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Jerzy J. LIPA, Klaus P. CARL and Errol W. VALENTINE

Blastocrithidia caliroae sp. n., a Flagellate Parasite of Caliroa cerasi (L.) (Hymenoptera: Tenthredinidae) and Notes on its Epizootics in Host Field Populations

Synopsis. A flagellate Blastocrithidia caliroae sp. n. was found for the first time in a field population of the pear slug, Caliroa cerasi (L.) in New Zealand during the season 1972/73. Since the insect host originated from Europe, subsequent investigation revealed that this flagellate infects natural populations of C. cerasi in the Federal Republic of Germany, in France, Austria and Switzerland. Field observations indicated that B. caliroae sp. n. is responsible for high mortality of its host and the collapse of the outbreaks of C. cerasi. In the life cycle of B. caliroae sp. n. the main four morphological forms occur: blastocrithidial, promastigote, opisthomastigote and epimastigote. Data on size and relative number of various forms are given.

The pear-slug, Caliroa cerasi (L.), is a pest of some fruit and other trees and shrubs (Carl 1972). It is a native of the palearctic region, but is now widely distributed in temperate and subtropical areas of all five continents. During a biological control programme to establish parasites of European origin in New Zealand, one of us (E.W.V.), recorded massive flagellate infections in the haemocoel of field populations of C. cerasi at Nelson, New Zealand, during the 1972/73 season. He also found infections by the same pathogen in host eonymphs originating from central Europe which were kept in quarantine for parasite emergence. Subsequently the pathogen has been recorded from several locations in Europe. In both areas there were instances of high larval mortality associated with the presence of the flagellate, suggesting that the protozoan in high numbers is pathogenic (Carl 1976).

Methods

For the detection of early stages of infection, crushed larvae in tap water were examined with dark field illumination. In heavily infected larvae the presence of the pathogen can be recognized with the stereomicroscope at low magnification because the water shimmers due to the high concentration of organisms. Morphological details were studied on smear preparations of haemolymph which were fixed in methanol for 5 min. and stained for 45 min. in 3% Giemsa's stain diluted with Weise's buffer (0.49 g KH_2PO_4 and 1.14 g $Na_2HPO_4 \times 2H_2O$ filled up to 1000 ccm with boiled distilled water) at pH 7.2.

Results

Morphology

According to present taxonomic concept for entomophilic *Trypanosomatidae* we recognize in the development of the studied flagellate the following morphological forms: blastocrithidial, opisthomastigote (= herpetomonad), promastigote (= leptomonad) and epimastigote (= crithidial) (Pl. I 1), besides the term haptomonad is used for slender and elongated forms without a free flagellum. Detailed measurements of the various morphological forms are listed in Table 1.

Blastocrithidial. Length without flagellum, 18.5–23.7 μm; exposed flagellum, 2.1–18.5 μm; overall length, 21.6–38.1 μm; width, 2.1–3.1 μm. The blastocrithidial form is elongated, with a slender body, characterized by the location of the kinetoplast close to·the posterior end of the body. The flagellum goes along the undulating membrane which is well seen in many specimens (Pl. I 1–4, II 5).

The anterior end from which the flagellum arises is pointed. The nucleus is located at the mid-length of the body and has a diameter of about 2–3 μm. The cytoplasm is dense and not vacuolated.

Opisthomastigote. Length without flagellum, 17.5–24.7 μm; exposed flagellum, 7.2–12.4 μm; overall length, 29.9–35.1 μm; width, 3.6–5.1 μm. The opisthomastigote form has a long spindle-shaped body characterized by the location of the kinetoplast either posterior to or lateral to the nucleus (Pl. II 6). The flagellum passes through a long reservoir. The cytoplasm is dense and not vacuolated. In some cases the flagellum arising from kinetoplast forms a loop instead of going directly toward front end (Pl. II 6).

Promastigote. Length without flagellum, 17.5–25.7 μm; exposed flagellum, 13.4–23.4 μm; width, 2.3–4.1 μm. The promastigote form differs from the opisthomastigote in that the kinetoplast is located anterior to the nucleus (Pl. II 7). The posterior end of the promastigote form is sharply pointed while the anterior end is broader and slightly

Table 1

Measurements (in micrometers) of ten flagellates, *Blastocrithidia caliroae* sp. n., of five main morphological forms

Forms	1	2	3	4	5	6	7	8	9	10
Blastocrithidial										
pe-mn	10.3	11.3	13.4	10.3	11.3	9.3	8.2	15.4	16.0	18.5
mn-k	−8.8	−8.2	−6.2	−8.2	−7.2	−7.2	−7.2	−4.1	−10.3	−3.1
k-ff	20.6	19.6	18.5	21.6	21.6	19.6	21.6	20.6	18.5	21.6
lff	8.2	2.1	6.2	18.5	6.2	3.1	10.3	3.1	3.1	13.4
gb	3.1	3.1	2.6	2.8	2.6	2.1	2.6	2.3	2.6	2.1
lap	23.7	20.6	18.5	19.6	19.6	18.5	19.6	22.7	19.6	23.7
oal	31.9	22.7	24.7	38.1	25.8	21.6	29.9	25.8	22.7	37.1
Opisthomastigote										
(herpetomonad)										
pe-mn	14.4	16.5	15.0	14.4	14.4	16.5	15.4	15.4	12.4	17.5
mn-k	±1.5	±2.1	±1.0	±2.1	±2.1	±1.5	1.0	±1.5	±1.5	±1.5
k-ff	18.5	16.5	17.5	18.5	19.6	18.0	17.5	16.5	18.5	13.4
lff	11.3	8.2	9.3	10.8	12.4	11.3	11.3	10.3	12.4	7.2
gb	4.1	4.1	4.1	4.1	4.1	4.1	3.6	4.1	4.1	5.1
lap	22.7	24.7	23.7	21.6	22.7	23.7	22.7	23.7	17.5	23.7
oal	34.0	32.9	33.0	32.4	35.1	35.0	34.0	34.0	29.9	30.0
Promatigote										
(leptononad)										
pe-mn	17.5	14.4	15.4	14.4	13.4	14.4	15.4	12.4	14.4	15.4
mn-k	4.1	3.6	4.1	7.2	2.6	7.2	4.1	3.1	3.1	6.2
k-ff	22.7	20.6	21.6	25.7	17.5	24.8	21.6	16.5	21.6	23.7
lff	19.6	18.0	18.5	23.2	15.4	21.6	18.5	13.4	18.5	20.6
gb	4.1	2.6	3.1	3.1	2.2	3.1	3.1	2.3	3.1	3.1
lap	25.7	20.6	22.7	24.2	23.2	24.8	22.7	17.5	20.6	24.8
oal	45.3	38.0	41.2	47.4	38.6	46.4	41.2	30.9	39.1	45.4
Haptononad										
pe-mn	12.4	6.2	10.3	11.3	10.3	10.3	9.8	9.3	10.3	11.3
mn-k	3.1	10.3	2.1	1.5	3.1	3.1	2.1	2.1	3.1	3.1
k-ff	3.1	2.1	2.6	2.1	3.1	1.0	1.5	2.1	2.1	1.0
lff	0	0	0	0	0	0	0	0	0	0
gb	2.1	3.1	2.1	2.1	2.1	2.1	2.6	2.1	2.1	2.1
lap	17.5	18.5	14.9	14.4	16.6	14.4	13.4	13.4	15.4	15.4
oal	17.5	18.5	14.9	14.4	16.5	14.4	13.4	13.4	15.4	15.4
Epimatigote										
pe-mn	8.8	6.2	6.2	8.2	7.2	6.2	7.2	7.2	8.8	8.2
mn-k	±1.0	±3.1	±2.6	±2.1	±1.5	±1.5	±1.5	±1.5	±1.5	±1.0
k-ff	17.5	12.4	14.4	11.3	14.4	12.4	7.2	15.4	16.5	17.5
lff	14.4	7.2	10.3	7.2	9.3	10.3	4.1	11.3	13.4	12.4
gb	6.2	8.2	6.2	7.2	6.2	6.2	7.2	8.2	5.1	7.2
lap	13.4	11.3	10.3	14.4	13.4	10.3	11.3	12.4	12.4	11.3
oal	27.8	18.5	20.6	21.6	22.7	20.6	15.4	23.7	25.8	23.7

Abreviations: pe-mn — posterior extremity to middle of nucleus; mn-k — middle of nucleus to kinetoplast; k-tff — kinetoplast to tip of free flagellum; lff — length of free flagellum; gb — greatest breadth; lap — length from posterior to anterior end; oal — overal length; — or +: indicate that kinetoplast is located posterior or laterally ± to the nucleus.

truncate. The nucleus is oval or elipsoidal and in most cases is located in the mid-length of the body. The kinetoplast is bean-shaped.

H a p t o m o n a d. Length, 14.4–18 µm; width, 2.1–3.1 µm. This form present on some preparations, is characterized by the lack of a free flagellum while in other features it is similar to the opisthomastigote form.

E p i m a s t i g o t e. Length without flagellum, 10.3–13.4 µm; exposed flagellum, 4.1–14.4 µm; overall length, 15.4–27.8 µm; width, 5.1–8.2 µm. This form has a pear-shaped, broad body (Pl. II 9–12). The kinetoplast is located anterior to the nucleus. The flagellum arising from the kinetoplast passes through a funnel shaped reservoir. The nucleus 2–3 µm in diameter is usually located in the center of the body.

A m a s t i g o t e. This form, which is characterized by the ovoid body and lack of the flagellum, was very scarce in the slides examined (Pl. III 10, 12).

Development

The life cycle of the studied flagellate includes various morphological forms typical of the genus *Blastocrithidia* within the family *Trypanosomatidae*. The proportion of various forms depends on the age of the flagellate population and the habitat. In the midgut the promastigote form is prevalent followed by the blastocrithidial form.

The relative number of the various forms was estimated on a few slides prepared from the body of *Caliroa cerasi* larvae (Table 2). Although the most common was the promastigote form the blastocrithidial form is the most typical of the flagellate studied.

Table 2

Number of various developmental forms of *Blastocrithidia caliroae* sp. n. in smeared bodies of *Caliroa cerasi* (sample of 170 flagellates)

Morphological forms	%
Blastocrithidial	15.3
Promastigote	71.8
Opisthomastigote	2.9
Epimastigote	10.0

Table 3

Incidence of *Blastocrithidia caliroae* sp. n. in larvae of *Caliroa cerasi* of different age groups. Alsace, 1974

Larval instar	No. healthy	Number with infection	
		slight	heavy
L_1	27	0	0
L_{2-4}	141	38	21
L_{5-6}	89	42	69

On Diagram 1 it is seen that the four main morphological forms, namely blastocrithidial, promastigote, opisthomastigote and epimastigote are well defined by their body lengths and the length of the free flagellum.

This flagellate divides into two cells by binary fission only; multiple fission was not observed. The division usually starts from the kineto-plast (Pl. IV 13, 14) and is followed by the division of the nucleus. One of the newly formed kinetoplast holds the old flagellum while the se-cond one immediately forms a new flagellum. Therefore, before the division is completed a dividing flagellate has two flagella, two kine-toplasts and two nuclei (Pl. IV 13, 14). The process of division is con-tinued and leads to the formation of two daughter-like flagellates (Pl. IV 14, 15).

The division takes place mainly in the promastigote form (Pl. IV 13–15), because it can be symmetrical only in this forms. However, some epimastigote forms undergoing division were also observed (Pl. IV 15).
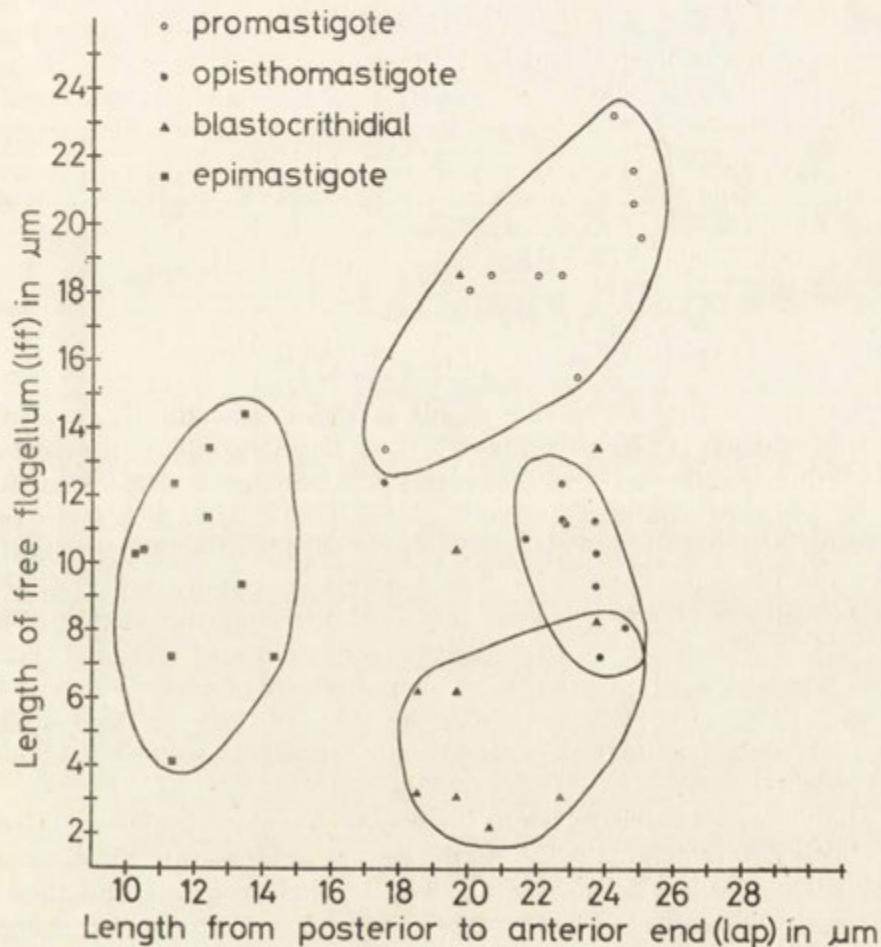
Diagram 1. Characterization of four morphological forms of *Blastocrithidia caliroae* sp. n. by the length of the free flagellum and length of the body

Taxonomic position

According to the present taxonomic concept in the family *Trypano-somatidae*, the flagellate found in *Caliroa cerasi* should be placed in the genus *Blastocrithidia* (W a l l a c e 1963, L i p a 1963 a, b). This conclusion is based on the fact that some morphological forms posses a short un-dulating membrane. In the life cycle of species of the related genera *Leptomonas* or *Herpetomonas* no forms with undulating membranes were observed.

This is the first record of a flagellate parasitic in *Caliroa cerasi*. W a l l a c e (1966) and S m i r n o f f and L i p a (1970) have tabulated all previous records of flagellates infecting *Hymenoptera* but there are no member of the genus *Blastocrithidia*. The flagellate studied is not iden-tical with any other *Blastocrithidia* species known from other insects because of morphological and host differences.

For the reasons given, we consider that the flagellate described by us from *Caliroa cerasi* is a new species, and the name *Blastocrithidia caliroae* sp. n. is proposed.

The holotype slide, stained with Giemsa, from host collected on Sep-tember 3, 1974, in Alsace, France is in the collection of the senior au-thor. Paratype slides are in the junior authors' collections.

Effect of the Pathogen on the Host

Except the first larval instar, all larval stages and the eonymphs have been found to be parasitised by the flagellate. Since the probabi-lity of infection increases with age, the proportion of diseased individuals is higher in mature than in young larvae (Table 3). While at an early stage of infection the infected larvae are indistinguishible from healthy ones and continue to move and feed normally, heavily infected larvae are recognizable by the dry apearance of their mucous coating. Their colour is a dull dark brown to blackish-brown, sometimes with yellow spots where the dried up slime has pealed off. Larvae showing this appearance eventually cease feeding and die and dry up before form-ing cocoons. Mortality occurs usually in the larval instars 4–6, rarely in younger larvae.

The disease is carried over to the eonymphal stage by larvae infected short before reaching maturity. As the disease progresses in the eonymphs their colour changes from bright yellow to reddish brown, and they die before changing to the pronymphal stage. From eonymphal samples hibernating under laboratory conditions, it would appear that although diseased individuals are eventually killed, the pathogen does not spread

during the coccon period to healthy individuals. It is likely that the disease is transmitted from one generation to the next through the adult stage, but infected adults have not yet been observed.

Observations on Epizootics

An epizootic of the flagellate was observed during an outbreak of the pear-slug in the Upper Rhine Valley near Waldshut (Federal Republic of Germany), where infected larvae were found at the peak of an outbreak in the autumn generation of 1973. Of 2500 larvae collected for shipment of parasites to New Zealand, 53% died in rearing before reaching the eonymphal stage. About 90% of the moribund or dead larvae examined contained heavy flagellate infections and showed external signs of the disease indicating that the pathogen had been the cause of death. The further development of this outbreak remained uncertain because all of the infected trees were removed in early 1974.

Near Vienna observations were carried out on an outbreak of *Caliroa cerasi* which developed since 1971 and collapsed in 1973. In 1972, mortality in eonymphs of the first generation of *C. cerasi* which were kept in cold storage at 8 + 1.5°C from July on, rose from 42% in August to 74% in November. About 40% of the larvae of the intermediate generation and about 60% of the eonymphal stage died of the disease. Simultaneously, the field population of the pear-slug started to decline in the autumn generation of 1972, when the larval population level dropped by about 80% as compared to the first generation. In 1973 *C. cerasi* occurred only at endemic level at which it continued in 1974. The sudden break-down of this population could neither be correlated with the action of hymenopterous parasites nor with abiotic factors. Winter conditions in 1972/73 were not severe, and although the total larval parasitism by hymenopterous parasites had increased from 0.6% in the summer generation of 1971 to 32% in 1972, it is known from similar instances observed elsewhere that this increase alone was insufficient to explain the sudden decline of the pear-slug population. Therefore, the principal cause of the retrogradation of this outbreak must be seen in the action of *Blastocrithidia caliroae* sp. n.

Conclusions

There is sufficient evidence from observations carried out on the flagellate so far that it is pathogenic and that it may be a regulating factor of pear-slug populations. At least in one instance, during an out-

break of *Caliroa cerasi* near Vienna, *Blastocrithidia caliroae* sp. n. was the principal cause of the break-down of the host population. There have been previous observations of high mortality at the peak of two outbreaks of *C. cerasi* near Waldshut and in the Alsace in 1966 where in individual samples 36–78% of the larvae and 45–80% of the eonymphs died. Both epizootics ceased the following year. The true cause of mortality was not recognized at that time, but it was evident that this unexplained mortality together with high parasitism by hymenopterous parasites had led to the collapse of these populations (C a r l 1972, 1976). From the symptoms observed in dying individuals in both these instances it can now be concluded that the unexplained mortality was due to the flagellate.

To date *Blastocrithidia caliroae* sp. n. has been recorded in Europe from western and southern parts of the Federal Republic of Germany, eastern France and Austria and from central Switzerland which suggests a fairly wide distribution. In Nelson, New Zealand, it probably arrived together with the original host population, or through parasite introductions from Europe carried out in the 1920s and again from 1970 onwards. The available information suggests that the pathogen is widespread and may be an important control agent of its host. It might be rewarding to obtain more information on the factors leading to epizootics in order to make use of the disease in biological control programmes of *C. cerasi* in geographic regions where the pathogen does not yet occur.

ZUSAMMENFASSUNG

Eine Flagellatenart, *Blastocrithidia caliroae* sp. n., wurde erstmalig in Neuseeland im Jahre 1972/73 in einer Feldpopulation der Kirschenblattwespe, *Caliroa cerasi* (L.), gefunden, Später wurde sie auch in Europa, dem ursprünglichen Verbreitungsgebiet des Wirtes, festgestellt. Infizierte Blattwespenpopulationen wurden in der Schweiz, der Bundesrepublik Deutschland, in Öesterreich und Frankreich nachgewiesen. Untersuchungen im Freiland zeigten, dass *B. caliroae* sp. n. epidemisch auftreten kann und dann eine sehr hohe Sterblichkeit der Wirtslarven und -eonymphen verursacht; die Ergebnisse deuten darauf hin, dass das Pathogen den Zusammenbruch von Massenvermehrungen der Kirschenblattwespe herbeizuführen vermag.

Im Entwicklungszyklus von *B. caliroae* sp. n. wurden die vier hauptsächlichen morphologischen Formen nachgewiesen, nämlich Blastocrithidia, Promastigoten, Opisthomastigoten und Epimastigoten. Die einzelnen Formen, ihre Grösse und relative Häufigkeit werden beschrieben.
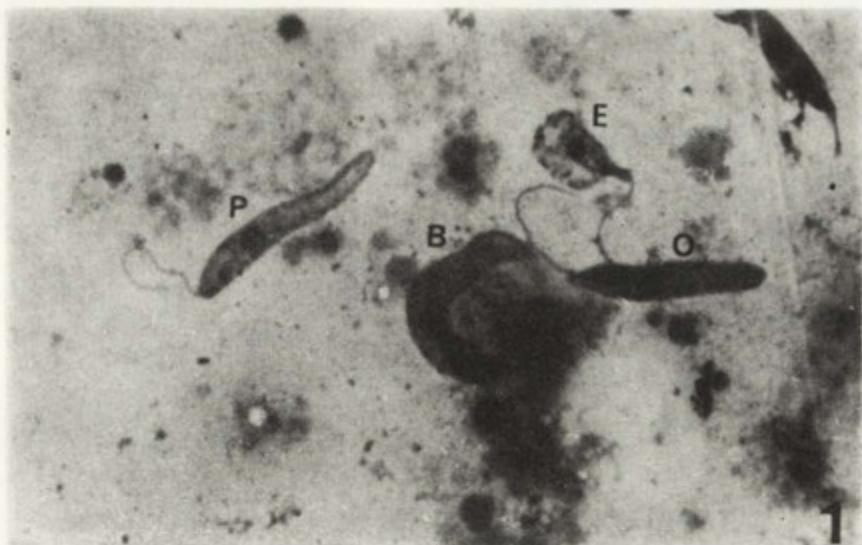
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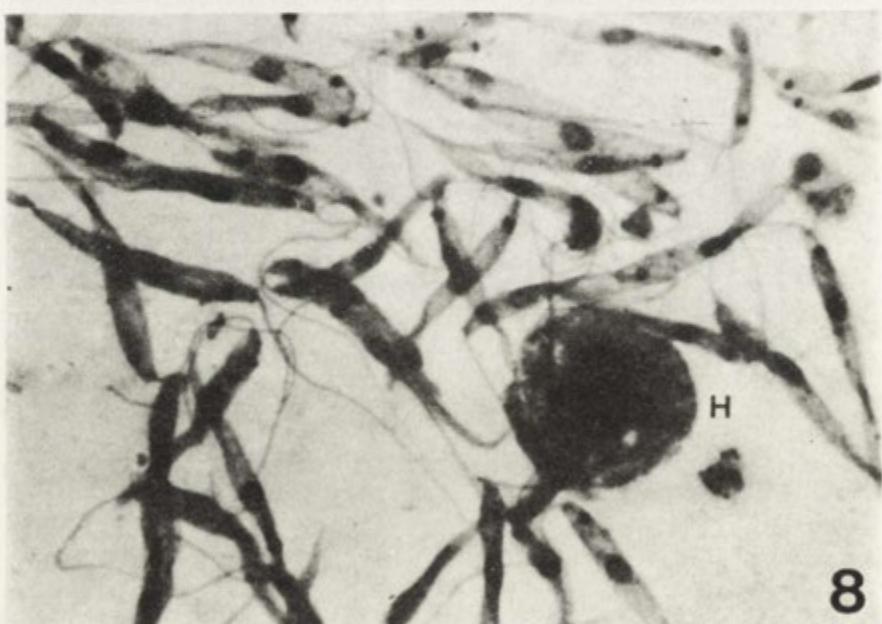
EXPLANATIONS OF PLATES I–IV

1: Various morphological forms of *Blastocrithidia caliroae* sp. n. P — promastigote,
B — blastocrithidial, E — epimastigote, O — opisthomastigote
2–5: Blastocrithidial forms with well seen undulating membrane and with kineto-
plast located posterior to the nucleus
6: Opisthomastigote with kinetoplast located posterior to nucleus and with flagel-
lum making a loop
7: Promastigote form with twisted body
8: A group of promastigote forms in the host haemolymph, H — haemocyte
9: A group of epistomastigote forms from the host haemolymph, H — haemocyte
10–11: Epimastigote forms, some being under binary fission
12: Two amastigote forms (A) and one epimastigote (E)
13–15: Promastigote forms in various stages of binary fission, notice that the
division of the body is preceded by the division of kinetoplast and nucleus
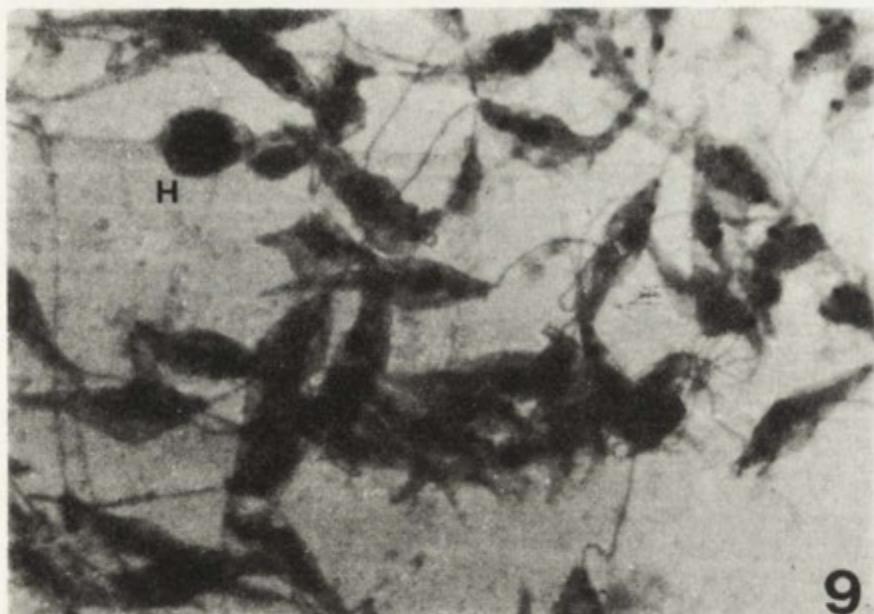
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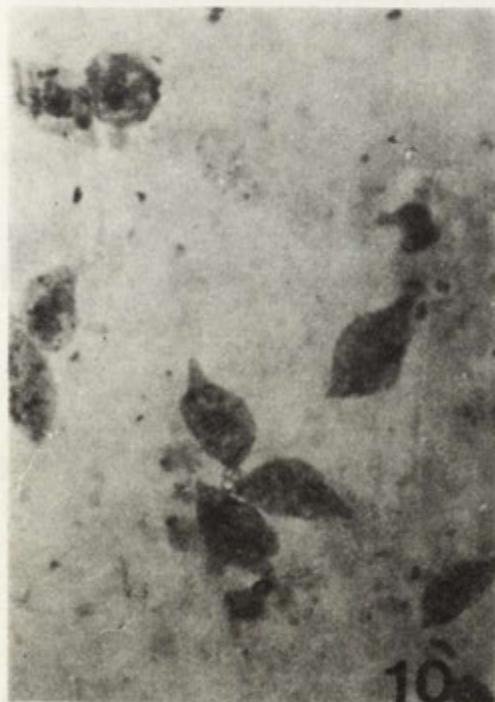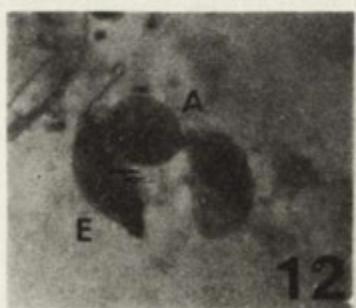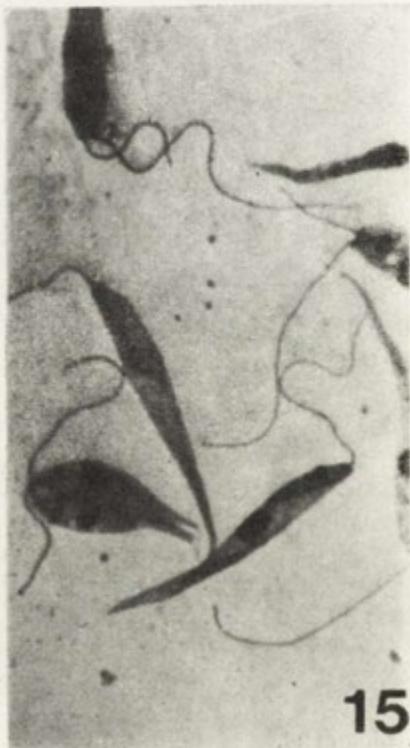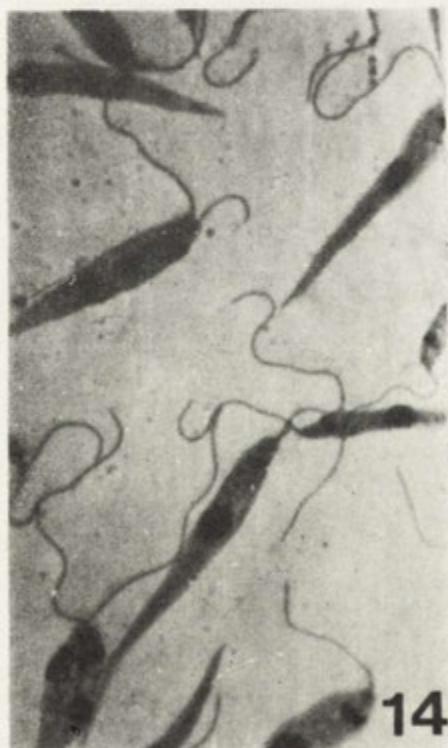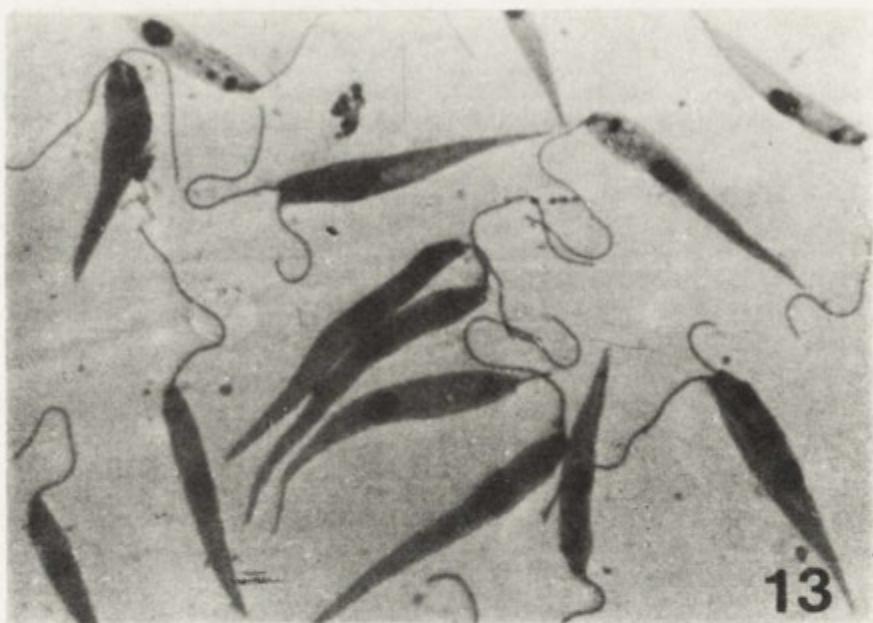
PLATE II

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J. J. Lipa et al.   auctores phot.

