer l'espèce sphaeroideus dans le genre *Lagenodifflugia* Medioli et Scott, 1983.

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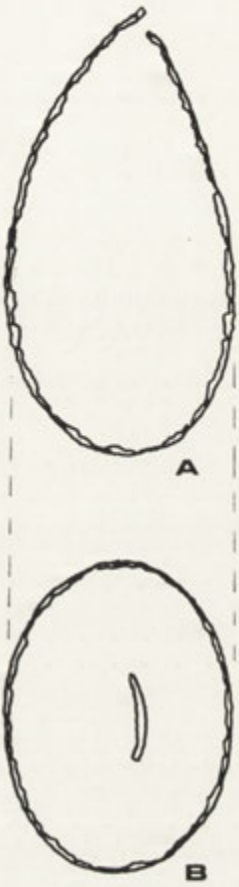
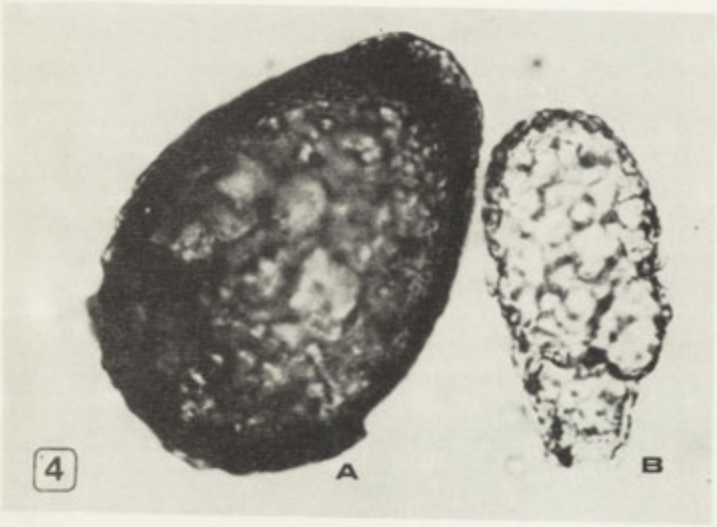
SUMMARY

In this work, we study Testate *Amoebae* from Fäsen Lake's boards (German Tyrol) and we describe two new taxa.

EXPLICATION DE PLANCHE

1—4A: *Centropyxis declivistoma* sp. nov. 1 — face dorsale, 2 — face ventrale, 3A — profil schématisé, 3B — vue apicala (×400)
4B—8: *Lagenodifflugia sphaeroideus* (Tarnogradsky) com. nov. (×400). 8A — aspect dans le xylol (les bulles d'air ne sont pas résorbées), 8B — vue apicale





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On a New Myxosporean Parasite (*Myxozoa*), *Myxidium cholecysticum* sp. n., from the Freshwater Fish *Astyanax scabripinnis* (Jenyns, 1842)

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Synopsis. A new species, *Myxidium cholecysticum* (*Myxozoa*: *Myxidiidae*) is described. The parasite was found in the gall bladder of the freshwater fish *Astyanax scabripinnis* (Jenyns, 1842) (*Pisces*, *Characidae*) caught in a water reservoir near Campinas, São Paulo State, Brazil. *M. cholecysticum* sp. n. is coelozic and disporablastic. Large trophozoites (3.4×0.4 mm) and mature spores (14.1×7.8 μm) were found floating in the bile without apparent pathogeny.

Only limited information is available on protozoans of the genus *Myxidium* Bütschli, 1882, parasites of fishes found in South America (Cunha and Fonseca 1917, Kudo 1920, Nemeczek 1926, Penido 1927, Pinto 1928 a, b, Guimarães 1931, Moser et al. 1976, Jayasri and Hoffman 1982). None of these forms have been reported from host genus *Astyanax*. In this paper, a myxosporidian parasite, *M. cholecysticum* sp. n. (*Myxozoa: Myxidiidae*), is described from the gall bladder of the freshwater fish *A. scabripinnis* (*Pisces, Characidae*).

