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On behalf of Editorial Board of ACTA PROTOZOOLOGICA I wish to express our deepest appreciation to Stanisław Dryl who acted as Editor of ACTA PROTOZOOLOGICA for almost a quarter of a century. His labor and persevering efforts in spreading the protozoological knowledge and consolidating protozoologists around the journal remains inestimable.

The Editorial Board informs that since 1990 Stanisław L. Kazubski, Jerzy Sikora and Anna Wasik have been nominated Editors of the journal ACTA PROTOZOOLOGICA.

Leszek Kuźnicki
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Inhibition of Potassium-Induced Ciliary Reversal in *Fabrea salina* by Inorganic and Organic Calcium Channel Blockers

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Synopsis. Inorganic calcium channel blockers $CoCL_2$, $MnCl_2$ and $NiCl_2$ caused in LD_{50} (at exp. 2h) concentrations complete inhibition of ciliary reversal which was induced in controls by 100 mM KCl solution. Under similar condition $CdCl_2$, $LaCl_3$ and organic calcium channel blockers (D-600, verapamil, nifedipine) caused marked decrease of duration of potassium-induced ciliary reversal. Similarly as in metazon cells, Ca channels blocking effects of tested substances were decreased at higher level of free calcium ions in external medium.

Under natural conditions *Fabrea salina* lives in highly concentrated salt waters, characterized by approximately 300-500 times higher content of electrolytes than medium inhabited by fresh water ciliates. In spite of the above mentioned important environmental differences the general motile behaviour of *Fabrea* is similar to that known in other ciliates. They shown left-spiraling forward movement at relatively high swimming rate (ca 500 μm per s) and short or longer lasting ciliary reversal (avoiding reactions) in response to various external stimuli. The large size (100-400 μm diameter) and typical rounded shape of *Fabrea* with anteriorly located beak-shaped proboscis are characters, which make easy behavioural studies of this ciliate.

Eckert (1972) and Naitoh (1974) brought evidence that in the ciliate protozoa ciliary reversal (CR) induced by external factors depends on calcium transmembrane current throghout voltage sensitive calcium chanels. It is known from recent studies on paramecia (Dryl and Hildebrand 1980, Hildebrand and Dryl 1983) than even

slight increase of free calcium ions within cilia (e.g. 10^{-6} M Ca^{2+}) is sufficient for evoking CR and that duration of induced CR is objective and reliable measure of increased level of calcium ions within cell membrane. It was shown (Dryl et al., 1982) that *Fabrea salina* belongs also to group of excitable unicellular organisms which show behavioral and electrophysiological response towards external stimulation based on calcium transmembrane current.

It is known that external potassium ions depolarize the cell membrane of cilitaes (Kinosita et al. 1964, Dryl and Jahn 1974, Machemer 1977), is inducing a short or longer lasting CR and that the duration of the above mentioned response is associated with opening of voltage sensitive calcium channels within cell membrane. More recently Dryl and Totwen-Nowakowska 1985 have shown that some inorganic and organic calcium channel blockers (CCB) can inhibit potassium-induced reversed beat of compound cilia (i.e. cirri) in *Stylonychia mitilus* and this evoked hope that behavioural tests based on induction of CR can be very useful tools for studying the effects of CCB on modulation of behaviour in ciliates. For that reason it appeared interesting to analyze how in case of marine ciliate *Fabrea salina* the potassium-induced CR will be influenced by inorganic and organic CCB.

Material and Methods

Strain of *Fabrea salina* (obtained by courtesy from the Laboratory of Zoology, Ecole Normale Superieure, Paris, France) was grown at temp. 22-24°C in the medium: 1 M NaCl + 0.1 M $MgCl_2$ + 0.024 $CaCl_2$ + 0.022 M KCl + 0.005 M Tris/HCl (pH 7.2) with addition of Aerobacter aerogenes suspension as a standard food.

The ciliates were kept at room temperature (22-24°C) and 2 h before starting experiments they were collected and depending on planned series of experiments — were washed three times in solution A or B, which had following compositions:

Solution A:	5 ml	1 M Tris	Solution B:	The same content of
	35 ml	0.1 N HCl		electrolytes, except
	5 ml	1 M $MgCl_2$		$CaCl_2$ which was
	2.5 ml	1 M KCl		increased five times
	5 ml	1 M $CaCl_2$		(i.e.: 25 ml 1 M
	947.5 ml	1 M NaCl		$CaCl_2$)

Solutions A and B were characterized by different content of calcium ions which according to known references are essential for action of CCB on the metazoan cells.

It should be added that KCl and CCB have been also prepared on the basis of the above mentioned solutions.

After 2 h of washing in soluton A or B the ciliates were incubated for 3 min in LD_{50} concentrations of applied CCB and then exposed to solution of 100 mM KCl in order to check the duration of potassium-induced CR. Potassium chloride solution contained the same concentration of CCB as incubation medium.

Observations were carried out on 40 ciliates in depression slides containing 0.5 ml of experimental medium. The motile behaviour of *Fabrea* was analysed under low power binocular microscope and the criterion of duration of observed CR response was the renormalization of movement in 50% of observed ciliates. Final calculations have been done within ten repeated measurements.

Control experiments were performed on cilites exposed to 100 mM KCl solutions devoid of CCB blockers.

Data concerning the duration of potassium-induced CR included in Table I (for inorganic CCB) and Table II (for organic CCB) were recalculated as percentage in relation to control mean value and were presented in diagram (Fig. 1).

Results and Discussion

It was necessary to determine the lethal effects of CCB in order to carry out experiments at conditions of 100% survival. The established LD_{50} concentrations of CCB are indicated in second column of Table I and II.

Table 1

Duration of potassium-induced ciliary reversal (in seconds) in *Fabrea salina* exposed and not exposed (control) to inorganic calcium channel blockers

Blockers	Applied concn.	Level of calcium ions in external medium	
		5 mM	25 mM
$MnCl_2$	0,775 M	NoCR (3110±96)	No CR (1840±108)
$CoCl_2$	0,500 M	No CR (3158±103)	No CR (1850±112)
$NiCl_2$	0.110 M	No CR (3140±100)	No CR (1850±110)
$CdCl_2$	3×10^{-2} M	390±22 (2950−22)	3666±18 (1870−120)
$LaCl_3$	1×10^{-3} M	1192±94 (3110−121)	707±58 (1802−160)

Second column includes LD_{50} (at 2 h exp.) concentrations of applied inorganic Ca channel blockers. Data in columns 3rd and 4th indicate duration of ciliary reversal induced by 100 mM KCl prepared on standard medium. In parentheses are data for controls, not exposed to Ca channel blockers.

Ciliates exposed for 3 min to above mentioned solutions of CCB showed reduced rate of forward swimming and intermittent short lasting CR responses.

In case of action of $MnCl_2$, $CoCl_2$ and $NiCl_2$ no CR could be induced after exposure to 100 mM KCl, while in case of $CdCl_2$, $LaCl_3$ and organic blockers (verapamil, D-600, nifedipine) marked decrease of duration of potassium-induced CR was noticed (Table I and II, Fig. 1).

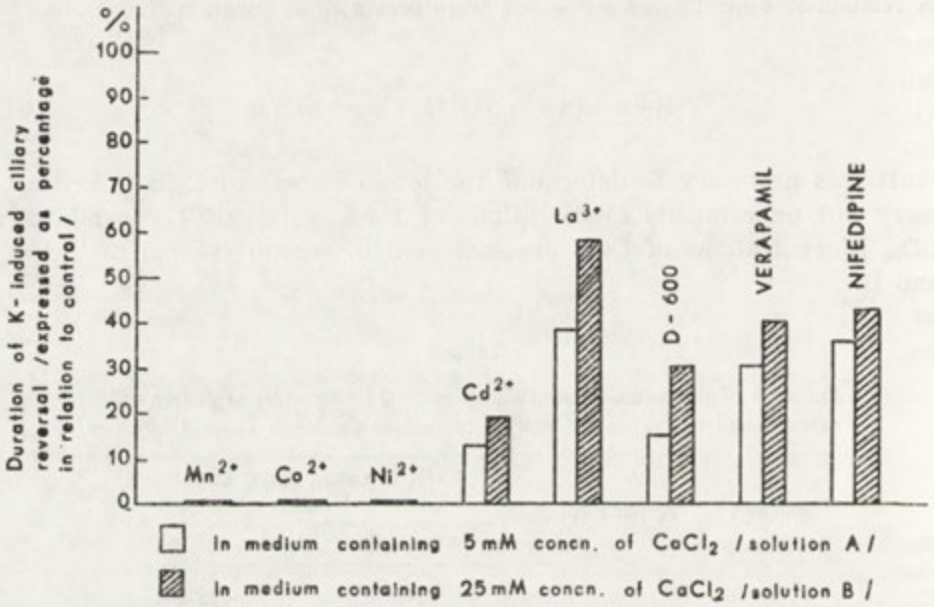

Fig. 1. Effects of inorganic and organic calcium channel blockers on duration of ciliary reversal in *Fabrea salina* induced by 100 mM KCl prepared on standard medium. Values included in columns are expressed as percentage in relation to mean valuea of controls given in Table 1 and 2

Many authors suggest that inorganic CCB are acting at region of calcium channel called "selectivety filter", whereas organic CCB can modulate function of "voltage sensitive gate" and it is suggested that they have different binding sites within interior of calcium channel on the side close to cell interior of calcium channel on the side close to cell interior (Fig. 2). It is evident from the present report that both inorganic and organic CCB are acting on *Fabrea*, and so it is tempting to conclude that at least the general functional organization of calcium channels in this ciliate does not differ much from that known in metazoan cells.

It should be pointed out that the effective concentrations of inorganic CCB are much higher in *Fabrea* than in case of fresh water ciliates and in heart or smooth muscle cells of vertebrates. On the other hand, the

Table 2

Duration of potassium-induced ciliary reversal (in sec.) in *Fabrea salina* exposed and not exposed (control) to organic calcium channel blockers

Blocker	Applied concn.	Level of calcium ions in external medium	
		5 mM	25 mM
D-600	0.75×10^{-4} M	450±18	560±16
		(2932±161)	(1842±133)
Verapamil	0.75×10^{-4} M	924±58	763±46
		(3058±208)	(1924±175)
Nifedipina	4.5×10^{-4} M	1095−44	917−48
		(3075−164)	(1959−122)

All explanations like for Table 1.

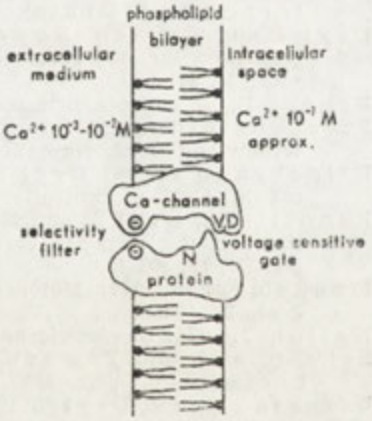

Fig. 2. Hypothetical schematic picture of proteinaceous calcium channel within cell membrane. Three regions of voltage sensitive gate indicate binding sites of nifedipine (N), verapamil (V) and D-600 (D). (Adapted from Schramm and Towart 1985)

corresponding effective concentrations of organic CCB were similar to those reported in metazoan cells.

Complete blockade of Ca channels caused by Co, Mn and Ni ions supports similar results reported in case of *Stylonychia mytilus* (Dryl and Totwen-Nowakowska 1985). It was interesting to state that lanthanium showed rather weak blocking effect on Ca channels and this is also in a good agreement with recent findings on *Paramecium octaurelia* (Ucieklak nad Dryl 1990) where lanthanium did not show any effect on duration of potassium induced CR.

Since it is well known that high level of external calcium is causing marked decrease of blocking effects of CCB on metazoan cells (Flac-

kenstein 1982) — it was interesting to check this phenomenon in ciliates. For that reasons experiments were performed at low (5 mM) and high (25 mM) level of external calcium (Solution A and B). The results are shown in Fig. 1, where quantitative data concerning potassium-induced CR from Table I and II were recalculated and expressed as percentage in relation to mean values of control. It is clear that in case of Cd^{++}, La^{+++}, D-600, verapamil and nifedipine the blocking effects of CCB were weaker in solutions with higher content of external calcium.

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Free Potassium and Membrane Potentials in Cells of *Blepharisma japonicum*

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Synopsis. Potassium selective microelectrodes (K^+-SMEs) were used to measure the intracellular ionic activity of potassium, a_i (K^+), and cell membrane potential, E_m, of protozoan cells of *Blepharisma japonicum*. The measurements yielded a mean a_i (K^+) value of 54.8 mM and resting E_m of −43.3 mV when the cells were bathed in standard Pringsheim solution. The observed cytosolic K^+ activity is significantly higher than could be predicted from the passive K^+ ion distribution across the cell membrane. The relationship between the E_m and the computed equilibrium potential for potassium, E_{eq} (K^+), suggests the existence of some specific active process, which is able to maintain the intracellular K^+ concentration of *Blepharisma* at a high level or else the cell membrane is noticeably permeable to ions other than potassium.

Ion movements between the cell interior and the outer environment play a very important role in the behaviour of all cells. Although much work has been done in recent years on the relationship between membrane potentials and the distribution of ions across the cell membrane in biological materials such as invertebrate and vertebrate neural and alimentary tissue, erythrocytes, muscles and even bacteria, the amount of information about ionic phenomena is still very sparse for protozoan cells. Among the few studies published on ion regulating mechanisms in *Protozoa* may be mentioned the work of Carter (1957) on the ciliate *Spirostomum*, and Stein (1960) or Dunham and Child (1961) on *Tetrahymena*, regarding the estimations of sodium, potassium, and chloride ion levels and transfer constants under various experimental

conditions. In *Blepharisma intermedium* (Hilden 1970) and *Tetrahymena* (Andrus and Giese 1963) the ionic contents of sodium and potassium were measured in different saline media and in the presence of metabolic inhibitors. However, in none of the above mentioned studies, the cytosolic ion activities have been analyzed concomitantly with transmembrane potential. Therefore the present work was undertaken to obtain elementary data on the origin of the membrane potential across the cell membrane in *Blepharisma* using conventional and potassium-selective microelectrode techniques.

Material and Methods

Stock cultures of *Blepharisma japonicum* were grown in glass containers in semi-darkness in Pringsheim solution at room temperature. The food source for the cells was the axenic culture of *Tetrahymena pyriformis*. The chosen cells for experiments were transferred to a fresh maintenance Pringsheim solution without nutritional component for a few hours, and after subsequent medium change, the cell sample was finally placed in an experimental teflon chamber mounted on a stereoscopic stage. During measurements the cells were kept at constant temperature assured by an electronic Peltier device.

Conventional glass microelectrodes were prepared from borosilicate microfilament capillaries (1B15OF from W.-P. Instruments, USA). The capillaries were pulled on a vertical needle/pipette puller (model 750 from David Kopf Instruments, USA) to a tip diameter of 1-2 μm. These micropipettes were then placed tip-up in a solution of 0.8 M potassium acetate to fill the tips by the wick action of the inner microfilament. The rest of the micropipette volume was made up with the same solution using a syringe with 30 gauge needle (Hamilton AG, Switzerland). The tip potentials of such microelectrodes ranged from 3 to 6 mV and tip resistances were about 30-40 Mohms. The microelectrodes were used for experiments immediately or stored for several weeks in the potassium acetate solution in a refrigerator. Micropipettes for potassium selective microelectrodes were the same as those used for the conventional one. After the pulling procedure the micropipettes were salinized by dipping the tips in a 2% soluton (v/v) of dimethylchlorosilane in carbon tetrachloride and sucked up to a height of 500-800 um from the tip. Then one batch of 15 micropipettes were heated with the tips u in a predried oven at 15°C for at least one hour. The completely salinized micropipettes were filled, by dipping the tips in potassium selective liquid exchanger (IE-190 from W.-P. Instruments, USA or 477317 from Corning, USA), and then by backfilling the remaining tapered portion with more ion exchanger if necessary and finally with reference solution of 150 mM of KCl. The electric potential, E'_m, obtained from K^+-SME in KCl or in maintenance solution is given by Eq.:

$$E'_m = V_0 + S \times \log a_0 (K^+), \quad (1)$$

where: V_0 is an empirically determined constant, S the slope of relationship of microelectrode response in different concentrations of KCl solution, and a_0 (K^+) is the ionic activity of the potassium cations. These values were determined by measuring the electrical output response of K^+-SME in pure KCl solutions of the

following concentrations or activities in brackets (mM/l): 100(75), 10(9), 1.0(1.0), 0.1(0.1) (Dean 1985). The obtained S ranged from 57 to 60 mV per 10-fold increase in a_0 (K^+) for both ion exchangers. The selectivity of potassium microelectrodes over sodium ions is sufficient in the high-K^+ and in low-Na^+ experimental solutions, thus only a_0 (K^+) significantly contributes to the K^+-SME response (Walker 1971). Typical electric ion-selective microelectrode characteristics for both ion exchangers are shown in Table 1.

Table 1

Physico-chemical characteristics of typical potassium-selective microelectrodes with different types of ion-exchangers (see: Djamgoz and Laming 1981)

Type of ionexchanger	Selectivity constant $k_{Na\text{-}K}$	$\frac{1}{k_{Na\text{-}K}}$	Resistance (Ohms)	Slope S(mV)
IE-190	0.020±0.001	50:1	7×10^9	59.1±0.1
No. 477313	0.022±0.002	46:1	5.4×10^9	57.6±0.1

Upon impaling a cell with an ion-selective microelectrode, the change in K^+-SME potential, E'_m, is a function of both the membrane potential and the electrical potential, E_K, due to the potassium ion activity gradient across the membrane as described by Eq.:

$$E'_m = E_m + E_K. \quad (2)$$

In a Donnan equilibrium for K^+ ions, E_m must equal E_K and, therefore E_m is expected to equal 0 mV. In independent measurements of E'_m and E_m, the potassium activity inside the cell, a_i (K), can be calculated from a rearrangement of Eq. 2 using the calibration data and the known composition of the bathing media. Calculations of potassium a_i (K) assume that the E_m detected by the potassium selective and conventional microelectrodes are the same.

Both microelectrodes, ion selective and conventional, were connected to the electronic recording system by nonpolarizable Ag-$AgCl_2$ wires. The bath solution was coupled with the circuit via $AgCl_2$-KCl-filled agar bridge. The fast and slow electrical responses of microelectrodes were displayed on an oscilloscope (5103N from Tetronix, USA) and/or digital multimeter (V-650 from Meratronic, Poland), respectively.

Results and Discussion

The application of potassium acetate-filled microelectrodes reduced greatly the filling solution leakage from conventional microelectrodes into the cell (Smith and Robinson 1981, Stoner et al. 1984). Stable cell membrane potentials were recorded for one hour or longer. During this time neither changes in membrane integrity nor cell morphology were observed in light microscopy inspection. Membrane potentials obtained in a series of individual measurements are shown in Fig. 1 A.

The average membrane potential in Blepharisma is -43.3 ± 8.1 mV (S. E., $n = 39$). The criteria for acceptance of ion activity measurements were: (a) initial sharp record deflecton upon cell impalement to new relatively stable potential, and (b) following withdrawal of the K^+-SME, a potential response return to its initial value. Based on these two criteria ion selective microelectrodes were calibrated to insure that no change in the electrical characteristics had occurred. In every ion-selective microelectrode impalement into the cell the value of the electrical potential measured was positive ranging from 5 to 15 mV (Fig. 1 B). This indicates directly that the potassium a_i (K) of the cell cytoplasm is higher than the equilibrium potential for K^+ since E_K exceeds zero. In resting cells of

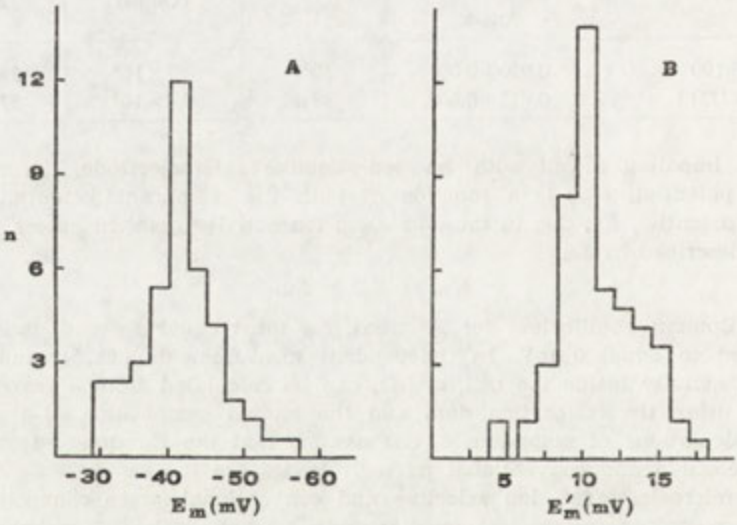

Fig. 1. Distribution of membrane potential E_m, A) and electrochemical ion potential (E'_m, B) values of *Blepharisma* cells

Blepharisma the E_K potentials were quite stable over an adequately long time, however, differences were observed from cell to cell. The obtained E'_m values like in the case of E_m appear to be close to normal distribution, around means of 10,9 ± 4,4 mV (S. E., $n = 54$) (Fig. 1). mean intracellular potassium activity, a_i (K), determined on the base of these measurements is equal to 54.8 ± 4.7 mM, what indicates a cytosolic potassium concentration, c_i (K), of 73.1 ± 6.3 mM, when assuming an intracellular ion activity coefficient for K^+ of 0.75 (Robinson and Stockes 1959, Dean 1985) (Table 2). The estimated concentration value is somewhat lower as compared with those reported by Hilden (1970) obtained by means of flame photometry in the cytoplasm of *Blepharisma*

intermedium. The differences in the potassium levels in those related cells probably result from the medium used, or reflect the experimental techniques applied. It has been frequently observed that cytosolic potassium as well as other ions are not entirely free and occur in the cytoplasm partially as a bound fraction (Klein 1959, 1964, Dunham and Child 1961, Chapman-Andresen and Dick 1962).

Table 2

Direct and computed intracellular parameters for *Blepharisma* cells

Membrane potential E_m(mV)	Electrical potential E'_m(mV)	Potassium activity $a_i(K^+)$ (mM)	Potassium concentration $c_i(K^+)$ (mM)	Potassium equilibrium potential, $E_{eq}(K^+)$(mV)
−43,3±8.1	10.9±4.4	54.8±4.7	73.1±6.3	−127±2.1

The K^+ equilibrium potential, E_{eq} (K), across the cell membrane in *Blepharisma* was calculated to be around — 127 mV from direct measurements of the extracellular (0.35 mM) and intracellular potassium ion activities (Table 2). Therefore, measured transmembrane potential is much lower as compared with the equilibrium potential in culture medium. This observation agree with Hilden's (1970) conclusion that potassium ion movement through the cell membrane of *Blepharisma* is probably regulated by an active process, which might be coupled to the sodium pump, as in other cells (Hodgkin 1958, Russel and Brown 1972, Akera et al. 1981). Carter (1957) was the first to suspect the existence of an active process in ionic regulation in protozoans. He found that the internal potassium level in *Spirostomum* exceeds the ion concentration in the surrounding medium at least ten times, and the internal sodium levels were markedly lower in comparison with those in the medium. The change of potassium concentration in the medium from 0.02 to 1.28 mM evokes its rise in the *Spirostomum* cytoplasm from 3.2 to 7.8 mM, indicating the ability of this ciliate to ionic regulation at a very low external concentration of potassium. A similar process has been observed by Dunham and Child (1961) and confirmed later by Andrus and Giese (1963) in the ciliate *Tetrahymena pyriformis*. These organisms incubated in low-potassium media had an internal/external concentration ratio of potassium of about 100 or more. Exposure to iodoacetic acid, sodium azide and 2,4-dinitrophenol induced a 30-50% potassium loss in these cells.

In *Tetrahymena* vorax cells an electrogenic ion transport contribution up to 30% of the cell membrane potential has been shown as well,

probably due to extrusion of Na ions from the cell cytoplasm (Connolly and Kerkut 1983). Therefore, it seems reasonable to suggest that an active ion transport exist in some protozoans as has been shown for a variety of cells of higher organisms (Adrian 1956, Hodgkin 1958, Tobias 1950, Glynn 1959, Keynes 1954).

On the other hand, the reasons of the observed discrepancy between the measured electrochemical potassium potential and membrane potential in *Blepharisma* cells might lie in the considerable contribution of other ions to the membrane potential, especially in a dilute medium. The failure of the *Blepharisma* cell membrane to behave as a true potassium electrode has been shown for some protozoan and various metazoan cells. It has been observed that the resting membrane potentials of ciliates such as *Stylonychia* and *Stentor* are almost independent of external potassium concentrations between 0.1 and 1.0 mM, attaining the Nernst dependence around of 8 mM only (DePeyer and Mechemer 1977, 1978, Wood 1982). In squid axons the relationship of membrane potential to external potassium is not consistent with the Nernst equation at lower concentrations of potassium (Hodgkin 1958). The same was shown for forg muscle cells where the membrane potential is appreciably lower than the K^+ equilibrium value (Tobias 1950, Adrian 1956).

In conclusion, the precise mechanism of ion regulation in cells of *Blepharisma* is difficult to outline at this point. Specifically oriented experiments with the use K^+ — and other ion-selective microelectrodes for routine measurements of net ion fluxes across the cell membrane of protozoan cells should provide further insight into this subject.

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Dependence of Contractile Vacuole Activity in the Ciliate *Blepharisma japonicum* on Changes of Calcium Concentration and Calcium Ionophore

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Synopsis. The dependence of the frequency of contractile vacuole expulsions in *Blepharisma japonicum* upon changes in the concentration of extracellular calcium and calcium ionophore, A 23187, were studied. An increase of calcium concentration in Pringsheim maintenance medium caused a substantial decrease in frequency of vacuole contractions as compared with the control. If the Ca^{+2} ion concentration is lowered in the medium with the EGTA/Ca^{+2} buffer to the level of 10^{-7} M, the pulsation rhythm of vacuole activity, is distinctly reduced. Cell treatment with A 23187, known to alter the cell membrane permeability to Ca^{+2} ions, in concentrations from 2.5 μM to 20 μM in the Pringsheim solution (0.85 mM Ca^{+2}) resulted in progressive dose-dependent stimulation of the frequency of vacuole contractions. These results indicate that the intracellular Ca^{+2} ions are likely to influence the rate of expulsion of the contractile vacuole in *Blepharisma* cells.

Recently it has been shown that the contractile vacuole activity in *Paramecium aurelia*, *Paramecium multimicronucleatum* and *Blepharisma japonicum*, is highly dependent on sodium concentration in the cell cytoplasm (Boggs and Wade 1972, Frixione and Perez-Olvera 1985, Fabczak and Fabczak 1989a). Drugs like monensin and ouabain, known for their ability to increase the Na^+ influx by selective enhancement of membrane permeability to sodium ions (Walkowiak and Chambers 1975, Pressman 1976) or depressing active Na^+ pumping (Gorman and Marmore 1970), respectively, produced the well displayed dose-dependent decrease of the frequency of contrac-

tile vacuole pulsations. An inverse relationship between Na^+ level in the cytoplasm and the pulsatory action of the vacuole was observed if amiloride, which is extensively used as a tool to decrease the passive permeability to sodium ions in cell membranes (Kleyman and Cragoe 1988), was present in the medium. The simplest hypothetical link between changes in the cytoplasmic Na^+ level in those cells and the vacuole contractile activity would be that the internal Na^+ change simply acted as a mediatory agent to modify the intracellular free Ca^{+2} concentration within the vicinity of the contractile structures of the vacuole. This sort of dependence has frequently been described at the plasma membrane in a number of excitable cells, in which at least part of the calcium efflux from the cell is directly regulated by a transmembrane gradient of Na^+ ions (Glitsch et al. 1970, Blaustein 1974, DiPolo and Beauge 1983, Liu et al. 1985, Jacob et al 1987).