PLATE IV

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auctores phot.

JERZY J. LIPA

Nosema porphyriniae n. sp., a New Microsporidian Parasite of *Porphyrinia amasina* Eversman (*Lepidoptera, Noctuidae*)

Synopsis. A new microsporidian *Nosema porphyriniae* n. sp. is described from larvae and pupae of *Porphyrinia amasina* Eversman. The spores fixed and stained with Giemsa's solution are 2.9–4.1 by 1.8–2.4 μm. This microsporidian caused a general infection attacking various host tissues and was evidently responsible for the collapse of laboratory rearing of *P. amasina* introduced to Canada for the biological control of a weed *Cirsium arvense* (L.) Scop.

The stem borer moth, *Porphyrinia amasina Eversman,* is being introduced from Japan to Canada with the purpose of biological control of a weed *Cirsium arvense* (L.) Scop. (*Compositae*). This weed originating from Europe has been introduced to Canada in 17th century and is known there as the Canadian thistle.

In this paper a new microsporidian *Nosema porphyriniae* n. sp. is described which caused a general infection of pupae and larvae of *P. amasina.*

Material and Methods

Diseased and dead larvae and pupae of *Porphyrinia amasina* were obtained from Dr. D. P. Peschken of the Canada Agriculture, Research Station, Regina, Saskatchewan, during my vistit at Regina, August 20 to 24, 1973. These larvae and pupae originated from a dying laboratory rearing maintained for three generations under a quarantine at the Research Station.

Larvae and pupae were microscopically examined and the smeared tissues were stained with 0.25% Giemsa's solution for 24 h.

Results

Out of 8 larvae and 5 pupae 2 and 3 were infected, respectively; two dead moths were free from infection.

Development

S c h i z o g o n y: Uninucleate, binucleate and tetranucleate schizonts were observed and their diameter varied from 3 to 6 μm. Mature schizonts divided into two daughter cells. The cytoplasm of schizonts was dense and stained deeply blue so the nuclei were difficult to see against the dark background (Pl. I 1).

S p o r o g o n y: Sporonts were elongate cells with lighter cytoplasm than schizonts. They were binucleate and their length was up to 5 μm.

S p o r e s: Spores are ovoidal and are stained well with Giemsa's stain in their central part (Pl. I 2). The spores fixed in methyl alcohol and stained with Giemsa's solution varied from 2.9 to 4.1 μm in length and from 1.8 to 2.4 μm in width. The measurements of a sample of 30 spores are given in Table 1.

Table 1

Frequency distribution of the length of one sample of 30 spores of *Nosema porphyriniae* n. sp.

Dimensionable groups in μm			
2.5–2.9	3.0–3.4	3.5–3.9	4.0–4.4
3	17	8	2

Pathology

This microsporidian caused a general infection attacking gut, silk glands (Pl. I 3), gonads (Pl. I 4), hemocytes (Pl. I 2) and other tissues.

The intensity of infection indicates that this microsporidian is a lethal pathogen of *P. amasina*. Evidently this parasite was the main cause or one of the causes of the collapse of the rearing stock of *P. amasina* at the Research Station at Regina.

Taxonomic Position

The type of development clearly indicates that the microsporidian involved belongs to the genus *Nosema*. Several microsporidians have been reported from *Noctuidae* but no one was mentioned to infect *P. amasina*.

Since this insect was collected in Japan, and after importation to Canada was kept under quarantine, it is obvious that the parasite was brought together with its host.

Careful comparison of the spore size of the studied microsporidian with the size of spores of other *Nosema* species known from *Noctuidae* indicates that it is a new species and a name *Nosema porphyriniae* n. sp.

is proposed for it. Such assumption is based on the fact that there are differences in the size distribution of spores of *Nosema porphyriniae* n. sp. comparing with other microsporidians involved.

Discussion

Several insects have been introduced to Canada with the purpose of biological control of *Cirsium arvense* (G o e d e n et al. 1974). One of them is a noctuid *Porphyrinia amasina* imported from Japan that was bred successfully in the laboratory on *C. arvense* in preparation for screening tests (H a r r i s a n d P e s c h k e n 1974).

Dr Peschken, who kindly supplied the insects for this study, has informed me that after three generations the laboratory colony of *P. amasina* has collapsed. It was suspected that the cause of that collapse was improper photoperiod. However, in the light of my studies it seems that an important role in the collapse of the laboratory rearing of *P. amasina* played *Nosema porphyriniae* n. sp. which infected 38% of the examined larvae and pupae.

ZUSAMMENFASSUNG

Beschrieben wurde eine neue Mikrosporidienart *Nosema porphyriniae* n. sp. aus Larven und Puppen der *Porphyrinia amasina* Eversman. Die fixierten und Giemsa-gefärbten sporen messen 2.3–4.1 × 1.8–2.4 µm. Diese Mikrosporidienart verursachte eine allgemeine Infektion und bewirkte das Aussterben der Laborzüchtung von *P. amasina*. Diese Insekte wurden nach Kanada aus Japan zwecks biologische Bekämpfung des Unkrautes *Cirsium arvense* (L.) Scop. eingeführt.

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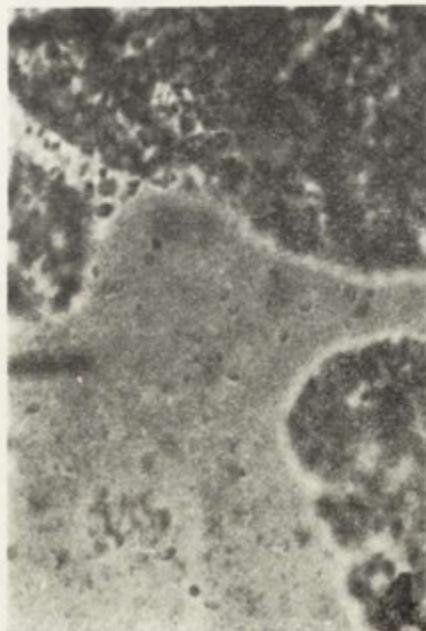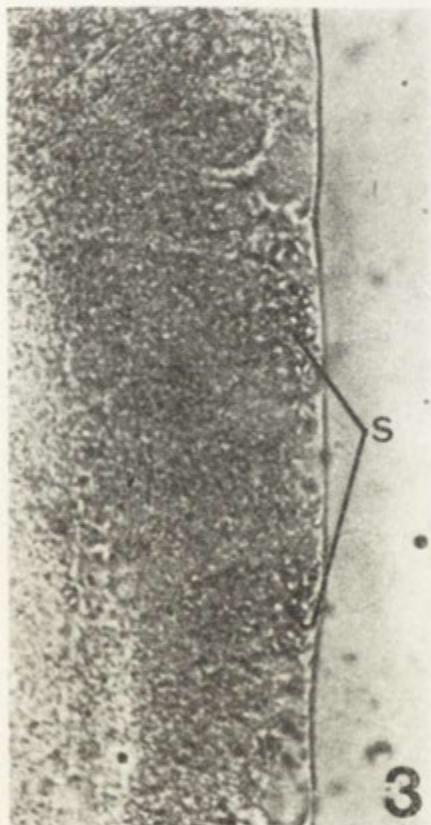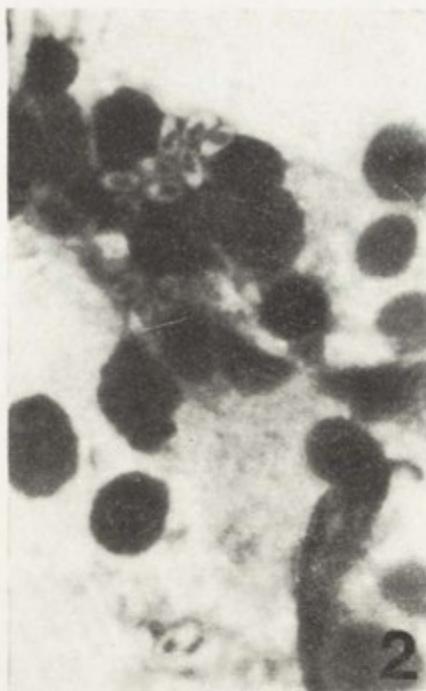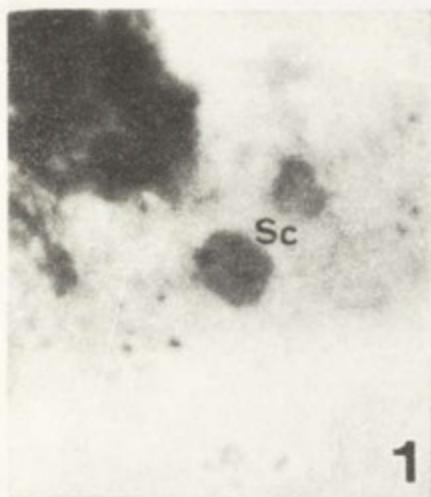
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EXPLANATION OF PLATE I

Nosema porphyriniae n. sp.

1: Tetranucleate schizont
2: Spores and hemocytes
3: Groups of spores in silk gland
4: Spores in male gonad

PLATE I

J. J. Lipa

JERZY J. LIPA

Nosema pyrrhocoridis n. sp., a New Microsporidian Parasite of Red Soldier Bug (*Pyrrhocoris apterus* L.) (*Heteroptera, Pyrrhocoridae*)

Synopsis. A new microsporidian *Nosema pyrrhocoridis* n. sp. is describ-
ed parasitizing in the red soldier bug (*Pyrrhocoris apterus* L.). The
parasite infects mainly the midgut epithelium and the Malpighian
tubules. The fixed and stained spores measured 3.0–6.0 $\times$ 1.8–3.0 μm.
The infection level was low since two adults and two fifth instar
larvae were infected out of 60 examined insects in the given population.
A list of microsporidians recorded in *Hemiptera* is given.

The red soldier bug (*Pyrrhocoris apterus* L.) is a very common insect
feeding on fruits of linden trees (*Tilia* spp.). It also feeds on other plants,
dead animals and sometimes is recorded as plant pest (L i p a and L i p o-
w a 1957). In many laboratories this insect is reared in mass for physiolo-
gical and biochemical experiments.

During my work on the flagellate *Leptomonas pyrrhocoris* (Zotta)
parasitic in the soldier bug I have examined several hundreds of larvae
and adults of *P. apterus* observing only the flagellate infection. However,
on July 15, 1967, during a field work at Białowieża National Park in
a few specimens of *Pyrrhocoris apterus* I have found a new parasitic
microsporidian *Nosema pyrrhocoridis* n. sp. that is described in this
paper.[1]

Material and Methods

Larvae and adults of red soldier bug (*Pyrrhocoris apterus*) collected on July
15 and 16, 1967 at Białowieża National Park were examined microscopically.
Smeared preparations were prepared from the tissues of infected insects, fixed
in methyl alcohol and stained with 0.5% Giemsa's solution for 24 h. Spores were
measured using an eyepiece micrometer and photographed using Zeiss Belichtungs-
automatik, Jena.

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[1] The microsporidian *Nosema pyrrhocoridis* n. sp. was also recorded in *Pyrrho-
coris apterus* adults and larvae collected in Bari, Italy, on November 10, 1976 by
Mgr. Jadwiga Ziemnicka of our Institute.

Results

Out of 60 examined specimens of *Pyrrhocoris apterus* only two adults and two fifth instar larvae were infected. This indicates that the level of microsporidian infection in the studied population of *P. apterus* was low. In spite of examining a large number of specimens collected in other localities this microsporidian was not observed.

Development

S c h i z o g o n y: Young schizonts are uninuclate and have 2–3 μm in diameter. Older schizonts have two or four nuclei and are 4 to 6 μm in diameter (Pl. I 1,2). The cytoplasm of schizonts is stained deeply blue and the nuclei deeply red.

S p o r o g o n y: Sporonts are elongate and their length is up to 8 μm (Pl. I 4). They are binucleated and are stained weaker than schizonts.

S p o r e s: Spores are oval and have well seen deeply stained two nuclei (Pl. I 1, 3, 4 II 5, 6). The spores fixed and stained are 3.0–6.0 by 1.8–3.0 μm. The measurements of a sample of 150 spores are given in Table 1.

Table 1

Frequency distribution of the length of one sample of 150 spores of *Nosema pyrrhocoridis* n. sp. and of 50 spores of *Nosema apis* Zander

Microsporidian	Dimensionable groups in μm						
	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0
Nosema pyrrhoco-ridis n. sp.	1	6	32	33	43	26	9
Nosema apis Zander					8	34	8

Infected Tissues

The spores and the developmental stages of *Nosema pyrrhocoridis* n. sp. were most frequently observed in the smears of midgut epithelium and of Malpighian tubules.

However, the parasite was also observed in hemocytes (Pl. II 5) and in fat body (Pl. II 6).

Taxonomic Position

The type of development of the microsporidian involved clearly indicates that it belongs to the genus *Nosema*. In the literature there is no provious record of the fully identified microsporidian infection of *Pyrrhocoris apterus*. However, G r o b o v (1967) reported that spores of *Nosema apis* Zander were present and survived in the gut of *P. apterus* but

he did not observed the presence of schizogonic or sporogonic stages of *N. apis* in the tissues of this insect.

As seen in Table 1 there is a clear and significant differences in the size of spores of *Nosema apis* Zander and *Nosema pyrrhocoridis* n. sp. which indicates that they are different species.

Table 2

Microsporidians recorded in insects of the order *Heteroptera*

Microsporidian	Host	Size of spores in μm	Infected tissues	References
Nosema adiei (Christophers)	*Cimex rotundatus* L.	3 × 1.7	gut, Malpighian tubules, salivary glands	Christopers (1922) Shortt and Swaminath (1924)
Nosema bialovesianae Lipa	*Nepa cinerea* L.	2.8–3.3 × 1.9–2.2	midgut, fat body	Lipa (1966)
Nosema nepae Poisson	*Nepa cinerea* L.	4.2–5.9 × 2.4–3.1	midgut, fat body and others tissues	Poisson (1928); Lipa (1966)
Nosema veliae Poisson	*Velia currens* F.	5.5–7 × 3	midgut, fat body, enocytes	Poisson (1928), Weiser (1961)
Nosema pyrrhocoridis n. sp.	*Pyrrhocoris apterus* L.	3.0–6.0 × 1.8–3.0	midgut, Malpighian tubules, fat body, hemocytes	Lipa, this paper
Nosema graphosomae Galli-Valerio	*Graphosoma italicum* Müll.	not given	not given	Galli-Valerio (1924); Weiser (1961)
Thelohania veliae Poisson	*Velia currens* F.	5.5–7 × 3	midgut, fat body, enocytes	Poisson (1928)
Thelohania mercieri Poisson	*Notonecta viridis* Del.	4.5 × 1.5	fat body	Poisson (1924)
Thelohania nepae Lipa	*Nepa cinerea* L.	2.0-3.0 × 1.4–1.8	fat body, midgut	Lipa (1966)
Toxoglugea gerridis Poisson	*Aquarius najas* Deg.	4.5 × 0.8	fat body	Poisson (1940)
Toxoglugea mercieri Poisson	*Notonecta viridis* Del.	4,5–5.0 × 1.5	fat body	Poisson (1940)

In Table 2 all microsporidian species recorded in *Heteroptera* are listed. It is seen that the size of spores and tissues infected differ *Nosema pyrrhocoridis* n. sp. from other *Nosema* spp. known from *Heteroptera*.

Therefore, I consider that the microsporidian I found in *Pyrrhocoris apterus* has not been described previously and I propose a name *Nosema pyrrhocoridis* n. sp. for it.

Discussion

The red soldier bug (*P. apterus*) is an extremely common insect and is occurring in a great number in the whole Europe. It would seem, therefore, that pathogens, predators and parasites that attack this insect should be numerous. This is not so.

The only protozoan known previously in *P. apterus* was a flagellate *Leptomonas pyrrhocoris* Zotta (Z o t t a 1926, L i p a 1963). However, G r o b o v (1967) when studying the role of insects in transmitting the spores of *Nosema apis* Zander in nature reported the presence of microsporidian spores in the gut of *P. apterus*. Out of 20 insects collected at the end of June and the beginning of July 1962, in the gut of two adult bugs G r o b o v found structures resembling *Nosema* spores. Since these red soldier bugs were collected close to bee hives where infection by *Nosema apis* Zander was occurring, G r o b o v considered that this microsporidian was involved.

G r o b o v infected healthy bugs with spores isolated from naturally infected *P. apterus* and obtained a positive infection. Spores were observed in midgut smears prepared on 3rd, 5th and 15th day after infection. The excrements of the infected bugs contained spores.

When spores isolated from the gut or excrements of *P. apterus* were fed to 65 adult honey bees (*Apis mellifera* L.) 35 bees exhibited microsporidian infection. They had a diarrhea and their midgut showed pathological changes typical to *Nosema apis* infection. Among 64 untreated bees infection was not observed.

In further experiments spores of *Nosema apis* were fed with honey solution to the following insects: *Hemiptera*: *Pyrrhocoris apterus* L.; *Hymenoptera*: *Lasius niger* L., *Pseudovespa vulgaris* L., *Vespa crabo* L.; *Diptera*: *Musca domestica* L.; *Orthoptera*: unidentified grasshopper. Although spores of *N. apis* were observed in the gut of all insects for several days after feeding only spores isolated from excrements or from the gut of *P. apterus* caused infections among healthy honey bees; spores isolated from other insects were not infectious.

The experiments conducted by G r o b o v (1967) indicate that spores of *Nosema apis* passing through the gut of insects listed above lost their viability except spores passing through the gut of *P. apterus*. It is rather surprising and these experiments should be verified. It should be taken

under consideration that in the gut and tissues of *P. apterus*, like in other hemipterans, are present symbiotic yeast which are very similar to *Nosema* spores. As a matter of fact in his paper G r o b o v (1967) refers to „Structures resembling microsporidian spores" present in the gut of *P. apterus*.

It is, at present, impossible to decide without examining the smeared preparations prepared by G r o b o v whether he observed in the examined specimens of *P. apterus* the yeasts, the spores of *Nosema apis* or the spores of *N. pyrrhocoridis* n. sp.

However, in the light of my previous studies on the feeding habits of *P. apterus* (L i p a and L i p o w a 1957) the possibility of the presence of spores of *Nosema apis* in the gut of this bug cannot be rejected. The red soldier bug feeds on dead animals and sucking the body of dead *A. mellifera* may swallow the spores of *Nosema apis*.

However, as seen in Table 2 the spores of *Nosema apis* are larger than spores of *Nosema pyrrhocoridis* n. sp. Therefore, both species can be easily recognized and separated basing on the size of spores and the presence of the developmental stages of *N. pyrrhocoridis* n. sp. in the tissues of *P. apterus*.

ZUSAMMENFASSUNG

Beschrieben wurde eine neue Mikrosporidien-Parasit *Nosema pyrrhocoridis* n. sp. von *Pyrrhocoris apterus* L. Der Parasit infiziert vornahmlich die Darmwand und Malpigische Gefässe. Die fixierten und Giemsa-gefärbten sporen messen 3.0–6.0 × 1.8–3.0 μm. Das Infektionsniveau was niedrig da von 60 untersuchten Insekten der gegebenen Population nur zwei Imaginen und zwei Larven des V Stadiums infiziert wurden. Eine Aufzälung der bei *Hemiptera* festgestellten Mikrosporidien wird angegeben.

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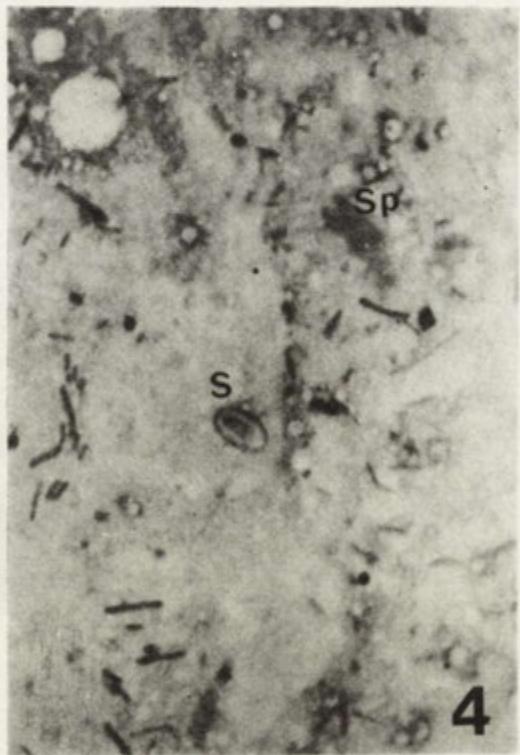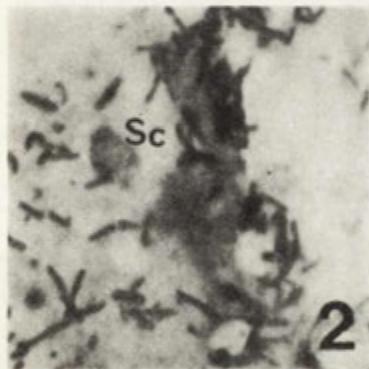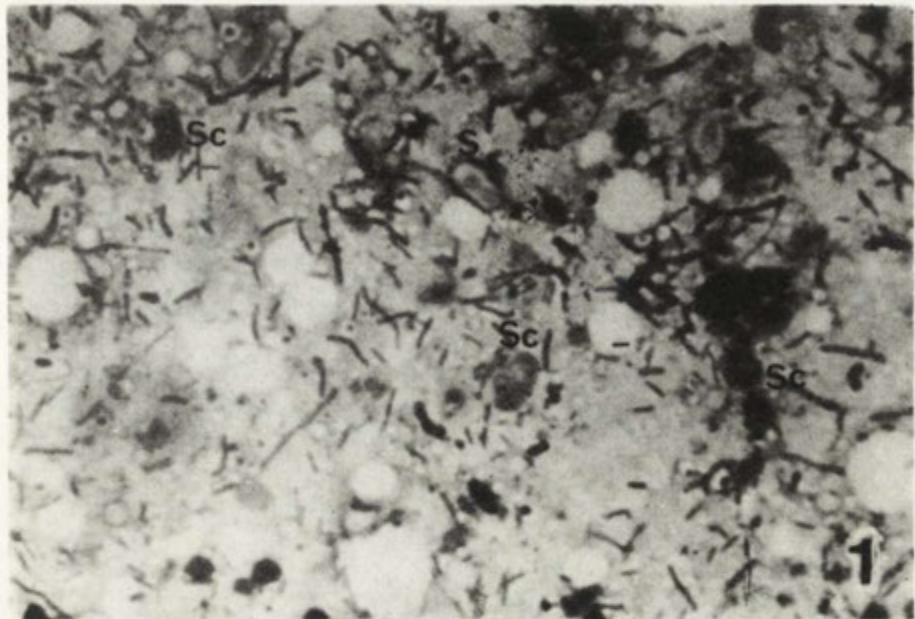
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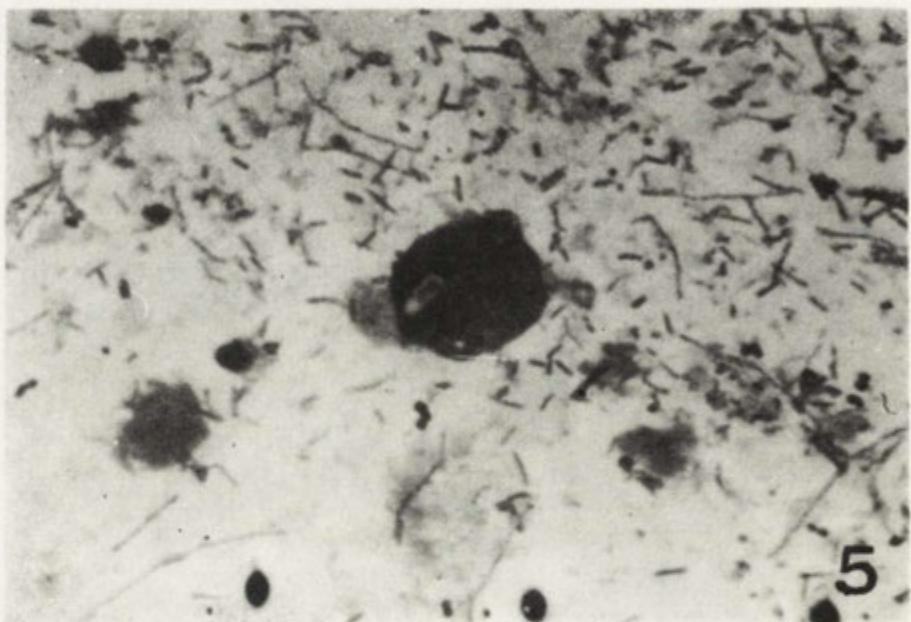
EXPLANATION OF PLATES I–II
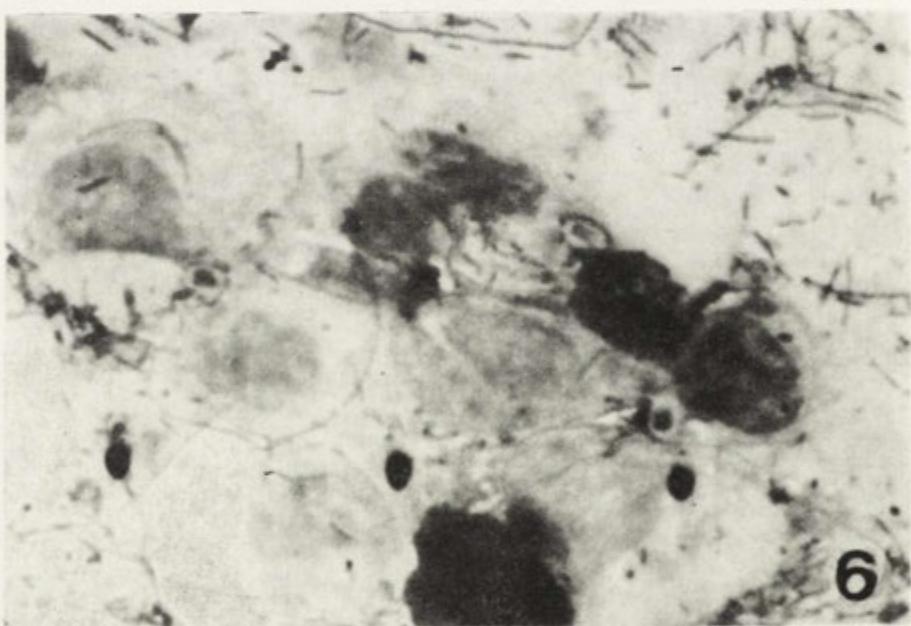
Nosema pyrrhocoridis n. sp.