Material and Methods

One hundred and twenty two *Astyanax scabripinnis* (Jenyns, 1842) were collected from a man-made water reservoir in Alpes Farm, Campinas Municipality, São Paulo State, Southern Brazil. Fishes (46—64 mm) were killed and immediately examined for the presence of parasites under binocular microscope. Examination of fresh spores obtained from trophozoites was made with a drop of sa-

line solution (1%). Permanent slides were mounted by staining trophozoites and spores with Giemsa after fixation in absolute methanol. The extrusion of the polar filaments was achieved with fresh spores treated with 5% KOH water solution (Hoffman et al. 1965). Thirty stained spores and trophozoites were used to obtain morphometric data and all measurements are given in micrometers (μm) unless otherwise stated. The illustrations have been drawn with the aid of a camera lucida.

Observations

Myxidium cholecysticum sp. n. (Fig. 1; Pl. I 1, 2)

Trophozoite. Coelozoic. Large elongate masses, visible to the naked eye, varying greatly in size and usually flatenned leaf-like structures floating in the bile of the gall bladder. The thickness of one indi-

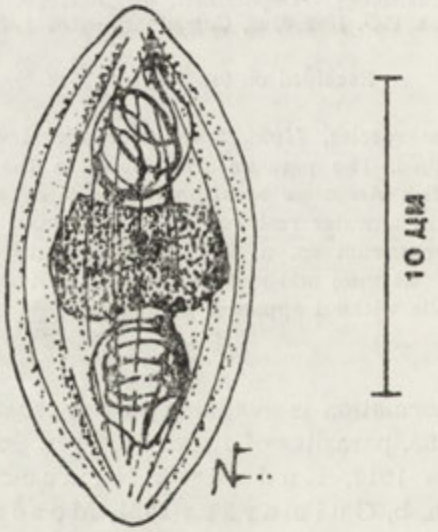

Fig. 1. Mature spore of *Myxidium cholecysticum* sp. n. (Giemsa stained)

vidual is uniform and the body is very flexible and easily folded, in the center of wich spores are differentiated. Fresh preparations show the protoplasm clearly definite into hyaline ectoplasm and greatly vacuolated endoplasm enclosing many fat globules (Pl. I 1). The parasite moves slowly with frequent emission of many lobose-like pseudopodia projections with uniform width and blunt extremities all around their surface (Pl. I 2). The endoplasm contains several nuclei in different stages of spore formation but mature spores are more concentrated in the center. Polysporous trophozoites, with many spores in pairs (Pl. I 2) can be found, suggesting disporous development.

Spore. (Fig. 1). Generally elipsoid in front view, with round ends. Shell with longitudinal striation; suture line slightly S-shaped, being oblique to the longitudinal axis of the spore. Two polar capsules, pyriform in shape, located at each extremity of the spore open in the more dilated part of the valves. Polar filaments coiled approximately 5-6 times and when extruded shows a uniform diameter along their extension. The nuclei of the sporoplasm appear widely separated in stained smears. The iodinophilous vacuole was not found.

Measurements. Thirty trophozoites and spores have been measured and range is given with arithmetic mean and its standard deviation within the parentheses.

trophozoite length:	379—9272	(3364.0 ± 2131.1)
trophozoite width:	30—967	(370.4 ± 302.5)
pseudopodia length:	111—205	(154.7 ± 23.7)
pseudopodia width:	5.7—11.4	(7.6 ± 1.5)
spore length:	12.0—15.9	(14.1 ± 1.1)
spore width:	6.4—9.6	(7.8 ± 0.9)
polar capsule length:	3.4—5.2	(4.2 ± 0.9)
polar capsule width:	3.0—4.1	(3.6 ± 0.3)
polar filament length:	42.8—60.0	(55.2 ± 5.7)

No. of trophozoites/host: 1 to 7 (2.42).

Site of infection: gall bladder.

Pathogeny: not apparent.

Incidence: 21 fishes infected out of 122 examined.

Host: *Astyanax scabripinnis* (Jenyns, 1842), lambari, (*Pisces, Characidae*).

Type locality: Alpes Farm (Atibaia River Basin), Campinas Municipality, São Paulo State, Southern Brazil.