To examine this suggestion, the effect of low external calcium concentration and calcium ionophore, A 23187 increasing membrane permeability to calcium ions, on the frequency of expulsions of the contractile vacuole in *Blepharisma* cells was studied. A short report on the dependence of the contractile vacuole rhythm on internal calcium changes is reported elsewhere (Fabczak and Fabczak 1989b).

Material and Methods

The ciliates *Blepharisma japonicum* were grown in Pringsheim solution with addition of *Tetrahymena pyriformis* cells as a food source in semi-darkness at room temperature. The cells chosen for each experiment were preincubated in fresh Pringsheim solution devoid of nutritional components for 2 h. For experiments the preincubated sample of about 20 cells was transferred from the culture medium to the test solution on a microscope slide and sealed under a cover glass. Contractile vacuole activity observations were made under low illumination with an Ergaval light microscope at a ×4 magnification. The frequencies of vacuole contractions were assessed by the use of a stop watch at 0.5 s accuracy as the time between consecutive contractions per minute. Observations usually lasted 30 min. of continuous viewing of single individuals chosen from the sample randomly. The test solution consisted of 1 ml of Pringsheim solution to which suitable aliquots of stock solutions of calcium, calcium ionophore, A 23187 (Calbiochem), or EGTA/Ca^{+2} buffer were added to obtain the required ionophore or calcium concentration (Portzehl et al. 1964) (Table 1). Stock solution of ionophore A 23187 (1 mg/1 ml) was prepared in dimethyl sulphoxide (DMSO) (Serva) and absolute ethanol (1:1 v/v) and stored in the dark at −10°C. EGTA (Sigma) was dissolved in Pringsheim solution and adjusted to pH of 7.4 with NaOH. Calcium concentration of experimental solutions was measured by the use of a calcium-selective minielectrode (no. 603, Diamond Electro-Tech. Inc., USA).

Results and Discussion

The contractile activity of the contractile vacuole complex (CVC) of *Blepharisma japonicum* cells incubated in higher concentrations of Ca^{+2} ions is presented in the diagram (Fig. 1). The variation of CVC pulsation

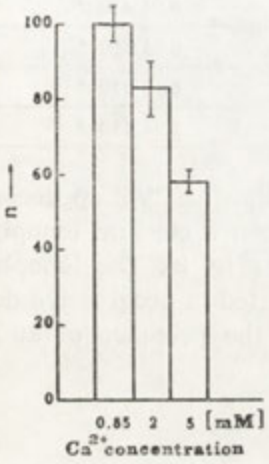

Fig. 1. Effect of Ca^{+2} ions added to the Pringsheim solution on contractile vacuole activity of *Blepharisma* cells

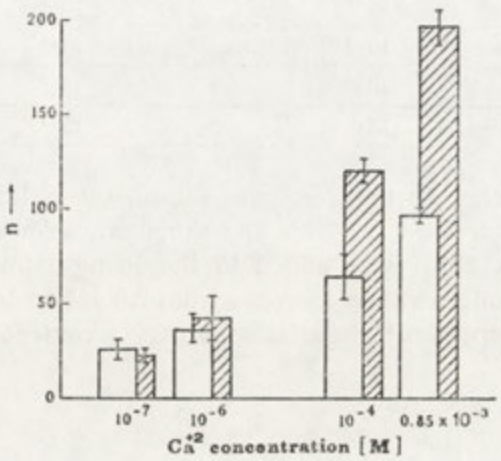

Fig. 2. Influence of concentrations of Ca^{+2} ions in the absence (clear) or presence (hatched) of the calcium ionophre, A 23187, on the cell contractile vacuole activity

frequency (n) is expressed as the percentage of the frequency in a control. Increasing concentrations of Ca^{+2} ions in Pringsheim solution, decrease of the rate of expulsion per minute that means that the time between each vacuole contraction is longer as the external Ca^{+2} concentration is raised. Approximately a five-fold increase of ion concentration (i.e. 5×10^{-3}M Ca^{+2}) caused frequency enhancement by about 50% of the control. This Ca^{+2} ion dependence of *Blepharisma* CVC behaviour is in agreement with the findings in *Paramecium* and *Tetrahymena* (Czarska 1964, Organ et. al. 1978). Treatment of both these species with Ca^{+2} of higher concentrations, as in the case of *Blepharisma*, elicited a marked elongation of the interval of CVC expulsion as the osmotic pressure of the medium was enhanced.

In order to change the cytoplasm Ca^{+2} ions level in *Blepharisma*, buffered Ca^{+2} was added to the basic Pringsheim solution (Table 1). At low Ca^{+2} concentration (10^{-7} M) the *Blepharisma* contractile vacuole functioned at a rather low frequency. The medium with lower Ca^{+2} concentration than 10^{-7} M seems to be rather toxic for the examined

Table 1

Concentrations of free Ca^{+2} in EGTA/Ca^{2+} buffers added to the Pringsheim solution

Free Ca^{2+} concentration (M)	Total EGTA concentration (M)	Total Ca^{2+} concentration (M)
10^{-4}	10^{-4}	2×10^{-4}
10^{-5}	10^{-3}	9.94×10^{-4}
10^{-6}	10^{-3}	8.63×10^{-4}
10^{-7}	10^{-3}	6.13×10^{-4}
10^{-8}	10^{-3}	5.94×10^{-4}

cells. As the Ca^{+2} was raised, the pulsatory rhythm of CVC appreciably increased (Fig. 2). The same can be observed when a calcium ionophore A 23187 was added to the Pringsheim solution (Fig. 3). The ionophore concentration increase from 2.5 μM to 20 μM resulted in progressive dose-dependent stimulation of CVC contractions. In the presence of 20 μM

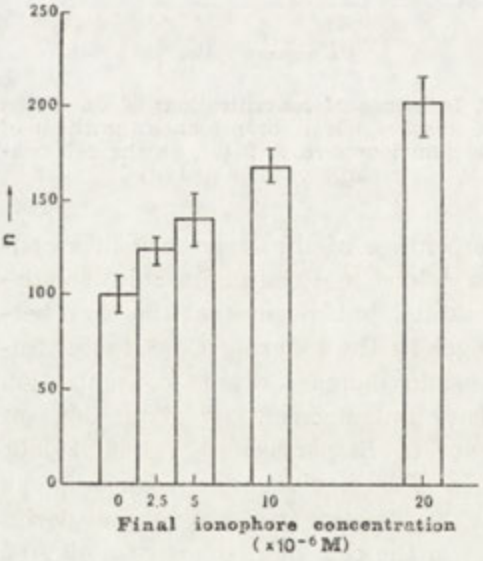

Fig. 3. Alteration of CVC activity with different Ca^{+2} ionophore concentrations in the Pringsheim medium

A 23187, low concentrations of Ca^{+2} influenced only slightly the CVC frequency (Fig. 2). With an increase of external free Ca^{+2} a marked increase of the frequency of CVC expulsion takes place as compared with media devoid of the ionophore et the same free Ca^{+2} level. The observed differences in CVC response in *Blepharisma*, incubatd in both experimental solutions (i.e. with or without ionophore) were greater if the external

free Ca^{+2} concentrations were higher. Addition of ionophore A 23187 to Pringsheim solution (0.85×10^{-3} M Ca^{+2}) almost doubled the rate of CVC activity (Fig. 2).

This specific action of externally applied Ca^{+2} ions indicates that the cell CVC activity is regulated by changes of the free Ca^{+2} concentration within the cell contractile vacuole complex. The lipophilic carrier ionophore, A 23187, which transports divalent cations across membranes tends to equilibrate the free concentrations of these cations in the aqueous compartments separated by membranes (Reed and Lardy 1972). This peculiarity seems to explain the observed much higher sensitivity of CVC activity to changes of Ca^{+2} in the external solutions in the presence of A 23187.

Recently it has been shown that the Ca^{+2} ionophore in conjunction with Ca^{+2} chelating agents can be used to control directly the internal free Ca^{+2} concentrations in intact red blood cells or change the membrane transport properties in cells or lecithin vesicles (Lew and Garcia-Sancho 1985, Pohl et al. 1980, Lee and Vidaver 1984). It was observed in several protozoan microorganisms that internal Ca^{+2} is able to modulate the ciliary or flagellar motions as well (Eckert et al. 1979, Doughty and Dryl 1981, Connolly and Kerkut 1983, Doughty and Diehn 1979. In solutions with low free Ca^{+2} concentration the cells of *Tetrahymena* or detergent *Paramecium* models do not exhibit ciliary reversal (Naitoh and Kaneko 1972, Brutkowska et al. 1977). A calcium ionophore suppresses greatly the phototactic orientation in *Stentor* and also induces a cell tumbling response of *Euglena gracilis* or *Bacillus subtilis* (Ordall 1977, Doughty and Diehn 1979, Chang et al. 1981, Prusti et al. 1984).

The results presented indicate that internal free Ca^{+2} ions are likely to regulate the frequency of contractile vacuole activity in *Blepharisma* cells. This supports the suggestion that the previously observed relationship in *Blepharisma* and other protozoans between the internal sodium and vacuole contraction rate may occur in fact through sodium-dependent changes in the Ca^{+2} concentration of the cell cytoplasm or within the contratile vacuole complex.

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Polymyxin B. Gentamycin and Neomycin Inhibit Phagocytic Activity of *Tetrahymena pyriformis*

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Synopsis. Food vacuole formation in starved *Tetrahymena pyriformis* cells and, in less degree, in the fed ones is inhibited under the influence of polymyxin B (PXB), gentamycin and neomycin, in a dose dependent manner. The strongest inhibition of phagocytosis was observed after 20 min. of incubation with any tested drugs. PXB was shown to be the most effective inhibitor of phagocytosis; about 60% of inhibition was found in the cells treated with 0.1 μM PXB. The same level of phagocytosis inhibition was observed in the presence of 1.0 μM gentamycin. Neomycin was shown to be rather weak phagocytic inhibitor, since comparable level of phagocytic activity inhibition was noted in the cells treated with 100 μM neomycin. In antibiotic-free buffer, the restoration of phagocytic activity took place.

Tetrahymena pyriformis, a ciliated protozoan cell is very convenient model to study phagocytic activity due to cell maintenance in axenic culture with high cell density attained. Therefore the experiments on phagocytosis it is possible to carry out with numerous cell samples in well controlled experimental conditions.

It is known that phagocytosis is a multistep process. The nutrition recognized, then concentrated in oral cavity is taken on by the cell. This is accompanied by generation of second messengers: phosphatidylinositol 4,5-bisphosphate (PIP_2) and arachidonic acid metabolites (Young et al. 1984). Invagination of the membrane entrapping the food and then the cutting off the phagosome takes place. In *Tetrahymena* food vacuole (phagocytic vacuole) is formed during 20-60 sec. (Nilsson and Weidner 1986). In the process calcium ions have been reported to play an

important role in a wide variety of cellular systems including *Tetrahymena vorax* (Sherman et al. 1982), *Amoeba proteus* (Prusch and Minck 1985), neutrophils (Greenberg et al. 1982) and mouse macrophages (Ito et al. 1981, Young et al. 1984).

In the present study we used antibiotics known to interact with the plasma membrane electrostatically due to highly positive charge of molecule and known to display calcium ions from the membrane constituents. Polymyxin B (PXB), an amphiphilic peptidolipid is characterized by the presence of highly charged positive head and a lipophilic tail (Langer 1984). Neomycin and gentamycin, both belong to the same class of aminoglicoside antibiotics, are strongly cationic molecules, either. At neutral pH a positive charge of gentamycin was estimated as + 4, whereas that of neomycin was estimated to be + 6 (Kirschbaum 1984, Gustin and Hennessey 1988, respectively).

The experiments described in the paper revealed the strong inhibition of the mentioned above drugs on the phagocytic activity of *Tetrahymena pyriformis*.

Material and Methods

The experiments were performed on *Tetrahymena pyriformis* cells. The cultures were maintained on 1% proteose peptone and 0.1% yeast extract (Difco) at 27° C. At early logarithmic phase of growth, the cells were harvested by centrifugation at 500 × g for 5 min on MSE oil centrifuge (England), and suspended in 50 mM Tris-HCl buffer, pH 7.2 either for 30 min, for washing out of the growth mediumn (this are the fed cells) or for 20 h starvation (this are starved cells). Afterwards, both kinds of cells were treated with the antibiotics such as polymyxin B (PXB), gentamycin and neomycin (all from Serva). Preliminary experiments have shown that *Tetrahymena* cells are very sensitive to the above mentioned drugs. Therefore, at first, we focused our attention on looking for concentration of antibiotics in which the cells have not been killed during 60 min of incubation with drug, as checked with trypan blue staining.

In the experiments on phagocytosis polymyxin B was used in concentration range from 0.1 μM to 10.0 μM, neomycin from 10.0 μM to 500.0 μM. The gentamycin was used in 1.0 and 5.0 μM concentration. The cells were treated with antibiotics during 60 min. At 10-th, 30-th and 50-th min of drug treatment, the 200 μl cell samples were taken off, in order to estimate the phagocytic activity. This was done by 10 min incubation of the cells with 20 μl of india ink used as marker of newly formed vacuoles. The phagocytosis was stopped by cell fixation with formaldehyde used at final concentration of 4%. After brief washing in the Tris buffer, in order to remove the particles of india ink from the cell surface, the cells were mounted on objective glass. The food vacuoles were counted in minimum 50 cells of each experimental cell sample under the contrast phase microscope PZO (Poland). The phagocytic activity of antibiotic treated cells was expressed as per cent of the control ones.

For recovery estimation, the cells treated with the antibiotics, were transferred into antibiotic-free buffer for 90 min incubation. At 80-th min. a 200 µl cell samples were taken off for phagocytic activity determination, as described above.

Results

Figure 1 presents the phagocytic activity of starved *Tetrahymena* cells under the influence of PXB. Phagocytosis decreased in the presence of PXB in a dose dependent manner. The lowest drug concentration used by us (0.1 µM) evoked decrease of phagocytic activity to about 40% of that found in the control. This level of phagocytosis is sustained until 60 min

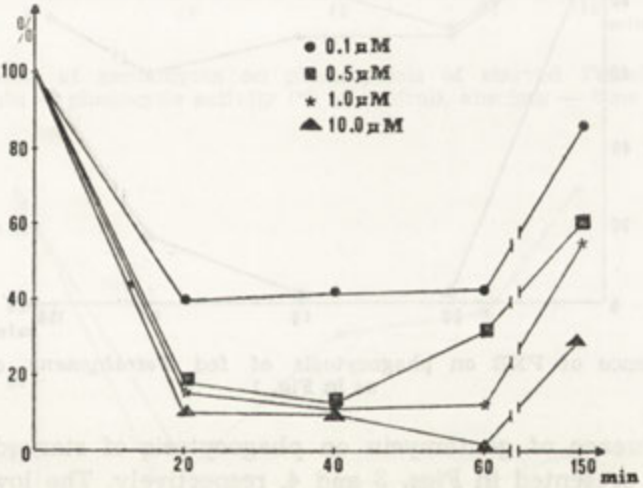

Fig. 1. Influence of PXB on phagocytosis of starved *Tetrahymena* cells. Ordinate — phagocytic activity (% of control), abscissa — time (min.)

of the drug incubation. However, after washing out of PXB, the formation of the food vacuoles is restored to about 85% of that found in control. In higher PXB concentrations (0.5 µM, 1.0 µM and 10.0 µM) the phagocytic activity decreased below 20% of that of the control value in the samples taken from 20 and 40 min of incubation with the drug. After 60 min of incubation, the phagocytic activity of *Tetrahymena* cells treated with 0.5 µM PXB rises to about 30% of the control level, indicating partial cell adaptation. Contrary to that, the formation of new food vacuoles by the cells treated with PXB in higher concentration than 1.0 µM, is arrested or even more inhibited as in the case of 10.0 µM PXB treated cells (Fig. 1). However, after cells transfer into PXB-free

medium, the restoration of phagocytic activity, depending on the used drug concentration, was observed (Fig. 1).

The well-fed cells are less sensitive to the action of PXB used in concentration of 0.1 μM and 1.0 μM, although higher PXB doses exert very strong inhibition of phagocytic activity after 20 min of treatment (Fig. 2). These all cell samples are able to restore their phagocytic activity after washing out of drug. In the case of cells treated with 0.1 μM PXB the phagocytic activity is even higher and exceeds that of the controls (Fig. 2).

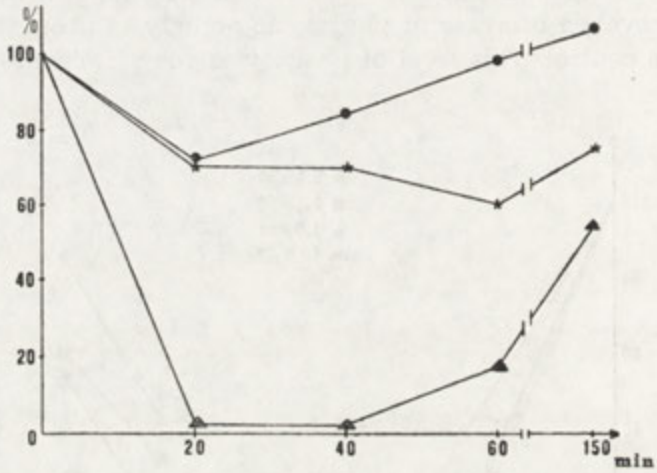

Fig. 2. Influence of PXB on phagocytosis of fed *Tetrahymena* cells. Legends as in Fig. 1

The influence of gentamycin on phagocytosis of starved as well as fed cells is presented in Figs. 3 and 4, respectively. The lowest concentration of gentamycin used by us (10 μM) strongly disturbed phagocytosis of the starved cells after 20 min of treatment (Fig. 3). The level of phagocytosis is estimated to be about 38% of that of the control one. After 40 min of incubation with the drug phagocytic activity of the sample increases to about 76% and after the next 20 min to about 84% of that of the controls. Gentamycin used in concentration of 5 μM inhibited phagocytosis of the starved cells much more effectively. The activity was estimated to be 12% after 60 min of treatment. However, in the gentamycin-free medium the phagocytic activity of that cells is restored to about 17% of the control level. Similar changes of phagocytosis inhibition are observed in the fed cells after 20 min of gentamycin treatment (Fig. 4). The inhibition is very strong and was accounted to be 37% of the control level in the cells treated with 1.0 μM and 17% with 5 μM gentamycin, respectively. As seen in Fig. 4, these cell samples are able

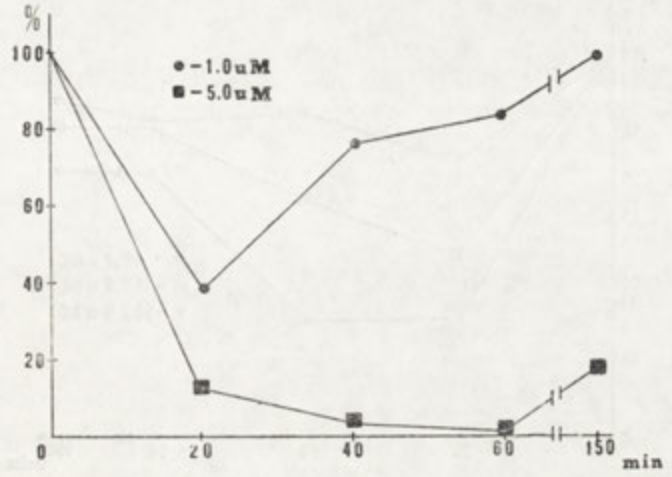

Fig. 3. Influence of gentamycin on phagocytosis of starved *Tetrahymena* cells. Ordinate — phagocytic activity (% of control), abscissa — time (min.)

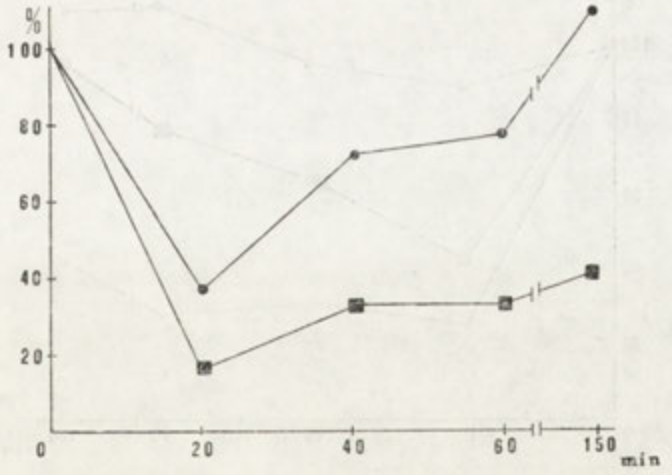

Fig. 4. Influence of gentamycin on phagocytosis of fed *Tetrahymena* cells. Legend as in Fig. 3

to restore their phagocytic activity, even the drug presence in the medium. When gentamycin is absent from the medium, the restoration is more pronounced, and, in the cells incubated with 1.0 μM gentamycin, exceeds even the control level.

The effect of neomycin on phagocytosis of *Tetrahymena* cells is presented in Figs. 5 and 6. The decrease of the amount of new vacuoles in

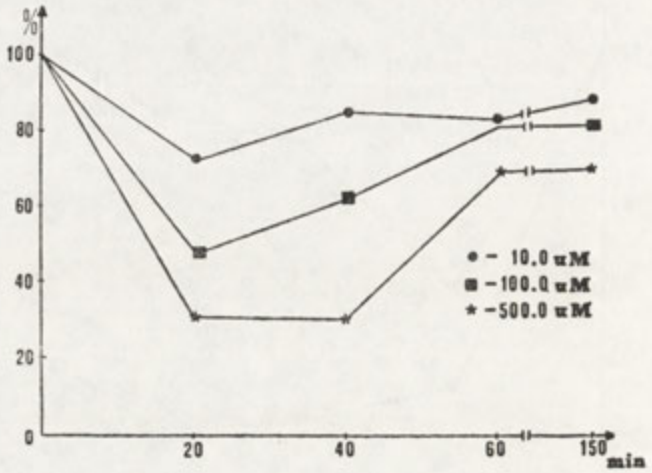

Fig. 5. Influence of neomycin on phagocytosis of starved *Tetrahymena* cells. Ordinate — phagocytic activity (% of control), abscissa — time (min.)

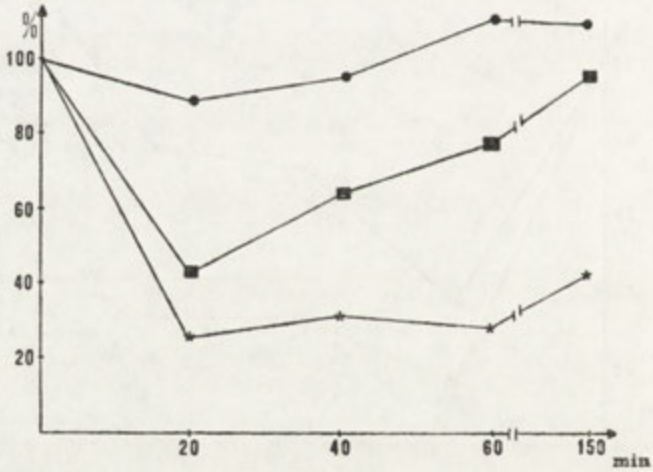

Fig. 6. Influence of neomycin on phagocytosis of fed *Tetrahymena* cells. Legend as in Fig. 5

starved cells is very rapid after 20 min of incubation. In the cells treated with 10.0 μM neomycin, the phagocytic activity is about 27% lower than that of the control. In the cells treated with 100 μM neomycin, the activity under study was inhibited by about 52% whereas in cells incubated with 500 μM neomycin, phagocytic activity was estimated to be about 32% of that noted in the control. After 40 min of incubation with the drug, phagocytic activity increases. In cells treated with 10.0 μM neo-

mycin the activity of vacuole formation rises to about 85% of that in control, whereas in the cells treated with 100.0 μM it increases to about 62% of the control level. In the cells treated with 500 μM, the phagocytosis remains at the same level as it was found after 20 min of treatment. Longer incubation with the drug results in the gradual increase of the food vacuole formation. After drug removing, the augmentation of phagocytosis is more pronounced. Similar pattern of neomycin influence on phagocytosis was also found in the well-fed cells (Fig. 6). Phagocytic activity of cells 20 min. treated with 10.0 μM neomycin was estimated to be about 10% lower than in controls. In the cells treated with 100.0 μM neomycin, it is about 43% of the control level, whereas, in the sample incubated with 500.0 μM neomycin it is estimated to be about 26% of the phagocytic activity of untreated cells. The phagocytosis of the cells increases partially after 40 and 60 min. of incubation with the antibiotic. The recovery is more pronounced in neomycin-free medium.

Discussion

The presented results revealed the decrease of phagocytic activity of *Tetrahymena pyriformis* in the presence of polymyxin B, gentamycin and neomycin. All of them disturbed formation of the food vacuoles in a dose dependent manner. The effect was more pronounced after short time treatment. The comparable level of phagocytosis inhibition (about 60%) was attained by the lowest drug concentration in subsequent order: PXB (1×10^{-7} M), gentamycin (1×10^{-6} M) and neomycin (1×10^{-5} M). PXB 5-fold more concentrated inhibited formation of the food vacuoles in about 83% in comparison to the control, whereas gentamycin, also 5-times more concentrated, inhibited phagocytic activity in about 87% Neomycin, was shown to be in lesser degree effective in inhibition. About 45% of phagocytic activity decrease was reached in 10-folds more concentrated drug. It was shown that neomycin in highest concentration (500 μM) is not more effective in inhibition of formation of food vacuoles then that used in 100 μM.

What is the mechanism of the antibiotic action?

It is known that PXB is inserted into membrane due to lipophilic part of the molecule, and that this binding is enforced by interaction of the positive charge head of PXB molecule with phospholipids of the membrane, mainly with phosphatidylserine (PS) and phosphatidylinositol (PI). These interactions evoke displacement of calcium ions from the membrane (Langer 1984). In the case of sarcolemma, the displacement of the calcium ions is accompanied by the decrease of membrane potential.

It was also shown that in human blood cells removing of the calcium ions under PXB treatment inhibits K^+ efflux via calcium regulated K^+ channels, changing therefore the hyperpolarization of the membrane (Varečka et al. 1987).

We can suggest that inhibition of *Tetrahymena* phagocytosis in the presence of PXB is a result of change of plasma membrane potential via direct inhibition of Ca^{2+} channels and/or via calcium regulated K^+ channels. Since, as low PXB concentration as 10^{-7} M is shown to be a very potent phagocytic inhibitor, we can suppose that in *Tetrahymena* the drug acts at a very specific sites. On the other hand, the other mechanisms of PXB phagocytic activity inhibition are not excluded. It was reported that phosphorylation of calcium binding proteins mediated by calcium dependent protein kinase was important in phagosome formation. (Vaux and Gordon 1982). The phosphorylation of proteins mediated by phospholipid-sensitive Ca^{2+} dependent protein kinase decreased in the presence of PXB (Wrenn and Wooten 1984). Therefore, it can be suggested, that in our experimental conditions the phosphorylation of calcium binding proteins can be inhibited under the presence of PXB.