1: Schizonts (Sc) and spores (S) in smeared preparation
2: Binucleate schizont
3: Binucleate spore
4: Sporont (Sp) and spore
5: Spore in a hemocyte
6: Spores in the smeared fat body

PLATE I

J. J. Lipa

auctor phot.

PLATE II

J. J. Lipa auctor phot.

Jerzy J. LIPA

Microsporidian Infections of *Galleria mellonella* (L.) (*Lepidoptera, Galleriidae*) with the Description of a New Species *Nosema galleriae* n. sp.

Synopsis. A new microsporidian *Nosema galleriae* n. sp. infecting the great wax moth (*Galleria mellonella* L.) is described. This microsporidian caused epizootics in laboratory cultures of *G. mellonella* and was recorded in the USA and in the Soviet Union. The parasite attacks various tissues and causes the general infection. The schizogony and sporogony of *N. galleriae* n. sp. was described. The living spores are 3.1–4.6 × 2.0–2.1 μm; fixed and stained spores are 2.5–5.1 × 1.8–3.0 μm.

A second microsporidian of the genus *Nosema* was observed in *G. mellonella* which has small spores 1.3–2.1 × 0.8–1.1 μm.

The great wax moth (*Galleria mellonella* L.) is frequently occurring pest in bee hives. The larvae feed on pollen and wax of combs spinning the silk tunnels and burrowing in them. Besides of the direct damage to combs this insect may serve as vector of European and American foulbrood, the last caused by *Bacillus larvae* White.

There are several known parasites, predators and pathogens of *G. mellonella* and they were listed by Borchert (1966). So far, however, there was no record of natural protozoan infection of *G. mellonella* except experimentally induced infections in the laboratory. The purpose of this paper is to present results of a taxonomic study on a new microsporidian *Nosema galleriae* n. sp. and a note on another *Nosema* sp. from *G. mellonella*.

This study was undertaken when the author worked at the Laboratory of Insect Pathology, University of California, Berkeley, USA, as the fellow of the Rockefeller Foundation (1958–1959) and completed at the Institute of Plant Protection, Poznań, Poland.

Material and Methods

From March to July of 1959 a heavy epizootic of a microsporidian infection in a laboratory culture of *Galleria mellonella* kept on Haydak's diet in insectary of the Division of Biological Control, University of California at Albany was observed. Similar epizootic was observed during 1966–1967 in the laboratory culture of *G. mellonella* kept on wax combs at the All-Union Institute of Plant Protection in Leningrad.

Insects from both sources were used for experimental work. Dead and diseased larvae of *G. mellonella* were examined microscopically to estimate the infection level and tissues attacked. Smeared preparations were fixed in absolute methyl alcohol for 2 min and then stained with 0.5⁰/₀ Giemsa's solution for 24 h.

Artificial infections of various insect species were performed by microinjection of microfeeding applying to each larva a known number of purified spores.

Results

Nosema galleriae sp. n.

Host: *Galleria mellonella* (L.)
Habitat: general infection
Locality records: Albany and Berkeley, California, USA, 3.III.1959–16.VII.1959; Leningrad, Soviet Union, 25.XII.1967.

Pathology

Microscopical examination of infected and dead larvae showed that *Nosema galleriae* n. sp. caused a general infection. The parasite infected silk glands (Pl. I 1), Malpighian tubules (Pl. I 2), fat body, nerves (Pl. I 1), hemocytes (Pl. III 7, 8) and gonads. In many larvae midgut epithelium was also infected. The fact that in some larvae midgut was not infected was evidently due to the transovarial infection as these larvae originated from the infected laboratory rearings. When larvae of *G. mellonella* were infected per os the midgut was always infected.

Development

S c h i z o g o n y: The typical schizonts are spherical (Pl. II 3–6). When treated with Giemsa's stain, its cytoplasm appears as deep blue and the nuclei as deep red. There are variations in size and shape among schizonts; their diameter varies between 3 and 5 μm. In most schizonts two red-stained nuclei can be observed (Pl. II 3 and 4). Schizonts ready to undergo division have four nuclei (Pl. II 5).

S p o r o g o n y: The sporonts stain relatively lightly with Giemsa's solution, having a pink stained cytoplasm and a bright red nucleus. They

are elongate and their size is about 5 to 7 μm in length (Pl. II 6). The sporonts turn into sporoblasts which mature into spores.

S p o r e s: The spores vary in shape and size (Pl. III 7, 8, 9 and Pl. IV 10). The great majority of the spores are ovoidal. The length of fresh spores varies in distilled water from 3.1 to 4.6 μm, the width from 2.0 to 2.1 μm. Spores fixed in methyl alcohol and stained with Giemsa's solution measured 2.8–4.2 × 1.8–2.0 μm.

Spores of *Nosema galleriae* n. sp. collected in the USA and in the Soviet Union showed slight variations in size (Tables 1 and 3).

The length of polar filament varied from 40 to 80 μm.

Table 1

Frequency distribution of the length of spores of *Nosema galleriae* n. sp., and *Nosema* sp., from *Galleria mellonella* L.

Microsporidian	Dimensionable groups (in μm)								
	1.1–1.5	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	1.5–5.5
Nosema galleriae n. sp. fixed and stained spores: USA									
strain			1	21	14	14	1		
USSR strain			3	6	21	13	3	3	1
fresh spores:					9	32	8	1	
Nosema sp. fixed and stained spores	4	44	2						

Epizootiology

First larvae of *G. mellonella* infected with *Nosema galleriae* n. sp. were noticed on March 3, 1959 in Albany and Berkeley. These larvae were obtained as test insects for experiments with various insect pathogens. However, due to the fact that infection with *N. galleriae* n. sp. was discovered the whole colony was not suitable for such purpose.

The infection was observed among all larval instars (I to V), pupae and adults. No attempts were made to eliminate the microsporidian infection from the rearing stock of *G. mellonella*. At the final stage of a microsporidian epizootic (June 1959) about 68⁰/o of all developmental stages of *G. mellonella* were infected (Table 2). At the beginning of the epizootic (March 1959) the infection level was 18⁰/o only.

The epizootic in the rearing stock of *G. mellonella* in Leningrad had a similar course and at the final phase about 45⁰/o of insects were infected.

Table 2

Results of diagnosis of examined specimens of *Galleria mellonella* (L.) from insectary stock in Albany, June 15, 1959

Developmental stage of *G. mellonella*	Number of examined specimens	Number of healthy insects	Number of infected insects
Larvae	39	9	30
Pupae	11	5	6
Moths	12	6	6
Total	62	20	42

Artificial Infections

A few experiments were conducted in which larvae of *Galleria mello-nella*, *Junonia coenia* Hbn., *Pseudaletia unipuncta* Haw. and *Tenebrio molitor* L. were infected with spores of *Nosema apis* Zander, but the results of these tests were negative. This is in agreement with results of other investigators (B o r c h e r t 1966, G r o b o v 1967) indicating that *N. apis* is not infectious to other insects except adults of honey bees (*Apis mellifera* L.).

In two tests the infectivity of *Nosema galleriae* n. sp. was tested to larvae of silkworm (*Bombyx mori* L.) and the great wax moth (*G. mello-nella*). Only larvae of *G. mellonella* become infected while larvae of *B. mori* were resistant.

In one experiment 10 larvae of *Galleria mellonella* were microfeed with 15 000 spores of *Plistophora californica* Steinhaus et Hughes from *Peridroma margaritosa* Haw. but all larvae examined 11 days after microfeeding were healthy.

Taxonomic Position

Since one spore is produced from one sporont the microsporidian found in *G. mellonella* belongs to the genus *Nosema*. The first assumption was that this microsporidian is identical with *Nosema apis* Zander which seemed to be logical since both insects, that is honey-bee (*Apis melli-fera* L.) and the great wax moth (*Galleria mellonella* L.) inhabit the same biotope that is the hives. Besides, in many laboratories *G. mello-nella* is reared on the bee combs which may be contaminated with spores of *Nosema apis* causing the infection of *G. mellonella* larvae. However, the simple comparison of the spore dimensions of both microsporidians indicated that spores of *Nosema galleriae* n. sp. are much smaller than spores of *Nosema apis* which are 4.8–6.0 × 3.0–4.0 μm. Furthermore,

special experiments showed that *Nosema apis* is not infectious to larvae of *G. mellonella.*

When I firstly recorded the microsporidian infection of *G. mellonella* in 1959 and later in 1967 there was no literature record on this subject except paper by C h o r i n e (1930). This insect has been extensively used in several studies and successful and unsuccessful attempts to infect it with several microorganisms were made.

The earliest available information as far as microsporidian artificial infections are concerned is that by C h o r i n e (1930). This author reported that when spores of *Thelohania vanessae* Chorine, described from *Vanessa urticae* L., were injected to larvae of *G. mellonella*, amoebulae were liberated but they were phagocytased by blood cells and therefore no infection developed.

In a series of papers K e l l e n and L i n d e g r e n (1968, 1969, 1973) described four microsporidians from *Plodia interpunctella* and *Cadra* spp. which were found by them and other authors (S p r e n k e l 1973, N o r d i n and M a d o x 1974) to be infectious to *Galleria mellonella* and other insects. The characteristics of these microsporidians are given in Table 3.

Dr J. W e i s e r (personal communication) has pointed out that since *Galleria, Plodia, Cadra* and *Ephestia* may occur together in stored products or in rearings they may harbour the same pathogens. Therefore it is necessary to compare carefully the microsporidians described from those insects, since microsporidians of *Galleria, Plodia* and *Cadra* were collected in California.

As seen in Table 3 *Nosema galleriae* n. sp. differs distinctly from such species like *Nosema heterosporum* and *Thelohania nana* by the size of its spores.

Although spores of *Nosema plodiae* are somewhat in the range of spore size of *N. galleriae* n. sp. but these two species differ in their pathogenicity to *G. mellonella.* K e l l e n and L i n d e g r e n (1968) obtained only subacute infections of *G. mellonella* caused by *N. plodiae.* On the other hand in my investigations *N. galleriae* n. sp. showed high pathogenicity to *G. mellonella* larvae.

Since no inflammatory reaction was observed in *G. mellonella* infected with *N. galleriae* n. sp. this microsporidian cannot be identified with *N. invadens* described by K e l l e n and L i n d e g r e n (1973).

A microsporidian *Octosporea ephestiae* (Mattes 1928) known from *Ephestia kühniella* Zell. differs from *Nosema galleriae* n. sp. by its development and attacking the fat body only.

Summarizing, I consider that *Nosema galleriae* n. sp. differs from microsporidians described in other insects and reported to be infectious

Table 3

Comparison of *Nosema galleriae* n. sp. and *Nosema* sp. with *Nosema apis* Zander and other microsporidians reported to be infections to *Galleria mellonella* L.

Microsporidian	Original host	Type of infection	Spore size in μm F – fresh S – stained	Remarks on pathogenicity to *Galleria mellonella*	References
Nosema galleriae n. sp. USA strain USSR strain	*Galleria mellonella* L.	General	F: 3.1–4.6 × 2.0–2.1 S: 2.8–4.2 × 1.8–2.0 S: 2.5–5.1 × 1.8–3.0	highly infectious and causing a general infection	Lipa, this paper
Nosema sp.	*Galleria mellonella* L.	unknown	S: 1.3–2.1 × 0.8–1.1	lack	Lipa, this paper
Nosema plodiae Kellen et Lindegren	*Plodia interpunctella* Hbn.	general	F: 4.09±0.24 × 1.89±0.03 S: 3.43±0.04 × 1.61±0.03 F: 3.67±0.37 × 1.79±0.22	subacute infection	Kellen and Lindegren (1968) Sprenkel (1973)
Nosema heterosporum Kellen et Lindegren	*Plodia interpunctella* Hbn.	mainly midgut and fat body	F: 5.46±0.65 × 1.99±0.03 S: 4.95±0.09 × 2.08±0.01	successful infection without comments	Kellen and Lindegren (1969)
Nosema invadens Kellen et Lindegren	*Cadra cautella* Wlk. *Cadra figulilella* Gregson	general	F: 4.37±0.54 × 1.80±0.14 S: 4.41±0.41 × 2.0±0.17	successful infection without comments	Kellen and Lindegren (1969)
Thelohania nana Kellen et Lindegren	*Plodia interpunctella* Hbn.	fat body and muscles	F: 2.07±0.03 × 1.86±0.02	successful infection without comments	Kellen and Lindegren (1969)
Thelohania vanessae Chorine	*Vanessa urticae* L.	fat body	4.2–6.0 × 3.0–4.0	heavy phagocytosis prevented infection	Chorine (1930)

to *G. mellonella*. The positive results of artificial infections does not necessarily mean that a specific insect is a natural host for a given microsporidian. Therefore, I consider that the microsporidian studied by me in *G. mellonella* has never been described previously and I propose a name *Nosema galleriae* n. sp. for it.

Nosema sp.

Host: *Galleria mellonella* (L.)
Habitat: unknown
Locality record: unknown; material obtained on August 8, 1959 from Dr. Jaroslav Weiser, Laboratory of Insect Pathology, Institute of Entomology, Prague, Czechoslovakia.

Spores of *Nosema* sp. recorded by Dr. W e i s e r in *G. mellonella* are much smaller than of *Nosema galleriae* n. sp. As seen in Tables 1 and 2 the spores were 1.3–2.1 by 0.8–1.1 µm (Pl. IV 11). As no more data are available about this species it is difficult to discuss its taxonomic status.

Discussion

As mentioned earlier the great wax moth (*G. mellonella*) is a well known pest in bee hives and frequently various control measures against this insect are undertaken. Therefore, the knowledge of the effective pathogens of *G. mellonella* may lead to the development of biological control of this pest.

However, *G. mellonella* is broadly used in various biochemical and pathological experiments. In such cases all pathogens from its laboratory rearings should be eliminated as they can interfere with some experiments or can make difficult to rear this insect in great number.

For the reasons mentioned above the finding of the microsporidian *Nosema galleriae* n. sp. described in this paper is of interest from the theoretical and practical standpoint.

As it was mentioned earlier *Nosema apis* is not infectious for *G. mellonella* larvae. This was proved in some tests during this study and besides nobody in the literature mentioned such successful infections.

It should be pointed, however, the possibility of the accidental presence of spores of *Nosema apis* in the gut lumen of *G. mellonella* larvae. This is quite possible when *G. mellonella* will feed on wax combs taken from hives where bee colony is heavily infected with *Nosema apis*. When such *G. mellonella* larvae would die due to various causes one could come to the false conclusion, at the microscopical examination, that this was due to infection with *Nosema apis*. Therefore, all cases of diseases

of *G. mellonella* should be carefully examined and verified by artificial infections.

As cultures of *G. mellonella* are maintained in many insect pathology laboratories one could expect that there should be several cases of the accidental infections of this insect with many pathogens including microsporidians. However, this is not the case. It may be emphasized that although in 1958–1959 several insect species were reared in laboratory and insectary in Berkeley and Albany only *G. mellonella* rearing was infected with *Nosema galleriae* n. sp.

Unfortunately, the spores of *N. galleriae* n. sp. stored since 1959 lost their viability and it is not possible at present to perform infections of *Plodia* spp., *Cadra* spp. and *Ephestia* spp.

N. galleriae is rather a rare parasite since it was discovered relatively recently in spite of intensive study of pathogens of *G. mellonella*. However, the fact that larvae of *G. mellonella* infected with *Nosema galleriae* n. sp. were recorded in the USA and in the USSR indicates that this microsporidian is widely distributed and evidently occurs everywhere its host *G. mellonella* occurs.

Unfortunately, the lack of material and its type — only the microscopic slides — obtained from Dr. W e i s e r and referring to *Nosema* sp. with small spores does not allow to make more detailed studies on this microsporidian. It is difficult to state whether this is a true pathogen of *G. mellonella* or whether these slides were prepared from larvae having spores of an unidentified *Nosema* in their gut lumen as a results of feeding on contaminated food. This is quite probable as no schizogonic or sporogonic stages were observed on examined smeared preparations.

ZUSAMMENFASSUNG

Beschrieben wurde neuer Mikrosporidien-Parasit *Nosema galleriae* n. sp. der *Galleria mellonella* L. Diese Mikrosporidien-Art verursachte Epizootien in Laborzuchten der *G. mellonella* in U.S.A. und U.S.S.R. Der Parasit infiziert verschiedene Gewebe und verursacht eine allgemeine Infektion. Beschrieben wurde ein Schizo-

gonien- und Sporogonien-Zyklus der *N. galleriae* n. sp. Lebende Sporen messen 3.1–4.6 $\times$ 2.0–2.1 µm; fixierte und gefärbte 2.5–5.1 $\times$ 1.8–3.0 µm.

Eine andere Mikrosporidien-Art derselben Gattung *Nosema* mit kleinen Sporen (1.3–2.1 $\times$ 0.8–1.1 µm) wurde bei *G. mellonella* beobachtet.

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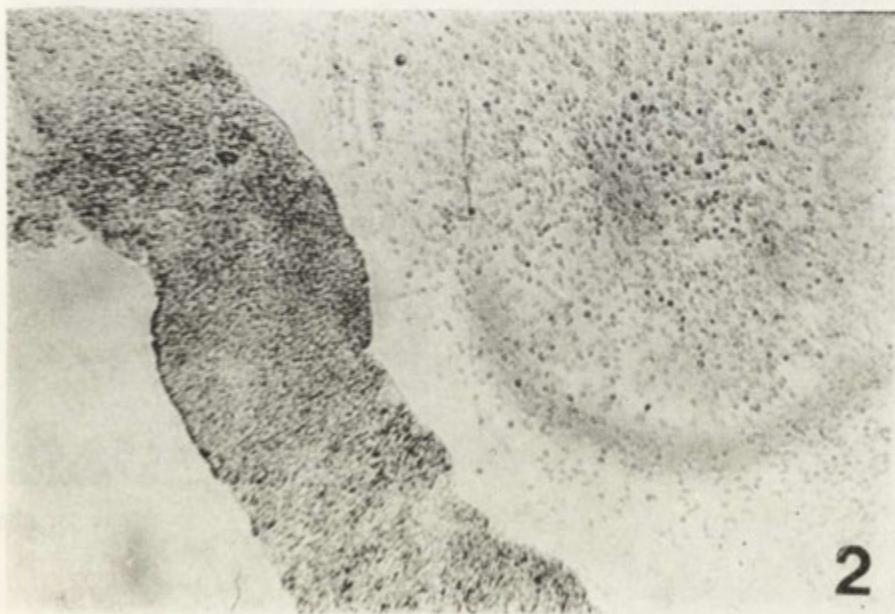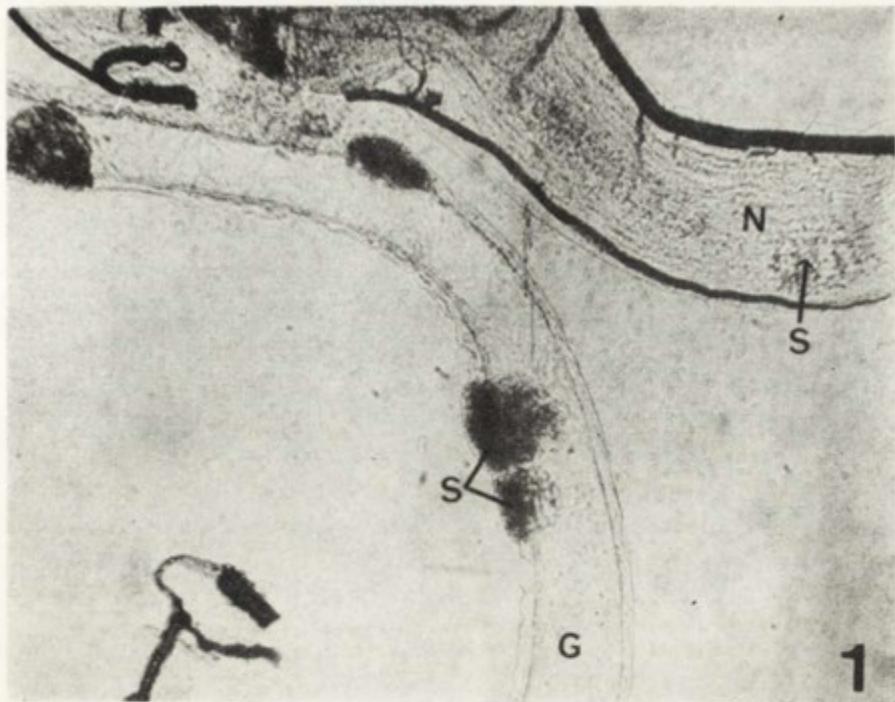
EXPLANATIONS OF PLATES I–IV

Nosema galleriae n. sp.

1: Masses of spores (S) of *Nosema galleriae* n. sp. in silk glands (G) and in nerve ganglions (N); fresh preparation
2: Malpighian tubules completely filled with spores of *Nosema galleriae* n. sp.; fresh preparation
3–5: Binucleate and tetranucleate schizonts of *Nosema galleriae* n. sp.
6: One sporont (Sp), schizonts (Sc) and spores of *Nosema galleriae* n. sp.
7–9: Sporonts (Sp), schizonts (Sc) and spores of *Nosema galleriae* n. sp. seen within hemocytes of *Galleria mellonella* L.
10: Spores of *Nosema galleriae* n. sp.
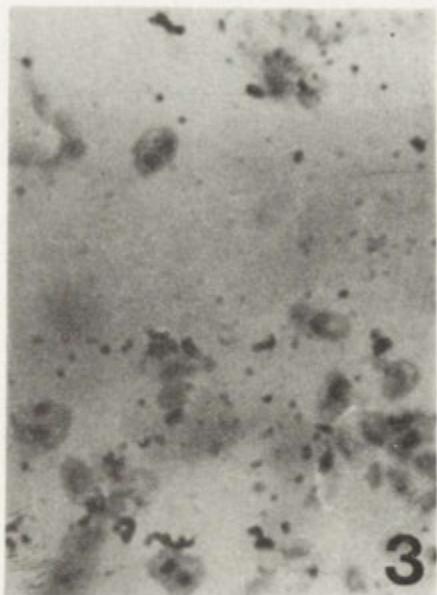
Nosema sp.

11: Spores of *Nosema* sp.; notice the small size of spores as compared with spores of *Nosema galleriae* n. sp. seen on Fig. 10
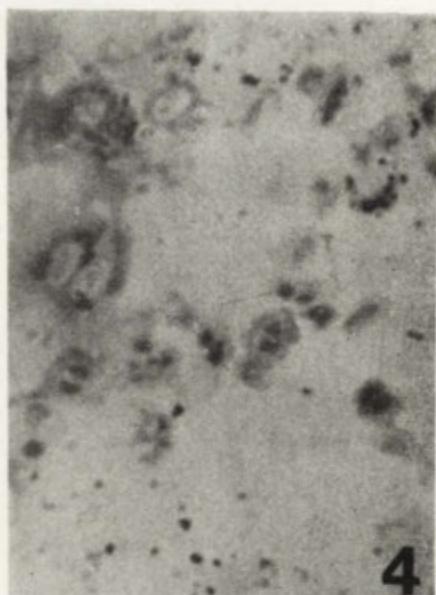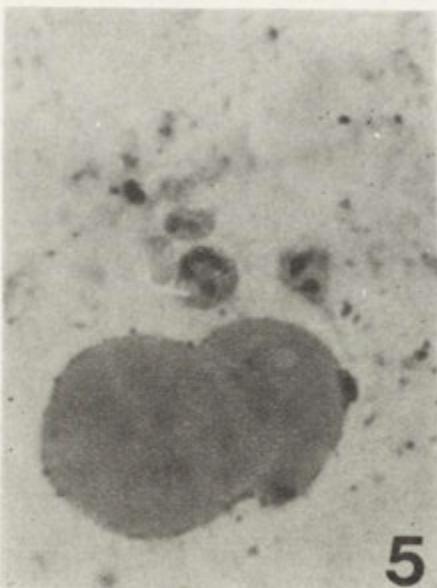
PLATE I

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PLATE II

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PLATE III

J. J. Lipa auctor phot.

PLATE IV
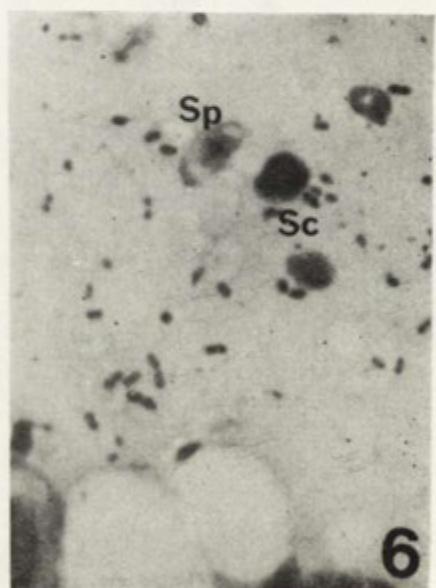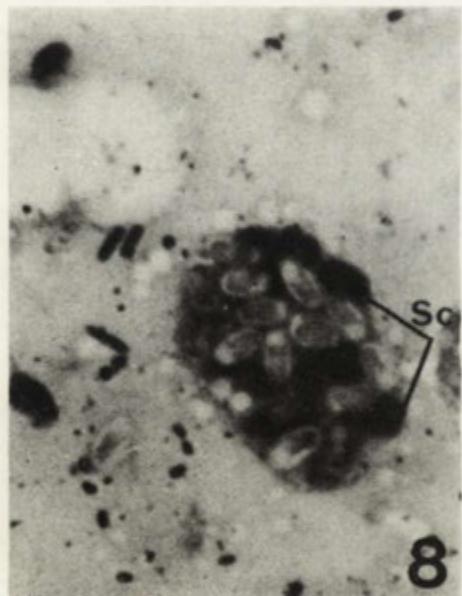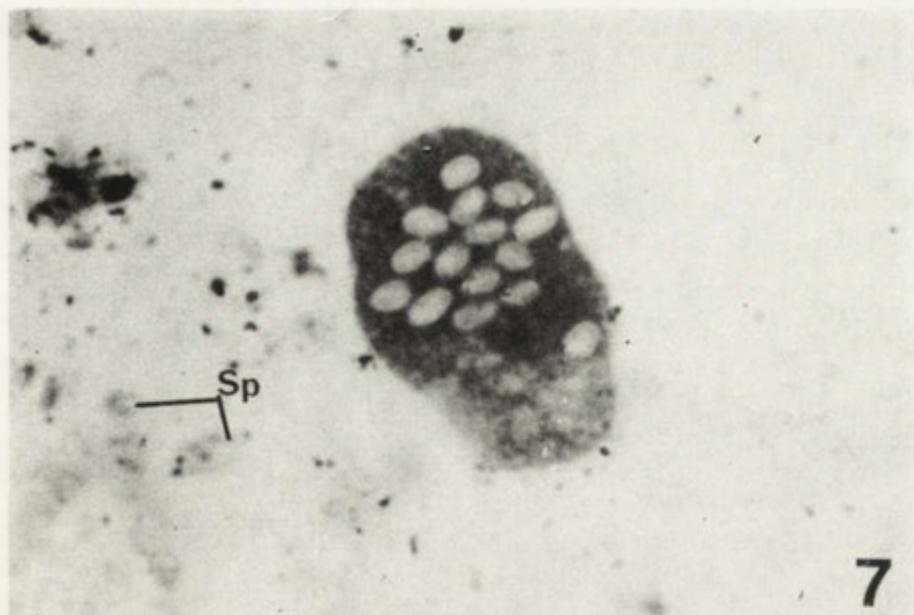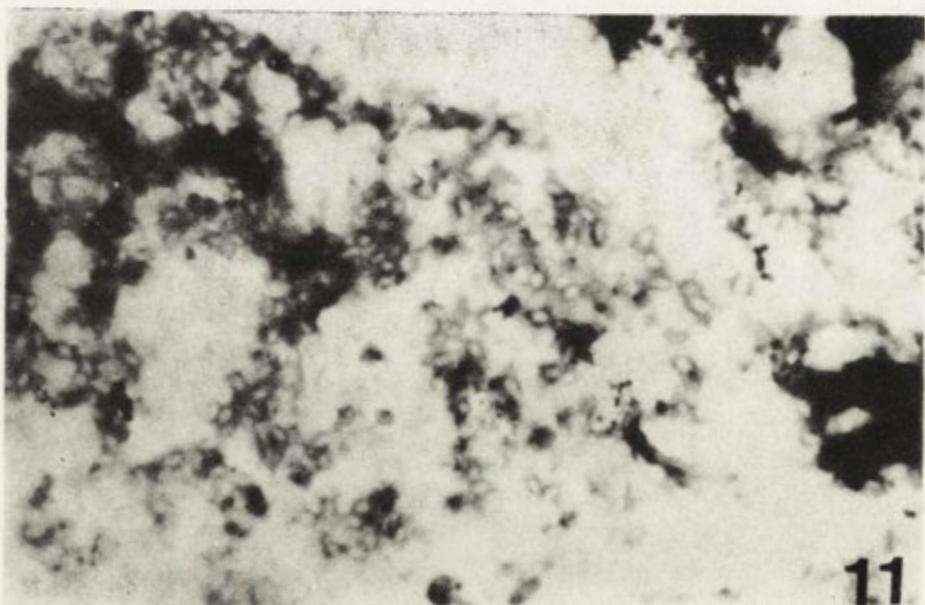
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Thelohania ostriniae n. sp., a New Microsporidian Parasite of the European Corn Borer *Ostrinia nubilalis* Hbn. (*Lepidoptera, Pyralidae*)

Synopsis. A new microsporidian *Thelohania ostriniae* n. sp. is described from the European corn borer, *Ostrinia nubilalis* Hbn. collected in southern France. The parasite invades the fat tissue of larvae and pupae of its host. The life cycle of *T. ostriniae* n. sp. is described. The fixed and stained spores measured 2.04–3.59 × 1.75–1.92 μm. The studied population of *O. nubilalis* was heavily infected by *Thelohania ostriniae* n. sp. (36%) and by *Nosema* (*Perezia*) *pyraustae* (Paillot) (36%).

