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Tayyaba SULTANA

Studies on Two New Species of Flagellates of the Genus *Polymastix* Bütschli, 1884 from Insects in India

Synopsis. Two new species of the genus *Polymastix* Bütschli 1884. *P. rayi* n. sp. and *P. ganapatii* n. sp. are described from the gut of Cockroaches and scarabaeid body and a deeply staining cap-like structure above it. It measures $11.8–25.8 \times \times 5.7–11.8 \mu$m (average $18.1 \times 8.5 \mu$m). *P. ganapatii* n. sp. lacks a parabasal body but has unequal flagella and an axostyle not longer than the body; it measures $12.9–24.2 \times 7.2–15.9 \mu$m (average $17.1–11.2 \mu$m). A key to the species of the genus is also included.

The genus *Polymastix* was erected by Bütschli (1884) for a flagellate which had been described earlier by Grassi (1879), as *Trichomonas melolonthae*. Later on, *Polymastix melolonthae* has been reported by several workers including Mackinnon (1913), Ludwig (1946) and Laird (1956). Travis and Backer (1931) described *P. phyllophagae* while Geiman (1932) named another species *P. wenrichi*. Grassé (1952) named *P. hystrix* with a very brief description and Qadri and Bhaskar Rao (1963) described *P. periplanetae* from the common cockroach. Thus there are five species known so far, all from insects. The parasites described herein constitute the sixth and seventh species of the genus.

Material and Methods

During the course of investigation numerous cockroaches (*Periplaneta americana*) were examined and 50% of them were found to harbour *Polymastix rayi* n. sp. in the hindgut, but it was heavy only in a few hosts. Simultaneously about 48 scarabaeid larvae were examined and almost all of them were found to be infected with another parasite of this genus, although the incidence was never heavy except in a few.

The parasites were observed in the living condition in hanging drop preparations. The addition of a drop of methylene blue or Lugol's solution was helpful in studying the parasite. Both dry and wet methods were employed for making permanent preparations. For the dry preparations the smears were exposed to osmic vapours, refixed in Methanol and stained with Giemsa stain. In the case of wet preparations, the smears were fixed in Schaudinn's fluid and stained with phospho-tungstic acid haematoxylin.

Polymastix rayi n. sp.

Morphology

The parasites are fairly large, elongated and somewhat spindle-shaped, with the anterior end broader and rounded and the posterior narrower and tapering (Fig. 1 *3–6, 9*). Some of the organisms are pyriform (Fig. 1 *1, 2*). The pellicle is covered with several ectocommensal bacteria. The cytoplasm contains bacteria or several deeply staining granules near the middle of the body (Fig. 1 *8*).

A pair of closely situated belpharoplasts is present near the anterior end (Fig. 1 *1, 2, 4*). The granules are almost at the same level (Fig. 1 *1*) or at slightly different levels (Fig. 1 *2*). One of these granules gives origin to a pair of flagella which are almost equal and shorter than the body (Fig. 1 *1, 4*). The other granule gives origin to the axostyle and a pair of unequal flagella. In some of the organisms, two of the four flagella appear to be thicker than the other two (Fig. 1 *8*).

The axostyle is very conspicuous because of its enormous length (Fig. 1 *2, 3, 6–9*). It appears to be thinner as it curves around the nucleus (Fig. 1 *5, 6, 8*). But the major portion of the axostyle is uniform in thickness, follows a wavy course (Fig. 1 *6–9*) and projects out of the posterior end for a considerable distance (3.6–6.2 μm with an average of 5.0 μm).

A characteristic feature of this organism is the presence of a deeply staining horizontal rod-like body between the nucleus and the blepharoplasts. It is slightly curved (Fig. 1 *3*) or straight (Fig. 1 *7, 9*) and extends right across the body surface. Just above this is an area which stains less intensely but forms a characteristic cap-like structure at the anterior tip (Fig. 1 *3, 7–9*). While the rod-like body possibly corresponds to the parabasal body described by earlier workers (Travis and Becker 1931), the significance of the cap-like body could not be made out.

The nucleus is rounded or slightly ovoid and situated a little behind the anterior end of the body. It measures: length 1.5–3.1 μm (average 2.7 μm) and width 1.5–3.1 μm (average 2.3 μm).

Measurements

Length of the body 11.8–25.7 μm (average 18.1 μm); breadth of the body 5.7–11.8 μm (average 8.4 μm); length of the axostyle 6.7–24.2 μm (average 16.9 μm); length of the spike 3.6–6.2 μm (average 5.0 μm); length of the flagellum I 6.7–21.1 μm (average 14.1 μm); length of the flagellum II 7.7–24.7 μm (average 14.3 μm); length of the flagellum III 5.7–25.7 μm (average 15.2 μm); length of the flagellum IV 13.3–28.8 μm (average 19.8 μm).
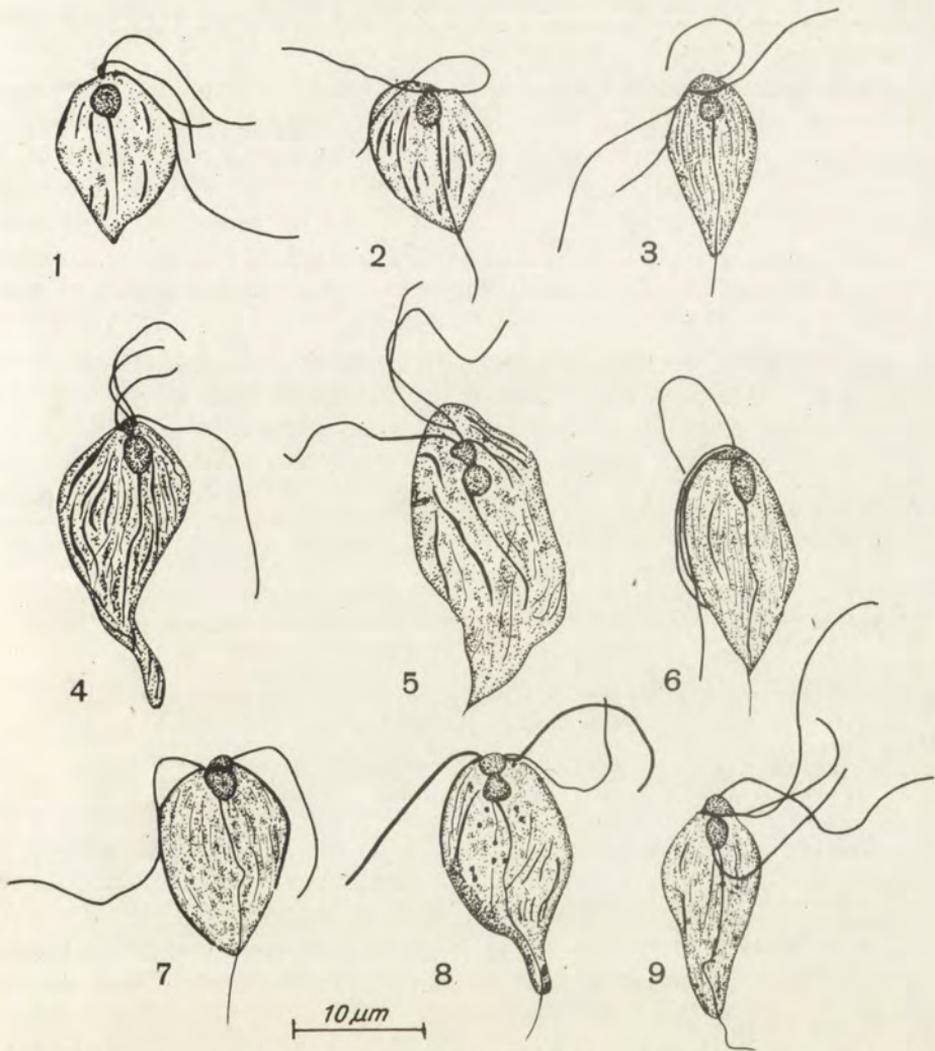
Fig 1. *Polymastix rayi* n. sp. *1, 2, 4* – Showing blepharoplasts and origin of flagella, *3* – Showing a curved parabasal body, cap-like structure and long axostyle, *5, 6* – Showing the anterior thickening of the axostyle, *7, 9* – Showing a rod-like parabasal body, cap-like body and long wavy axostyle, *8* – Showing a pair of flagella thicker than the other. (All figures from smears exposed to osmic vapours, fixed in methanol and stained with Giemsa stain)

Discussion

The genus *Polymastix* contains five species so far described from insect hosts. These are *P. melolonthae* (Grassi, 1879); *P. phyllophagae* Travis et Becker, 1931; *P. wenrichi* Geiman, 1932; *P. hystrix* Grassé, 1952 and *P. periplanetae* Qadri et Bhaskar Rao, 1963. Of these *P. melolonthae* has been recorded and described by several workers including Mackinnon (1912)

and Laird (1956). Two species *P. wenrichi* and *P. hystrix* have been rather inadequately described.

The organism described above is reported from the same host (*Periplaneta americana*) as *P. periplanetae* and resembles it in several features. However, the nucleus of the latter species is close to the anterior end while in the present form it is a little away from the anterior end of the body. Further while a parabasal body is absent in the latter species, a horizontal rod-like parabasal body is present above the nucleus in this organism. It also has an additional cap-like body at the anterior tip, the significance of which is not clear.

In possessing a parabasal body it resembles all the species except *P. wenrichi*. However, the nature of the parabasal body is different. Also its axostyle is much longer than any of these species.

In view of these differences the parasite is considered distinct and named *Polymastix rayi* n. sp. after the late Dr. H. N. Ray of the School of Tropical Medicine, Calcutta.

Species: *Polymastix rayi* n. sp.
Host: *Periplaneta americana*
Habitat: Hindgut
Locality: Khuldabad, District Aurangabad, Maharashtra, India.

Polymastix ganapatii n. sp.

Morphology

In stained preparations the parasites are relatively stumpy, having a very broad and rounded anterior half and a narrower, conical posterior half (Fig. 2 *1, 3, 6, 9, 10*). Some of the organisms appear elongated, with a bend or a twist just behind the middle of the body (Fig. 2 *2, 5*). The pellicle is fairly well developed and is covered by elongated ectocommensal bacteria. The cytoplasm appears to stain almost uniformly and very few inclusions or granules were seen.

A pair of small blepharoplasts is situated at the extreme anterior end of the body. The granules are placed fairly close together, at slightly different levels (Fig. 2 *2, 3-5, 8*). In preparations stained with phospho-tungstic acid haematoxylin the blepharoplasts in some forms are elongated and bilobed in nature and placed horizontally above the nucleus (Fig. 2 *4*). The posteriorly placed granule gives origin to a pair of flagella which are almost equal in length (Fig. 2 *5, 8*) and about as long as the body. The other granule gives rise to a pair of distinctly unequal flagella (Fig. 2 *3, 5, 8*), a shorter one directed forwards along with the other pair and a longer one which trails behind. While the shorter flagellum is almost of the same length as the body, the trailing one is about one-and-a-half times the body length. Some of the flagella terminate in acromenes (Fig. 2 *5*).
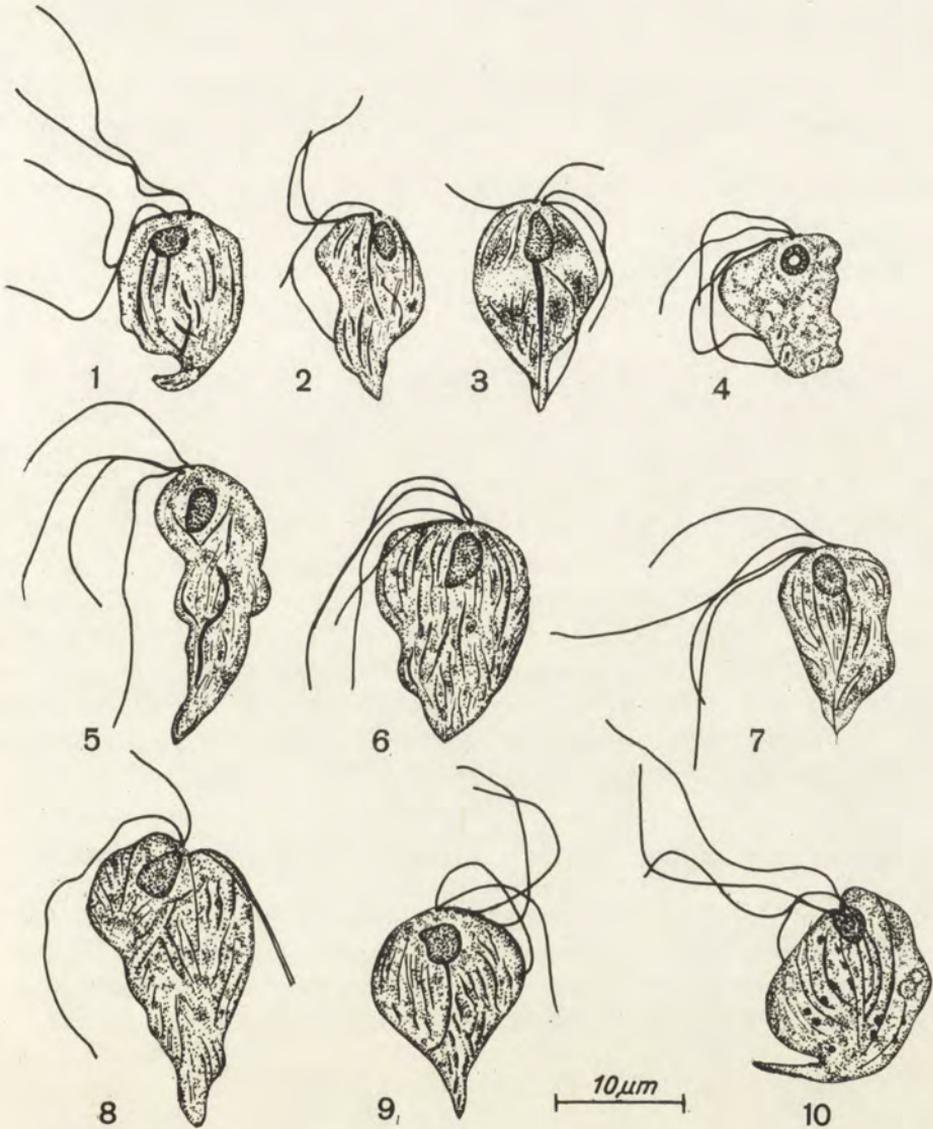
Fig. 2. *Polymastix ganapatii* n. sp. *1, 2, 6* — Showing general structure, *3, 8* — Showing blepharoplasts, origin of flagella and axostyle, *4* — Showing bilobed blepharoplasts and ring of chromatin granules in nucleus, *5* — Showing an elongated form with acronematic flagella, *7* — Showing a long axostyle of varying thickness, *9* — Showing a shorter axostyle, *10* — Showing uniformly distributed chromatin granules in nucleus. (Figs. *1–3, 5–9* from smears exposed to osmic vapours, fixed in methanol and stained with Giemsa stain. Figs. *4, 10* from smears fixed in Schaudinn's fluid and stained with phospho-tungstic acid haematoxylin)

The axostyle seems to originate from the posteriorly placed blepharoplast (Fig. 2 *2*). It runs below (Fig. 2 *3, 7, 9*) or besides (Fig. 2 *1, 2*) the nucleus. The anterior part of the axostyle is thicker than the posterior half

(Fig. 2 *2*, *3*, *7*, *9*). The axostyle is as long as or slightly shorter than the body (Fig. 2 *1*, *3*, *7*, *9*) and runs a somewhat curved course.

The nucleus is longer than broad and situated just behind the blepharoplasts. It is characteristic in having the chromatin distributed uniformly (Fig. 2 *10*) or in a ring around a central halo (Fig. 2 *4*) and in not having a distinct endosome.

Measurements

Length of the body 12.8–24.2 μm (average 17.1 μm); breadth of the body 7.2–15.9 μm (average 11.2 μm); length of the nucleus 2.1–5.1 μm (average 3.5 μm); width of the nucleus 1.5–4.1 μm (average 2.6 μm); length of the flagellum I 6.7–26.2 μm (average 16.9 μm); length of the flagellum II 6.7–28.3 μm (average 17.1 μm); length of the flagellum III 8.2–26.7 μm (average 18.4 μm); length of the flagellum IV 13.9–36.5 μm (average 23.5 μm).

Discussion

A comparison of the structure of the present organism with those of the six reported species shows differences in many respects. In its general appearance the present organism comes close to *P. wenrichi* and *P. periplanetae* due to the absence of a parabasal body. However, it has a much lesser range in body dimension that *P. wenrichi*, unequal flagella as against approximately equal ones and a nucleus with the chromatin distributed

Table 1

Comparative Body Dimensions of the Various Species from Insects of the Genus
Polymastix Bütschli, 1884
(All measurements in microns)

Sr. No	Species	Length of the body	Width of the body
(1)	*P. melolonthae* (Grassi, 1879) Laird, 1956	7.9–19.6 (13.9)	2.9–6.9 (5.3)
(2)	*P. phyllophagae* Travis and Becker, 1931	4.7–12.4	3.8–5.4
(3)	*P. wenrichi* Geiman, 1932	12.0–35.0	8.0–22.0
(4)	*P. hystrix* Grasse, 1952	5.0–15.0	–
(5)	*P. periplanetae* Qadri and Bhaskar Rao, 1963	12.0–24.0 (19.18)	5.5–9.5 (7.5)
(6)	*P. rayi* n. sp.	11.8–25.7 (18.1)	5.7–11.8 (8.4)
(7)	*P. ganapatii* n. sp.	12.8–24.2 (17.1)	7.2–15.9 (11.2)

either uniformly or in a ring around a central halo. It is contrasted from *P. periplanetae* in being relatively much broader (17.1×11.2 μm as against 19.2×7.5 μm), in the presence of a much shorter axostyle and in the absence of a distinct endosome in its nucleus.

It differs from *P. melolonthae*, *P. phyllophagae*, *P. hystrix* and *P. rayi* n. sp. in not possessing a parabasal body. It has a shorter axostyle than *P. melolonthae* and is larger in its body dimension than *P. phyllophagae* and *P. hystrix* (as shown in Table 1). The axostyle of this species is as long as or slightly shorter than the body while that of *P. rayi* n. sp. is much longer and projects out of the body to a distance of 4.97 μm on an average.

These differences justify the erection of a new species for this parasite and hence it is designated *Polymastix ganapatii* n. sp. after Dr P. N. Ganapati, Emeritus Professor of Zoology, Andhra University, Waltair (A. P), India.

Species: *Polymastix ganapatii* n. sp.
Host: Scarabaeid Larvae
Habitat: Hindgut
Locality: Aurangabad, Maharashtra, India.

The type slides are deposited in the Protozoology Section, Zoology Department, Marathwada University Aurangabad (M. S) India.

Key to the species of the genus *Polymastix* from insects

1. Parabasal body absent	...	2
Parabasal body present	...	4
2. Broad and leaflike in shape: all flagella approximately equal	... *P. wenrichi* Geiman, 1932	
Body elongated and narrower; flagella unequal ...	...	3
3. Axostyle with long spike	... *P. periplanetae* Qadri et Bhaskar Rao, 1963	
Axostyle as long as shorter than body	... *P. ganapatii* n. sp	
4. Deeply staining cap-like structure present above parabasal body ...	... *P. rayi* n. sp.	
Deeply staining cap-like structure absent ...	...	5
5. Axostyle thick, darkly staining and short ...	... *P. melolonthae* (Grassi, 1879)	
Axostyle thread-like and as long as body ...	... *P. phyllophagae* Travis et Becker, 1931	

(*P. hystrix* Grassé, 1952 is not included in the key as it is not adequately described).

ACKNOWLEDGEMENT

RÉSUMÉ

Deux espèces nouvelles du genre *Polymastix* Bütschli, 1884 sont décrites, *Polymastix rayi* n. sp. et *Polymastix ganapatii* n. sp., provenant respectivement de l'intestin de la blatte et des larves des *Scarabaeidae*. *P. rayi* n. sp. est caractérisé par la présence d'un corps parabasal et d'une structure en forme de calotte au dessus prenant une forte coloration. Ses dimensions sont de $11.8-25.8 \times 5.7-11.8$ μm, en moyenne 18.1×8.5 μm. *P. ganapatii* n. sp. est privé de corps parabasal, ses flagelles diffèrent en longueur, et l'axostyle ne dépasse pas longueur du corps, ses dimensions sont de $12.9-24.2 \times 7.2-15.9$ μm, en moyenne $17.1-11.2$ μm. Un tableau systématique des espèces appartenant à ce genre est inclu.

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Tayyaba SULTANA

Rhizomastix dastagiri n. sp.
(Mastigophora: Rhizomastigida) a New Flagellate from the Gut of the Cockroach Periplaneta americana

Synopsis. A new flagellate Rhizomastix dastagiri n. sp., is described from the cockroach Periplaneta americana in India. It is characterized by the presence of a distinct blepharoplast near or behind the nucleus, and a short rhizostyle of variable thickness and a free flagellum slightly longer than the body. Dimensions $5.7-12.3 \times 4.1-8.7$ μm (average 8.6×5.7 μm).

Alexeieff (1911) established the genus Rhizomastix to accommodate a uniflagellate protozoan which he obtained from the alimentary canal of axolotls. He named it R. gracilis. Identical forms were recorded from tipulid larvae by Mackinnon (1913), Geiman (1932) and Ludwig (1946). Two more species from insects, R. periplanetae from the cockroach Periplaneta americana and R. gryllotalpae from the mole cricket Gryllotalpa africana, were described by Bhaskar Rao (1963, 1970), besides one species R. ranae described from frogs by Krishnamurthy (1969). Thus there are altogether four species described so far in this genus, two from insects, one from amphibians and one (R. gracilis) occurring in both insects and amphibians.

