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New Flagellate *Hexamastix marathwadensis* sp. n. from an Insect *Polyphaga indica*

Synopsis: A new flagellate *Hexamastix marathwadensis* sp. n. (*Mastigophora* : *Trichomonadida*) is described from the gut of an insect *Polyphaga indica* in Maharashtra, India. It measures 8.2–10.3 × 6.7–9.8 μm (average: 9.0 × 8.5 μm) and is distinguished by the presence of a slender axostyle projecting behind the body for a considerable distance, distinctly unequal flagella and a band-shaped, comma-shaped or cap-like pelta.

Flagellates of the genus *Hexamastix* were first recorded by Alexeieff (1911) from the intestine of newts and referred to the genus *Polymastix*. However, in 1912 the same author separated them and designated the genus *Hexamastix* to accommodate them. Grassé (1926) listed two more species of this genus namely *H. termitis* (Grassi, 1879) and *H. grylotalpae* Grassé, 1926. Six more species, all from termites, were added by Kirby (1930), while another species from termites was described by De Mello (1946). Geiman (1932) named a form from tipulid larvae while Bhaskar Rao (1970) recorded two more species from the cockroach and the mole cricket respectively. One species each from a blattid *Pycnoscelus surinamensis* and *Polyphaga indica* were described by Sultana (1975, 1976). Thus this genus has, at present, 12 species from insects seven of them being from termites.

Material and Methods

The flagellates described were found in the hind gut of the insect *Polyphaga indica*, during the course of a survey of the flagellate fauna of insects of Maharashtra state, India. The organisms were studied in the living condition with the help of vital stains like methylene blue and toluidene blue. Permanent preparations were made either with Giemsa's stain after fixation in methanol or by Phospho-tungstic haematoxylin following fixation in Schaudinn's fluid.

Description of *Hexamastix marathwadensis* sp. n.

The flagellate is fusiform (Fig. 1 *1, 2, 7, 11*), ovoidal (Fig. 1 *4, 6*) or somewhat rounded (Fig. 1 *3*) in shape. The cytoplasm shows several deeply staining granular and bacterial inclusions (Fig. 1 *1–5, 8, 9, 11*).

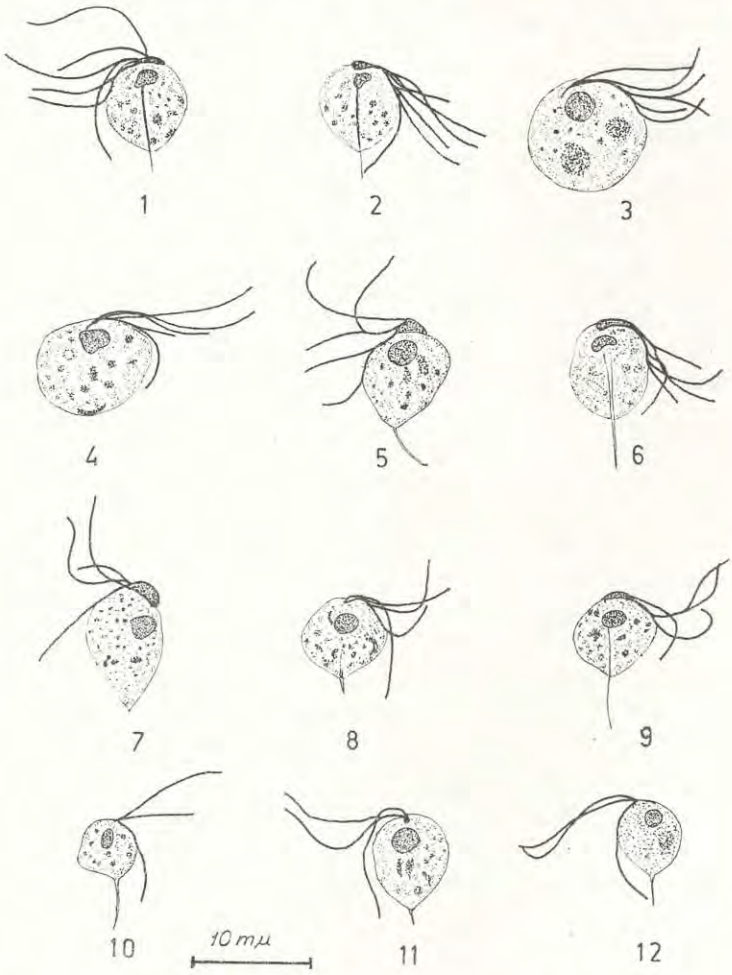

Fig. 1. *1—11. Hexamastix marathwadensis* sp. n. *1, 2* — Fusiform individuals with six flagella, band-like pelta and uniform axostyle, *3* — Rounded individual with six flagella, *4* — Ovoidal form with five flagella, *5* — Form with five flagella and cap-like pelta, *6* — Form with five flagella and comma-shaped pelta and axostyle with slightly expanded anterior end, *7* — Four flagellate form with a cap-like pelta, *8* — Four flagellate form with a short spike, *9* — Four flagellate form with a long spike and band-like pelta, *10—12* — Three flagellate forms with long spike, *11* — Three flagellate form showing blepharoplast
(All figures from smears exposed to osmic vapours, fixed in methanol and stained with Giemsa stain)

Close to the anterior end of the body is a single blepharoplast, from which arise the flagella and the axostyle. The granule is masked by the pelta in most cases but is clearly seen in some cases (Fig. 1 *11*). The fully developed individual has six flagella, of which five are forwardly or laterally directed and one backwardly directed (Fig. 1 *1, 2, 4*). All the flagella are distinctly unequal in their length, the shortest being about as long as the body and the longest about one-and-a-half times the body length. The axostyle is very slender, running through the body and projecting behind in the form of a spike. While it is of uniform thickness in most cases (Fig. 1 *1, 2, 9*), in some the anterior end appears to be slightly expanded (Fig. 1 *6*). However, there is no distinct capitulum. The posterior spike tapers sharply and is conspicuous for its length, measuring 1.0–7.2 μm with an average of 4.8 μm.

The nucleus is located a little behind the anterior end of the body. It is spherical (Fig. 1 *3, 8, 11, 12*) or somewhat transversely elongated (Fig. 1 *1, 2, 6, 9*) and measures 2.1 × 2.3 μm on the average. Lying above the nucleus is a distinct pelta. It is variable in its shape an size, being somewhat thick and cap-like (Fig. 1 *5, 7*), elongated and comma-

Table 1

Dimensions of *Hexamastix marathwadensis* sp. n. (All measurements in μm)

S. No.	Particulars	6-flagellate form	5-flagellate form	4-flagellate form	3-flagellate form
(1)	Length of the body	8.2–10.3 (9.0)	5.7–9.3 (7.9)	5.1–10.3 (7.4)	4.6–8.2 (6.3)
(2)	Maximum width of the body	6.7–9.8 (8.5)	4.1–9.8 (6.8)	3.6–7.2 (5.5)	3.1–7.2 (5.3)
(3)	Size of nucleus	1.0–2.6 × 2.1–2.6 (2.1 × 2.3)	1.0–2.6 × 1.5–3.6 (1.8 × 2.2)	1.0–2.1 × 1.5–2.6 (1.6 × 2.0)	1.0–2.6 × 1.5–2.6 (1.8 × 2.0)
(4)	Length of flagellum 1	6.7–9.3 (8.3)	6.7–10.3 (8.6)	5.1–11.3 (8.1)	5.7–12.3 (8.9)
(5)	Length of flagellum 2	8.7–12.9 (10.7)	8.2–11.3 (9.5)	7.2–12.3 (9.7)	6.7–12.3 (9.9)
(6)	Length of flagellum 3	9.8–13.4 (11.8)	8.2–15.4 (10.3)	8.2–13.4 (10.7)	7.7–13.8 (11.3)
(7)	Length of flagellum 4	10.8–13.4 (12.5)	8.7–16.5 (11.4)	10.3–16.5 (12.1)	—
(8)	Length of flagellum 5	10.8–15.4 (13.5)	9.8–16.5 (12.1)	—	—
(9)	Length of flagellum 6	11.8–15.9 (14.5)	—	—	—

shaped (Fig. 1 *6*) or thin, short and band-like (Fig. 1 *1*, *2*, *9*). Neither a cytostome nor a parabasal body was seen.

Besides the fully developed six-flagellate forms, the host harboured several developing stages of this organism. Forms with five flagella (Fig. 1 *4–6*) and four flagella (Fig. 1 *7–9*) showed the typical characters of the fully developed forms except for the flagellar number and slightly smaller size. Those with three flagella (Fig. 1 *10–12*) were distinctly smaller in size and also lacked the pelta, besides having the axostyle almost inconspicuous but for the long projecting spike. The dimensions of the flagellate in its various forms is shown in Table 1.

Discussion

This flagellate is recorded from the same host and locality as *H. polyphagae* Sultana, 1976. However, it is easily marked off from that species by the presence of a distinct pelta which is absent there. Also while that species has six flagella which are almost equal or subequal and about as long as body, this species has distinctly unequal flagella, many of which are longer than the body. The posterior spike of the axostyle is also much longer here averaging 4.8 μm as against 3.0 μm.

In possessing a pelta this species comes close to *H. singhi* Bhaskar Rao, 1970 and *H. alexeieffi* Sultana, 1975, but is distinguished from them by other characters. *H. singhi* has a well developed hyaline and twisted axostyle with a broad spoon-shaped calyx and six flagella which are more or less equal in length, as against the slender axostyle devoid of calyx and clearly unequal flagella here. There is single granule in the species described here as against two distinct granules in the blepharoplastic complex in *H. alexeieffi*, besides the presence of a delicate and straight axostyle with a long spike, compared with a twisted and better developed axostyle with a shorter spike in *H. alexeieffi*.

From the rest of the species, it differs in the presence of a clear pelta. Further, the presence of a delicate axostyle marks it off from *H. termitis* Grassi, 1879, *H. claviger* Kirby, 1930, *H. conclaviger* Kirby, 1930, *H. disclaviger* Kirby, 1930 and *H. periplanetae* Bhaskar Rao, 1970. The presence of six flagella all of which are unequal distinguish it from *H. termitis*, *H. termopsidis* Kirby, 1930, *H. laticeps* Kirby, 1930, *H. tipulae* Geiman, 1932 and *H. singhi*. In view of these differences and its distinctness from all the species hitherto described, the organism is designated *Hexamastix marathwadensis* sp. n., after the region where it was found.

Species: *Hexamastix marathwadensis* sp. n.
Host: *Polyphaga indica*
Habitat: Hind gut
Locality: Aurangabad, Maharashtra, India.

The type slides are deposited in the Protozoology Laboratory, Department of Zoology, Marathwada University, Aurangabad 431002, India.

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RÉSUMÉ

Une nouvelle flagellée est décrite: *Hexamastix marathwadensis* sp. n. (*Mastigophora* : *Trichomonadida*), provenant de l'intestin de l'insecte *Polyphaga indica* de Maharashtra aux Indes. Elle mésure 8.2–10.3 × 6.7–9.8 μm (9.0 × 8.5 μm en moyenne) est se distingue par la présence d'un axostyle mince qui s'étend à une distance considérable derrière le corps, par les flagelles clairement inégales, et par les pelta en forme de ruban, de virgule ou de calotte.

REFERENCES

Alexeieff A. 1911: Notes sur les flagelles I et II. Archs. Zool. exp. gén., 6, 491–529.

Alexeieff A. 1912: Sur quelque noms de genres des flagelles qui doivent disparaitre de la nomenclature pour cause de synonymie on pour toute autre raison. Diagnoses de quelques genres recomment etudies. Zool. Anz., 39, 674–680.

Bhaskar Rao T. 1970: An account of the genus *Hexamastix* Alexeieff, 1912 from insects of India. Proc. Indiana Acad. Sci., 71, 13–18.

Geiman Q. M. 1932: The intestinal protozoa of the larvae of the crane fly *Tipula abdominalis*. J. Parasit., 19, 173.

Grasse P. P. 1926: Contribution a l'etude des flagelles parasites. Archs. Zool. exp. gén., 65, 345–602.

Grassi B. 1879: Dei protozoi parassiti e specialmente di quelli che sono nell 'uomo'. Gaz. Med. Ital. Lombardi, 39, 445–448.

Kirby H. 1930: Trichomonad flagellates from termites. I. *Tricercomitus* gen. nov. and *Hexamastix* Alexeieff. Univ. Calif. Publ. Zool., 33, 393–444.

Mello de, F. I. 1946: Further studies on the intestinal protozoa of Indian *Cryptotermes*. An. Inst. Med. Trop., 3, 53–80.

Sultana T. 1975: The morphology of *Hexamastix alexeieffi* sp. n. a new flagellate from the gut of a blattid *Pycnoscelus surinamensis* (Linnaeus). Marathwada Univ. J. Sci., 14, 223–227.

Sultana T. 1976: On a new species of *Hexamastix* Alexeieff, 1912 from the gut contents of an insect *Polyphaga indica* in India. Riv. Parassit., (in press).

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New Flagellate *Hypotrichomonas venkataramiahii* sp. n. from the Gut of *Varanus* sp. from Warangal, A. P., India

Synopsis. A new flagellate *Hypotrichomonas venkatramiahii* is described from the rectal contents of the *Varanus indica*. It is characterized by the presence of crescent shaped pelta, robustus axostyle and the tailing flagellum with 2–3 feebly developed undulations, ending in a conspicuous and distinct acroneme.

The genus *Hypotrichomonas* was established by Lee (1960) for a flagellate found in the intestine of reptiles, which had an undulating membrane and without a costa. This flagellate was first described as *Trichomonas* sp. by Das Gupta (1936) in a Culture from the intestinal contents of *Boa*. In 1951 Moskowitz gave the detailed description of this organism from various reptiles under the name *Trichomonas acosta*. Lee (1960) added some details to Moskowitz's organism and created a new genus *Hypotrichomonas* under the family *Monocercomonadidae* Kirby (1944). Later, in 1963 Honigberg made a new sub-family *Hypotrichomonadinae* included the above species.