Gentamycin, due to its polybasic character aggregates the anions situated on the surface membrane. This was connected with Ca^{2+} replacement from anionic phospholipids mainly phosphatidylserine (PS). The displacement of ^{45}Ca from PS monolayer as well as red cell ghost and plasmalemma of heart muscle cells was nearly completely in the presence of gentamycin (Lullmann and Vollmer 1982). As low concentration of gentamycin as 0.11 μM is able to remove Ca^{2+} from brush border membrane (Kirschbaum 1984). Depression of the action potential and contraction of cultured cardiocytes evoked by the presence of gentamycin as well as their rapid restoration when gentamycin was removed from the medium were also reported (De la Chapelle-Groz and Athias 1988). In higher concentration of gentamycin (1 mM) the interaction with phosphatidylinositol 4,5-bisphosphate (PIP_2) as well as the inhibition of protein kinase C redistribution were also observed (Ramsammy et al. 1988). In our experimental conditions, the decreased of phagocytic activity of *Tetrahymena* in gentamycin and its recovery are probably connected with calcium replacement from the membrane.

Neomycin, similary to gentamycin, is cationic molecule. The low concentration of the drug (80 μM) disturbes the inward Ca^{2+} current of *Paramecium*, and this effect can be negated by external calcium ions (Gustin and Hennessey 1988). Moreover, neomycin interacts with anionic lipids like PS, phosphatidylinositol (PI), phosphatidylglicerol (PG)

and PIP_2 (Gabev et al. 1989). It was also reported that neomycin binding to PIP_2 prevents its hydrolysis by phospholipase C, disturbing trombin-stimulated cell proliferation (Carney et al. 1985). In higher drug concentration (5 mM), the Ca^{2+} pump can be disturbed, especially in the presence of insufficient amount of phospholipids (Missiaen et al. 1989).

We can suppose that inhibition of phagocytic activity of *Tetrahymena* cells observed in the presence of antibiotics is probably dealing with changes of membrane potential. Ca^{2+} replacement from the negatively charged membrane constituents could disturbe transmembrane ion fluxes, changing intracellular calcium ion concentration. On the other hand, interactions of the antibiotics with phosphatidylinositides, the presence of which in Ciliates have been reported (Kaneshiro 1987) are of special importance in the transduction of signal from external medium into the cell. Inhibition of PIP_2 resulted in blocking of phosphatidylinositol triphosphate (IP_3). The role of IP_3 in releasing of calcium from intracellular stores is well documented now (Berridge 1985 and Boughriet et al. 1988). Therefore, the inhibition of phagocytosis of *Tetrahymena* cells in the presence of these antibiotics seems to be evoked by arrest of intracellular Ca^{2+} release via inhibition of IP_3 generation due to inhibition of PIP_2. The restoration of phagocytic activity in the presence of drugs indicates the existence of some mechanisms of cell protection against the drug action, which seems to be more pronounced in fed cells.

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Study of Insulin-Provoked Endocytosis in Different Taxa of *Tetrahymena*

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Synopsis. Investigation into the physiological nutrition of different *Tetrahymena* taxa with the Quin 2 technique, and follow-up of phagocytosis of Chinese ink in the same taxa demonstrated that the underlying mechanisms of physiological and non-physiological food intake were dissimilar. The phylogenetically closely related taxa responded by a similar feeding behaviour to the presence of insulin. The behaviour of the secretion-inhibited mutant strain differed from that of the "wild" strain. The phylogenetical relationship is hardly, if at all, demonstrable in the aphysiological system and the involvement of hormonal influence seems to be a chance coincidence in physiological conditions of nutrition.

Unicellular organisms are able to respond to hormones of higher animals. The response is frequently specific. For example, the unicellular organism, *Tetrahymena* responds to insulin (Csaba and Lantos 1975) and epinephrine (Csaba and Lantos 1976) by alteration of its sugar metabolism, to thyroxin and its precursors by increased mitotic activity (Csaba and Németh 1980), and to histamine and serotonin by increased phagocytotic activity (Csaba and Lantos 1973). The non-specific membrane structures which serve as binding sites transform in the presence of the hormone to specific receptors with change the hormone binding capacity and responsiveness of the target cell (Csaba 1980, 1981). This phenomenon has been termed hormonal imprinting, and has been shown to take place also in higher organisms during the perinatal period (Csaba 1986).

The "memory" of primary interaction with the hormone is not limited to the unicellular organism originally involved in imprinting, but is transmitted to the offspring generations and persists after several hundreds of generations (Csaba and Németh 1982). Imprinting can be induced in many unicellular organisms, but its trend and intensity may vary between the taxa of the same species. For example, we demonstrated earlier that primary interaction with insulin induced a positive binding and functional imprinting in *Tetrahymena pyriformis*, but a negative one in *T. thermophila* (Kovács and Csaba 1987).

The unicellular eukaryotes possess not only potential receptors for vertebrate hormones (Csaba 1985, Legros et al. 1975, Fawell et al. 1988, Fawell and Lenard 1988, McKenzie et al. 1988, Feldmann et al. 1982, Loose et al. 1981) but contain active molecules which are immunologically similar to hormones (Le Roith et al., 1980, 1983, Roth et al. 1982). Among others, *Tetrahymena* contains several polypeptide and steroid hormones or hormone-like materials, which are presumably by-products of the protein and steroid synthesis of this ciliated cell (which occupies a high systematic position within its order) and which probably also have an as yet unknown functional importance. In this light, it seemed to be of interest to investigate whether the unicellular organism which operates a hormone producing and hormone-responsive (receptor) system, would change its endocytotic activity in response to exposure to a self-contained hormone. Since insulin is present in *Tetrahymena*, and exogenous insulin induces receptor formation in its membrane (Csaba 1985), we examined the influence of this hormone on the spontaneous and particle induced endocytotic activity in five taxa and one mutant strain of *Tetrahymena*, to cover also possible intra-species variations in the physiological reactions.

Material and Methods

The following taxa of *Tetrahymena* were used: *Tetrahymena pyriformis* GL (Gelei, Hungary); *T. pigmentosa* (Nozawa, Japan); *T. hegewischi* KP 7 (Cech, USA); *T. thermophila* B VIII (Tiedtke, FRG); *T. thermophila* MS-1 (Tiedtke, FRG); *T. malaccensis* MP 75 (Cech, USA).

All cells were cultured in 0.1% yeast extract containing 1% Bacto tryptone medium (Difco, Michigan, U.S.A.) at 28°C, and were used in the logarithmic phase of growth.

Vacuole counting by means of the Quin 2 technique (Tsien 1981)

Part of the mass cultures was not treated, to serve as control, part was treated with 10^{-6} M insulin (Semilente, MC, Novo, Copenhagen) for 1 h, for imprinting.

After imprinting the cells were washed in plain medium and were returned to plain medium for 24 h.

Subsequently each imprinted and not imprinted culture was subdivided into two groups, of which one was exposed to 10^{-6} M insulin, the other was not. Thirty min later 5 ml of each subgroup-culture was combined with 50 μM Quin 2/AM (Calbiochem, La Jolla, California, U.S.A.) solution in DMSO. Twenty five min later the cells were spread on slides, dried in warm (45°C) air flow, washed in PBS for 5 min and rinsed in distilled water. The endocytotic vacuoles were then counted under a Zeiss Fluoval microscope.

Testing for phagocytosis of Chinese ink

The cells were imprinted with insulin as described above. After their return to plain medium for one day, the imprinted and not imprinted cultures were equally washed and "starved" for 4 h in Losina solution. Subsequently one of two subgroups was treated with 10^{-6} M insulin, and Chinese ink, suspended in Losina solution, was added to all cultures. Ten min later phagocytosis was arrested by addition of a 4% formalin solution.

The nutrient vacuoles were counted in 50 cells in each group and the significance of inter-group variations was determined with Student's *t*-test.

Results

Vacuole counting with the Quin 2 technique demonstrated the appearance of extraordinarily numerous vacuoles in the *T. thermophila* MSl cells under the influence of insulin. The vacuole count tended to decrease, yet was still high one day later. Reexposure to insulin accounted again for an enormous increase in the vacuole count. A significant increase over the control also occurred in the *T. hegewischi*, *T. pigmentosa* and *T. pyriformis* cells on primary exposure but, unlike MSl cells, no further increase could be observed after reexposure (Fig. 1.)

Insulin imprinting enhanced the phagocytosis of Chinese ink only in the *T. hegewischi* cells, and decreased it in the *T. thermophila* MSl cells. The changes observed in the strains of the other taxa were not significant statistically. One day after imprinting again *T. hegewischi* alone showed an appreciable decrease in phagocytotic activity. Reexposure to insulin increased considerably the phagocytotic activity in *T. hegewischi* and *T. thermophila*, but decreased it considerably in *T. pigmentosa* and *T. malaccensis*. The other taxa showed no significant change in response to reexposure (Fig. 2).

Reexposure to insulin increased significantly the vacuole count detectable with Quin 2 in the *T. thermophila* MSl mutant strain (Pl.I: 1). While

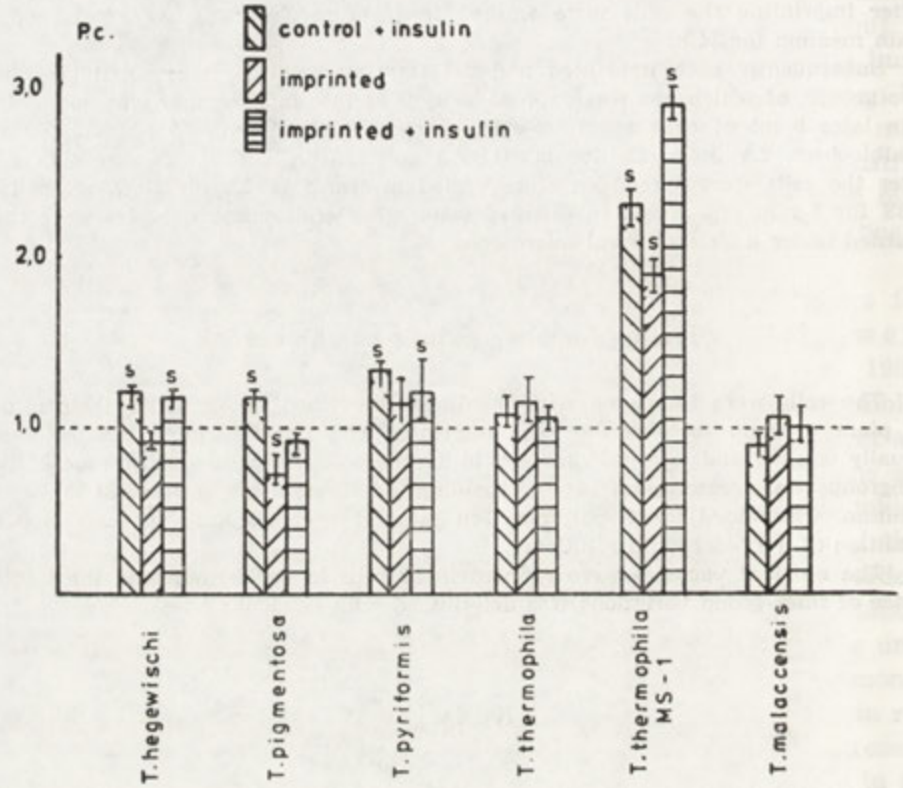

Fig. 1. Phagocytotic activity of different taxa of *Tetrahymena* determined by counting of fluorescent vacuoles after Quin 2/AM treatment. Phagocytotic coefficient (PC) related to the control as 1. $s = p < 0.01$

vacuoles were quite innumerous in the control cells, many vacuoles made appearance in cells of the other taxa in response to insulin treatment. Chinese ink phagocytosis did not follow this response pattern.

The size of Quin 2-detectable vacuoles was considerably increased in *T. hegewischi* cells after insulin exposure (Pl. I: 2).

Discussion

The broad-range action of insulin on vertebrate cells also occurs to a certain extent at the unicellular level, in which insulin influences not only glucose metabolism, but also the intake, incorporation and elimination of amino acids and to a lesser degree also the mitotic activity. The present experiments have thrown light on two aspects of endocytosis,

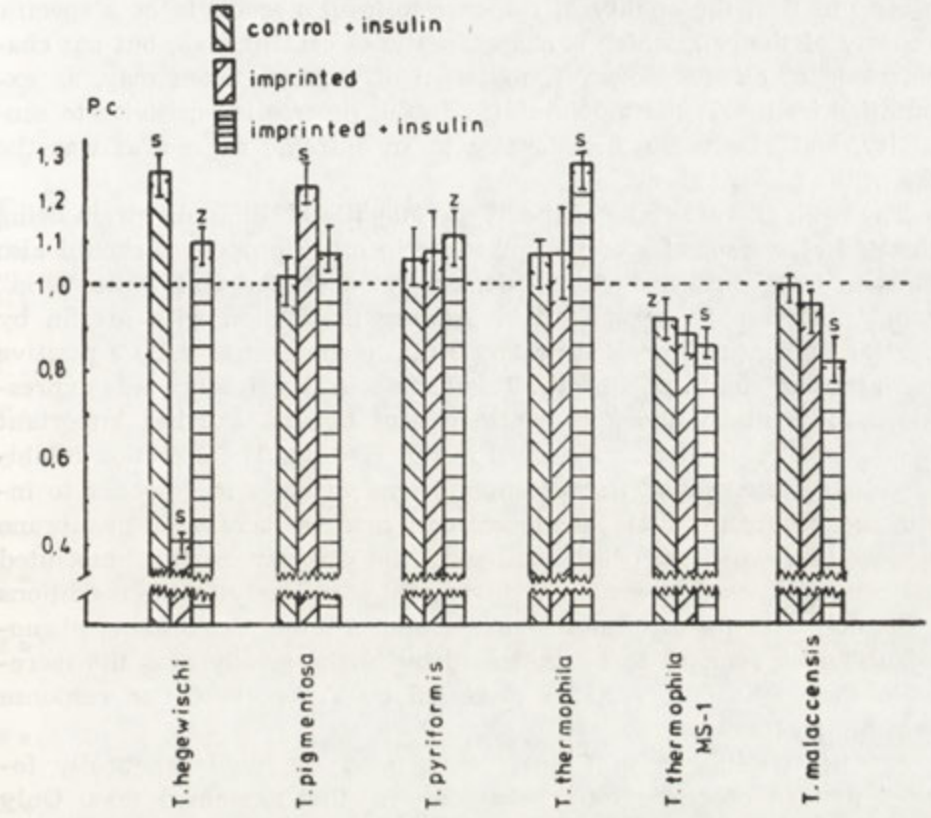

Fig. 2. Phagocytotic activity of different taxa of *Tetrahymena* determined by counting of Chinese ink containing food vacuoles. Phagocytotic coefficient (PC) relative to the control as 1. $s = p < 0.01$ and $z = p < 0.05$

food intake in plain nutrient medium, as assessed in terms of Quin 2-detectable vacuole counts, and intake of particles (Chinese ink) artificially fed in conditions of relative starvation. The two endocytotic mechanisms differed considerably under the given conditions of the experiment.

Quin 2 is known to detect the free Ca^{2+}, which is always present in the nutrient vacuoles (Tsien 1981). Thus the vacuole counts characterized the extent of normal food intake, as well as its change under the influence of insulin immediately after primary exposure, one day later, and on reexposure.

Primary treatment with insulin increased considerably the "appetite" of the three phylogenetically closely related taxa *T. hegewischi*, *T. pigmentosa* and *T. pyriformis*, but had no such effect on the phylogenetically relatively distant, interrelated taxa *T. thermophila* and *T. malaccensis*.

In view of this, the quality of response to insulin seems to be a specific property of the cell, which is characteristic of certain taxa, but not characteristic of others. However, mutation of a given taxon may, as exemplified by the *T. thermophila* MSl strain, reverse insensitivity to sensitivity, and vice versa, occasionally to an extreme degree, as was the case in the present study.

The main characteristic of the *T. thermophila* MSl mutant strain being inhibited of lysosomal secretion, changes in other properties should also be taken into consideration. We demonstrated earlier that while "wild" type *T. thermophila* responded to primary interaction with insulin by negative imprinting, the MSl mutant strain responded to it by a positive imprinting as, e.g. *T. pyriformis*. This behavioural difference was expressed in food intake under the influence of insulin. Further important phenomenon could also be observed in this context. The secretion-inhibited cells secreted many fluorescent nutrient vacuoles in response to insulin reexposure (Pl I: 1). This indicated a marked increase in membrane permeability, which accorded well with the observation that imprinted MSl cells reexposed to insulin disintegrated completely in the conditions of fixation with formalin and PAS reaction. Another remarkable phenomenon, which remains to be explained by further study, was the increase in the size of the vacuoles produced by *T. hegewischi* in response to insulin treatment.

Artificial feeding with Chinese ink biased the phylogenetically founded pattern of endocytotic behaviour in the examined taxa. Only a single taxon, *T. hegewischi*, showed an appreciable increase in Chinese ink phagocytosis under the influence of insulin, and another single taxon, *T. thermophila*, showed an opposite (negative) response. Significant imprinting also occurred in a single case only. It follows that the cells would respond differently to their normal food than to artificially offered particles.

The Quin 2 fluorescence technique used in the present study made it possible to follow the physiological feeding mechanism of the cell. The experimental observations strongly suggest that the mechanisms of physiological and non-physiological food intake may differ considerably, particularly if the influence of regulators is also involved. Another conclusion emerging from the present study is that the response of unicellular organisms to regulators — hormones — is taxon-dependent, to judge from variations in the trend of response to, and resistant or sensitive behaviour towards, the regulator. Identical trends of response in phylogenetically closely related taxa can be substantiated by cytochemical analysis of the physiological index. The aphysiological system is in itself not conclusive of these interrelationships.

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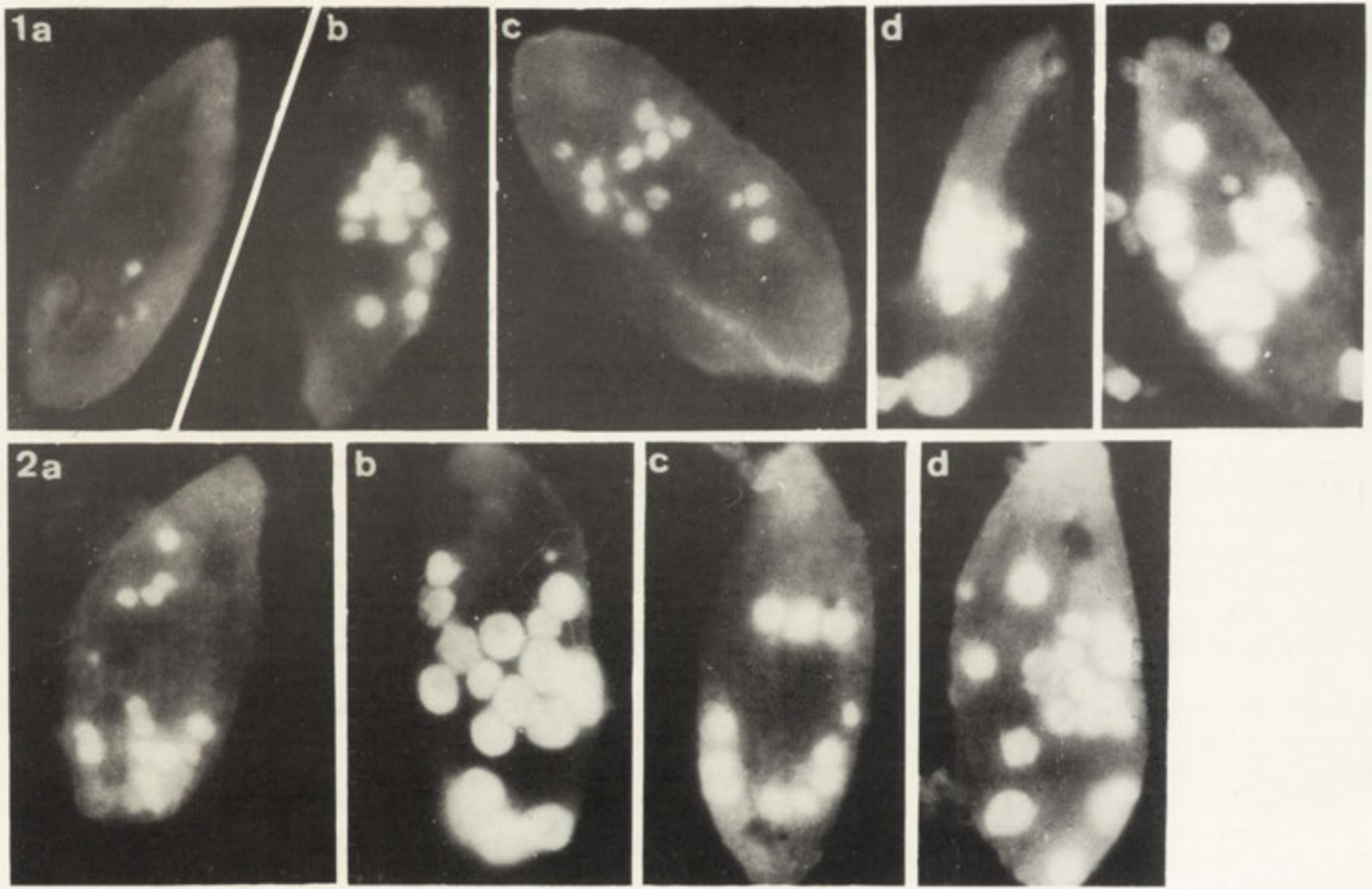
EXPLANATION OF PLATE I

1: *Tetrahymena thermophila* MS-1 mutant strain treated by Quin 2/AM; a — control, b — control + insulin, c — imprinted, d — imprinted + insulin. (1200X)
2: *Tetrahymena hegewischi* treated by Quin 2/AM; a, b, c, d as in 1. (1200X)



P. Kovács and G. Csaba auctores phot.

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The Distribution of Ciliates through the Reticulo-Rumen of Sheep

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Synopsis. Distribution of ciliates through the reticulo-rumen was examined in two sheep fed hay-concentrate diet every 12 h. It was found that protozoa of the family *Ophryoscolecidae* were most numerous in the middle region of the rumen and holotrichs at the bottom of the ventral sac. A reduction in ciliate concentration was observed during their passage from the rumen to the reticulum. The density of *Entodinium* was reduced by 29-36%, of *Dasytricha ruminantium* by 48-57%, of *Polyplastron multivesiculatum* — 42-62%, of *Diplodinium* sp. — 53-61%, of *Isotricha* sp. — 58-76% and of *Ophryoscolex caudatus* by 70-74%. The ratio of sequestration of ciliates on the solid particles was related to the dimensions of the ciliate cells. About 60% of total *Entodinium* and 47% of *Dasytricha ruminantium* were found in the liquid fraction of rumen content whereas over 74% of *Polyplastron multivesiculatum* was found in the particulate fraction. A spatial structure of the solid fraction in the rumen is discussed as a factor affecting the movement of ciliates through the reticulo-rumen.

Ciliate protozoa are usually present in the rumen at high numbers. There is an opinion that the division rate of ciliates is inadequate for maintenance of ciliates in the rumen (Hungate 1966) and thus the presence of protozoa depends upon a slow rate of their removal from the forestomachs (Abe and Kumeno 1973). It has been confirmed in numerous experiments that the outflow rate of ciliates from the rumen is much lower than the outflow rate of rumen fluid (Weller and Pilgrim 1974, Czerkawski and Breckenridge 1977, Harrison et al. 1979, Michałowski et al. 1986) and sequestration of protozoa in the rumen is postulated to explain this phenomenon.

The data supporting this theory are limited and attachment of ciliates to food particles is considered as a mechanism of sequestration (Bauchop and Clarke 1976, Orpin and Hall 1977, Clarke 1977, Orpin and Letcher 1978). On the other hand, however, it can be easily imagined that solid particles in the rumen form some sort of a spongy matrix of different channel dimensions. It is also well known that dimensions of rumen ciliates varied from about 10 to over 500 μm (Dogiel 1927). Thus it can not be precluded that a possibility of the movement of protozoa through the solids is related to their cell dimensions. If it is true, the particulate fraction of the rumen content may be considered as a filter influencing the passage of ciliates from the rumen to the reticulum and hence influencing also their removal from the forestomachs. Very simple experiments presented here were undertaken in order to confirm the above suggestion.

Material and Methods

Two adult sheep sheep "W" and sheep "S" equipped with large rumen fistulae (10-12 cm internal diameter) were used. The animals were kept in separate pens and fed 400 g concentrate and 450 g hay every 12 h. Water was available all the time.

The following samples were taken: of rumen content and rumen fluid from the middle region of the rumen; fluid from the ventral sac of the rumen and from the reticulum. All the samples were taken during relaxing phase of the muscles of the rumen and reticular wall. The samples were collected every 2 h over a 10 h period and the first sample was taken 2 h after morning feeding. The sampling was repeated three times on three different days. Portions of fluid from middle region of the rumen and from its ventral sac were always taken from three different sites. For collecting the fluid from the ventral sac and reticulum, the sampling tube was always introduced up to surface of their wall. Samples of rumen content were taken by hand using a large ladle. Samples of the fluid from all three regions of the reticulo-rumen were withdrawn by suction using a steel tube about 50 cm long and of 0.8 cm external diameter, connected to a syringe via a rubber tube. The wall of the sampling end of the steel tube (about 5 cm in length) possessed numerous holes of about 0.2 cm diameter.

Samples of rumen content of sheep "W" were used for preparation of liquid and particulate fractions of this digesta. Its portions (about 100 g) were weighed and put on a layer of surgical gauze spread over the top of a beaker and the fluid was allowed to effuse at 38°C. The remaining material was enveloped with the same gauze and put on the bottom of the beaker. A portion of warm (38°C) "caudatum type" salt solution (Coleman et al. 1972) was poured carefuly on the enveloped digesta and then this material was squeezed by pressing down with hand. The squeezing procedure was repeated twice. The obtained liquid was pooled while solids from the gauze were carefully taken off. All fractions were weighed and samples (5 g) were fixed with 4% formaldehyde solution.

Ciliates were counted under light microscope using a Fuchs-Rosenthal chamber. Each sample of the fixed material was counted 6 times.

The ciliates were measured using an eyepiece micrometer. Length and breadth of fifty cells from each group of protozoa were measured.

All statistical calculations were made according to Ruszczyc (1970).