Material and Methods

During the course of investigation of the intestinal flagellates of Arthropods, flagellates of the genera Rhizomastix and Monocercomonoides were encountered in the gut of the cocroach Periplaneta americana. The former was found only in one lot of about 30 cockroaches collected from Paithan, Maharashtra. The infection was moderate in most of them.

The parasites were examined in the living condition with the help of vital stains such as Methylene blue and Lugol's solution. Permanent preparations were stained with Giemsa's stain following exposure to Osmic vapours and fixation in methanol or with phospho-tungstic acid Haematoxylin after fixation in Schaudinn's fluid. The drawings were made with a camera lucida at a magnification of about 2000×.

Rhizomastix dastagiri n. sp

Morphology:

The parasite is variable in its shape being ovoidal (Fig. 1 *1, 2, 4*) or
irregular (Fig. 1 *6, 8, 9*). Some of the forms are elongated with one end
rounded and the other somewhat narrower and pointed (Fig. 1 *5, 7*).

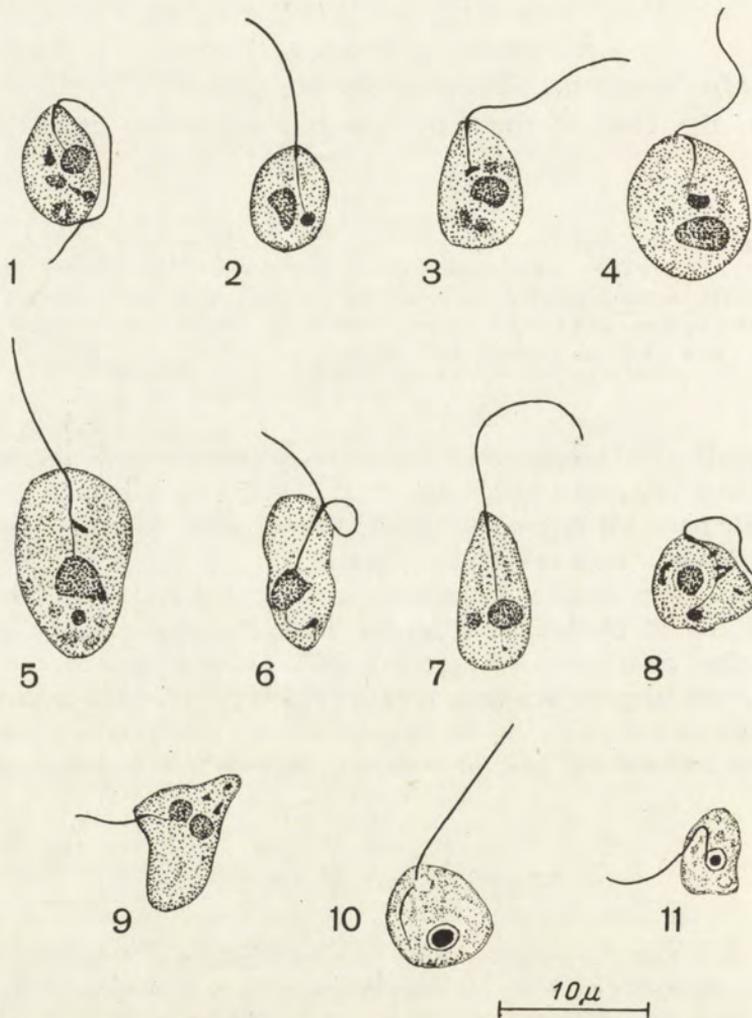The blepharoplast is clearly seen in most of the preparations. It is

Fig. 1. *Rhizomastix dastagiri* n. sp. *1* – Ovoidal form showing general structure, *2* – Showing
a large spherical blepharoplast and uneven rhizostyle, *3* – Showing rod-like blepharoplast above
nucleus and thickening of rhizostyle at its distal end, *4* – Showing kidney-shaped blepharoplast
and uneven rhizostyle, *5* – large form with an irregularly shaped nucleus, *6* – Elongated form
with a posteriorly placed blepharoplast, *7* – Showing a dot-like blepharoplast behind nucleus,
8 – Showing an irregular form with cytoplasmic inclusions, *9* – Dividing form, with two nuclei,
10–11 – Showing nuclear structure

variable in size, shape and position. It is very small and dot-like (Fig. 1 *7*) or somewhat bigger and spherical (Fig. 1 *2, 8*). It is a horizontal rod-like structure in some (Fig. 1 *3*) or large and kidney-shaped (Fig. 1 *4*) in others. It is located behind the nucleus (Fig. 1 *6–8*) or by the side of the nucleus (Fig. 1 *2*) or in front of the nucleus (Fig. 1 *3–5*). From this arises a well defined rhizostyle, which runs forward to the body surface, from where it continues as a single free flagellum. The rhizostyle is characteristic in being of variable diameter through its length, being relatively slender near the basal portion and thicker towards the distal end (Fig. 1 *2, 4–7*). The rhizostyle passes straight beneath the nucleus (Fig. 1 *6, 7*) or slightly curves around the nucleus (Fig. 1 *8*) and is apparently stiffer than the free flagellum. There is a thicker portion near the point where the flagellum becomes free (Fig. 1 *2, 3, 6, 7*). The rhizostyle measures about half of the body length on the average, while the free flagellum is a little longer than the body, measuring 4.6 to 17.5 μm with an average of 10.8 μm.

The nucleus is large and spherical (Fig. 1 *1, 7, 8*), elongated (Fig. 1 *3, 4*) or irregular (Fig. 1 *2, 5, 6*) in shape. It is situated either near the middle of the body (Fig. 1 *1, 8*) or slightly posterior to it (Fig. 1 *2, 4, 6, 7*). In well differentiated specimens, it has a distinct nuclear membrane and a conspicuous central endosome, with a clear space in between (Fig. 1 *10, 11*).

The cytoplasm stains uniformly and has well stained granules and bacteria (Fig. 1 *1, 8*). The periplast is thin.

A few binucleate forms were found (Fig. 1 *9*), probably representing division forms.

Measurements: Length of the body 5.7–12.3 μm (average 8.6 μm); breadth of the body 4.1–8.7 μm (average 5.7 μm); length of the nucleus 1.5–3.6 μm (average 2.1 μm); breadth of the nucleus 1.5–3.6 μm (average 2.2 μm); length of the rhizostyle is 1.0–9.2 μm (average 4.1 μm); length of the free flagellum 4.6–17.5 μm (average 10.8 μm).

Discussion

As mentioned in the introduction, there are three species of the genus *Rhizomastix* described from insect hosts. They are *R. gracilis* Alexeieff, 1911 from Axolotls and Tipulid larvae, *R. periplanetae* Bhaskar Rao, 1963 from *Periplaneta americana* and *R. gryllotalpae* Bhaskar Rao, 1970 from *Gryllotalpa africana*. The species described here constitutes the fourth species from insects, and second from the cockroach, *Periplaneta americana*, in India.

R. gracilis has a thick rod-like rhizostyle as long as the body and a free flagellum about thrice body length. Contrasted with this, the parasite described above has a rhizostyle of variable thickness (i. e., slender near the base and

thicker distally) and shorter length, averaging about half the length of the body. Alexeieff (1911) suggested the presence of a small basal granule at the point of the emergence of the flagellum while Mackinnon (1913) redescribing this species from Tipulid larvae noticed that "this basal granule mentioned by Alexeieff appears rather as a thickening of the rhizostyle than as a granule". In the present species, the thickening is very conspicuous and extends up to half of the length of rhizostyle. The organism is also much larger in size, measuring 5.7–12.3 μm $\times$ 4.1–8.7 μm (8.6 $\times$ 5.7 μm) as against 3.7–10.0 μm $\times$ 2.0–4.0 μm (7.5 $\times$ 2.5 μm) in *R. gracilis*. The free flagellum is only slightly longer than the body.

The new flagellate comes close to *R. gryllotalpae* in its dimensions but differs in other characters. The rhizostyle in *R. gryllotalpae* is uniform in diameter and about as long as the body, while in this form it is variable in diameter and relatively shorter. The free flagellum is also slightly longer in the new species than in *R. gryllotalpae*.

The parasite under discussion is described from the same host as *R. periplanetae*. However, the two forms show several differences. The rhizostyle is large and sometimes looped around the nucleus in *R. periplanetae*, whereas in this species it is shorter, straight and of varying thickness. The free flagellum is a little more than the body length in the latter, while it is about thrice the body length in the former. The parasite is slightly smaller in size than *R. periplanetae* (8.6 $\times$ 5.7 μm as against 9.6 $\times$ 7.3 μm) and does not show the dimorphism described in the other species.

Thus this parasite shows distinct differences from the other described species. Besides possessing a rhizostyle of variable diameter, this species is characterized by the presence of a distinct blepharoplast. The blepharoplast is located near or behind the nucleus and is either small and dot-like or somewhat larger and spherical, rod-like or kidney-shaped.

In view of these distinct differences this parasite is considered new to science and named *R. dastagiri* n. sp. after the author's father, for his constant encouragement throughout the course of this work.

     Species: *Rhizomastix dastagiri* n. sp.
     Host: *Periplaneta americana*
     Habitat: Middle and hindgut
     Locality: Paithan, Maharashtra, India

The type slides are deposited in the Protozoology section, Zoology Department, Marathwada University, Aurangabad (M. S.) India.

ACKNOWLEDGEMENT

RÉSUMÉ

Une nouvelle espèce des Flagéllés, *Rhizomastix dastagiri* n. sp., est décrite en provenance de la blatte *Periplaneta americana* aux Indes. Elle est caracterisée par un blépharoplaste distinct présent à côte ou derrière le noyeau, par un rhizostyle court de l'épaisseur variable, et par une flagelle libre dont la longueur légérement dépesse celle du corps.

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Eimeria bateri Bhatia et al., 1965 from the Common Grey Quail in Iran

Synopsis. Eimeria bateri Bhatia et al., 1965, is described from the common grey quail for the first time in Iran. The oocysts are ellipsoidal 14.4–28.2 × 12.0–21.0 μm with a mean of $20.60 \pm 0.0978 \times 15.90 \pm 0.085$ μm. Sporocysts are elongated ovoid $10.53 - 16.6 - 5.85 - 8.20$ μm with a mean of 12.8×6.75 μm with a prominent Stieda body. Sporocystic residuum are present.

Up to date three species of *Eimeria* have been reported from the common grey quail (*Conturnix conturnix conturnix*); namely *E. dispersa* Tyzzer, 1929, *E. coturnicis*, Chakravarty et Kar, 1947 and *E. bateri* Bhatia, et al. 1965. Tsunoda and Muraki (1971), reported the presence of *E. uzura* as a new species from Japanese quail (*Coturnix coturnix japonica*).

During the survey on parasites of wild birds, common grey quail (*Coturnix coturnix*) were collected for parasitical studies.

The faecal material of these birds was searched for coccidial oocysts and helminth eggs.

Oocysts were found in the faeces and subsequent studies revealed that they were *Eimeria bateri*.

This is the first report of this parasite from the common grey quail in Iran. In addition the biometrical studies on the size of oocysts were done to give detail on size variation in this Coccidium.

Materials and Methods

The birds used in this investigation were bought from the local pet shops, but were originally caught in North West Iran.

The birds were brought to the laboratory and kept in cages. Droppings were examined under the microscope either directly or after concentration using saturated solution of common salt. For further studies the faecal material was mixed with 2% potassium bichromate ($K_2C_rO_4$) solution in petri dishes and was oxygenated by stirring vigorously twice a day.

The measurements were made using an eye piece micrometer each division of which represented 3 μm. Readings were made to the nearest fifth a division. Since all measurements were made by the writer it was considered that operator bias, if any, was constant in all cases.

Results

The oocysts found in naturally infected birds were ellipsoidal and in 500 measurements only 25 (5%) were spherical. Oocyst wall colourless, smooth and 1.17–1.4 μm thick. Micropyle and oocyst residuum were absent but large polar granules were present. In 8% there were no polar granules and in 67% there was a single granule and in 25% there were two or three granules. Oocysts measured 14.4–28.2 × 12.0–21.0 μm with a mean of $20.60 \pm 0.0978 \times 15.90 \pm 0.085$ μm.

The shape index (length divided by width) was 1.028–2.0 with a mean of 1.295. A relatively wide variation in length and width was observed (Fig. 1).
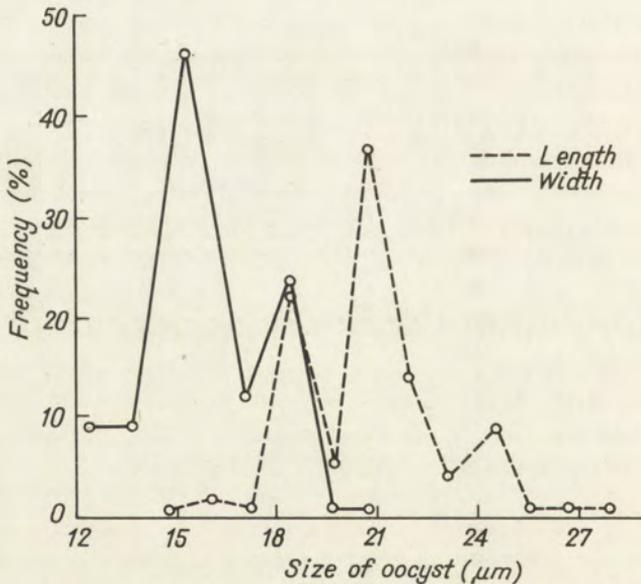

Fig. 1. Distribution of length and width of *E. bateri*

There were two peaks in width, 15.3 and 18.3 μm, and two in length, 18.3 and 20.7 μm.

To find the relationship between length and width, mean and standard deviation of width in different size were plotted (Fig. 2). As shown in

Fig. 2 when length was 20.4 μm the mean width fell to 15.4 μm. Otherwise increasing width corresponded with increasing length.

Sporocysts elongated ovoid measured 10.53–16.6 × 5.85–8.20 μm with a mean of 12.8 × 6.75 μm with a prominent Stieda body. Sporocystic residuum always present.

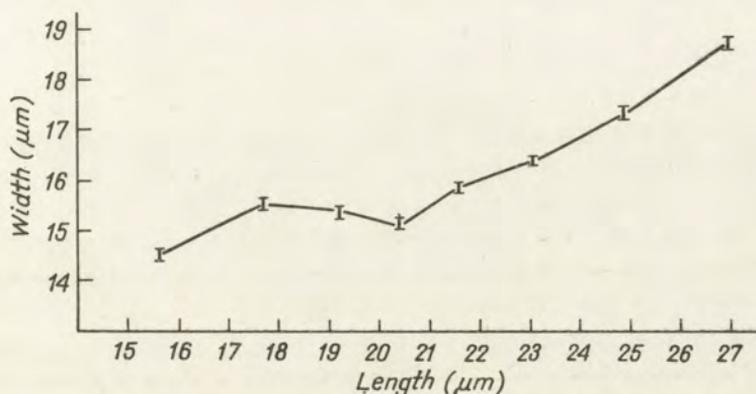The sporulation time was 42 h at 24°C.

Fig. 2. Correlation between length and width of *E. bateri*

Discussion

Four species of *Eimeria* have been described from *Coturnix* quail. Of these species *E. bateri* has been studied in more detail than the others. Norton and Peirce (1971) isolated the parasite from common grey quail, then they studied its life cycle and size variation in Japanese quail (*Coturnix coturnix japonicum*). In In the same year Shah and Johnson reported this parasite from quail in the USA.

The present studies on the coccidia of this birds showed that this species of *Eimeria* can also be found in Iran.

The oocysts found in common grey quail were compared with previous reports of *Eimeria* in this bird. The description of oocyst in the present studies is quite similar to the description of *E. bateri*. Although the size of this species reported by different authors (Bhatia et al. 1965, Norton and Peirce 1971, and Shah and Johnson 1971) is not exactly the same, the size variation has been reported in other species of coccidia from different hosts. Jones (1932) working with *E. acervulina* and *E. maxima* found great variation in the size of occyst and attributed this to the crowding of the parasite, the age and inherent differences in the host birds. Becker et al. (1955) working with *E. brunetti* found that in the

later stages of the patent period the oocysts were significantly large. Boughton (1930) in a biometrical study of *Isospora lacazei* in sparrow found that the oocysts become progressively smaller as the sexual phases of cycle continued. Anwar (1964) found that the size of *I. lacazei* not only varies in different hosts species but also shows a variation in individual host.

In the present studies the size of oocysts resembles those reported by Shah and Johnson (1971) who gave the size as 14–28 by 12–19 μm (mean 20.5 by 15.3 μm), but in the present work the wall was found thinner and the shape index differed, being more variable.

The length and width of oocysts in these studies show two distinct peaks. Jones (1932) found that the distribution curves of *E. acervulina* and *E. maxima* show two peaks and as she emphasized it is the result of host effect on parasites. Norton and Peirce (1971) showed that a wider variation in size and shape of *E. bateri* can be obserwed when the bird is infected with 1000 oocysts than when infected with a single oocyst.

I found that generally, the width increased in proportion to the length. However, with a length of 20.4 μm (approximately mean length) there was not the proportionate increase in width. In fact the width was approximately mean width. This shows that the oocysts are elongated ellipsoids at their mean size.

РЕЗЮМЕ

Eimeria bateri Bhatia et al., 1965 описывается впервые от обыкновенного серого перепела из Ирана. Ооцисты элипсоидальной формы о размерах 14.4–28.2 × 12.0–21.0 μm со средней 20.60 ± 0.0978 × 15.90 ± 0.085 μm. Спороцисты удлиненно яицевидной формы, о размерах 10.53–16.6 × 5.85–8.20 μm со средней 12.8 × 6.75 μm, с выдающимся штидовским тельцом. Остаточное тельцо спороцисты присутствует.

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K. K. MISRA

Haemogregarina gangetica a New Name for *Haemogregarina simondi* of a River Turtle, *Trionyx gangeticus*

Synopsis. The name *Haemogregarina simondi*, proposed by Misra et al. (1974) from a river turtle *Trionyx gangeticus* Cuvier was preoccupied. Originally, *H. simondi* is a blood parasite of *Solea vulgaris*. Therefore, a new name *Haemogregarina gangetica* is being proposed for the blood parasite of river turtle, *Trionyx gangeticus* Cuvier.

A paper was published, "*Haemogregarina simondi* sp. n. a new haemogregarine from a river turtle, *Trionyx gangeticus* Cuvier", by Misra et al. (1974) in this Journal in Vol. XII, fasc. 30. Dr. Marshall Laird informed the author that the name *H. simondi* is preoccupied. The literature shows that "*Haemogregarina simondi*" is a parasite of common sole *Solea vulgaris* (after Wenyon 1926). *Haemogregarina simondi* of *Solea vulgaris* differs from the *Haemogregarina* of *Trionyx gangeticus* in morphology and mensural detail. Besides, hosts of these two haemogregarines are from different vertebrate classes. Therefore, a new name *Haemogregarina gangetica* is being proposed for the blood parasite of river turtle, *Trionyx gangeticus* Cuvier.

RÉSUMÉ

Le nom *Haemogregarina simondi* proposé par Misra et al. (1974) pour une espèce nouvelle provenant de la tortue de rivière *Trionyx gangeticus* Cuvier, c'est avéré être nomen preoccupatum. Son porteur, *H. simondi* est parasite de sang de *Solea vulgaris*. Par conséquence, un nom nouveau: *Haemogregarina gangetica*, est proposé pour le parasite de sang de la tortue de rivière, *Trionyx gangeticus* Cuvier.

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D. P. HALDAR and N. CHAKRABORTY

Studies on the Morphology and Cytochemistry of the Ciliate, *Nyctotherus chatterjeei* Chakravarty et Chatterjee (*Plagiotomidae–Ciliophora*) from a Mole Cricket, *Gryllotalpa* sp.

Synopsis. The paper contains the description of *Nyctotherus chatterjeei* Chakravarty et Chatterjee from the mid-gut content of the mole cricket, *Gryllotalpa* sp. collected in Kalyani, West Bengal, India. The ciliate is pear-shaped and measures 120.0 to 137.5 μm by 57.5 to 87.5 μm. Cytochemical studies have been carried out on this ciliate and the results have been described in this paper.

While studying the cephaline gregarines from insects of Kalyani, we obtained a ciliate from the mid-gut contents of the mole cricket, *Gryllotalpa* sp. Upon detailed study of live specimens as well as stained preparations, the ciliate was identified as belonging to the genus *Nyctotherus* Leidy (*Plagiotomidae*). Chakravarty and Chatterjee (1957, 1959)[1] described *Nyctotherus chatterjeei* from *Gryllotalpa vulgaris* collected in Calcutta; their description of the parasite was too inadequate and at first we thought that as it differed from *N. chatterjeei* in size, structure of the cytopharynx, position of the micronucleus as well as arrangement of ciliary lines, the organism should be given a separate specific status. The ciliate has been under our observation for the last two years, and during this period we have thoroughly compared the two organisms. We are now convinced that the parasite belongs to the same species, as the differences are not too sharp. We, therefore, propose to present a detailed account of the ciliate in this paper.

To understand the cytochemical make-up of the ciliate, we conducted some cytochemical reactions to demonstrate DNA, RNA, polysaccharides including glycogen, protein and lipid in it. The paper has been presented in two parts: first dealing with the general morphology, and the second, with cytochemical observations.

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[1] Chakravarty and Chatterjee (1957) first named the ciliate as *Nyctotherus pyriformis*. Later on it was communicated to them that the specific name *pyriformis* was already preoccupied. Therefore, they proposed a new name in 1959, *Nyctotherus chatterjeei*.