Material and Methods

These parasites were observed in the intestinal contents of *Varanus* lizard from the suburbs of Warangal. The incidence of infection was heavily associated with *Monocercomonas*. In living condition the parasites were elongated with a characteristic jerky movement. It resembles mostly with *Monocercomonas*.

pH of the alimentary tract was recorded and pH in stomach it was — 7, in duodenum — 8 in intestine — 9 and in rectum — 9. The infection was heavy in the intestinal and rectal contents, but in stomach and duodenum parasites were not seen. Duodenum was slightly alkaline while the intestine and rectum were of more alkaline in nature. The above data elucidates that the parasites can survive better in more alkaline media (pH 9) rather than in less alkaline (pH 8) or acidic.

To study the parasite in living condition. 0.85% normal saline was used. For permanent preparations methanol and Schaudinns fluid were used as fixatives and later on Giemsa stain for former and hematoxylin stain for the later were used. All the drawings were made with the aid of Camera lucida at a magnification of 2200 ×.

Description of *Hypotrichomonas venkataramiahii* sp. n.

In stained preparation the parasite is elongated (Fig. 1 *1*, *2*, *3*) or pyriform (Fig. 1 *4*, *5*) with anterior and posterior ends blunt and round. There is a clear district knotch at the anterior end from where the mastigont elements arise (Fig. 1 *2*).

The nucleus is elongated somewhat triangular in shape (Fig. 1 *3*). The anterior end is round while the posterior end is pointed. A clear spherical or elongated endosome situated in the centre of the nucleus (Fig. 1 *3*, *6*).

The axostyle is robustus distinguished into three parts capitulum, stem and spike. The stem is slightly narrower towards posterior region

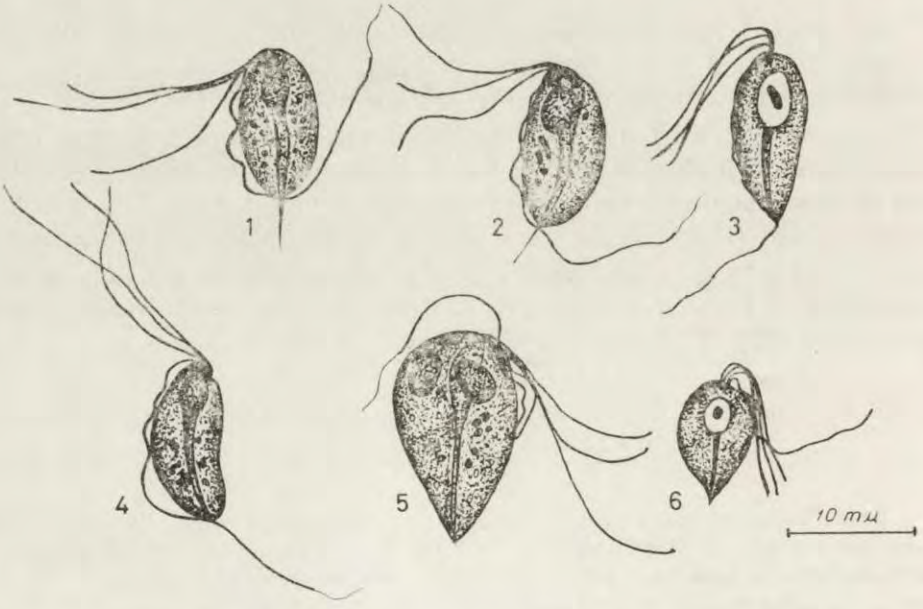

Fig. 1–6. *Hypotrichomonas venkatramiahii* sp. n. *1–2* Elongated form showing crescentic shaped pelta and an acroneme in the trailing flagellum. *3* — Showing triangular nucleus with an elongated endosome. *4* — Pyriform showing an axostyle projecting out. *5* — Divisional form showing two nuclei and a double set of flagella. *6* — Showing spherical endosome in the nucleus. Fig. *1*, *2*, *4* and *5* from smears fixed in methyl alcohol and stained with Giemsa's stain. Fig. *3* and *6* from smears fixed in schaudinn's fluid and stained with haematoxyline

Table 1

The dimensions of 75 individuals *Hypotrichomonas venkatramiahii* sp. n.

	Min. in μm	Max in μm	Average in μm
Body length	7.5	13.63	11.79
Body breadth	5.22	9.31	7.23
Nucleus length	2.72	4.31	3.51
Nucleus breadth	1.81	3.63	2.53
Axostyle	6.81	14.77	11.03
Trailing flagellum	18.87	26.13	24.06
Acroneme	1.13	5.56	2.27
Anterior flagella 1	11.22	16.59	14.75
Anterior flagella 2	11.81	18.18	16.14
Anterior flagella 3	13.4	19.5	17.8

and leads to a fine spike which projects outside the body (Fig. 1 *1*, *2*). The anterior part of the capitulum is in continuation with a crescent shaped 'pelta' (Fig. 1 *1*, *2*).

There is a single blepharoplast situated in the anterior end just above the nucleus. Three unequal flagella arise from the blepharoplast. One is always longer and the other is shorter and the third one is of moderate size.

The undulating membrane originates from the blepharoplast and travels towards the posterior end. It is thrown into 2–3 feebly developed folds and continues posteriorly as a trailing flagellum (Fig. 1 *1–6*). It always ends with a prominent acroneme measuring 1.13–5.56 μm (Fig. 1 *1–5*).

Discussion

So far four species, three from reptiles and one from bird, have been reported from the genus *Hypotrichomonas*, which was created by Lee (1960). *H. acosta* was described by Moskowitz (1951) and redescribed by Lee (1960) from squmate host. *H. osmaniae* from *Varanus indicus* was described by Krishnamurthy (1967). The third species *H. avium* described by Susan Navarathnam (1970) from the bird *Cryptoplectron erythryorhynchum*. The fourth species *H. hemidactyli* from *Hemidactylus* sp. by Bhaskar Rao et al., 1975. The present species in the fifth species of the genus *Hypotrichomonas* described from a reptile *Varanus* sp. from Warangal region.

H. acosta is ovoidal or spherical in shape and measures 4.00–17.00 μm × 2.50–15.00 μm, while the present species is elongated or pyriform with a distinct knotch at the anterior end measuring 7.5–13.63 μm × × 9.31–5.22 μm. The anterior flagella are longer than the known species. Axostyle is straight, without twists projects as a fine spike in the new species. Pelta is present in new form while absent in *H. acosta*.

H. osmaniae is spherical or fusiform, measures 7.20–13.88 μm × × 4.11–12.85 μm whereas the new form is elongated and differs in size. The anterior flagella are unequal in length in known species and also in new species, but new species differs in the length of flagella from the *H. osmaniae*. The known species has a tube like axostyle and straight with uniform thickness while the new form can be distinguished into three distinct parts. The undulating membrane in known form is having 1 or 2 folds while the new form has 2–3 folds with acronematic — trailing flagellum. The new form has a round or elongated nucleus which is larger than the neither to known species.

The new species differs from the *H. avium* which is spherical or pear shaped body measuring (4.5–9.5 μm) 6.4 μm × (3.5–7.5 μm) 5.5 μm. The known species possesses clear cytostome while in the new form it is absent. Axostyle is tubular without capitulum always curved in the cytoplasm, and no pelta, while in the new form it is conspicuous, and differentiated in three parts. Undulating membrane is feebly developed in known-species while in new form it has 2–3 folds and trailing flagellum posesses acroneme.

H. hemidactyli is round in shape measures (10.9–5.9) 8.0 × (10.9–5.2) 7.8 μm which differs from the new species. Axostyle in known form is uniform, tubular while in new species it is having three distinct parts. Acroneme to the trailing flagellum is absent in known species, while in new form it is more prominent. There are two clear speces in the known species while in new form they are absent.

In view of these characters this species is considered to be new to science and designed as *Hypotrichomonas venkataramiahii* sp. n. after Prof. K. Venkata Ramaiah, Vice-Chancellor of Kakatiya University, Warangal, A. P., India.

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RÉSUMÉ

Une nouvelle flagellée est décrite: *Hypotrichomonas venkataramiahii* provenant du contenu du rectum de *Varanus indica*. Elle est caractérisée par la présence de pelta en forme de croissant, d'un axostyle robuste et d'une flagelle trainante qui est équipée de 2–3 ondulations faiblement développés et terminée par un acronème prominent et distinct.

REFERENCES

Geiman Q. M. and Wichterman R. J. 1937: Intestinal protozoa from Galapagos Tortoises (with description of three sp. n.). Parasitology, 23, 331.

Lee J. J. 1960: *Hypotrichomonas acosta* (Moskowitz) gen. nov. from reptiles 1. Structure and division. J. Protozool., 7, 393.

Moskowitz N. 1951: Observations on some intestinal flagellates from reptilian host (*Squamata*). J. Morphol., 89, 257–309.

Honigber B. M. 1963: Evolutionary and systematic relationships in the flagellate order *Trichomonadida* Kirby. J. Protozool., 10, 20.

Krishnamurthy R. 1967: *Hypotrichomonas osmaniae* sp. n. from a Varanid lizard. Current Sci., 21, 553.

Navarathnam S. E. 1970: *Hypotrichomonas avium* from the bird *Cryptoplectron erythrorhynchum*. Rev. Parassit., 31.

Bhaskar Rao T. et al., 1976: On a sp. n. of the genus *Hypotrichomonas* Lee, 1960 (Sub-family *Hypotrichomonadinae*) from *Hemidactylus* sp. 251 Current Sci., 45, 263–264.

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Morphologie der Zooide und Schwärmer von *Heteropolaria colisarum* gen. nov., spec. nov. (*Ciliata, Peritrichida*), einer symphorionten *Epistylidae* von *Colisa fasciata* (*Anabantoidei, Belontiidae*)

Synopsis. Es wurde die Morphologie und Ökologie der Zooide und Schwärmer von *Heteropolaria colisarum* spec. nov., einer symphorionten *Epistylidae* von *Colisa fasciata*, untersucht. Das Myonemsystem und der Oralapparat wurden mit Hilfe von Protargolversilberungen analysiert. Die biometrischen Daten des Silberliniensystems werden angegeben. Die Lebendbeobachtungen und die Analyse von Protargol- und Silbernitratpräparaten zeigten klar, daß die Zooide einen epistyliformen Bauplan besitzen. Die Schwärmer sind dagegen horizontal polarisiert, ähnlich wie jene von *Heteropolaria horizontalis* Chatton (1936) und *Heteropolaria lwoffi* Fauré-Fremiet (1943). Dieses Merkmal veranlaßte uns, jene drei Arten aus der Gattung *Epistylis* Ehrenberg (1838) herauszunehmen und in einer neuen Gattung, *Heteropolaria* gen. nov., zu vereinigen. Dieser Schritt wird ausführlich diskutiert. Danach sehen wir im Genus *Heteropolaria Epistylidae*, die von *Peritrichida* des Typus *Platycola* abstammen könnten.

Einige Beobachtungen über die Konjugation und Morphogenese von *Heteropolaria colisarum* werden mitgeteilt. Während der Morphogenese ist das Myonemsystem nicht mehr nachweisbar.

Nach Scheubel (1973) sind bisher nur drei *Epistylidae* bekannt, die auf den Kiemen und der Körperoberfläche von Fischen und Amphibienlarven leben: *Epistylis lwoffi* Fauré-Fremiet, 1943, *Epistylis apiosomae* Scheubel, 1973 und *Epistylis phoxini* Scheubel, 1973. Wir fanden nun auf der Körperoberfläche mehrerer Exemplare von *Colisa fasciata* eine neue sehr interessante *Epistylidae*, die hier bereits makroskopisch sichtbare bäumchenartige Überzüge bildet. Wegen der besonderen heteropolaren Schwärmerbildung, ein phylogenetisch sehr schwierig zu deutendes Merkmal, das auch bei zwei anderen Arten der Gattung *Epistylis*

Ehrenberg, 1838 beobachtet worden ist, schien es uns notwendig, solche *Epistylidae* in einer eigenen Gattung, *Heteropolaria* gen. nov., zusammenzufassen.

Untersuchungsmethoden

Um ein möglichst vollständiges Bild über die Morphologie dieser neuen *Epistylidae* zu bekommen, wurden neben einer gründlichen Lebendbeobachtung verschiedene Silberimprägnationsverfahren angewendet. Die Darstellung des Silberliniensystems erfolgte mit einer trockenen Versilberungsmethode (Foissner 1976), die der Oralstrukturen und des Myonemsystems mit der Protargolmethode nach Wilbert (1975). Es wurde allerdings nicht mit Natriumhypochlorit gebleicht, sondern mit 0.5%-iger Kaliumpermanganatlösung (10 Minuten) und 5.0%-iger Oxalsäurelösung (s. Zagon et al. 1970). Zur Darstellung des Kernapparates diente Orcein-Essigsäure.

Morphologie der Zooide

Körperform: Ein sehr auffälliges Merkmal dieser 180–260 µm langen und 30–50 µm breiten *Epistylidae* ist die fast rechteckige Körperform der völling gestreckten Zooide (Abb.1, Taf. I 1,2, Taf. II 9), die an der Stielbasis etwa halb so breit wie beim Peristomkragen sind. Längere Zeit in Kultur gehaltene Individuen verkleinern sich auf 130–180 µm und werden etwas breiter. Diese auffällige Variabilität der Länge und Breite mag zum Teil darin begründet sein, daß auch Zooide gemessen worden sind, die nicht vollständig gestreckt waren, da es bei dieser Species schwer zu entscheiden ist, ob die Zooide völlig gestreckt sind oder nicht. Bei der Kontraktion bilden sich nämlich in der ganzen Länge des Tieres sehr viele Falten, die aber so dicht hintereinander liegen, daß der Umriß der kontrahierten Zooide fast glatt erscheint (Abb. 1, Taf. I 1). Die Länge der völlig kontrahierten Zooide beträgt 95–120 µm. Eine Überwallung des Stieles bei der Kontraktion, wie sie bei anderen großen Epistyliden beobachtet worden ist (Matthes et al. 1970), tritt nicht ein.