The European corn borer *Ostrinia* (= *Pyrausta*) *nubilalis* Hbn. is an important pest of corn, hop, millet and other plants in Europe, Asia and in North America. Intensive studies on natural enemies of *O. nubilalis* resulted in describing several predators, parasitic insects and pathogenic microorganisms which reduce the populations of this pest.

The European corn borer (*O. nubilalis*) was known to be parasitized by a flagellate *Leptomonas pyraustae* Paillot (Paillot 1927) and a microsporidian *Nosema* (*Perezia*) *pyraustae* (Paillot 1927). The latter plays an important role in the dynamics of the insect host (Kramer 1959, Decker 1960).

In this paper I present results of observations on a new microsporidian species *Thelohania ostriniae* n. sp. which I found in *O. nubilalis* collected in France, during my visit at the Station de Zoologie Agricole, INRA, Montfavet (Avignon) in 1974.

Material and Methods

Larvae, pupae and adults of *Ostrinia nubilalis* used in these studies were obtained from Dr. Gilberte Guenellon, INRA, Montfavet. These larvae were originally collected in the corn fields at Manduel (Gard) by Mr. Guy Schlatter of the Societe d'Interet Collective Agricole, Les Angles.

Insects were dissected and their tissues were examined under the microscope in order to find and identify the pathogens.

Smeared preparations of various tissues were routinely fixed in methyl alcohol and stained in 0.5% Giemsa's solution for 24 h.

Results

Larvae and pupae of *O. nubilalis* examined during this study were found to be infected by *Nosema (Perezia) pyraustae* (Paillot) and by a new microsporidian *Thelohania ostriniae* n. sp.

Thelohania ostriniae n. sp.

Development

S c h i z o g o n y: Schizonts are spherical and measure 4–6 μm. When treated with Giemsa stain two to four red nuclei appear against a bule cytoplasm.

S p o r o g o n y: Sporogonic stages stain weaker than schizonts. In the course of sporogony eight sporoblasts are formed and consequently eight spores are produced from each sporont (Pl. I 1, 2, II 3). In some cases only four sporoblasts were produced and consequently four spores were formed (Pl. I 2).

S p o r e s: The spores occur in pansporoblasts that are oval or ellipsoidal in shape.

The mature spores are slightly elongated. They have a thick wall and deeply stained sporoplasm with red nucleus and metachromatic granule (Pl. I 1). The length of fixed and stained spores varies from 2.04 to 3.59 μm and the width from 1.75 to 1.92 μm. The spores produced in number of four in the pansporoblast are slightly larger and have a pearlike shape. Their length varied from 3.65 to 3.78 μm and the width from 2.29 to 3.1 μm (Pl. I 2).

P a t h o l o g y: This microsporidian infected fat body of larvae and pupae of *O. nubilalis* and in some insects the infection was fairly heavy.

Incidence of Infection

Out of 17 examined larvae and 13 pupae of *O. nubilalis* five and six were infected with *Thelohania ostriniae* n. sp., respectively.

The data given above indicate that the field population of *O. nubilalis* from which were taken the examined larvae and pupae was infected at 36% with *Thelohania ostriniae* n. sp.

Taxonomic Position

This is the first record of a microsporidian infection of *O. nubilalis* caused by a species of the genus *Thelohania* which differs from other

Thelohania species known from pyralids. From *Thelohania nana* K e l l e n et L i n d e g r e n (1969) described from *Plodia interpunctella* Hbn. differs by almost twicely larger spores. From *Octosporea ephestiae* (M a t - t e s 1928, originally described as *Thelohania ephestiae* and transferred by W e i s e r (1961) to the genus *Octosporea*, differs by the type of development and morphology of spores.

Summarizing, I consider that the *Thelohania* recorded in *O. nubilalis* has not been previously described. Therefore, for the microsporidian herein described, the name *Thelohania ostriniae* n. sp. is proposed.

Nosema pyraustae (Paillot)

This microsporidian was observed in four dead and three living pupae and in four living fourth instar larvae out of 30 examined specimens. The schizogonic and sporogonic stages observed on smeared preparations (Pl. II 4) were typical for this species. The diameter of schizonts varied from 3.0 to 7.0 μm depending on the number of nuclei. Only binucleate and tetranucleate schizonts were observed.

The spores of *Nosema pyraustae* were 3.0–6.9 μm in length and 1.4–2.5 μm in width. They are longer than spores of *Thelohania ostriniae* n. sp. (Table 1) and therefore they appear as narrower than spores of

Table 1

Frequency distribution of the length of two samples of 50 spores each of *Thelohania ostriniae* n.sp. and *Nosema pyraustae* (Paillot)

	Dimensionable groups in micrometers										
	1.6–2.0	2.1–2.5	2.6–3.0	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0
Thelohania pyraustae sp. n.	1	7	31	9	1						
Nosema pyraustae (Paillot)			3	25	19	1			1		1

Thelohanie ostriniae n. sp. what is well seen in Pl. I 1, 2, II 3. The spores of *Nosema pyraustae* are stained by Giemsa's stain much deeper than spores of *Thelohania ostriniae* n. sp.

A single abnormally large spore (a macrospore) of *Nosema pyraustae* was observed on one smear only (Pl. I 1). The large variations in size and shape among the spores of *N. pyraustae* was reported by K r a m e r (1960).

Discussion

The European corn borer (*Ostrinia nubilalis*) is an economic pest of corn and other plants in many countries. Therefore, the studies of the biotic factors reducing its populations may contribute to the developing of the control measures against this pest.

Several authors reported on the role of *Nosema pyraustae* in reducing the population of *O. nubilalis* (Z i m m a c k et al. 1954. Z i m a c k and B r i n d l e y 1954; K r a m e r 1959, D e c k e r 1960; R a u n et al. 1959), v a n D e n b u r g h and B u r b u t i s 1962). K r a m e r (1959) and M c L a u g h l i n (1972) has pointed out several factors deciding about the effective spread of *N. pyraustae* in its host population and in some cases the diseases increased up to 85⁰/₀ or more. In my observations out of 30 larvae and pupae of *O. nubilalis* examined at Montfavet in September of 1974, *Nosema pyraustae* infected 11 insects (36⁰/₀), while the other (36⁰/₀) were infected by *Thelohania ostriniae* n. sp.

It is interesting to notice that among about 900 larvae of *O. nubilalis* collected and examined in Poland in the period 1963–1976 in Poland no one was infected with *Nosema pyraustae* or *Thelohania ostriniae* n. sp. These larvae were collected in many regions of Poland by the author or Prof. Dr. Czesław Kania, Institute of Plant Protection, Academy of Agriculture, Wrocław.

Dr. Alois Huger from the Institute of Biological Control, Darmstad informed me that in the Federal Republic of Germany, *Nosema pyraustae* was found only in one population of *O. nubilalis* in a locality close to France.

My observations as well as the data from the literature indicate that the microsporidian infections occur in *Ostrinia nubilalis* populations in Southern Europe only. The reasons of that remain unknown.

The short visit at Station de Zoologie Agricole INRA at Montfavet, September 6 to 9, 1974 did not allow to make more detailed studies on the epizootics of *Thelohania ostriniae* n. sp. and *Nosema pyraustae* in *Ostrinia nubilalis* populations. Such studies would be greatly needed especially in relation to *T. pyraustae* n. sp. This microsporidian would be a perspective pathogen to be introduced to the North America and to other regions with the purpose to supplement the effect of *Nosema pyraustae* on the populations of *Ostrinia nubilalis*.

ZUSAMMENFASSUNG

Beschrieben wurde eine neue Mikrosporidienart *Thelohania ostriniae* n. sp. von Maiszunsler (*Ostrinia nubilalis* Hbn.). Der Parasit infiziert die Fettkörper der Raupen und Puppen des Wirtes. Angegeben wurde der Lebenszyklus des *T. ostri-*

niae n. sp. Die fixierten und Giemsa-gefärbten sporen messen 2.04–3.54 × 1.57–1.92 μm. Die untersuchte Population der *O. nubilalis* war stark durch *Thelohania ostriniae* n. sp. und *Nosema (Perezia) pyraustae* (Paillot) befallen.
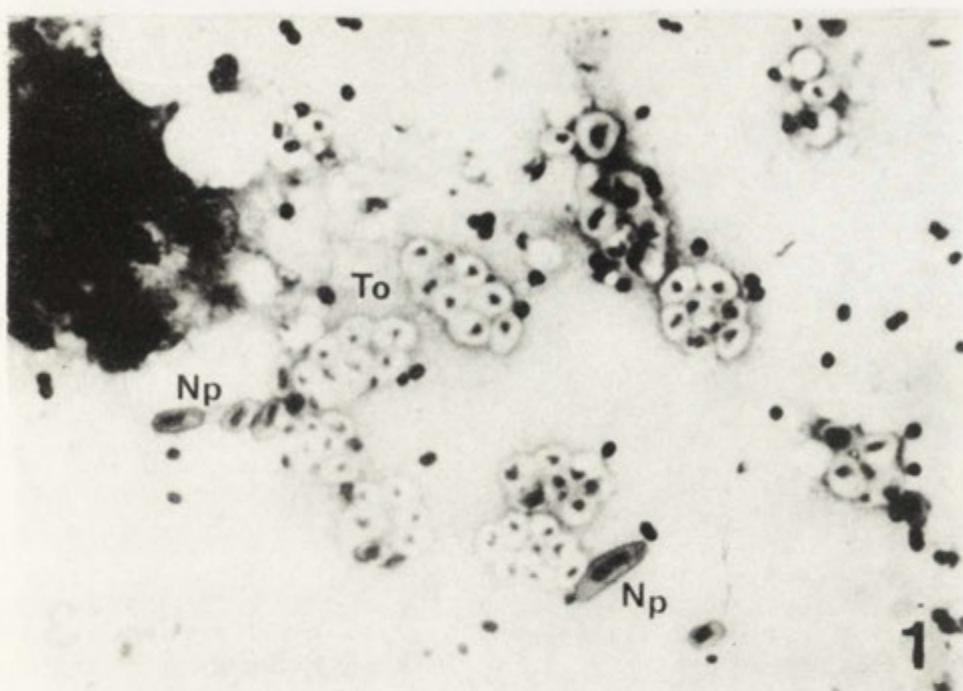
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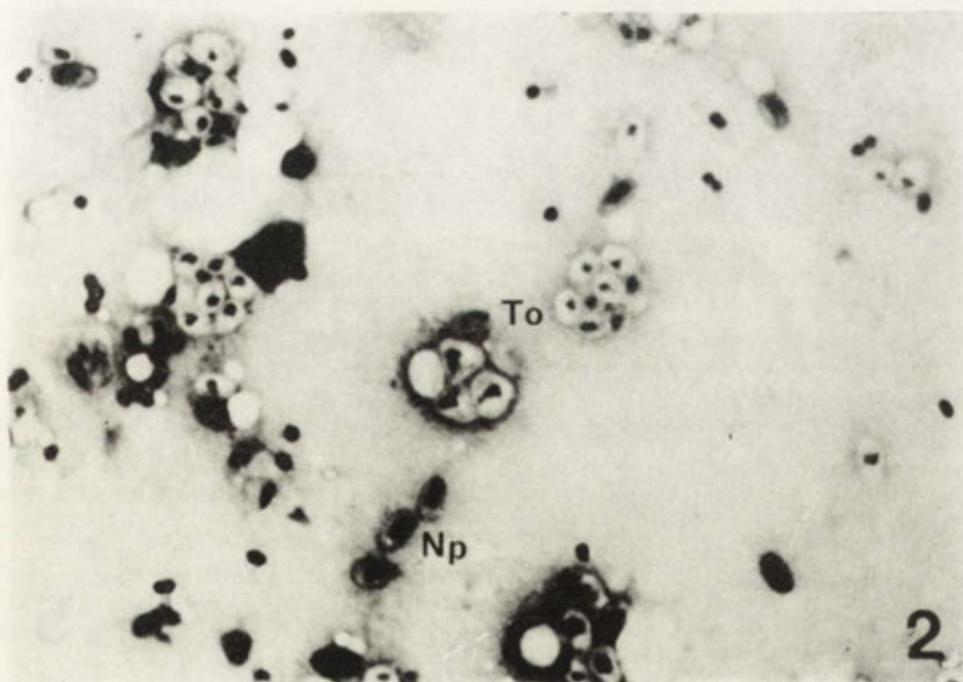
EXPLANATIONS OF PLATES I-II
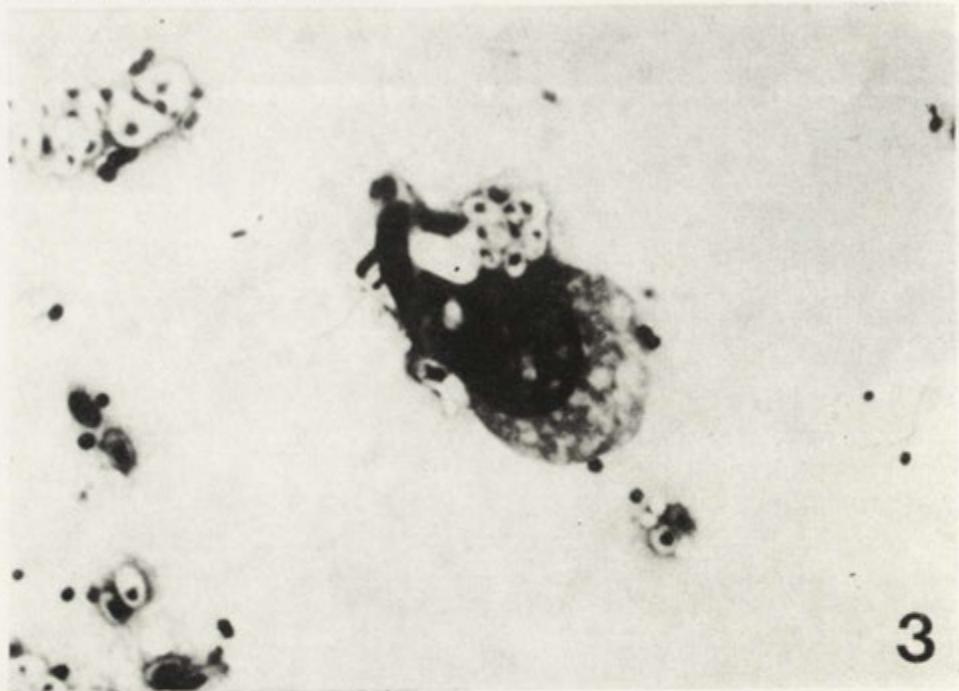
1: Spores of *Thelohania ostriniae* n. sp. (To) in pansporoblasts and single spores of *Nosema pyraustae* (Paillot) (Np)
2: Pansporoblast with four or eight spores of *Thelohania ostriniae* n. sp. (To) and single spores of *Nosema pyraustae* (Paillot) (Np)
3: Spores of *Thelohania ostriniae* n. sp. in the host hemocyte
4: Schizonts of *Nosema pyraustae* (Paillot)
5: Spores of *Nosema pyraustae* (Paillot)

J. J. Lipa   auctor phot.

PLATE II

J. J. Lipa auctor phot.

Anna CZAPIK et Anna JORDAN

Deux ciliés psammophiles nouveaux: *Hippocomos loricatus* gen. n., sp. n. et *Pleuronema tardum* sp. n.

Synopsis. En studiant les ciliés psammophiles de la mer Baltique on a trouvé deux espèces nouvelles: *Hippocomos loricatus* gen. n., sp. n. et *Pleuronema tardum* sp. n. On a examiné attentivement leur morphologie et surtout du peristome en s'appuyant sur les préparations imprégnes à l'argent. On a placé le genre *Hippocomos* dans la famille *Pleuronematidae* à causede structures buccales qui montrent une ressemblance avec celles de *Pleuronema*.

Deux ciliés que nous allons décrire ici ont été trouvés dans le sable submergé de la mer Baltique à deux metres de profondeur. Après les avoir étudié *in vivo* nous avons fait des préparations imprégnées d'argent d'après la méthode de Chatton ($AgNO_3$) et de Tuffrau (proteinate d'argent). En examinant les structures de la bouche revelées par l'imprégnation nous sommes arrivés à la conviction qu'elles ne correspondent à aucune déscription et que les ciliés trouvés représentent des espèces nouvelles.

Hippocomos loricatus gen. n., sp. n.

Le corps ovoide, legèrement aplati mesure environ 80 µm de long. La partie antérieure est foncée, remplie de grains refringeantes en forme de grains de riz, la partie postérieure claire. Les vacuoles digestives qui sont situées parmi les grains refringeants contiennent de petites algues vertes. La vacuole contractile est située près du pôle posterieur à droite. Le macronucleus est oval. La surface du corps est couverte de plaques ectoplasmiques carrées, incolores, grâce auxquelles le profil de l'animal est dentelé. Cette structure nous a fait d'abord supposer que nous avons à faire au genre *Pseudoplatynematum* décrit par B o c k (1952). Pourtant après avoir examiné la structure de la bouche il fallait renoncer à cette conception et admettre qu'il s'agit d'un genre nouveau.

La ciliature somatique comporte 21 cinéties dont une postorale composée de 5 cinétosomes fort serrés. La première cinétie à gauche du péristome commence à peu prés au niveau du cytostome et finit avant le pôle postérieur. Entre chacunes de deux cinéties il y a une fibrille parallèle à eux et de même longueur. Les cinétosomes sont groupés en paires, qui s'unissent entre eux par des fibrilles perpendiculaires aux cinéties. Ces fibrilles transversales forment avec les fibrilles longitudinales un réseau dont les mailles, carrées dans la partie antérieure et équatoriale du corps deviennent irregulières dans la partie postérieure (Fig. 1-3, Pl. I 1). Les cils sont assez longs (Pl. I 2); au bout du corps il y a un cil caudal.
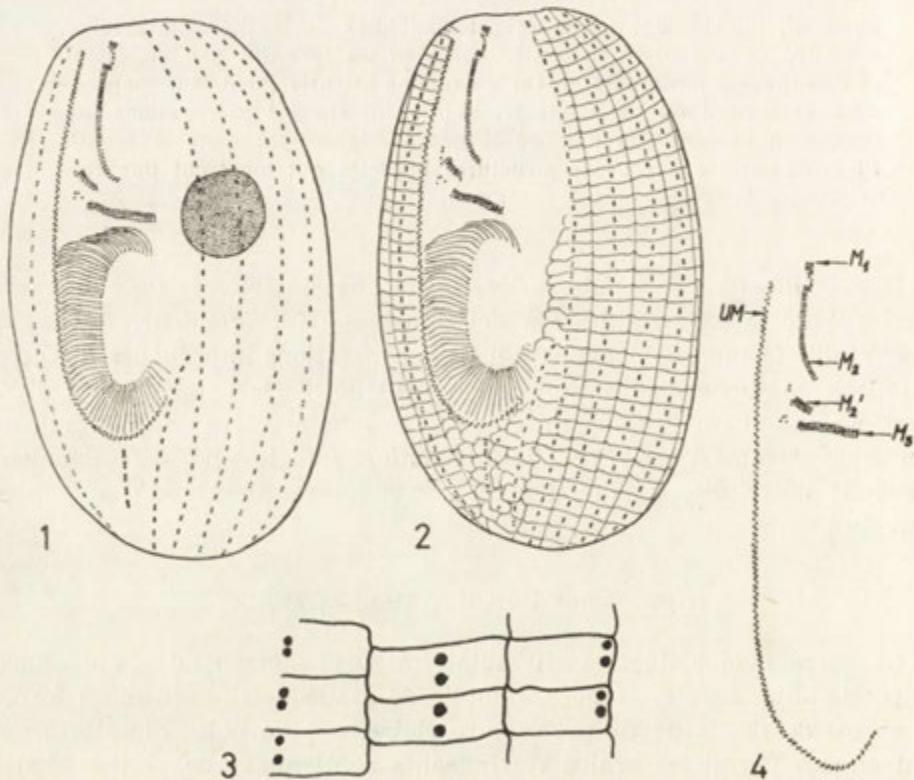
Fig. 1-4. *Hippocomos loricatus*. 1 — la structure du péristome et les cinétie, 2 — la structure de la pellicule, 3 — le fragment de la pellicule, 4 — la structure des membranelles

Le péristome allongé commence près du pôle antérieur et s'étend le long de deux tiers du corps (Fig. 1-2, Pl. I 3). Il contient une longue parorale et trois membranelles. Sa forme générale rappelle fort le péris-

tome de *Pleuronema*. C'est pourquoi il nous parait logique de nous référer aux structures buccales de *Pleuronema*, pour identifier les éléments de la bouche de notre cilié.

La partie descendante de la parorale (UM) qui mesure 45 µm contient les cinétosomes disposés en zig-zag; dans la partie transversale les cinétosomes sont alignés. Cela correspond à ce qu'on trouve chez *Pleuronema*: là-bas la ligne en zig-zag devient simple dans sa partie distale. La différence entre les parorales de deux genres consiste en ce que chez *Hippocomos loricatus* la parorale reste en bas ouverte, sans contourner l'aire du cytostome.

La première membranelle buccale (M₁) qui est située un peu plus haut que le commencement de la parorale, est formée de trois rangées très courtes: les rangées extérieures comptent quatre cinétosomes et la rangée intérieure en a trois. M₂ consiste, comme chez *Pleuronema*, en deux fragments: le premier, parallel à UM est long (15 µm) et contient deux rangées de cinétosomes. Le deuxième (M₂′) qui se trouve entre le premier et la parorale est situé obliquement par rapport à eux (Pl. I 4). Il est composé de trois rangées courtes, chacune contenant 7 cinétosomes. La troisième membranelle (M₃), située au-dessous de M₂′ forme une ligne transverse, perpendiculaire à l'axe longue du corps. (Fig. 4). Elle mesure 7.5 µm de long et contient trois rangées de cinétosomes. C'est elle qui, observée sur l'animal vivant, ondule le plus fort en ressemblant à une crinière (Pl. II 5). Entre UM et M2′ nous avons observé quelques cinétosomes dispersés. L'anse cytostomienne contient environ 46 fines stries courbés. (Fig. 1, 2).

En décrivant le péristome nous avons adopté le nom M2′ introduit par G r o l l i è r e et D e t c h e v a (1974) pour la courte membranelle qui jusqu'ici était considerée comme la partie de M₃. En étudiant la stomatogenèse de *Pleuronema* G r o l l i è r e a constaté que ce fragment a l'origine commune avec M2 donc il appartient à cette dernière. Si l'on regarde le stade où se forme la première membranelle adorale chez *Pleuronema* on voit que la disposition des structures buccales dans cette période ressemble au péristome de *Hippocomus loricatus,* en confirmant l'affinité entre les deux genres. *Hippocomus loricatus* représenterait en ce cas une forme plus ancienne au point de vue de la phylogenèse; ses structures buccales ressemblant à celles de *Pleuronema* sont pourtant plus simples. Pour mieux éclairer ces problèmes il serait nécessaire d'observer la stomatogenèse. Malheureusement, le matériel dont nous disposions était peu abondant et difficile; les animaux ne restaient vivants dans les prélèvements que pendant quelques heures. Nous n'avons pas réussi à trouver les stades de division. Donc il faut attendre à former les conclusions finales jusqu'à ce qu'on trouve ces étapes.

Pleuronema tardum sp. n.

Ce cilié rappelle au premier coup d'oeil par ses mouvements et par sa forme l'espèce *Schizocalyptra magna* décrite par Dragesco (1968). Il nage lentement entre les grains du sable en tournant autour de son axe. La partie antérieure du corps observée sous une loupe paraît foncée à cause d'inclusions réfringeantes. Le corps mesure 140–160 μm de long; il est plus large dans sa partie antérieure (75–85 μm) et se rétre-cit vers le bout jusqu'à 45–40 μm (Pl. II 6).

La ciliature somatique est constituée par 40–50 cinéties dont une est préorale (Fig. 5) (Pl. II 7–8). On trouve parfois une courte cinétie posto-
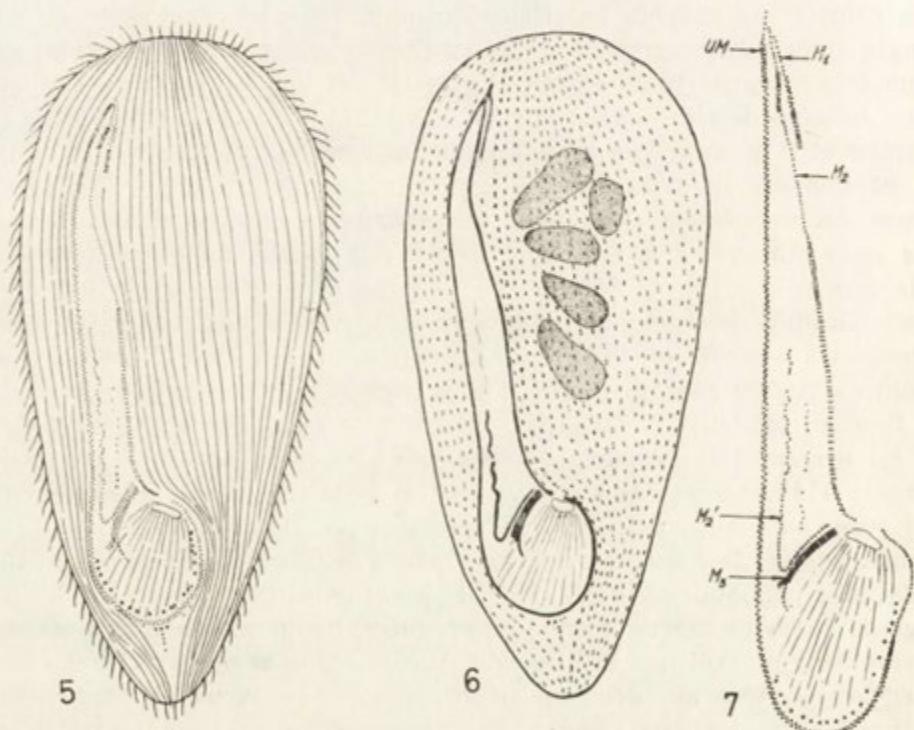
Fig. 5–7. *Pleuronema tardum*. 5 — vue générale du péristome et les cinéties, 6 — macronucleus et les cinéties, 7 — les membranelles

rale composée de 6–7 cinétosomes. Les cinétosomes dans les fragments antérieurs des cinéties sont groupés par deux, mais au niveau de la bouche ils deviennent simples.