Material and Methods

The material for the study was obtained from the mid-gut of the mole cricket, *Gryllotalpa* sp. collected from moist places within the University campus. Observations on the live materials were made in smears prepared in 0.5 per cent saline solution. For routine examinations Schaudinn's fluid-fixed specimens were stained in Heidenhain's iron-alum haematoxylin. The argentophilic system was studied by dry silver method after Klein.

The methods used in cytochemical localization were applied after Pearse (1968). The Feulgen reaction and Pyronin-Methyl green technique were followed for DNA and RNA respectively. General polysaccharides were detected by the PAS-technique; saliva-digestion at 37° C prior to this was carried out for glycogen. Mercury-bromophenol blue method and Sudan black B method were followed for studying the localization of general protein and simple lipid in the parasite. Control slides were maintained with all cytochemical tests whenever necessary.

Observations

General Morphology

The body of the ciliate (Fig. 1, Pl. I 1) is more or less pear-shaped with a gradually tapering anterior extremity that sometimes ends in a blunt point, and a rounded posterior end. The body is broadest near its posterior two-third portion and from this part it gradually begins to taper to its anterior extremity. It is lined by a pellicle which is highly flexible, as the organism is able to squeeze through the narrow spaces between the solid contents within the gut. This has been observed in fresh preparations in saline solution as well as in fixed and stained slides where organisms with various contours are seen (Fig. 1). The body is more or less compressed and measures 120.0 to 137.5 μm (with a mean of 128.1 μm) in length and 57.5 to 87.5 μm (with a mean of 70.2 μm) in breadth. The body is covered by relatively short and uniform cilia.

The peristome starts at the anterior end of the body, runs posteriorly up to a short length, turns slightly towards the right and ends in the cyto-
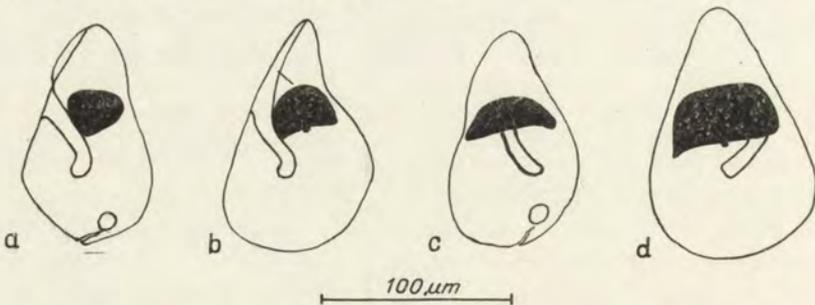

Fig. 1. Outline drawings under the camera lucida showing variations in shape of body of the *Nyctotherus chatterjeei* as well as that of macronucleus

stome which curves inwards as the cytopharynx leading up to two-third of the body. The cytopharynx is stout and ends in a rounded tip. The total length of the peristome and cytopharynx is 70.0 to 95.0 μm (with an average of 80.2 μm) and the width of the cytopharynx is 7.0 to 9.0 μm (with a mean of 8.6 μm). The cilia lining the dorsal wall of the cytopharynx are shorter and stouter.

The cytopyge is located slightly above the posterior apex of the body and enters into the endoplasm making an oblique angle. There is a single contractile vacuole situated near the anal tube. The vacuole sometimes reaches a diameter of 30.0 μm when fully distended.

The endoplasm can be differentiated into a prenuclear and a postnuclear regions. The former contains very fine granules and shows greater deposition of lipid materials (vide infra). The postnuclear region contains a few food vacuoles and is full of many coarse granules.

The macronucleus, located in front of the cytopharynx, is relatively massive, elongated and usually reniform with a depression on the ventral side in which generally the micronucleus is lodged. Variations in the outline of macronucleus are found in the form of a rectangular, triangular, bandlike or biconvex structure (Fig. 1). It measures 35.0 μm in length and 20.0 μm in breadth on the average. The macronucleus is enveloped by a membrane. The lateral tips of this membrane are connected with the pellicle on the two sides by ectoplasmic fibrils in the form of a suspensor apparatus called 'karyophore'. The structure can be observed only when viewed from the ventral aspect. The diameter of micronucleus is 5.0 μm on the average.

Good dry silver preparation following the method of Klein reveals the presence of very close-set longitudinal kineties (silver line) (Pl. I 2). The kineties originate at a point in the anterior end of the body and then move downwards, running parallelly with each other in the form of 'S' covering the entire length of the body. Ultimately they converge at the posterior extremity of the body. The number of kineties on the dorsal side of the body is about 60; the number on the ventral side could not be counted from our preparations.

Unfortunately, no division stage of the parasite or cystic condition could be found out from a good number of preparations so far. Studies in this line are in progress at present in the laboratory.

Cytochemistry

Feulgen positive substance (DNA) (Pl. I 3). This is found both in the macro- and in the micronucleus of the ciliate. The former shows the presence of many deep purple stained chromatin granules, a few of which

may even take a diameter of 2.0 μm. The granules are more or less compact with little non-staining patches between them. The micronucleus takes up a diffuse homogeneous stain.

Pyronin-methyl green positive substances (Pl. I 4). In this reaction the cytoplasm demonstrates a reddish violet stain indicating presumably the presence of RNA. The distribution of pyroninophilic granules is more or less uniform throughout the cytoplasm. In the macronucleus the Feulgen positive granules also take up a strong positive reaction with methyl green which is, in all probability, responsible for the presence of DNA. Since the intensity of reaction in the macronucleus is too strong, a detailed study of the staining behaviour of the micronucleus could not be made out from our preparations.

PAS-positive substances (Pl. I 5). The cytoplasm of the ciliate exhibits a strong purple colour indicating the localization of general polysaccharides. The positive granules are in the form of spherical and discoid bodies. The intensity of the reaction is greater in the prenuclear region. Both macro- and micronucleus are free from any PAS-positive granules.

After digestion with saliva at 37°C the cytoplasm becomes practically devoid of coarse PAS-positive granules. The saliva-digested granules are considered as glycogen.

Mercury-bromophenol blue positive substances (Pl. I 6). Both the nuclei and the cytoplasm stain blue with this reaction indicating the sites of deposition of total protein. The intensity of reaction in the nuclei is very high. In the cytoplasm the positive granules are more concentrated in the endoplasm. The dorsal and the ventral walls of the cytopharynx are strongly positive, while the pellicle, basal granules and cilia are moderately positive with this stain.

Sudanofilic substances (simple lipid) (Pl. I 7). The whole of the cytoplasm reveals the presence of this substance as indicated by deposition of black granules in these areas. In the prenuclear region the lipid granules are very fine and densely accumulated forming a cap-like structure while in the other regions the granules are more or less coarse. The pellicle also gives a faintly positive reaction. Both the nuclei are free from any positive granule.

Discussion

The ciliate described herein has a close resemblance to *Nyctotherus chatterjeei* Chakravarty et Chatterjee in respect to the general shape of the body. The hosts of the two ciliates also belong to the same genus *Gryllotalpa*. But since the descriptions given by Chakravarty and Chatterjee (1957) are too inadequate and there exist some differences in measurements of body, position of the micronucleus and disposition of the cytopharynx,

it was thought earlier that the present one might be a new species. Later, the idea of creating a new species for the ciliate was dismissed on the arguments that the differences are mainly concerning the dimensions and also that it is hardly possible that there could exist two so similar parasitic species in one host. It is, therefore, proposed to give a redescription of *Nyctotherus chatterjeei* Chakravarty et Chatterjee in this paper, with the information on the holotype and paratype materials.

Researches are now in progress in the laboratory to study the infraciliature of the ciliate in the light of the recent publications of Albaret (1970, 1973) and the results will be published later.

The probable interpretation for the presence of various cytochemical substances in the ciliate may be made in the light of discussions by Ghosh et al. (1970).

Diagnosis of *Nyctotherus chatterjeei* Chakravarty et Chatterjee:

Pear-shaped body; length 120.0 to 137.5 μm (mean 128.1 μm); breadth 57.5 to 87.5 μm (mean 70.2 μm); macronucleus reniform with a depression on the ventral side for the micronucleus, may also be rectangular, triangular, band-like or biconvex and measures 35.0 μm × 20.0 μm; karyophore in association with the macronucleus; cytopharynx prolonged up to posterior two-third of the body; single contractile vacuole attached to cytopyge at the posterior end; ciliary lines arranged parallelly in the form of 'S' covering the entire length of the body.

Holotype: Trophozoite on slide number A11/1 prepared from the midgut content of the mole cricket, *Gryllotalpa* sp., collected in Kalyani, West Bengal, India, by N. Chakraborty on 11.3.73 deposited at present at Zoology Department, Kalyani University; Paratype: Many on slide number A11/1 as well as on other slides, other particulars as for the holotype slide.

RÉSUMÉ

L'étude offre la description du *Nyctotherus chatterjeei* (Chakravarty et Chatterjee) de l'ntenstin de la Courtilière *Gryllotalpa* sp. trouvé à Kaylani, Bengal Occidental, Indes. Le Cilié est piriforme et ses dimensions s'élèvent à 120–137.5 μm sur 57.5–87.5 μm. Une étude cytochimique de ce Cilié a été effectuée dont les résultats sont décrits dans le travail présent.

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EXPLANATION OF PLATE I

1: Photomicrograph of *Nyctotherus chatterjeei.* × 625
2: Photomicrograph of the ciliate showing ciliary lines over the body using dry silver/ver method. × 406
3: Feulgen-positive substance in the macro- and micronuclei. × 450
4: Pyronin-methyl green-positive substance in the nuclei and cytoplasm. × 354
5: PAS-positive granules in the cytoplasm. Negative reaction in the nuclei is well-mark-rk-ed. × 385.
6: Mercury-bromophenol blue positive substances. × 554
7: Deposition of simple lipid granules. Pattern of localization of positive granules in the/he prenuclear region may be noted. × 550

PLATE III
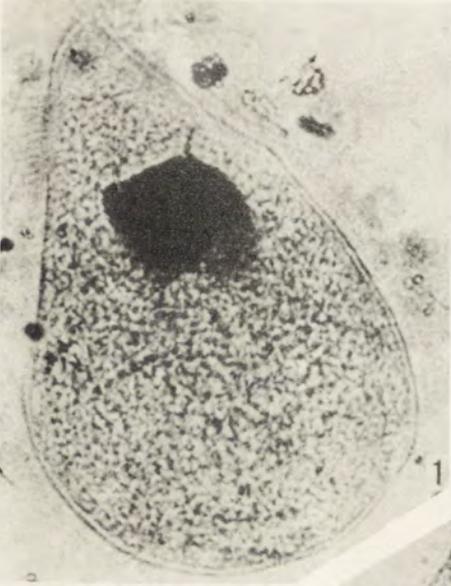
D. P. Haldar
et N. Charaborty

Stanisław L. KAZUBSKI

On the Variability of a Parasitic Ciliate *Semitrichodina sphaeronuclea* (Lom) (*Urceolariidae*) According to the Altitude of its Habitats above Sea Level

Synopsis. In the ciliate *Semitrichodina sphaeronuclea* f. *macrodentata* (Lom) (fam. *Urceolariidae*) parasitizing the slug *Bielzia coerulans* (Bielz) (fam. *Limacidae*) some morphological variability has been observed in correlation with the altitude of habitats above sea level. The morphological changes concern body dimensions, diameter of the adhesive disc and of the denticulate ring, and the number of denticles. It is suggested that these changes are correlated with climatic differences, especially with temperature decreasing with altitude. These changes show the same direction as it has been observed by the present author in the case of seasonal and geographical variability in other ciliate species.

The problem of morphological variability of ciliates under the influence of various factors of external environment has been undertaken by the present author on several occasions (Kazubski 1967, 1968, 1969, 1974, Kazubski and Migała 1968). In these studies mainly seasonal variation has been concerned, however, some geographical aspects of the variability have been revealed also. The temperature factor has been regarded as responsible for observed differences in the morphology of ciliates, either in particular seasons of the year, or in various parts of the distribution area of species. Then, it has been suggested (Kazubski 1969, 1974) that the influence of temperature might have direct bearing on the morphology of ciliates in correlation with the altitude of their habitats above sea level if, of course, climate zonation is involved. The present paper is devoted to verification of this hypothesis.

Material and Methods

A ciliate *Semitrichodina sphaeronuclea* f. *macrodentata* (Lom), family *Urceolariidae*, has been used as a model for study. This ciliate inhabits mantle cavity of terrestrial slugs of the family *Limacidae*. The material has been collected on 23rd to 28th July 1974 in forests

on northern slopes of the Babia Góra mount (1725 m of altitude, situated in West Beskids, southern Poland), in the territory of the Babia Góra National Park, and the local Forest District of Zawoja. In this territory, the greatest difference of height in Poland occurs between the foot and the top of the mount in a relatively small area devoid of any natural barrier.

For comparison 12 subpopulations of ciliates were used (subpopulation = parasites from single host specimen); they were collected from *Bielzia coerulans* (Bielz), fam. *Limacidae*. Five of them originated from slugs gathered near the lower border of the forest, in the dreinage basin of the torrents Markowy Potok and Cylowy Potok, near the village Zawoja (Kwatery). This site was situated at the height of about 760 m above sea level (site 1). Four subpopulations originated from slugs gathered in the fir and spruce forest association (*Abieti Piceetum montanum*) with beech *Fagus silvaticus* in admixture, near the tarn Marków Stawek and at the sides of the neighbouring path, so called Górny Płaj, in its west part. These sites were situated at about 1050–1125 m a. s. l. (site 2). The last three subpopulations originated from slugs found in the forest of upper mountain zone (*Piceetum excelsae carpaticum*) below the Brona Pass near red-marked tourist path leading from the hostel Markowe Szczawiny to the pass. The altitude of this site was 1225–1300 m a. s. l. (site 3). Total distance between extreme sites did not exceed 2.5 km in straight line and the maximum difference in height was 540 m.

The ciliates were mounted in permanent preparations according to Klein's dry method of silver impregnation. From each subpopulation a sample of about 40 specimens, directed upwards with their adhesive discs, were measured. After proving the lack of essential differences between them, the particular subpopulations of *S. sphaeronuclea* were grouped into three classes depending on the altitude of their habitats above sea level, corresponding to three mentioned sites. In further study only these three classes were compared. The number of ciliates measured in each class is given in Table 1. The differences in numbers of measurements in particular columns are due to the fact that not all the structural elements were satisfactorily visible for measuring in each specimen of the ciliate. All numerical data were statistically checked and the significance of the differences between the data obtained at the different sites were calculated.

Results

The results of statistical analysis of some more important metric and meristic features of the ciliate *Semitrichodina sphaeronuclea* f. *macrodentata* with respect to the altitude of its habitats above sea level are presented in Table 1.

The comparison of these data has shown that the dimensions of the body, of the adhesive disc and of the denticulate ring, as well as the number of denticles become greater with the increase of altitude. These differences are striking when the material from two extreme sites is compared; all differences are highly significant statistically. Relatively great differences have been found also between the ciliates from middle and upper sites. Smaller differences occur between the ciliates from lower and middle sites, but most of them are statistically significant. No visible differences have been found in the shape of denticles in ciliates from particular sites, but in the specimens with greater number of denticles they are more tightly arranged.

Table 1

Metric and Meristic Data on the Ciliate *Semitrichodina sphaeronuclea* (Lom) from Three Sites of Different Altitude above Sea Level

	Sites			Significance of differences***** between the data from sites:		
	1 760 m	2 1050–1125 m	3 1225–1300 m	1:2	2:3	1:3
Diameter of the body	45–86* 62.4** ± 0.53*** 7.1**** n = 180	50–82 64.6 ± 0.51 6.4 n = 154	45–87 66.7 ± 0.64 7.0 n = 118	**2.933**	**2.671**	**5.169**
Diameter of the adhesive disc with the border membrane	38–59 45.6 ± 0.28 3.7 n = 176	35–57 46.2 ± 0.31 3.6 n = 136	36–61 49.0 ± 0.40 4.1 n = 106	1.389	**5.579**	**7.065**
Diameter of the adhesive disc	32–55 39.9 ± 0.29 4.1 n = 198	31–53 41.7 ± 0.30 3.8 n = 150	34–54 44.6 ± 0.34 3.9 n = 128	**4.127**	**6.176**	**10.181**
Diameter of the denticulate ring	18.5–31.0 22.7 ± 0.17 2.5 n = 206	18.0–30.0 23.4 ± 0.19 2.4 n = 162	18.0–33.0 25.2 ± 0.26 3.0 n = 133	**2.683**	**5.819**	**8.491**
Length of the denticle	9.0–13.0 10.65 ± 0.06 0.8 n = 160	9.0–13.0 11.10 ± 0.09 0.9 n = 116	9.0–13.0 11.07 ± 0.09 0.8 n = 86	**4.248**	0.231	**3.934**
Number of the denticles	25–33 28.3 ± 0.09 1.3 n = 210	26–31 28.8 ± 0.09 1.2 n – 168	27–34 30.3 ± 0.12 1.5 n = 137	1.180	**3.092**	**6.033**

* Range, **mean value, ***standard error of mean, ****standard deviation, n – number of measurements ***** Significance of differences between the means are calculated according to the Student's test. The data overpassing the critical value $t_{0.01}$ are bold faced.

Discussion

The material used in the present study is in many respects homogeneous to the greatest possible degree. The ciliates were collected during a short period of time (6 days) from the same host species and therefore indirectly, from the same or very similar biotopes. Their host, *Bielzia coerulans*, shows rather restricted niche requirements; it lives on soil surface, under stones or under the bark of fallen down trees. This species is fairly common in forests of Babia Góra. Particular sites from which the slugs were collected,

are situated close together (up to 2.5 km in straight line) in the same complex of forest. It would be difficult to imply the existence of any strict isolation between local populations of slugs, from which the samples have been taken, and between particular subpopulations of their ciliate parasites. In consequence, it may be admitted that the subpopulations of ciliates used for study were genetically uniform.

The greatest differences in the material under study concern the altitude of habitats above sea level and some climatic differences involved.

The lowest site (760 m) was situated in lower part of the lower mountain forest zone. In this part of Babia Góra, in July, summer conditions occur with mean day temperature of air exceeding 15°C. In this zone mean annual temperature (according to meteorological station in Zawoja) is slightly over 6°C, and the number of days with mean day temperature above 0°C is 278 in a year.[1]

The middle site (1050–1125 m) is situated in a transitory zone between the lower and the upper mountain forest zone. At this altitude the day temperatures do not exceed 15°C, so the summer conditions do not occur at this altitude. Mean annual temperature is about 3–3.5°C. According to the data of nearby situated meteorological station Markowe Szczawiny (1180 m), mean annual temperature is equal to 3.2°C, and the number of days with temperature above 0°C is 225 in a year.

The uppermost site (1225–1300 m) is situated in the upper part of the upper mountain forest zone. Mean day temperature is only slightly over 2.5°C. The number of days with mean day temperature above 0°C was not noted for this place but probably it does not exceed 200.

Taking into account mezoclimatic regions distinguished by Hess (1966), the lower site in the present study may be placed near the upper border of the warm temperate region, the middle site in the temperate cool region, and the upper site in the cool region.

A consideration of all above mentioned data leads to a conclusion that the changes in morphology observed in *S. sphaeronuclea* f. *macrodentata*, consisting in the increase of body dimensions and number of denticles, rely on the altitude of habitats above sea level involving climatic zonation. These changes show the same pattern as the seasonal and geographical variability (Kazubski 1969, 1974), consisting in the increase of body dimensions and of the number of cortical elements in protozoans in cooler seasons and in northern parts of their area. All kinds of changes

[1] The data on meterological conditions of Babia Góra are taken from "The climate of Babia Góra" by Obrębska-Starklowa, published in a monograph: "Babia Góra National Park" edited by Szafer 1963, and from unpublished article of the same author, entitled "Climate", comprising the data for the years 1954–1962 and prepared for purposes of the National Park.

discussed above, confirm the conclusion about correlation between some morphological differences in ciliates and temperature of their environment.

It is worthy to note that the temperatures characterizing particular sites in the present paper, were obtained from routine observations in meteorological posts and concerned air temperature measured in standard conditions. Such data may serve for comparison of particular sites but they give only indirect information about real microclimatic conditions in the proper habitats of slugs and their ciliate parasites. So, greater comparability of these data has been achieved by taking into consideration only the ciliates from one host species, originating from the same or similar habitats characterized by similar microclimate. It cannot be excluded that the same species of ciliate taken from the same localities but from other host species living in other habitats and microclimate, would also display similar morphological differentiation.

In addition, some comments have to be done concerning my earlier paper on *S. sphaeronuclea* f. *macrodentata* (Kazubski 1971). In Table 1 of the cited paper the metric and the meristic data are arranged according to the dates of collection of the material without attention to the altitude of localities. The lowest locality from which the material has been collected is situated at about 450 m, while the uppermost one at 1080 m above sea level. So, this material ought to be analyzed once more and the correlation of some morphological changes with the altitude taken into account as well as the seasonal changes.

ACKNOWLEDGEMENTS

RÉSUMÉ

Chez le Cilie *Semitrichodina sphaeronuclea* f. *macrodentata* (Lom) (fam. *Urceolariidae*) parasite du gastropode *Bielzia coerulans* (Bielz) (fam. *Limacidae*), on a constaté une variation morphologique dépendante de l'altitude au dessus du niveau de la mer, des habitats. Les différences morphologiques concernent les dimensions du corps, le diamètre du disque adhésif et de l'anneau denticulé, et le nombre de denticules. On suggère que ces différences sont co-ordonnées aux changements climatiques plus particulièrement à la baisse de la température avec l'altitude. Ces différences présentent la même tendance qui a été observie par l'auteur auparavant au cas de la variation saisonnière et géographique d'autres espèces des Ciliés.

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Note added in proof.