Oralapparat: Die Zooide sind im Bereich des ziemlich schmalen Peristomkragens merkbar verbreitert. Der kleine Peristomdiskus ist beim strudelnden Tier deutlich über den Peristomkragen herausgehoben und besitzt in seiner Mitte eine stets deutlich sichtbare zapfenartige Aufwölbung ("Nabelung") (Abb. 1). Die kontraktile Vakuole liegt an der rechten Wand des Vestibulums, etwa in der Höhe des Peristomkragens. Die Bewimperung gleicht der anderer *Epistylidae* (vgl. Lom 1964, Foissner 1977) (Abb. 3): Haplo- und Polykinete beschreiben am

Peristomdiskus etwa $1^1/_2$ Umdrehungen (etwa 560°), bevor sie in das steil nach dorsal gerichtete Vestibulum hinabsteigen. Dort zweigt sich die Polykinete in einen 1. und 2. Peniculus auf, von der Haplokinete zweigt sich die germinale Kinete ab. Das Vestibulum geht in den großen, nun wieder nach ventral gerichteten Cytopharynx über, in dessen unterem Abschnitt sich von der Polykinete der 3. Peniculus abzweigt. Der zweite Peniculus endet etwa 10µm vor dem ersten und dritten, erreicht also das Cytostom nicht. Die Haplokinete wird im Gebiet des Cytopharynx von einer kräftig imprägnierbaren Struktur begleitet. Haplo- und Polykinete beschreiben im Vestibulum und Cytopharynx $1^3/_4$ Umgänge, bevor sie beim Cytostom enden.

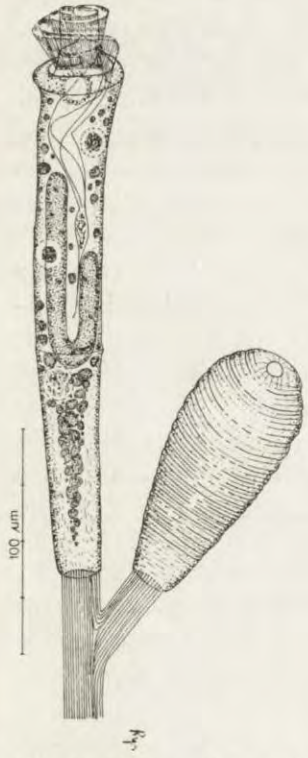


Abb. 1. *Heteropolaria colisarum* gen. nov., sp. nov. Typische Form eines schlanken Zooids im gestreckten und kontrahierten Zustand

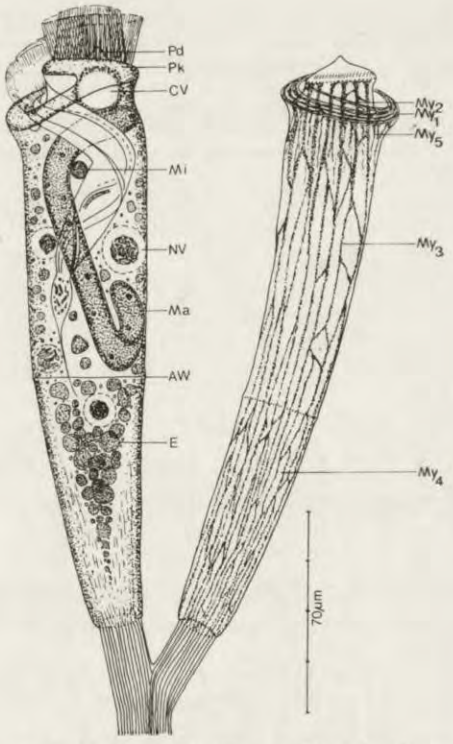


Abb. 2. Typische Form eines breiteren Zooids. Beim rechten Tier ist nur das nach Protargolimprägnation sichtbare Myonemsystem eingezeichnet worden. AW — Anlage des aboralen Wimperkranzes, CV — kontraktile Vakuole, E — cytoplasmatische Einschlüsse, Ma — Makronucleus, Mi — Mikronucleus, My_{1-5} — Teile des Myonemsystems, NV — Nahrungsvakuole, Pd — Peristomdiskus, Pk — Peristomkragen

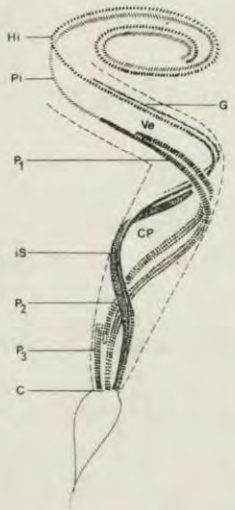


Abb. 3. Oralstrukturen nach Protargolimprägnation. C — Cytostom, Cp — Cytopharynx, G — germinale Kinete, Hi — Haplokinete, iS — imprägnierbare Struktur, Pi — Polykinete, P_1–P_3 — 1.–3. Peniculus, Ve — Vestibulum

Heteropolaria colisarum unterscheidet sich also hinsichtlich des Oralapparates ganz deutlich von *H. lwoffi*, bei der der 2. Peniculus bis zum Cytostom reicht (s. Lom 1966). Auch sonst ist eine Identität dieser Art mit *H. lwoffi* nicht anzunehmen, selbst unter Einbeziehung der großen Neigung von *H. lwoffi* sehr unterschiedliche Ökoformen auszubilden (Lom et al. 1961).

Kernapparat: Der Makronucleus ist etwa 130 µm lang, 10–12 µm breit und liegt stets in der vorderen Hälfte des Tieres. Seine Form ist auch beim völlig gestreckten Zooid ziemlich wechselnd, indem meist ein Ende hakenförmig gekrümmt ist, wobei die Krümmung aber an jeder beliebigen Stelle auftreten kann, so daß auch U-förmige Kerne vorkommen (Abb. 1,2). Beim kontrahierten Tier ist seine Form mannigfaltig gekrümmt und verschlungen (Taf. II 10). Er enthält stets ziemlich viele 1–3 µm große Nucleolen. Der 4–5 µm große Mikronucleus liegt nahe des Makronucleus (Taf. II 10).

Stiel, Scopula, aboraler Wimperkranz: Der dichotom verzweigte Stiel ist 15–20 µm breit und wird bis 2000 µm lang. Er besteht aus vielen deutlich erkennbaren Fibrillen, die sein gesamtes Inneres ausfüllen (Taf. I 8). An seiner Oberfläche war er häufig dicht mit Bakterien besetzt, besonders im basalen Abschnitt (Taf. I 1). An der Epidermis des Wirtes ist er durch eine haftscheibenartige Verbreiterung befestigt. Die Zooide sitzen am Ende Aufzweigungen in bündelförmigen Aggregaten (Taf. II 9).

Die Scopula ist von einem Kranz kleiner, stark argyrophiler Körnchen umgeben. Die Ansatzstellen der Stielfibrillen erscheinen ebenfalls als kleine argyrophile Körnchen, die durch kleine Silberlinien verbunden werden, so daß ein feines Netzwerk entsteht (Taf. I 7).

Die Anlage des aboralen Wimperkranzes ist auch beim lebenden Tier an einer leichten Verbreiterung des Körpers erkennbar (Abb. 2). Nach trockener Silberimprägnation finden sich hier zwei sehr nahe nebeneinanderliegende stark argyrophile Silberlinien. In den Protargolpräparaten zeigen sich dagegen viele etwa 1 µm lange, schräggestellte, argyrophile Stäbchen, von denen nicht festgestellt werden konnte, ob es Basalkörper sind oder nicht (Taf. I 6).

Myonemsystem: Das Myonemsystem von *Heteropolaria colisa-*

rum besteht aus zwei beinahe vollständig isolierten Teilen (Abb. 2). Der erste Teil des Systems, der offensichtlich der Kontraktion und Retraktion des Peristomkragens dient, ist ein die ganze Breite des Peristomkragens einnehmendes, dicht unter der Pellicula verlaufendes Myonemband, das sich aus vier bis fünf dicht nebeneinanderliegenden Myonemsträngen aufbaut (Abb. 2 My_1). Von diesem Myonemband zweigen viele kleine, schwach imprägnierbare Myoneme ab, die hauptsächlich in die lippenartige Vorwölbung des Vestibulums ziehen (Abb. 2 My_5, Taf. III 13) und sich dann mit dem zweiten Teil des Myonemsystems (My_3) vereinigen. Von den Myonemen des Peristomkragens zweigt sich außerdem am Eingang zum Vestibulum ein mäßig dicker, sich verjüngender, im Uhrzeigersinn verlaufender Myonemstrang ab und zieht zur Mitte des Peristomdiskus, wo er sich manchmal etwas auffasert (Abb. 2 My_2, Taf. III 16). Der Peristomdiskus ist aber ansonsten frei von Myonemen. Dieses besondere Myonemband ermöglicht vermutlich eine koordinierte Kontraktion und Retraktion von Peristomkragen und Peristomdiskus. Der zweite Teil des Myonemsystems besteht aus den meridional dicht unter der Pellicula verlaufenden Körpermyonemen, die bei der Scopula beginnen (Abb. 2 My_4), unter der Anlage des aboralen Wimperkranzes hindurchziehen (Taf. I 6) und bei der Haplokinete enden (Abb. 2 My_3, Taf. III 12, 14). Unmittelbar unterhalb der Haplokinete sind die Myoneme, die eine fasrig-körnige Struktur aufweisen, am stärksten ausgebildet (Taf. III 12). Am Eingang zum Vestibulum sind sie torbogenartig ausgespart (Taf. III 12); hier verlaufen ja die oben erwähnten sehr feinen, vom Peristomkragen abzweigenden Myoneme. In dem Bereich vom aboralen Wimperkranz bis zur Scopula werden die Myoneme durch ausgeprägte Anastomosierung so zahlreich, daß fast eine zusammenhängende plattenförmige Schicht entsteht (Taf. I 8). Dieser zweite Teil des Myonemsystems dient offensichtlich der Retraktion und Kontraktion des Körpers und des Peristomdiskus, da ja die Myoneme direkt bei der Haplokinete beginnen und dort auch sehr kräftig ausgebildet sind.

Pellicula, Cytoplasma: Die Pellicula ist derb (etwa 0.6 µm dick) und so fein gestreift, daß der Rand der Tiere glatt erscheint (Taf. I 1, Taf. II 9). Dicht unter der Pellicula liegen in einer Zone von sehr zähem Ektoplasma die etwa 1 µm großen Mitochondrien und darunter die Myoneme.

Das Cytoplasma ist von leicht gelblichen Granula erfüllt, die besonders in der Umgebung der kontraktilen Vakuole gehäuft auftreten. Neben den 10–15 µm großen Nahrungsvakuolen, die im Cytoplasma eine deutliche Cyclose zeigen, enthalten gut ernährte Zooide noch viele schollenartige, stark lichtbrechende 2–8 µm große Einschlüsse, die besonders

im Basalteil der Zooide gehäuft auftreten. Die trichterförmige Anordnung dieser Einschlüsse (Abb. 1, 2, Taf. I 1, 2, Taf. II 9) ist sehr auffällig und typisch für gut ernährte Individuen. Bei schlecht ernährten Zooiden oder solchen, die sich zu Schwärmern umbilden, sind diese Einschlüsse nicht feststellbar. Dagegen sind sie bei sich teilenden Tieren noch vorhanden. Sie werden auch dann über die Cytopyge entfernt, wenn die Tiere längere Zeit unter dem Deckglas beobachtet und etwas gequetscht worden sind.

Silberliniensystem: Das Silberliniensystem ist ein typisches Engstreifensystem und gleicht dem anderer *Epistylidae* (vgl. Foissner 1977, Foissner et al. 1974). Die Silberlinien verlaufen in konzentrischen Kreisen um das Tier und liegen sehr nahe nebeneinander, da es bei der Präparation häufig zu einer starken Kontraktion der Zooide kommt (Taf. I 4). Zwischen, an und auf den Silberlinien, die sich manchmal auch gabelförmig aufzweigen oder blind in der Pellicula enden, d. h. keinen vollständigen Kreis bilden, liegen die Pelliculaporen (Taf. I 4). Infolge der eigenartigen Kontraktionsweise (s. oben) und des sehr geringen Abstandes der Silberlinien (s. unten) kommt es sowohl in den trockenen Silberpräparaten als auch in den Protargolpräparaten zu einer uns bisher unbekannt gewesenen Erscheinung: Die Silberlinien kommen in den gefalteten Pelliculateilen so nahe nebeneinander zu liegen, daß mehrere Silberlinien als *eine* etwas dickere Silberlinie erscheinen (Taf. I 5). Auf dieses Phänomenon, das wir als *Pseudostreifung* bezeichnen, ist bei der Untersuchung des Silberliniensystems und der Bestimmung der biometrischen Daten unbedingt zu achten. Für die biometrische Analyse der argyrophilen Strukturen dürfen nur solche Tiere verwendet werden, die wenig kontrahiert sind und daher keine Pseudostreifung zeigen.

Biometrische Charakteristik: Für die Errechnung und Bestimmung der unten angeführten Werte s. Foissner et al. (1974). Anzahl der untersuchten Individuen: 15. Länge der lebenden Tiere in µm: 180–260. Silberliniensystemtyp: Engstreifensystem (EST). Anzahl der Silberlinien vom Oralapparat (0) bis zum aboralen Wimperkranz (W) (Extremwerte): 180–206. Anzahl der Silberlinien vom 0 bis zum W (Mittelwert): 194.0. Anzahl der Silberlinien vom W bis zur Scopula (S) (Extremwerte): 123–150. Anzahl der Silberlinien vom W bis zur S (Mittelwert): 130.3. Abstand der Silberlinien in µm: 0.1–0.7. Errechneter Abstand der Silberlinien in µm: 0.7. Durchschnittliche Gesamtanzahl der Silberlinien (DGS): 324.3. Anzahl der Pelliculaporen pro 100 µm² (Extremwerte): 13–25. Anzahl der Pelliculaporen pro 100 µm² (Mittelwert): 16.6.