La cavité buccale, très longue (80–100 μm) occupe environ trois quarts de la cellule. La membrane ondulante formée par les cinétosomes dis-

posés en zig-zag encercle complétement la région cytostomienne (Pl. II
9–10). Les cinétosomes situés dans sa partie distale sont plus serrés et
alignés. La première membranelle buccale (M_1 ou alpha membranoide)
débute au même niveau que la parorale par 12 cinétosomes groupés en
2 et 3; plus bas on voit 10 cinétosomes disposés en zig-zag et finalement
15 paires de cinétosomes. La deuxième membranelle (M_2' ou beta mem-
branoide) consiste comme chez toutes les espèces de *Pleuronema* en deux
fragments très distincts; le premier, appelé M_2 débute par 9–10 paires
de cinétosomes suivis d'une rangée de 46–50 cinétosomes simples. La
partie distale de M_2 formée par 40 paires de cinétosomes possède une
courbure caracteristique pour le type *Pleuronema marinum* (Pl. II 9).
Le deuxième fragments de M_2 appelé M_2' présente une cinétie en forme
de V ouvert qui ressemble par sa forme M_2' de *Pleuronema roscoffensis*.
Ce n'est pas pourtant M_2 typique parce qu'elle est formée par des cinéto-
somes repartis anarchiquement les uns contre les autres. Chez toutes les
autres espèces de *Pleuronema* sauf *P. puytoraci* les auteurs precedants
(D r a g e s c o 1968, B o r r o r 1963) ont observé toujours M_2' constituée
par une cinétie double. La troisième membranelle buccale (M_3) consiste
en trois rangées de cinétosomes. La partie distale de la première rangée
à gauche est plus allongée et courbée à gauche; cette courbure contient
toujours 6 cinétosomes (Pl. II 10). Les deux autres rangées sont plus
courtes (11 μm contre 14 μm de la première rangée). Chez toutes les
autres espèces de *Pleuronema* M_3 est aussi constituée de 3 rangées de
cinétosomes mais celle de droite a dans sa partie distale une courbure
dirigée à droite de la cellule. Les deux autres, parallèles entre elles, se
dirigent postérieurement vers la gauche (Fig. 7).

L'aire buccale est striée par les côtes qui convergent vers le cytosto-
me. Ils forment deux champs: "rib field I" qui contient 10–11 et "rib
field II" qui contient 3–4 stries. Le cytopyge est situé au-dessous du
péristome. L'argyrome rappelle bien celui de *Pleuronema coronatum*
(Pl. II 8).

Le macronucleus de cet animal est très caracteristique; en général
il y a un seul macronucleus chez les espèce de *Pleuronema* à l'exception
de *P. grassei* et *P. arenicola* décrites par D r a g e s c o (160), mais ces
déscriptions sont en somme trop inexactes pour qu'on puisse identifier
l'animal d'après eux. Le macronucleus de notre bête consiste en 4–7
fragments (Fig. 6). La forme du noyau est ici le caractère le plus
important pour l'identification du cilié *in vivo*. La vacuole pulsatile est
en position postérieure.

En étudiant la litterature concernante le genre *Pleuronem* nous avons
trouvé dans le travail d'A g a m a l i e v (1971) une déscription, qui cor-
respond à notre cilié. L'auteur russe considére les animaux qu'il a trouvé

Tableau 1

La comparaison de *Pleuronema tardum* sp. n. avec certain espèce du genre *Pleuronema*

l'espèce		*Pleuronema coronatum* Kent, 1881	*Pleuronema borrori* Dragesco, 1968	*Pleuronema puytoraci* Groliere et Detcheva, 1974	*Pleuronema marinum* Dujardin, 1841	*Pleuronema roscoffensis* Dragesco, 1968	*Pleuronema tardum* sp. n.
les dimensions du corps	longueur	58–80 μm (64)	95–122 μm (109)	70–120 μm (97)	95–140 μm (124)	85–124 μm (107)	140–160 μm (145)
	largeur	30–50 μm (36)	71–76 μm (74)	45–70 μm (62)	72–100 μm (85)	55–72 μm (66)	45–75 μm
nombre des cinéties		35–40 (35)	41–46 (43)	28 (29)	58–66 (62)	36–38 (37)	40–50 (45)
cinéties préorales		2–4	2–6	3	1–2	2	1
les dimensions du peristome	longueur	32–52 μm	60–65 μm	48–75 μm (61)	62–80 μm (70)	68–72 m (70)	80–100 μm
	largeur	12–16 μm	21–23 μm	18–25 μm	18–24 μm (22)	20–22 μm (21)	15–25 μm
"Rib-field I'"		10–13	18–24	12–14	10–13 (11)	9–13 (11)	10–11
"Rib-field I'''"		1–4	14–21	3–4	1–4 (3)	3–6 (5)	2–4
le macronucleus		unique	unique	unique	unique	unique	composé de 4–7 fragments

dans la mer Caspienne comme une forme de *Pleuronema marinum*. Or, d'après D r a g e s c o (1968) qui a fondé la systematique de *Pleuronema*, l'espèce *Pleuronema marinum* possède toujours un macronucleus unique. Les autres différences entre notre cilié et tous les autres représentants de ce genre y compris *Pleuronema marinum*, concernent la forme et les dimensions du corps, le nombre de cinéties et les détails dans la construction de la bouche. Elles sont présentées sur le tableau cité d'après D r a g e s c o (1968) et complété par deux espèces nouvelles: *Pleuronema puytoraci* Groliére et Detcheva, 1974 et *Pleuronema tardum*. On ne prend en considération sur ce tableau que les espèce décrites à l'aide de techniques modernes.

SUMMARY

During a study on psammophilic ciliates of the Baltic Sea two new species have been found, namely *Hippocomos loricatus* gen. n., sp. n. and *Pleuronema tardum* sp. n. Description of their morphology, especially of the structure of peristome, is based on silver impregnated preparations. The genus *Hippocomos* is placed within the family *Pleuronematidae* due to great similarity of the buccal apparatus to these structures in the genus *Pleuronema*.

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EXPLICATION DES PLANCHES I–II

Hippocomos loricatus
1, 2: vue générale de l'animal (impregnation en protargol)
3: vue générale du péristome (impregnation argentique AgNO₃)
4: les membranelles du péristome (protargol)
5: l'appareil buccale vu de côte (protargol)
Pleuronema tardum
6: vue générale de l'animal
7: les cinéties et des cils dans la partie antèrieure du corps
8: l'argyrome (protargol)
9–10: la partie porterieure du péristome (protargol)
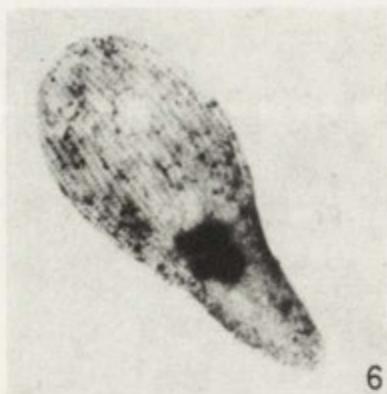
A. Czapik et A. Jordan

auctores phot.

A. Czapik et A. Jordan

auctores phot.

Anna CZAPIK et Anna JORDAN

Les ciliés psammophiles de la mer Baltique aux environs de Gdańsk. Partie II

Synopsis. La continuation des recherches sur les ciliés psammophiles apporte de nouveaux details concernant la morphologie de certaines espèces insuffisament connues à savoir la construction de l'appareil buccal de *Plagiopogon loricatus, Cardiostomatella vermiformis* et *Paraspathidium fuscum.*

Cet ouvrage représente la continuation du travail Czapik et Jordan (1976). Après avoir donné une revue générale des espèces trouvées dans le sable submergé nous nous bornons maintenant aux quelques espèces plus intéressantes ou décrites peu précisement.

Plagiopogon loricatus Kahl, 1933

Nous avons redécrit ce cilié dans notre travail précédent (Czapik et Jordan 1976), en s'appuyant sur les préparations faites d'après la méthode de Chatton et sur les observations *in vivo*. Cette fois nous avons réussi à imprégner ce cilié en utilisant le proteinate d'argent. Nous avons obtenu l'image de la structure ectoplasmique (Pl. I 1) décrite exactement l'année dernière, ainsi que celle du faisceau de nemadesmes (Pl. I 2) représentant une véritable nasse composée de 10 éléments qui s'enfoncent dans le corps jusqu'à 2/3 de son longueur.

Paraspathidium fuscum (Kahl, 1928)

Les préparations faites d'apres la méthode de Tuffrau à l'aide du protargol ont démontré les nouveaux détails de ce cilié cité aussi dans notre travail précédent, à savoir la structure de la pellicule composée de petits champs pentagonales et les nemadesmes entourant l'ouverture buccale (Pl. I 3–6).

Porpostoma notatum Moebius, 1888

K a h l (1935) a identifié le genre *Porpostoma* avec *Helicostoma* mais F a u r é - F r e m i e t (1935) a retabli le nom ancien en accentuant les différences dans la structure de la bouche et la forme différente du macronucleus. M a z o u é (1935) a étudié la nutrition de *Porpostoma notatum* en notant son polymorphisme. Enfin M u g a r d (1949) dans son étude sur les ciliés histophages a décrit la morphologie ainsi que le cycle vital de cette espèce. *Porpostoma notatum* n'est pas une espèce psammophile sensu stricto. Nous l'avons trouvé en grand nombre dans le vieux lit de la Vistule parmi les colonies de *Cordylophora lacustris*. Les bêtes measuraient 120–150 µm de long. La structure de la bouche était exactement comme l'a présenté M u g a r d: nombreux champs ciliaires en long de la fente buccale. Le macronucleus était aussi typique, en ruban (Pl. II 7–9).

Cardiostomatella vermiformis (Kahl, 1928)

K a h l, qui a décrit ce genre et l'espèce n'a pas vu distinctement la structure de la bouche, qui est très petite. Il constate seulement qu'à côté gauche de la bouche se trouve une petite membranelle tandis que le côté droit est bordé par des cils ordinaires. D r a g e s c o qui a retrouvé cette espèce dans la Manche en 1960 n'a reussi non plus de définir la ciliature buccale avec précision. Il avait impression qu'elle consistait en deux fortes membranelles antérieures et peut-être une postérieure. Mais dans la publication suivante (1963) l'auteur prétend qu'il s'agit plutôt des cinéties qui "contournent la bouche, recouvrent partiellement l'ouverture buccale, prenant ainsi l'apparence des membranelles". D r a g e s c o en conclût que ce cilié ne peut être placé parmi les Hymenostomes mais plutôt parmi les Gymnostomes. B o r r o r (1963), qui en même temps a trouvé ce cilié à Alligator Harbor, constate la présence de trois membranelles mais ne voit aucune structure qui corresponde à UM.

Les structures buccales chez *Cardiostomatella* sont en effet très difficiles à impregner mais après plusieurs échecs nous avons obtenu une bonne préparation à l'aide du protargol. La petite bouche triangulaire du cilié est entourée du côté droit par une parorale composée d'une rangée de cinétosomes. Le côté gauche du cytostome est occupé par trois petites membranelles dont chacune contient trois courtes rangées des cinétosomes (Pl. II 10–11). La structure de la bouche représente donc le tetrahymenium typique et il est évident que ce genre appartient aux Hymenostomes, comme supposait F a u r é - F r e m i e t déjà en 1957.

Sur les préparations on ne voit pas une cinétie ou un grupe de cinétosomes qui pourrait être consideré comme scuticus. On en peut conclure que le cilié devrait être placé dans la famille Tetrahymenidae mais la décision finale doit être basée sur la stomatogénèse dont nous n'avons trouvé aucun stade.

Le corps des individus trouvés mesurait 200–300 μm de long; le nombre des cinéties somatiques oscillait environ 50; il y avait 4 cinéties postorales. Le macronucleus était composé de 5–7 fragments mais dans certains individus il était rubané, présentant probablement le stade précedant la division cellulaire.

SUMMARY

Further study on psammophilic ciliates furnished new data concerning the morphology of some little known species, especially the structure of buccal apparatus in *Plagiopogon loricatus*, *Cardiostomatella vermiformis* and *Paraspathidium fuscum*.

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EXPLICATIONS DES PLANCHES I–II

Plagiopogon loricatus

1: la structure de la surface
2: la nasse

Paraspathidium fuscum
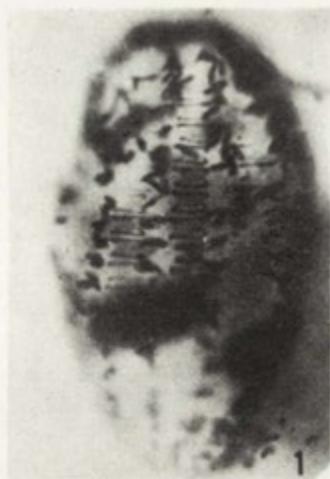
3: vue générale de l'animal
4: structure de la pellicule
5: l'ouverture buccale
6: les nemadesmes entourant la bouche

Porpostoma notatum
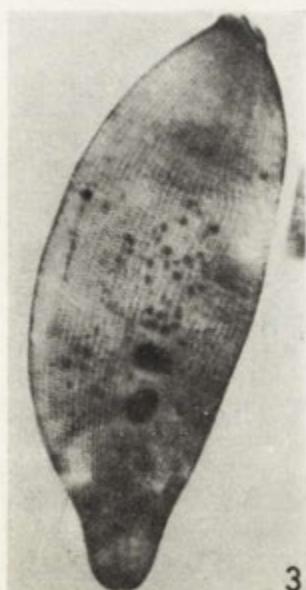
7: vue générale de l'animal
8: la macronucleus
9: l'appareil buccale

Cardiostomatella vermiformis
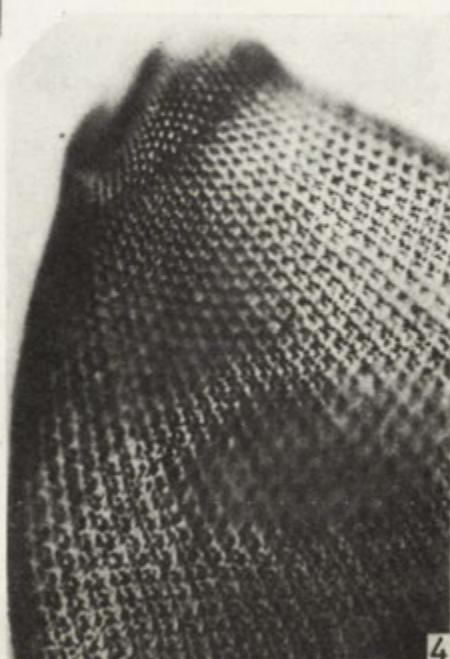
10–11: l'appareil buccale
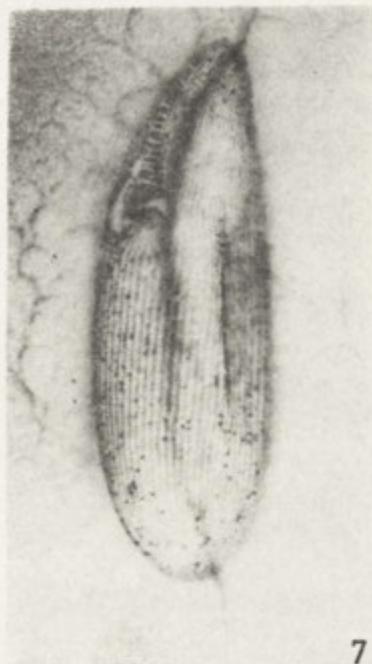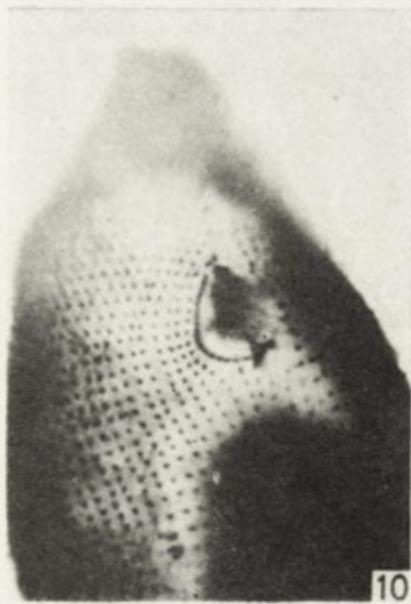
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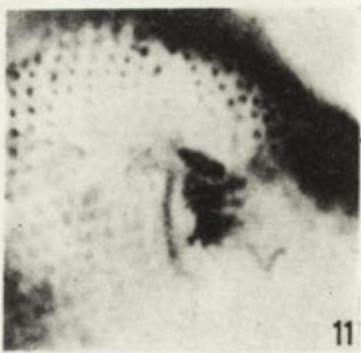
A. Czapik et A. Jordan

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Jan TESAŘIK and Roman JANISCH

The Effect of Merotomy on RNA Distribution in *Paramecium caudatum*

Synopsis. The effect of merotomy on RNA distribution during regeneration in *Paramecium caudatum* was studied. RNA levels were measured by microspectrophotometry in sections from regenerating fragments stained by the method of Unna and Pappenheim. In control intact animals RNA was distributed evenly throughout the cell. As early as 2 min. after merotomy RNA was found at high levels at the periphery of the fragment, the highest levels being found at the site of injury at 10 min. following merotomy, then began to decline. At 2 h after merotomy slightly increased levels of RNA were detected only at the site of injury. The level of total cytoplasmic RNA was also increasing till 10 min. after merotomy and then it began to fall. Also in anuclear fragments, transport of RNA towards the periphery and the site of injury was observed during the first 5 min. after merotomy.

The ability of *Paramecium* fragments to regenerate has been reported in many studies (Chen-Shan 1966, 1969, 1970, Janisch 1965, 1967, Peebles 1912, Tatar 1938). The synthesis of new proteins required for the regeneration of missing structures depends on the presence of nucleic acids. So far no data have been available on RNA synthesis and RNA distribution in the cytoplasm of regenerating paramecia. Ellwood and Cowden (1966), who studied RNA metabolism in *Stentor coeruleus* by microspectrophotometry, found increased levels of RNA, particularly near the end of regeneration. By means of various antimetabolites they demonstrated the dependence of regeneration on the RNA synthesis. Pelvant et al. (1972), using autoradiography, detected the highest level of RNA synthesis approximately at the end of the first regeneration period in *Stentor coeruleus*.

The present study is concerned with changes in RNA distribution and in the rate of RNA synthesis immediately after sectioning and at the early stages of regeneration of the fragments in *Paramecium caudatum*.

Material and Methods

Culture

Paramecium caudatum strain was isolated from hay infusion collected in
the neighborhood of Brno. The infusoria were grown in medium containing wheat
grains and inoculated with *Enterobacter aerogenes* according to Villeneuve-
-Brachon (1940).

Merotomy

Merotomy was carried out by free hand with a steel microscalpel in indivi-
dual cells under the dissecting microscope. Fragments having approximately 2/3
of the original cell length were allowed to regenerate in hanging drops on glass
slides. Fixation was made with a $3:1:1$ mixture of alcohol: acetic acid: formal-
dehyde at 2, 5, 10, 30, 60 and 120 min. after merotomy.

Staining

Oriented embedding of fragments (Janisch 1974) ensured that on sectioning
the plane of a section runs parallel to the longitudinal axis. Sections, 5 µm thick
were stained with methyl green and pyronin by the method of Unna and Pappen-
heim. The specificity of the staining was checked up with ribonuclease.

Measurements

Absorption was measured at $\lambda = 5650$ A using a Zeiss microspectrophotometer.
The diameter of each field measured was 2.5 µm. For measuring, each section
of fragments as well as that of control animals was divided according to a stand-
ard pattern into 11 fields which included the following areas: fields 1–3 at the
site of injury, fields 4–8 at the periphery, fields 9–11 in the central area of the
fragment or cell (Fig. 1).

Results and Discussion

Intact nonregenerating cells schowed even distribution of RNA
throughout the cytoplasm (Pl. I 1). Absorption of RNA varied from 60%
to 65%, the mean value being 62.8%. These minor differences may be
accounted for by quantitative variations in RNA synthesis during the
life cycle and by individual variations. However, after merotomy ir-
regular distribution of RNA was detectable as early as at 2 min. High
concentration of RNA was found all along the periphery of the fragment,
with the maximum level at the site of injury (Pl. I 2 and Fig. 2). In
50% of specimens a light band, 1 µm thick, with a very low absorption
was observed about 5 µm behind the precipitation membrane covering
the surface of the wound at 2 to 5 min. after merotomy. This band was
located at the site which corresponded to a distinct osmiophilic band,
perhaps a boundary between the necrotic and intact cytoplasm, observed
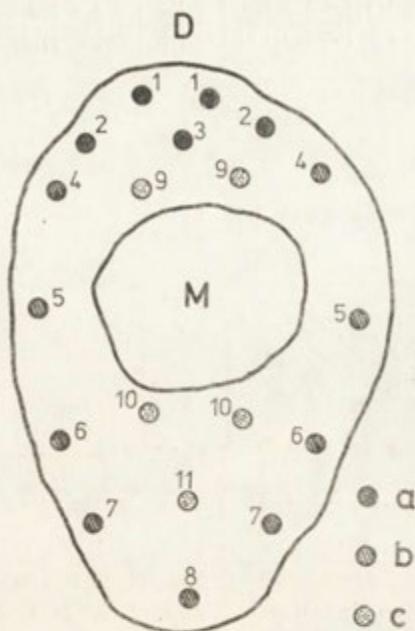
Fig. 1. A diagram of localization of the measured fields in the regenerating frag-
ments. a — site of injury, b — periphery, c — central area

in ultrathin sections (Janisch 1964). The amount of RNA in the high
RNA concentration area grew with the time of regeneration the absorp-
tion reaching 95% at the injury site and 85% at the periphery at 10 min.
after merotomy. Then RNA level became to decline and at 2 h a slightly
increased absorption was found only at the site of injury (Fig. 3). Absorp-
tion of RNA in the central part of the cell showed that total amount of
cytoplasmic RNA rose during the first 10 min. of regeneration, then
began to fall, and dropped to the normal level at 2h after merotomy
(Pl. I 3).

The paramecia that have lost a part or the whole macronucleus upon
merotomy do not regenerate. Nevertheless, they are able to survive for
a certain period, the length of which varies in each animal, and even to
repare some structures of the cell. In anuclear fragments fixed at 5 min.
after merotomy the same mode of RNA movement towards both the
periphery and the injured parts of the cell was found as in the frag-
ments containing the macronucleus (Pl. I 4). This suggested that the
macronucleus was not involved in controlling the movement of RNA
towards the periphery and to the site of injury.

It has been reported for some protozoa that the ability to regenerate
as well as the rate of regenerative processes are related, apart from other

| Field No.	1	2	3	4	5	6	7	8	9	10	11
Absorption %	92	85	81	81	80	80	79	80	71,5	71	73
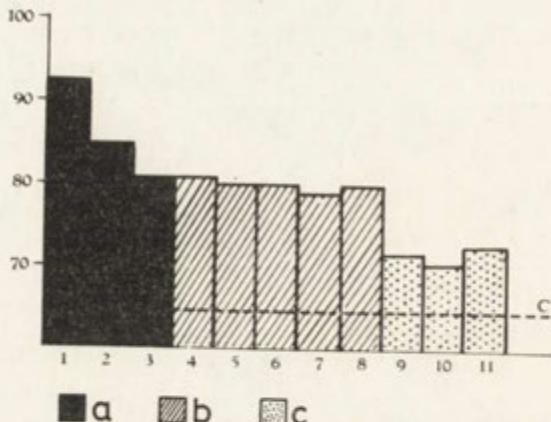
Fig. 2. Absorption of RNA in various areas of fragments stained 5 min. after merotomy. Abscissa: numbers of measured fields, ordinate: absorption (⁰/₀), a — site of injury, b — periphery, c — central area, solid line (C) indicates absorption in control intact animals

aspects, also to the size of fragments. By comparing RNA distribution in fragments of two different sizes (2/3 and 9/10 of the original length respectively) no differences were found between the zones of the high RNA concentration. On the other hand RNA levels in the central area, in other words, the total amount of RNA in the cell appeared to be inversely proportional to the size of fragment (Fig. 4). No variations were found in the distribution of RNA between the anterior and posterior fragments. A partial loss of the cytoplasm seems to activate the synthesis of RNA in the nucleus, with the degree of RNA synthesis increasing proportionally to the increasing amount of the cytoplasm lost. Consequently, in large fragments the normal level of cytoplasmic RNA is attained soon, i.e., as soon as the cell has recovered its original shape and size.

Recent autoradiographic studies indicate that in infusoria RNA is synthetized at an almost constant rate during the interphase (Kimball 1964, Kimball and Perude 1962, Rao and Prescott 1967). Our conclusion that RNA is synthetized at an increased rate for the first two hours of regeneration, with the maximum at 10 min. after merotomy, is based on a comparison with values obtained for RNA levels in intact, nonregenerating paramecia. Differences in the amount of RNA
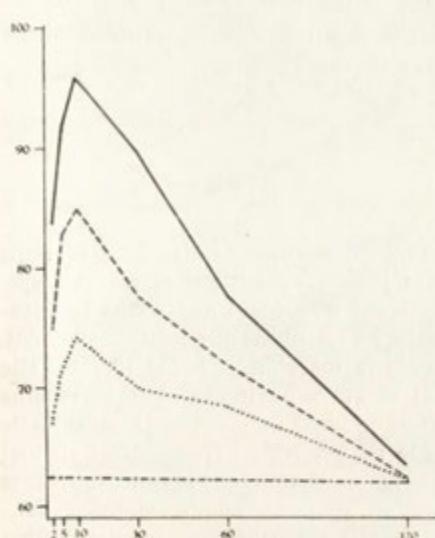
Fig. 3. RNA distribution in different areas of the fragment related to the time of regeneration. Abscissa: time of regeneration (min.), ordinate: absorption (%), absorption at the site of injury _____ , at the periphery ____ , in the central area .... Absorption in intact controls —.—.—. The highest optical density was found in all parts of the fragment 10 min. after merotomy
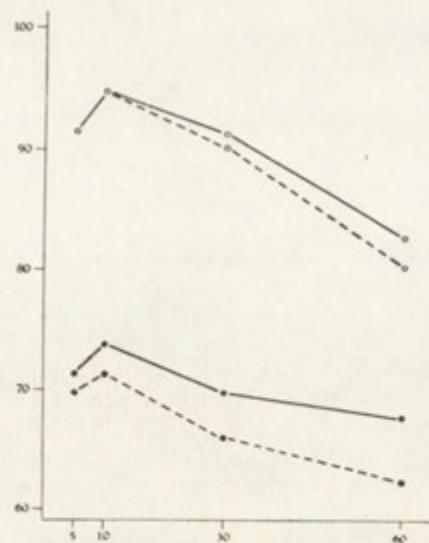
Fig. 4. Absorption of RNA at the site of injury (○) and in the central area (●) of two fragments of different sizes during regeneration. Abscissa: time of regeneration (min.), ordinate: absorption (%). The larger fragment, the lower optical density. Large fragments (9/10 of the original length) _____ , small fragments (2/3 of the initial length) _____ .

were minimal in intact control organism as well as in various fragments fixed in the same period.

Similar results have been reported for a number of other objects. In *Acetabularia mediterranea*, for instance, RNA is transported to the tip and accumulated at the site of injury where also increased levels of protein synthesis can detected (B r a c h e t 1965, W e r z 1965). However, it has not been decided yet whether also in *Paramecium* the metabolic events leading to reparative morphogenesis are localized to the injury site and, if so, to what extent. Our finding increased levels of RNA at the site injury lends support to the affirmative answer. On the other hand, the increased RNA concentration at this site could be accounted for by a mere passive transport of RNA, which occurs as a result of physicochemical changes following merotomy. Elucidation of this question requires further experiments determining the location of protein synthesis in regenerating fragments. It should also be decided which

kind of RNA is involved in this processes. Investigation into the ultra-
structure of regenerating fragments, which is in progress, should show
to what extent ribosomal RNA participates in the events.

RÉSUMÉ

On a examiné à l'aide de la méthode microphotométrique la structure de dans
des fragments en voie de régénération de *Paramecium caudatum*, et cela en sui-
vant l'orientation des tranches de paraffine colorées d'après l'Unna. Dans le cyto-
plasme des infusoires indemnes l'acide ribonucléique est divisé proportionnellement.
La synthèse de RNA augmente déjà 5 min. après la mérotomie, après 10 min. elle
atteint le maximum, puis elle va en diminuant et après avoir complété le volume
perdu du cytoplasme elle reprend les valeurs normales. Peu après la mérotomie
(2 min.) s'effectue la distribution de RNA vers la périphérie du fragment et surtout
vers le lieu du défaut où il atteint, là aussi, le maximum en 10 min. après la
mérotomie. La même façon de la distribution de RNA se manifeste aussi dans
des fragments sans noyaux qui ne régénèrent pas. La synthèse et la distribution
de RNA représentent des procès indépendants l'un de l'autre et la distribution de
RNA est gérée indépendamment du noyau.