The study has been repeated to a lesser extent in August 1975 on the ciliates *Semitrichodina
sphaeronuclea* (Lom) originating from the same host and places as in the present paper. One
examined subpopulation was collected from site *1* (lowest) and two others from the site *3*
(uppermost). Similar differences to those described in the present paper were observed in the
body dimensions and the diameter of the adhesive disc and the denticulate ring, as well as
in the number of denticles between the subpopulations from both sites.

Vassil GOLEMANSKY

Contribution à l'étude des Rhizopodes et des Héliozoaires du psammal supralittoral de la Méditerranée

Synopsis. Les résultats des recherches sont présentés concernant la faune des Rhizopodes et des Héliozoaires de quelques régions de la Mediterrannée, à savoir de la Mer Adriatique (près de Kjozga) de la Mer Thyrrénienne (environs du Lago Lungo) et du Golfe de Tunis. En somme, 21 espèces des Rhizopodes et 5 espèces des Héliozoaires étaient trouvées. Une nouvelle espèce des Rhizopodes: *Pseudodifflugia andreevi* sp. n., est décrite. On a completé les données sur la morphologie, l'ecologie et la répartition géographique de quelques autres espèces dont la connaissance était faible et la position taxonomique pas très claire. On a proposé une classification ecologique des espèces des Rhizopodes connues jusqu'à présent du psammal supralittoral de la Mediterrannée.

Les premières informations sur la faune des rhizopodes psammobiontes de la Méditerranée ont été publiées par Decloitre (1972). Dans le psammal supralittoral de la plage Bonne-Grâce à Six-Fours (département de Var, France) il a trouvé les espèces *Antarcella atava* (Collin) Deflandre et *Cochliopodium granulatum* Penard. Mais les deux espèces trouvées n'appartiennent pas au groupe écologique des psammobiontes obligatoires. Ce sont plutôt des formes eurybiontes, apportées probablement fortuitement dans ce biotope. Plus tard Laminger (1973) signale quarte espèces de théca-moebiens dans les eaux souterraines littorales des côtes yougoslaves de la mer Adriatique dans la région du Rab. Les espèces trouvées par Laminger sont psammobiontes obligatoires, repandus dans le psammal de presque toutes les mers étudiées jusqu'à présent (Golemansky 1969, 1970 a, b, c, d, 1971, 1973, Valkanov 1970). En même temps Chardez (1973) signale d'avoir trouvé aussi sept espèces de thécamoebiens dans trois plages sablonneuses du littoral espagnol de la Méditterranée dans la région de Costa Brava. Deux des espèces trouvées, notamment *Pseudocorythion mannei* et *Micramphora hellebauti* sont décrites par l'auteur cité pour la première fois. Toutes les espèces trouvées, à l'exception des taxons nouveaux, sont des psammobiontes obligatoires à une distribution cosmopolite.

Le matériel pour la présente étude à été recolté en 1974 dans trois régions de la Méditerranée par le Dr. T. Marinov de 'Institut d'océanographie de Varna et le Dr. St. Andreev de Musée national d'histoire naturelle à Sofia. Que ses deux collègues trouvent ici l'expression de ma sincère gratitude pour le matèriel qu'ils ont si aimablement mis à ma disposition.

(1) Mer Adriatique. Plage sablonneuse près de Kjozga, Italie. 24.IV.1974. Leg. Dr. T. Marinov.

(a) — distance de la mer (D.): 1 m; profondeur dans le sable (Pr.): 0.20 m.

(b) — D.: 5 m; Pr.: 0.40 m.

(2) Mer Thyrrhénienne. Plage sablonneuse près de Lago Lungo, Italie. (120 km au nord de Naples). 7.V.1974. Leg. Dr. T. Marinov.

(a) — D.: 1 m; Pr.: 0.20 m.

(b) — D.: 3 m; Pr.: 0.40 m.

(c) — D.: 6 m; Pr.: 0.60 m.

(3) Golfe de Tunis. 10.XII.1974. Leg. St. Andreev.

(a) — plage sablonneuse près de Sidi Bou Said. D.: 3 m; Pr.: 0.50 m; S $^0/_{00}$ = 36.81$^0/_{00}$.

(b) — plage sablonneuse "La Marsa". D.: 1 m; Pr.: 0.30 m; S $^0/_{00}$ — 9,19$^0/_{00}$.

En résultat des études effectuées furent trouvées au total 26 espèces de psammobiontes, dont 21 espèces de rhizopodes et 5 espèces de héliozoaires. La liste complète des espèces trouvées et leur répartition dans les stations étudiées sont présentées au Tableau 1.

L'étude de la faune psammobionte de la Méditerranée a permis de découvrir quelques rhizopodes et héliozoaires inconnus ou peu connus jusqu'à présent, dont la déscription est faite dans le travail présent. De plus on a obtenu de nouvelles données sur la morphologie, la répartition et la biologie de quelques-uns des rhizopodes et des héliozoaires déjà connus, en complétant ainsi nos connaissances actuelles de ces espèces.

<div align="center">

Notes taxonomiques et biologiques
Rhizopoda von Siebold, 1845

</div>

Amphorellopsis taschevi Golemansky, 1970. Fig. 1 a, b. Cette espèce, connue jusqu'à présent uniquement du littoral cubain de la mer des Caraïbes (Golemansky 1970 d) fut trouvée aussi dans deux des stations prospectées de la Méditerranée (2b et 3a). Au cours de l'étude présente des nombreux exemplaires observés permirent d'élargir et de compléter la description de l'espèce. Il faut avant tout souligner, que les dimensions de la thèque d'*Amphorellopsis taschevi* de la Méditerranée varient dans un plus vaste diapason et que l'on trouve souvent des exemplaires, dont la longueur atteint 46 µm. Un grand nombre d'individus, surtout de la station 3a ont la partie postérieure de la thèque effilée en forme de lance qui a, dans certains cas, la tendance de former une courte épine. Dans un cas nous

Tableau 1

Tableau de la composition d'espèces et de la répartition des Rhizopodes et des Heliozoaires trouvés dans les stations explorées de la Méditerranée

Espèces trouvées	Kjozga, Italie 24. 4. 1974		Lago Lungo, Italie 7. 5. 1974			Golf de Tunis 10. 12. 1974	
	a	b	a	b	c	a	b
Rhizopoda							
Psammonobiotus communis Gol.	+		+	+	+	+	+
Psammonobiotus minutus Gol.						+	+
Pseudocorythion acutum (Wailes) Valk.				+	+		+
Chardezia caudata Gol.						+	
Corythionella acolla Gol.	+						
Corythionella minima Gol.	+				+		+
Cyphoderia littoralis Gol.	+	+				+	+
Micramphora pontica Valk.						+	
Amphorellopsis elegans Gol.			+		+		
Amphorellopsis taschevi Gol.				+		+	
Centropyxiella arenaria Valk.						+	
Micropsammella retorta Gol.						+	
Cryptodifflugia lanceolata Gol.				+	+	+	+
Difflugiella psammophila Gol.	+	+					
Pseudodifflugia andreevi sp. n.						+	
Pseudodifflugia sp.				+		+	
Microchlamys patella Clap. Lachm.					+		
Hyalosphaenia cuneata Stein					+		
Phryganella sp.	+						
Lagenidiopsis valkanovi Gol.				+		+	
Lagenidiopsis elegans (Gruber) comb. n.						+	
Heliozoa							
Actinophrys sol Ehrenberg							+
Actinophrys sp.	+						
Acanthocystis pectinata						+	
Oxnerella maritima Dobell							+
Genus sp.							+
Au total	7	2	3	5	7	14	9

avons eu la possibilité d'observer aussi les pseudopodes de l'animal, qui sont de type filopode.

A. taschevi est un psammobionte obligatoire, habitant les eaux souterraines supralittorales avec un haut degré de salinité (S = $36.81^0/_{00}$).

Pseudodifflugia andreevi sp. n. Fig. 2 a, b.

Description: La thèque est allongée, piriforme, avec la partie postérieure arrondie. Dans la région du pseudostome elle est rétrécie et forme un court col

qui s'élargit de nouveau dans la région du pseudostome et forme un élargissement caractéristique en forme d'entonnoir. La section transversale de la thèque et du col est ronde. Le pseudostome, situé au centre de l'élargissement en forme d'entonnoir, est également rond. La symétrie de la thèque est radiale monaxonne. Le revêtement est formé d'une base chitinoïde, sur
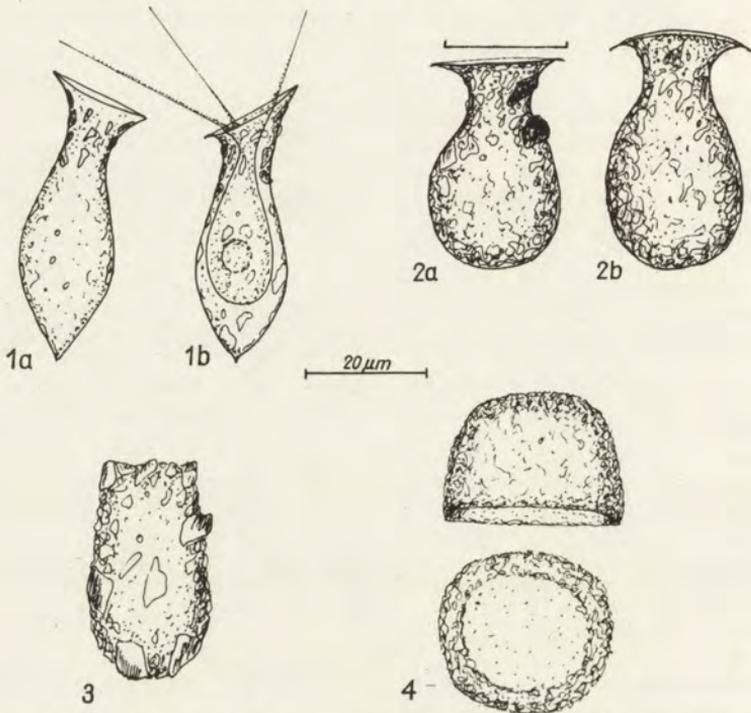

Fig. 1. 1–4. 1 a–b – *Amphorellopsis taschevi* Golemansky, a – thèque vide, b – exemplaire vivant, 2 a–b – *Pseudodifflugia andreevi* n. sp., deux exemplaires différents, 3 – *Pseudodifflugia* sp., 4 – *Phryganella* sp., vue latérale et vue ventrale

laquelle sont agglutinées de nombreux éléments polygonaux d'origine endogène et exogène. Parfois on observe dans la région du col quelques xénosomes plus gros. La périphérie de l'élargissement du pseudostome est mince et transparente, de couleur jaunebrun. Les dimensions varient dans les limites suivantes: longueur: 36–40 μm; diamètre: 20–25 μm; col: 10–13 μm; élargissement du pseudostome: 15–19 μm.

Matériel: 2 préparations microscopiques dans la collection de l'auteur à l'Institut de Zoologie à Sofia.

Localité: Le psammal supralittoral de la plage "La Marsa" du littoral tunisien de la Méditerranée (Station 3a).

Nous sommes heureux de dédier cette espèce à Dr. St. Andreev, l'auteur du matériel contenant le nouveau taxon.

Discussion: D'après la morphologie de la thèque *Pseudodifflugia andreevi* sp. n. ressemble à l'espèce *Nadinella tenella* Penard, décrite du lac de Génève (Penard 1890), mais chez la nouvelle espèce la thèque n'est pas aplatie latéralement dans la région du col et du pseudostome, comme c'est le cas de *N. tenella*. *Ps. andreevi* sp. n. a quelques ressemblances morphologiques à l'espèce *Amphorellopsis maximus* Golemansky, bien que les dimensions de cette derniere espèce sont considérablement plus grandes et les thèques sont latéralement aplaties.

Ps. andreevi sp. n. diffère de toutes les espèces du genre *Pseudodifflugia*, décrites jusqu'à présent, par l'élargissement caractéristique en forme d'entonnoir dans la région du pseudostome, que l'on trouve chez un grand nombre de psammobiontes obligatoires du groupe des thécamoebiens. C'est pourquoi nous supposons, que *Ps. andreevi* sp. n. est aussi un psammobionte obligatoire, n'habitant que les eaux souterraines littorales des plages sablonneuses.

Pseudodifflugia sp. Fig. 3. Des thèques vides de *Pseudodifflugia* sp. ont été trouvées dans deux stations explorées (2a et 3a). Leur forme est allongée, presque cylindrique, avec la partie arrière arrondie. La section transversale est ronde. La thèque est formée d'une base chitinoïde incolore, sur laquelle sont agglutinés des éléments différents de forme, de grandeur et d'origine. Les dimensions des éxemplaires observés sont les suivantes: hauteur: 30–36 μm; diamètre: 17–20 μm; pseudostome: 10–13 μm.

Au point de vue morphologique les thèques des exemplaires trouvés dans la Méditerranée sont proches de ceux découverts par nous dans le psammal supralittoral de la mer des Caraïbes et dans l'océan Atlantique (Golemansky 1970 d, 1975 in litt.). De leur côté les deux formes montrent certaines ressemblances morphologiques avec les espèces *Pseudodifflugia gracilis* Schlumberger et *Ps. fulva* Archer. Bien que l'on trouve relativement souvent des thèques de *Pseudodifflugia* dans les eaux souterraines littorales des plages sablonneuses on n'a pas trouvé jusqu'à présent des exemplaires vivants. Ce fait, ainsi que certaines ressemblances morphologiques des thèques n'excluent pas la possibilité d'avoir une pénétration fortuite d'espèces dulçaquicoles du genre *Pseudodifflugia* dans le psammal supralittoral des mers. Et cela d'autant plus, que les espèces d'eau douce du genre ont été déjà trouvées dans les biotopes salés et saumâtres par Schultze (1874), Valkanov (1936) etc.

Phryganella sp. Fig. 4. La thèque a la forme d'une hémi-sphère régulière et est formée d'une matière chitinoïde incolore, avec de petits éléments exogènes collés sur elle. Le pseudostome est large (environ 2/3 du diamètre) de forme ronde. Nous n'avons observé que seulement deux thèques vides,

provenant de la station 1a dont les dimensions variaient dans les limites suivantes: hauteur: 22–23 μm; diamètre: 24–25 μm; pseudostome: 16 μm.

Chardez (1973) a trouvé dans le psammal supralittoral de la mer du Nord de pareilles thèques qu'il décrit comme un nouveau taxon: *Phryganella marinus* sp. n. Toutefois, *Phryganella* sp. diffère de *Phryganella marinus* par sa forme hémisphaerique et la structure de la thèque, qui est formée chez cette dernière espèce d'une base chitinoïde avec des gros et nombreux xenosomes polygonaux agglutinés. Dans notre cas il n'est pas exclu aussi d'avoir trouvé des thèques vides de *Phr. hemisphaerica* Penard — espèce bien répandue dans l'eau douce et dans le sol, qui peut penetrer fortuitement dans les eaux souterraines.

Lagenidiopsis elegans (Gruber, 1884) comb. n. Fig. 5 a, b.

Syn.: *Lagena elegans* Gruber, 1884; p. 499.

Dans son travail sur les protozoaires du Golfe de Gênes en Méditerranée Gruber (1884) décrit un rhizopode, qu'il rapporte au genre Lagena Reuss. Mais les caractères morphologiques des thèques de l'espèce décrite, ainsi que le type du corps cytoplasmique et des pseudopodes nous permettent de ranger ce taxon dans le genre *Lagenidiopsis*, décrit recemment par nous (Golemansky 1974).
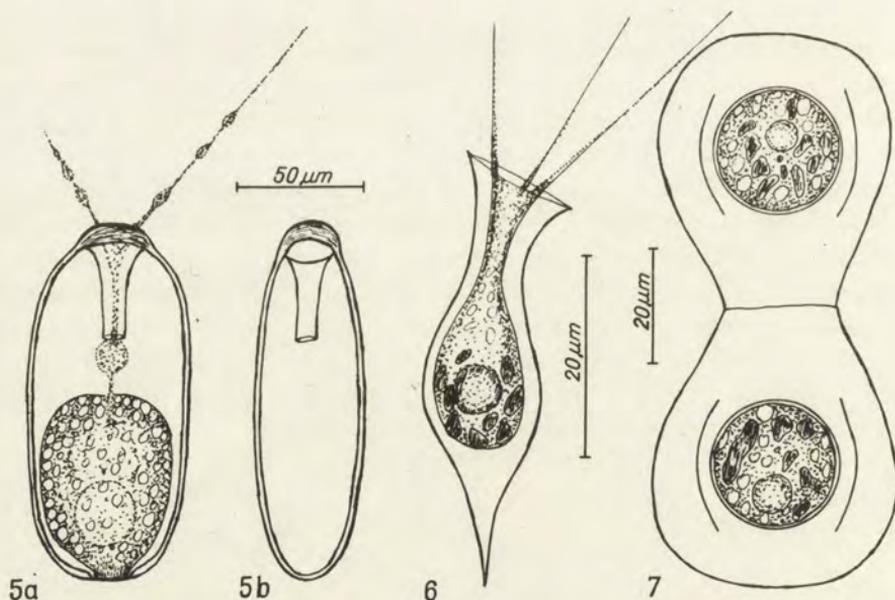

Fig. 5–7. 5 *a–b* — *Lagenidiopsis elegans* (Gruber) comb. n., *a* — exemplaire vivant, vue latérale, *b* — vue de profil, 6 — *Chardezia caudata* Golemansky, exemplaire vivant, 7 — *Hyalosphaenia cuneata* Stein, deux exemplaires enkystés après la division

L. elegans ne fut trouvé que dans une seule des stations explorées (3a). Les thèques de l'espèce ont la forme elliptique allongée, latéralement aplatie, incolore et transparente. La paroi chitinoïde est épaisse d'environ 2.5 μm. Dans la région du pseudostome elle est épaissie complémentairement d'une matière chitinoïde plus sombre. Le pseudostome rond s'ouvre à l'intérieur de la thèque au sommet d'un tube mince et droit. Les dimensions des exemplaires observés sont en moyenne comme suit: longueur: 112 μm; largeur: 50 μm; épaisseur: 25 μm; le tube: 20 × 5.5 μm; Selon G r u b e r (1884) les dimensions de *L. elegans* sont en moyenne de 120 × 80 μm.

Le corps cytoplasmique est situé dans la deuxième moitié de la thèque. Il est fixé à sa base avec l'aide d'un épipode court et épais. Les animaux émettent un, rarement deux ou plus filopodes. Le noyau est grand, environ 20 μm de diamètre.

L. elegans se distingue facilement du *L. valkanovi* Gol. par ses dimensions plus élevées et l'absence des épines latérales caractéristiques des thèques.

Heliozoa Haeckel, 1866.

Dans le psammal supralittoral de la Méditerranée nous avons trouvé aussi 5 espèces de héliozoaires, dont 2 ne purent pas être identifiés pour le moment. Nous croyons utile de faire une brève description de ces espèces, en espérant aider de cette façon les futures recherches sur la faune héliozoaires du psammal supralittoral des mers.

Actinophrys sp. ? Fig. 8. Le corps cytoplasmique est rond et incolore, son diamètre allant de 10 à 13 μm. Sur la pèriphérie on observe de
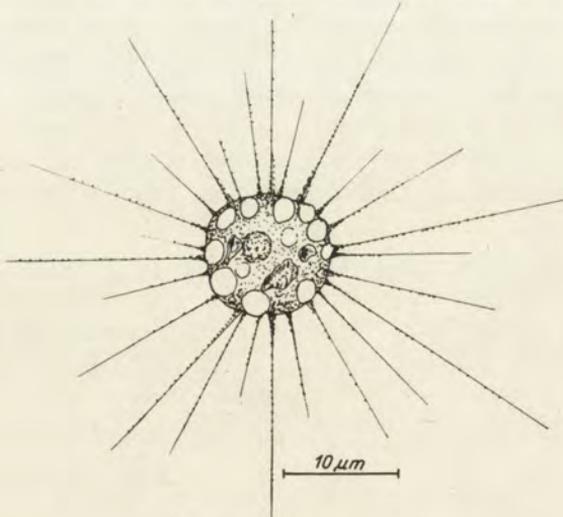

10 μm

Fig. 8. *Actinophrys* sp. ?

nombreuses vacuoles disposées en un ou deux rangs. Le noyau unique est situé excentriquement. Sur les actinopodes de différentes longueurs on observe souvent des granules cytoplasmiques. Assez grand nombre d'exemplaires d'*Actinophrys* sp. furent trouvés dans les eaux souterraines de la station 1a.
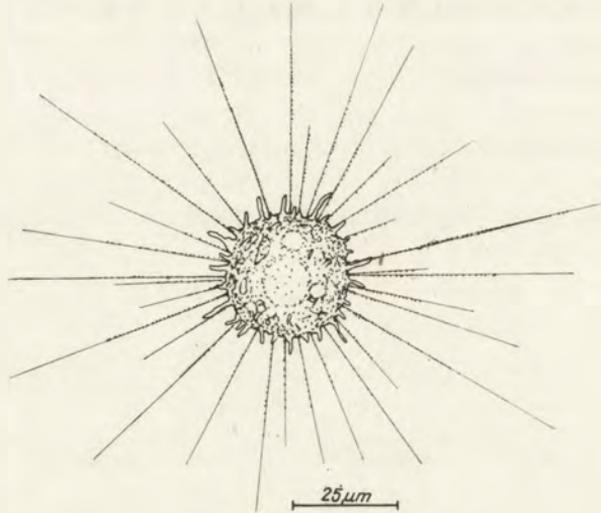

Fig. 9. Genus sp.