An den biometrischen Daten ist vor allem die große Anzahl der Silberlinien (DGS) auffällig, wie sie bisher bei anderen *Peritrichida* nicht festgestellt worden ist. Dazu muß allerdings bemerkt werden, daß andere große *Epistylidae*, z. B. *Epistylis galea*, bisher nicht auf ihre DGS untersucht worden sind.

Locus typicus und Ökologie: *Heteropolaria colisarum* wurde zweimal auf mehreren Exemplaren von *Colisa fasciata* festgestellt, wo sie vorwiegend unterhalb des Maules, oberhalb der Augen und an den Spitzen der Flossen bis 1 cm große weißlich-graue Kolonien bildete. In der Aquaristik wird diese Fischart als *Colisa labiosa* bezeichnet, wobei aber sehr strittig ist, ob sie nicht mit *Colisa fasciata* synonym ist. Die Flossenformel unserer Exemplare betrug: D XV–XVIII/8–10, A XVI–XVIII/17-20, mLR 29-31. Die Fische stammten sicher von Nachzuchten aus Ostasien, das Herkunftsland war aber nicht zu erfahren.

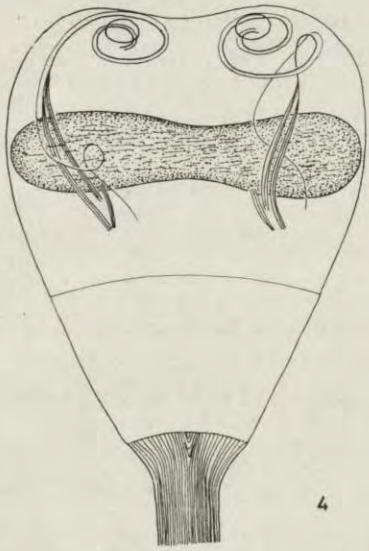
Nach vorsichtiger Entfernung der Kolonien, zwischen deren Stielen sich massenhaft Bakterien und *Tetrahymena sp.* befanden, wurde eine weißlich-rötliche Veränderung der Epidermis festgestellt. Diese Schädigung muß aber nicht zwangsläufig durch *H. colisarum* hervorgerufen worden sein, da derzeit die Frage nicht zu beantworten ist, ob primär (a) eine bakterielle Infektion vorlag und sich *H. colisarum* nur sekundär auf der geschädigten Hautstelle ansiedelte, oder (b) es umgekehrt war oder (c) beide Infektionen sekundär waren und auf eine andere, vielleicht mechanische Noxe zurückgehen.

Vom Wirt mitsamt den Schuppen herabgelöste Kolonien von *H. colisarum* konnten tagelang im Blockschälchen am Leben erhalten werden. Fast alle Tiere gingen jedoch im Verlaufe der Zeit in das Schwärmstadium über und setzten sich dann auf den Boden des Blockschälchens fest. Eine Vermehrung wurde aber nie beobachtet. Ebenso versuchten wir vergeblich, die Tiere zur Cystenbildung zu bringen (vgl. Lom 1966 bei *H. lwoffi*).

Morphologie der Schwärmer

Die ersten Anzeichen der Schwärmerbildung sind eine merkbare Kontraktion des Zooiden, die Ausbildung einer Einschnürung bei der Anlage des aboralen Wimperkranzes, eine Vergrößerung und erhöhte Pulsationsfrequenz der kontraktilen Vakuole und die Defäkation der cytoplasmatischen Einschlüsse, wodurch die Tiere ziemlich durchsichtig werden (Abb. 6). Das Anfangsstadium der Schwärmerbildung gleicht also im wesentlichen dem vieler anderer *Peritrichida*.

Im mittleren Stadium der Schwärmerbildung (Abb. 7) setzt nun



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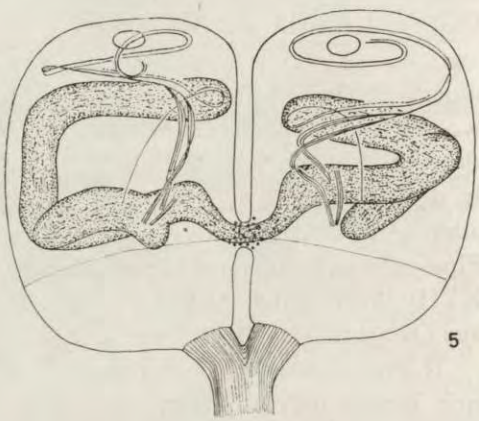

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Abb. 4, 5. Mittleres und spätes Teilungsstadium. Der Makronucleus zeigt fädige Strukturen. Das Myonemsystem ist nicht mehr nachweisbar. Protargolimprägnation

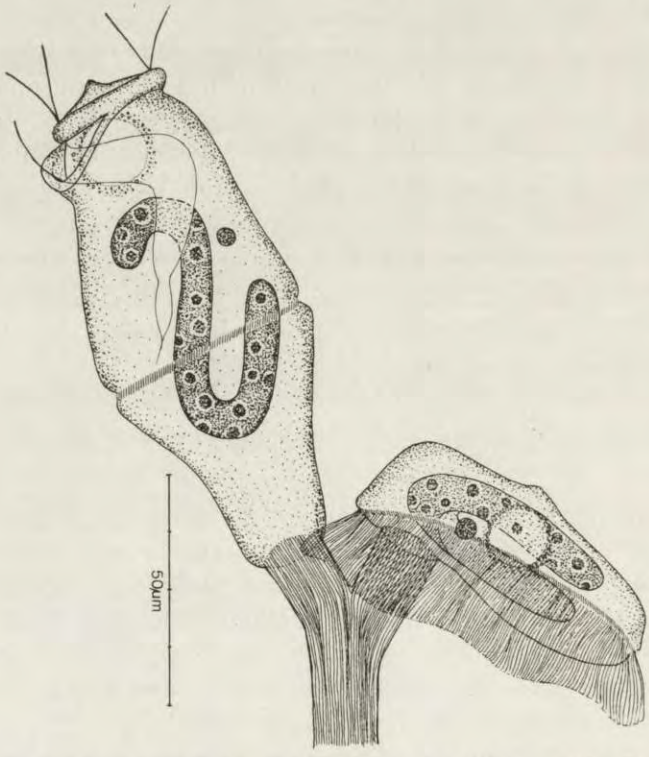

Abb. 6. Frühes und spätes Stadium der Schwärmerbildung. Die Scopula wird an den Rand des Schwärmers verlagert. Nach Lebendbeobachtungen

ein eigenartiger, bisher nur von wenigen Peritrichida bekannter Prozeß ein, der dazu führt, daß die Scopula ihre zentrale Lage verliert und immer mehr an den Rand des Schwärmers verlagert wird. Der aborale Wimperkranz, der mittlerweile schon Cilien trägt, die aus sehr vielen, eng nebeneinanderliegenden, schräg gestellten Basalkörperreihen entspringen, verschiebt sich nämlich dorsal in Richtung des Peristomkragens, so wie dies K r a l i k (1961) bei den Schwärmern von *Platycola truncata* beschrieben hat. Während dieses Vorganges kon-

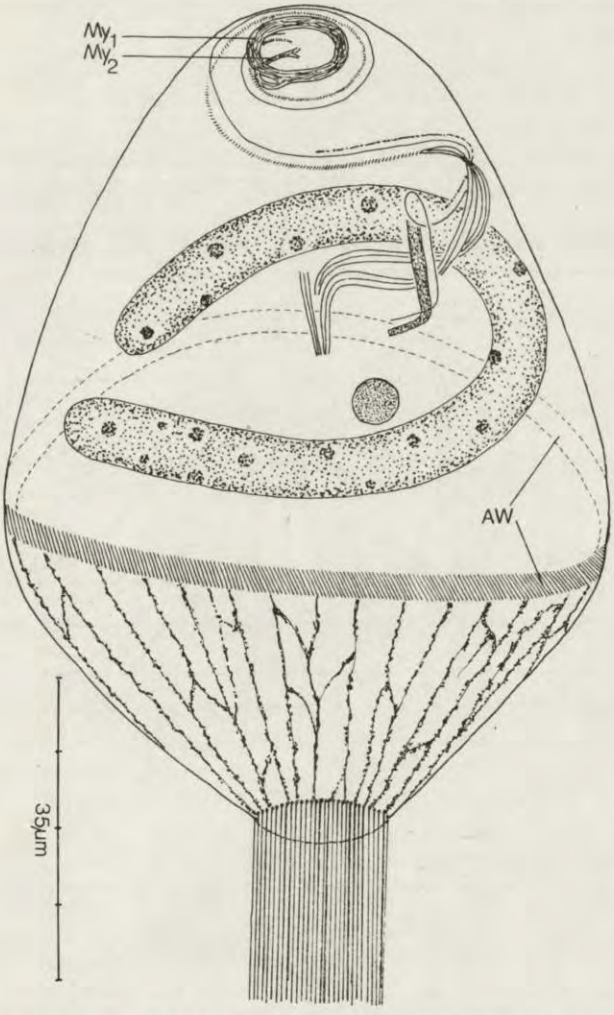

Abb. 7. Mittleres Stadium der Schwärmerbildung. Die Dezentralisierung der Scopula erfolgt durch Verschiebung des aboralen Wimperkranzes in Richtung des Peristomkragens (AW). Das Myonemsystem erscheint im aboralen Teil des Tieres aufgelockert, bleibt aber im wesentlichen unverändert erhalten (My_1, My_2). Protargolimprägnation

trahiert sich das Tier immer mehr, bis es die in Abb. 6 dargestellte Form erreicht hat. Der aborale Wimperkranz verläuft beinahe diagonal um den Schwärmer.

Kurz vor der Ablösung des Schwärmers erscheint der Stiel dicht unterhalb der Scopula eingeschnürt. Die Ablösung selbst erfolgt unter heftiger Rotation in der Horizontalen. Der fertige Schwärmer schwimmt

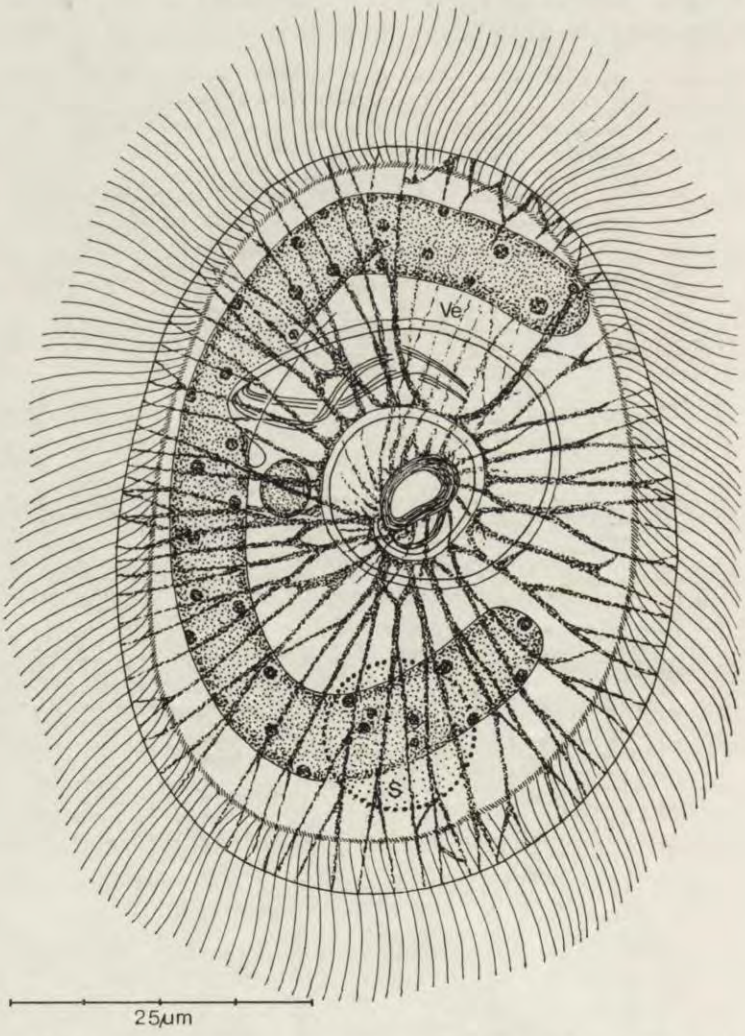

Abb. 8. Ansicht eines Schwärmers von der oralen Seite. Der aborale Wimperkranz und die Scopula (S), die sich auf der aboralen Seite des Schwärmers befinden, sind ebenfalls eingezeichnet worden. Der Oralapparat bleibt unverändert erhalten. Der Eingang zum Vestibulum ist durch eine torbogenartige Aussparung im Myonemsystem gekennzeichnet (Ve). Die das Vestibulum begrenzende Peristomlippe wird aber von feinen Ausläufern des Myonembandes des Peristomkragens durchzogen. Protargolimprägnation

sofort nach der Ablösung vom Stiel mit so ungeheurer Schnelligkeit umher, daß es unmöglich ist, seine Bewegungsweise zu beobachten. Stößt er aber auf eine feste Unterlage, so sistiert die Bewegung oft augenblicklich und er kriecht mit Hilfe der aboralen Wimpern umher, wobei die Scopula am Bewegungspol ist.

Die Form des 50–75 μm langen, 40–60 μm breiten und etwa 30 μm dicken Schwärmers ist in Aufsicht unregelmäßig oval (Abb. 8 Taf. I 1, Taf. III 17, 18), die breiteste Stelle ist meist der mittlere Teil. Das Vorderende und Hinterende sind merkbar verschmälert. Der bis auf eine winzige Öffnung geschlossene Peristomkragen ist etwas nach aussen gewölbt, so daß auch der Schwärmer genabelt erscheint (Abb. 6).