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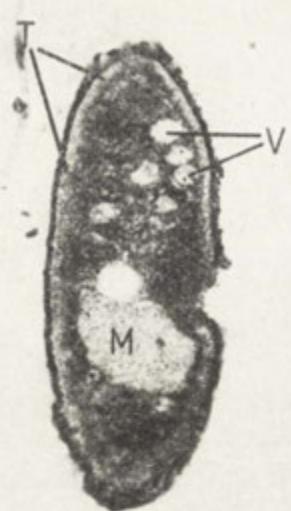
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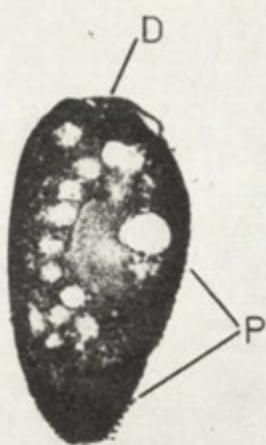
EXPLANATION OF PLATE I

1: Longitudinal parafin section of control intact *Paramecium caudatum* stained by the method of Unna and Pappenheim. Red stained cytoplasmic RNA is envenly distributed through out the section. Light band (T) below the surface is formed by the layer trichocysts. There are also food vacuole (V) and macronucleus (M)

2: Longitudinal section of a fragment stained 5 min. after merotomy. The periphery of the fragment (P) and the site of injury (D) are intensively stained

3: Longitudinal section of a fragment stained 120 min. after merotomy. Increased density remains only at the site of injury (D)

4: Longitudinal section of a regenerating anuclear fragment 5 min. after merotomy. The periphery (P) and the site of injury (D) stain more intensively
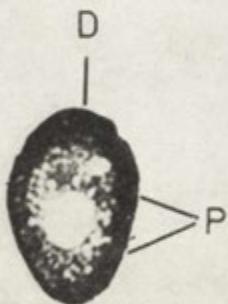
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Studies on the Electrical Stimulation of Contraction in Spirostomum. III. The Effect of Polycations on Cell Membrane Excitability

Synopsis. The interaction of poly-1-lysines (PL) of two molecular weights (188 000 and 400 000) with *Spirostomum* cell membrane was studied experimentally with respect to the excitability of the cells. These investigation were carried out by means of electrically induced contraction of cell body with the extracellular electrodes. The experimental solutions containing 5 mM Tris-HCl with some simple cations were tested in the concentration range of 5 to 10 mM. Although PL of both molecular weights decrease the net negative charge of membrane surface similarly at low concentration, the lower molecular weight PL had less effect on excitability of *Spirostomum* than higher molecular weight one. Application of PL results in rapid fall in threshold of electrical excitation of concentration. The treatment with polyglutamate had no appreciable effect on the excitability. Relationship between PL binding to the cell membrane surface and membrane functions in contractile phenomena of protoplasmic structures is discussed.

It is well known that polycations such as polylysine are able to bind to the membrane surface of animal cells (Mehrishi 1969, Sela and Katchalsky 1959) and may change the physiological properties of membrane (Mayhew et al. 1973, Brandt and Freeman 1967, Wolpert and Gingell 1968). The treatment with PL can cause the alteration in membrane impedance of amoeba cells and hence the change in ionic permeability of membrane (Gingell 1967, Brandt and Freeman 1967, Bruce and Marshall 1965). On the other hand there is evidence which support the existence of close relationship between membrane impedance changes and pattern of cellular motile behaviour (Braatz-Shade and Stockem 1972). Local application of poly-1-lysine or ribonuclease causes localized contraction at the surface of the eggs of *Xenopus laevis* (Gingell 1967). The contraction induced by poly-1-lysine is reversed by treatment with the negative

polyions polyglutamate and heparin as well as by increasing in the ionic strength of the external solution. From the above data effect of poly-cations on the impedance of egg or amoeba membrane seems to be strictly correlated with contraction being associated with a fall and relaxation with a rise in impedance (G i n g e l l and P a l m e r 1968).

The present communication describes detailed experimental treatment of influence of some simple cations on contractile excitability in the presence of the adsorbed polycations on cell membrane.

Materials and Methods

Spirostomum ambiguum has been cultured in lettuce infusion medium. The ciliates were collected for the experiments by low speed centrifugation and wash-ed twice with 5 mM Tris-HCl buffer at pH 7.1. After that the cells were left in the same buffer for about 12 h. Incubation of organisms was carried out in the solution containing poly-l-lysine in concentration from 1 to 10×10^{-6} g/ml. The duration of one incubation time was 5 min. and it was repeated five times. This procedure allows reduction of the cytotoxic effect of polycations. In the experiments following solutions were used:

(1) 5 mM Tris-HCl buffer at pH 7.1. as a control solution,

(2) 5 mM Tris-HCl with pH in the range of 3.6 to 7.3 for preparation of incubation and testing solutions,

(3) solutions of NaCl, KCl, $MgCl_2$, $CaCl_2$, $NiCl_2$ and $BaCl_2$ in concentration between 0.5 and 10 mM buffered with 0.005 M Tris-HCl at various pH for testing influence of simple cations on cell previously incubated in solution with poly-l-lysines,

(4) solution of poly-l-lysine of different molecular weights: 188 000 and 400 000 with various values of pH,

(5) solutions of glutamate with molecular weights: 15 000 and 98 000 in final concentrations from 1 to 20×10^{-5} g/ml at various pH values.

The experiments were carried out in the room temperature (18–20°C), on cells in antidromic-parallel position in respect to electric field (F a b c z a k et al. 1973). All solutions were prepared on the basis of three times glass-distilled water.

Results

The experiments reported in this paper show that the addition of substances, which alter the surface potential, to the medium in which *Spirostomum* are moving bring about a striking changes in the cell membrane excitability. The threshold of electrical stimuli depends upon concentration of polycation in external solution, its molecular weight and pH of medium. The results are experessed in terms (of relative excitability (E_{max}^{-1}), which characterizes the cell excitability (F a b c z a k 1974). At constant concentration of poly-l-lysine the cell excitability
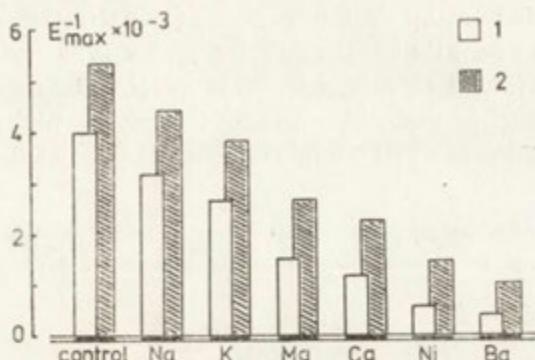
Fig. 1. The diagram showing the comparison of the value of relative excitation in different external solutions, 1 — without poly-l-lysine and 2 — with poly-l-lysine (m.w. 188 000) in concentration 5×10^{-6} g/ml
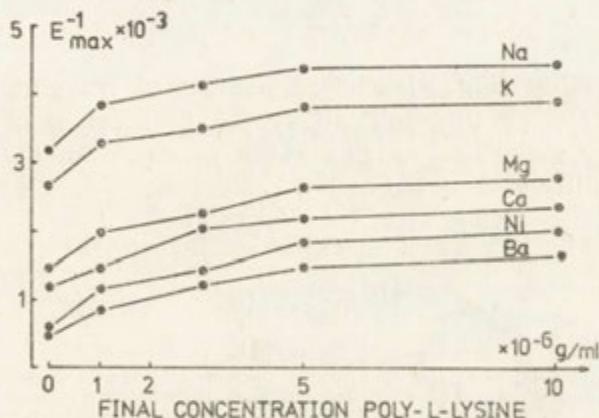
Fig. 2. Changes in excitability for some cations in external solutions resulting from exposure to various concentration of poly-l-lysine (mol.w. 188 000)
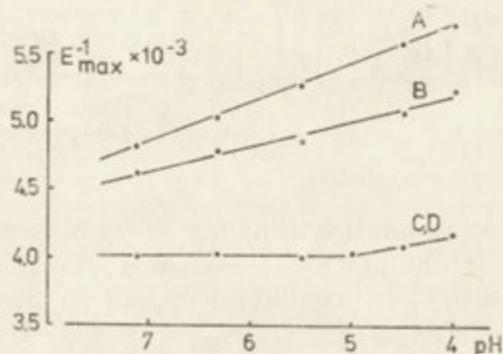
Fig. 3. The influence of poly-l-lysine (A — 400 000 and B — 188 000) and poly-glutamate (C — 15 000 and D — 98 000) on the relative excitability of *Spirostomum*, at different pH of external solution (concentration 5×10^{-6} g/lm)

decreased with increasing cation concentration in the range of 0.5 to 10 mM in medium for all simple cations (Fig. 1 and Table 1). The greatest effect on the threshold of excitation was exerted in medium containing poly-l-lysine of higher molecular weight and at its higher concentration (Fig. 2 and 3). Increase of H^+-ions concentration caused the rise of con-

Table 1

The values of constants of strength-duration curve and relative values of excitation of *Spirostomum* at different concentration of poly-l-lysine

The kind of constant	Concentration of poly-l-lysine ($\times 10^{-6}$)					
	0 g/ml		1 g/ml		5 g/ml	
	NaCl	MgCl$_2$	NaCl	MgCl$_2$	NaCl	MgCl$_2$
a (V msec cm^{-1})	22.5	27.3	32.5	29.4	31.5	37.8
b (V cm^{-1})	2.2	4.41	2.1	4.0	1.8	3.8
n	0.82	0.82	0.89	0.89	0.96	0.96
$E_{max}^{-1} \times 10^{-3}$	3.18	1.48	3.8	2.1	4.4	2.7

tractile excitability (Fig. 4). The application of polyglutamate had no appreciable effect on threshold at physiological values of pH independently of their molecular weights. As it is shown in Fig. 4 the small rise of excitability is observed at low pH values.
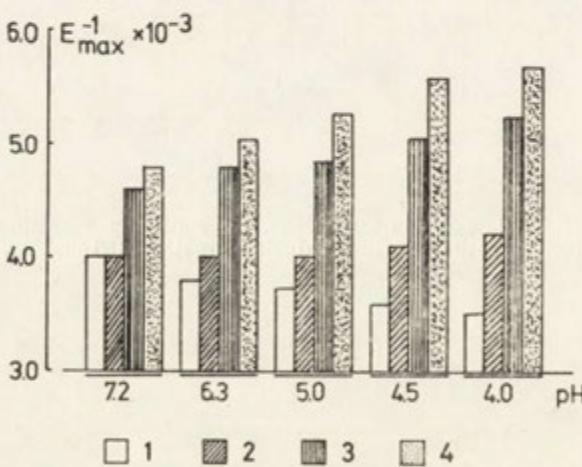

Fig. 4. The comparison of contractile excitability in external medium with different pH and different molecular weight of polyions (in concentration 5×10^{-6} g/ml), 1 — control solution, 2 — polyglutamate (C, D), 3 — poly-l-lysine (B), 4 — poly-l-lysine (A)

The values of constants (a, b, n) for strength-duration curve which describe the cell excitability are presented in Table 1. Results of the experimental work suggest that the interaction of polycation with outer membrane surface changes the curvature coefficient (n) of this curve (Table 1). On the contrary it should be stressed that the application of different kinds of simple cations at various concentration in spite of affecting constants (a, b) have no effect on the curvature coefficient (n).

Discussion

The assumption that the localization of the system controlling the triggering of contractile protoplasmic phenomena is within the cell plasmalemma has been first formulated long time ago by L i l l i e (L i l- l i e 1906, 1916). He suggested that protoplasmic contraction wherever it takes place is always stimulated by electrical depolarisation of plasmalem- ma. The direct evidence which supported the idea that the membrane pro- perties determine contractile reactions of internal cellular structures was obtained from the experiments on muscle and nerve cells (H o d g- k i n and K a t z 1949, H u x e l y 1958, 1964, K e y n e s 1967, N a c h- m a n s o n 1967, T o b i a s 1959 a, b, H o d g k i n and C h a n d l e r 1965). From some recent data for nonmuscle cells it seems that this phenomen- on plays fundamental role in the basic processes of cellular behaviour. (B i n g l e y et al. 1962, B i n g l e y and T h o m p s o n 1962, B r a n d t and F r e m a n 1967, B o r s e l l i n o et al. 1973, J e o n and B e l l 1955, K o r o h o d a et al. 1970, N a c h m i a s 1964, S e i f r i z 1953, S t e w a r d 1964).

The functional properties of cell membrane which are characterized by resting or bioelectrical potential difference across the membrane is due mainly to the unequal distribution of diffusible ions across the membrane and is detectable between the internal and external bulk phases. Since the cell membrane is in permanent contact with the external medium, it has to react on changes in external factors through the alterations of membrane potential. This was shown for muscle and nerve cells and for other nonmuscle cells as well (J e n e r i c k and G e r a r d 1953, K i t a 1966, K o k e t s u 1965, L o w e n s t e i n 1966, B r u c e and M a r s h a l l 1965, H o d k i n and C h a n d l e r 1965). Increase in cation concentration in medium results in the membrane depolarisation. It is connected with change of the threshold of electrical excitation. In the case of cells which possess the contractile properties it is related to the contraction triggering. For nonmuscle cells, however, this is not experimentally documented as yet. The indirect evidence in favour of this hypothesis was given by B r u c e and M a r s h a l l (1965).

In *Spirostomum* the alteration of different external factors leads to changes in excitability (Sleigh 1970, F a b c z a k 1974). In this paper it was shown that the change of excitability can be evoked by application of polycation as well. Poly-l-lysine in physiological pH range is positively charged particle and it is adsorbed by negatively charged ionic groups of cell membrane surface (M e h r i s h i 1969, S e l a and K a t c h a l s k i 1959). Under the same conditions polyglutamate is negatively charged

and, therefore, it does not exhibit affinity to the cell surface, having no effect on membrane physiological properties (Fig. 4). Since poly-l-lysine is tightly bound to the cell surface, its physiological effect can be reversed by washing with polyanion only. So the functional membrane properties can be changed not only by the decrement or increment of the medium ionic strength but also by the change of net negative surface charge, caused by adsorption of polycations. It was observed that the binding of polycations altered the impedance of membrane which modified the motile behaviour of amoeba cells (Wolpert and Gingell 1968).

It seems that there exists a direct relationship between membrane surface charge and bioelectric membrane potential. Adsorption of electrically charged particles on cell membrane surface changes the value of electrical surface potential, causing alteration in ionic permeability. Resulting transmembrane ionic fluxes would influence the bioelectric potential across membrane (Mayhew et al. 1973). This statement is strengthened by the existing close correlation between artificial membrane permeability and the value of transmembrane potential (Bangham and Standish 1965, Mueller and Rudin 1968, Papahajopoulos and Bangham 1966). The change of coefficient of curvature for strength-duration curve gives the evidence in favour of that idea.

In general terms, on the ground of experimental data presented in this article it may be suggested that alterations in surface potential can act as a transducing mechanism whereby environmental influences can bring about changes within the cytoplasm and thus evoke cellular motile responses.

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Endocytosis in *Paramecium* III. Effect of Cytochalasin B and Colchicine

Synopsis. The treatment of *Paramecium caudatum* with cytochalasin B inhibits food vacuoles separation. This phenomenon is not due to filament disordering or disruption. The effect is reversed after 24 h of treatment or after the drug removing. Colchicine causes the decrease of endocytosis rate influencing neither filaments nor microtubules of the oral apparatus.

Cytochalasin B (CB) is a very well known agent influencing many of cell functions (for literature see Wessel et al. 1971, Miranda et al. 1973, Miranda and Godman 1974, Rathke et al. 1975). The mechanism of its action is not yet elucidated and results are often contradictory (Burnside and Manasek 1972, Holtzer and Sanger 1972), but there are a lot of data indicating that it interacts with filaments (Spooner and Wessels 1970, Malawista 1971, Goldman 1972, McGuire and Moellmann 1972). On the other hand a lot of filaments can be observed near the buccal cavity of *Paramecium*. The influence of CB on the food vocuole formation has been also establi- shed. It inhibits phagocytosis in *Metazoa* cells (Williams and Wolf 1971, Zigmond and Hirsch 1972, Axline and Reaven 1974) and also in ciliate *Tetrahymena pyriformis* (Nilsson et al. 1973, Hoff- man et al. 1974, Rothstein and Blum 1974). However, the lack of the effect on the endocytosis has been also stated (Wills et al. 1972). The important role in the process of endocytosis in *Paramecium* can be also played by microtubular structures of oral apparatus (Lund 1941, Mast 1947, Schneider 1964). The using of colchicine which speci- fically binds to microtubular subunit (Borisy and Taylor 1967a,b, Shelansky and Taylor 1967) can help to solve this problem.

Material and Methods

Paramecium caudatum cells growing in lettuce infusion were collected by centrifugation and washed three times with phosphate-citrate buffer pH 6.3. They remain in this buffer overnight. 2 ml samples containing about 400 cells each were treated with CB in concentrations 2-100 µg/ml or by 1-5 mg/ml colchicine in phosphate-citrate buffer pH 6.3. CB was prepared from the stock solution containing 10 mg CB per 1 ml of dimethylsulfoxide (DMSO). Phosphate-citrate buffer pH 6.3 and 10 µl/ml DMSO were used as control media. After 5 min. 24 h treatment the cells were exposed for latex particles of 0.81 µ diameter. They were fixed with 10% neutralised formaline after 15 min. exposition and the number of latex containing vacuoles was counted. The cells treated with 20-100 µg/ml CB were washed after 30 or 60 min. treatment. Their ability to food vacuoles formation was tested after 30 min. 24 h in the same way as in non-washed cells. Each experiment was repeated three times and the mean number of food vacuoles and standard deviation were calculated. The observations of individual cells in small oil droplets were also carried out. In order to exclude the possibility of the lack of CB activity in experimental medium, after 24 h the "naive" cells (i.e., taken from buffer medium) were added to the centrifugated, experimental medium. Their ability to phagocytosis was observed.

Preparation for Electron Microscopy

The cells were fixed in 2% glutaraldehyde followed by 2% osmium tetroxide in cacodylate buffer pH 7.1 and than dehydrated in increasing ethanol series and two changes of propylene oxide. The material was embeded in Epon and sectioned with LKB ultramicrotome. Contrasted with uranyl acetate and lead citrate sections were examined in JEM 100 B electron microscope. Some cells were glycerinated in 50% glycerol during 24 h before fixation.

Results

The observations of the living cells indicate that the endocytosis inhibiting concentrations of CB have no effect on the other functions of the cells. After 5 min. treatment no food vacuoles formation in 100 and 50 µg/ml CB was observed. The mean number of vacuoles in 20 and 10 µg/ml was on the same level as in control cells. After 30 min treatment no phagocytosis in 20 µg/ml CB occurred either (Table 1). In observed CB treated living cells the movement of the oral cilia was not altered. The normal accumulation of latex particles was also observed. The abnormal enlargement of the formed food vacuole took place, and separation of food vacuole was inhibited. The effect of CB could be reversed after washing the treated cells with phosphate-citrate buffer, although the mean number of vacuoles in cells which had been previo-

Table 1*

Mean number of food vacuoles formed in *P. cuadatum* after different time of CB treatment

Concentration CB (μg/ml)	Time of treatment			
	5 min.	30 m in.	60 min.	24 h
100	0.61±0.57	0.07±0.27	0.17±0.36	4.00±1.11
50	0.72±0.51	0.33±0.39	0.54±0.25	4.16±1.25
20	2.03±1.88	0.75±0.67	0.73±0.23	8.15±0.99
10	4.75±1.75	3.20±1.26	2.85±1.37	6.58±0.90
5	8.35±1.99	5.93±1.27	4.90±1.07	7.90±1.08
2	9.83±1.47	8.25±0.74	6.56±1.02	8.62±1.09
10 μl/ml DMSO	9.01±1.08	6.38±1.60	7.34±0.84	7.69±0.68
Phosphate-citrate buffer pH 6.3	9.11±0.96	7.68±0.91	7.00±1.30	7.75±0.96

* Each mean number was calculated from about 100 cells.

usly treated with 100 μg/ml CB, was lower than in control paramecia (Table 2). DMSO even in concentration 10 μg/ml had no effect on vacuole formation (Table 1). The observations in electron microscope indicate that filaments which underly almost the whole oral apparatus of *Paramecium* are not disrupt or disordered during CB treatment. It can be very well seen in glycerinated cells (Pl. I 1–5).

After 24 h of CB treatment the inhibiting effect of CB on the vacuole formation is reversed. The control with "naive" cells indicates that the phenomenon is not due to the lack of CB activity in experimental medium since their phagocytosis is arrested.

The treatment of *P. caudatum* with 1, 2 or 5 mg/ml colchicine causes the decrease of food vacuole formation rate (Table 3).

The observations of the living cells revealed that neither larger nor more filled vacuoles were formed. It suggests that the decrease of particle accumulation rate and slower enlargement of the food vacuole formed occurred during colchicine treatment. The examination of electronomicrographs shows no differences in ultrastructure of oral microtubules in treated and untreated cells (Pl. II 6–10).

Discussion

The arresting of food vacuole formation in various cytochalasin treated cells was stated by many authors (W i l l i a m s and W o l f 1971, Z i g m o n d and H i r s c h 1972, N i l s s o n et al. 1973, H o f f m a n et al. 1974, R o t h s t e i n and B l u m 1974). The present study reveals that CB causes the inhibition of food vacuole formation in *P. caudatum* influencing neither accumulation of ingested particles nor the growing

Table 2

Mean number of food vacuoles formed in *P. caudatum* after removing of CB

Concentration of CB (mg/ml)	Prior washing		Washed after 30 min. CB treatment			Washed after 60 min. CB treatment		
	Time of treatment		Time after washing			Time after washing		
	30 min.	60 min.	30 min.	60 min.	24 h	30 min.	60 min.	24 h
100	0.18±0.39	0.10±0.31	2.67±1.57	2.54±0.10	6.95±1.61	2.80±0.76	3.60±0.86	8.90±1.83
50	0.50±0.36	0.64±0.29	4.86±0.74	3.91±0.66	7.50±1.32	3.76±0.94	4.00±0.92	9.16±1.05
20	0.95±0.44	2.80±0.98	4.71±0.26	3.80±0.98	7.83±0.91	2.82±0.74	4.09±0.59	7.76±0.15
DMSO 10 µl/ml	5.78±0.76	4.00±0.98	5.53±0.80	3.71±0.84	7.66±1.70	4.71±0.87	3.68±0.57	7.08±1.40
phosphate-citrate buffer pH 6.3	4.90±0.43	4.08±0.52	6.15±1.27	4.97±1.06	7.90±1.17	5.32±0.71	4.68±0.70	8.12±1.44

Table 3

Mean number of food vacuoles formed in *P. caudatum* after different time of treatment with colchicine

Concentration of colchicine	Time of treatment			
	5 min.	30 min.	60 min.	24 h
	2.50±0.25	2.16±0.23	2.32±0.23	2.19±0.27
5	4.36±0.26	3.13±0.21	2.60±0.20	2.21±0.35
2	4.86±0.32	3.80±0.26	3.48±0.22	3.30±0.37
1hosphate-citrate buffer ppH 6.3	8.07±0.53	6.86±0.45	7.60±0.51	6.10±0.30

of formed vacuole. Since no interaction of the drug with microfilaments was stated it can be speculated (on the contrary to suggestions of G a v i n 1976) that its effect is due to the disturbances in the mechanism of membrane fusion. Similar hypothesis has been also put forward by E s t e n s e n (E s t e n s e n 1971, E s t e n s e n et al. 1971). It is supported by investigations of other authors indicating the lack of cytochalasin effect on microfilaments in different cells (B l u e m i n k 1971, a, b, F o r e r et al. 1972, G o l d m a n 1972, G o l d m a n and K n i p e 1972, M i r a n d a et al. 1973, 1974) and experiments with tritated cytochalasins suggesting that they act directly on the cell membrane (C o h n et al. 1972, K r i s h a m 1972, K e l l y and S a m b r o c k 1973, N e l s o n and J a f f e 1973, R a t h k e et al. 1975). It has been supposed that CB penetrates hydrophobic regions of membrane (H a u s c h k a 1973, M a y h e w et al. 1974). In erythrocytes the CB binding site may be associated with one of the major membrane proteins (L i n et al. 1974).

The reversibility of CB effects on the food vacuole formation in *P. caudatum* can be explained by experiments of other authors stating loose of the whole (H a u s c h k a 1973) or almost whole drug (T a n n e n b a u m et al. 1973, M a y h e w et al. 1974, R a t h k e et al. 1975).

The problem of lack of effectiveness after 24 h CB treatment seems to be more complicated. The control experiments with naive cells indicate that there is no degradation of CB in experimental medium, so the effect can be due to the adaptation of the cells to the environment. Similar phenomena have been observed in *T. pyriformis* after antimitotic agents treatment (G a v i n and F r a n k e l 1966, M a z i a and Z e u t e n 1966, S t o n e 1969, N e l s o n 1970). S a r a s and B u r c h i l l (1975) have stated that endogenous recovery of *Stentor coeruleus* from inhibition of oral regeneration by antimitotic agents is due to the formation of intracellular resistance. *In vivo* reassembly of microtubules in *T. pyri-*

formis in presence of colchicine can take place after the subunits of microtubules lack the ability of colchicine binding (W u n d e r l i c h and H e u m a n 1974).

The results obtained in experiments with colchicine are in agreement with N i l s s o n (1973) studies on *T. pyriformis*. In both cases the significant decrease of food vacuole formation rate can be observed. Since no changes in microtubule ultrastructure were observed in this study it seems probable that the colchicine binding to membrane can take place, similar as in *Metazoa* cells (F e i t and B a r r o n d e s 1970, S t a d l e r and F r a n k e 1972, P i t m a n et al. 1972, B e r l i n and U k e n a 1972, H i n d e l a n g - G e r t n e r et al. 1976) and in *T. pyriformis* (W u n d e r l i c h and H e u m a n 1974). Colchicines role in membrane fusion has been also suggested (C h a j e k et al. 1975).

Thus the effect of both, used in present paper, chemicals is due to their interaction with membranes rather, than action on filaments or microtubules.

ZUSAMMENFASSUNG

Die Behandlung von *Paramecium caudatum* mit Cytochalasin B (CB) aufhaltet die Abreissung von der Nahrungsvakuolen. Die Vakuolen sind nach der 24 Stunden dauernten Behandlung oder nach der Beseitigung von CB wieder gebildet. Nach der Behandlung mit Kolchicine das Tempo von der Nahrungsvakuolen Ausbildung ist langsamer als in der Kontrole. Die Filamenten und Mikrotubulen des Cytopharynx sind nicht verändert während der Behandlung mit CB und Kolchicine.