Genus sp. Fig. 9. Le corps est rond et incolore, de diamètre environ 30 µm. Le cytoplasme est hyalin et contient 1–2 vacuoles difficilement percevables. La partie centrale est plus claire. L'appareil nucléaire n'est pas observé. Radialement du corps sortent un grand nombre d'actinopodes minces et longs. Le diamètre total de l'animal avec les actinopodes atteint 200 µm. De la zone périphérique du cytoplasme, entre les actinopodes, émergent aussi de nombreux pseudopodes en mouvement incessant. Leur longueur dépasse rarement 5–7 µm. La plupart des pseudopodes ne survivent que quelques secondes, mais rapidement à leur place apparaissent des nouveaux pseudopodes. Les actinopodes sont permanents et sur leur longueur on observe souvent des grains de cytoplasme finement granulé.

Des exemplaires isolés de cette espèce ont été trouvés dans la station 3b, ensemble avec les espèces *Actinophrys sol* Ehrenberg (diamètre environ 42 µm) et *Oxnerella maritima* Dobell (diamètre environ 13 µm).

Conclusion

A la suite des recherches présentes, ainsi que des recherches de Decloitre (1972), Laminger (1973) et Chardez (1973) on a trouvé dans le psammal supralittoral de la Méditerranée au total 21 espèces de rhizopodes et 5

espèces de héliozoaires. Bien que préliminaires les recherches effectuées montrent, que les eaux souterraines littorales des plages sablonneuses de la Méditerranée, sont habitées par une faune psammobionte relativement riche, ressemblant à la faune des autres mers et océans, étudiés de ce point de vue. En resultat des recherches, effectuées jusqu'à présent, on a découvert aussi 3 taxons inconnus pour la science (*Pseudocorythion mannei* Chardez, *Micramphora hellebauti* Chardez et *Pseudodifflugia andreevi* sp. n. qui sont, d'apres nous, des habitants spécifiques du psammal supralittoral.

Au point de vue écologique, les rhizopodes observés peuvent être rangés dans trois catégories écologiques (Tableau 2). Le plus nombreux est le groupe des psammobiontes obligatoires (20 espèces), tandis que le nombre des psammobiontes facultatifs et des eurybiontes est fort limité. A notre avis ce fait est dû à ce que la Méditerranée est caractérisée par une salinité considérablement plus élevée que celle d'autres mers intercontinentales, comme par exemple la mer Noire et la mer Baltique, dans lesquelles le nombre des psammobiontes facultatifs et des eurybiontes est becaucoup plus grand. La haute salinité est une barrière limitant la pénétration de certains

Tableau 2

Repartition des rhizopodes psammobiontes méditerranéens d'après leurs exigéances écologiques

Psammobiontes obligatoires	Psammobiontes facultatifs	Eurybiontes et ubiquistes
Psammonobiotus communis Gol.	* *Microchlamys patella* Clap. Lachm.	*Antarcella atava* (Collin) Deflandre
Ps. minutus Gol.	* *Hyalosphaenia cuneata* Stein	*Cochliopodium granulatum* Penard
Pseudocorythion mannei Chardez		* ? *Pseudodifflugia* sp.
Ps. acutum (Wailes) Valk.		* ? *Phryganella* sp.
* *Chardezia caudata* Gol.		
Corythionella acolla Gol.		
C. pontica Gol.		
* *C. minima* Gol.		
* *Cyphoderia littoralis* Gol.		
Micramphora pontica Valk.		
M. hellebauti Chardez		
* *Amphorellopsis elegans* Gol.		
* *Amph. taschevi* Gol.		
* *Centropyxiella arenaria* Valk.	Note: Les espèces précedées d'un astérisque sont signalées pour la première fois dans le présent travail.	
* *Micropsammella retorta* Gol.		
Cryptodifflugia lanceolata Gol.		
* *Difflugiella psammophila* Gol.		
* *Pseudodifflugia andreevi* sp. n.		
* *Lagenidiopsis valkanovi* Gol.		
L. elegans (Gruber) comb. n.		

rhizopodes eurybiontes d'eau douce ou du sol dans le psammal supralittoral de la Méditerranées, surtout dans les zones à proximité de la mer.

La découverte de 5 espèces d'héliozoaires dans le psammal supralittoral de la Méditerranée indique, que cet intéressant biotope offre des conditions aussi favorables au développement de ce groupe d'organismes, considérés surtout comme habitants des vastes superficies d'eau.

SUMMARY

The results of investigations are presented concerning the fauna of *Rhizopoda* and *Heliozoa* in some regions of the Mediterrannean Sea, namely of the Adriatic Sea (Kjozga area), the Tyrrhenian Sea (close to Lago Lungo), and the Gulf of Tunis. In all, 21 species of *Rhizopoda* and 5 of *Heliozoa* have been found. A new species of *Rhizopoda*: *Pseudodifflugia andreevi* sp. n., is described. Data are completed on the morphology, ecology and geographical distribution of some other species which were not very well known and had a rather uncertain taxonomical position. An ecological classification is proposed of the *Rhizopoda* species presently known from the supralitoral psammal of the Mediterrannean coast.

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Stefan RADZIKOWSKI

DNA and RNA Synthesis in the Nuclear Apparatus of *Chilodonella cucullulus* O.F.M.

Synopsis. Investigations concerned the process of DNA and RNA synthesis in the nuclear apparatus of *Chilodonella cucullulus* (O.F.M.). It was found that DNA replication in the micronucleus and the macronucleus is different and that in the macronucleus there are two phases of replication and some chromatine, included in endosome, does not replicate at all.

The main producer of RNA is the macronucleus, particularly its peripheric parts. The endosome chromatine is not active in this synthesis.

It is assumed that chromatine of the paramer, replicating earlier is euchromatine while chromatine of the orthomer replicating later is heterochromatine; the endosome might be a peculiar kind of chromatine containing defective copies totally blocked to synthesis.

Introduction

In most of ciliates the "S" synthesis periods are not simultaneous in micronucleus and in macronucleus. Even in the same species both periods may occur at different points of interphase (Mc Donald 1958, Prescott 1960). Duration of these periods is to some extent species-specific. The synthesis does not start at the same time in the macro- and micronucleus (Prescott et al. 1962), and its duration is different in both kinds of nuclei.

Considering data obtained up to now it seems highly probable that the initiation of the replication in the macro- and micronucleus occurs independently.

DNA synthesis in the macronuclei occurs: either (1) during the passage of replication bands or (2) simultaneously in numerous points of the macronucleus (*Stentor, Paramecium*). Replication bands occur both in *Hypotricha* (Gall 1959, Kimball and Prescott 1962) and in *Holotricha* (Kaneda 1960, 1961). In *Holotricha* they pass only through the orthomer zone, while in *Hypotricha* — through the whole macronucleus.

The macronucleus of *Chilodonella cucullulus*, of heteromeric type, con-

sists of two different zones: the orthomer — rich in chromatine and the paramer which has little chromatine.

The aim of the present study was to show the difference: (a) in DNA replication between the micronucleus and both zones of macronucleus; (b) in the course of RNA synthesis between both nuclei.

Material and Methods

The methods of culture have been described in previous papers (Radzikowski 1965, 1969). For autoradiographic investigations H³-thymidine (Spec. Act. 20.1 Ci/mM) and H³-urydine (Spec. Act. 27 Ci/mM) were used, from the Amersham Radiochemical Center, England. H³-thymidine, of the concentration of 60 μm Ci/ml to 100 μm Ci/ml, was added to the culture medium. H³-urydine, was added through pulsation for a long time, in a concentration of 30 μm Ci/ml. For isolation of nuclei 1% triton solution was used: isolation of the middle part of the macronucleus paramer was done by shaking macronuclei in a 3% triton solution. The cells were fixed for 10 min with a mixture of alcohol and acetic acid at the ratio of 3:1. In order to remove not incorporated precursors cells were rinsed for 10 min in 5% trichloroacetic acid at 4°C. The specimens were next covered with emulsion (Ilford L-4) and exposed for 8 to 15 days. Then the film was developed and fixed and finally they were dyed for 1 min with acetoorceine and closed in DPX. During the whole interphase cycle the protozoa were fixed at regular one-hour intervals.

For cytophotometric study the protozoans were fixed for 10 min with a 3:1 mixture of alcohol and acetic acid, 10 min hydrolysed in 1 n HCL at 59—60°C. For Feulgen's reaction Pararosalinine (Merck, Darmstadt) following 10 min hydrolysis with 1 n HCL at 59—60°C was used, the DNA content was determined[1] by means of a next Universal Micro-Spectral Photometer UMSPI (Zeiss).

Results

The nuclear apparatus of *Chilodonella cucullulus* (O. F. M.) consists of one micronucleus and a macronucleus of heteromeric type with two different zones: the outer zone called orthomer, rich in chromatine which occurs as numerous Feulgen positive granules, and the inner zone — called paramer which is non-granular and reacts weakly in Feulgen's reaction, with the exception of chromatine of the central part, called endosome (Fig. 1). Such a distribution of chromatine is characteristic during about 80% of the whole interphase. In the first hour after division no differentiation of zones can be observed. Only during the second hour the characteristic difference appears.

Cytophotometric linear measurements of a macronucleus with separate zones show that the concentration of DNA is identical in the outer zone and the endosome but remarkably lower in the inner zone (Pl. II 11).

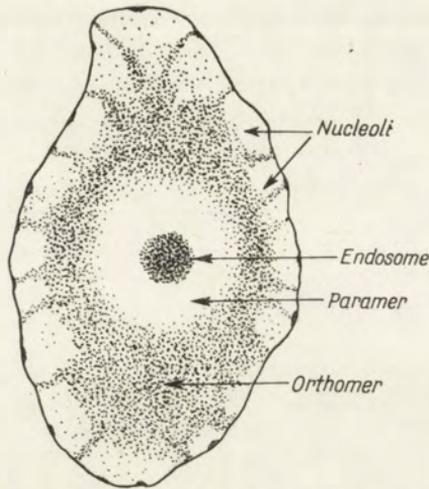
[1] DNA content given in conventional units.

Fig. 1. Structure of the macronucleus of *Chilodonella cucullulus*

However, cytophotometric measurements given in Table 1 indicate that DNA content is lowest in the endosome and highest in the outer zone of the macronucleus. The DNA content in the endosome varies and, apparently, it does not depend on the DNA content in the whole macronucleus.

Duration of the interphase, at 16°C, is usually 12 h. Phase G_1 begins in the micronucleus after its division but before the fission of the macronucleus and cytokineses; it lasts during the whole fission and for an hour

Table 1

DNA Contents* in the Particular Parts of the Macronucleus of
Chilodonella cucullulus

Whole macronucleus	Central part with endosome (paramer)	Endosome
144.59	26.93	4.8
121.18	31.93	5.13
90.90	32.17	4.36
102.45	15.51	3.39
84.46	17.58	3.97
97.25	17.09	2.50
103.82	28.04	6.85
95.34	34.39	9.45
130.99	9.57	1.94
81.17	15.44	4.46
97.54	32.16	4.74

* values coming from various macronuclei; presented in conventional units.

after it (the total duration of phase G_1 in the micronucleus amounts to ca. 2 h (Fig. 2). The phase of replication S lasts for about one hour in the micronucleus and does not coincide with the replication phase in the macronucleus (Fig. 2, Pl. I 1–3). Period G_2, however, is the longest one and its duration amounts to ca. 80% of the time of the whole interphase.
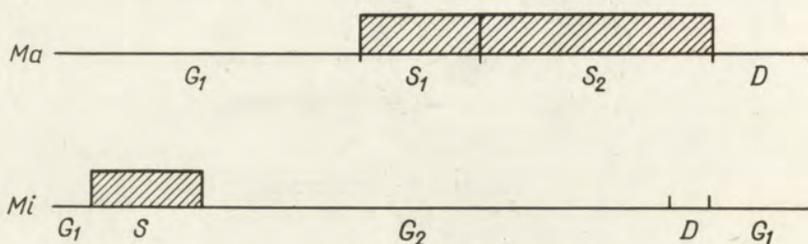

Fig. 2. Phases of DNA replication in the macronucleus and micronucleus of *Chilodonella cucullulus*

The period of division D lasts no more than 20–30 min and does not coincide with the fission time of the macronucleus (Fig. 2). Unlike the micronucleus, phase G_1 of the macronucleus lasts for nearly 50% of the interphase. Phase S begins in the second half of the interphase and lasts without interruption until the fission, but two different steps of DNA synthesis were found. First the DNA replication proceeds in the inner zone excluding the endosome and that period may be called S_1 (Pl. I 1). Next the DNA replication occurs in the outer zone and this phase may be
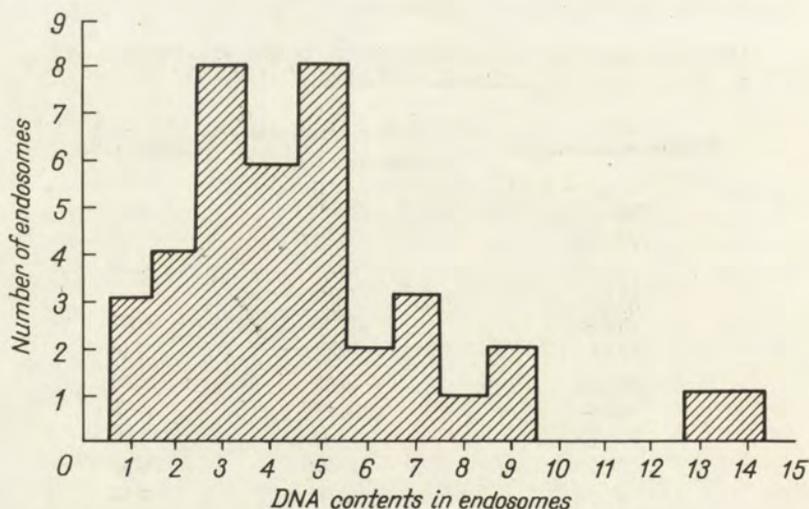

Fig. 3. DNA contents in endosomes from different macronuclei of *Chilodonella cucullulus*

called S_2 (Pl. I 2). According to previous cytophotometric studies (Radzikowski 1969) and to the present results it may be established more firmly that the macronucleus has no phase G_2 and the fission ends the process of replication. If the incubation is continuous i.e., if the protozoa are in H³-thymidine during the whole interphase, the incorporation of the precursor may be observed in the whole macronucleus except the endosome (Pl. I 5, 6).

Also cytophotometric measurements of DNA in the endosome show that its replication does not occur (Fig. 3). In spite of a great variability of DNA content in the particular endosomes, the distribution of this variability approximates normal distribution with only one maximum. Instead, in case of replication a second maximum, corresponding with endosomes of a double quantity of DNA, would occur.

In one of the experiments the cells were incubated with H³-thymidine for 8 h, next dividers were isolated, incubated again for 3–4 h in a medium without any labelled precursor and then the progeny was fixed. In such a case it was observed that the label is uniformly distributed over the whole macronucleus (Pl. I 4) including also endosome! Apparently, following the fission, the whole chromatine is thoroughly mixed and the endosome is formed with sorted chromatine which has undergone replication either in the paramer or the orthomer in the previous interphase!!! (Fig. 4).
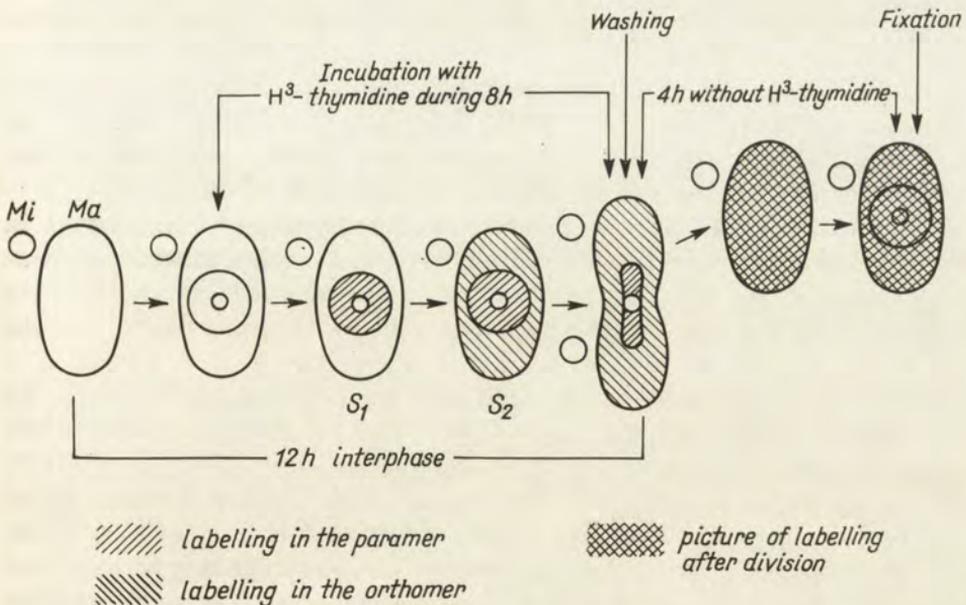

Fig. 4. Scheme of experiment showing the absence of DNA synthesis in the endosome of *Chilodonella cucullulus* (detailed explanation in the text)

RNA synthesis

It is difficult to obtain a univocal answer as to RNA synthesis in the micronucleus without using ME since its intensity seems to be so small that, in principle, the number of silver grains in the emulsion over the micronucleus does not differ from their number in the background. To the contrary, the rate of RNA synthesis in the macronucleus is very high during the whole interphase. When the precursor is added by pulsation a label appears in the nucleus as soon as after 15 min and over the cytoplasm — after 15–20 min. If the protozoa are incubated during the whole interphase it can be observed that incorporation occurs in the whole macronucleus except the endosome! (Pl. II 7, 8). Particularly intense incorporation occurs under the nuclear membrane in spots corresponding with nucleoli. Visible slowing down of synthesis may be observed during the fission of the macronucleus.

Discussion

There are two different patterns of DNA replication in the macronuclei of ciliates: replication bands and scattered replication. Hence the question whether there is any chromatine arrangement into an earlier replicating and later replicating one. Ammermann (1972) has excluded such possibility for *Stylonychia mytilus*. Hanson and Twichell (1962) have shown two-stage replication in *Paramecium trichium*, and Kudrjavtsev (1966) — in *Paramecium putrinum*, but they have not discussed the nature of this phenomenon. Nilsson and Leick (1973) have also found some difference in time between the replication of "micronucleic DNA" and that of the remaining parts, in the macronucleus of *Tetrahymena pyriformis*.

The macronucleus of *Chilodonella cucullulus* is highly differentiated in its morphology and functions, there are two zones, one rich and one poor in chromatine, the DNA content and its concentration in these zones are different, there is a two-step replication, and no replication at all in the endosome.

Relevant to the problem in hand are the following data: Lima de Faria (1959), Pelc and La Cour (1960), Evans (1964) on plants where the uptake of H^3-thymidine into heterochromatine occurs later. Baer (1965) on replication in x and y chromosomes of insects where the heterochromatine parts are replicated later. Taylor (1960), Lima de Faria et al. (1965) on heterochromatic chromocentres of chromosomes in man, Ammermann et al. (1947) on polythenic chromosomes of *Stylonychia mytilus*. Comparing my results with all those cited above, it seems highly probable that the earlier replicating DNA in the inner zone of the macronucleus

of *Chilodonella* (Pl. I) may be considered as euchromatine while DNA of the outer zone, replicating later — as heterochromatine (Pl. I 3).

It results from Lima de Faria studies (1959) that the conventional unit of heterochromatine contains three times as much DNA as in the same unit of euchromatine. Cytophotometric results obtained from investigations on *Chilodonella* show that a unit from the paramer always contains less DNA than a unit from the orthomer (Pl. II 11), so these results, too, suggest that euchromatine occurs in the central part (the endosome excluded) while heterochromatine — in the outer zone.

Experiments with H³-thymidine and H³-urydine as well as the results of cytophotometric measurements show a total absence of synthetic activity of the chromatine enclosed in the endosome (Pl. I 5, 6, Pl. II 7, 8, Fig. 3). It is highly probable that macronuclear DNA may have varying replication ability including its complete absence as in the chromatine of the endosome. In reference to metazoa Frenster (1965) and Allfrey and Mirsky (1963) have formulated the opinion that nuclear chromatine may occur in various states of blockade. It may be concluded from Hsu and Lockhart's (1965) investigations on man's chromosomes that differences in the time of replication not only concern the differences between euchromatine and heterochromatine but they also occur within the heterochromatine of the same nucleus, e.g.: autosome heterochromatine undergoes earlier replication than heterochromatine of the x and y chromosomes.

It cannot be excluded that it is precisely the chromatine of the orthomer and the endosome which is of the most differentiated heterochromatine: (a) that occurring in the orthomer with later replication than in euchromatine (b) that of the endosome — totally inactive.

Andersen (1974) has stated that in *Tetrahymena pyriformis* the replication of all macronuclear DNA is not necessary for the division to take place. The non-replicating part penetrates into the cytoplasm and undergoes replication there, in the next interphase. A similar mechanism occurs in *Chilodonella* but the non-replicating chromatine (endosome) remains in the nucleus all the time, and during the division it is eliminated into the cytoplasm only in some cases (Radzikowski 1965). The examples quoted above show that both in plant and animal cells there occurs chromatine, blocked to a varying degree, manifesting itself in varying synthesizing ability. It is not excluded that the phenomenon of non-replication of a part of chromatine before the division is possible only in macronuclei which are characterized by a high content of multiplied chromatine material which probably occurs in the form of chromosome fragments, Ammermann (1970), Kloetzel (1970), Radzikowski (1973). Then replication would concern only the complete part of the genome while defective copies could be totally blocked or eliminated into the cytoplasm.

The fact of asynchronous replication in the macro- and micronucleus (Pl. I 1, 2, 3) has probably no connection with the cases quoted above. It seems highly probable that the beginning of replication in either of these nuclei is differently controlled and independent of the other. This might be evidenced by such facts: the absence of a micronucleus does not affect DNA replication in the macronucleus; experimentally prolonged replication in the macronucleus results in an increased chromatine content but does not in the least affect the replication in the micronucleus of *Tetrahymena pyriformis* (Jeffery et al. 1970).