Die Protargolimprägnationen zeigten klar, daß der Schwärmer die Oralstrukturen und das Myonemsystem des vegetativen Individuums unverändert beibehält (Abb. 8, Taf. III 17, 18). Lediglich die Myoneme im aboralen Teil erscheinen wegen dessen vergrößerter Oberfläche mehr aufgelockert (Taf. III 15). Die Lage des Oralapparates scheint beim Schwärmer nicht streng fixiert zu sein, sehr im Gegensatz zu den Angaben von Chatton (1936). Er kann längs, quer oder schräg zur Längsachse orientiert sein. Der Makronucleus nimmt die in den Abbildungen eingezeichnete, dreiviertelkreisförmige Gestalt an. Der Mikronucleus liegt meist im Zentrum dieses Kreises.

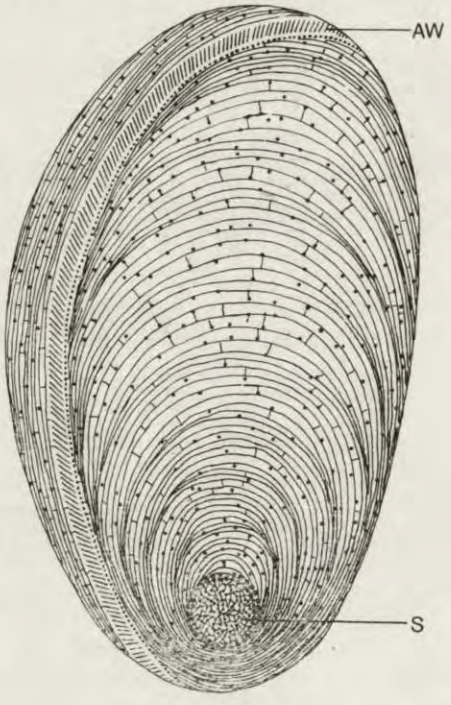

Abb. 9. Seitliche Ansicht eines Schwärmers nach trockener Silberimprägnation. Die Scopula (S) wird von stark argyrophilen Körnchen begrenzt. In ihrem Inneren findet sich ein sehr engmaschiges Silberliniennetz. Unterhalb des aboralen Wimperkranzes (AW) verläuft eine Reihe sehr regelmäßig angeordneter argyrophiler Körnchen. Aus Gründen der Übersichtlichkeit wurden nur etwa die Hälfte der Silberlinien und Pelliculaporen eingezeichnet

Das Silberliniensystem des Schwärmers bietet das in Abb. 9 dargestellte Bild. Die azentral verlagerte Scopula wird von den Silberlinien elliptisch umgeben, wobei diese am Bewegungspol so nahe nebeneinanderliegen, daß sie eine homogene argyrophile Schicht bilden. Ob es in diesem Gebiet zu einem Verschmelzen oder einer Reduktion der Silberlinien kommt, konnte nicht entschieden werden.

Die Schwärmer schwimmen mehrere Stunden umher, bis sie sich am Boden des Glasschälchens festsetzen. Dabei wird zuerst eine scheibenförmige Haftplatte, die etwa den halben Durchmesser des Schwärmers besitzt, abgeschieden. Hierauf erfolgt die Sekretion des Stieles, auf dem der Schwärmer zuerst noch rechtwinkelig orientiert ist, ähnlich wie bei der Endphase der Schwärmerbildung (Abb. 6). Dann beginnt die Rückverwandlung zum normalen Zooid, die genau umgekehrt wie die Schwärmerbildung verläuft. Der Schwärmer bzw. das junge Zooid wird dabei immer mehr vertikal zum Stiel orientiert, der 50–100 µm lang ist.

Der Schwärmer bietet somit insgesamt den Eindruck eines extrem abgeflachten und verkleinerten vegetativen Zooiden (vgl. Chatton 1936). Die Verkleinerung wird wohl durch die Defäkation der cytoplasmatischen Einschlüsse und eine erhöhte Flüssigkeitsabgabe erreicht bzw. ermöglicht. Dagegen konnte der Mechanismus, der zur Dezentralisation der Scopula führt, nicht entdeckt werden. Die zunächst sehr wahrscheinliche Hypothese, daß die Dezentralisation durch eine besondere Ausbildung des Myonemsystems ermöglicht wird, bestätigte sich nicht, da dieses im wesentlichen dem anderer *Epistylidae* gleicht (s. Foissner 1977). Bei *H. horizontalis* entsteht die Asymmetrie des Schwärmers durch eine ringförmige Wulstbildung um den Stiel herum, wobei eine Hälfte dieses Ringwulstes sehr viel mehr an Größe zunimmt als die gegenüberliegende. Über die der Wulstbildung zugrunde liegenden Strukturen machte Chatton (1936) keine Angaben.

Morphogenese

Die Morphogenese konnte leider nicht vollständig studiert werden, da in den Präparaten nur mittlere und späte Teilungsstadien gefunden worden sind und sich die Tiere in Kultur nicht vermehrten. Aus diesen Stadien (Abb. 4, 5) ist zu entnehmen, daß sich die Morphogenese des Oralapparates wohl so, wie von Lom (1964) bei *Opisthonecta minima* (s. Foissner 1975) beschrieben, vollzieht. Der Teilungsvorgang gleicht in den Grundzügen dem von *Epistylis galea* (s. Matthes et al. 1970). Es konnte jedoch nicht festgestellt werden, ob sich der Makronucleus dabei zu einer Kugel kondensiert (Matthes et al. 1970), Oder ob er

seine Form behält (s. Dass 1953). Die Tochtertiere bleiben bis zuletzt über eine schmale Plasmabrücke verbunden, durch die der Makronucleus zieht. Diese Brücke enthält viele etwa 0.5 µm große argyrophile Körnchen (Abb. 5).

Eine sehr bemerkenswerte, in der Literatur unseres Wissens bisher noch nicht beschriebene Beobachtung muß hervorgehoben werden: Bei allen gefundenen Teilungsstadien (15) waren keine Myoneme mehr nachweisbar! Auch das Myonemband im Peristomkragen verschwindet. Diese Beobachtung stimmt gut mit der Tatsache überein, daß die mit Teilungsbeginn birnenförmig kontrahierten Individuen keine oder eine sehr stark herabgesetzte Kontraktilität zeigen.

Konjugation

In den Protargolpräparaten wurden auch mehrere konjugierende Tiere festgestellt. Die Mikrogamonten sind etwa 30 µm groß, besitzen nur ein spärlich entwickeltes Myonemsystem und ließen keinerlei Oralstrukturen erkennen, was darauf hinweist, daß sie durch multiple Teilung aus vegetativen Zooiden entstehen (Taf. II 11). Der Makronucleus liegt zu Beginn der Konjugation horizontal im Mikrogamonten (Taf. II 11, Pfeil), löst sich später aber in viele Bruchstücke auf. Ein Mikronucleus liegt im Zentrum des vom Makronucleus gebildeten Kreises. Da nur festgeheftete Mikrogamonten beobachtet worden sind, konnte nicht festgestellt werden, ob ihre Scopula zentral oder azentral gelagert ist. Die Mikrogamonten waren in allen vier beobachteten Fällen stets etwas oberhalb der Mitte des Makrogamonten festgeheftet.

Diskussion

Die Verbreitung von heteropolaren Schwärmern unter den peritrichen Ciliaten

Heteropolare Schwärmer wurden erstmals von Chatton (1936) bei *Heteropolaria horizontalis* beschrieben, die epizoisch auf *Balanus* lebt. Diese Art wurde kürzlich von Jankowski (1967), ebenfalls auf *Balanus*, mit allen typischen Merkmalen wiedergefunden. Von Fauré-Fremiet (1943) wurde später *Heteropolaria lwoffi* entdeckt, die epizoisch auf *Gasterosteus aculeatus* und anderen Fischen lebt. Lom (1966) und Scheubel (1973) haben dann beobachtet, daß diese Art ebenfalls heteropolare Schwärmer ausbildet. Wie bereits erwähnt (s. S. 239) hat Kralik (1961) bei *Platycola truncata* ebenfalls hetero-

polare Schwärmer festgestellt, deren Genese sehr der von *Heteropolaria colisarum* gleicht. Bei *Platycola* wird allerdings nicht nur die Scopula, sondern auch das Peristom und der Makronucleus verlagert, so daß diese drei Strukturen fast übereinander liegen (Kralik 1961). Chatton (1936) will auch bei *Zoothamnium* sp. heteropolare Schwärmer festgestellt haben. Eine Heteropolarität weisen auch die Schwärmer von *Ellobiophrya donacis* (Chatton et al. 1929) auf, indem es bei dieser Art durch eine starke Vorwölbung auf einer Seite des aboralen Abschnittes zu einer Verlagerung der Scopula kommt. Weitere Berichte über heteropolare Schwärmer sind mir ebensowenig wie Chatton (1936) und Kralik (1961) bekannt geworden.

Begründung der Errichtung einer eigenen Gattung für *Epistylidae* mit heteropolaren Schwärmern

Die heteropolare Schwärmerbildung stellt bei den *Epistylidae* offensichtlich ein phylogenetisch sehr schwierig zu deutendes Merkmal dar, da sie, wie schon Chatton (1936) bemerkte, nicht nur als Anpassung an ihre, wie es scheint, obligat symphorionte Lebensweise verstanden werden kann. Chatton (1936) betrachtete daher diesen Typus als primitiv und gründete darauf seinen Ableitungsversuch der *Peritrichida* von gymnostomen Vorfahren. Raabe (1952) sah dagegen in den horizontal polarisierten Schwärmern das Ergebnis einer sekundären Anpassung an die symphorionte Lebensweise, ohne dies allerdings zu begründen. Diesen Schwärmertyp primär nur als das Ergebnis einer ökologischen Gegebenheit verstehen zu wollen, erscheint uns verfehlt, wenn man bedenkt, daß viele andere symphorionte peritriche Ciliaten diesen nicht besitzen (s. z. B. Nenninger 1948, Matthes 1950, Lust 1950). Freilich existieren auch keine stichhaltigen Hinweise dafür, daß dieses Merkmal als primitiv und ev. als phylogenetisches Relikt im Sinne gymnostomer Vorfahren zu deuten ist. Wir neigen eher der Auffassung zu, daß es sich hier um gehäuselos gewordene Peritrichida vom Typus der *Platycola* handelt, die in ihrer Ontogenie die Dezentralisation der Scopula noch beibehalten haben. Bei *Peritrichida* des Typus *Platycola* ist eine Verlagerung der Scopula als Folge des ziemlich flachen Gehäuses schon leichter verständlich, da durch die damit verbundene dorsale Verschiebung des aboralen Wimperkranzes die Abflachung des Schwärmers in Richtung der Abflachung des Gehäuses erfolgt (s. Abb. 9 bei Kralik 1961) und so die Schwärmerbildung ohne räumliche Behinderung vollzogen werden kann.

Welche der oben diskutierten Hypothesen auch immer zutreffen mag, so scheint es doch gerechtfertigt, Epistylidae mit horizontal polarisierten

Schwärmern als eigene Gattung abzutrennen, da sie zweifellos eine besondere Entwicklungslinie innerhalb der *Epistylidae* darstellen.

Die hier durchgeführte generische Trennung auf Grund der Schwärmermorphologie wurde bereits früher von Lust (1950) und Matthes (1958) erfolgreich angewandt. So betont Lust (1950), daß sich die Genera *Opercularia*, *Orbopercularia* und *Operculariella* hinsichtlich der Schwärmermorphologie ganz deutlich unterscheiden. Aus diesem Grund halten wir es auch für ungerechtfertigt, das Genus *Operculariella* Stammer, 1948 aufzulösen, wie dies Matthes et al. (1973) vorgeschlagen haben. Matthes (1958) hat ja selbst bei seinem Genus *Cyclodonta* als wesentlichstes Genuskriterium den eigenartigen Schwärmer, der einen besonderen Haftring ausbildet, angeführt.

Diagnose von *Heteropolaria* gen. nov. (*Epistylidae* Kahl, 1930–1935)

Epistylidae, deren Scopula beim Schwärmer eine deutlich azentrale Lage einnimmt, so daß dieser horizontal polarisiert wird.

Genotypus: *Heteropolaria horizontalis* (Chatton, 1936).

Das neue Genus enthält bisher drei sichere Arten: *Heteropolaria horizontalis* (Syn.: *Epistylis horizontalis* Chatton, 1936), *Heteropolaria lwoffi* (Syn.: *Epistylis lwoffi* Fauré-Fremiet, 1943) und *Heteropolaria colisarum* nov. spec.

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SUMMARY

The morphology of the zooids and telotrochs of *Heteropolaria colisarum* spec. nov., an *Epistylidae* which lives symphoriontly on *Colisa fasciata* is described. The myonem system and the oral structures were analyzed by means of protargol silvered specimens. The biometrical values of the silverline system are stated. The observations of living animals and the analysis of protargol and silvernitrat stained specimens show clearly that the zooids are epistyliform. However the telotrochs are horizontally polarized like the telotrochs of *Heteropolaria horizontalis* (Chatton, 1936) and *Heteropolaria lwoffi* (Fauré-Fremiet, 1943). This feature motivated us to take out this three species from the genus *Epistylis* (Ehrenberg, 1838) and to unite them into a new genus, *Heteropolaria* gen. nov. This step is discussed in detail. Accordingly, we think that the genus *Heteropolaria* includes *Epistylidae* whose ancestors could have been *Peritrichida* of the typus *Platycola*.

Some observations on the morphogenesis and conjugation of *Heteropolaria colisarum* are noticed. The myonem system is not demonstrable during the morphogenesis.