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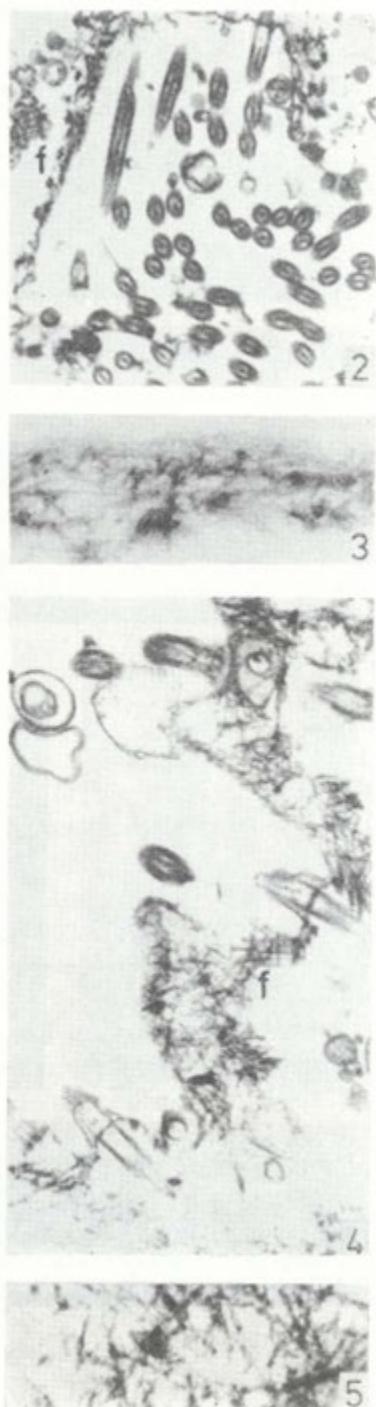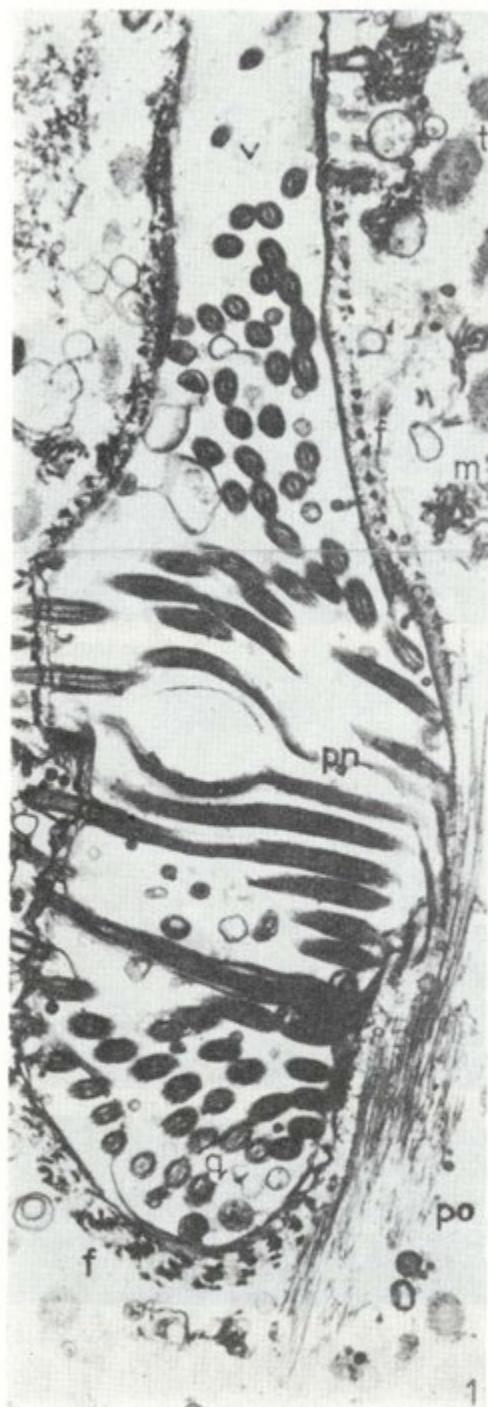
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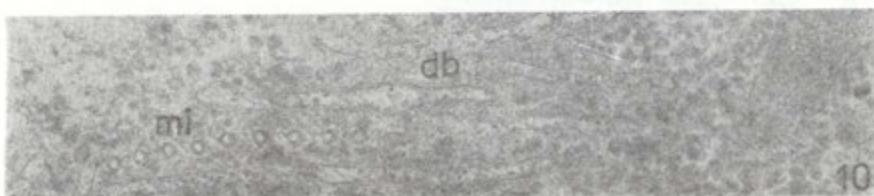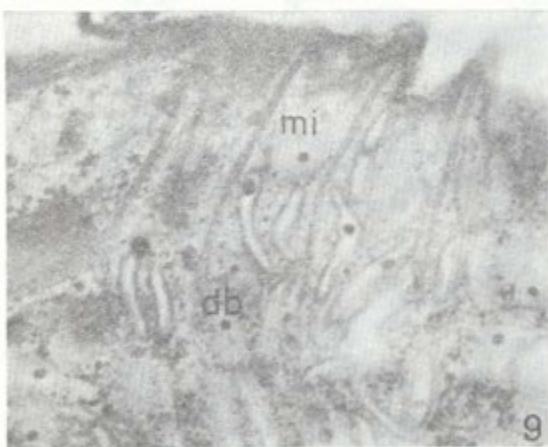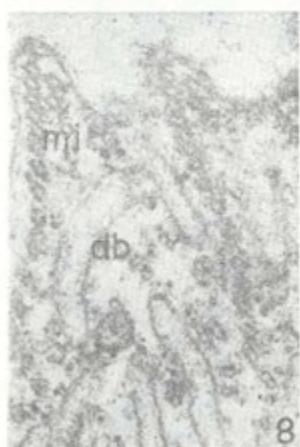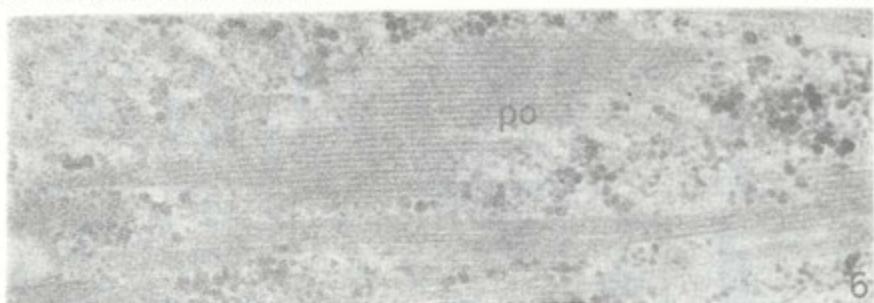
1: Oral apparatus of *P. caudatum* in glycerinated cell. f — filaments, po — postoral fibers, pn — peniculus, q — quadrulus, m — mitochondria, t — trichocysts, v — vestibulum ($\times$ 12 000)

2: Fragment of the oblique section of oral apparatus in glycrinated *P. caudatum* after 1 h treatment with 10 µl/ml DMSO. f — filaments ($\times$ 8000)

3: Fragment of filaments from fot. 2 ($\times$ 45 000)

4: Fragment of the oblique section of oral apparatus of *P. caudatum* treated with 100 µg/ml CB during 1 h. f — filaments ($\times$ 15 000)

5: Fragment of filaments from fot. 4 ($\times$ 30 000)

6: Postoral fibres (po) in control *P. caudatum* ($\times$ 75 000)

7: Similar section of postoral fibres (po) in colchicine treated cell (5 mg/ml, 1 h) ($\times$ 30 000)

8: Area of nacked ribbed wall in control *P. caudatum*, mi — microtubules, db — disc bodies (80 000)

9: The same area in colchicine treated cell (5 mg/ml, 1 h) ($\times$ 15 000)

10: Cross section of microtubules (mi) in colchicine treated cell (5 mg/ml, 1 h) (60 000)

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The Influence of EGTA/Ca Buffers on Food Vacuole Formation by *Tetrahymena pyriformis* GL

Synopsis. It is shown that *Tetrahymena pyriformis* GL does not loose the capacity for suspension uptake within wide range of free calcium ions concentrations in external meedium (10^{-7}–10^{-3} M Ca^{2+}). Only in 10^{-2} M Ca^{2+} concentration the process of phagocytosis is to some extent suppressed. Changes of the motile behaviour of *Tetrahymena* appearing parallely to variations of Ca^{2+} in external medium influence only slightly the phagocytic activity.

Preliminary observations made by one of the authors (Brutkowska, unpublished) showed that EGTA added in different concentrations to the medium with *Tetrahymena* could disturb greatly both phagocytosis and contractile vacuole activity, and also the motile behaviour of this ciliate. This effect was dependent on the concentration and the time of action of EGTA on *Tetrahymena*. From these data it was concluded that the amount of calcium ions bound by EGTA increased steadily in the external medium, while on the membrane and probably inside of the cell calcium content was considerably reduced; this caused morphophysiological disturbances, including rupture of pellicle, followed by disintegration and death of the cells. However, during the course of these experiments, the concentrations of calcium in external medium and in the cell were not established. Then, the problem arose, what is the influence of different known concentrations of external calcium on physiological functions of *Tetrahymena pyriformis*.

Material and Methods

The experiments were carried out on *Tetrahymena pyriformis* GL, cultivated in test tubes at 27°C. 1% proteose peptone (Difco) and 0.01% yeast extract (Difco), was used as culture medium. The stock cultures of ciliates were maintained by

inocculation of new culture medium with one drop of the old culture every third day. From such three day old stock cultures the animals were taken for experiments; 0.5 ml was transferred to 5 ml of fresh culture medium and kept for 24 h. 18 hours before each experiment the ciliates were washed with 1 mM Tris/HCl buffer (Fluka) without calcium ions, or with 1 mM $CaCl_2$, 10^{-3} M of Ca^{2+} at pH 7.10–7.15 and collected by two centrifugations using a hand centrifuge.

Ciliary movement and phagocytosis were again taken as test functions in these experiments. Calcium buffers with a known level of free calcium ions in the medium were prepared according to the calculations of P o r t z e h l et al. (1964). For each buffer the same concentrations of 2.5 mM EGTA diluted by NaOH, and of 5.0 mM Tris HCl buffer were used; the concentration of calcium chloride was changed. This procedure gave us the range of solutions where the final concentrations of calcium ions in the experimental media were 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} M, respectively. Ionic strength was 0.05. The stable, 7.10–7.15 pH was obtained by adjustment with HCl. Each sample contained 150–250 specimens in 1 ml solution. Culture medium was diluted in each experiment about 1:2000 times.

To measure the phagocytic activity, polystyrene beads (PLP) 0.794 μ were used. They were added to the medium containing *Tetrahymena* together with one of the calcium buffers, or with 1 mM Tris HCl buffer (control medium). After 10 min of observation the *Tetrahymena* were fixed and the number of food vacuoles in 50 specimens was counted using 200 × magnification of Zeiss microscope, normal light optics. The mean number, standard deviation and percentage of control values were calculated. On parallel sample observations of the motile behaviour of *Tetrahymena* were made, complemented by photographic registration of movement according to the method of D r y l (1958) in the third minute of immersion.

Two series of experiments were carried out. It was interesting to compare the response of animals preadapted to this lower calcium level with those, preadapted to higher, used normally in control media, i.e., 10^{-3} M Ca^{2+} concentration. For that reason the animals were washed with the diferent, so-called "preadapting" media, of the same composition as control media. Two concentrations of calcium ions were used: approximately $10^{-6.5}$ M and 10^{-3} M. For estimation of Ca^{2+} concentration **Electroselenium Evans** Atomic Absorption Spectrophotometer was used [1].

Results and Discussion

The medium with the lowest concentration of free calcium ions, (10^{-8} M) appeared very toxic to *Tetrahymena*. In every experiment all specimens died within 5–7 min. and those kept in preadapting medium with a lower calcium level always died earlier.

The next lowest external calcium concentration 10^{-7} M, also killed the animals. They survived about 40–45 min. after immersion.

None of the other concentrations tested was toxic to *Tetrahymena*. The animals survived in all these solutions without visible sings of damage.

At Ca^{2+} concentrations of 10^{-8} M, *Tetrahymena* showed only very slight motility immediately prior to death and desintegration.

When the calcium concentration was 10^{-7} M the forward movement of *Tetrahymena* was predominant (Pl. I).

At all other concentrations, many types of the movements appeared, but spontaneous ciliary reversal was the most characteristic for the solutions at 10^{-5} and 10^{-4} M.

At free calcium ions concentrations of 10^{-2} M, the reversal movement was very seldom observed. Immediately after transfer to this medium the animals started to swim forward very fast. This movement could last for a long time, in some cases longer than 6–7 min.

No significant difference was found between the number of food vacuoles formed by *Tetrahymena* in the two different preadapting media. These values were used as controls to compare with those found in experimental solutions (Table 1).

Table 1

The effect of two different Ca^{+2} concentrations preadapting media on food vacuole formation by
Tetrahymena pyriformis GL

Groups of experiments	Concentration of free calcium ions in preadapting media	
	10^{-3} M Ca^{2+}	$10^{-6.5}$ M Ca^{2+}
I	3.88±0.82*	3.78±0.96
II	3.86±1.10	3.76±0.92
III		3.82±0.98

* Mean number and SD of food vacuoles formed in 10 min. (calculated in 150 specimens of each group).

Since the medium containing 10^{-8} M calcium killed *Tetrahymena* quickly, it was difficult to calculate the rate of food vacuole formation. After repeated observations, we came to the conclusion that the ciliates were able to ingest PLP but only for a very brief time (variable, but up to 2 min.) after immersion in this solution.

A summary of our results on the influence of different amounts of external calcium on phagocytosis in *Tetrahymena pyriformis* is presented in Fig. 1. The preadapting conditions, i.e., the concentrations of calcium
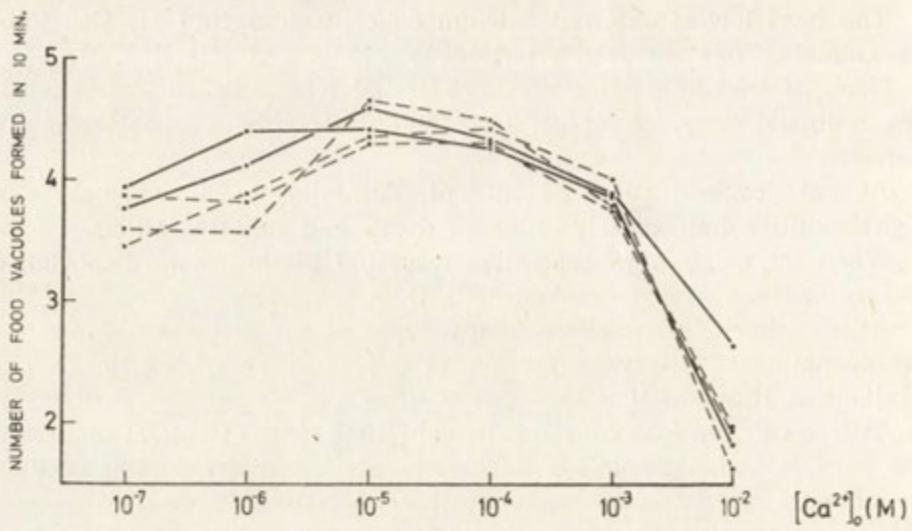
Fig. 1. The effect of EGTA/Ca buffers on the food vacuole formation in *Tetra-hymena pyriformis* GL. Each point is a mean number of food vacuoles formed during 10 min. by 50 animals. Continuous lines represent 2 series of experiments carried out with cells preadapted in solution 10^{-3} M Ca^{2+}, whereas discontinuous lines represent 3 series of experiments with cells preadapted in solution $10^{-6.5}$ M Ca^{2+}

in which the animals stayed after washing with Tris-HCl solutions (control medium), seems to have special significance for phagocytosis only in one case, namely when calcium concentration in experimental medium is 10^{-6} M. Here the number of food vacuoles formed in 10 min. was significantly less in animals taken from the medium of lower calcium concentration, than the number of vacuoles formed by animals preadapted to the medium more rich in calcium ions.

In all other cases both curves are very similar, showing that the number of food vacuoles is either similar, or higher, than in animals tested in control media, with the exception of the medium with 10^{-2} M of external calcium in which the number of food vacuoles decreases to about 50% of the other values. On the contrary the highest number of food vacuoles is seen at calcium ions concentrations of 10^{-5} and 10^{-4} M.

The results of these experiments showed that decreased concentration of calcium ions in the external medium is toxic to *Tetrahymena*. Solutions containing 10^{-8} and 10^{-7} M are lethal to the ciliates.

In solutions with low calcium concentrations, *Tetrahymena* is not able to show ciliary reversal, and this is in agreement with findings of N a i t o h and K a n e k o (1972) in their experiments on detergent models of *Paramecium*.

Despite these disturbances, *Tetrahymena* is not deprived of the capacity for food vacuole formation, at least during the first minutes after transfer to the lowest calcium concentration, 10^{-6} M. In higher calcium concentrations formation of food vacuoles takes place without difficulty.

An excess of calcium in the external medium, more than 10^{-3} M, does not appear to affect *Tetrahymena* adversely, although it can cause partial inhibition of phagocytosis. At the highest concentration of calcium ions (10^{-2} M) fast forward movement is always observed.

The higher concentrations of calcium ions (5–20 mM) are known as factors inhibiting both pinocytosis (C h a p m a n - A n d r e s e n 1962, C o o p e r 1968) and phagocytosis (B r u t k o w s k a 1967, N i l s s o n 1972). However, J o s e f s s o n (1975) suggest that competitive inhibition of pinocytosis by calcium does not explain the role of this ion which also facilitates the formation of channels in *Amoeba* (C o o p e r 1968). Similar phenomenon concerning phagocytosis in *Paramecium caudatum* is known from previous publication (B r u t k o w s k a 1967). In KCl medium with immediate presence of 0.01 mM of $CaCl_2$ a higher level of phagocytic activity than in KCl alone was observed. Hence, the mechanism of the action of calcium is, in this respect, still unclear.

In media with 10^{-5} and 10^{-4} M concentrations of calcium ions distinct acceleration of phagocytosis can be seen independently of the concentration of calcium in preadapting media. In these cases spontaneous ciliary reversal movement is more often observed than at other concentrations. The explanation of this fact can be made merely if we assume the inducting properties of sodium chloride, in our experiments at about 5 mM concentration. The phenomenon is very well known from other researches (C h a p m a n - A n d r e s e n 1958, 1962, 1972, J o s e f s s o n 1968 and C o o p e r 1968 on *Amoeba*, B r u t k o w s k a 1967 on *Paramecium* and N i l l s o n 1976 on *Tetrahymena*). J o s e f s s o n (1966) found Tris as compound inducing pinocytosis similarly to Na^+. Stimulating effect of Tris ion cannot be excluded also in our experiments. In this intermediate level at which the concentration of calcium ions in the external media is neither too low, nor too high (i.e., 10^{-5} and 10^{-4} M), the stimulation of phagocytosis together with specific character of ciliary movement has been observed.

ACKNOWLEDGEMENTS

RÉSUMÉ

Il est démontré que *Tetrahymena pyriformis* GL garde toujours la faculté d'absorber les suspensions dans un large diapazon des concentrations de Ca^{2+} libre dans le milieu ambiant (10^{-7}–10^{-3} M Ca^{2+}). Seulement avec 10^{-2} M Ca^{2+} la phagocytose devient réduite dans une certaine mésure. Les changements des réactions motrices correspondant à des concentrations variées du Ca^{2+} dans le milieu ambiant, n'exercent qu'une influence légère sur l'activité phagocytique.

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EXPLANATION OF PLATE I

Motile behaviour of *Tetrahymena pyriformis* GL in EGTA/Ca buffers at changing level of Ca^{2+} ions in external medium. The movement of *Tetrahymena* in two different preadapting media
1: At concentration of $10^{-6.5}$ M Ca^{2+}
2: At concentration of 10^{-3} M Ca^{2+}
No special differences are seen in the movement of *Tetrahymena* staying in these media
The movement of Tetrahymena in changing level of Ca^{2+} ions in EGTA/Ca buffers medium
3: The movement of *Tetrahymena* immersed in 10^{-7} M Ca^{2+} concentration. The animals move slowly forward, or even lay on the bottom of the vessel
4: Fast chaotic, partially also circling movement of *Tetrahymena* immersed in 10^{-5} M Ca^{2+} concentration
5: Rather fast forward movement of *Tetrahymena* in the medium of 10^{-2} M concentration of Ca^{2+}

M. Brutkowska et al.   auctores phot.

Jerzy J. LIPA

Infection of *Notocellia uddmanniana* L. (*Lepidoptera: Tortricidae*) by the Microsporidian *Nosema carpocapsae* Paillot

Synopsis. The microsporidian infection of *Notocellia uddmanniana* L. is recorded for the first time. The causative agent was identified as *Nosema carpocapsae* Paillot a well known parasite of codling moth (*Carpocapsa pomonella* L.). The parasite caused a general infection and very heavily attacked Malpighian tubules, silk glands, midgut epithelium and other tissues of *N. uddmanniana*. The size of fresh spores varied 3.4–7.3 by 2.0–3.0 μm. Large and abnormal spores were also observed. In 1968 the infection level in population of *N. uddmanniana* on *Rubus caesius* L. was low but in 1969 amounted 25%.

A microsporidian *Nosema carpocapsae* Paillot is a well known pathogen of codling moth (*Carpocapsa pomonella* L.) causing a general infection of larvae, pupae and adults. It is transmitted transovarially and plays an important role in the dynamics of *C. pomonella* (Lipa 1963).

In this paper I present results of studies on natural infection of *Notocellia uddmanniana* L. by *Nosema carpocapsae*. A tortricid *Notocellia uddmanniana* is a common feeder occurring on plants belonging to *Rosaceae* family. In some countries it is known as pest of raspberry, dewberry and strawberry.

Material and Methods

On June 8, 1968 Mgr. S. Lewandowski has brought to our laboratory five diseased caterpillars and pupae of *Notocellia uddmanniana* L. with the request for a diagnosis of some dead specimens. These insects were collected in Poznań Dębina on May 28, 1968 on a plant *Rubus caesius* L.

A year later on June 6, 1969 Mgr. S. Lewandowski supplied several diseased and dead larvae of *N. uddmanniana* which were also found to be infected by the microsporidian found a year earlier. These specimens were collected in the same place as those in 1968.

Healthy and diseased larvae, pupae and adults of *N. uddmanniana* were dissected and their tissues were examined under the phase and light microscope.

The life cycle of the microsporidian involved was studied on the smeared preparations fixed in methyl alcohol and stained in Giemsa stain 0.25% for 24 h.

Results

Pathology

The infected larvae greatly differed from the healthy ones. The heavily infected larvae of *N. uddmanniana* are slugish and show low apetite. They have abnormal appearance and through their cuticule on the ventral side a white intestine is seen. This milky appearance of the midgut was due to a huge number of spores of *Nosema*. At the microscopical examination it was found that the midgut epithelium, silk glands (Pl. I 1 and 2) tracheal matrix (Pl. I 3) and Malpighian tubules were heavily infected and damaged by the parasite. The silk gland and Malpighian tubules were frequently so heavily infected by the parasite that practically no part of these organs was free from the pathogen (Pl. I 1 and 2).

In the light of observed pathological changes in the infected larvae of *N. uddmanniana* there is no question that the studied microsporidian is a lethal parasite causing the death of insects.

Development

S c h i z o g o n y: Typical schizonts are spherical and their size depends on their age. Young schizonts are mainly binucleate and have 2 to 4 μm in diameter; uninucleate schizonts were rarely observed. The cytoplasm of schizonts stained with Giemsa's solution is deeply blue and the nuclei are deep red.

Older schizonts are tetranucleate (Pl II 5) and their size differs according to their development. The largest ones have up to 8 μm in diameter. The nuclei of schizonts are mainly round but many of them are reniform. Tetranucleate schizonts divide into two daughter cells. Chains of schizonts were not observed on examined preparations.

S p o r o g o n y: The begining of the sporogonic phase is manifested first in the nuclei and cytoplasm of schizonts. They both become more diffused and stain weakly with Giemsa's solution as compared in schizonts.

The sporogonic stages are spindle shaped binucleate bodies and

usually have 6 to 8 µm although some of them are up to 10 µm long. The twin nuclei are located close one to another (Pl. II 6).

The sporoblasts are fusiform and always solitary. The size of the sporoblast decreases as maturation progresses and its shape tends to become oval. Each sporoblast produces a single spore that is characteristic for the genus *Nosema*.

Spores

A great majority of spores is oval (Pl. II 4 and 5). The length of living spores measured in water ranged from 3.4 to 7.3 µm (typically 3.9–5.1) and their width 2.0 to 3.0 µm. Spores fixed in methyl alcohol and stained with 0.25% Giemsa's solution were slightly smaller (Table 1).

Table 1

Frequency distribution of the length of two samples of 50 spores each of *Nosema carpocapsae* Paillot in smeared preparation of tissues of *Notocellia uddmanniana* L.

Sample of spores	Dimensionable groups (in µm)								
	3.1–3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0	7.1–7.5
Alive in water	1	9	20	9	2	1	1	1	1
Fixed and stained	3	23	18	1	2	–	1	1	1

Among typical spores there were some considerably elongate or spindle shaped (Pl. II 7 and 8). The largest observed spores were up to 8.5 µm long. The number of such spores is not high as one abnormal spore is observed per 15–20 normal spores.

The polar filament was easily extruded by applying pressure to the cover glass. Its length was up to 80 µm.

Taxonomic Position

This is the first record of a microsporidian infection in *Notocellia uddmanniana*. As mentioned above each sporoblast produces a single spore what means that the microsporidian discovered in *N. uddmanniana* belongs to the genus *Nosema*. This microsporidian attacks various tissues and causes a general infection. The life cycle and pathology of the studied *Nosema* make this species very similar to *Nosema carpocapsae* Paillot (P a i l l o t 1939, L i p a 1962). Also, when shape and spore size of studied *Nosema* was compared with spore size of *Nosema carpocapsae* no important differences were observed.

Both insects *Notocellia uddmanniana* and *Carpocapsa pomonella* belong to the same family *Tortricidae* and besides develop on the plant species of the family *Rosaceae*. Since they inhabit the same biotopes they can be parasitized by the same pathogens.

Therefore, basing on the similarities in the life cycle, pathology and spore dimension as well as on ecological considerations I assume that the microsporidian observed in *Notocellia uddmanniana* is identical with *Nosema carpocapsae* Paillot.

Discussion

The lepidopterans of the *Tortricidae* family form a large group of economic pests of plants mostly decidous trees. Therefore the study of pathogens of tortricids have practical implications as they may lead to the development of biological control of economic pests.

Since, *Notocellia uddmanniana* is feeding on *Rubus* spp. and in some cases was recorded as an economic pest the studies of its pathogens are of value for the general knowledge of pathogens of insects belonging to the *Tortricidae* family as well as for biological control.

Although, *Nosema carpocapsae* Paillot is known as common parasite of *Carpocapsa pomonella* L. this is the first case of recording it in another insect species belonging to the *Tortricidae* family. In its new host *N. carpocapsae* preserve the same type of development, spore size and pathological effect.

ZUSAMMENFASSUNG

Eine Mikrosporidien-Infektion wurde das ersten Mal bei *Notocellia uddman-niana* L. festgestellt. Als Infektionserreger wurde *Nosema carpocapsae* Paillot identifiziert — ein gut bekannter Parasit der *Carpocapsa pomonella* L. Der Parasit verursacht eine allgemeine Infektion und infiziert vor allem die Malpigischen Gefässe, die Spinnendrüsen, den Mitteldarm und andere Gewebe. Lebende Sporen messen 3.4–7.3 $\times$ 2.0–3.0 µm; fixierte und gefärbte. Der Infektionsgrad der *Notocellia uddmanniana*-Population war im Jahre 1968 nich gross, dagegen im Jahre 1969 betrug er schon 25%.