On the basis of previous research on RNA synthesis in the nuclear apparatus of ciliates it may be stated that macronuclei are the main producers. There are data indicating that in micronuclei some synthesis occurs but in small quantities and in certain periods only (Ammermann (1970, Murti and Prescott 1970, Pasternak 1967). Anyway, results obtained on *Chilodonella cucullulus* in general do confirm the rule, no synthesis in the micronucleus and a very active macronucleus. An intense label is particularly characteristic of its peripheric parts, corresponding to nucleoli (Radzikowski 1973). Synthesis occurs during the whole interphase (not in the endosome) without interruption, including the period of replication; it is connected with the fact that in the period S not all parts of the genome undergo simultaneous replication, hence there are always free spots for RNA synthesis which serve as matrices.

RESUMÉ

On a étudié le cours de la synthèse de l'ADN et de l'ARN dans l'appareil nucléaire de *Chilodonella cucullulus* (O. F. M.). On a constaté que la replication de l'ADN dans le micronoyau ne coincide pas avec sa replication dans le macronoyau et que cette dernière s'effectue à deux temps sans entrainer la totalité de la chromatine nucléaire (l'endosome). La formation de l'ARN dans la cellule est réalisée en prémier lieu par le macronoyau, en particulier par sa zone périphérique. La chromatine de l'endosome ne participe pas à la synthèse. Il est suggéré que la chromatine du paramère qui se replique la prémière constitue l'euchromatine, tandis que celle qui se replique plus tard dans l'orthomère représente l'hétérochromatine. L'endosome serait constitué d'une chromatine particulière contenant les copies défectives dont la synthèse serait entièrement bloquée.

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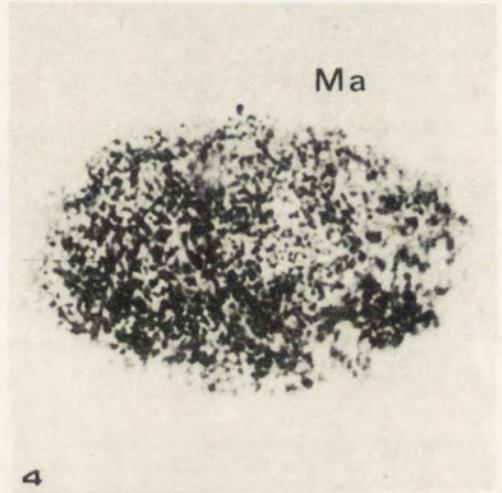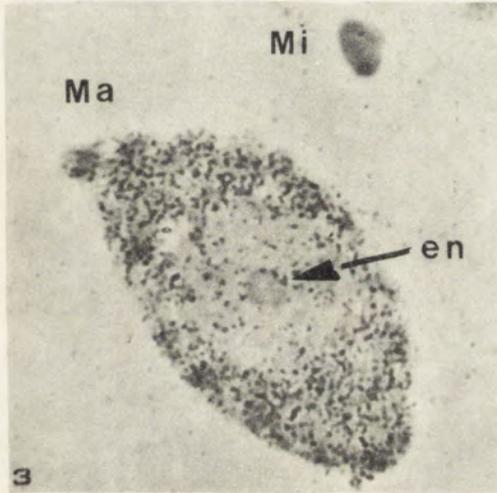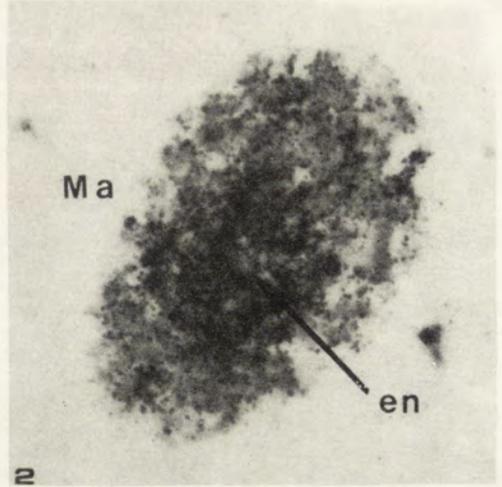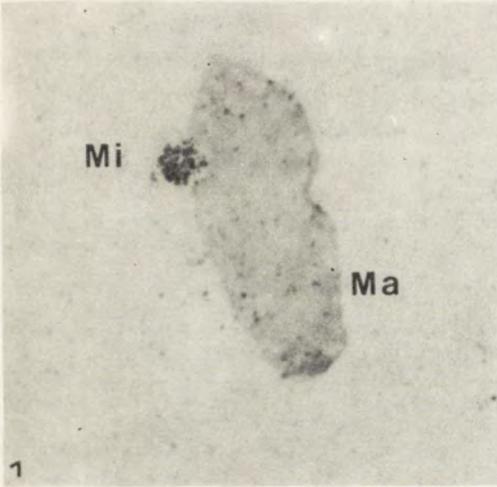
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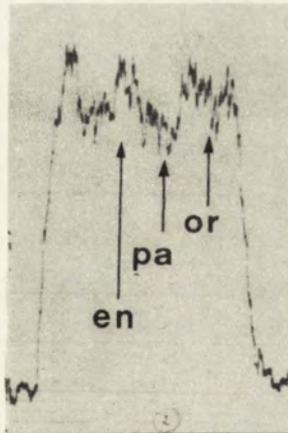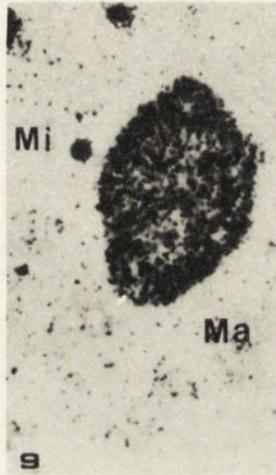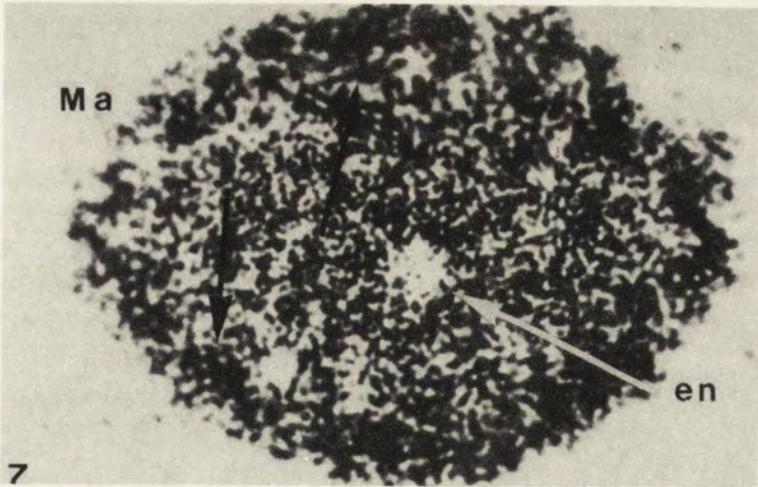
EXPLANATION OF PLATES I–II

Uptake of H^3-thymidine and H^3-urydine in the nuclear apparatus of *Chilodonella cucullulus*.

1: One hour after division. DNA replication in the macronucleus magn. $1600\times$
2: Six hours after division. DNA replication only in the central part of the macronucleus. magn. $1600\times$
3: Nine hours after division. DNA replication only in the outer part of the macronucleus. magn, $1600\times$
4: H^3-thymidine during 8 h before division. Since the division the ciliates were incubated during four hours in non-radioactive medium. Labelling evenly distributed. magn. $1600\times$
5: Macronucleus isolated from specimens remaining in H^3-thymidine during the whole interphase. No incorporation in the endosome!!! magn. $1200\times$
6: Isolated central part of macronucleus with the endosome. Incubation with H^3-thymidine during the whole interphase. magn $1600\times$
7: Incubation with H^3-urydine as above. Strong label over the peripheric part of the macronucleus. No labelling over the endosome!!! magn. $1600\times$
8: Incubation with H^3-urydine as above. Central part isolated. No labelling over the endosome. magn. $1600\times$
9: Incubation with H^3-urydine as above. Macro- and micronucleus. magn. $1000\times$
10: Incubation with H^3-urydine. Macronucleus during division. magn. $1000\times$
11: Linear DNA distribution in the macronuclear cytophotometric measurement, in the endosome (en), the paramer (pa), the orthomer (or.). magn. $1000\times$

PLATE I

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Effect of pH on *Blepharisma intermedium*.
3. Changes in the Levels of Some Macromolecules, Free Amino Acids and Protein Pattern

Synopsis. The total carbohydrate, glycogen and protein contents of *Blepharisma intermedium* vary quantitatively with the change in pH and molarity of different buffered media, compared to the organisms grown in unbuffered hay infusion media. Under these conditions there are significant changes in the free amino acid pattern of the ciliate and that of proteins as determined by disc electrophoresis.

Living cells have perfected specific transport or carrier systems to carry certain types of molecules across the cell membrane. These membrane transport systems participate in the regulation of metabolic steady states, by their ability to adjust to wide fluctuations in the composition of the external environment of cells. Depending upon the nature and extent of entry of substance from the growth medium, the conditions within the cell are altered. Therefore metabolic processes depend upon the concentration gradients between the environment and the cell or cell organelle.

Protozoans display an amazing diversity of habitat and this may be due to their biochemical make up which allows them to adjust themselves to their environment. It is now known that change in external conditions is reflected in a change in metabolic activity (Hogg and Kornberg 1963, Ryley 1962). Researches on protozoa have shown that the pH of the growth medium alters the normal cell functioning. Our previous work has shown that in *B. intermedium*, the external pH has an effect on fission rate, oxygen consumption and content of some cellular constituents as determined cytochemically (Shadaksharaswamy and Jyothy 1973 a, b).

In the present investigation quantitative estimations of glycogen, total carbohydrate and protein of the ciliate grown in different types of buffer of varying pH and molarity have been carried out. Observations have also been made on the free amino acid and disc electrophotoretic protein pattern of the organism grown under similar conditions.

Material and Methods

B. intermedium was grown as described in our earlier communication (Kasturi Bai et al. 1969).

Conditions chosen for estimation of the cellular constituents and determination of free amino acid pattern were those chosen for cytochemical investigations (Shadaksharaswamy and Jyothy 1973 b), viz. tris, acetate and citrate buffers between pH values of 5.0 and 9.0 and 1 to 10 mM concentrations. A pH of 6.0 with respect to acetate buffer in the text should be considered as 5.6.

Total protein was determined using the method of Lowry et al. (1951). A known number of organisms varying from $2-5 \times 10^4$ were collected in the form of a pellet by centrifugation and washed with 0.015 M KCl. Finally the pellet was resuspended in 5% TCA for 20 min at room temperature. The contents were centrifuged and the acid insoluble material was washed twice with 5% TCA and used for protein analysis.

The total carbohydrates were estimated by the phenol–sulphuric acid method of Dubois et al. (1956) using organisms dried to constant weight.

Method of glycogen assay was that of Cook (1962). A pellet containing about $2-5 \times 10^4$ organisms was obtained as before. This was then repeatedly extracted with boiling 55% ethanol until the final extract did not give colour with anthrone reagent. The residue obtained was hydrolyzed at room temperature for 10 min with 2.0 ml of 1 N NaOH. To this hydrolyzate 2 ml of 50% perchloric acid were added, the contents mixed and chilled to 0–5° C. To aliquots of this adjusted to 2 ml, 10 ml of anthrone reagent (0.1% anthrone in 72% v/v H_2SO_4) were squirted and the developed colour was read at 630 nm.

Protein pattern was studied by the disc electrophoretic technique of Ornstein (1964) and Davis (1964) using a discontinuous gel system (Cyanogum 41). Cells of *B. intermedium* (3×10^5) washed with 0.015 m KCl were extracted with water, phosphate buffer pH 7.0, and 0.6 M KCl separately. The KCl extract gave the maximum number of protein bands and therefore it was used for extraction in all cases. The extract thus obtained was freed from low molecular weight components by gel filtration in Sephadex G 25, concentrated and electrophorized. During electrophoresis a current of 6.5 ma was imposed along each gel column. Tris-glycine buffer pH 8.4 was used as electrode buffer. At the end of the electrophoretic run the gels were stained for 1 h in a 0.5% solution of amido black 10 B in 6% acetic acid followed by destaining in 6% acetic acid.

For the detection of free amino acids the 55% ethanol extracts obtained in glycogen assay were pooled and evaporated to dryness in vacuum. The residue was then dissolved in minimum amount of 80% ethanol and the solution was subject to two dimensional paper chromatography using solvent systems butanol: acetic acid: water (4:1:5 v/v/v) for the first dimension and propanol: water (85:15 v/v) for the second dimension.

Results

Changes in the total carbohydrate, glycogen and protein contents of *B. intermedium* with changes in nature of buffer, its pH and molarity are given in Tables 1, 2 and 3. Results are expressed on dry weight basis (mg%).

From Table 1 it can be seen that in tris, citrate and acetate buffers at pH 6.0, where the rate of fission is 2 per day, the total carbohydrate content is 9.0, 13.8 and 21.0 mg% respectively. However, the glycogen content which

is 93.5% of the total carbohydrate in the control is only 64.3% in tris,
75.0% in citrate and 52.0% in acetate buffer. In citrate and acetate buffers
the rate of fission is 2 even at pH 5.0. At this pH also similar buffer depen-
dence of the carbohydrate and glycogen contents is observed. Under these
conditions, changes in protein content is appreciable in tris buffer where
it is less than the control by 27.0%.

Table 1

Effect of Buffer (1 mM Concentration)

pH	Buffer	Total Carbohydrate (mg %)	Glycogen (mg %)	Protein (mg %)	No. of Protein bands
–	Control	9.0	8.4	48.0	10
	Tris	9.0	5.8	35.1	10
6.0	Citrate	13.8	10.3	52.1	7
	Acetate	21.0	10.9	42.9	9
5.0	Citrate	10.0	7.8	48.5	11
	Acetate	17.2	8.0	45.7	13

Results in Table 2, where buffer and molarity are kept constant, show
that the total carbohydrate and glycogen contents decrease with increase in
pH from 5.0 to 9.0. The glycogen content is least at pH 9.0, accounting
for only 35.9% of the total carbohydrate and maximum at pH 5.0 (84.3%).
Protein content is less than the control in all cases, the maximum decrease
(60.0%) being at pH 5.0.

The level of macromolecular components with change in molarity of

Table 2

Effect of pH (Tris Buffer of 5 mM Concentration)

Buffer	pH	Total Carbohydrate (mg %)	Glycogen (mg %)	Protein (mg %)	No. of Protein bands
Control	–	9.0	8.4	48.0	10
	5.0	11.1	9.4	19.3	14
Tris	6.0	10.5	8.1	41.6	10
	9.0	9.8	3.5	27.5	10

the buffer at constant pH is given in Table 3. Increase in buffer concentration from 1 to 10 mM increases the carbohydrate content with a corresponding increase in glycogen content. Compared to control the protein content is less at all molarities, while the most marked decrease is with 1 and 10 mM buffers, being 33.0 and 27.0% respectively.

Table 3

Effect of Molarity (Tris Buffer at pH 6.0)

Buffer	Molarity	Total Carbohydrate (mg %)	Glycogen (mg %)	Protein (mg %)	No. of Protein bands
Control	–	9.0	8.4	48.0	10
	1	9.0	5.8	35.1	10
Tris	5	10.5	8.1	41.6	10
	10	11.4	10.0	32.0	10

From Fig. 1 it can be seen how the protein pattern of the organism varies under different growth conditions. The maximum number of bands are obtained with tris and acetate buffers at pH 5.0. In all cases the band with maximum intensity is fast moving and the slow moving ones are of low intensity. In all the three buffers, at pH 5.0, there are two bands moving faster than the darkest band, similar to control. The same is not observed at pH 6.0 and 9.0. Variations are mostly observed with the bands lying in the middle of the zymogram.

The free amino acid pattern of *B. intermedium* is given in Table 4. The free amino acids present in the control are: histidine, lysine, glycine, serine, cysteic acid, citrulline, aspartic acid, glutamic acid, alanine, tyrosine, valine, leucine and/or isoleucine. Some of the significant changes observed in the amino acid pattern are: (1) Cysteic acid present in the control is absent in the organisms grown in tris and its presence is doubtful in the other two buffered media, (2) Threonine absent in the control is present in all cases, (3) Proline absent in the control is present only in organisms grown in acetate buffered media and (4) the number of unidentifiable ninhydrin positive spots are more than in control in all cases.

Discussion

The results of Table 1, 2 and 3 show that the protein content of *B. intermedium* varies with the nature of buffer, its pH and molarity. Unlike in tris its concentration does not change appreciably in acetate and citrate
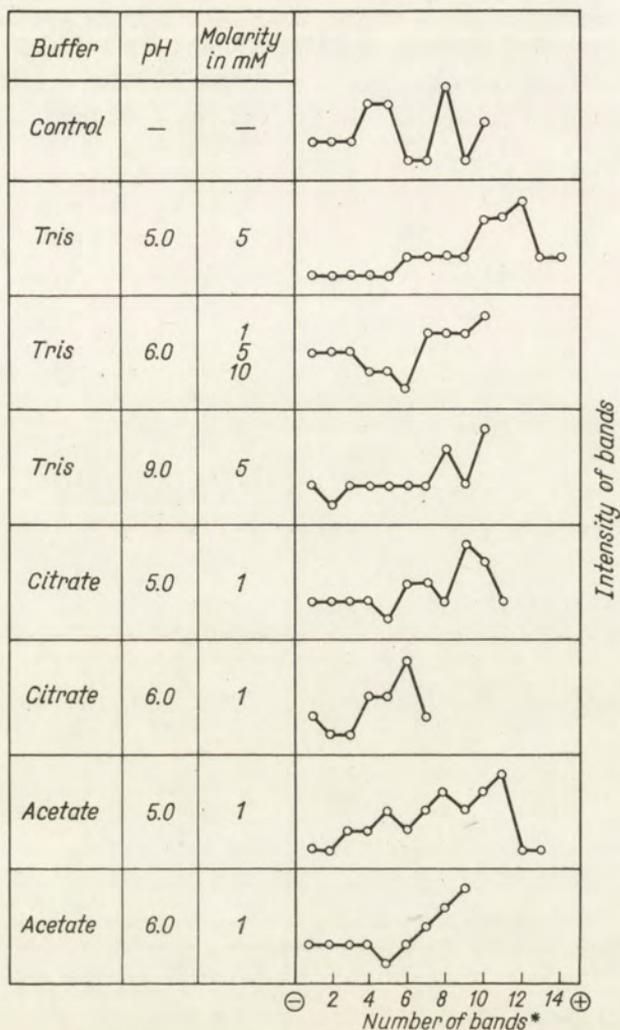
Fig. 1. Number and intensity of protein bands of *B. intermedium* grown under different conditions and separated by disc electrophoretic procedure

* The serial number of bands of the different buffer systems do not correspond with one another.

buffers. But the pH and molarity have more effect on the protein content than the nature of the buffer. At pH 5.0 (tris, 5 mM) the protein content is less by 53.6% when compared to pH 6.0. This decrease in protein content at pH 5.0, where the glycogen content is more than the control, shows that protein is metabolized in preference to carbohydrate, while generally it is the carbohydrate that is utilized before the breakdown of protein. At pH 9.0 there is breakdown of both glycogen and protein, their concentration being 41.7% and 57.3% that of control respectively. This pH may

Table 4

Effect of Nature of Buffer, pH and Molarity on the Free Amino Acid Pattern of *B. intermedium*

Amino-acids and Related Conditions	His	Lys	Gly	Ser	CySO3H	Cit	Asp	Glu	Ala	Tyr	Val	Leu/Ileu	Thr	Pro	Un-known
Control	+++	+++	+++++	++++	++	++	+	++	++++	+	+	++	-	-	1
Tris, pH 5.0, 5 mM	++	++	+++	+++	-	++	++	+++++	+++++	+	+	++	+++	-	6
Tris, pH 6.0, 10 mM	++	++	+++	+++	-	++	++	+++++	+++	+	+	+	+++	-	5
Tris, pH 6.0, 5 mM	++	++	+++	+++	-	++	++	+++++	+++	+	+	+	+++	-	5
Tris, pH 6.0, 1 mM	+++	+++	+++	+++	-	++	++	+++++	++++	+	+	+	+	-	5
Tris, pH 9.0, 5 mM	+++	+++	++	++	-	++	++	+++++	+++++	+	+	++	+	-	5
Citrate, pH 5.0, 1 mM	+++	++	++	++	?	++	++++	+	+++	+	+	++	+	-	5
Citrate, pH 6.0, 1 mM	++	++	+++	+++	?	++	++	+	+++	+	+	+	++	-	5
Acetate, pH 5.0, 1 mM	++++	+++	+++	+++	?	++	++	+	++++	+	+	++	+++	+	5
Acetate, pH 6.0, 1 mM	++++	+++	++	++	?	++	++	++	++++	+	++	++	+++	+	3

not be suitable for the growth of the organism and therefore to sustain growth there is utilization of both glycogen and protein. In tris buffer (pH 6.0) at 10 mM concentration, protein is used in preference to carbohydrate, while at 1 mM concentration there is utilization of both glycogen and protein. With 5 mM buffer the changes are minimal. Thus, this molarity seems optimal for growth.

Glycogen constitutes a major portion of the carbohydrate content of the ciliate. A similar observation is made with *Tetrahymena pyriformis* (Koehler and Fennell 1964). The carbohydrate content is either the same or more than the control under various conditions studied, its concentration being very much more than control in acetate and citrate buffers. The glycogen content, however, varies under experimental conditions. Its content is less than in control in tris buffer at a pH of 9.0 (5 mM) and pH 6.0 (1 mM) showing its greater utilization under these conditions. In citrate and acetate buffers the glycogen content is more at pH 6.0 than at pH 5.0. This indicates that undissociated molecules penetrate into cells more readily than the corresponding ions and exibit toxicity by altering the internal pH.