LITERATUR

Chatton E. 1936: Les migrateurs horizontalement polarisés de certains péritriches: De leur signification. Mem. Mus. Hist. Nat. Belg. (Ser. 2), 3, 913–940.

Chatton E. et Lwoff A. 1929: *Ellobiophrya donacis* Ch. et Lw. péritriche vivant sur les branchies de l'acéphale *Donax vittatus* Da Costa. Bull. Biol. France et Belg., 63, 321–349.

Dass C. M. S. 1953: Studies on the nuclear apparatus of peritrichous ciliates. Part I. The nuclear apparatus of *Epistylis articulata* (From.). Proc. Nat. Inst. Sci., 19, 389–404.

Ehrenberg C. G. 1838: Die Infusionsthierchen als vollkommene Organismen. Leipzig.

Fauré-Fremiet E. 1943: Commensalisme et adaptation chez une vorticellide: *Epistylis lwoffi* n. sp. Bull. Soc. Zool. France, 68, 154–157.

Foissner W. 1975: *Opisthonectidae* (*Ciliata, Peritrichida*) nov. fam. und Revision der Genera *Telotrochidium* (Kent) und *Opisthonecta* (Fauré-Fremiet). Protistologica, 11, 395–414.

Foissner W. 1976: Erfahrungen mit einer trockenen Silberimprägnationsmethode zur Darstellung argyrophiler Strukturen bei Protisten. Verh. Zool.-Bot. Ges. Wien, 115, 68–79.

Foissner W. 1977: Revision der Genera *Astylozoon* (Engelmann) und *Hastatella* (Erlanger) (*Ciliata, Nantantina*). Protistologica (im Druck).

Foissner W. und Schiffmann H. 1974: Vergleichende Studien an argyrophilen Strukturen von vierzehn peritrichen Ciliaten. Protistologica, 10, 489–508.

Jankowski A. W. 1967: Infuzorii iz mantijnoj polosti *Balanus* v Barencevom Morie. Parazitologja, 1, 82–93.

Kahl A. 1930–35: Wimpertiere oder *Ciliata*. In: Die Tierwelt Deutschlands. (ed. Dahl F), G. Fischer, Jena.

Kralik U. 1961: Ein Beitrag zur Biologie von loricaten peritrichen Ziliaten, insbesondere von *Platycola truncata* Frommentel 1874. Arch. Protistenk., 105, 201–258.

Lom J. 1964: The morphology and morphogenesis of the buccal ciliary organelles in some peritrichous ciliates. Arch. Protistenk., 107, 131–162.

Lom J. 1966: Sessiline peritrichs from the surface of some freshwater fishes. Fol. Parasitologica, 13, 36–56.

Lom J. and Vavra J. 1961: *Epistylis lwoffi* (?) from the skin of Perches. Acta Soc. Zool. Bohem., 25, 273–276.

Lust S. 1950: Symphorionte Peritriche auf Käfer und Wanzen. Zool. Jb. (Syst.), 79, 353–463.

Matthes D. 1950: Die Kiemenfauna unserer Landasseln. Zool. Jb. (Syst.), 78, 573–640.

Matthes D. 1958: Das peritriche Ciliat *Cyclodonta bipartita* (Stokes) nov. gen. Arch. Protistenk., 102, 481–500.

Matthes D. und Scheubel J. 1970: *Epistylis galea* Ehrenberg, ein polymorphes Peritrich. Arch. Protistenk., 112, 30–64.

Matthes D. und Guhl W. 1973: Adaptive Reduktion des Wimperapparates in der Gattung *Orbopercularia* (*Ciliata, Peritricha*). Protistologica, 9, 349–352.

Nenninger U. 1948: Die Peritrichenfauna der Umgebung von Erlangen mit besonderer Berücksichtigung der Wirtsspezifität. Zool. Jb. (Syst.), 77, 167–266.

Raabe Z. 1952: *Ambiphrya miri* g. n., sp. n. — eine Übergangsform zwischen *Peritricha — Mobilia* und *Peritricha — Sessilia*. Ann. Univ. M. Curie-Skłodowska, Sect. C, 6, 339–358.

Scheubel J. 1973: Die sessilen Ciliaten unserer Süßwasserfische unter besonderer Berücksichtigung der Gattung *Apiosoma* Blanchard. Zool. Jb. (Syst.), 100, 1–63.

Stammer H. J. 1948: Eine neue eigenartige entoparasitische Peritriche, *Operculariella parasitica* n. g. n. sp. Zool. Jb. (Syst.), 77, 163–168.

Wilbert N. 1975: Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos, 6, 171–179.

Zagon I. S. and Small E. B. 1970: *Carchesium polypinum*: Somatic and buccal structure analysis after protargol staining. Trans. Amer. Microsc. Soc., 89, 443–449.

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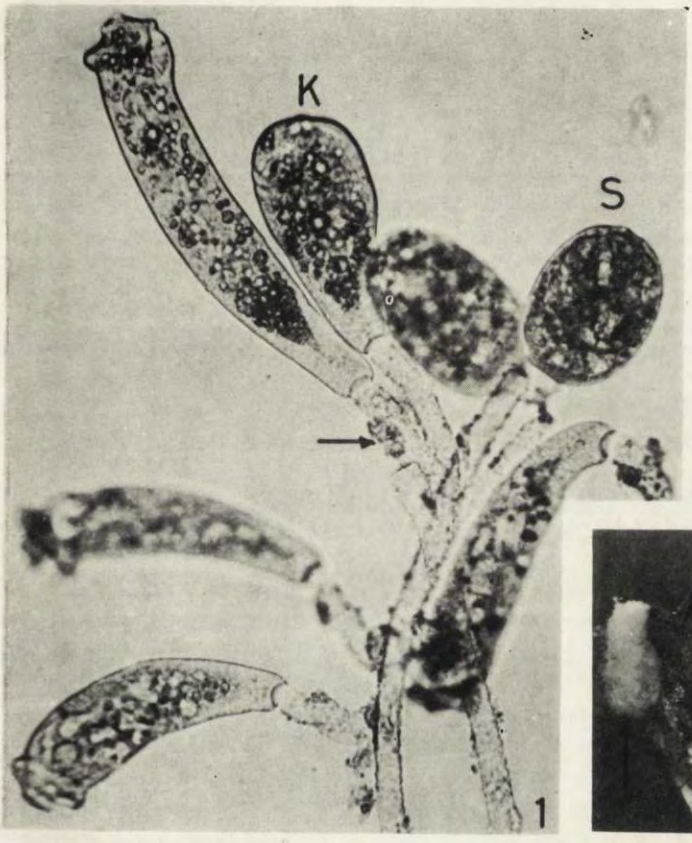

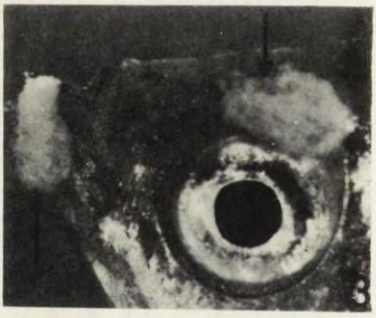

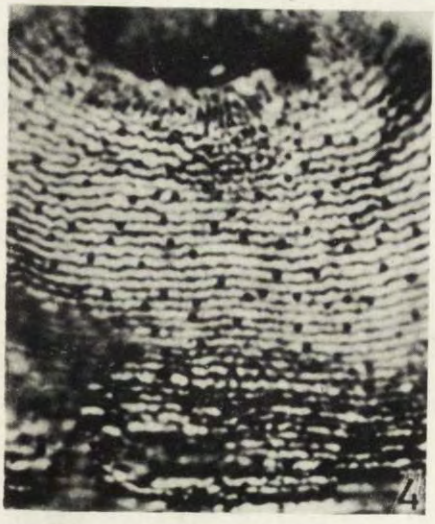

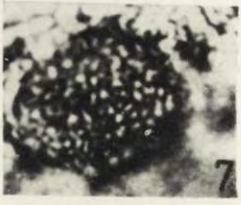

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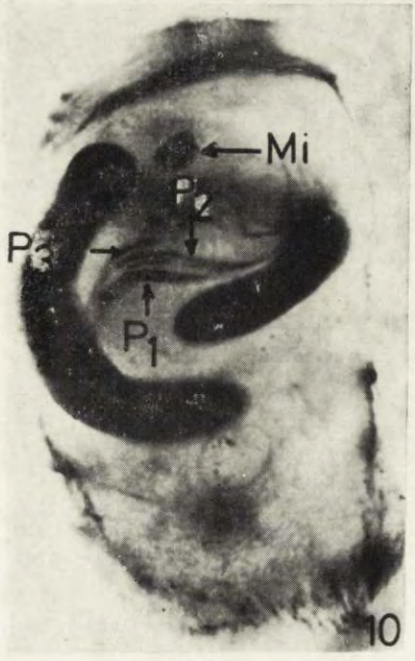

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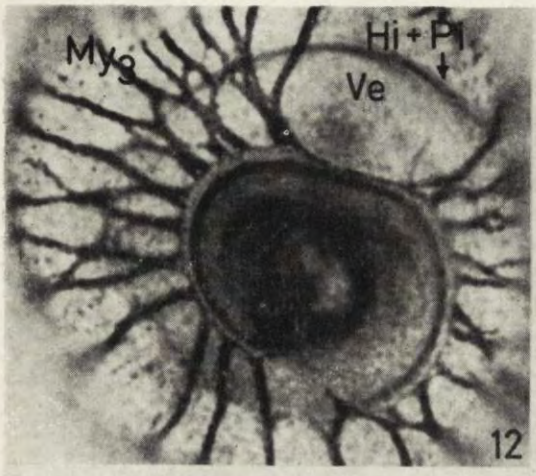

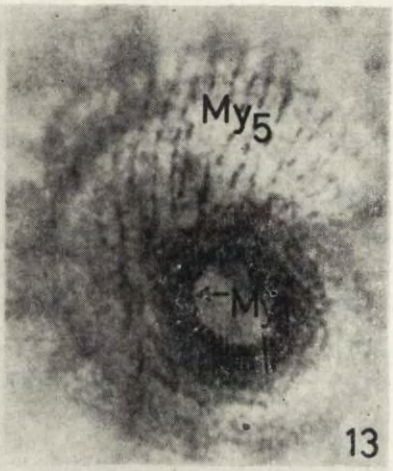

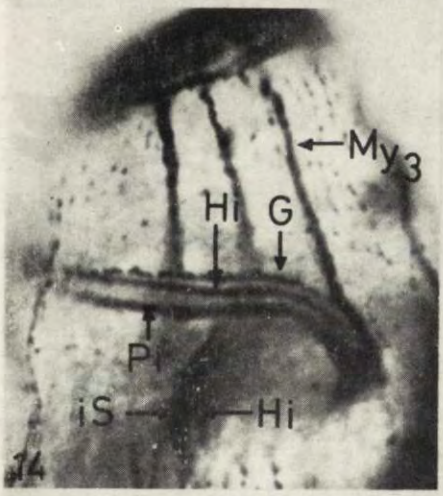

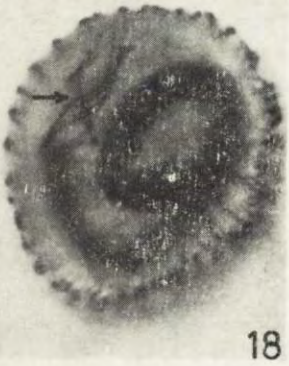

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LEGENDEN ZU DEN TAFELN I–III

1: Teil einer Kolonie von *Heteropolaria colisarum* mit gestreckten und kontrahierten (K) Zooiden und einem Schwärmer (S). Der dichte Bakterienbesatz (Pfeil) an den Stielen ist deutlich erkennbar. Lebendaufnahme
2: Gestrecktes Zooid, das die trichterförmige Anordnung von cytoplasmatischen Einschlüssen klar zeigt. Lebendaufnahme
3: Mundregion von *Colisa fasciata* mit zwei Kolonien (Pfeile) von *Heteropolaria colisarum*. Lebendaufnahme
4: Oraler Teil des Silberliniensystems, der die den Silberlinien anliegenden Pelliculaporen zeigt. Im unteren Bildteil findet sich ein Übergang zur Pseudostreifung. Trockene Silberimprägnation
5: Typische Pseudostreifung, die durch das sehr enge Zusammenrücken der Silberlinien bei der Kontraktion des Tieres entsteht. Trockene Silberimprägnation
6: Anlage des aboralen Wimperkranzes (Pfeil) nach Protargolimprägnation. Die Myoneme ziehen dicht unterhalb der Anlage ununterbrochen hindurch
7: Die Scopula läßt nach trockener Silberimprägnation ein sehr engmaschiges Silberliniennetz erkennen
8: Nach Protargolimprägnation sind auf der Scopula die Ansatzstellen der Stielfibrillen als kleine argyrophile Körnchen erkennbar. Die Myoneme sind in der Umgebung der Scopula sehr dicht angeordnet
9: Kleine Kolonie mit schlanken, wurmförmigen Zooiden. Lebendaufnahme
10: Übersichtsaufnahme, die den Makro- und Mikronucleus (Mi) und die drei Peniculi (P_{1-3}) zeigt. Protargolimprägnation
11: Konjugierende Individuen. Der Makronucleus des Mikrogamonten (Pfeil) ist noch unverändert, der des Makrogamonten hat sich in viele voluminöse Teile aufgelöst. Das Myonemsystem des Makro- und Mikrogamonten ist gut erkennbar. Protargolimprägnation
12, 13: Aufsicht auf ein Zooid bei verschiedener Fokussierung, um die einzelnen Teile des Myonemsystems ($My_{1, 3, 5}$) zu zeigen. Am Eingang zum Vestibulum (Ve) sind die My_3 torbogenartig ausgespart. Haplo- und Polykinete (Hi+Pi) steigen nach etwa eineinhalb Umgängen in das Vestibulum hinab. Protargolimprägnation
14: Verlauf von Haplo- und Polykinete im Vestibulum (nähere Erklärung s. Textfigur 3). Protargolimprägnation.
15: Ansicht eines Schwärmers von der aboralen Seite. Die deutlich azentral verlagerte Scopula (S) und das Myonemsystem sind erkennbar. Protargolimprägnation
16: Oraler Teil des Myonemsystems. Das vom Peristomkragen in den Peristomdiskus ziehende Myonem (My_2) ist hier besonders gut sichtbar. Protargolimprägnation
17, 18: Ansicht zweier Schwärmer von der oralen Seite. Der gesamte Oralapparat (Hi+Pi, Pfeil) sowie das Myonemsystem bleiben unverändert erhalten. Protargolimprägnation

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Die Ciliatenfauna der Savanne von Lamto (Elfenbeinküste) [1]

Synopsis. Der Artenbestand der bodenbewohnenden Ciliaten aus drei afrikanischen Standorten („savane brulée", „savane non brulée", „Galeriewald" bei Lamto, Elfenbeinküste) wurde ermittelt. Insgesamt wurden 40 Ciliatenarten gefunden, darunter vier bisher nicht bekannte Formen (*Holosticha distyla* spec. n., *Lamtostyla lamottei* gen. n. spec. n., *Oxytricha tricirrata* spec. n. und *Spathidium bonneti* spec. n.). 17 Arten werden zeichnerisch dargestellt, von *Cyrtolophosis elongata* Kahl wird die Morphogenese beschrieben. Die Untersuchung der Infraciliatur erfolgte mittels der Protargoltechnik.