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EXPLANATION OF PLATES I–II

Nosema carpocapsae Paillot in *Notocellia uddmanniana* L.
1–2: Silk glands of *Notocellia uddmanniana* heavily infected with *Nosema carpocapsae*
3: Spores of *Nosema carpocapsae* in the tracheal matrix
4–5: Smears of tissues of *Notocellia uddmanniana* infected with *Nosema carpocapsae*; notice the huge number of spores and few schizonts (S)
6: Binucleated sporont of *Nosema carpocapsae*
7–8: Normal and abnormal spores of *Nosema carpocapsae*
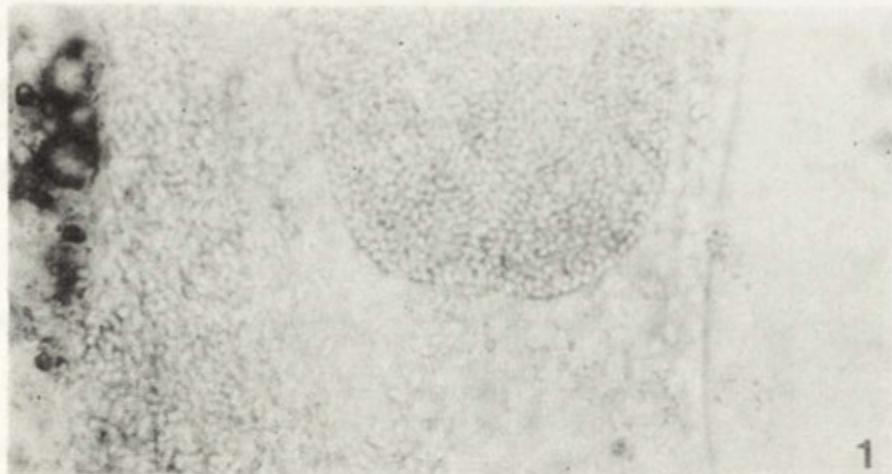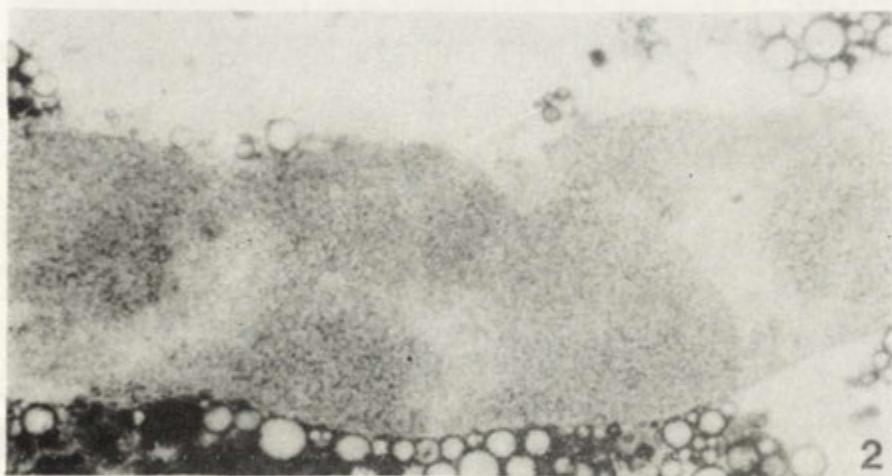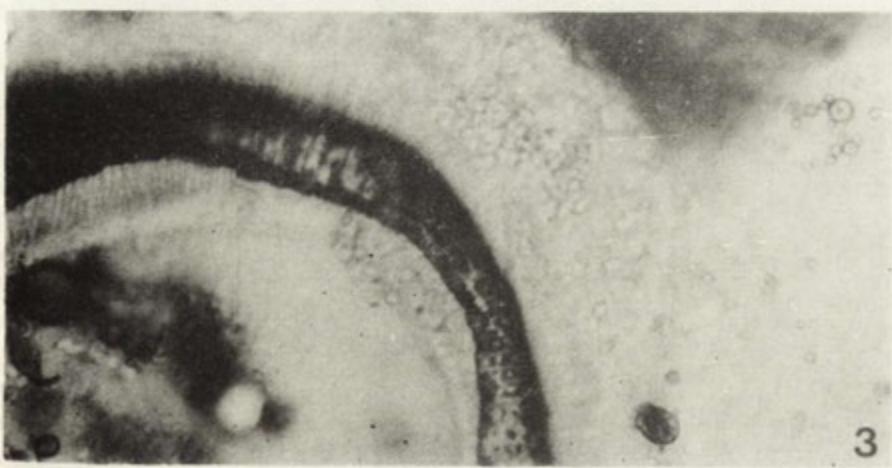
PLATE I

PLATE II
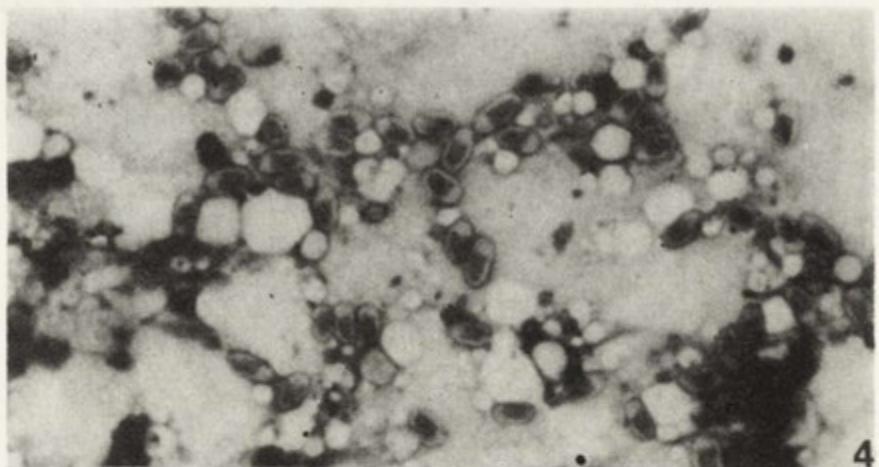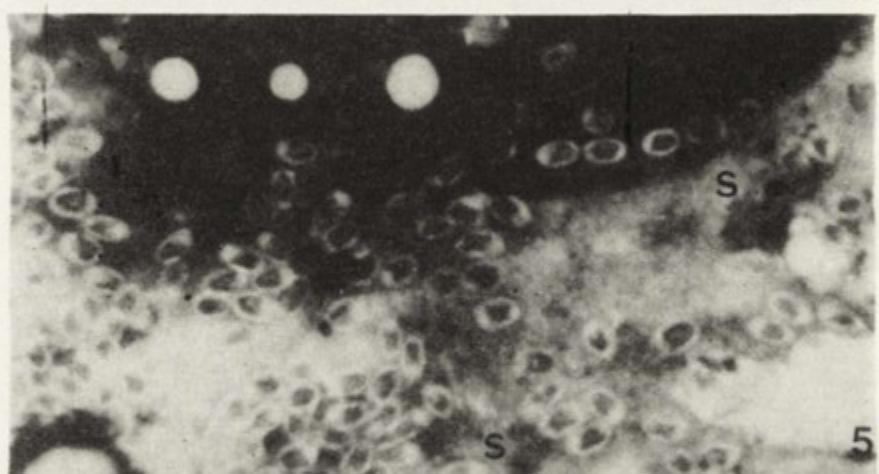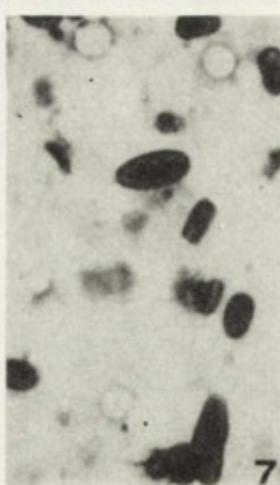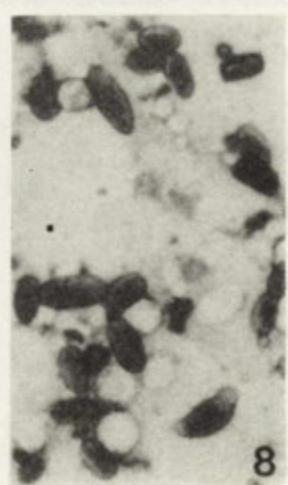
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and H. Ray GAMBLE

Cultivation of *Naegleria* Using Alcohol Killed Bacteria

Synopsis. Growth of *Naegleria* has been achieved using a medium containing alcohol killed bacteria suspended in a yeast extract-glucose solution. Under these conditions amebae grow with a shorter mean generation time (MGT) than reported in the past for other methods of axenic cultivation. In addition, this medium avoids the destruction of essential thermolabile factors present in the bacteria. The use of protein hydrolysates and serum fractions is unnecessary.

A newly isolated strain of naegleroid type ameboflagellate (Cl-1) and *Naegleria gruberi* (Pringsheim strain) have been grown successfully using this method.

Media reported for the axenic cultivation of ameboflagellates have included various serum fractions (B a l a m u t h 1964, C e r v a 1969, 1971, F u l t o n 1970, F u l t o n and G u e r r i n i 1969, O'D e l l and B r e n t 1974, O'D e l l and S t e v e n s 1973, O u t k a and K l u s s 1967) or heat killed bacteria (B a l a m u t h 1964, B r e n t 1954, C h a n g 1960, F u l t o n 1970, O'D e l l and S t e v e n s 1973, O u t k a and K l u s s 1967, S c h u s t e r 1961). Although the shortest mean generation times (MGT) reported for amebae grown under these conditions range from 7.7–9.4 h they do not approach those reported for amebae grown under bacterized conditions (F u l t o n 1970, F u l t o n 1974, O'D e l l and S t e v e n s 1973, O u t k a and K l u s s 1967, S c h u s t e r 1961). We therefore consider these media to be nutritionally adequate, but not equivalent, to that available to amebae grown in bacterized cultures.

In an attempt to obtain axenic growth of the ameboid stage of ameboflagellates with a MGT closer to that obtained in bacterized cultures we considered that bacteria killed in ways other than by heat might retain a greater nutritional adequacy. Attempts to achieve this by killing bacteria with common histological fixatives were unrewarding. However, it was found that the simple method of killing bacteria with a 50%

ethyl alcohol solution is efficient in its ability to kill bacteria, and that these bacteria are nutritionally more adequate than heat killed bacteria as judged by shortened MGT.

This report describes the axenic growth of *N. gruberi* on a medium containing yeast extract, glucose, and alcohol killed bacteria (AKB). A preliminary report of this work has been presented (N a p o l i t a n o and L a V e r d e 1975).

Materials and Methods

Strains

Two strains of ameboflagellates were used in this study. The first, designated Cl–l, was newly isolated from Long Island soil following techniques described earlier (N a p o l i t a n o et. al. 1970). It is "typically neagleroid" inasmuch as its cyst contains plugs, and transformation of the amebae yields flagellates with 2 flagella. The second strain was the Pringsheim strain of *Naegleria gruberi* CUCC No. 1518/11.

Stock Cultures

Stock clonal cultures of amebae were maintained on NM Agar (F u l t o n 1970), or 12 YA (N a p o l i t a n o et al. 1970) in Petri dishes. Stocks were grown as monoxenic culture lines using either *Enterobacter aerogenes* or *Escherichia coli* as the bacterial supplement. Transfers were made at weekly intervals. Axenic stock cultures of amebae grown on AKB yeast extract-glucose medium were transferred at 2–7 day intervals for routine maintenance under these conditions. Bacteria used in this study included two strains of *E. coli*, Adelphi University strain No 16 and ATCC No 11775; and two strains of *Enterobacter aerogenes*, Adelphi University strain No 2 and ATCC No 13048; and *Proteus mirabilis*, Adelphi University strain No 41. All bacterial stocks were maintained on slants of nutrient agar and transferred to either nutrient broth or Penassay, Antibiotic Medium No. 3 (DIFCO) for use in experiments. Bacteria used as food for amebae were grown at 30°C.

Preparation of Solutions

Four solutions were used extensively. A standard 2×10^{-3} M tris buffer pH 7.4 was used for routine handling of protozoa. This solution was modified by the addition of 1×10^{-3} $MgSO_4$ (tris–Mg buffer) for

washing and harvesting bacteria. The solution used to kill bacteria was prepared as a 1 : 1 mixture of 100% ethanol with tris-Mg buffer. An antibiotic mixture consisting of penicillin and streptomycin was prepared in sufficient quantities to achieve a final concentration of 25 µg/ml each in final culture media.

Preparation of the Axenic Medium

Preparation of the axenic medium was accomplished by selecting 24–48 h nutrient broth or 12–24 h. Penassay cultures of either *E. aerogenes* or *E. coli* grown in 50 ml round bottom centrifuge tubes containing 30 ml of broth. Following centrifugation for 2–3 min. the supernatant was discarded and replaced with 5 ml of the alcohol-tris-Mg solution. Cells were resuspended by mixing on a Vortex Genie mixer at setting No. 10 until the pellet was completely disrupted (varies from 1–2 min. with different strains of bacteria). These cells were allowed to stand for 2–3 min. after which the alcoholic suspension was centrifuged again, the supernatant decanted, and two washes performed using 5 ml of tris-Mg buffer for each. The number of cells suspended in the second wash was determined by hemocytometer counting and adjusted to achieve a bacterial concentration of 10^7-10^9 cells/ml in particular experiments. The bacteria were centrifuged once again, the supernatant poured off, and the remaining fluid above the pellet removed with a pasteur pipette. The pellet was then suspended in 2.7 ml of a tris solution containing yeast extract and glucose (each 0.01% W/V) and the antibiotic solution (25 µg/ml). Antibiotic was added to inhibit bacteria associated with the inoculation of stock bacterized amebae.

Inoculation of Protozoa

Amebae taken from 24–48 h bacterized tris-suspensions were inoculated to tubes or flasks containing AKB media as described above. The inoculum consisted of an aliquot of 0.3 ml adjusted to achieve a concentration of 10^3 or 5×10^4 cells/ml in a final volume of 3 ml. Inoculated cultures were maintained at selected temperatures in a constant temperature bath with shaking (72 strokes — 2.5 cm/min).

Counting

Enumeration of cells grown in tubes and flasks was determined by direct counting of protozoa in a Spencer Brightline Hemacytometer. Two methods were used. In the earlier method, fixation was accomplished

by adding a dilution fluid consisting of Carnoy's fixative and Lugol's iodine solution (1 : 1) thereby fixing, staining and diluting the population in one step. The entire contents of flasks or tubes were fixed for counting of the amebae at 6, 12, 24 and 48 h intervals. Following fixation cultures were vortexed for 90 s on a Vortex Genie at setting No. 7. After allowing uptake of stain the cells were vortexed for an additional 30 s. Aliquots were immediately removed with a pasteur pipette and multiple counts performed using the counting chamber. In the second method a drop of culture fluid was aseptically removed with a pasteur pipette and placed in the hemacytometer chamber without fixation. This approach allowed the same flask or tube to be used throughout the experimental period. Counting of amebae was simpler and more direct in this method.

Storage of Alcohol Killed Bacteria

Experiments to determine the adequacy of AKB as food for the amebae following a period of storage at refrigeration or freezing temperatures were performed. The bacteria were prepared as usual, except that, AKB suspensions were placed at 5°C or —5°C in the yeast extract glucose medium for periods of 2–6 weeks. Following strorage, AKB bacterial suspensions were returned to room temperature, antibiotic solution was added and amebae were inoculated, after which cultures were maintained as described above.

Long-term Viability of Protozoa in AKB Medium

Studies to test viability of amebae following long-term maintenance on AKB media were performed by removing cultures after 3 days of growth at 32°C and setting them aside at room temperature for periods of at least 2 months. Cysts from these cultures were inoculated to freshly prepared AKB media and were also tested for their ability to grow again in bacterized cultures by direct transfer of cysts to Petri dishes containing bacterized NM agar.

Sterility Testing

Routine sterility testing of axenic cultures included inoculation of test samples to nutrient agar and broth, Sabouraud's agar and broth, Penassay broth and Thioglycollate medium at 22, 30 and 37°C with

examination after 3–5 days. Sterility testing to determine the efficiency of the alcohol killing method was achieved by transferring samples after the final wash to nutrient broth or Penassay. In some experiments (ATCC strains), testing was performed immediately after suspension of treated bacteria in alcohol.

Results

Adequacy of the Alcohol Killing Method

The AKB method was tested using 5 strains of bacteria in modules of 18–22 tubes for a total of 560 tests apart from routine sterility testing of bacteria prepared as food for the protozoa in particular experiments. A summary of these trials given in Table 1, suggests that this method

Table 1

Adequacy of the alcohol killing method using three species of bacteria

Bacteria	Strain	Trials	Growth	No growth
E. coli	AU 16	213	0	213
	ATCC 11775	36	0	36
E. aerogenes	AU 2	75	1	74
	ATCC 13048	36	0	36
P. mirabilis	AU 41	100	1	99

is reliable. Similar reliability has been obtained throughout all our experiments in which bacteria were killed in this way in preparation for their use in growth experiments.

Bacterial Concentration

The concentration of AKB has proved to be of critical importance. The MGT of amebae was profoundly influenced by the concentration of bacteria in the yeast extract-glucose medium as shown in Table 2. Whereas a MGT of 2.5 $\pm$ (0.2) h was obtained for Cl-1 in the presence of 10^9 bacterial cells/ml, the MGT increased to 3.8 $\pm$ (0,3) h at a bacterial concentration of 10^8 cells/ml and increased to 19.1 $\pm$ (11.7) h in the

Table 2

MGT of strain Cl-1 in the presence of 3 different
bacterial concentrations in AKB medium*

Replications	Bacterial concentration	MGT (h)
9	5.0×10^9 cells/ml	2.5 ± 0.2
6	5.9×10^8 cells/ml	3.8 ± 0.3
6	6.0×10^7 cells/ml	19.1 ± 11.7

* Mean Generation Time (MGT) is calculated as follows: MGT $= t/n$, where t — time in hours; $n = 3.3 \log (b/B)$, where B — number of cells at initial time and b — number of cells at later time.

presence of 10^7 bacterial cells/ml. Amebae became smaller, divided less frequently, and in some cases failed to form cysts in the presence of the lower concentrations of bacteria.

Growth under Varying Conditions

Growth of Cl-1 amebae was studied at different temperatures, pH, and in the presence or absence of antibiotic supplements. Flasks containing amebae growing on AKB yeast extract-glucose medium were maintained at 26, 32, and 38°C as described above. The growth of amebae at these temperatures is shown in Fig. 1. The shortest MGT (2.5 h) was
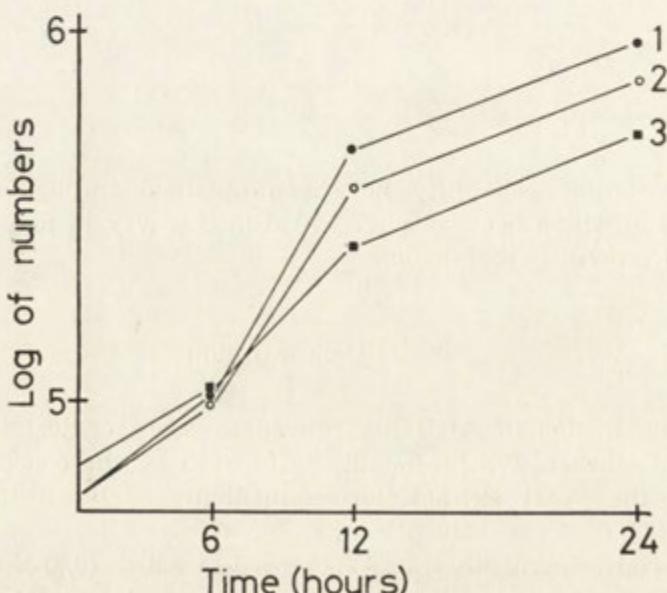
Fig. 1. Growth of Cl-1 at three temperatures. 1 — 32°C (2.5), 2 — 26°C (3.4), 3 — 38°C (4,5). Numbers in brackets are MGT in hours. S. E. < 0.8 p < 0.05

obtained at 32°C. All subsequent growth studies were performed at this temperature.

Results obtained when the pH was varied (Fig. 2) indicated that optimum growth was obtained at pH 7.4 in 2×10^{-3} M tris buffer. Growth of Cl-1 amebae at pH 7.4 in phosphate buffer, or at pH 6.9 in either phosphate or tris buffer occurred with a longer MGT and yielded fewer cells than obtained in those cultures grown in tris buffer at pH 7.4.
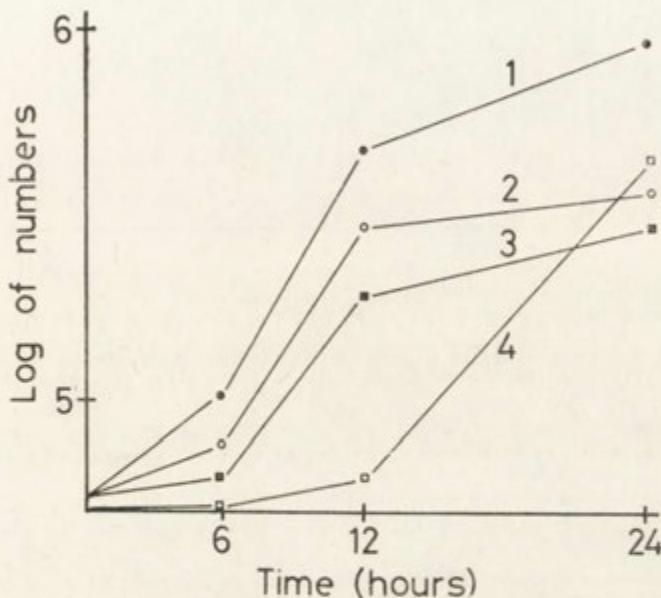
Fig. 2. Growth of Cl-1 amebae on AKB yeast-glucose medium prepared in phosphate or tris buffer at selected pH values. 1 — tris buffer pH 7.4 (2.5), 2 — phosphate buffer pH 7.4 (2.9), 3 — phosphate buffer pH 6.7, 4 — phosphate buffer pH 6.9 (4.5). Numbers in brackets are MGT in hours. S. E. < 0.2. $p < 0.05$

Growth in the presence of the penicillin–streptomycin antibiotic mixture was also studied. As indicated in Fig. 3 no significant change in growth of Cl-1 amebae was obtained when the antibiotic was present as compared to growth in its absence.

Growth of *N. gruberi* on AKB yeast extract-glucose medium was studied using several inoculum sizes. All growth experiments were performed at 32°C, at pH 7.4 and with bacterial concentrations greater than 10^9 cells. Results are shown in Fig. 4 and indicate that smaller inoculum sizes produce yields similar to large inocula with similar MGT during log phase of growth.
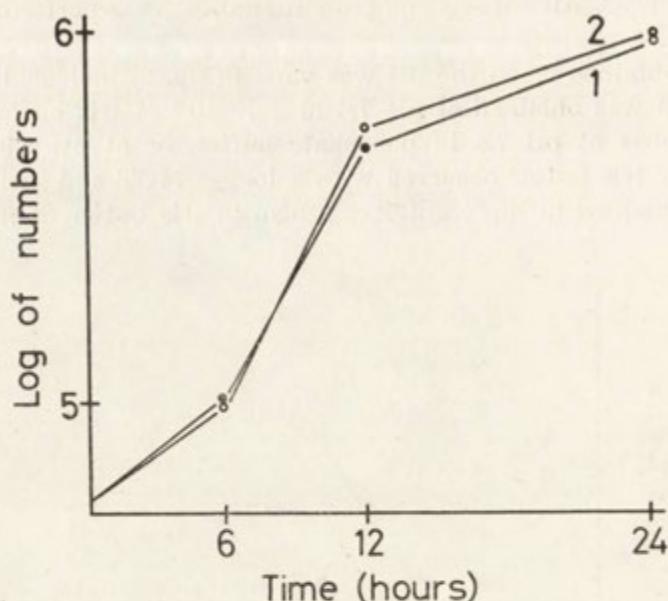
Fig. 3. Growth of Cl-1 amebae using AKB yeast extract-glucose in the presence
(1) or absence (2) of antibiotic, S. E. < 0.12, $p > 0.05$
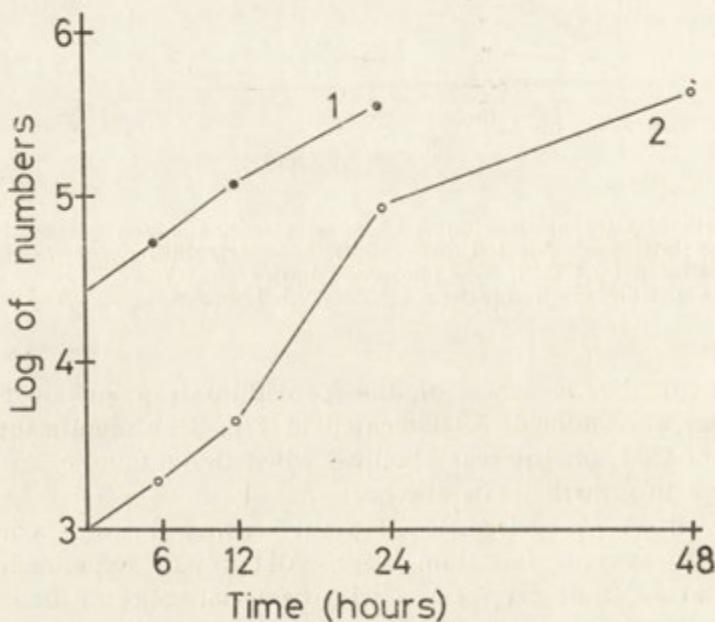
Fig. 4. Growth of *N. gruberi* on AKB yeast extract-glucose medium using two
different sized inocula. *N. gruberi* (large inoculum) (1), (4.4), *N. gruberi* (small
inoculum) (2), (3.7). Numbers in brackets are MGT in hours

Storage of AKB

Growth of amebae fed stored bacteria was tested as described above. As shown in Table 3 these bacteria retained their nutritional adequacy. Some loss of bacterial cells did occur using this method due to additional manipulations.

Table 3

MGT of strain Cl-1 at 32°C grown using *E. coli* stored at different temperatures. Time of storage was 21 days

Preparation	Replications	Bacterial Concentration	MGT (h)
Unstored	9	5.0×10^9 cells/ml	2.5
Frozen	5	4.3×10^8 cells/ml	5.4
Refrigerated	2	5.0×10^8 cells/ml	6.7

Stages in the Life History

Encystment of amebae occurred regularly and began between 24–72 h in axenic cultures of Cl-1, and later in *N. gruberi*. The cysts are viable inasmuch as they readily excyst when transferred to fresh AKB yeast extract-glucose medium, or to bacterized cultures. Long term storage of cysts did not alter their excystment behavior.

At first we observed that attempts to transfer amebae grown on AKB yeast extract-glucose medium to bacterized solid surface media were not always successful. In some trials amebae failed to grow, and in others they grew slowly with encystment in less than 24 h. However, it was found that when amebae were transferred first to a tris suspension of living bacteria immediately following growth on AKB yeast extract-glucose medium and then transferred to solid surface media they grew as well as those routinely maintained on solid surface media.

Discussion

The AKB yeast extract-glucose medium used in this study differs from other media reported for the axenic growth of ameboflagellates in that it contains no serum fractions, protein hydrolysates, or heat killed bacteria. Destruction of thermolabile factors present in bacteria is avoided in the use of this method. The MGT of two strains of ameboflagellates on AKB yeast extract-glucose medium resembles more closely that

obtained with bacterized cultures than those reported for ameboflagellates grown on other axenic media. Although this medium supported growth of these two strains of ameboflagellates, differences were noted between them. This is not surprising as considerable variation in the growth of other strains of ameboflagellates has been clearly reported by others (O u t k a and K l u s s 1967, O'D e l l and S t e v e n s 1973; O'D e l l and B r e n t 1974).

The growth of Cl-1 amebae is of particular interest to us since its MGT on this medium is shorter than that reported for other strains grown on other media. For example, S c h u s t e r (1961) reported a value of 9.4 h for *N. gruberi* grown on heat killed bacteria. O'D e l l and B r e n t (1974) reported 7.7, 8.6 and 8.8 h for three different strains of *N. gruberi* in media containing autoclaved *E. coli*. F u l t o n (1974) reported a doubling time of 8–10 h for *N. gruberi* in a medium containing a macromolecular fraction of fetal calf serum.

The concentration of bacteria available to the amebae was critical in determining the growth characteristics observed in these studies. This information is of particular interest in view of the report by D a n s k o and A l e x a n d e r (1975) whose study of protozoan–prey relationships among various soil amebae indicated that protozoan multiplication ceases when the concentration of bacteria falls to 10^6–10^7 cells/ml. Results obtained in this study using AKB yeast extract-glucose medium suggest that the mean generation time becomes extended when the concentration of bacteria is dropped to 10^7 cells/ml or below.

In summary, a medium using alcohol killed bacteria suspended in a yeast extract-glucose solution for the growth of ameboflagellates is described. This medium is simply prepared, lacks heat killed bacteria, protein hydrolysates and serum fractions, may be stored at refrigeration (5°C) or freezing temperatures (—5°C) and supports the growth of ameboflagellates with a shorter MGT than those reported for the growth of these protozoa using other axenic media. A future report will include modification of this medium to replace the yeast extract fraction.

RESUMÉ

La croissance de *Naegleria* a executé en employant une agent intermédiare qui contient la bacterie tuée à l'accol quia suspendue à la solution de la levure de l'extrait du glucose. Les amibes croitent avec un temps génération moyenne plus

court que ces conditions rapportent au temps passé pour les autres methodes. En plus de, cette moyenne évite la destruction des agents thermolaibile essentiels assitent à la bacterie. L'emploi des hydrolysates de la proteine et les fractions de serum est peu nécessaire.

Une souche isolé nouvellement de la sorte naegleroid amoeboflagellate et *Naegleria gruberi* (la souche Pringsheim) a cru avec succes employé cette methode.

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R. Krishnamurthy and T. Sultana: New Flagellate *Hexamastix marathwadensis* sp. n. from an Insect *Polyphaga indica* — T. S. Bhaskar Rao, P. Dayakar, A. Devi, M. Damodhar Reddy, T. Bhaskar Rao: *Hexamitus pipiensae* sp. n. (*Flagellata*) from the frog *Rana pipensi* from Warangal, Andhra Pradesh, India — P. Dayakar, A. Devi, T. S. Bhaskar Rao and T. Bhaskar Rao: New Flagellate *Hypotrichomonas venkataramiahii* sp. n. from the Gut of *Varanus* sp. from Warangal, A. P., India — U. Buitkamp: Die Cilaitenfauna der Savanne von Lamto (Elfenbeinküste) — E. Sousa e Silva: Some Ultrastructural Variations of the Nucleus in Dinoflagellates throughout the Life Cycle — Д. В. Осипов и С. А. Подлипаев: Электронномикроскопическое исследование ранних стадий заражения *Paramecium caudatum* симбионтами макронуклеуса (йота- бактериями) — L. N. Seravin and E. E. Orlovskaja: Feeding Behaviour of Unicellular Animals. I. The Main Role of Chemoreception in the Food Choice of Carnivorous *Protozoa* — A. A. Karpenko, A. I. Railkin and L. N. Seravin: Feeding Behaviour of Unicellular Animals. II. The Role of Prey Mobility in the Feeding Behaviour of *Protozoa* — S. Dryl and I. Totwen-Nowakowska: Contribution to Studies on Motor Response of *Stylonychia mytilus* to Mechanical and Chemical Stimuli — A. Grębecki and G. Nowakowska: On the Mechanism of Orientation of *Paramecium caudatum* in the gravity Field. I. Influence of Ciliary Reversal and of External Ca Deficiency on the Geotactic Behaviour — G. Nowakowska and A. Grębecki: On the Mechanism of Orientation of *Paramecium caudatum* in the Gravity Field. II. Contributions to a Hydrodynamic Model of Geotaxis — A. T. W. Cheung: Reactivation of Tritonated Models of *Opalina* — J. E. Swenson, S. M. Gittleson and I. I. Isquith: Observations of the Effect of Rotation on *Tetrahymena pyriformis* Aggregations (Pattern Swimming).

CONTENTS

Index 35133