It has been reported (Shadaksharaswamy and Jyothy 1973 a) that with tris buffer, the fission rate is 2 at pH 6.0 irrespective of the molarity of the buffer used. From Table 3 it is seen that there is also no significant change in the total protein concentration of the organism grown in this buffer of different molarities. The electrophoretic pattern of the proteins are also similar. However, the change of pH to 5.0 with this buffer where the rate of fission is 1, affects both protein content and its pattern. The protein content is about half of what it is at pH 6.0 and the electrophoretic pattern indicates 14 bands as compared to only 10 at pH 6.0. At pH 5.0 the rate of fission is 2 in acetate and citrate buffers while it is 1 in tris (Shadaksharaswamy and Jyothy 1973 a). But the protein content of the organisms grown in the first two buffers is more than twice of those grown in tris. The number of bands, however, are 13, 11 and 14 respectively. Therefore there is no correlation between protein content and its pattern.

Determination of free amino acid pattern is important for it forms a suitable means of tracing genetic differences hidden under identical morphological features (Hawkins and Danielli 1961). Finley and Williams (1955) have discovered in *Vorticella microstomata* a change in the composition of amino acids during conjugation. The number of amino acids detected by the chromatographic method increases during this process from 10 in the vegatative to 15 in the conjugants. Changes in the amino acids and proteins have observed in asexual and sexual stage of *Blepharisma* by Seshachar and Padmavathi (1956). The number of amino acids increased from 14 in the vegetative to 18 in the conjugants. Maintaining

the nutritional state of the organism constant and using the conditions used for the various estimations, it can be seen from Table 4 that there are variations in the free amino acid pattern of *B. intermedium*. Free proline has been reported in many ciliates (Lee and Rene 1955, Loefer and Scherbaum 1960, Scherbaum et al. 1959, Wu and Hogg 1956) and has never been detected in any species of *Blepharisma*. However, its presence has been shown in the hydrolyzate of the conjugants of *B. undulans* (Seshachar and Padmavathi 1956). The detection of this amino acid in free state only in organisms grown in acetate buffered media is significant. Various species of *Blepharisma* differ with respect to threonine (Seshachar and Saxena 1963). The presence of this amino acid in all cases except in the control is also significant. Cysteic acid is the only sulphur containing amino acid in the control and the disappearance of this amino acid under various experimental conditions is of interest. It is only the study of the metabolism of these amino acids that can explain the changes observed.

ZUSAMMENFASSUNG

Die Gesamtgehalte an Kohlehydraten, Glykogen und Protein in *Blepharisma interme-dium* andern sich quantitativ mit der Veranderung des pH und des Grammolekuls von verschiedenen Puffermedien, im Gegensatz zu den in ungepufferten Heuinfusionsmedien gezuchteten Organismen. Unter diesen Bedingungen ergeben sich wesentliche Unterschiede im Freiaminosaurenmuster der ziliaten sowie im Proteinmuster, das durch Scheibenelektrophorese bestimmt wurde.

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Effects of Ionic Detergents on Phagocytic Activity of *Tetrahymena pyriformis* GL and *Paramecium caudatum*

Synopsis. The anionic detergent SDS and cationic detergent CTAB show similar effects on phagocytic activity in *Tetrahymena pyriformis* and *Paramecium caudatum*. Low concentrations of both compounds ($5 \times 10^{-7} - 2 \times 10^{-6}$ g/ml) may cause a slight increase of the number of food vacuoles formed in 10 min with marked decrease of food vacuole volume, it is suggested that the stimulation of the process of phagocytosis may be caused in the case of SDS by higher level of the cell surface potential, while CTAB may induce similar effect as a result of possible interaction with mucous layer of the cell surface. Higher concentrations of SDS and CTAB (in the range $4 \times 10^{-6} - 8 \times 10^{-6}$ g/ml) inhibit the formation of food vacuoles probably due to observed contraction phenomena within cytoplasm.

The influence of detergents as surface acting agents on protozoan cells was known from the research of Chaix and Baud (1947), Bailenger and Troadec (1953), Bailenger et al. (1953) and Rockstroh (1967). These compounds show a high chemical affinity to the lipid components of the cell membrane and can disturb greatly the normal function of organism. In high, lethal concentrations their action causes disintegration of the cell membrane and death. Apart from concentration, the electric charge of detergents used is of great importance in their biological activity. The immobilization and cell lysis appears earlier and in lower concentrations of cationic detergents than that of anionic ones (Brewer and Bell 1969). The most toxic among the anionic compounds seems to be sodium dodecyl sulphate.

In sublethal concentrations the action of many kinds of detergents on protozoan cells consists in characteristic changes in body shape and in

motility (Bujwid-Ćwik and Dryl 1971). These effects are, however, reversible, the rate of recovery according to concentration used.

Several types of reversal movement have been recognized in *Paramecium caudatum* by Dryl and Bujwid-Ćwik in sublethal media of cationic detergent cetyl trimethyl ammonium bromide (CTAB) and anionic sodium dodecyl sulphate (SDS): periodical ciliary reversal (PCR), continuous ciliary reversal (CCR), then partial ciliary reversal (PaCR) in addition to other uncoordinated types of ciliary reversal (Dryl and Bujwid-Ćwik 1972, Bujwid-Ćwik and Dryl 1974). These authors have also shown that induced by potassium/calcium ions ciliary reversal is inhibited by CTAB, but SDS stimulates it greatly. Similar results were obtained in experiments with *Tetrahymena pyriformis* GL (Brutkowska et al. 1974).

The experiments of Butzel et al. (1960) showed that the galvanotactic response of *Paramecium caudatum* was inhibited by CTAB, while the negatively charged sodiumhexylsulfosuccinate and neutral Tween 80 were inactive in this respect.

These observations indicate changes in the level of the cell membrane excitation of *Protozoa* (Jahn 1962).

For that reason it seemed of interest to study the influence of the same detergents on such important function as the formation of food vacuoles and to test their possible effect considered as a response to cell membrane excitation.

Material and Methods

The experiments were carried out with *Tetrahymena pyriformis* GL and a wild strain of *Paramecium caudatum*. 2% proteose peptone (Difco) was used for the culture of *Tetrahymena*, with daily transfer of 1 ml of cell suspension to 10 ml of nutrient medium. *Paramecium* was cultured on lettuce infusion with *Areobacter areogenes* according to method of Sonneborn (1950).

18–24 hours before each experiment, the animals were collected geotactically in 1 mM Tris HCl buffer containing 1 mM $CaCl_2$ at pH 7.3–7.4. The ciliates were washed carefully until the culture solution was diluted about 100–200 times.

All solutions were diluted with the same Tris buffer, except the stock solutions of detergents, which were prepared with distilled water. The influence of both SDS and CTAB was tested in the following final concentrations: 5×10^{-7}, 1×10^{-6}, 2×10^{-6}, 4×10^{-6} and 8×10^{-6} g/ml. Each component was prepared in a concentration four times higher than that used in the experiments.

Polystyrene latex particles, diameter 0.8 μm (PLP, Difco) were used as test particles for ingestion after dilution with Tris buffer to a concentration of 1:200 of the stock suspension.

The rate of phagocytic activity of the ciliates was measured as the total number of food vacuoles (whether containing PLP or not) formed during 10 min of exposure of the animals to the test solution. They were transferred to 10 mM $NiCl_2$ to immobilize them for counting the number of food vacuoles in 50 specimens at a magnification of 18–400 using

Zeiss normal light optics. The mean number and standard deviation of food vacuoles per ciliate was then calculated, and the Kolmogorov-Smirnov test was used to present significance, probability and diagrams of the experimental data (Siegel 1956). Five or six groups, each of 50 animals, were tested in each experiment, together with control group, immersed in Tris buffer without detergent. The total number of animals in which food vacuoles were counted comprised 250 for each concentration of the two detergents because each experiment was repeated five times.

Additional observations on the motile behaviour of ciliates were always made during the experiments. These observations, carried out on experimental group of animals, were then compared with observations carried out on control groups.

Results and Discussion

Tetrahymena pyriformis and *Paramecium caudatum* remained alive for about one hour at 8×10^{-6} g/ml concentration of SDS. However, the changes of body shape and motile behaviour were then found. The animals became shorter and thicker than usual and different types of ciliary reversal occurred. All these changes disappeared after some time and animals normalized the movement and their body shape, although they were still left in the same concentrations of detergent. Similar disturbances were observed at lower, 4×10^{-6} and 2×10^{-6} g/ml concentrations of SDS, but they lasted shorter, proportionally to decreasing concentrations. The response of animals on the lowest, 1×10^{-6} and 5×10^{-7} g/ml concentrations of this compound did not differ so much from their normal behaviour observed usually after transferring them to the new media. They could survived in these concentrations for many hours.

Since both ciliate species remained alive at 8×10^{-6} g/ml concentration of CTAB for only a few minutes, hence we paid the attention above all to 4×10^{-6} g/ml and lower concentrations of this detergent. Similar changes of body shape and of motility as in SDS were then observed. However, these changes lasted for longer time than when the same concentrations of SDS were used. They were also dependent on concentration, as the process of recovery. The influence of lowest, 5×10^{-7} g/ml concentration of CTAB exerted rather weak action on the behaviour of *Paramecium* and *Tetrahymena*. They survived in this concentration for more than two hours. At the beginning of the action of this concentration the short-lasting PCR could be observed.

The above observations are in agreement with the findings of Dryl and Bujwid-Ćwik. They seem to indicate the more toxic effect of CTAB than of SDS on both species of *Protozoa*.

The action of SDS and CTAB on phagocytosis of *Tetrahymena pyriformis* and of *Paramecium caudatum* was dependent on the concentrations of these detergents in the medium.

The phagocytic activity of *Tetrahymena* was considerably inhibited by 8×10^{-6} g/ml concentration of SDS. There were about 20% of cells devoid of food vacuoles, the remaining 80% contained only a few of them. At a concentration of 4×10^{-6} g/ml, SDS showed only a slight inhibitory effect, while at lower concentrations a slight stimulation of suspension uptake was observed (Fig. 1).
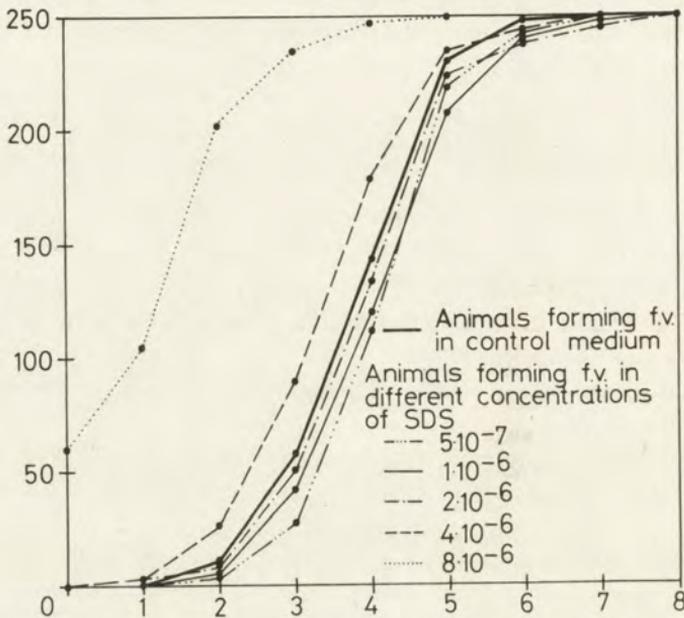

Fig. 1. Cumulative frequency distributions of the food vacuoles formed by *Tetrahymena pyriformis* GL in 10 min dependent on concentration of SDS. Abscissa: Number of food vacuoles, Ordinate: Cumulative frequency of animals

Paramecium responded to the highest, 8×10^{-6} g/ml concentration tested by a complete inhibition of vacuole formation, while the concentration of 4×10^{-6} g/ml resulted in a considerable stimulatory effect. Lower concentrations also increased the number of vacuoles formed (Fig. 2).

It is interesting to note that when food vacuoles were very small their number was as a rule very high. In these cases the values of standard deviation were also usually very high (Table 1).

The statistical significance of these results is shown on Table 2 and 3.

The highest concentration of CTAB in which particles uptake could be observed both in *Tetrahymena* and *Paramecium* was 4×10^{-6} g/ml (Fig. 3 and 4). However, even at this concentration the uptake of particles was disturbed, especially in *Paramecium*, in which about 30% of the cells were devoid of vacuoles. At concentration of 2×10^{-6} g/ml the influence of CTAB on *Paramecium* was more pronounced than on *Tetrahymena*; in the latter
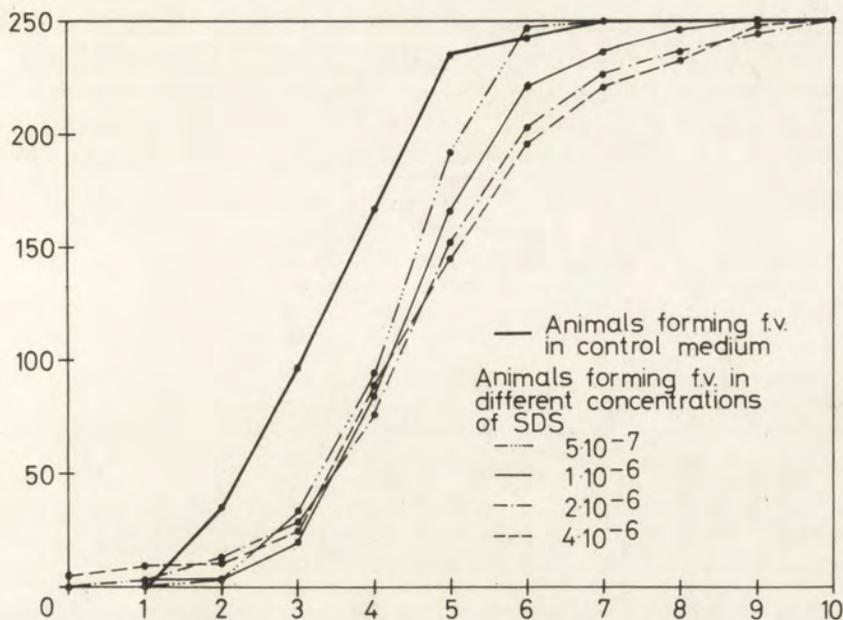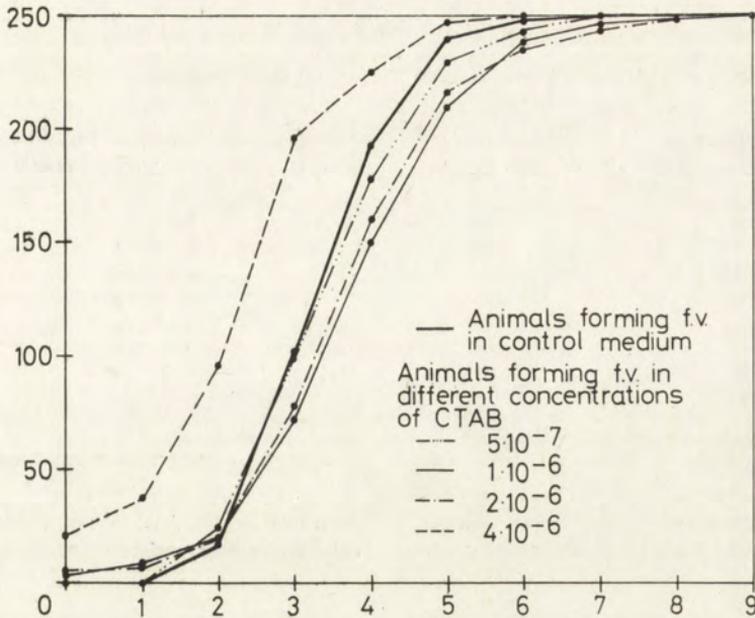
Fig. 2. Cumulative frequency distributions of the food vacuoles formed by *Paramecium caudatum* in 10 min dependent on concentration of SDS. Abscissa: Number of food vacuoles, Ordinate: Cumulative frequency of animals

Table 1

Tetrahymena (T) and *Paramecium* (P) in the Medium of SDS and CTAB
Results of One Experiment

Control Tris buffer medium	Final concentrations of both compounds mg/ml				
	0.0008%	0.0004%	0.0002%	0.0001%	0.00005%
		SDS			
T. 3.5 ± 0.93	2.1 ± 1.24	4.5 ± 1.47	4.8 ± 1.22	4.8 ± 1.22	4.2 ± 1.16
P. 4.4 ± 0.97	0	6.1 ± 2.02	5.0 ± 1.79	5.1 ± 1.11	4.9 ± 1.04
		CTAB			
T. 3.9 ± 1.01	0	2.0 ± 1.32	3.7 ± 1.10	4.2 ± 1.07	4.1 ± 1.31
P. 4.2 ± 0.92	0	0.9 ± 0.67	3.8 ± 0.77	4.7 ± 0.45	4.9 ± 1.08

species stimulation of food vacuole formation was observed. In the two lowest concentrations stimulation of food vacuole formation in both species was observed. The statistical analysis indicates that the differences found in all these concentrations were significant (Table 4 and 5).

If we compare the influence of both detergents on phagocytic activity in *Tetrahymena* and *Paramecium* with the changes in its cell membrane excitation

Table 2

The Effect of SDS on Food Vacuole Formation by *Tetrahymena pyriformis* GL

Concentration of SDS in g/ml	Mean number of food vacuoles	Statistical significance of the differences between control and experimental groups
0	4.3	0
5×10^{-7}	4.5	$p < 0.05$
1×10^{-6}	4.4	$p < 0.10$
2×10^{-6}	4.4	$p < 0.10$
4×10^{-6}	3.9	$p < 0.025$
8×10^{-6}	1.6	$p < 0.001$

Table 3

The Effect of SDS on Food Vacuole Formation by *Paramecium caudatum*

Concentration of SDS in g/ml	Mean number of food vacuoles	Statistical significance of the differences between control and experimental groups
0	3.8	0
5×10^{-7}	4.8	$p < 0.001$
1×10^{-6}	4.9	$p < 0.001$
2×10^{-6}	5.2	$p < 0.001$
4×10^{-6}	5.2	$p < 0.001$
8×10^{-6}	0	0

we can see that the process of food intake is to some extent independent and not correlated directly with these changes. SDS in concentrations which evoke the pronounced excitation of the cell membrane of both species (8×10^{-6} and 4×10^{-6} g/ml) effects the inhibition of particles uptake. However, this process is also inhibited by the concentrations of CTAB decreasing greatly the level of cell membrane excitation (4×10^{-6} and 2×10^{-6} g/ml, Dryl and Bujwid-Ćwik 1972, Bujwid-Ćwik and Dryl 1975, Brutkowska and Dryl unpublished).

On the contrary, the uptake of particles is not, or only slightly disturbed in lower concentrations of both compounds where the level of cell membrane excitation of *Tetrahymena* and *Paramecium* is very closed to the level found in animals in control Tris buffer medium. The stimulation of phagocytosis appearing in such concentrations of detergents could therefore indicate some discoordination of the cell membrane function.

However, the causes evoking such changes in SDS media certainly differ from that in CTAB.

Fig. 3. Cumulative frequency distributions of the food vacuoles formed by *Tetrahymena pyriformis* GL in 10 min dependent on concentration of CTAB. Abscissa: Number of food vacuoles, Ordinate: Cumulative frequency of animals
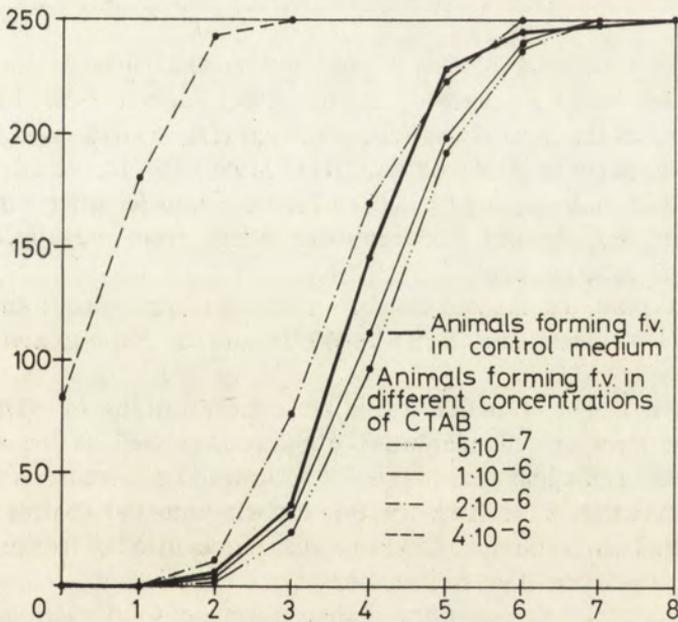

Fig. 4. Cumulative frequency distributions of the food vacuoles formed by *Paramecium caudatum* in 10 min dependent on concentration of CTAB. Abscissa: Number of food vacuoles, Ordinate: Cumulative frequency of animals

Table 4

The Effect of CTAB on Food Vacuole Formation by *Tetrahymena pyriformis* GL

Concentration of CTAB in g/ml	Mean number of food vacuoles	Statistical significance of the differences between control and experimental groups
0	3.7	0
5×10^{-7}	3.9	$p < 0.10$
1×10^{-6}	4.1	$p < 0.001$
2×10^{-6}	4.1	$p < 0.001$
4×10^{-6}	2.8	$p < 0.001$

Table 5

The Effect of CTAB on Food Vacuole Formation by *Paramecium caudatum*

Concentration of CTAB in g/ml	Mean number of food vacuoles	Statistical significance of the differences between control and experimental groups
0	4.3	0
5×10^{-7}	4.8	$p < 0.001$
1×10^{-6}	4.5	$p < 0.10$
2×10^{-6}	4.0	$p < 0.01$
4×10^{-6}	1.0	$p < 0.001$

A negatively charged SDS not only lowers the surface tension of the cell membrane similar to other detergents (Pethica and Schulman 1953), but also increases the negative surface potential (Duszyński and Wojtczak 1974). As suggested by Brewer and Bell (1969) the increased number of negative electric charges on the cell surface of *Amoeba* after immersion in SDS medium may induce the repulsive effect from negatively charged polysaccharide mucous coat.