Material: Syntypes on slide No. 4023 deposited in the Collection of the Department of Parasitology, State University of Campinas (Unicamp), Brazil and Nos. 4024, 4025, 4026, 4027 kept in author's collection. Ten hosts were fixed in formol 10% and preserved in alcohol 70° GL for further reference.

The species name refers to the localization in the host.

Discussion

The genus *Myxidium* have been reported from a variety of marine and freshwater fishes from different parts of the world. Jayasri and Hoffman (1982) related 109 species. Only seven species occurs in South American fishes: *M. stratum* Cunha et Fonseca, 1917, *M. cruzi* Penido,

1927, *M. fonsecai* Penido, 1927, *M. gurgeli* Pinto, 1928, *M. melanocetum* Noble, 1966, *M. coryphaenoidium* Noble, 1966, *M. iwamotoi* Moser, Noble et Lee, 1976. The genus *Myxidium* has not previously been reported from the fish genus *Astyanax*; most of them are identified by spore morphology, based in shape, size and the position of the polar capsules in the spore. This report presents aspects of trophozoite characteristics of *M. cholecysticum* sp. n. — the most distinguishing features. Compared to spore morphology of other *Myxidium* species, *M. cholecysticum* sp. n. resembles *M. capsulare* Auerbach, 1910, *M. striatum* Cunha et Fonseca, 1917, *M. moxostomatis* Kudo, 1921, *M. aplodinoti* Kudo, 1934, *M. folium* Bond, 1938, *Myxidium* sp. Guilford, 1965 and *M. macrocheili* Mitchell, 1967. The leaf-like trophozoite features correspond well with those of the new species, with the exception of pseudopodia with lobose-like projections. Trophozoites are not described for *M. capsulare* and *M. aplodinoti*. However, except for the most important morphological differences of the vegetative stages and host species, *M. cholecysticum* sp. n. differs from the above myxosporidians by the following aspect: the polar filaments are significantly longer (42.8-60.0 μm) than those of *M. moxostomatis* (30-35 μm), *M. folium* (34-42 μm), *M. macrocheili* (20-30 μm and *M. striatum* (30 μm).