Die Savanne von Lamto (Elfenbeinküste) wird seit 1961 umfangreichen ökologischen Untersuchungen unterzogen, die bis in jüngster Zeit im Rahmen der Internationalen Biologischen Programms (IBP) durchgeführt wurden. In dieser Region findet man einen Feuchtsavannentyp vor („Guineasavanne", Abb. 1), weite Gebiete mit hohem Grasbewuchs, die von zahlreichen Galeriewäldern durchzogen werden.

In der Umgebung der Station für Tropische Ökologie Lamto wurden drei Standorte ausgewählt, die auf ihren Bestand an Ciliatenarten hin untersucht wurden. Zum einen wurde ein Gebiet bearbeitet, das alljährlich durch Buschfeuer abgebrannt wird („savane brulée", Plateau du Grand Nord). Das zweite Untersuchungsgebiet war das Areal um die Station selbst, das seit vielen Jahren vor der Passage des Buschfeuers geschützt wird („savane non brulée"). Zum dritten war der Boden eines Galeriewaldes (Plateau du Grand Nord), der an die „savane brulée" angrenzt, Objekt meiner Untersuchung. Es wurden nur Bodenproben aus dem Horizont 0–5 cm Tiefe entnommen. Zur Bearbeitung wurden diese luftgetrocknet und nach Bonn überführt. Präparate der unten neu aufgeführten Arten befinden sich im Institut für Landwirtschaftliche Zoologie, Universität Bonn.

[1] Mit Unterstützung der Deutschen Forschungsgemeinschaft.

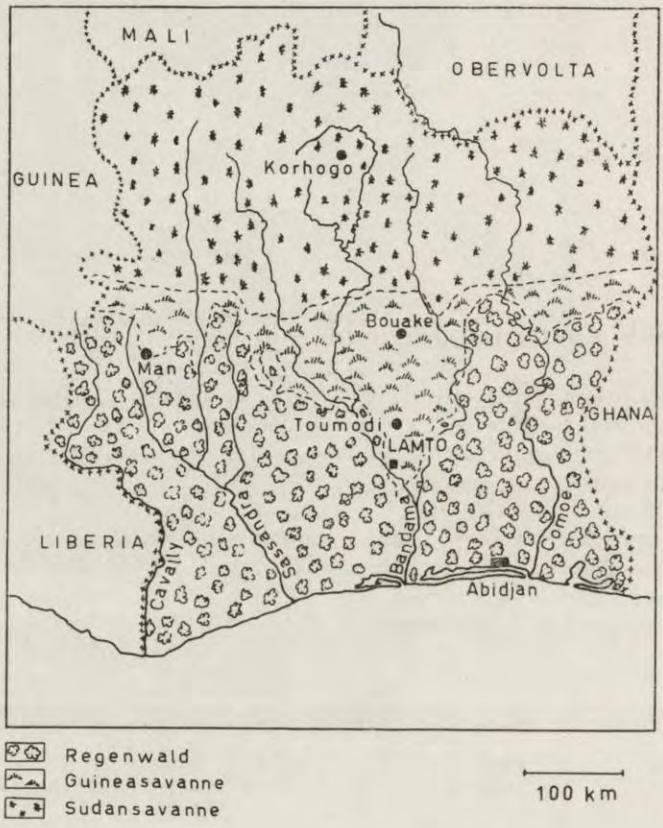

Abb. 1. Vegetationstypen der Rep. Elfenbeinküste und die geographische Lage der Station für Tropische Ökologie Lamto. (nach: „Bulletin de liaison des chercheurs de Lamto", num. spec. 1974, fasc. 1)

Methoden

Zur Determination der in den drei Standorten vorkommenden Ciliatenarten wurden die Bodenproben in Petrischalen mit Wasser aufgeschwemmt. Daraufhin erfolgte die Excystierung der Organismen und häufig eine Massenvermehrung der einen oder anderen Form. Arten, die sich nicht derart entfalteten, wurden mittels Kapillarpipetten von den übrigen isoliert und in kleinen Kulturschälchen meist erfolgreich zur Vermehrung gebracht. Das Filtrat einer Bodenaufschwemmung des jeweiligen Standortes diente als Kulturmedium, nachdem ein Reiskorn zur Entfaltung einer Bakterienflora hinzugefügt wurde.

Die Infusorien wurden nach der Protargolmethode präpariert (Bodian 1936; Dragesco 1962; Tuffrau 1967; Wilbert 1975), die erlaubt, die Infraciliatur der Organismen auf das Genaueste darzustellen.

Vorbemerkungen zur systematischen Einordnung der Ciliaten

Im Laufe der letzten Jahrzehnte mussten aufgrund immer neuer morphologischer Erkenntnisse Taxonomie und Nomenklatur mehrmals geändert werden. In einer vergleichenden Betrachtung stellt Corliss (1974 a) die vergangenen Perioden (1880–1930, 1930–1950, 1950–1970) zusammen, in denen sehr unterschiedliche Klassifikationsschemata ihre Gültigkeit hatten.

Die Anwendung der modernen Silberimprägnationstechniken, vor allem zusammen mit vielen ultrastrukturellen Untersuchungen an Zellorganellen, brachten eine Fülle neuer Befunde, die eine erneute Revision der Ciliatenklassifikation notwendig machte (siehe hierzu: Jankowski 1967 und 1973, Corliss 1974 a, b und 1975, de Puytorac et al. 1974). Während in nächster Zeit noch einige Unklarheiten bezüglich systematischer Untereinheiten ausdiskutiert werden müssen, stehen die höheren Kategorien bereits fest: Die noch von Jankowski (1973) in einem Unterstamm zusammengefassten Ciliaten werden zum „Phylum *Ciliophora* Doflein" erhoben. Nach Corliss (1975) wird dieser neue Stamm unterteilt in drei Klassen: (1) *Kinetophragminophora* de Puytorac et al., (2) *Oligohymenophora* de Puytorac et al. und (3) *Polyhymenophora* Jankowski. Diese Unterteilung wird sowohl von der Arbeitsgruppe um de Puytorac (1974) als auch von Corliss (1975) vorgeschlagen. Die noch unterschiedlichen Beurteilungen der zum grössten Teil zu den Kinetophragminophora gehörenden Infusorien sollen hier nicht Gegenstand einer Diskussion sein. Die in meiner Arbeit aufgeführten Klassifikation entspricht der von Corliss (1975), ohne dass ich damit einer Wertung vornehme.

Ergebnisse

In den 3 Böden konnten insgesamt 40 Ciliatenarten nachgewiesen werden (Tab. 1), wobei die „savane brulée" das artenreichste Untersuchungsareal ist (33 Arten). Vergleichsweise artenarm ist der Galeriewald (22 Arten). Die wenigsten Formen treten in der „savane non brulée" auf (19 Arten). Die „savane non brulée" ist im übrigen auch relativ arm an Thecamöben (Bonnet persönl. Information).

Auffallend ist bei der Betrachtung der Artenliste, dass die meisten auftretenden Ciliaten als kosmopolitische Bewohner von Böden und Gewässern Europas, Asiens und Amerikas bekannt sind. Offensichtlich bilden die verschiedenen Arten Standortvariationen aus, die an die ent-

Tabelle 1

Liste der nachgewiesenen Ciliatenarten

Art	Sb	Snb	Gw
1. *Blepharisma hyalinum*	×		
2. *Bryometopus pseudochilodon*	×	×	×
3. *Bryophyllum* spec.	×		
4. *Chilodonella gouraudi*	×	×	×
5. *Chilodonella uncinata*	×		
6. *Colpoda acuta*	×		
7. *Colpoda colpidiopsis*	×		
8. *Colpoda cucullus*	×	×	×
9. *Colpoda inflata*	×	×	×
10. *Colpoda steini*	×	×	×
11. *Cyclidium glaucoma*	×		
12. *Cyrtolophosis elongata*	×	×	×
13. *Cyrtolophosis mucicola*	×	×	×
14. *Dileptus americanus*(?)	×		
15. *Drepanomonas revoluta*	×		
16. *Enchelyodon* spec.	×		
17. *Euplotes muscorum*		×	
18. *Histriculus similis*		×	
19. *Holosticha distyla*		×	×
20. *Holosticha multistilata*			×
21. *Homalogastra setosa*	×	×	×
22. *Lamtostyla lamottei*	×		
23. *Leptopharynx sphagnetorum*	×		×
24. *Mycterothrix tuamotuensis*	×	×	
25. *Oxytricha agilis*	×	×	×
26. *Oxytricha platystoma*		×	
27. *Oxytricha setigera*	×		
28. *Oxytricha tricirrata*	×		×
29. *Platyophrya angusta*	×		×
30. *Platyophrya spumacola*		×	
31. *Sathrophilus muscorum*	×		×
32. *Spathidium longicaudatum*	×		×
33. *Spathidium bonneti*	×	×	×
34. *Spathidium muscicola*	×	×	×
35. *Sphaerophrya* spec.	×		
36. *Tetrahymena pyriformis*			×
37. *Trachelostyla affine*	×	×	×
38. *Uroleptus kahli*	×	×	×
39. *Uroleptus muscorum*	×		
40. *Vorticella microstoma*	×		×
Gesamtartenzahl:	33	19	22

Sb = "savane brûlée", Snb = "savane non brûlée", Gw = Galeriewald.

sprechenden Temperaturverhältnisse ihrer Umgebung angepasst sind (Buitkamp 1976).

Folgende in den Zeichnungen und teilweise im Text erscheinenden Abkürzungen fanden Verwendung:

AZM — Adorale Membranellenzone
Bo — Borsten
CC — Caudalcirren
Cp — Cytopyge
cV — kontraktile Vakuole
Cy — Cytostom
Db — Dorsalborsten
eM — endorale Membran
FC — Frontalcirren
$M_{1\ 2\ 3}$ — Membranelle 1, 2, 3
Ma, Mi — Makro-, Mikronukleus
Mbr — Membranelle(n)
pM — parorale Membran
Sc — Scuticus
TC — Transversalcirren
UM — Undulierende Membran
VC — Ventralcirren, -reihe
VPK — ventraler Teil der peripheren Kinete

Klasse: *Kinetophragminophora*
Unterklasse: *Gymnostomata*
Ordnung: *Prostomatida*
Unterordnung: *Prorodontina*
Familie: *Prorodontidae*

Platyophrya angusta Kahl (Abb. 2 a, b)
(= *Telostoma ferroi* R. und Grandori)

Grösse: 20–40 µm. Gestalt nach links gekrümmt, dorsoventral abgeflacht, Körper sehr biegsam. Der Schlund liegt auf der linken Seite unter dem apikalen Pol, ist spaltförmig und mit einer kräftigen Reuse ausgestattet. Der Ma und der Mi liegen zentral. Die Ventralseite ist dichter bewimpert als die Dorsalseite: ventral stehen einheitlich 3 Kineten mit etwa 18 Cilienpaaren, dorsal liegt eine uneinheitliche, lockere Bewimperung vor. Die Cilien sind 4 µm lang. Die cV liegt terminal. Eine deutliche Mundbewimperung fehlt bis auf eine rechts vom Mundspalt stehende Kinete aus 8 Cilien. Allerdings verdichtet sich die Körperbewimperung in der Mundregion.

Der Ciliat wurde in der „savane brulée" und im Galeriewald angetroffen. Nach meinen Beobachtungen ernährt sich der Ciliat von Bakterien; Kahl (1930) gibt sehr kleine farblose Flagellaten als Nahrung an.