Similar to that in *Amoeba* mucous coat has been also found both in *Tetrahymena* (Nilsson and Behnke 1971) and in *Paramecium* (Wyroba and Przełęcka 1973).

Then, as a result of action of lower concentrations of SDS on these *Protozoa*, the level of cell membrane excitation, as well as the acceleration of the process of phagocytosis may arise following an increase of the surface potential. Similarily, a decrease of the surface potential in low pH media was interpreted as a factor inhibiting the pinocytosis of cationic dyes in *Paramecium caudatum* (Grębecki 1963).

On the contrary, the slight stimulative effect on food vacuole formation observed in lower concentrations of positively charged CTAB is probably related with chemical affinity of this compound to outside negatively charged mucous layer. This suggestion is consistent with all data concerning the

interaction of the surface potential and the electric charge of ions in the induction of pinocytosis in *Amoebae* (Chapman-Andresen 1958, 1962).

In higher concentrations of both SDS and CTAB an interesting fact of recovery the phagocytic activity was observed. In the first minutes after transferring the animals into the medium containing detergents the animals formed no vacuoles. They started to do so after some time, simultaneously they returned to normal motile behaviour. This effect seemed to be correlated with body length and shape. Food vacuoles were never formed if the body of animals was visibly contracted, shorter than usual. The electrically induced body contraction in *Paramecium* also prevents the formation of food vacuoles (Dryl and Brutkowska unpublished). Similar effect of detergents used in high concentrations may be one more evidence of deep penetration of these compounds affecting in some cases the contractile ectoplasmic microfibrillar system and disturbing the functions of organism (Allen 1971).

However, the mechanism of the action of these two detergents on phagocytosis of *Tetrahymena* and *Paramecium* is so far unclear, and further studies are needed the elucidate the underlying causes.

RÉSUMÉ

Les effets du SDS et du CTAB sur la phagocytose chez *Tetrahymena* et chez *Paramecium* plutôt se ressemblent, sans être pourtant identiques. Les concentrations élévées sont très toxiques pur les deux espèces, mais une renormalisation des cellules touchées par leurs effets reste néanmoins possible. Dans ces concentrations la phagocytose se trouve complètement ou partiellement arrêtée ce qui semble être lié à la contraction du corps, car les vacuoles alimentaires ne sont jamais produites par un animal déformé qui perd de longueur et gagne en diamètre. La formation des vacuoles alimentaires est légèrement stimulée par les faibles concentrations du SDS ce qui peut être expliqué par une augmentation du potentiel éléctrocinétique de la membrane céllulaire due. à cette substance. Le CTAB qui porte une charge positive peut accélérer la formation des vacuoles alimentaires dans des certaines concentrations, probablement grâce à une interaction avec la couche mucoïde présente à la surface des cellules.

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Motor Response of Fragments of *Stylonychia mytilus* (O. F.M.) to Treatment with Potassium Ions

Synopsis. Investigations on the sensibility of *Stylonychia mytilus* to potassium ions have been performed on cell fragments of this ciliate. It has been proved that differences occur in chemoreceptive sensibility between the fore and back as well as between the left and right zone of *Stylonychia* cell. Differences in duration of movement reversal in particular groups of locomotive organelles show that there exists a physiological differentiation of definite zones of the *Stylonychia* cell membrane. The similarity in the duration of cirri reversal in the right component of a doublet and of the left marginal cirri of a single *Stylonychia* cell confirms previous suggestion on the derivation of the right component of a doublet from the left part of the *Stylonychia mytilus* cell.

Stylonychia mytilus (O. F. M.) possesses relatively few cirri, several groups of which may be distinguished, according to morphological and physiological differences. The resistence of the cell of this ciliate to microsurgical operations permits to isolate its fragments without destroying their movement or response to outer stimuli. This renders possible investigations of motor responses in the particular groups of locomotive organelles in *Stylonychia*. Former studies of the response of *Stylonychia* to potassium ions, carried out on whole indivividuals (Dryl and De Peyer 1970, Dryl and Totwen-Nowakowska 1975) seem to suggest that within the cell of this ciliate, the same as in *Euplotes* (Okajima and Kinosita 1966), the differences occur in physiological features of the cell membrane of body surface and cirri. This may be concluded from the differences of the duration of reversal in particular groups of locomotive organelles.

In *Stylonychia*, an initial reversal of movement of all locomotive organelles in a threshold concentration of 6–8 mM KCl, with 1 mM of $CaCl_2$ concentration in the outer medium, is followed by a gradual renormalization of movement, first appearing in the Adoral Zone of Membranelles (AZM) and in the left marginal cirri, while the frontal and right marginal cirri are still in reversal. The differences of motor responses of particular groups of cirri in *Euplotes*, according to Naitoh and Eckert (1969) are due to local

differences in concentration of calcium in the cell membrane, caused by the excitation of the cell. According to one of the latest hypotheses (Dryl 1975) the potassium reversal, accompanying the depolarization of cell membrane in ciliates, consists in displacing, by potassium, calcium ions from the sites of their adsorption in the chemoreceptive zone of the cell membrane; this displacement of Ca ions may cause conformative changes of the cell membrane and its greater permeability to external calcium ions. This process results in an increase of the calcium level in the region of structural elements responsible for the movement of cilia.

One of the reasons for carrying out investigations on fragments of *Stylonychia* cells was the fact that in an unoperated cell it was difficult to establish the direction of beat of frontal, ventral and caudal cirri and to distinguish normal motion from its reversal. Besides, those investigations aimed at excluding an interaction of groups of motor organelles and at examining any possible influence of the impairment of the pellicle on the way in which the fragments respond to stimuli. Additional investigations carried out on fragments of a doublet and particularly on its right component, the symmetry of which is inversed, aimed at getting a confirmation or a rejection of former opinions suggesting the derivation of the right component from the left part of the Stylonychia cell (Dryl and Totwen-Nowakowska 1972).

Material and Methods

Experimental material consisted of single individuals of *Stylonychia mytilus* (O. F. M.) and of its doublets formed by means of thermic shock (Totwen-Nowakowska 1965). Both forms of *Stylonychia* were cultured in Pringsheim medium and fed on *Tetrahymena pyriformis*. Single cells and doublets were cut by hand under a stereomicroscope with the use of metal and glass micro-scalpels or by putting the ciliates through a thin pipette.

The direction of movement of locomotive organelles was determined basing on the direction and character of movement of the fragments. Observations were carried out with the use of a stereomicroscope and an inversed P. Z. O. microscope. Besides, the evaluation of velocity and direction of movement of AZM and cirri was based on the observation of the movement of carmine particles suspended in Pringsheim liquid. In a series of investigations on the movement of AZM membranelles the fragments were immobilized by means of a micro-needle connected with a Zeiss micro-manipulator. The duration of the reversal was measured with a watch.

The Ca level in the Pringsheim medium used, with pH 7.3, was adjusted to 1 mM by adding an adequate quantity of $CaCl_2$. In all experiments potassium of 16 mM KCl concentration was used. This concentration was obtained by adding 0,5 ml of 32 mM KCL concentration to 0.5 ml of Pringsheim medium containing the examined fragment. The time of observation of a single fragment after adding potassium was 15 min for each experiment. KCl was added 10 min after the operation. The numbers of observations in particular experiments have been presented in Table 1.

Mean values and standard deviations of the duration of reversal in locomotive organelles
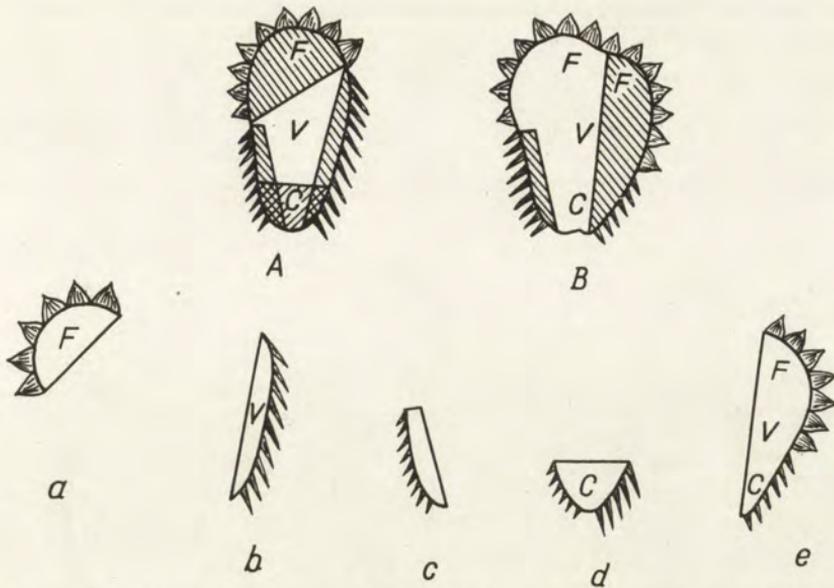
Fig. 1. Type of performed operations on *Stylonychia mytilus*. A – single form of *Stylonychia*, B – "flat" doublet of *Stylonychia*, a – front fragment of single form, b – right-side fragment of single form, c – left posterior fragment of single form and doublet, d – posterior fragment of single form, e – right-side fragment of doublet.
Letters on fragments denote groups of cirri preserved in them: F – frontal cirri, V – Ventral cirri, C – Caudal cirri

(Table 1) were calculated separately for each type of fragments if the reversal lasted less than 15 min. The duration of long reversals (over 15 min) has not been closely examined.

Operations were performed according to the scheme shown in Fig. 1. The authors applied the classification of motor organelles of *Stylonychia* according to Machemer (1965) and observed their motor responses in the following fragments of *Stylonychia*:

(a) the frontal fragment of a single form: the movement of AZM and frontal cirri was observed;

(b) the right-side fragment of a single form: the movement of right marginal cirri and ventral cirri was examined;

(c) the left-posterior fragment of a single form and a doublet: the movement of left marginal cirri was observed;

(d) the posterior fragment of a single form: the movement of caudal cirri was observed;

(e) the right-side fragment of a doublet: observation concerned the movement of: the right AZM, right frontal cirri, ventral and caudal cirri, right-side marginal cirri.

Results

Motor responses of fragments in Pringsheim medium.

Ten minutes after the operation, a period considered necessary for the reaction to the postoperative shock to be out, observations were carried out on *Stylonychia* fragments isolated on microscopic slides – or watch-glasses.

(a) Frontal fragment of a single form

Directly after the operation the fragment detached itself from the substratum and swam forwards rotating round the section plane, then it fell on the substratum and turned clockwise in one spot (Fig. 2 a^1). After fixing on
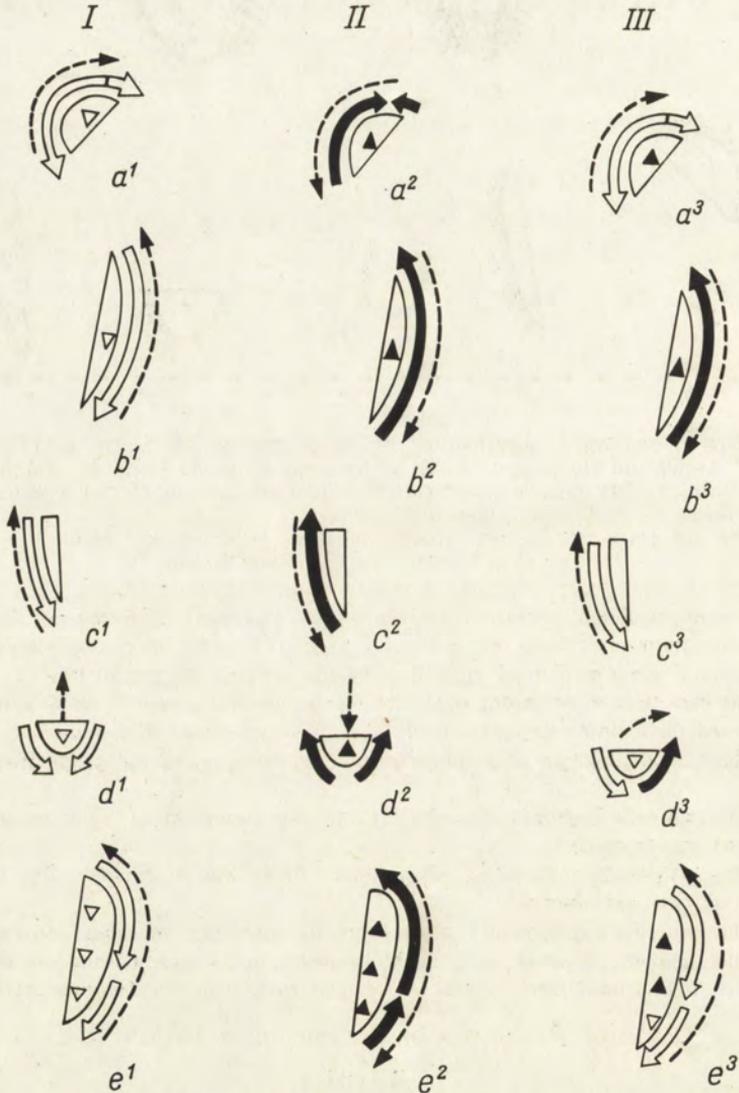
Fig. 2. Motor response of *Stylonychia mytilus* fragments: I — during normal movement (a^1, b^1, c^1, d^1, e^1), II — in the first phase of reversal caused by KCl (a^2, b^2, c^2, d^2, e^2), III — after 10 min of adaptation in KCl (a^3, b^3, c^3, d^3, e^3)

Triangular arrows – direction of movement of F. V. C. cirri. Long and broad arrows – direction of movement of marginal cirri and AZM.

White arrows – normal movement of locomotive organelles. Black arrows – reversal of movement of locomotive organelles. Narrow dotted arrows – direction of movement of fragment.

the micro-needle followed a reversal of movement of AZM membranelles which lasted some 20 s and made the fragment turn to the left; then the normal movement of AZM reappeared and the fragment turned to the right with short pauses, lasting ca 1 s each, and occurring at irregular intervals.

(b) Right-side fragment of a single form

After the operation, fragment stayed on the substratum where it moved forward turning arcwise to the left Fig. 2 *b*. During the spontaneous reversal of the cirri movement, which occurred every several seconds, the fragment withdrew along an arc turning left.

(c) Left-posterior fragment of a single form and of doublet

The fragment, which was detached from the substratum after the operation and swam forward rotating round its section plane, subsequently dropped onto the substratum. During its forward movement it described arcs to the right (Fig. 2 *c*[1]). Unfrequent spontaneous reversals of the cirri movement made the fragment move back along an arc directed to the right.

(d) Posterior fragment of a single form

After the operation the fragment stayed on the substratum. Frequent spontaneous reversals of the cirri movement caused violent backward movement of the fragment and its separation from the bottom. After some rotation in the liquid medium the fragment dropped again onto the substratum, where it stayed motionless for some seconds then moved back again, etc. This type of response lasted for 5–6 min, then the first forward movements of the fragment appeared (Fig. 2 *d*[1]). Spontaneous reversals of the cirri movement causing backward movements of the fragment on the substratum occurred less and less frequently and then forward movement returned.

(e) Right-side fragment of doublet

The fragment became detached from the substratum after the operation and swam forward and backward rotating round its section plane; then it dropped onto the substratum. Here, it moved forward along a left turning arc (Fig. 2 *e*[1]). Frequent spontaneous reversal of AZM and cirri movement caused backward movement of the fragment along a left turning arc.

Motor Responses of Fragments in 16 mM KCl

(a) Frontal fragment of a single form

The reversal of movement of AZM membranelles lasted for 100–130 s (Table 1 a) causing leftward rotations of the fragment (Fig. 2 *a*[2]). With gradual renormalization of movement of AZM membranelles the rotations of the fragment became slower until a short period of complete immobility;

Table 1

Duration of Movement Reversal of Particular Groups of Locomotive Organelles of *Stylonychia mytilus* in 16 mM KCl/1 m CaCl₂ in Pringsheim medium

Kind of Fragment	Number of Experiments	Duration of Reversal			
(a) front fragment of single form	15	AZM	70–180*	102**	29.7*** s
		FC	over 15 min		
(b) right-side fragment of single form	10	RMC	over 15 min		
		VC	,,	,,	,,
(c) left posterior fragment of single form	10	LMC	50–120	81	27.1 s
left posterior fragment of doublet	10	LMC	60–140	90	24.7 s
(d) posterior fragment of single form	15	CC	55–90	65	10.0 s
(e) right side fragment of doublet	10	AZM	60–150	110	27.7 s
		MC	90–120	104	15.0 s
		FC	over 15 min		

Denotations: FC — Frontal cirri   MC   — Marginal cirri
               VC — Ventral cirri   LMC — Left marginal cirri
               CC — Caudal cirri   AZM — Adoral Zone of Membranelles
    * Range, ** mean value, *** standard deviation

then the fragment began to move rightwards i.e., clockwise (Fig. 2 *a*³). The frontal cirri kept the reversal of movement during the whole experiment.

(b) Right-side fragment of a single form

The right-marginal cirri, as well as the few ventral cirri occurring in the fragment, kept a long-lasting reversal of movement. Owing to this the fragment moved backwards describing circles of ca. 2 mm of diameter. During the whole experiment the outline of its track was unchanged but its movement grew slower and slower (Fig. 2 *b*² *b*³) (Table 1 b).

(c) Left-posterior fragment of single form and of doublet

The reversal of movement of the left marginal cirri both of the single form and of the doublet lasted for 100–130 s (Table 1 c). The fragment, which detached itself from the substratum in the first phase and swam backward rotating round its section plane parallel to the longer axis of the cell, dropped onto the bottom and moved backward along an arc turning to the right (Fig. 2 *c*²). With gradual renormalization of the cirri movement the fragment moved more and more slowly until it stopped. After a short time of immobility it began to move forward along an arc turning to the right (Fig. 2 *c*³).

(d) Posterior fragment of a single form

The fragment performed short backward movements and detached itself from the substratum. After several rotations in the liquid medium it dropped again to the bottom and stayed there motionless for ca. 1 s, then it executed another backward movement, etc. (Fig. 2 d^2). After some 80 s (Table 1 d) the first straight forward movements appeared alternating with short rightward rotations in one spot due to the action of marginal cirri of which the left ones returned to normal movement while the right ones kept being in reversal (Fig. 2 d^3).

(e) Right-side fragment of doublet:

Owing to strong reversal of movement of AZM and cirri the fragment detached itself from the substratum at first and it swam backward rotating round the section plane. After dropping onto the bottom it performed slower and slower clockwise rotations in one spot (Fig. 2 e^2). After 100–130 s (Table 1 e) and a short time of immobility the fragment moved forward along an arc turning to the left (Fig. 2 e^3). AZM and marginal cirri returned to normal movement while frontal and ventral cirri kept being in reversal.

Discussion of Results and Conclusions

The spontaneous reversal of movement of the locomotive organelles which persisted in fragments of *Stylonychia* was stronger in the right marginal cirri and in the frontal ventral and caudal cirri than in the AZM and the left marginal cirri. Response to KCL occurred in all groups of locomotive organelles appearing as the reversal of the direction of their movement. The same as it has been established in undamaged cells (Dryl and De Peyer 1970), normal movement of AZM membranelles and of the left marginal cirri in the fragments reappeared sooner than in the frontal and right marginal. The reversal of movement of caudal cirri had a periodical character alternating with moments of complete immobility; it lasted for ca. 80 s. The duration of reversal of the marginal cirri of the right component of the doublet was approximately the same as that of its left marginal cirri as well as of the left marginal cirri of a single form of *Stylonychia*: 100–130 s.

Conclusions based on investigations of *Stylonychia* fragments are in concordance with the results of investigations of whole cells of this ciliate. They prove that there exist differences of sensibility to potassium ions between the front and the back zone as well as between the left and the right zone of the cell of *Stylonychia*.

The lack of correlation of movement of the particular groups of

organelles also proves that chemoreceptive properties of the surface of *Stylonychia* mean a physiological differentiation of some definite zones of the cell membrane. The similarity of the duration of reversal of the marginal cirri in the right component of a doublet and the left marginal cirri of the intact *Stylonychia* cell confirms previous conclusions concerning the possible derivation of the right component of a doublet from the left part of the *Stylonychia* cell.

RÉSUMÉ

On a étudié la sensitivité de *Stylonychia mytilus* aux ions de potassium sur les fragments des cellules de ce Cilié. Il a été mis en évidence qu'il existe de différences de sensitivité à la réception des stimulants chimiques entre la partie antérieure et postérieure aussi ben comme entre les zones gauche et droite de la cellule de *Stylonychia*. Les différences de durée du mouvement rebroussé dans les groupes distinctes d'organelles locomotrices montrent qu'il existe une différentiation physiologique entre les zones diverses de la membrane céllulaire de *Stylonychia*. La durée du rebroussement des cirri est pareille chez la composante droite d'un animal double et chez les cirri marginaux de l'individu simple ce qui confirme les suggéstions antérieure à savoir que la composante droite de l'animal double est originaire de la partie gauche de la cellule de *Stylonychia mytilus*.

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