Results and Discussion

Concentration of ciliates of family *Ophryoscolecidae* was the highest in middle region of the rumen while holotrichs were most numerous in the ventral sac (Table 1 and 2). The highest concentration of *Holotricha*

Table 1

The concentration of ciliates ($\times 10^3$/g) in the rumen content (A) and in the fluid from middle region (B), and ventral sac of the rumen (C) and reticulum (D) of sheep „W". (Mean values from 18 observations ± Standard error)

Population	A	B	C	D
	a	a,b	b,c	c
Entodinium sp.	436.9±33.17	379.4±27.06	320.3±22.67	278.6±19.01
	a	b	b	b
Diplodinium sp.	45.1±2.78	22.6± 1.69	22.1± 1.74	21.3± 1.67
	a	b	b	b
Polypl. multivesiculatum	5.3± 0.53	2.6± 0.45	3.0±0.52	2.0±0.31
	a	b	b	b
Ophryoscolex caudatus	10.6± 1.16	4.6±0.47	4.8± 0.80	3.2± 0.34
	a	a,b	b	a
Dasytricha ruminantium	5.3± 0.54	7.9± 1.63	9.1± 1.52	4.7± 0.54
	a	a	b	a
Isotricha sp.	5.3± 0.72	6.3± 1.01	13.2± 1.78	4.4± 0.44

Values in a row with different letters differ significantly ($p < 0.05$).

was observed at the bottom of ventral sac over all 12 h feeding period (Fig. 1). These results suggested that ophryoscolecids and holotrichs occupied 2 different regions of the rumen. The presence of large numbers of these ciliates in regions distant from these mentioned above may be a consequence of the passage of protozoa with the stream of the liquid fraction of digesta after contraction of the wall of an appropriate part of the rumen (Titchen and Reid 1965, Deswysen 1985) and the locomotor activity of the ciliates themselves.

The lowest number of ciliates was found in the reticulum and this concerns especially to the large ciliates i.e. *Polyplastron multivesiculatum*

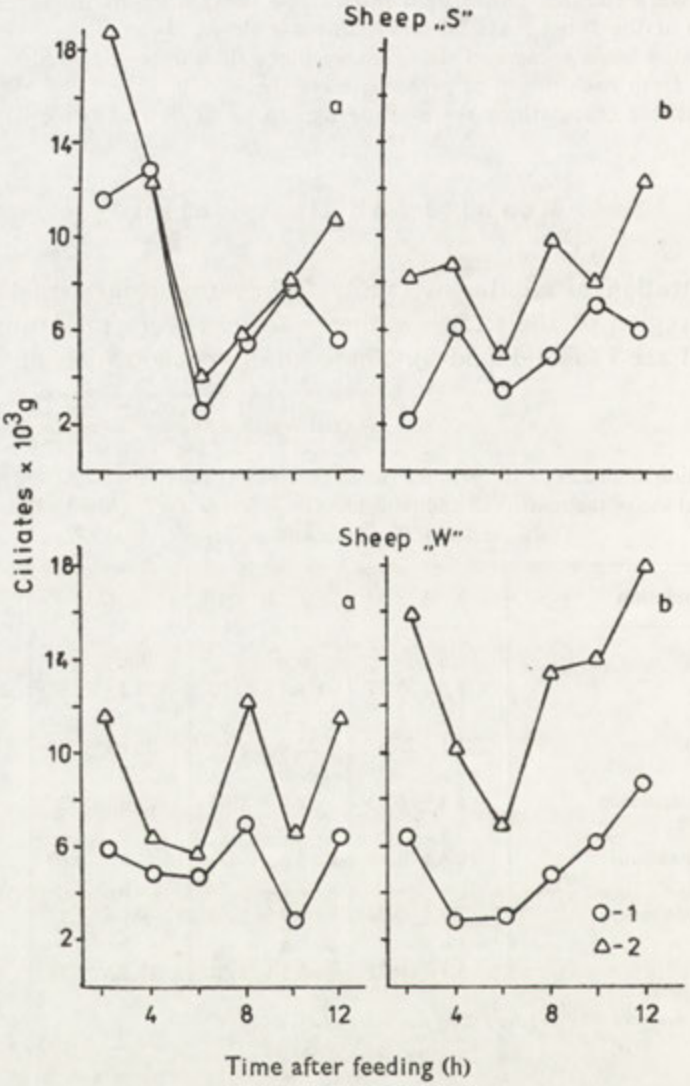

Fig. 1. The concentration of *Dasytricha ruminantium* (a) and *Isotricha* sp. (b) in the rumen content (1) and in the fluid from ventral sac of the rumen (2) at different time after feeding of sheep

and *Ophryoscolex caudatus*. They were about 42-74% less than in the rumen content (Table 1 and 2). Concentration of *Entodinium* was lower by 20-36% and *Diplodinium* sp. by about 53-59%. *Entodinium* group consisted mainly of *Entodinium exiguum*, *Entodinium simplex*, *Entodinium caudatum* and *Entodinium longinucleatum* while the *Diplodinium* group contained *Anoplodinium denticulatum*, *Diploplastron affine* and

Table 2

The concentration of ciliates ($\times 10^3$/g) in the content (A) and the fluid from medium region of the rumen (B), in the fluid from the ventral sac of the rumen (C) and in reticular fluid (D) of sheep "S". (Mean values from 18 observations ± Standard error)

Population	A	B	C	D
Entodinium sp.	a 463.0±25.34	a 406.5±20.05	b 317.6±19.81	b 329.5±19.83
Diplodinium sp.	a 65.8± 6.54	b 35.1± 3.77	c 23.5± 2.48	c 25.6± 2.56
Polypl. multivesiculatum	a 3.1± 0.23	b 1.9± 0.33	b 1.8± 0.31	b 1.8± 0.41
Ophryoscolex caudatus	a 11.7± 1.67	b 5.3± 0.84	b 3.5± 0.54	b 3.0± 0.49
Dasytricha ruminantium	a,b 6.7± 0.91	a 8.5± 1.15	a 9.1± 1.29	b 5.7± 0.64
Isotricha sp.	a 4.9± 0.48	a 5.1± 0.80	b 8.0± 0.91	a 3.4± 0.28

Values in a row with different letters differ significantly ($p < 0.05$).

Ostracodinium triloricatum. However, total counts of both groups was only given (Table 1 and 2) because identification of individual species of ciliates after feeding the sheep was practically impossible.

A b e et al. (1981) suggest that holotrichs sequester on the reticulum wall and migrate into the rumen only for few hours after feeding. The highest concentration, however, of these protozoa in sheep of the experiments presented here, was found at the bottom of the ventral sac and the lowest in the reticulum. Thus, the results obtained suggested that *Holotricha* occupied rather a region near the wall of the ventral sac. Microscopic observations, however, of the rumen and reticulum walls were not carried here.

It is well documented that contraction of the reticulum precedes the contraction of the rumen (T i t c h e n and R e i d 1965, D e s w y s e n 1985) and the result of this is the passage of the reticular digesta into the rumen and omasum. On the other hand, a flow of rumen fluid to the reticulum is the consequence of contraction of the rumen wall. The obtained results showed that the lowest concentration of ciliates was in the reticulum (Table 1 and 2). This suggest that some part of the ciliates was retained within the rumen presumably due to sequestration or arresting between the solid particles of digesta. It is also evident that sequestration (arresting) of *Entodinium* sp. and *Dasytricha ruminantium* was less than of other *Ophryoscolecidae* and isotrichs. The results presented in Table 3 showed that the passage of ciliates to the liquid fraction of

the rumen content during its effusing and squeezing was related to the dimensions of protozoa cells. Statistical analysis of the obtained results showed a negative correlation between the volume of ciliate cells, calculated according to Harmeyer and Hill (1964), and their number in the liquid fraction, expressed as proportion of the total count in the rumen content (Fig. 2). As it was said in "Introduction", large solid par-

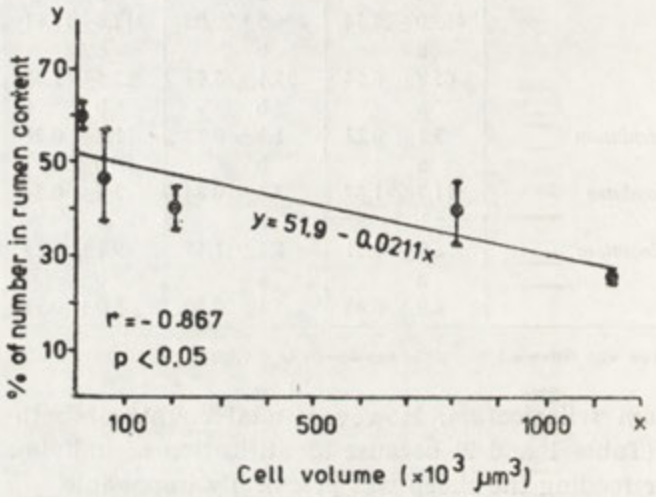

Fig. 2. Straight-line correlation between volume of the ciliate cells and the number of protozoa (expressed in % of the total counts in rumen content) passing to the liquid fraction of rumen digesta

ticles of rumen content form a sort of spongy matrix or a filter with different dimensions of channels. It is possible that some of them may be too small for the large protozoa to pass through, but wide enough for the small ones e.g. entodinia and *Dasytricha ruminantium*. Hence a relatively large part of the population of *Polyplastron multivesiculatum* and *Ophryoscolex caudatus* was arrested between the solid particles while an abundant proportion of *Entodinium* sp. and *Dasytricha ruminantium* was passing with the stream of rumen fluid into reticulum. It is also possible that the high flexibility of the *Isotricha* cells made their passage through the solids easier in spite of the large dimensions of these protozoa (Table 3). The reason presented here is not in contradiction to the importance of an attachment of ciliates to food particles as a factor preventing their removal from the rumen. This last, however, was confirmed only in the case of *Epidinium ecaudatum* (Bauchop and Clarke 1976) and *Isotricha intestinalis* and *Isotricha prostoma* (Orpin and Hall 1977, Orpin and Letcher 1978). Of these

Table 3

Dimensions (μm) as well as numbers of ciliates ($\times 10^4$) in 1 g of rumen content and distribution (%) over a liquid and particulate fraction of rumen digesta. (Mean values from 50 and 3 observations respectively ± Standard error)

Population	Dimensions		Numbers	Fractions	
	Length	Width		Liquid	Particulate
Entodinium sp.	41± 4.28	27± 3.60	97.4±17.38	59.8±1.61	40.2±1.58
Diplodinium sp.	94± 5.43	65± 5.10	8.9± 1.51	40.4±2.20	59.6±2.22
Polypl. multivesiculatum	184±13.00	106±11.03	1.0± 0.13	25.3±0.52	74.7±0.55
Dasytricha ruminantium	61± 5.10	45± 3.60	0.4± 0.04	46.7±5.75	53.3±5.73
Isotricha sp.	168±14.30	90± 8.82	1.0± 0.31	39.6±3.69	60.4±3.69

three species, only both isotrichs were present in the rumen of sheep used in the experiments reported here.

It was found in other experiments (in preparation) that an influx of digesta into the omasum occured only during the second phase of contraction of the reticulum. This suggests that the omasum influent is in fact the reticular fluid. Thus it is also possible that the ciliate concentration in omasal influent is equal to their concentration in reticular fluid. If this is the case then the ciliate density in the reticulum can be an appropriate basis for calculation of the removal of protozoal matter from the reticulo-rumen. This suggestion, however, needs an experimental confirmation.

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Euplotide Ciliates in Sea Ice of the Weddell Sea (Antarctica) *

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Synopsis. Euplotes algivora n. spec., *Cytharoides australis* n. spec. and *Cytharoides balechi* are euplotide from the sea ice of Antarctica. Their morphology and infraciliature are described and a new definition of the genus *Cytharoides* is given.

To a cursory glance the sea ice of the polar oceans seems to be a biotope which is hostile to life and unfavourable to colonization. However, this impression is misleading, since also here, upon a closer inspection, a population is present. Distinctly delimitable biotopes are open to colonization. These are the snow-ice interface, and the capillary system of the ice further subdivided into an inner system of gaps which is open to the sea. In this connection one has to mention the fact that these biotopes are differently developed in Arctic and Antarctic ice (Spindler, in press). Their inhabitants are algae, fungi, bacteria, protozoa and metazoa, which in their entirety are labelled "sympagic organism" Horner (1985). Studies on foraminifers by Spindler and Dieckmann (1986), and on ciliates by Fenchel and Lee (1972), Tuffrau (1974), and Corliss and Snyder (1986) show that here, as in other aquatic biotopes, protozoa are rich in species. In this paper, further ciliates will be described which have been found in sea ice of the Weddell-Sea.

Material and Methods

The material originates from the Antarctic expedition (ANT 5/3) of the Polarstern in austral spring (Oct.-Nov. 1986). Cf. Fig. 1 for the location of the sampling sites. Sea ice cores were taken from which ciliates were obtained by the method

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of Spindler and Dieckmann (1986). The ciliates were cultured at the laboratory in climatic chambers at +3°C, and were prepared for taxonomic description with silver nitrate according to Chatton and Lwoff (1930) and with protargol according to Wilbert (1975).

Type slides are at the Zoologisches Institut der Universität, Poppelsdorfer Schloss, 5300 Bonn, BRD.

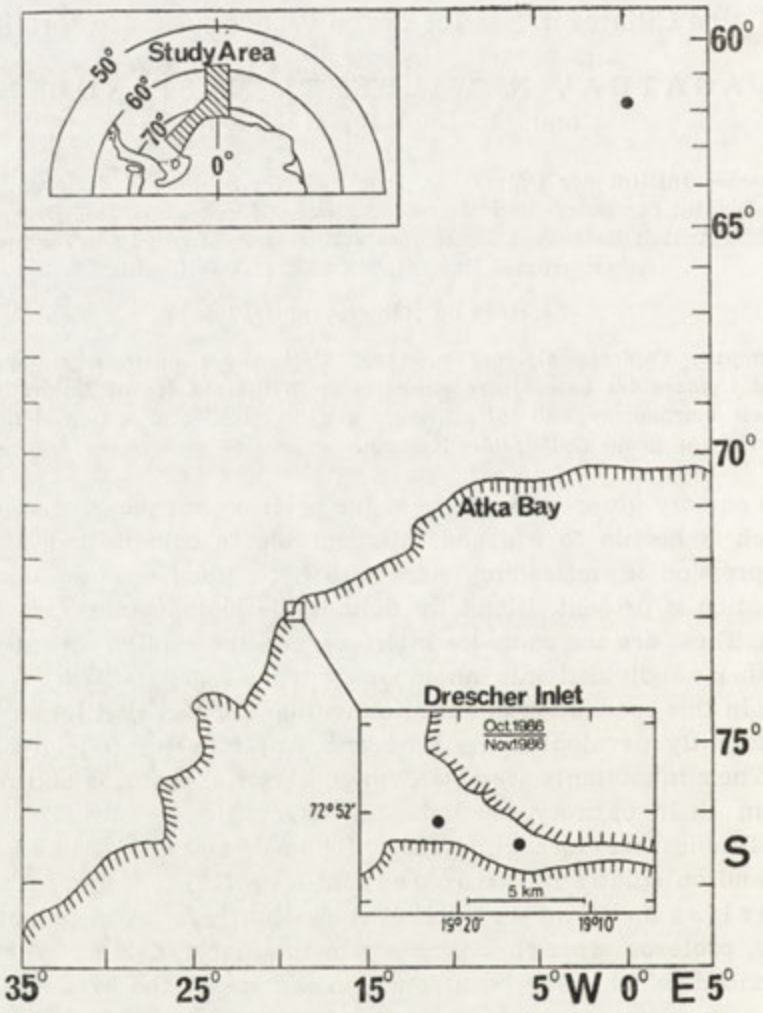

Fig. 1. Location of the sample sites

Abbreviations

AMZ — adoral zone of membranelles
Bk — basal body
Cc — caudal cirrus
CVP — contractile vacuole pore
Dc — dorsal cilium
Dk — dorsal kinety

FV — frontoventral cirrus
Ma — macronucleus
Max — maximum
Mi — micronucleus
Min — minimum
Mr — marginal cirrus
n — sample size
pM — paroral membranelles
S — standard deviation
Sx — standard error
Tr — transversal cirrus
Vr — variation coefficient
x — average

Results and Discussion

Cytharoides balechi, *Cytharoides australis* n. spec. and *Euplotes algivora* n. spec. are associated with *Pleuronema* spec., *Didinium* spec., *Lacrymaria* spec., *Holosticha diademata* and *Parauronema virginianum*.

The ciliates marked spec. could be determined down to their genus only, since their populations were decidedly small.

Parauronema virginianum and *Holosticha diademata* were present in large number. However, their infraciliature is sufficiently known, so that their description will be dispensed with.

Cytharoides balechi Tuffrau, 1974 (Fig. 2, Table 1)

Morphology: scutiform, small peristome lip, keeled elevation right of every dorsal kinety (Dk).

Length — 138-214 μm, width — 107-145 μm.

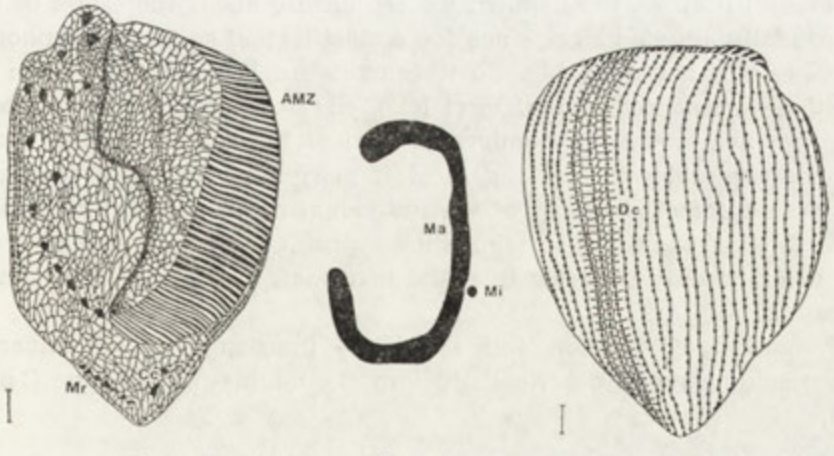

Fig. 2. ***Cytharoides balechi*** **Tuffrau, 1974: a — ventral view, b — nuclear apparatus, c — dorsal view. After protargol and silver nitrate impregnation. Silver-line system in figured exemplary for three kineties. Scale is 10 μm**

Table 1

Biometrical characteristics of *Cytharoides balechi* Tuffrau

Character	Min	Max	x	S	Sx	V	n
Length in μm	139	214	183	23.8	7.54	13	10
Width in μm	107	145	131	12.17	3.85	9.29	10
Length of AMZ in μm	121	149	133	9.00	3.00	6.8	9
Number of AMZ	80	101	90.14	8.32	3.14	9.22	7
Number of FV	10	10	10	0	0	0	5
Number of Tr	5	5	5	0	0	0	5
Number of Cc	2	2	2	0	0	0	5
Number of Mr	12	13	12.5	0.5	0.35	4	2
Number of Dk	27	28	27.5	0.5	0.35	1.8	2
Number of Bk of centre Dk	34	34	34	0	0	0	1

Somatic infraciliature: 27-28 dorsal kineties (Dk) with 34 cilia, 10 frontoventral cirri (FV), 5 transversal cirri (Tr), 2 caudal cirri (Cc), 12-13 marginal cirri (Mr) on the right side of the body.

Buccal infraciliature: very wide AMZ consisting of 80-101 membranelles, the peristome extends over about 4/5 of the length of the body.

Nuclear apparatus: macronucleus (Ma) is C — shaped, micronucleus (Mi) is located on the left side somewhat below the centre of the macronucleus.

Discussion. Tuffrau (1974) described a *Cytharoides balechi* corresponding exactly to the type found by us except for the absence of the 2 caudal cirri (Cc). At the moment we are unsure about the origin of these two caudally situated cirri, since for a clarification suitable morphogenetic stages are not available. So it remains unclear whether these cirri should be considered caudal cirri (Cc), cirri of a left marginal row of cirri, or whether they are emigrated cirri of the right marginal row.

Since euplotides are lacking the left marginal row and a comparison of the cinetosomal structure of the individual right marginal cirri to that of the two cirri under discussion shows quite considerable differences — they are markedly stronger than the marginals — they will be considered caudal cirri.

By means of Chatton and Lwoff — impregnation the silver-line system could now be described. It is of the double-patella type C u r d s (1975).

Cytharoides australis n. spec. (Fig. 3, Table 2)

Morphology: widely oval, frontoventral scutum is carinate, keeled elevation right of every dorsal kinety (Dk).

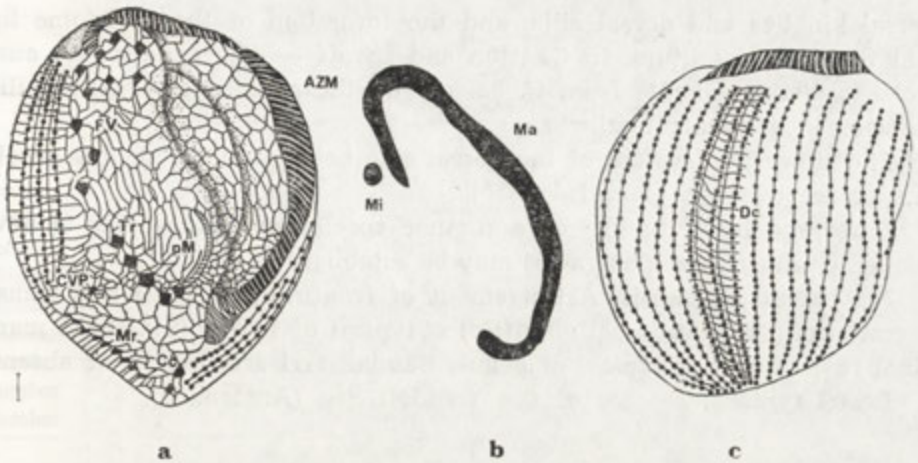

Fig. 3. *Cytharoides australis* n. spec.: a — ventral view, b — nuclear apparatus, c — dorsal view. After protargol and silver nitrate impregnation. Silver-line system is figured exemplary for three kineties. Scale is 10 μm

Table 2

Biometrical characteristics of *Cytharoides australis* n. spec.

Character	Min	Max	x	S	Sx	V	n
Length in μm	110	189	140.39	23.4	6.75	16.67	12
Width in μm	88	126	106.37	9.81	2.83	9.22	12
Length of AMZ in μm	96	109	101.76	3.8	1.1	3.74	12
Number of AMZ	73	97	82.92	7.62	2.2	9.19	12
Number of FV	10	10	10	0	0	0	11
Number of Tr	5	5	5	0	0	0	12
Number of Cc	2	2	2	0	0	0	11
Number of Mr	8	11	10	0.7	0.2	7.1	13
Number of Dk	18	25	22.08	2.18	0.6	9.87	13
Number of Bk of centre Dk	21	29	25.33	2.35	0.68	9.27	12

Length — 109-189 μm, width — 88-126 μm.

Somatic infraciliature: 18-25 dorsal kineties (Dk) with 21-29 dorsal cilia (Dc), 10 frontoventral cirri (FV), 5 transversal cirri (Tr), 2 caudal cirri (Cc), 8-11 marginal cirri (Mr) on the right side of the body.

Buccal infraciliature: the AMZ consisting of 73-97 membranelles extends over about 4/5 of the length of the body.

Nuclear apparatus: macronucleus (Ma) is C — shaped, micronucleus (Mi) lies closeto it.

Discussion. On account of its cirrial equipment *C. australis* is a typical representative of its genus. It is distinguished from *C. balechi* by the size of the body, the number of membranelles of the AMZ, number of

dorsal kineties and dorsal cilia, and the formation of the peristome lip and the frontal scutum. In Chatton and Lwoff — impregnation *C. australis* is distinguishable from *C. balechi* by the absence of argentophilic particles in the ventral pellicle.

The silver-line system of the dorsal side belongs to the double-patella type according to Curds (1975).

Since the infraciliature of a further species of *Cytharoides* is now available, a new genus diagnosis may be established.

New genus diagnosis. Arrangement of frontoventral (FV) and transversal cirri (Tr) and dorsal cilia (Dc) is typical of the family. Right marginal row of cirri is typical of genus. Caudal cirri are present or absent.

Locus typicus: sea ice of the Weddell Sea (Antarctica).

Euplotes algivora n. spec. (Fig. 4, Table 3)

Marphology: oval, conspicuous projection on the left front, two dorsal ribs.

Length — 40-59 μm, width — 24-40 μm.

Somatic infraciliature: 6 dorsal kineties (Dk) with 7-12 cilia. 10 frontoventral cirri (FV), 5 transversal cirri (Tr), 3 caudal cirri (Cc).

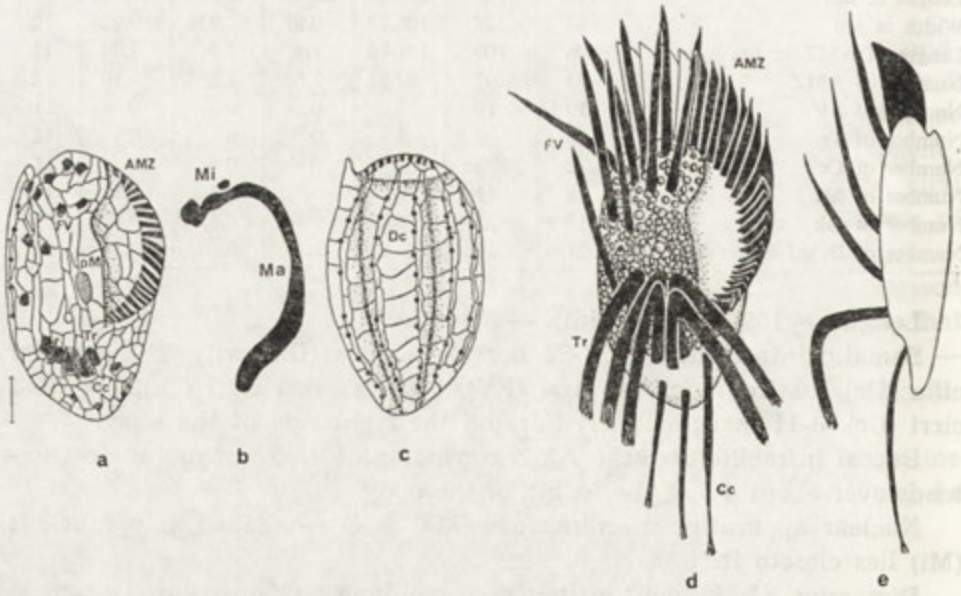

Fig. 4. *Euplotes algivora* n. spec.: a — ventral view, b — nuclear apparatus, c — dorsal view. After protargol and silver nitrate impregnation; d — after observation in vivo, e — longitudinal view. Scale is 10 μm

Table 3

Biometrical characteristics of *Euplotes algivora* n. spec.

Character	Min	Max	x	S	Sx	V	n
Length in μm	40	59	52.25	5.53	1.34	10.58	17
Width in μm	24	40	33.31	4.04	1	12.13	16
Length of AMZ in μm	32	40	35	2.51	0.72	7.16	12
Number of AMZ	28	37	32.41	3.14	0.67	9.69	22
Number of FV	10	10	10	0	0	0	17
Number of Tr	5	5	5	0	0	0	23
Number of Cc	3	3	3	0	0	0	6
Number of DK	6	6	6	0	0	0	16
Number of Bk of centre Dk	7	12	9.25	1.61	0.4	17.43	16

Buccal infraciliature: the AMZ consists of 28-37 membranelles, the peristome extends over about 4/5 of the length of the body.

Nuclear apparatus: macronucleus (Ma) is C — shaped, micronucleus (Mi) is situated anterior of the macronucleus.

In vivo the plasma is clear and uncoloured. The food consists of algae which lie in food vacuoles situated exclusively in the region of the frontoventral scutum. The end of the body is flat, set off from the frontoventral scutum in a stepwise fashion. The frontoventral cirri (FV) measuring about 30 μm are comparatively long. Transversal cirri (Tr) are decidedly strong and longer than the caudal cirri (Cc), their ends are reduced to fine fibres. The morphological division of the AMZ into two sections is conspicuous. The frontal membranelles, up to about the dorsoventral turning point of the AMZ, are strong and appear to be isolated from each other. In the following section, however, the membranelles are markedly shorter and joined to a band of membranelles.