ACKNOWLEDGEMENTS

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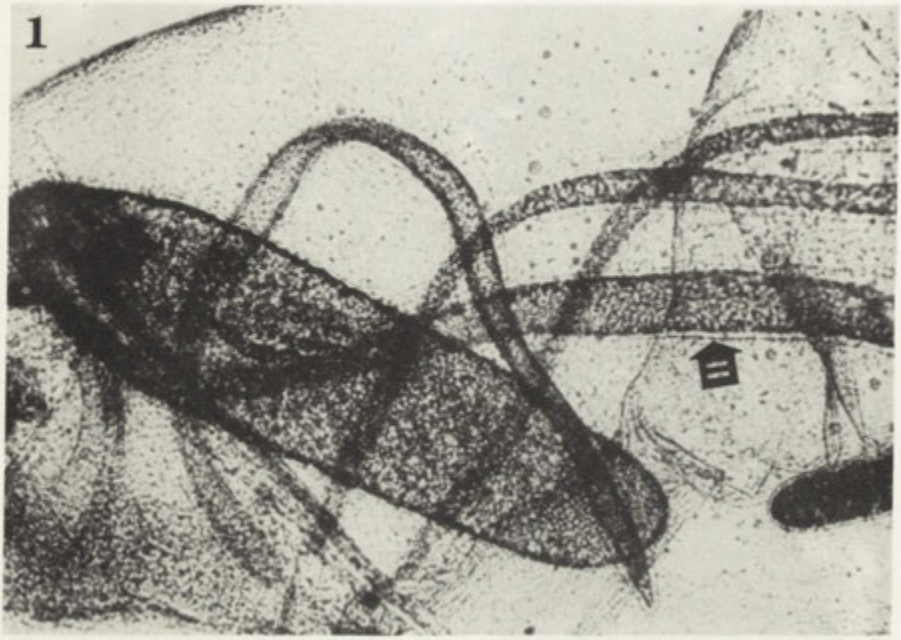
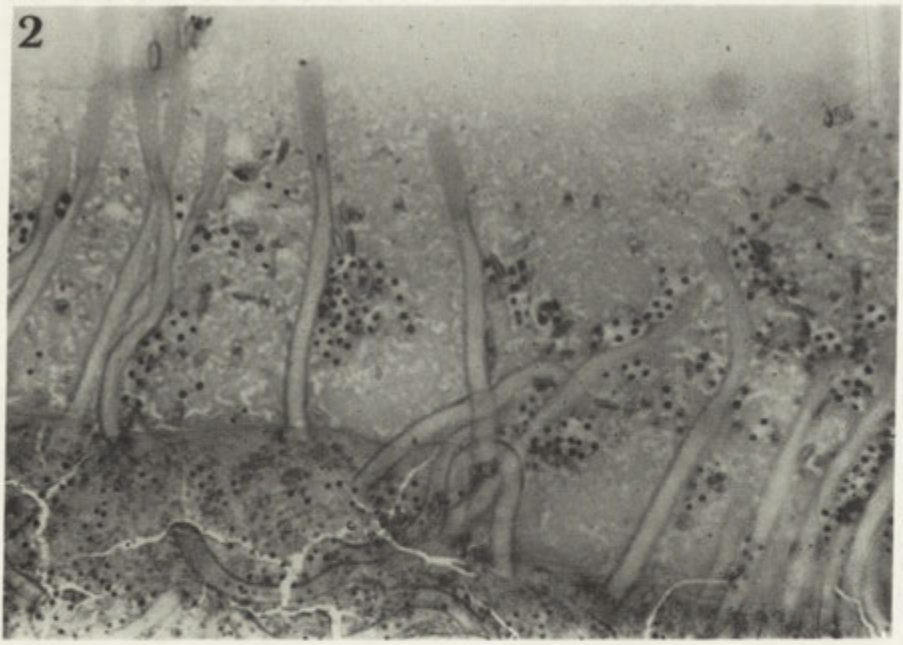
EXPLANATION OF PLATE I

Myxidium cholecysticum sp. n.

1: Several trophozoites floating in bile (fresh). Note the hyaline ectoplasm (arrow)
2: Ruptured trophozoite showing mature spores, disporous developing spores and lobose-like pseudopodia (Giemsa stained)





N. S. Cordeiro and I. Gioia auctores phot.

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Etude d'une infection microsporidienne naturelle chez *Anopheles gambiae* Giles (*Diptera, Culicidae*), moustique vecteur du paludisme au Sénégal

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Synopsis. La microsporidie *Vavraia* sp. (*Microspora, Pleistophoridae*) a été trouvée chez des larves de *Anopheles gambiae* (*Diptera, Culicidae*) récoltées à Dakar (Sénégal, Afrique de l'Ouest). Elle détruit essentiellement le tissu adipeux. Tous ses stades de développement sont à noyaux isolés. Les plasmodes sporogoniaux subissent, dans des vésicules sporophores, une plasmotomie et des divisions multiples en rosette pour donner des nombreux sporoblastes. Les vésicules sporophores ont une enveloppe épaisse. Elles contiennent souvent 8, 16 ou 32 spores. De grands nombres de spores sont parfois observés. Ces spores sont ovoides et mesurent 6,40 à 7,90 μm de long (moyenne 7,08 ± 0,19 μm) sur 4,60 à 6,20 μm de large (moyenne 5,50 ± 0,17 μm).

Les microsporidies infectent de nombreuses espèces des moustiques. Elles se transmettent aussi bien par la voie orale que par la voie transovarienne et jouent un rôle important dans la réduction des populations naturelles des moustiques (Weiser et Prasertphon 1981). Selon de nombreux auteurs, dont Kelly et al. (1981), Canning (1982) et Vavra et al. (1984), ces microsporidies présentent un intérêt certain en lutte biologique.

Les prospections effectuées chez des moustiques de la région de Dakar nous ont permis de trouver une microsporidie chez *Anopheles gambiae*, vecteur du paludisme au Sénégal. Dans ce travail, nous décrivons (en microscopie photonique) les lésions occasionnées par cette microsporidie ainsi que son cycle de développement.