Discussion. *E. algivora* shows the cirrial equipment which is typical of the genus, consisting of frontoventral (FV), transversal (Tr) and caudal cirri (Cc). The silver-line system of the dorsal side belongs to the double-patella type according to Curds (1975).

Locus typicus: sea ice of the Weddell Sea (Antarctica).

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Tintinnids near Pack-Ice between South Shetland and the South Orkney Islands (26 Dec. 1988 — 18 Jan. 1989)

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Synopsis. In total 19 species of suborder *Tintinnina* were identified in the Admiralty Bay, Bransfield Strait and in the region between Elephant Island and the South Orkney Islands. *Cymatocylis convallaria* was not only the most frequent but also the most abundant species at the particular station and at the whole studied area. *Tintinnina* cells occurred mainly in the upper 50 m of the water column. In the sea ice samples only empty loricas were recorded. The *Tintinnina* community showed no correlation with the location of the edge of the ice.

The Antarctic has been known as very rich region in biological production, where protozooplankton plays an important role (Buck and Garrison 1983, Garrison and Buck 1989). According to many authors the protozooplankton biomass of this region is dominated by flagellates and ciliates (Hentschel 1932, Hasle 1969, Fay 1973, Sieburth et al. 1978, Brockel 1981, Buck and Garrison 1983, Garrison and Buck 1989).

Antarctic ciliates can attain high cell densities what suggests that they may be an important food reserve for animals consuming microplankton (Hewes et al. 1985). Some ciliates are also known to be the epibionts of krill, commonly inhabiting this region (Rakusa-Suszczewski and Nemoto 1989).

Tintinnids are one of the most numerous group among marine ciliates (Littlepage 1968, Loeblich and Tappan 1968, Cosper 1972, Smetacek 1981, Brandini and Kutner 1987, Boltovsky et al. 1989, Garrison and Buck 1989). They are free living

specimens with protoplast protected by the lorica (Kofoid and Campbell 1929, 1939, Marshal 1969, Gold and Morales 1976 a, b, Sassi and Melo 1986). These cells are numerically important second trophic level feeders (Zeitzschel 1967) consuming small diatoms and other constituents of the ultra- and nano-plankton. Sherr et al. (1986) report that tintinnids can consume approximately 60% of the overal primary production of oceanic ekosystem, thanks to their metabolic and reproduction rates (Taniguhi and Kawakami 1985, Verity 1985, 1986). It should be taken under consideration that the significant primary production is associated not only with an open water but also with the sea ice (pack-ice and land-fast ice) (Ackley et al. 1979, Garrison and Buck 1986, Garrison et al. 1983, Kottmeier and Sullivan 1987).

Tintinnids are believed to be the cosmopolitan group since they are present at many regions of the world, for example in White Sea (Burkovsky et al. 1974, Burkovsky 1976), in Mediterranean Sea (Jorgensen 1924, Balech 1959), Western Pacific (Hada 1938), Eastern Pacific and the South Atlantic (Balech 1948, 1962). The Antarctic region is very rich in tintinnids too. Littlepage (1968) reported this group of cells in waters of Mc Murdo Sound, Alder (1989) in Scotia Sea and Boltovsky et al. (1989) and Alder (1989) in the Weddell Sea. However the suborder *Tintinnina* is very common around the world, the distinct differences in the species occurance in relation to the particular zone are observed. Even in the Antarctic region some species are common (*Cymatocylis convallaria, Laackmanniella naviculaefera*, and *Codonellopsis gaussi*) while several of them are restricted to the definite area (Alder 1989, Boltovsky et al. 1989). Alder (1989) reported differences in the specific species composition between Scotia and Weddell Sea, whereas Boltovsky et al. (1989) observed species variability even within the Weddell Sea.

The purpose of our study was to report an abundance and distribution of tintinnids in the edge zone of sea ice between Elephant Island and the South Orkney Island as well as in the Admiralty Bay of the King George Island and Bransfield Strait.

Material and Methods

The *Tintinnina* cells were sampled during the cruise of the "Profesor Siedlecki" from December 26, 1988 to January 18, 1989 in three regions: the central part and outlet of the Admiralty Bay of the King George Island, the Bransfield Strait and between Elephant Island and the South Orkney Islands. The stations between Elephant and the South Orkney Islands were located near the fast ice and

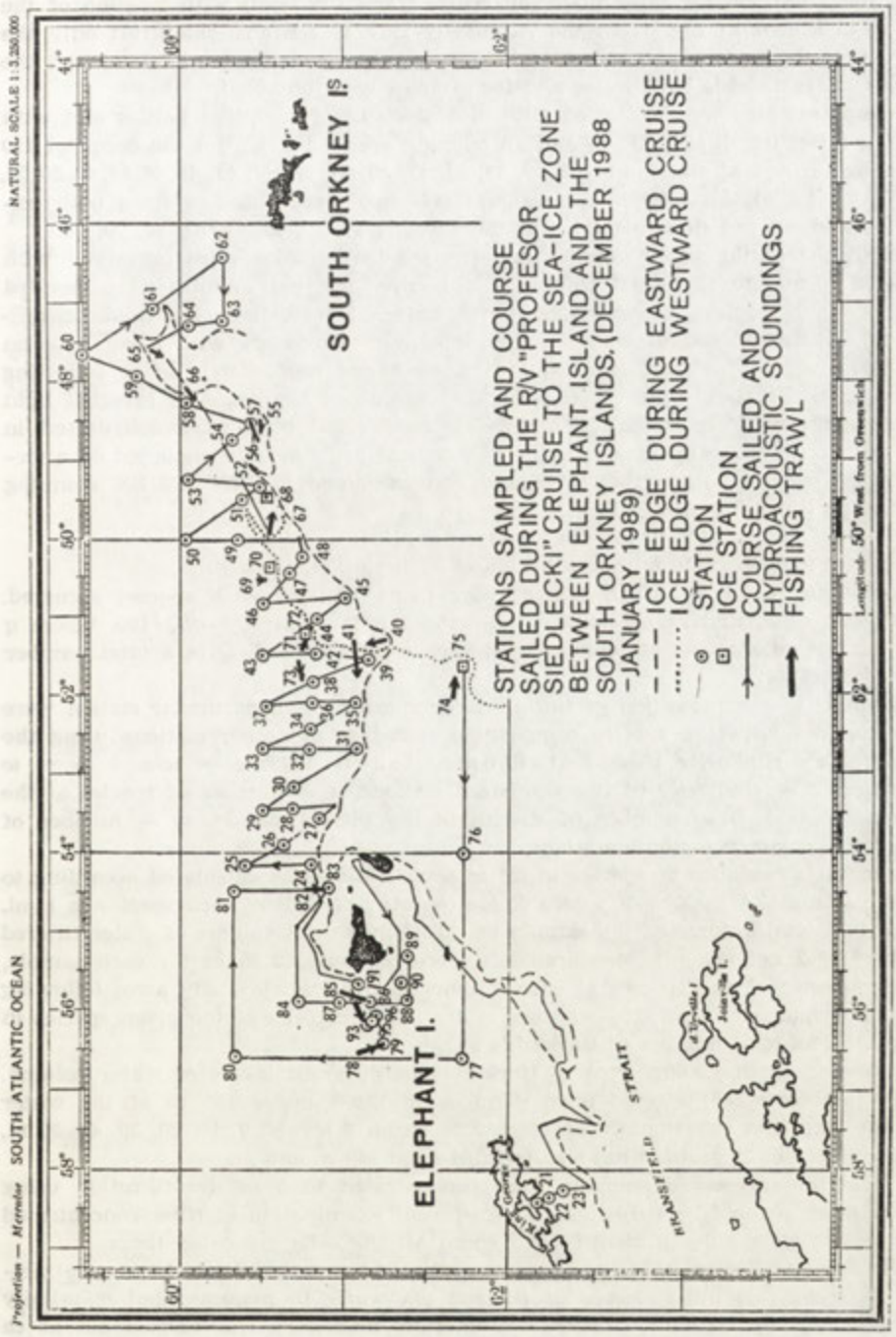

Fig. 1. Cruise track and stations

15-30 nM from the ice edge zone. The cruise track (transect) with location of the station is shown in Fig. 1. In the Admiralty Bay and Bransfield Strait only the water samples were collected, while in the area between Elephant Island and the South Orkney Islands both water and ice samples were obtained.

Water samples were collected with "Nansen" water sampling bottles and with the WP 2 net (mesh size 35 μm and an opening area 0.2 m^2) from the depth of 150 m to the surface, at stations 21-27, 29, 30, 32, 33, 35, 37, 38, 42, 43, 45, 46, 48, 49, 50, 52, 54, 57, 58, 59, 61, 62, 63. Sea ice samples (50 cm^3) were obtained from both uncolored and colored (brownish) ice at the stations 27, 30, 48, 52, 57, 68, 70, 75.

Both the bottle water samples and the sea-ice samples were preserved with buffered formaline (4% final concentration) only. The net samples were devided into two subsamples: on was fixed with buffered formaline (4% final concentration), while the second was fixed for electron microscopy with a combination of OsO_4 and $HgCl_2$. Precooled fixatives were added immediately after collecting the samples. Formaline preserved cells were examined by means of inverted light microscope (Carl-Zeiss, Jena). Osmium-sublimate fixed cells were dehydrated in ethanol and amyl acetate, dried by the CO_2 critical point method, mounted on a metal "stub", coated with carbon and gold and examined in JEM 120 EX scanning microscope operating at 40 kV.

Net samples were used for the following studies:

— species identification and distribution at each station,

— constancy count which means at how many stations each species occurred. It was calculated in percentage following the formula: $C = q/Q \cdot 100$, where q is a number of stations at which the species were recorded, Q is a total number of stations (27),

— similarity — composition of tintinnid communities at a particular station were compared with the species composition found at the other stations using the Jaccard's similarity coefficient (Romaniszyn 1972) $s = w/a + b - w$ where s — similarity of two compared stations, a — number of species at the first station, b — number of species at the second station, w — number of species common for both stations,

— general abundance of species in m^3 at each station was calculated according to the formula $A = X \cdot Y/V$ where X is a number of cells of each species in 1 ml, Y is a total volume of the sample in ml, V in a total volume of water filtered by WP 2 net (30 m^3). Measurements were repeated 20 times for each sample,

— dominance of the species at each station and the whole study area, following the formula $D = s/S \cdot 100$ where s is a number of cells of the given species in m^3, S is a total number of tintinnids in m^3.

To estimate the abundance of tintinnids in different layers of water column, bottle samples were selected from depth within the upper 150 m of the water column. The first subsample contained water from 6 levels: 0, 10, 20, 30, 40, 50 m, the second from 75 and 100 m and the third from 150 m only.

Formaline preserved samples were concentrated to 5 ml by filtration using 30 μm mesh nylon filter. By light microscopical examination of filter-concentrated samples we were able to identify and count all the cells presented there.

Some of the ice samples were simply allowed to melt, while others were melted in larger (500 ml) volumes of filtered sea water to maintain buffer salinity and prevent any osmotic changes. The melting procedure was carried out at ca 3°C. The qualitative and quantitative studies were carried out on filterconcentrated samples (see bottle samples).

Light microscopic photographs were taken using Biolar microseope (PZO),

Results and Discussion

Tintinnids from the Net Samples

The whole studied area: Admiralty Bay, Bransfield Strait and region between Elephant Island and the South Orkney Islands, appeared to be rich in ciliated protozoan planktonic community of the suborder *Tintinnina*. Using the combination of light and electron microscopy 19 species were there recorded. They belong to 5 families: *Codonellopsidae, Codonellidae, Cyttarocylididae, Ptychocylididae* and *Undellidae*. Pictures of some species are presented on plates I-IV. Table 1 shows the systematic of tintinnids based on Corliss (1979).

Table 1

Systematics of tintinnids by Corliss (1979).

Suborder	*Tintinnina*	Kofoid and Campbell, 1929
A. Family	*Codonellopsidae*	Kofoid and Campbell, 1929
	Codonellopsis balechi	(Hada, 1970)
	Codonellopsis gaussi 1	(Laackmann, 1907)
	Codonellopsis gaussi 2	
	Codonellopsis glacialis	(Laackmann, 1907)
	Laackmanniella prolongata	(Laackmann, 1907)
	Laackmanniella naviculaefera	(Laackmann, 1907)
B. Family	*Cyttarocylididae*	Kofoid and Campbell, 1929
	Cyttarocylis conica	Brandt, 1906
C. Family	*Codonellidae*	Kent, 1881
	Tintinnopsis lata	Meunier, 1910
	Tintinnopsis major	Meunier, 1910
D. Family	*Ptychocylididae*	Kofoid and Campbell, 1929
	Cymatocylis convallaria	Laackmann, 1909
	Cymatocylis ovata	Laackmann, 1909
	Cymatocylis folliculus	(Laackmann, 1909)
	Cymatocylis ecaudata	(Laackmann, 1909)
	Cymatocylis cylindrus	Laackmann, 1909
	Cymatocylis typica	Laackmann, 1909
	Cymatocylis drygalski	(Laackmann, 1909)
	Cymatocylis vanhoffeni	(Laackmann, 1907)
E. Family	*Undellidae*	Kofoid and Campbell, 1929
	Undella parva	(Brandt, 1906)
	Undella declivis	(Brandt, 1906)

In our studies the Admiralty Bay and Bransfield Strait were taken into consideration as a common region. Nine tintinnidspecies were found at all stations (21, 22, 23) of this region thus having 100% constancy. These were: *Codonellopsis balechi, Laackmanniella prolongata, Cymatocylis convallaria, C. ovata, C. ecaudata, C. cylindrus, C. typica, C. drygalski*

and *Tintinnopsis lata*. *Cymatocylis folliculus* was recorded nowhere else but in this particular region (at the station 21 and 23).

Table 2 shows values of constancy which represent the frequency of the species occurrence in the second experimental area between Elephant Island and the South Orkney Islands. *Cymatocylis convallaria* was the most commonly found species (present at 27 stations). The next one was *Codonellopsis balechi* which was found at 24 stations and *Laackmanniella naviculaefera* — at 22 stations. The second group of included species had the values of constancy within the range of 50-70%. They were: *Tintinnopsis lata*, *Cymatocylis typica*, *Laackmanniella prolongata* and *Codonellopsis gaussi*. The remaining species occurred with lower frequency. The minimal value of constancy was demonstrated for *Cyttarocylis conica* which was present at one station (38) only.

Table 2

The values of constancy for *Tintinnina* cells in the region between Elephant Island and the South Orkney Islands

Taxon	Constancy	
	q	*c*
C. convallaria	27	100
C. balechi	24	88,9
L. naviculaefera	22	81.5
T. lata	18	66.7
C. typica	17	63.0
L. prolongata	16	59.3
C. gaussi 2	14	51.9
C. cylindrus	13	48.1
T. major	11	40.7
C. ecaudata	11	40.7
C. gaussi 1	11	40.7
C. glacialis	10	37.0
C. ovata	10	37.0
C. vanhoffeni	10	37.0
C. drygalski	5	18.5
C. conica	1	3.7

$C = q/c$; q — number of stations on which a species was recognized, c — total number of stations.

Based on Jaccard's similarity coefficient, species frequency at a particular station was compared with the species composition of other stations. As it is shown in Fig. 2 there is no distinct species similarities between stations. The majority of the stations exhibited similarity from 0.251 to 0.5.

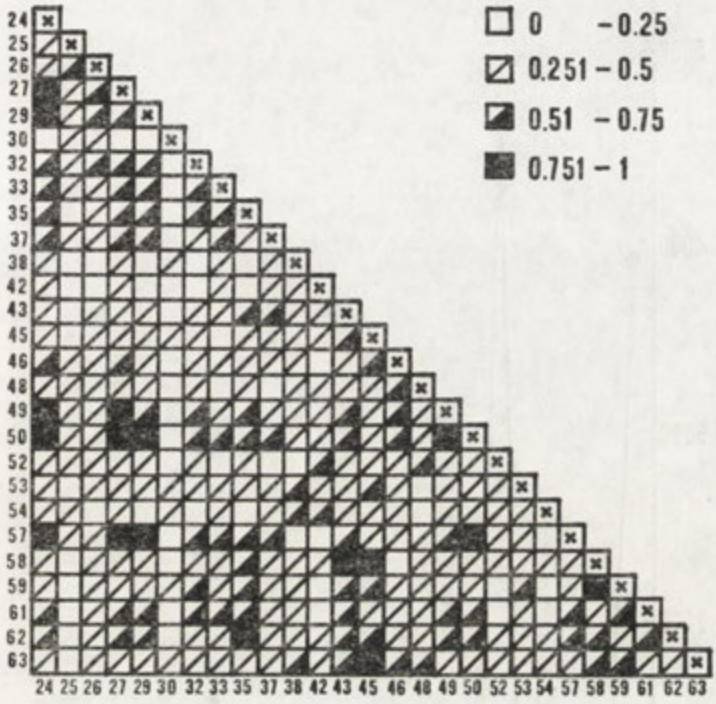

Fig. 2. Jackard's similarity coefficients of the species composition for stations between Elephant Island and the South Orkney Islands

Total number of tintinnids collected at each station is shown in Fig. 3. Both intact cells (lorica with protoplast) and empty loricas were taken into consideration. In the central part of the Admiralty Bay the density of cells reached almost 4000 species per m^3, decreasing rapidly towards the outlet of the Bay. The lowest density was observed in the Bransfield Strait (approximately 500 sp. per m^3). In spite of such differences in the cell density the species composition in the Bay and Bransfield Strait is rich and exhibits high similarity. Between Elephant Island and the South Orkney Islands the maximal values of cell density were ascertained at first 5 stations in the region close to the Elephant Island (Fig. 1). According to oceanographic studies (Tokarczyk et al. person. comm.) in the surface layer of this area, water originated in the Drake Passage was found. This water was homogenous and characterized by low stability. The second peak of density of tintinnids was observed at the station 49 and 50, where a very thin layer (about 30 m in depth) of relatively warm water with low salinity was found, which was probably due to the influence of melting ice (Tokarczyk et al. person. comm.). At the rest

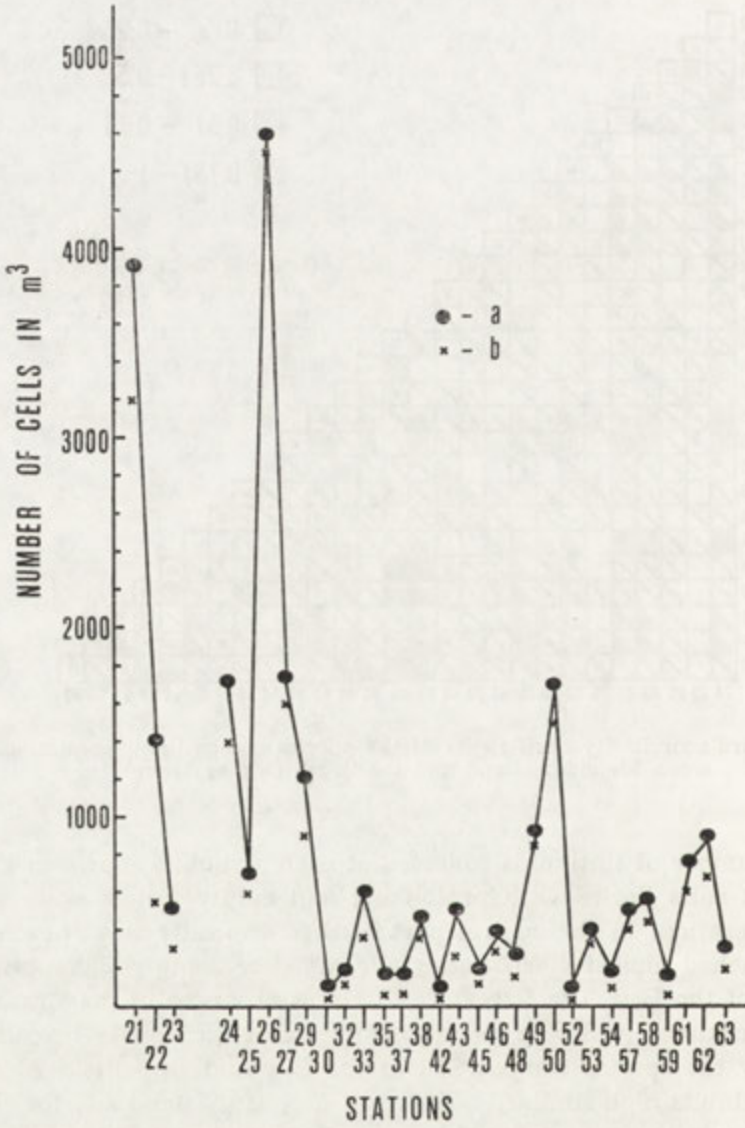

Fig. 3. Total number of tintinnids at each station: a — intact cells and empty loricas, b — intact cells

of the stations the density of tintinnids remained at lower level and did not exceed 1000 cell per m^3. These results suggest that there is no clear relationship between the number of *Tintinnina* cells and the distance from the ice edge.

The increased abundance of tintinnids which was observed in the

western part of the experimental area *Codonellopsis balechi* was probably caused not only by the water from the Drake Passage, but could be also influenced by the vicinity of the Elephant Island. The increased of the number of *Tintinnina* cells in the White Sea near the land was observed by Burkowsky (1976). It is difficult to determine environmental conditions causing the increase of the density of tintinnids in the central part of the studied area (station 49 and 50) since Burkowsky (1976) suggested that the accessibility of food, temperature, salinity, interspecies concurrence and presence of predators are the important factors controlling the abundance of the population.

On the basis of the species abundance we have calculated the species dominance index for each station (Table 3). In the Admiralty Bay (stations 21,22) *Cymatocylis convallaria* was the dominant species. Although this species was a very abundant also in the Bransfield Strait (station 23), *Codonellopsis balechi* had the highest value of the dominance index.

The species dominance for each station between Elephant Island and the South Orkney Islands are presented in Fig. 4. *Cymatocylis convallaria* is a dominant species at 25 stations, while *Codonellopsis gaussi* dominates at two stations in the western part of the studied area (25 and 26).

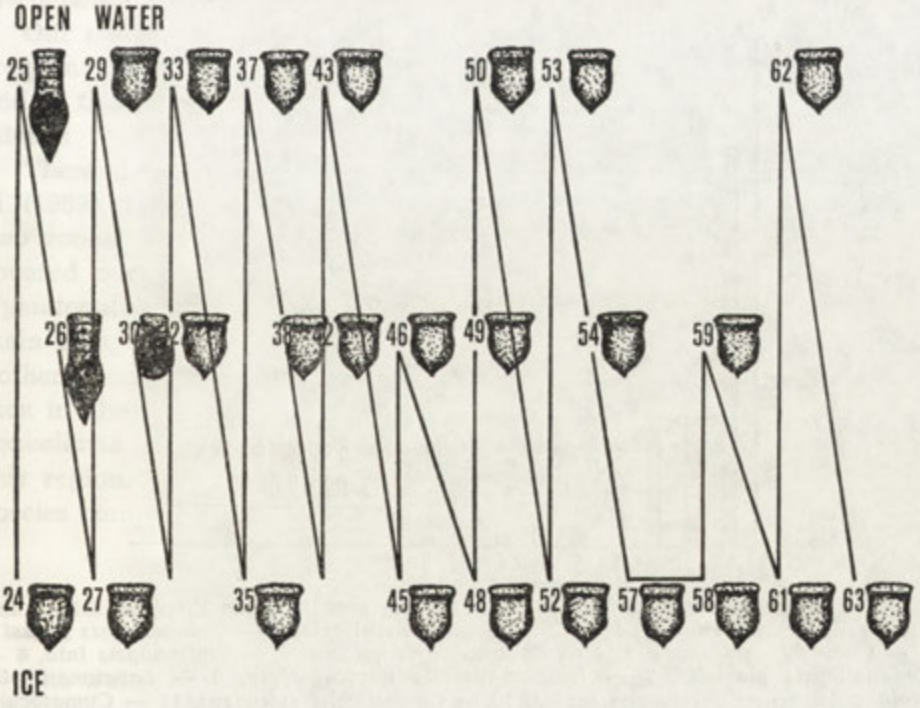

Fig. 4. The dominant species for each station between Elephant Island and the South Orkney Islands

Table 3

Dominance index of *Tintinnina*

Stations / Species	21	22	23	24	25	26	27	29	30	32	33	35	37
C. balechi	14.9	0.3	24.4	6.6	1.0	0.2	13.5	17.9	62.5	14.3	7.1	8.0	8.8
C. gaussi 1	0.1	0	1.2	2.1	36.1	51.1	4.4	2.0	0	7.1	3.4	0	8.8
C. gaussi 2	0	0	1.2	0.9	38.3	40.1	3.8	2.0	0	14.4	0	0	0
C. glacialis	0.1	0	0	0.3	14.3	5.1	0.6	0.3	0	0	0	8.0	0
L. prolongata	0.2	0.3	1.2	0.3	0	0	0.3	0	0	0	0	4.0	0
L. naviculaefera	0	0	3.5	0.9	0	0.7	0.6	0.3	0	7.1	0.1	4.0	0
C. convallaria	67.7	89.5	40.6	83.0	10.3	12.6	66.3	63.4	37.5	42.9	77.6	48.8	28.6
C. ovata	1.5	1.2	1,2	0.9	0	0	1.8	2.8	0	0	3.5	8.0	8.8
C. folliculus	0.1	0	2.3	0	0	0	0	0	0	0	0	0	0
C. ecaudata	0.9	1.2	1.2	0.3	0	0	1.1	0.4	0	3.5	1.7	4.0	0
C. cylindrus	2.4	2.2	5.7	0.9	0	0	1.8	2.8	0	3.5	1.7	4.0	0.8
C. typica	4.8	3.5	8.1	0.9	0	0.2	1.8	3.2	0	7.1	0	8.0	8.8
C. drygalski	0.6	1.2	2.3	0.3	0	0	0	1.6	0	0	0	0	0
C. vanhoffeni	0.6	0	0.2	0	0	0	0	0.1	0	0	1.4	3.2	8.8
C. conica	0.1	0	0	0	0	0	0	0	0	0	0	0	0
T. lata	5.9	0.3	6.9	2.1	0	0	3.4	3.2	0	0	0	0	17.8
T. major	0.1	0.3	0	0.5	0	0	0.6	0	0	0	3.5	0	8.8

The species dominance index calculated for the whole experimental area is shown in Fig. 5. *Cymatocylis convallaria* outnumbered the rest of the species. Values of the dominance index for this species was higher

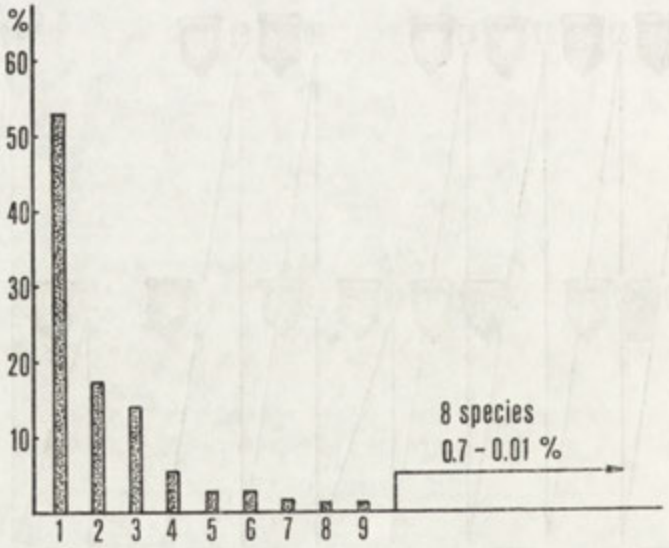

Fig. 5. The species dominance index (%) for the area between Elephant Island and the South Orkney Islands: 1 — *Cymatocylis convallaria*, 2 — *Codonellopsis gaussi* 1, 3 — *Codonellopsis guassi* 2, 4 — *Codonellopsis balechi*, 5 — *Tintinnopsis lata*, 6 — *Codonellopsis glacialis*, 7 — *Laackmanniella naviculaefera*, 8 — *Laackmanniella prolongata*, 9 — *Cymatocylis typica*, 10 — *Cymatocylis cylindrus*, 11 — *Cymatocylis ovata*, 12 — *Tintinnopsis major*, 13 — *Cymatocylis ecaudata*, 14 — *Cymatocylis vanhoffeni*, 15 — *Cymatocylis drygalski*, 16 — *Cymatocylis conica*

cells on each station (%)

38	42	43	45	46	48	49	50	52	53	54	57	58	59	61	62	63
9.5	0	10.2	4.0	2.8	0	7.7	2.7	0	5.0	7.2	1.9	7.8	7.5	1.6	2.5	16.7
0	0	0	0	0	0	2.1	0.4	0	0	0	0.2	0	0	0	0	0
0	0	0	0	2.8	3.6	0.7	0.1	16.6	0	0	0.2	0	0	1.6	0	0
0	0	0	0	2.8	3.6	0.7	0.1	0	0	0	0.2	0	0	0	0	0
4.8	0	3.7	2.0	2.8	3.6	1.5	2.4	0	0	0	1.8	2.6	3.7	1.6	1.3	3.3
0	1.6	3.7	2.0	5.5	3.6	2.1	2.7	0.1	2.5	0	1.8	6.6	3.7	1.6	1.2	3.3
76.1	65.5	77.8	76.1	77.7	82.0	78.3	88.5	66.6	89.0	71.4	78.7	74.8	70.1	85.6	86.1	60.0
0	0	0	0	2.8	0	0	0.4	0	0	0	1.9	0	0	0	1.3	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0.3	0.2	0	0	0	1.9	0	0	0.8	1.3	0
0	0	0.3	0	0	0	0.3	0.2	0	0	0	3.8	0	0	0.8	2.5	0
0	0	0.3	4.0	0	0	0.7	0.4	0	1.0	0	1.9	1.3	7.5	1.6	1.3	0
0	0	0	0	0	0	0	0	0	0	0	1.9	0	0	1.6	1.2	0
0	0	0.3	0	0	0	0	0.4	0	0	0	1.9	0.3	7.5	1.6	0	0
0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.7	16.4	3.7	11.9	2.8	3.6	4.9	1.1	16.7	0	14.2	1.8	6.6	0	0	1.3	16.7
4.8	16.4	0	0	0	0	0.7	0.4	0	2.5	7.2	0	0	0	1.6	0	0

than 50%. It was followed by *Codonellopsis gaussi* and *Codonellopsis balechi*. The abundance of the other species remained on the level below 2%.

Our results indicate that *Cymatocylis convallaria* is not only the most frequent but also the most abundant species at any particular station and in the whole region between Elephant Island and the South Orkney Islands.

These data are similar to the results obtained by Boltovsky et al. (1989). Their experimental area on the Weddell Sea was devided into two zones: a sothern sector and a nothern sector. The last one partly covered our study area. In total they recorded 14 species of tintinnids. *Cymatocylis convallaria*, *Codonellopsis gaussi* and *Co. glacialis* were the main components of the protozooplankton in the ice-free waters in the nothern zone. Alder (1989) who also studied the occurence of tintinnids in the nothen part of the Weddell Sea reported that *Cymatocylis convallaria* and *Codonellopsis gaussi* were the most abundant species in this region. Thus, the species composition recorded by us confirm the species composition for the Weddell water south of the Scotia Front.

Tintinnids from the Bottle Samples

The water samples collected from different levels of the water column were examined. The *Tintinnina* cells with the protoplast inside the loricas were found mainly within an upper 50 m layer. They were not nu-

merous and belonged to 3 species: *Cymatocylis convallaria, Codonellopsis balechi* and *Tininnopsis lata*. More often empty loricas of different species were found. In the samples from 75-100 m and from 150 m empty loricas were present exclusively. These results were similar for the Admiralty Bay, Bransfield Strait and the area between Elephant and the South Orkney Islands. The present results confirmed previous observations done by Burkovsky (1976) for *Tintinnina* cells in the White Sea. He postulated that the richest tintinnids population was found in the upper 15 m of the water column. According to him this layer was rich in food and had the optimal temperature for growth.

Kofoid and Campbell (1939) observed that there was marked change in the Ciliate fauna associated with depth of water. According to them, a wide variety of orders was present. As a depth increased, few in any orders other than *Heterotrichida* could be found, with tintinnids predominating.

Sea Ice Samples

Our studies of the uncoloured and coloured sea ice revealed complete lack of intact *Tintinnina* cells. Moreover, only a few empty loricas belonging to *Laackmanniella naviculaefera, Cymatocylis convallaria, C. cylindrus* were found. These species were common for both water and ice communities. The representatives of the genus *Undella* (*U. parva* and *U. declivis*) which were also found in the ice samples, appeared to be the species present in the ice exclusively. This genus is not specific for an antarctic region, because it was found in Florida current (*Undella declivis*) and in the Western Tropical Pacific Bay of Naples (*Undella parva*) (Kofoid and Campbell 1929). It has been suggested (Bunt 1968, Bunt and Lee 1970, Garrison et al. 1982) that ice crystals forming in the water column concentrate algal cells including heterotrophic protozoans (Horner 1976). Thus, it can not be excluded that empty loricas of tintinnids found in the ice samples were trapped during the ice formation.

The present study was carried out on board of R/V "Profesor Siedlecki" in the course of the Polish Academy of Sciences expedition (26 Dec. 1988 — 18 Jan. 1989) headed by Prof. S. Rakusa-Suszczewski.

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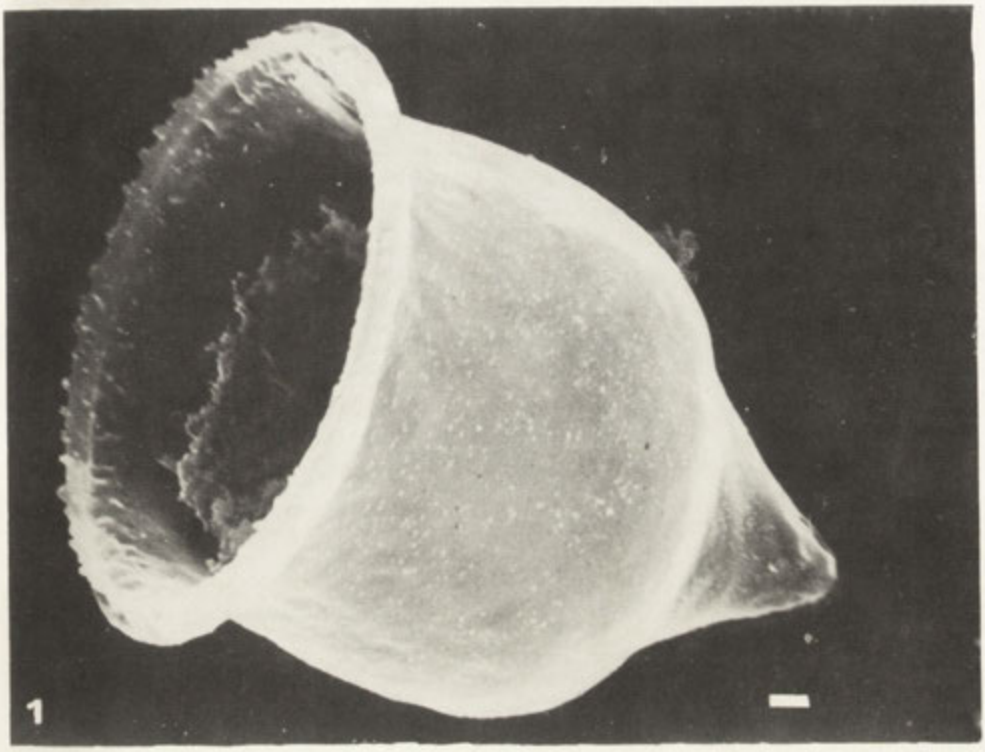
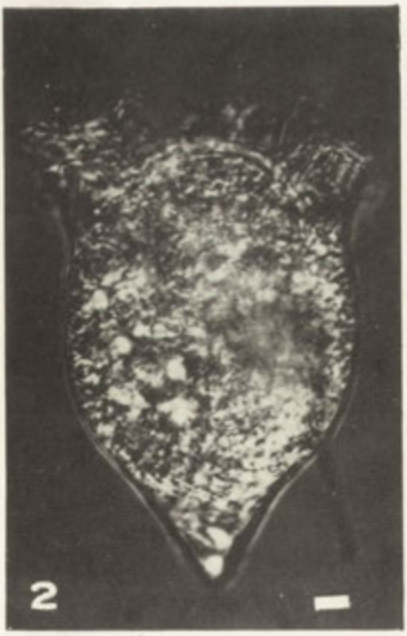
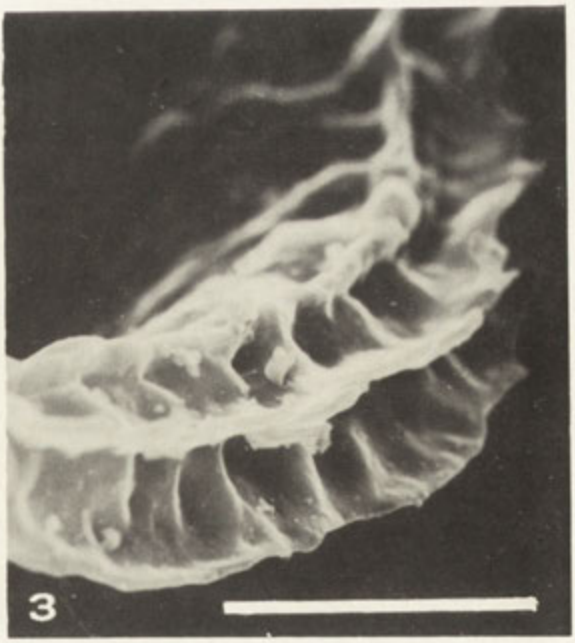
EXPLANATION OF PLATES I-IV

1: Scanning electron micrograph of *Cymatocylis convallaria*. Scale bar 10 μm
2: Light micrograph of *Cymatocylis convallaria* with protoplast inside the lorica. Scale bar 10 μm
3: Part of the anterior region of *Cymatocylis convallaria* lorica. Scale bar 10 μm
4: Scanning electron micrograph of *Cymatocylis vanhoffeni* lorica. Scale bar 100 μm
5: Anterior region of *Cymatocylis vanhoffeni* lorica. Scale bar 100 μm
6: Scanning electron micrograph of *Cymatocylis cylindrus* lorica. Scale bar 100 μm
7: Anterior region of *Cymatocylis cylindrus* lorica. Scale bar 100 μm
8: Light micrograph of *Laackmanniella naviculaefera* with protoplast inside the lorica. Scale bar 10 μm
9: Scanning electron micrograph of *Laackmanniella naviculaefera* lorica. Scale bar 10 μm
10: Anterior region of *Laackmanniella naviculaefera* lorica. Scale bar 10 μm
11: Light micrograph of *Tintinnopsis lata* lorica. Scale bar 10 μm
12: Light micrograph of *Tintinnopsis major* lorica. Scale bar 10 μm
13: Light micrograph of *Undella declivis* lorica. Scale bar 10 μm
14: Light micrograph of *Cyttarocylis conica* lorica. Scale bar 10 μm
15: Scanning electron micrograph of *Codonellopsis balechi*. Cilia in the anterior region are seen. Scale bar 10 μm
16: Scanning electron micrograph of *Codonellopsis gaussi* (form 1) lorica. Scale bar 10 μm







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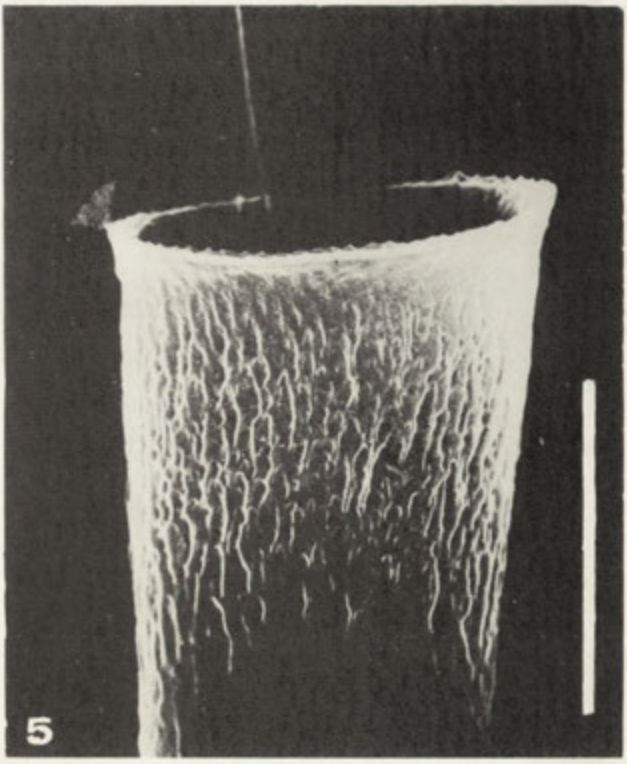

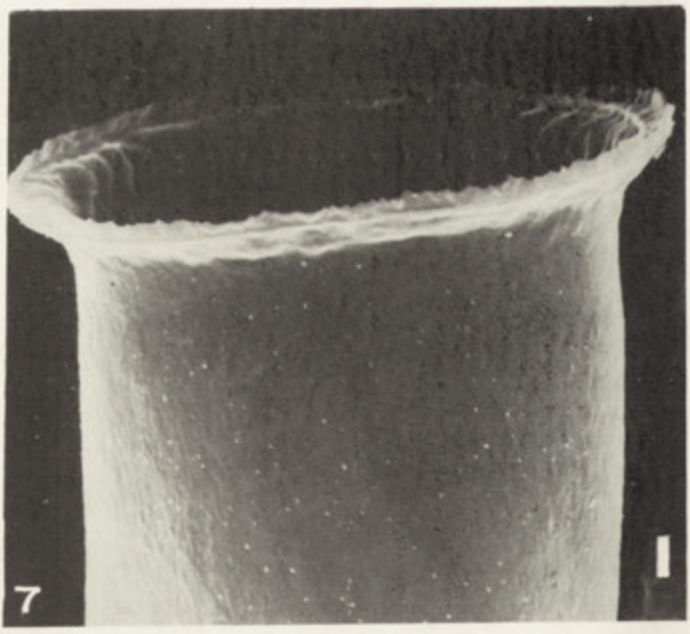

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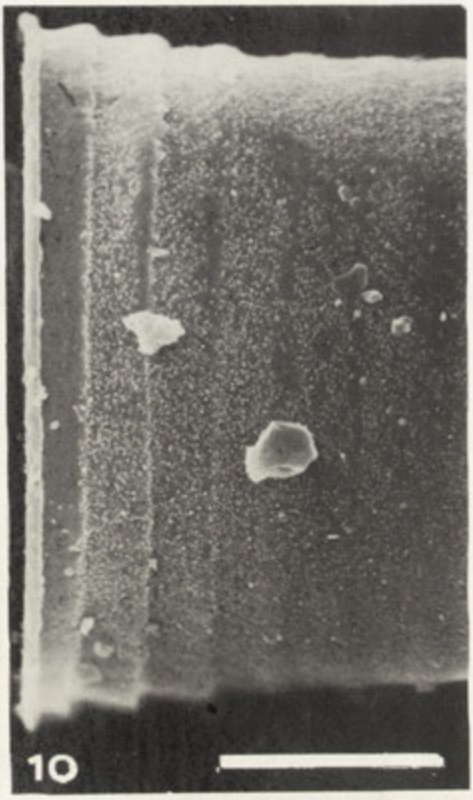

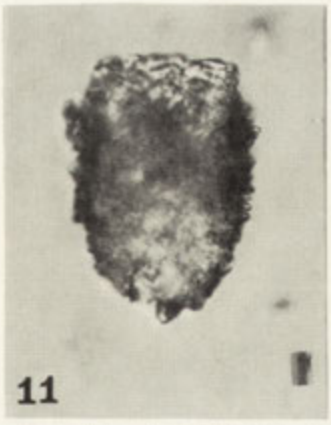

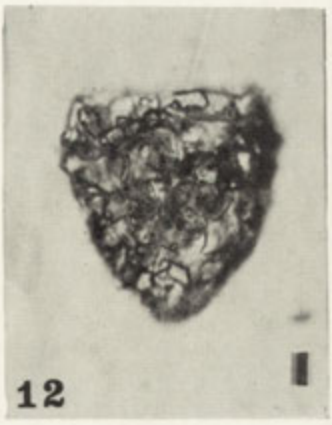

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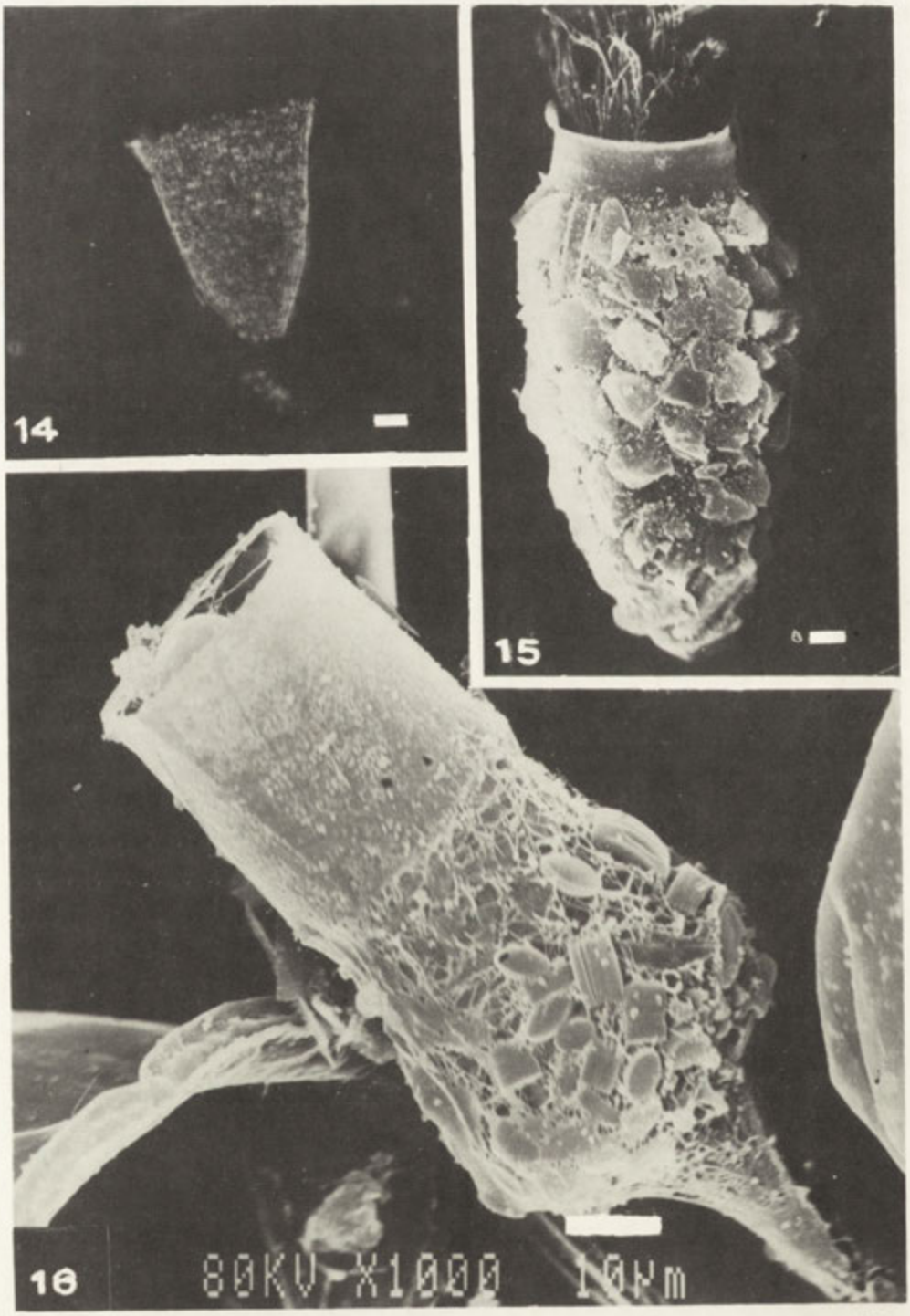

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Gregarina alphitobii sp. n. and *Mattesia alphitobii* sp. n., Parasitizing *Alphitobius diaperinus* Panz. (*Tenebrionidae, Coleoptera*)

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Synopsis. Two protozoan parasites, *Gregarina alphitobii* sp. n. and *Mattesia alphitobii* sp. n., parasitizing in the natural and laboratory populations of *Alphitobius diaperinus* Panz. in Africa (Nigeria), Europe (Poland, German Democratic Republic, West Berlin, Federal Republic of Germany), North America (USA) and Asia (India, Tonga Island) are described. The life cycle of the eugregarine *G. alphitobii* sp. n. parasitizing in the gut and the neogregarine *M. alphitobii* sp. n. infecting the fat body of *A. diaperinus* is described. Some data on the prevalence of parasitization and host-parasite relationship are also presented.

Alphitobius diaperinus (Panz.) has a world-wide distribution and is known as pest of various stored products. Originating from tropical regions (Africa, Asia) it has been introduced on all continents finding especially favourable conditions inside the broiler-houses in Europe and USA. It attacks mainly chicken feed but also makes same damage to some construction materials e.g. isolation foams. This insect has also veterinary importance as it is known as vector of Marek's disease of chicken.

For these reasons studying of natural enemies of *A. diaperinus* has practical significance as it may lead to the development of biological control methods against this important pest.

In this paper we describe an eugregarine *Gregarina alphitobii* sp. n. and a neogregarine *Mattesia dispora* sp. n. causing mortality and behavioural disorders in infected insects.

Material and Methods

Specimens of *A. diaperinus* for microscopic examination were collected on four continents: Europe, Asia, North America and Africa.

Asia. In Punjab (India) adults and larval stages of *A. diaperinus* were collected from various groceries, and they were reared in the laboratory.

Europe. Specimens for studies were collected in France, German Democratic Republic, West Berlin, Federal Republic of Germany, and Poland.

France. Specimens were received in 1981 from laboratory culture kept at the Laboratoire de Recherches sur les Insectes des Denrees I.N.R.A., Pont de La Maye (Bordeaux) 33140.

German Democratic Republic. Specimens for studies were received in 1984, 1986 and 1987 from the laboratory rearing kept at the Institut fur Pflanzenschutzforschung, Kleinmachnow. In 1985 insects for studies were also collected at chicken house at the VEB Broiler in Königs Wusterhausen.

West Berlin and Federal Republic of Germany. Insects for studies were received in 1984, 1985, 1986 from the laboratory culture kept at the Institut für Vorratsschutz BBA, West Berlin. Insects studied in 1984 originated from Tonga Island, while that studied in 1986 originated from insects collected in chicken house in Rechterfeld close to Oldenburg (F.R.G.).

USA. Insects for studies were received in 1985 from the laboratory rearing kept at the USDA Stored Product and Household Insects Laboratory, University of Wisconsin Madison, Wisconsin.

Poland. Insects for studies were collected in 1982 in chicken house close to Środa Wielkopolska.

Nigeria (Africa). Insects for studies were received in 1986 from the laboratory rearing kept at the Department of Biological Sciences, Rivers State University of Science and Technology, Port Harcourt, and from Nigerian Stored Products Research Institute, Ibadan Substation, at Onirelle-Ibadan.

The gut of the larvae and adults of *A. diaperinus* were dissected in Ringer's (0.75% saline) solution. The midgut, hindgut and the fat body heavily infected by parasites were fixed in Schaudin's fixative and stained with Heidenhain's iron hematoxylin or fixed in methanol and stained with Giemsa's stain. The larvae were fixed in Carnoy's fluid for histological studies and then sectioned to 5-6 μm and stained with Heidenhain's iron hematoxylin and eosin.

The following abbreviations are used in this paper: TL — total length, LE — length of epimerite, LP — length of protomerite, LD — length of deutomerite, WE — width of epimerite, WP — width of protomerite, WD — width of deutomerite.

Results

Gregarina alphitobii sp. n.

Habitat: Midgut and hindgut.

Localities: France — Pont de Le Maye (Bordeaux) 13.X.1981; Poland — Środa Wielkopolska 13.III.1982; German Democratic Republic —

Kleinmachnov 18.V.1984, 21.VII.1986, 13.VIII.1987; Königs Wusterhausen 30.V.1985; West Berlin — 26.VI.1984 (insects from Tonga Island 4.VI. 1984), 25.II.1986 (insects from Rechterfeld, F.R.G. coll. Nov. 1985); USA — Madison (Wisconsin) 21.XI.1985; Nigeria — Onirelle-Ibadan 30.IX 1986; Port Harcourt 6.X.1986.

Holotype: The holotype slides with cephalonts, sporonts, and cysts of *G. alphitobii* sp. n. on slide No. AD — 1/a, 2/b, 3/c are kept at the Zoology Department, Punjabi University, Patiala. Paratype slides are kept at the Institute of Plant Protection, Poznań, Poland.

Morphology

Sporonts in associations, elongate (Pl. I:5, 6). Maximum length of observed sporonts 294 μm; maximum witdth 86 μm. Ratio LP: TL = 1:3.0-5.8; ratio WP:WD = 1:0.9-2.0 (Table 1).

Table 1

Measurements of 5 cephalonts of *Gregarina alphitobii* sp.n.

TL	LE	LP	LD	WE	WP	WD	LP:TL	WP:WD	WP:LP
14.4	2.7	2.7	9.0	3.6	5.4	6.3	1:4.3	1:1.2	1:0.5
15.2	3.6	3.6	8.0	4.5	5.4	6.3	1:3.2	1:1.2	1:0.7
14.8	2.2	3.6	9.0	3.6	4.9	5.9	1:3.5	1:1.2	1:0.7
23.4	2.7	6.3	14.4	4.5	5.4	7.2	1:3.3	1:1.3	1:1.2
39.6	1.8	9.0	28.8	3.6	5.4	6.3	1:4.2	1:1.2	1:1.7

Development

The earliest stages in the epithelium of the host's midgut as revealed in the sections, is a roughly spherical body (6.3 × 4.5 μm) (Pl. I: 1) with vacuolated cytoplasm, having spherical nucleus near the periphery. While it grows, it may involve more than one adjoining cell. Later, the body of the parasite (Pl. I: 1b) is partitioned by a septum into protomerite (1.8 × 3.6 μm) and deutomerite (7.2 × 6.6 μm). As it grows further, an epimerite develops at the tip of small neck and the parasite is embedded in the epithelial cell. Afterwards it becomes a young cephalont and begins to live freely within the gut lumen of its host.