Matériel et techniques

Les larves de *Anopheles gambiae* ont été récoltées dans les plans d'eau de la région de Dakar (Sénégal, Afrique de l'Ouest). Sur 121 larves disséquées, 8 hébergeaient une microsporidie au niveau de leur tissu adipeux, soit un taux de parasitisme de 6,6%. Les lésions tissulaires provoquées par la microsporidie et les différents stades de son cycle de développement ont été étudiés en microscopie photonique. Les tissus adipeux infectés ont été fixés au Carnoy, puis colorés à l'Azan de Heidenhain. Les frottis des stades de développement de la microsporidie ont été colorés au Giemsa.

Résultats

Histopathologie

Les larves infectés ne présentent aucun signe clinique extérieur. Des coupes histologiques réalisées chez ces larves révèlent que la microsporidie infeste essentiellement le tissu adipeux. L'accumulation des spores dans les cellules adipeuses provoque l'hypertrophie de celle, puis leur désagrégation. Le tissu adipeux est alors transformé en une aggrégation de masses plus ou moins désorganisées (Pl. I 1).

Les stades de développement

Les mérontes et la mérogonie (Pl. I 2-5)

Les stades mérogoniaux sont des éléments plurinucléés (plasmodes mérogoniaux) mesurant en moyenne 7 à 21 µm de diamètre. Leur cytoplasme est plus ou moins clair et leurs noyaux sont souvent en division (Pl. I 4, 5). Des plasmodes mérogoniaux à 3, 4, 8, 16, 22 et 26 noyaux isolés ont été observés (Pl. I 2-5).

Les sporontes et la sporogonie (Pl. I 6-8)

En fin de mérogonie, chaque plasmode mérogonial se retracte dans une vésicule sporophore et devient ainsi un plasmode sporogonial (Pl. I 6). Les plasmodes sporogoniaux sont plurinucléés et leur diamètre varie entre 10 et 22 µm. Leur cytoplasme est souvent dense et intensément coloré. Ils subissent une plasmotomie et des divisions multiples en rosette (Pl. II 7, 8).

Les sporoblastes et les spores (Pl. II 9-14)

Les sporoblastes sont unincléés et souvent au nombre de 8, 16, 32 ou parfois plus de 32 dans une vésicule sporophore (Pl. II 9, 10). Ils sont

d'abord sphériques puis s'allongent et se transforment directement en spores. Les spores sont ovoïdes et uninucléées (Pl. II 11-13). Elles mesurent 6,40 à 7,90 µm de long sur 4,60 à 6,20 µm de large (moyenne 7,10 ± 0,19 × 5,50 ± 0,17 µm). Les vésicules sporophores ont une enveloppe épaisse (Pl. II 14).

Discussion

L'examen histologique des larves atteintes montre que le tissu adipeux est souvent totalement détruit. Ceci prouve que le développement de la maladie dans ce tissu est trop rapide pour permettre une régénération compensatrice des adipocytes.

La microsporidie responsable de cette maladie est polysporée, sa sporogonie se déroule dans une vesicule sporophore et tous ses stades de développement sont à noyaux isolés. Deux genres de microsporidies présentant ces caractères sont connus chez les mousiques. Ce sont les genres *Pleistophora* Gurley, 1893 et *Vavraia* Weiser, 1977 (Chapman et Kellen 1967, Pillai 1974, Canning et Hazard 1982).

Selon Canning et Hazard (1982), les critères génériques des *Pleistophora* et des *Vavraia* sont les suivants.

Pleistophora: noyaux isolés à tous les stades de développement; stades mérogoniaux limités par une paroi épaisse qui sera à l'origine de l'enveloppe de la vacuole sporophore; division des plasmodes mérogoniaux par plasmotomie; sporogonie généralement polysporée se déroulant dans une vésicule sporophore et se faisant par divisions successives du plasmode sporogonial pour donner des sporoblastes uninucléées qui évoluent en microspores et en macrospores.