Cephalont (Pl. I: 2). The cephalonts are solitary, broad and pale in colour. The youngest cephalonts observed in the gut or encountered in the smears of the gut contents measured from 13 to 40 μm (Table 1). They are attached to the midgut epithelial cells by their epimerites. The epimerite is a rounded, transparent, knoblike structure, having a diameter of 8 μm. The protomerite is relatively large, longer than wide

(Pl. I :2). The deutomerite is broad, short and saddle-shaped, having less breadth in the middle. The length of the deutomerite is from 9 to 28.8 μm. The nucleus is not seen in living specimens but when cephalonts are fixed and stained the nucleus is dark, oval in shape and situated in central part of the deutomerite.

Sporonts (Pl. I: 3,4). The sporonts are characteristically biassociative but solitary sporonts are also commonly observed within the gut lumen or on smeared preparations. The size of sporonts greatly varies depending on their age. In most cases the protomerite is broader at the apex but tends to grow narrow downwords. In some cases, it gives the appearance of a disc, set upon a short neck. In most cases the protomerite is greater in length than in breadth and the septum, separating it from the deutomerite, is with clear constriction (Table 2).

Table 2

Measurements of 15 single sporonts of *Gregarina alphitobii* sp.n.

TL	LP	LD	WP	WD	LP:TL	WP:WD	WP:LP
97	20	77	19	29	1:4.8	1:1.5	1:1.1
135	27	108	16	16	1:5	1:1	1:1.6
141	38	103	24	38	1:3.7	1:1.6	1:1.6
172	43	129	57	64	1:4	1:1.1	1:0.75
180	45	135	27	31	1:4	1:1.6	1:1.6
189	44	145	31	36	1:4.3	1:1.2	1:1.4
189	36	153	18	18	1:5.2	1:1	1:2
193	40	153	22	36	1:4.7	1:1.6	1:1.8
193	45	148	31	45	1:4.3	1:1.4	1:1.4
200	43	157	27	31	1:4.6	1:1.4	1:1.6
202	40	162	16	18	1:5.0	1:1.1	1:2.5
211	42	169	24	36	1:5.0	1:1.5	1:1.7
225	50	175	31	36	1:4.5	1:1.2	1:1.6
234	54	180	27	27	1:4.3	1:1.0	1:2
234	54	180	27	54	1:4.3	1:2.0	1:2

The deutomerite is elongated, increasing in width posteriorly and having a rounded posterior extremity. The pellicle is thin and clear. Distinct epicyteal striations are also observed in some of the sporonts. The nucleus is rounded, with well defined nuclear membrane with chromatin granules densely arranged at the periphery, and less concentrated in the middle. The nucleus is located centrally or closer to the posterior end.

Associations, Gametocysts, Sporocysts. The association of two sporonts is caudo-frontalin nature (Pl. I: 5-8). The primite (Pl. I: 5,6) differs

markedly from the satellite and is larger from the latter. The protomerite of the primite is like dilated disc (Pl. I: 6). Resting on a short thin neck and that of the satellite is sub-spherical and shorter (Table 3).

Table 3

Measurements of 15 sporonts in associations of *Gregarina alphitobii* sp. n.

	TL	LP	LD	WP	WD	LP:TL	WP:WD	WP:LP
prim.	70	14	56	16	20	1.5	1:1.2	1:0.9
sat.	50	10	40	14	16	1.5	1:1.4	1:0.7
prim.	76	16	60	12	12	1:4.7	1:1	1:1.3
sat.	56	8	48	12	12	1:7	1:1	1:0.6
prim.	82	22	60	24	24	1:3.7	1:1	1:0.9
sat.	72	12	60	12	16	1:4.3	1:1	1:1
prim.	114	30	84	20	24	1:3.8	1:1.2	1:1.5
sat.	74	10	64	12	20	1:4.2	1:1.6	1:0.8
prim.	142	36	106	26	55	1:3.9	1:2.1	1:1.4
sat.	89	22	67	29	38	1:4.0	1:1.3	1:0.8
prim.	165	43	122	50	46	1:3.8	1:0.9	1:0.9
sat.	129	43	86	43	43	1:3.0	1:1	1:1.0
prim.	175	31	144	22	22	1:5.6	1:1	1:1.4
sat.	144	31	113	18	31	1:4.6	1:1.7	1:1.7
prim.	179	50	129	46	56	1:3.6	1:1.2	1:1.1
sat.	126	40	86	40	50	1:3.1	1:1.2	1:1.1
prim.	185	40	145	36	58	1:4.6	1:1.6	1:1.1
sat.	164	32	132	32	49	1:5.7	1:1.5	1:1
prim.	193	36	157	18	27	1:5.4	1:1.5	1:2
sat.	130	31	99	20	31	1:5.1	1:1.5	1:1.5
prim.	200	38	162	28	31	1:5.3	1:1.1	1:1.3
sat.	145	31	114	26	29	1:4.7	1:1.1	1:1.2
prim.	202	36	166	22	20	1:5.6	1:0.9	1:1.6
sat.	121	27	94	32	27	1:4.5	1:1.2	1:0.8
prim.	211	36	175	20	22	1:5.8	1:1.1	1:1.7
sat.	137	29	108	22	27	1:4.7	1:1.2	1:1.3
prim.	281	57	224	49	85	1:4.9	1:1.7	1:1.2
sat.	176	42	134	41	79	1:4.2	1:1.9	1:1.0
prim.	294	63	231	36	96	1:4.6	1:2.7	1:1.7
sat.	191	43	148	40	82	1:4.4	1:2.1	1:1.1

The immature cyst is rounded and pale but with age turns dark brown (Pl. I 9). The cyst wall is thin and transparent, with a thick mucoid coat. The mature cyst measures from 55 to 90 μm.

The sporocyst (Pl. I: 10) is ovoidal with two coats and two knobs, it measures 8.5 × 6.3 μm.

Taxonomic Position

The eugregarine recorded in *A. diaperinus* belongs to the genus *Gregarina* Dufour as sporonts are biassociative, the epimerite is globular and cysts have the sporoducts. Although sporonts of different size are observed, but their shape and WP:WD and LP:TL coefficients are identical; this indicates that they represent one species. This is confirmed by the following facts: (a) — the smaller sporonts are often attached as satellites to a larger primite, (b) — the intermediate sizes are found between the small and the large sporonts.

The eugregarine from *A. diaperinus* is similar to the *Gregarina cuneata* Stein known from *Tenebrio molitor* L. and also recorded in *Alphitobius ovatus* Herbst (G e u s 1969). However, eugregarine from *A. diaperinus* differs from *G. cuneata* by having smaller width of protomerite and deutomerite and smaller size of cysts (Table 4). For this reason and considering that it is a first record of eugregarine infection in *A. diaperinus* we consider that the eugregarine we recorded in this insect has not been previously known and we propose for it a name *Gregarina alphitobii* sp. n.

Distribution and Parasitization

The eugregarine *G. alphitobii* sp. n. has a world-wide distribution as it was observed in populations of *A. diaperinus* in India, Europe, Africa and North America.

The parasitization level varied and the detail data are given in Table 5.

Pathogenicity of *G. alpitobii* sp. n. for its host is not greatly manifested by the level of mortality in *A. diaperinus* populations. Histological studies indicate that at heavy infection (Pl. I: 11) developing cephalons destroy gut epithelial cells.

Mattesia alphitobii sp. n.

Habitat: Midgut, fat body.

Locality: India — Patiala, German Democratic Republic — Kleinmachnow, 22. VII. 1986.

Holotype: The holotype slides AD-5/a, 6/a with various life stages of *M. alphitobii* sp. n. are kept at the Department of Zoology, Punjabi University, Patiala. Paratype slides are kept at the Institute of Plant Protection, Poznań, Poland.

Morphology and Development

Sporozoites (Pl. II: 12). The sporozoites within the sporocysts are elongated, sausage-shaped organisms found freely lying in the smears.

Table 4

Comparison of measurements of sporonts of *Gregarina alphitobii* sp.n. and *Gregarina cuneata* Stein

	TL	LP	LD	WP	WD	LP:TL	WP:WD	WP:LP
G. alphitobii sp. n.								
Sporonts solitary								
Range	97–234	20–54	77–180	16–57	16–64	1:3.7–5.2	1:1.0–2.0	1:0.75–2.5
Mean (n = 15)	186.3	41.4	144.9	26.5	34.3	1:4.5	1:1.3	1:1.6
Sporonts in syzygies								
Primite								
Range (n = 15)	70–294	14–63	56–231	12–50	12–96	1:3.6–5.8	1:0.9–2.7	1:0.9–2.0
Mean	171.2	36.5	134.7	28.3	39.9	1:4.7	1:1.4	1:1.28
Satellite								
Range (n = 15)	50–191	18–43	40–148	12–43	12–82	1:3.0–7.0	1:1.0–2.1	1:0.6–1.3
Mean	120.2	27.4	92.8	26.2	36.6	1:4.4	1:1.4	1:1.04
G. cuneata Stein								
Sporonts solitary								
Range (n = 10)	152–310	42–68	126–246	32–54	36–72	1:3.2–4.6	1:1.05–1.85	1:1.11–1.75
Mean	231.4	58	183.4	42	59.1	1:4.0	1:1.4	1:1.4
Sporonts in syzygies								
Primite								
Range (n = 15)	170–230	46–60	124–170	32–42	40–60	1:3.63–3.88	1:1.17–1.68	1:1.35–1.68
Mean	207.6	54.8	152.8	37.2	53.6	1:3.8	1:1.4	1:1.47
Satellite								
Range (n = 15)	134–222	28–48	106–178	30–50	36–72	1:4.37–6.93	1:1.20–1.50	1:0.8–1.0
Mean	189.6	36.8	152.8	41.2	55.6	1:5.15	1:1.3	1:0.89

Table 5

Examined laboratory and natural populations of *Alphitobius diaperinus* for *Gregarina alphitobii* sp. n. infection

Origin of insects and date of examination	Number of examined insects	Infection (%)
Laboratoire de Recherches sur les Insectes des Denrees INRA, Pont de la Maye (Bordeaux) 33140 (France)		
laboratory culture: 13. X. 1981	8	20
Chicken house, Sroda Wielkopolska (Poland)		
natural population: 13. III. 1982	11	35
Institut für Pflanzenschutzforschung, Kleinmachnow (GDR)		
laboratory culture: 18. V. 1984	6	33
VEB Broiler, Konigs Wusterhausen (GDR)		
natural population: 31. V. 1985	7	14.3
Institut für Vorratsschutz BBA, Berlin (Berlin West)		
laboratory culture: 26. VI. 1986 (Tonga Island)	7	42
25. II. 1985 (Rechterfeld, FGR)	5	40
USDA Stored Product and Household Insect Laboratory University of Wisconsin, Madison, Wisconsin (USA)		
laboratory culture: 21. XI. 1985	20	40%
Department of Biological Sciences, Rivers State University of Science and Technology, Port Harcourt (Nigeria)		
laboratory culture: 6. X. 1986	11	27
Nigerian Stored Products Research Institute, Ibadan Substation, Onirelle-Ibadan (Nigeria)		
laboratory culture: 30. IX. 1986	9	22
Department of Zoology Punjabi University, Patiala (India)		
laboratory culture: 20. IX. 1984	7 (adults)	40
	18 (larvae)	75
Groceries in Patiala (Punjab, India)		
natural population 12. III. 1985	10	32

They measure 8 μm in length and 1.5 μm in breadth, pointed at the both ends, with transparent and hyaline cytoplasm. The nucleus is not clearly visible. As seen in the Pl. II: 12 there is often a definite clear area around the sporozoite, probably resulting from shrinkage in fixation.

Schizogony and Gametogony. The sporozoite enters the epithelial cell and rounds up to become the schizont. The schizont (Pl. II: 13) get divided into bundles of the merozoites, their number being proportional to the size of the schizont. The merozoites (Pl. II: 13, 14) are rounded structures, measuring 2.5-3 μm in diameter and giving rise to the trophozoites. After growth, the trophozoites develop into gametocytes. Two gametocytes (Pl. II: 15) in syzygy show no sexual differentiation. They are rounded and have vacuolated cytoplasm. They associate in pairs and encyst. The cyst (Pl. II: 16) measures 14 × 10 μm in diameter.

The spores (Pl. II: 17, 18) measure 10.8 × 4.5-6.3 μm and are elongated or oval-shaped with protuberance at each pole. Each polar protuberence consists of two annular ridges and plug. When examined with the aid of the phase-contrast optics, the polar plugs are seen to protrude into the interior of the spores. The mature spores (Pl. II: 18) contain eight randomly distributed nuclei.

The gametocyst membrane of *M. alphitobii* sp. n., appears to be very fragile. Consequently, even the immature spores are rarely observed in pairs.

Taxonomic Position

The neogregarine observed in *A. diaperinus* belongs to the genus *Mattesia* Naville which is characterized by cycles of micronuclear and macronuclear merogony, and by gametogony, resulting in the formation of two spores within a gametocyst. This is the first record of a neogregarine infection in *A. diaperinus* and evidently a new neogregarine species is involved.

The genus *Mattesia* consists 7 described species and 3 identified to genus known from insects (Purrini 1977, Lipa and Wohlgemuth 1986). From *Coleoptera* the following species are known:

Mattesia trogodermae Canning (1964) from *Trogoderma granarium* Ev. (*Dermestidae*);

Mattesia grandis McLaughlin (1965) from *Anthonomus grandis* Boh. (*Curculionidae*);

Mattesia oryzaephili Ormieres et al. (1971) from *Oryzaephilus surinamensis* L. (*Cucujidae*);

Mattesia schwenkei Purrini (1977) from *Dryocoetes autographus* Ratz. (*Scolytidae*);

Mattesia sp. (Purrini 1976) from *T. granarium* Ev. (*Dermestidae*) and *Calandra granaria* L. (*Curculionidae*);

Mattesia sp. (Lipa and Wohlgemuth 1986) from *Prostephanus truncatus* (Horn) (*Bostrychidae*).

Besides, *Mattesia dispora* Naville, being a typical parasite of *Lepidoptera*, was found to be infectious to beetles *Laemophloeus ferrugineus* Steph. and *L. minutus* Oliv. (Finlayson 1950, Manning 1965).

Mattesia sp. found in *Alphitobius diaperinus* can be differentiated from other species by a spore size and shape, the prominence of the polar tuberances, the presence of polar plugs and two annular ridges. For this reason we consider that this neogregarine represents a new species for which we propose the name *Mattesia alphitobii* sp. n.

Distribution and Pathogenicity

M. alphitobii sp. n. was found in populations of *A. diaperinus* in Punjab (India) and in the German Democratic Republic (Europe). In the laboratory culture of *A. diaperinus* kept at the Institut für Pflanzenschutzforschung, Kleinmachnow (GDR) the infection level examined on 22.VII.1986 was 16% (12 examined insects). Among dead larvae collected in VEB Broiler in Königs Westerhausen and examined on 25.VI. 1985 the infection level was 87% (8 larvae examined).

Pathogenicity of *M. alphitobii* sp. n. to its host is high since the whole fat body can be completely destroyed by the parasite (Pl. II: 19, 20). The parasitized insects are often chalky in appearance due to destruction of the fat body by spores produced in a large number. Adults exposed to contaminated food become easily infected and their longevity and fecundity of females is greatly reduced. The rate of mortality is the highest among the youngest larval stages.

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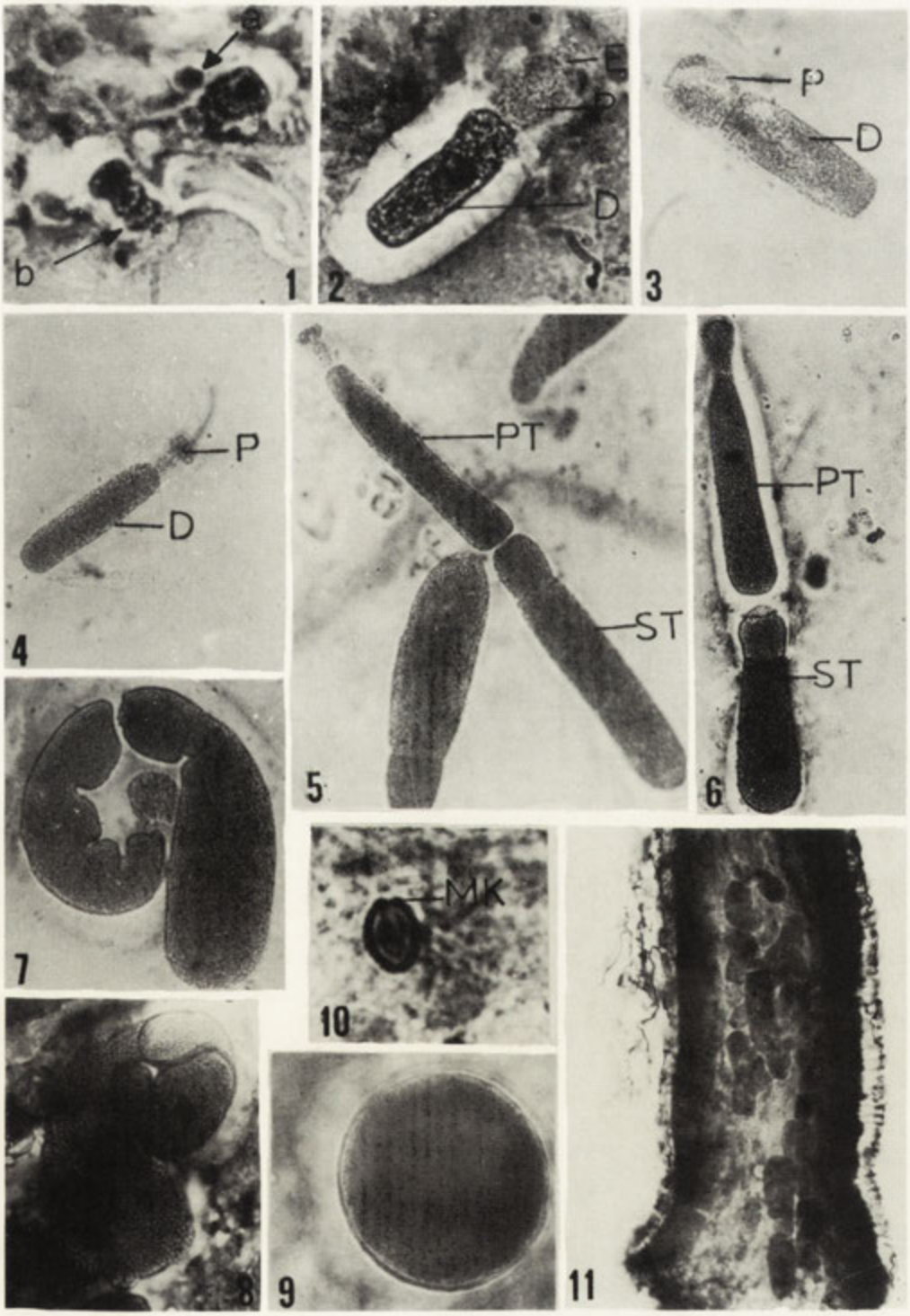
EXPLANATION OF PLATES I-II

1-11: *Gregarina alphitobii* sp. n.

1: Sporozoite (× 1000)
2: Fully developed cephalont (× 1000)
3-4: Sporonts (× 400)
5-6: Sporonts in syzygies (× 400)
7-8: Rotation of sporonts to form the cyst (× 400)
9: Cyst (×400)
10: Sporocyst (× 1000)
11: Gregarines in the lumen of the sectioned midgut (× 100)

12-20: *Mattesia alphitobii* sp. n.

12: Sporozoite (× 400)
13: Micronuclear plasmodium (× 400)
14: Macronuclear plasmodium (× 400)
15: Two gamecocytes in syzygy (× 400)
16: Gametocyst with binucleate sporoblast and residual bodies (× 400)
17: Mature spores (× 400)
18: Mature spores with remnants of sporozoites (×1000)
19-20: Spores in the alimentary tract of infected larvae (× 400)



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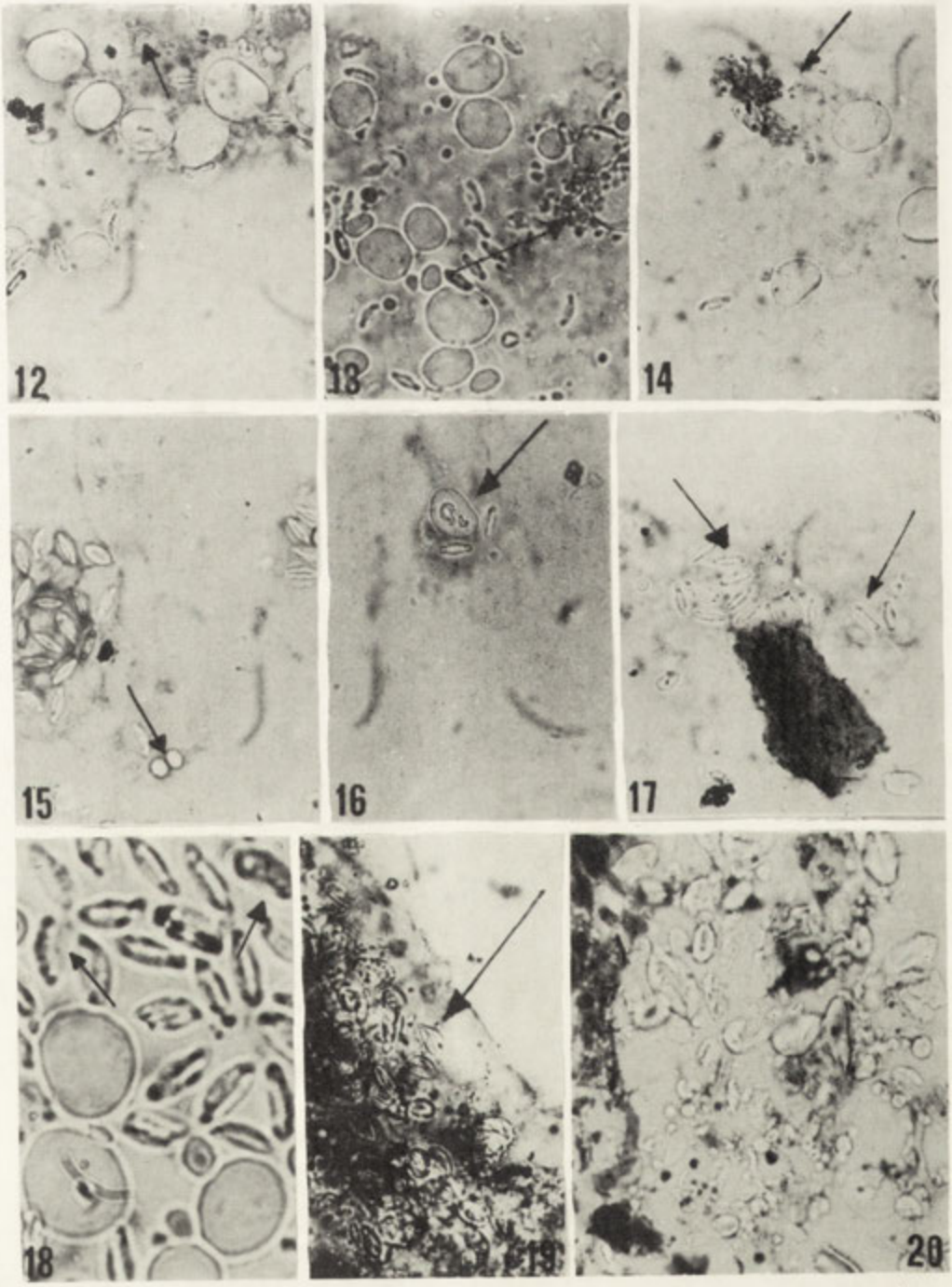

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Experimental Application of *Microsporidium sitophili* Ghose, to a Major Stored Grain Pest, *Oryzaephilus mercator* (F.) Infesting Nuts, *Anacardium occidentalis* and *Arachis hypogea*

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Synopsis: In a study of microbial control of *O. mercator* (F.), the microsporidian, *Microsporidium sitophili* was experimentally applied to 952 larvae and 2012 adults using a dose of 4×10^3, 4×10^4, 4×10^5 and 4×10^6 spores/ml. The incidence of infection and rate of mortality were noted and found significantly higher with a dose of 4×10^6 spores/ml.

Microbial control of pests is one of the most important parts of integrated pest control. Microbial agents include in addition to bacteria and viruses, microsporans deemed promisingly more effective as short and long-term control agents (Lipa 1976, Johnson and Pavlikova 1986, Fazairy and Ahlam 1987, Andreadis 1984, Erlandson et al. 1986, and Mercer and Wigley 1987).

Stored grain pests cause serious damage to stored grains and although the stored products present a prime opportunity to use protozoans as a preinfestation prophylactic, by distributing infecting qualities of pathogens, most research work has been done on field pests. This study, however, describes the application of *Microsporidium sitophili* Ghose for the control of the stored grain pest, *Oryzaephilus mercator* (F.) infesting the nuts *Anacardium occidentalis* and *Arachis hypogea*.

Material and Methods

The spores of *Microsporidium sitophili* Ghose were collected from *Sitophilus oryzae* (L), a common pest of rice grain. The microsporan was used as inoculum on

another pest, i.e., *Oryzaephilus mercator* infesting the nuts, *Anacardium occidentalis* and *Arachis hypogea*.

Collection of spores

Infected adults and larvae of *S. oryzae* were homogenized separately in 0.005M solution of ascorbic acid in 0.135M NaCl. The homogenates were washed in a progressively diluted solution of ascorbic acid until the material was transferred to distilled water (Vávra and Maddox 1976).

The homogenates were then strained through double thickness cheesecloth, which allowed the spores to pass through it but filtered out the larger body particles of the host. This filtrate was next centrifuged at 1000 rpm for 30 min to concentrate the spores. The spores were then suspended in 5% glucose solution and stored at 4°C.

Artificial infection

Uninfected 1000 larvae and 2100 adults were retrieved from the stock culture, starved for 25 h to induce hunger and to eliminate weak individuals. About 0.3 ml to 0.4 ml of the spore suspension was uniformly mixed with a measured amount of food material and was placed in a cavity slide (1 ml of spore suspension contains about $4 \times 10^8 - 4 \times 10^9$ spores). The slide was then kept in a petri dish containing some larvae (3rd and 4th instar) and adults of *O. mercator*. After allowing a feeding time of 2-3 h the larvae and adults were transferred to fresh vials containing sterilised food. A total of 952 larvae and 2012 adults were fed in this way in four different trials.

Simultaneously with the experimental feeding of the host larvae and adults, separate control batches were fed with sterile glucose solution. The control batches were subsequently reared in the same manner as the experimental batches and examined periodically.

Smear preparations

Smears of the infected tissue of inoculated and control larvae and adults of *O. mercator* were prepared by following method: a small piece of infected tissue was dissected from the host, placed in a drop of distilled water on a microscopic slide, a coverslip was applied and pressed lightly with the nail. Two drops of liquid paraffin oil were next added to a second slide, enough to cover an area just under the size of a coverslip. The coverslip from the first slide was carefully removed, making sure that some of the infected tissue adhered to the coverslip. The coverslip was gently lowered to the paraffin oil on the second slide and dropped on the top of the oil. The preparation was allowed to stand until the oil was evenly distributed beneath the coverslip. Examination under phase contrast microscope then followed (Hazard et al. 1981).