Vavraia: noyaux isolés à tous les stades; stades mérogoniaux limités par une membrane plasmique recouverte d'une paroi amorphe qui sera à l'origine de l'enveloppe de la vacuole sporophore; division des mérontes par plasmotomie ou par division multiple; sporogonie se déroulant dans une vésicule sporophore et se faisant par division multiple en rosette du plasmode sporogonial pour donner des sporoblastes uninucléés qui donnent un seul type de spores.

Certains auteurs ne sont pas d'accord avec ces critères. Ainsi par exemple, Faye et al. (1990), après des observations faites chez *Pleistophora senegalensis*, pensent qu'il est délicat de garder comme critère générique chez les *Pleistophora*, la double séquence sporogonique qui donne à la fois des microcpores et des macrospores. Larsson (1986, 1988), après des observations faites chez *Vavraia holocentropi*, propose qu'il faut supprimer de la diagnose du genre *Vavraia* le caractère "sporo-

gonie par division multiple en rosette" et y ajouter le caractère "sporogonie aboutissant à la formation de microspores et de macrospores".

Comme on peut le constater, les critères génériques chez les microsporidies font encore l'objet de beaucoup de débats. En tout cas, la microsporidie trouvée chez *A. gambiae* que nous venons de décrire présente les caractères des microsporidies du genre *Vavraia* tel qu'il a été défini par Canning et Hazard (1982).

A l'heure actuelle, a notre connaissance, seules 3 espèces de microsporidies du genre *Vavraia* ont été décrites. Ce sont: *Vavraia culicis* Weiser, 1974, *Vavraia cyclocypris* Voronin et Melnikova, 1984 et *Vavraia holocentropi* Larsson, 1986.

V. culicis se développe chez de nombreuses espèces de moustiques dont *Aedes aegypti, A. taeniorynchus, Aedes triseriatus, Anopheles albimanus, A. dureni, A. franciscianus, A. gambiae, A. quadrimaculatus, A. stephensi, A. triseriatus, Culex fatigans, C. pipiens* (hôte type), *C. pipiens quinquefasciatus, C. salinarius, C. tarsalis, C. territans* et *Culiseta longiareolata* (Weiser et Coluzzi 1972, Sprague 1977, Weiser 1977, Kelly et al. 1981, Canning et Hazard 1982, Purrini et al. 1986). Cette espèce produit des spores ovales dont les dimensions varient légèrement selon les espèces hôtes. Chez *A. gambiae* plusieurs dimensions ont été notées: 3,7 × 2,2 µm (microspores vivantes), 4 × × 2,6 µm (microspores en frottis), 4 × 2,5 µm, 5,1 × 3,7 µm (macrospores en frottis), 5,9 × 3,7 (macrospores). Chez *Culiseta longiareolata* les dimensions suivantes ont été notées: 3,8-4,8 × 2,4 µm (Weisser et Coluzzi 1972, Sprague 1977).

Vavraia cyclocypris a été décrite par Voronin et Melnikova (1984) chez un Ostracode, *Cyclocypris ovum*. Ses vacuoles sporophores ont une enveloppe fine et ses spores mesurent 3,4 × 1,7 µm (3,3-3,5 µm × 1,6-1,8 µm).

Vavraia holocentropi a été signalée chez un Trichoptère, *Holocentropus dubius* (Larsson 1986). Sa sporogonie se fait par fragmentation du plasmode sporogonial et aboutit à la formation de microspores (1,7-1,8 × 2,2-3,0 µm) et de macrospores (2,0-2,1 × 3,8-6 µm).

V. cyclocypri et *V. holocentropi* peuvent être séparées définitivement de notre espèce cae elles diffèrent d'elle par des caractères fondamantaux tels que "polymorphisme sporal", et "vacuole sporophore à enveloppe fine". Par contre *V. culicis*, ne diffère de notre espèce que par la taille de ses spores. Ce caractère discriminant est insuffisant pour créer une nouvelle espèce. C'est pourquoi nous appelons momentanément la microsporidie trouvée chez *Anopheles gambiae* du Sénégal *Vavraia* sp.

REMERCIEMENTS

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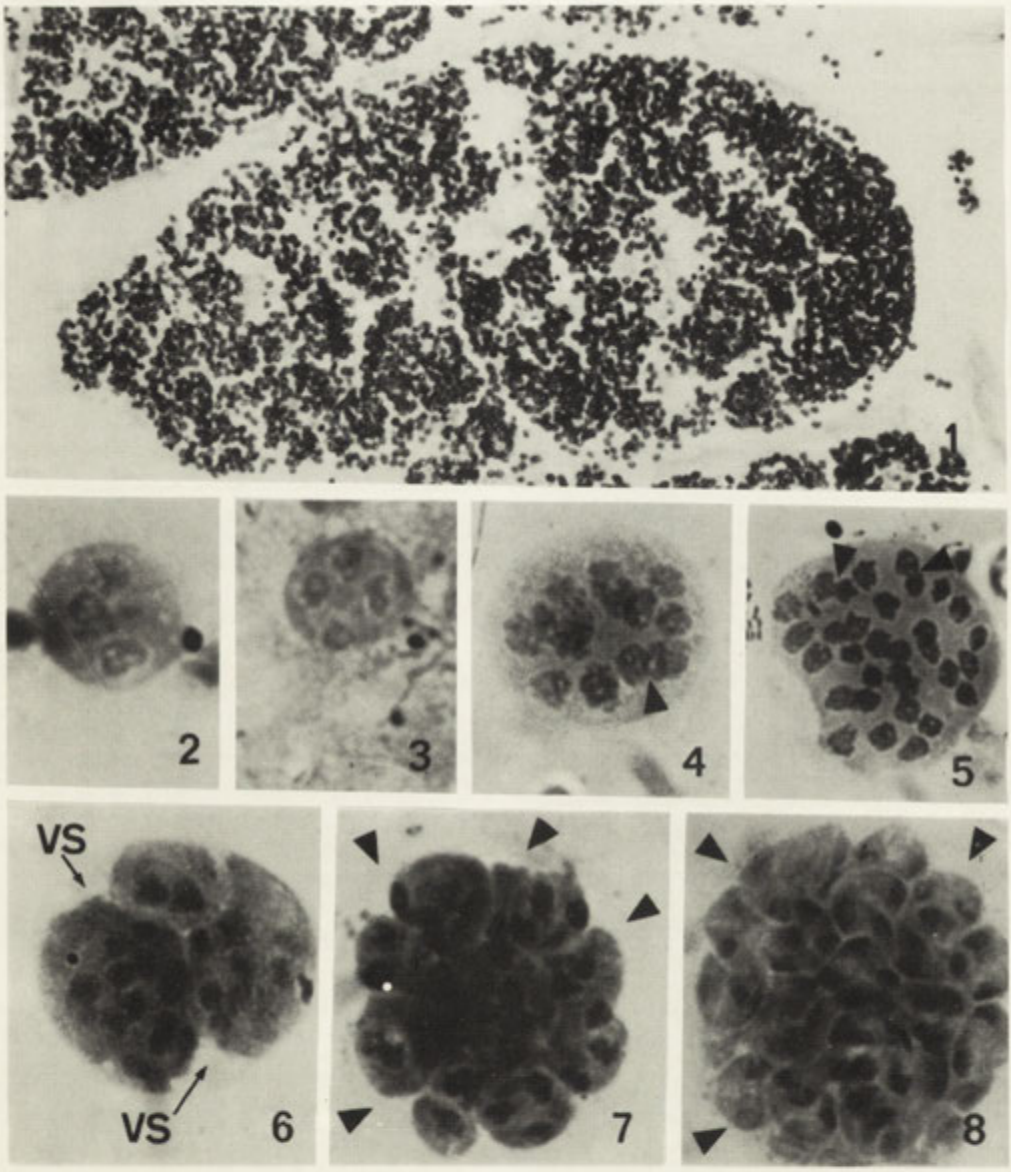
SUMMARY

The microsporidia *Vavraia* sp. (*Microspora, Pleistophoridae*) was found in larvae of *Anopheles gambiae* (*Diptera, Culicidae*) collected in Senegal. It destroyed the adipose tissue. All its developmental stages had isolated nuclei. Sporogonial plas-

modia underwent plasmotomy and multiple fission by rosette formation into numerous spororoblasts within spotophorous vesicles. The sporophorous vesicle has a thick wall. They usually contained 8, 16 or 32 spores. Sometimes large numbers of spores were observed. The spores were ovoid and measured 6.40—7.90 μm × 4.60—6.20 μm (mean 7.08 ± 0.19 μm × 5.50 ± 0,17 μm).