Histopathology

The experimental and control larvae and adults were fixed in Bouin-Duboscq-Brasil solution and subsequently 5-8 μm thick sections were stained by the modified polychromatic staining method of Vetterling and Thompson (1972).

Study of mortality rate

The mortality rate of the larvae and adults of *O. mercator* were recorded along with the prevalence of microsporan spores in them and the histopathological changes produced due to infection. The data regarding the rate of mortality of larvae and adults are given in Tables 2 and 3.

Results

Experimental infection

After feeding the spores of *M. sitophili* to the larvae and the adults of *O. mercator* examination began at 48 h and continued up to 168 h of post-application at regular intervals. It was observed that 120 h after the infective feed the polar filament was released in 60% of the spores.

At 120 h small schizonts with a variable number of nuclei measuring 8-10 μm were found in the epithelial tissue of the gut. 144 and 168 h after the infective feed the gut epithelial cells showed hypertrophy with vacuolated cytoplasm. Sporonts with a variable number of nuclei were observed inside the vacuolated cytoplasm. The gut epithelial cells greatly increased in size and the nuclei became fragmented. Smears prepared at 144 and 168 h post application showed sporogonial plasmodia after staining with Giemsa's solution, measuring 14.2 to 17.5 × 11.5 to 14.3 μm.

Sections of the gut of the infected host showed that the lumen also contained some spores. This may be due to the rupture of infected cells and to the abnormal regeneration process in the infected midgut epithelium (Lipa et al. 1983).

The fat bodies of the hosts are heavily infected at 144 and 168 h post application. In most cases the fat cells lost their shape and fragmented.

Incidence of infection

Table 1 indicates that both the larvae and the adults are more or less equally prone to infection by the microsporan spores. It was clearly observed that with increase of the spore dose the intensity of infection steadily increased, the highest incidence of infection being obtained using an inoculum of 4×10^6 spores/ml at 168 h post application.

Rate of mortality of larvae and adults

Larvae and adults of *O. mercator* receiving an inoculum of 4×10^6/ml microsporan spores were maintained in the laboratory for

Table 1

Infection rate in *Oryzaephilus mercator* (F.) infected experimentally with *Microsporidium sitophili* (figures in brackets indicate percentages of infection)

	48 h post application				72 h post application				96 h post application			
	no. examined		no. infected		no. examined		no. infected		no. examined		no. infected	
	adults	larvae	adults	larvae	adults	larvae	adults	larvae	adults	larvae	adults	larvae
4×10^3 spores/ml	47	25	1 (2.13)	1 (4.0)	70	29	6 (4.57)	4 (13.79)	50	28	12 (24.0)	7 (25.0)
4×10^4 spores/ml	58	45	4 (6.89)	4 (8.89)	49	37	4 (8.16)	4 (10.8)	80	44	20 (25.0)	16 (36.36)
4×10^5 spores/ml	76	46	4 (5.26)	6 (13.04)	57	25	5 (8.77)	5 (20.0)	104	30	30 (28.9)	10 (33.33)
4×10^6 spores/ml	54	19	2 (3.7)	3 (15.79)	72	25	12 (16.66)	6 (24.0)	58	27	18 (31.03)	10 (37.03)

120 h post application				144 h post application				168 h post application			
no. examined		no. infected		no. examined		no. infected		no. examined		no. infected	
adults	larvae	adults	larvae	adults	larvae	adults	larvae	adults	larvae	adults	larvae
108	40	24 (22.22)	14 (35.0)	86	20	24 (27.9)	7 (35.0)	100	48	28 (28.0)	17 (35.41)
80	45	24 (30.0)	15 (33.33)	104	20	32 (30.76)	7 (40.0)	97	44	32 (32.99)	17 (38.63)
122	21	38 (31.14)	7 (33.33)	49	25	16 (32.63)	9 (36.0)	82	40	32 (39.02)	15 (37.5)
82	35	32 (39.02)	12 (34.28)	80	29	36 (45.0)	10 (34.48)	87	45	40 (45.98)	18 (40.0)

studying the mortality rate. The larvae and the adults were divided into four batches of 40 each and kept in separate vials with sterilised food. In all the four batches it was found that the larvae succumbed to infection earlier than the adults. In all the batches of larvae taken together 3.75% died at the end of 48 h, 4.86% at the end of 72 h, 11.67% at the end of 96 h, 19.0% at the end of 120 h, 31.63% at the end of 144 h and 43.28% at the end of 168 h (Table 2). Microscopical examination of dead larvae showed large numbers of hypertrophied epithelial tissue and fragmented fat bodies. Death of the larvae was probably due to the damage of the gut epithelial cells and fat body cells caused by the heavy infection. In the control batch only 3 larvae died during the same period.

Table 2

Rate of mortality of *O. mercator* (larvae) after experimental infection with *Microsporidium sitophili* (4×10^{-6} spores/ml) (figures in brackets indicate percentages of mortality)

Hours post application	48	72	96	120	144	168
1st batch	2 (5.0)	1 (2.5)	3 (7.5)	6 (15.0)	7 (17.5)	8 (20.0)
2nd batch	1 (2.5)	2 (5.0)	4 (10.0)	5 (12.5)	6 (15.0)	8 (20.0)
3rd batch	2 (5.0)	2 (5.0)	4 (10.0)	5 (12.5)	9 (22.5)	7 (17.5)
4th batch	1 (2.5)	2 (5.0)	5 (12.5)	7 (17.5)	9 (22.5)	6 (15.0)
Control	—	—	—	1 (2.5)	1 (2.5)	1 (2.5)

Similar experiments were made on the adults and it was found that about 3.75% died at the end of 72 h, 7.8% at the end of 96 h, 14.79% at the end of 120 h, 19.83% at the end of 144 h and 31.95% at the end of 168 h (Table 3). 4 adults died in the control batch during the same period.

In the experiment on mortality rate it was observed that about 70% larvae and 60% adults succumbed to death after 168 h of experimental aplication of microsporan spores. Among the survivors the majority showed symptoms of infection viz., consumed a smaller amount of food, mated less often, laid fewer eggs and tended to aggregate.

Table 3

Rate of mortality of *O. mercator* (Adults) after experimental infection with *Microsporidium sitophili* (4×10^{-6} spores/ml) (figures in brackets indicates percentages of mortality)

Hours post application	48	72	96	120	144	168
1st batch	—	2 (5.0)	3 (7.5)	5 (12.5)	5 (12.5)	7 (17.5)
2nd batch	—	2 (5.0)	2 (5.0)	5 (12.5)	5 (12.5)	8 (20.0)
3rd batch	—	1 (2.5)	3 (7.5)	5 (12.5)	8 (20.0)	9 (22.5)
4th batch	—	1 (2.5)	4 (10.0)	6 (15.0)	6 (15.0)	7 (17.5)
Control	—	—	2 (5.0)	—	—	2 (5.0)

Discussion

When protozoan infections are considered as potential agents for insect control, the effect on both the mortality and fecundity of insects must be evaluated. For example: Weiser (1961) suggested that *Thelohania hyphantriae* could be useful because it caused 80% mortality among certain lepidopteran larvae and caused the remaining insects to die as adults or to produce abnormal eggs. Henry (1971) demonstrated that the application of *N. locustae* is useful for control of the density of *Melanoplus gladstoni*. Zimmack et al. 1954, Zimmack and Brindley 1957, Kramer 1959 a,b, Van Denburgh and Burbutis 1962, Peairs and Lilly 1974, Windles et al. 1976 and Hill and Gary 1979 suggested that *N. pyrausta* produces chronic infections that are frequently panzootic in the European corn borer, *Ostrinia nubilalis* and reduces adult longevity and fecundity.

Weiser (1976) suggested that the time taken for the establishment of the parasite for completion of its life cycle and production of spores varies considerably depending on the age of host, species of the parasite, size of the inoculum and other environmental factors.

In the present investigation it was observed that infection was first noticed at 48 h (in larvae) and 72 h (in adults) and histopathological changes at 144 h and 168 h after the infective feed. The rate of mortality was maximum at 144 h and 168 h, when masses of spores were released into the lumen of the host gut. It was also found that 4×10^6 spores/ml is

highly effective in both larvae and adults and cause 60-70% mortality and that the remaining adults and larvae showed various pathological symptoms.

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Studies on Histopathology and Development of a Microsporan Pathogen, *Nosema* sp. Infecting *Palorus ratzaebergii* Wissmann

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Synopsis. A microsporan, tentatively indentified as *Nosema* sp., was found in living and dead larvae and adults of *Palorus ratzaebergii* (*Coleoptera*: *Tenebrionidae*), following the mortality of this insect in West Bengal, India. It produces a generalized infection, accompanied by extensive tissue destruction, but was noticed especially in gut epithelium, fat body, hemocytes and muscles. Spores were ellipsoidal in shape and 5.1 μm — 8.5 μm × 4.0 μm — 5.9 μm (Unifixed); 4.9 μm — 8.2 μm × 3.2 μm — 5.7 μm (Fixed and stained). The polar filament was up to 105.95 μm ($\bar{x}$ = 84.76 μm). This parasite plays a significant role in mortality of *Palorus ratzaebergii*. The desirability of exploring its potentiality as a biological control agent is suggested.

Palorus ratzaebergii Wissmann (*Coleoptera*: *Tenebrionidae*) is one of the most serious pests of stored grains and the damage caused by this pest is extremely high. A knowledge of the pathogens of this pest is therefore important for the development of biological control methods.

From 1987-1988 I reared a large population of this pest in laboratory conditions. After some fays I found that a large number of insects were dead. Microscopic examination of these dead insects showed that the mortality of the hosts was due to protozoan parasites belonging to the phylum *Microspora*.

In this communication a report has been made on the histopathology and development of pathogenic microsporan, *Nosema* sp. infecting larvae and adults of *P. ratzaebergii*.

Material and Methods

Material examined in the present study was collected from a storehouse at Chinsurah (West Bengal, India) in 1987 and reared in the laboratory. The freshly collected hosts were brownish in color, moved actively and consumed sufficient amounts of food, but interestingly enough many adults and larvae were found dead after 14 days. Microscopic examination of dead larvae and adults revealed the presence of microsporan spores.

For light microscopical observation smears of the infected host organs were made following the method of Hazard et al. (1981). For permanent preparation smears of the infected host organs were air dried, fixed in methanol and stained in Giemsa's solution after hydrolysis for 10 min in 1 N HCl (Weiser 1976). For haematoxylin staining the smears were fixed in Bouin-Duboscq-Brasil solution for 1-6 h, washed in distilled water, keep in mordant of 1% aqueous solution of iron alum overnight, stained in Heidenhain's haematoxylin for 5 h and destained in iron alum solution to the desired intensity. Some of the wet smears were fixed in Carnoy's fluid and stained in Schiff's reagent. The smears were mounted in DePeX.

For histological studies whole larvae were fixed in Carnoy's fluid, washed in 100% ethanol, transferred three times through butyl alcohol and embedded in paraffin. 5-6 μm thick sections were cut and stained in Heidenhain's haematoxylin. Some larvae were fixed in Bouin-Duboscq-Brasil solution overnight, washed in water, dehydrated in graded alcohol, washed in butyl alcohol and embedded in paraffin. 6-8 μm thick sections were cut and stained in modified polychromatic staining method of Vetterling and Thompson 1972. To improve the staining of spores the original nuclear staining was supplemented with abbreviated haematoxylin staining method (2.5% iron alum for 1 h, haematoxylin for 1 h) and the original procedure was followed from point 4 onwards (Vetterling and Thompson 1972).

Abbreviations

Ic — infected cell
IFb — infected fat bodies
IMv — infected microvilli
Sp — spore
Va — vacuola
ICot — infected connective tissue
He — hemocyte
Im — infected muscle
Sc — schizont
Fsp — fresh spore
Spn — nucleus of the spore
Pf — polar filament

Observations

Gross pathology

After a lapse of twenty five days in the laboratory container the infected larvae and adults show certain symptoms. The adults become smaller in size and the beetles tend to aggregate. The larvae are more

readily identifiable, being characterised by a brownish ventral side which thus loses its original creamy white color. The voracious larvae diminish their food consumption and in heavily infected cases pupation does not occur.

Histopathology

Midgut epithelium

The midgut epithelium of healthy larvae is thin and consists of one layer of cells. The nuclei of healthy cells are smaller in size and have a moderate number of karyosomes.

In the weakly infected larvae only some cells are infected and the shape of the cells remain unchanged. In the haevily infected cases the midgut epithelium has a quite different appearance. Spores of *Nosema* sp. occur in great numbers in the cell cytoplasm. The cell wall becomes transparent and the volume of the cell greatly increases due to the presence of a large number of spores. In most cases the nuclei of host epithelia become fragmented (Pl. I: 1). The microvilli of the intestine of the infected hosts also become fragmented (Pl. I: 2).

In the gut lumen of the haevily infected larvae a great number of spores are noticed (Pl. I: 3,4). This may due to the rupture of infected cells and due to abnormal regeneration processes in the infected midgut epithelium (Lipa et al. 1983). Heavy vacuolization is frequently seen infected hemocytes become enlarged (Pl. II: 7).

Connective tissue

The connective tissue of the host is also affected by the parasite (Pl. I: 6).

Hemocyte

The hemocytes of the infected hosts are filled up with parasites. The infected hemocytes become enlarged (Pl. II: 7).

Fat body

The fat body of the larvae is one of the major sites of infection. The normal fat body cells are whitish in color with large nuclei. In most cases of infection no fat cells without infection came to my notice. In haevily infected form the fat cells are totally fragmented by the parasites infection (Pl. I: 6, Pl. II: 8).

Muscles

Dissection of heavily infected larvae shows group of spores in the muscles (Pl. II: 9).

Presporal stages

Schizogony: The microsporan involved belongs to the genus *Nosema*. This is indicated by the fact that binucleate spores are distributed singly within the host's tissues and do not occur in groups within the sporophorous vesicle.

The schizonts very greatly in size. Small schizonts are 3-4 μm in diameter (Pl. II: 10), while the largest ones are 7-9 μm. All schizonts have 2-4 nuclei. The diplokaryotic nuclei of the schizonts take a deeper stain than the cytoplasm (Pl. II: 10).

Stages in sporogony were not recognised in detail.

Spore: In sections the spores are always isolated. The spores are ellipsoidal in shape (Pl. II: 11) and internally contain the usual organelles. A polar cap is found at the anterior end of the spore after staining with PAS technique. Backward from the polar cap a uniformly thin polar filament (Pl. II: 12) (observed only after Heidenhain's haematoxylin) extruded through a clear area (polaroplast) by the application of H_2O_2 or urea. Girdle-shaped sporoplasm with a pair of nuclei is frequently observed after staining by the Weiser (1976) technique (Pl. II: 13).

Fresh spores are varied in size. Unfixed spores measure: n = 70, length 5.1-8.5 μm, $\bar{x}$ = 7.0 μm, SD = 0.62; width 4.0-5.9 μm, $\bar{x}$ = 4.2 μm, SD = 0.35. Fixed and stained spore measure: n = 70, length 4.9-8.2 μm, $\bar{x}$ = 6.5 μm, SD = 1.1; width 3.2-5.7 μm, $\bar{x}$ = 4.41 μm, SD = 0.75. The length of the polar filament is 65.2-105.95 μm, $\bar{x}$ = 84.76 μm, SD = 16136.

The measurements of the one sample of 70 spores are given in Table 1.

Table 1

Frequency distribution of the spore size in fixed and stained spores of *Nosema* sp.

Sample (N = 70)	Dimensionable groups in microns							
	length				width			
	4.9-6.2	6.5-6.9	7.0-7.8	8.0-8.2	3.2-4.0	4.1-4.7	4.9-5.05	5.2-5.7
Number of spores	20	27	13	10	18	11	36	1

Taxonomic position

The presence of binucleate spores and absence of sporophorous vesicles are consistent with the essential characters of the genus *Nosema*. Several microsporean have been reported from coleopteran insects but none of them were found to infect *Palorus ratzaebergii* Wissmann

Table 2

Showing comparative characters of *N. longifilum*, *N. lepturae*, *N. coccinellae*, *N. weiseri* and *Nosema* sp.

	N. longifilum Hesse, 1905	*N. lepturae* Lipa, 1968	*N. coccinellae* Lipa, 1968	*N. weiseri* Lipa, 1968	*Nosema* sp. (present study)
Shape of the spore	oval (larger size ellipsoidal)	oval or ellipsoidal	ellipsoidal	ellipsoidal	ellipsoidal
Size of the spore (μm)	4–5×3 and 6×4	4.6–6.1×2.2–3.5	4.4–6.7×2.3–3.4	3.0–4.1×1.9–2.5	fresh: 5.1–8.5($\bar{x}$−7.0) ×4.0–5.9($\bar{x}$−4.2) stained: 4.9–8.2($\bar{x}$−6.5) ×3.2–5.7($\bar{x}$−4.4)
Length of polar filament (μm)	85–90	≤ 117	≤ 170	60	65.2–105.95($\bar{x}$−84.76)
Principal infected tissue	fat body	general infection but especially tracheal matrix, fat body and midgut epithelium	midgut epithelium malpighian tubules, gonads, muscles, nerves	fat body	midgut, epithelium, fat body, hemocyte, muscle, connective tissue
Host species	*Otiorrhynchus fuscipes*	*Leptura rubra* L.	*Coccinella septempunctata* *Hippodamia tredecimpunctata* *Myrrha octodecimgutata*	*Rhizopertha dominica* F.	*Palorus ratzaebergii*
Host family	*Curculionidae*	*Cerambycidae*	*Coccinellidae*	*Rhizophagidae*	*Tenebrionidae*

Table 2 indicates that the species described here exhibits close resemblances to *N. longifilum* Hesse (1905), *N. lepturae* Lipa (1968a), *N. coccinellae* Lipa (1968b) and *N. weiseri* Lipa (1968c) in the shape of the spore and the site of infection, but differ in the size of the spore and host species. The large size of the spore (5.1 μm to 8.5 μm × 4.0 μm to 5.9 μm) definitely separates the present form from the previously described species of *Nosema* reported from the insects belonging to the order *Coleoptera*. On the basis of the above fact the present form can be designated as a new species. Since now a days it is practically impossible to given the correct species status of a microsporan sp. without electron microscopy, I propose the microsporan as *Nosema* sp.

Discussion

Infection of the gut epithelia and fat bodies followed by the passing of spores into the gut lumen and, presumably, to the outside with the feces, suggest that the insect may become infected by ingesting spores. Heavy infection in larvae suggests that transmission may also occur by the transovum passage, a method known in several other insects.

The parasite reported in the study is definitely destructive to its host tissues and possibly causes the unexplained mortalities of *Palorus ratzaebergii*. A very similar species, *Nosema gasti*, is known to be highly pathogenic to its host and significant steps have been taken toward producing it in large quantities and testing it as a biological control agent (Mc Laughlin and Bell 1970). Experimental application of the presently described species for the control of *Lesioderma sericorne* found that about 46.1% larvae and 22.2% adults show abnormilities (Ghose 1988). Further studies on this *Nosema* sp. are necessary to establish its potentiality in controlling economically important and undesirable insects in the field conditions.

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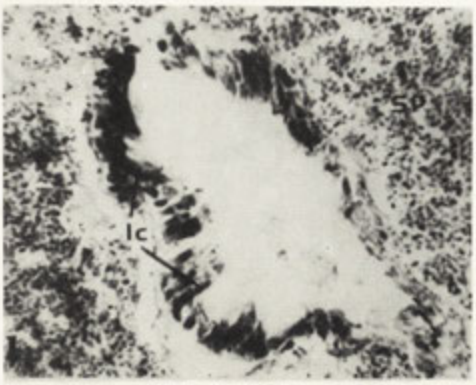
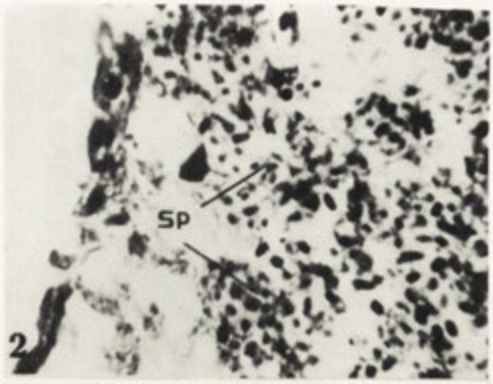
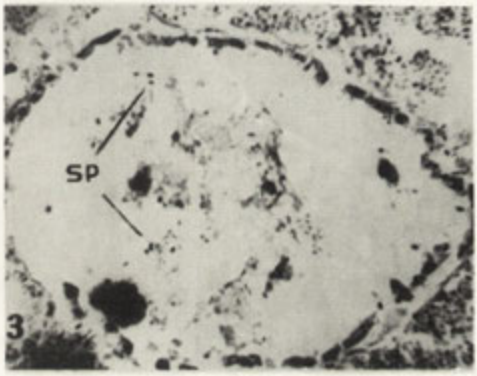
EXPLANATION OF PLATES I-II

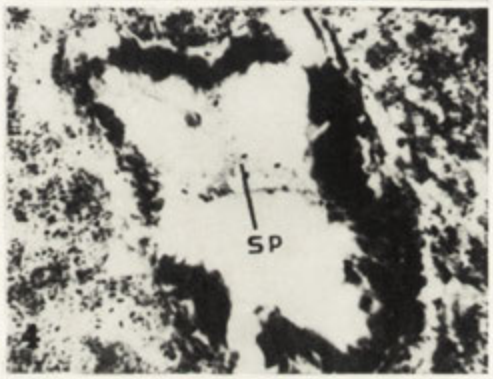
Nosema sp. infection of *Palorus ratzaebergii* larvae and adults

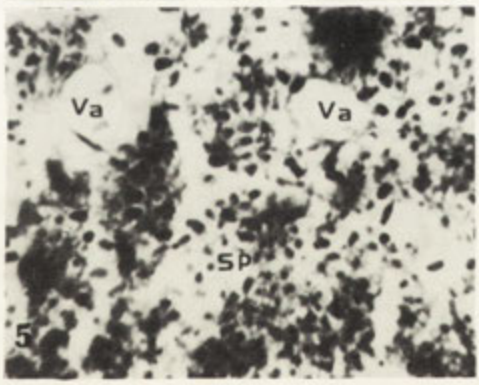
1: Infected gut epithelial cells
2: Infected microvilli
3-4: Spores within the lumen of the gut
5: Heavy vacuolization in strongly infected gut epithelium
6: Infected connective tissue and fat bodies
7: Infected hemocytes
8: Strongly infected fat bodies
9: Sagittal section showing muscles infected by the parasites
10: Schizonts and spores stained with Giemsa's solution
11: Fresh spores
12: Spore with extruded polar filament
13: Binucleate spores

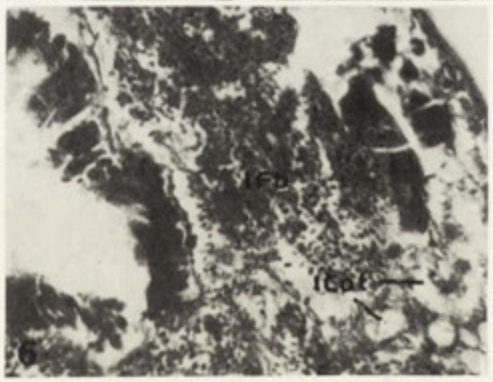












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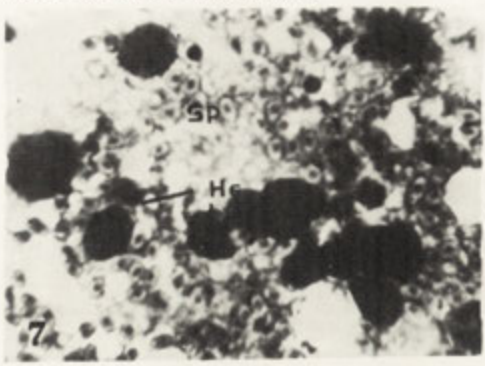

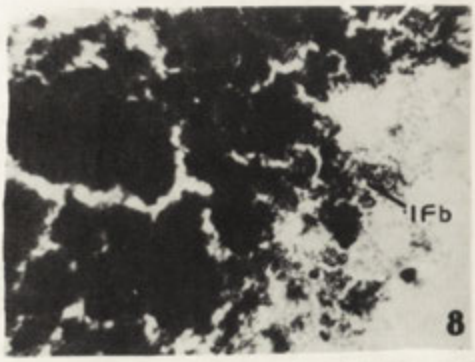

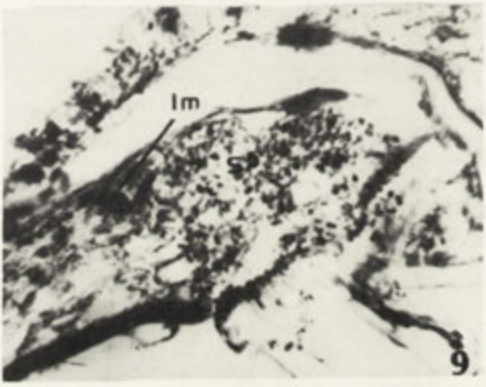

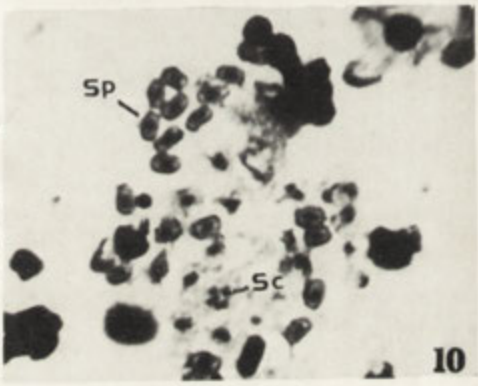

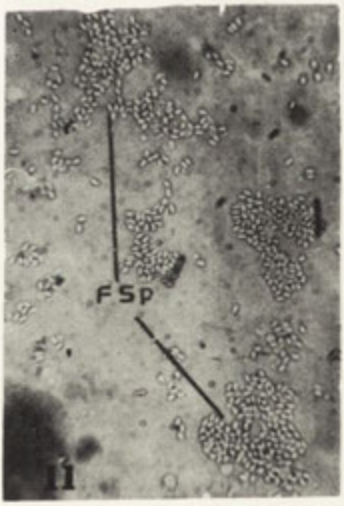

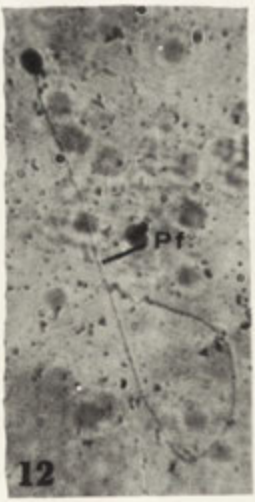

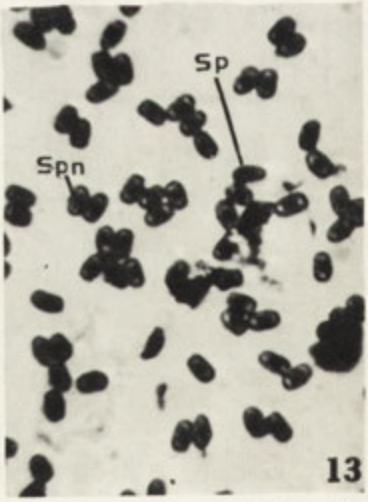

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