EXPLICATION DE PLANCHES I—II

La microsporidie *Vavraia* sp.
1: Tissu adipeux totalement envahi. Les points noirs correspondent aux stades de développement de la microsporidie (×450)
2—5: Plasmodes mérogoniaux. 2 — plasmode à 3 noyaux (× 1500), 3 — plasmode à 4 noyaux (×1200), 4—5 — plasmodes plurinucléés dont certains noyaux sont en division (têtes de flèche) (×1200)
6—8: Plasmodes sporogoniaux (×1200). 6 — formation de la vacuole sporophore (VS), 7—8 — division des plasmodes sporogoniaux. L'enveloppe de la vacuole sporophore (têtes de flèche) est épaisse
9—10: Vacuoles sporophores contenant des sporoblastes uninucléés dont le nombre varie (×1200)
11—13: Vacuoles sporophores contenant 8, 16 et environ 32 spores uninucléés (×1200)
14: Vacuole sporophore vivante. Son enveloppe (EV) est épaisse (×1200)



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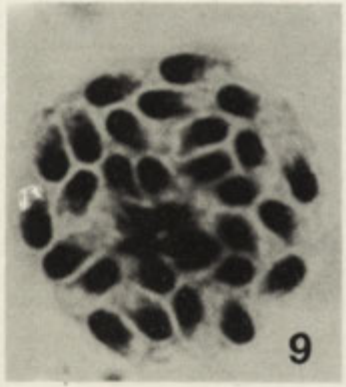

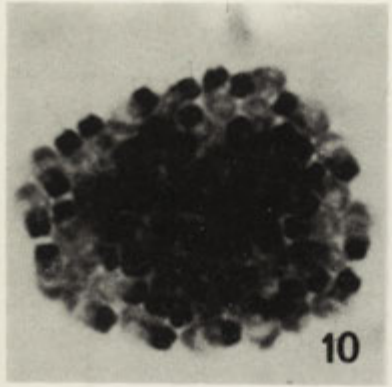

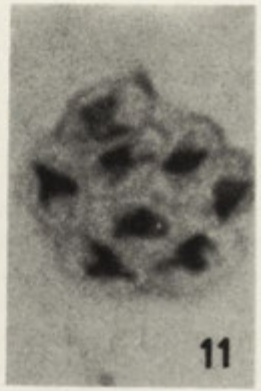

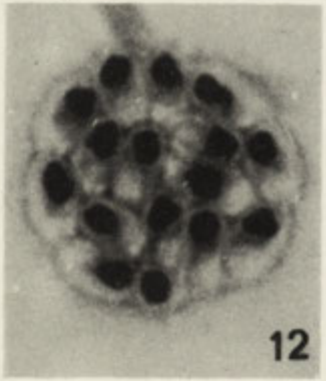

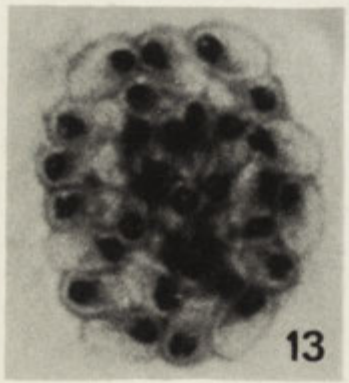

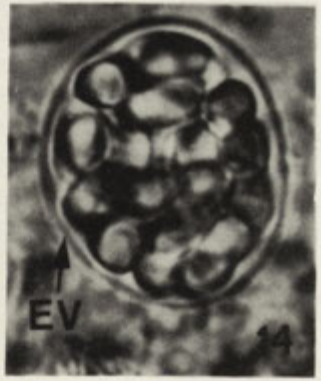

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