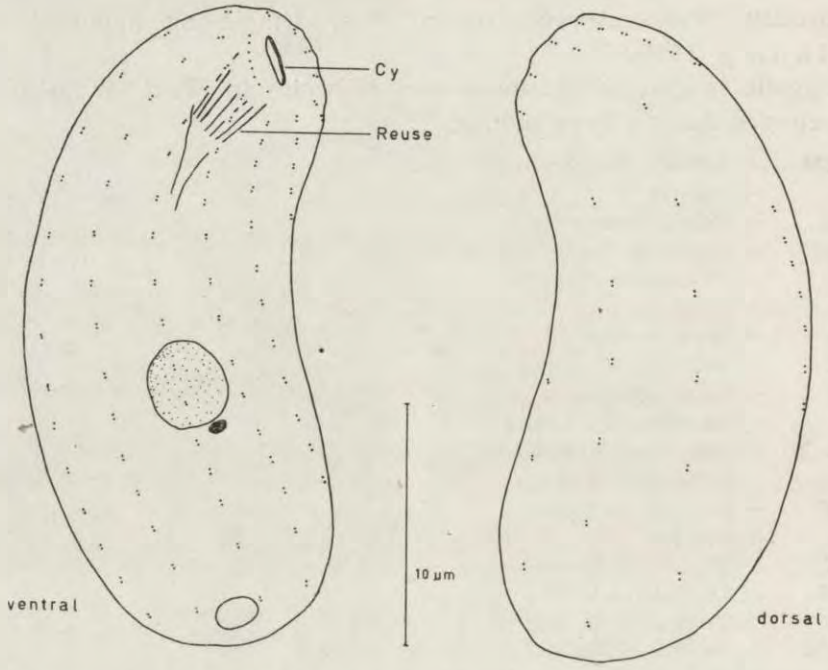

Abb. 2. *Platyophrya angusta* Kahl, Kinetom der Ventralseite (a) und der Dorsalseite (b), (Protargol)

Über die zur Gattung *Platyophrya* zu stellenden Formen bestehen offenbar noch Unklarheiten, besonders über die Einordnung der folgenden Art *P. spumacola* Kahl. Die beiden hier beschriebenen Arten zeigen eindeutige Verwandtschaftsmerkmale: eine kräftige Reuse, eine rechts vom Cytostom stehende Kinete als Mundbewimperung (UM?), doppelt stehende Cilien sowie eine sehr grosse Biegsamkeit des Zellkörpers. Nach Kahl (1935) weist die Form *Telostoma ferroi* R. und L. Grandori erhebliche Ähnlichkeit mit *P. angusta* Kahl auf. Corliss (1961) reiht diese Form sicherlich zu Recht in die Gattung *Platyophrya* ein.

Platyophrya spumacola Kahl (Abb. 3)
(= *Woodruffia sinistromembranellata* Gellért)

Länge: 80–110 µm. Gestalt ähnlich wie *P. angusta* Kahl, stark metabolisch. Ma und Mi sind oft auffallend weit nach caudal verlagert. Die cV liegt nicht ganz terminal, sondern ist leicht nach links verschoben. Die Anzahl der etwas spiralig verlaufenden Kineten, in denen die Cilien doppelt stehen, variiert von 24–30, je nach Grösse des Tieres. Die Wimpern sind 7 µm lang. Rechts vom Cytostom findet man eine feine, 3 µm

hohe UM. Links stehen 10 Membranellen, die etwa 5 µm hoch sind. Im Anschluss daran, fast schon dorsal gelegen, bilden verdichtete Körperwimpern ein Cilienfeld, dass anscheinend ebenfalls im Dienste der Nahrungsaufnahme steht. *P. spumacola* Kahl ist ein omnivorer Ciliat, der sich von Bakterien, Blaualgen, kleinen Flagellaten und Ciliaten ernährt; ich konnte auch Kannibalismus beobachten. Der Ciliat wurde nur in der „savane non brûlée" angetroffen.

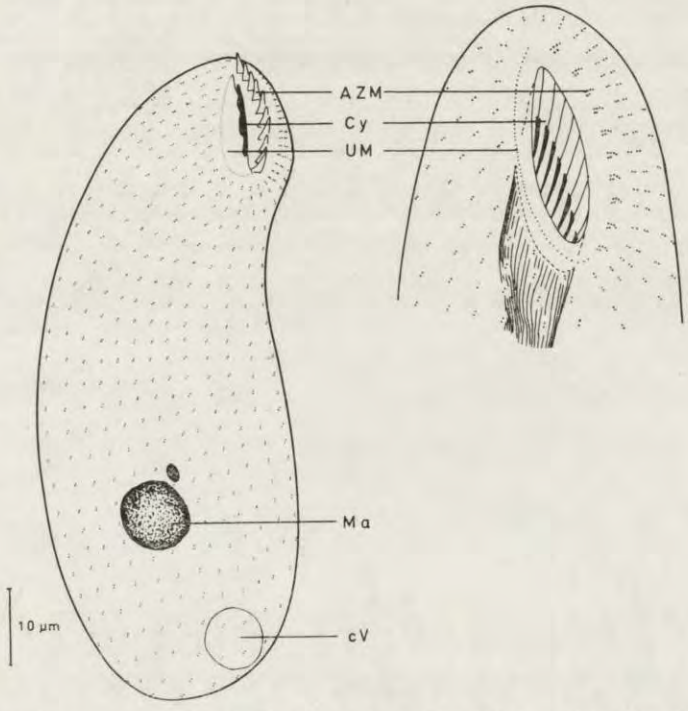

Abb. 3. *Platyophrya* spumacola Kahl, Gesamtaspekt (links), rechts Mundregion, nach Protargolpräparation

P. spumacola wurde zuerst von Kahl (1930) entdeckt. Gellért (1954) beschrieb die gleiche Form unter dem Namen *Woodruffia sinistromembranellata*. Diese falsche Artdiagnose ist sicherlich auf die seinerzeit unzureichenden Untersuchungstechniken zurückzuführen. Einige interessante Beobachtungen zur Biologie dieses Ciliaten werden von Czapik (1971) wiedergegeben. Die neueste Beschreibung der Morphologie sowie der Morphogenese stammt von Groliére (1975), jedoch weicht der von ihm beschreibene Aufbau der AZM von dem der von mir gefundenen Form ab (Abb. 3, Mundregion). Groliéres Vorschlag der Aufhebung der Gattung *Woodruffia* ist meiner Meinung nach berechtigt.

Im übrigen sei darauf hingewiesen, dass *Platyophrya* eine sehr ähnliche Morphogenese aufweist wie die weiter unten aufgeführte Gattung *Cyrtolophosis* (siehe Abb. 8 a–f).

Ordnung: *Haptorida*
Familie: *Spathidiidae*

Spathidium bonneti n. spec.[2] (Abb. 4)

Länge: 150 µm, Breite: ca 5 µm; Gestalt fadenförmig. Der Ma besteht aus 15 ovalen bis runden Fragmenten, während die Mi nicht entdeckt werden konnten. Die cV liegt ein wenig vom caudalen Pol abgesetzt. Die Bewimperung ist sehr locker. In 5 Kineten stehen die 8 µm langen Cilien in einem Abstand von 5 µm voneinander entfernt. Die ebenfalls

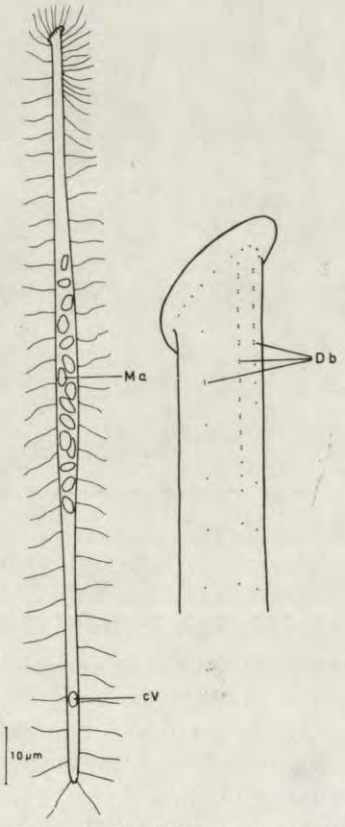

Abb. 4. *Spathidium bonneti* n. spec., Gesamtaspekt (links) und Dorsalansicht (rechts); Protargolimprägnation

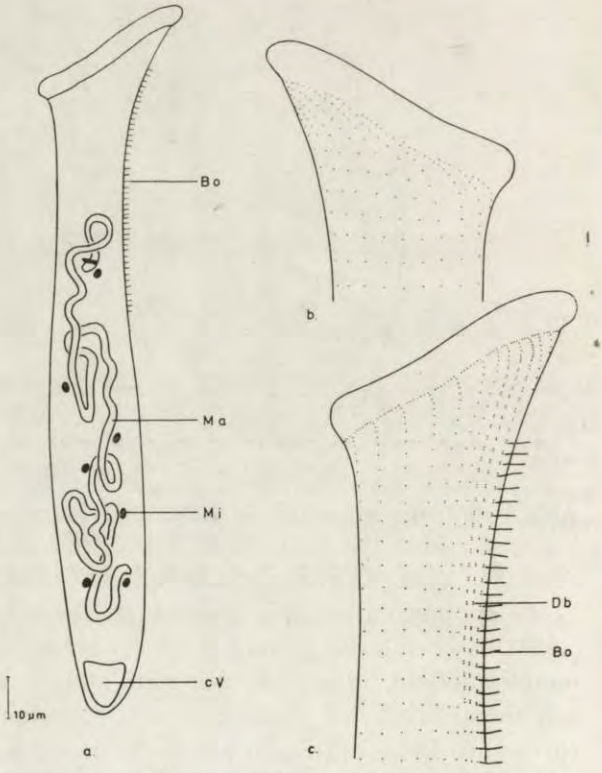

Abb. 5. *Spathidium muscicola* Kahl (n. Protargol), Gesamtansicht (a) und Infraciliatur der rechten (b) und linken Frontalregion (c)

[2] Diese Art benenne ich in freundschaftlicher Erinnerung nach dem Spezialisten für Thecamöben, Prof. Dr. Louis Bonnet (Toulouse).

8 µm langen Db sind hingegen dichter zusammengerückt. Sie stehen paarweise in drei Reihen, wobei die linke Reihe auf ein einziges Borstenpaar reduziert ist (Abb. 4 rechts). Der Mundwulst ist in typischer Weise ausgebildet. Das Bewegungsverhalten ist ganz ähnlich wie das der übrigen Vertreter dieser Gattung. Der Ciliat ist ein Räuber, der sich von kleinen Ciliaten ernährt. Ich habe ihn regelmässig in den Standorten „savane brûlée" und Galeriewald angetroffen. Durch seine Gestalt sowie seine ausserordentliche Biegsamkeit ist dieser bisher unbekannte Ciliat ausgezeichnet an das Leben im Boden angepasst.

Spathidium muscicola Kahl (Abb. 5 a–c)
Syn.: *S. atypicum* Wenzel, *S. alpinum* Gellért

Länge: 160–200 µm, Breite: ca 25 µm. Das Vorderende ist lateral mehr oder weniger abgeflacht, während die Gestalt im übrigen nahezu zylindrisch mit abgerundetem Hinderende ist. Der Ma ist langgestreckt und gewunden, Mi liegen in Mehrzahl vor. Das vom Mundwulst umgebene Cytostom ist spaltförmig und 25–30 µm lang. Es sind 23 Kineten vorhanden, in denen die 10 µm langen Cilien einzeln, aber ziemlich dicht stehen, besonders im Bereich des Wulstes und der Db. Die Db selbst ist dreireihig mit doppeltem Cilienbesatz. Die linke Reihe weist 29 Paare, die mittlere sowie die rechte Reihe weisen je 30 Paare von 3 µm langen Dorsalborsten auf. Dabei fällt im Präparat auf, dass die rechte Reihe durch besonders kräftige Borsten gekennzeichnet ist (Abb. 5c), welche immer dem jeweils hinteren Basalkörper entspringen. Man findet etwa 44 derartige Borsten vor, die sich etwa bis zur Körpermitte erstrecken. Diese rechte Dorsalreihe weist aber nur — wie erwähnt — 30 Kinetosomenpaare auf, die restlichen 14 Borsten stehen einzeln.

Die Somakineten enden nicht vor dem Mundwulst, sondern jede einzelne zieht noch ein kleines Stück parallel zu ihm. Diesen eigentümlichen Verlauf entnehme man den Abbildungen 5 b + c.

Die sich von Ciliaten räuberisch ernährende Art kommt in allen drei Untersuchungsarealen vor. Sie tritt selten in grösseren Anzahlen auf.

S. muscicola Kahl ist meiner Meinung nach identisch mit *S. atypicum* Wenzel (Wenzel 1953). Der Autor beschrieb eine Form, die von der Stammform *S. muscicola* hinsichtlich der Kernverhältnisse (1 Mi, 2 Ma) differiert. Kahl beobachtete offensichtlich die gleiche Variante und reiht sie unter *S. muscicola* ein. Die von Dragesco (1970) in Kamerun gefundene Form besitzt vier Kernteile. Ich fand ebenfalls bei Varianten aus Lamto vier Kernteile vor, die allerdings „kaffeebohnenartig" geformt waren. Der Ma der von Gellért (1954) beschriebenen Modifikation

ist U-förmig. Seine *S. alpinum* Gellért hingegen zeigt starke Ähnlichkeit mit der hier beschriebenen Form (Gellért 1955). Sie muss daher als selbständige Art fallengelassen werden.

Die Variationsbreite von *Spathidium* ist bekanntermassen sehr gross, und über hundert Arten sind bereits beschrieben worden (Dragesco 1970). Die Gattung bedarf einer dringenden Revision — ein sicherlich ausserordentlich schwieriges Unternehmen.

Unterklasse: *Hypostomata*
Ordnung: *Nassulida*
Unterordnung: *Microthoracina*
Familie: *Microthoracidae*

Drepanomonas revoluta Penard (Abb. 6)

Grösse: konstant 20–22 µm, Gestalt langgestreckt-oval, dorsoventral abgeflacht. Die Mundgrube ist klein und liegt links in der Mitte. Ein Ma und ein Mi vorhanden. Zwischen ihnen und der Mundgrube findet man eine cV. Der präorale Körperabschnitt ist auf der linken Seite mit 3 Rippen versehen. Dazwischen stehen einzelne 5 µm lange Cilien. Die übrigen Wimpern der frontalen Hälfte stehen paarweise zusammen. Ventral verlaufen 3 lockere Kineten, deren Cilien in der Mitte einzeln, im hinteren Bereich sowohl als Paare als auch einzeln stehen. Eine weitere Gruppe von Cilienpaaren befindet sich am rechten Rande der Mundgrube. Dorsal ist die Bewimperung sehr locker, die Cilien sind hier vereinzelt angeordnet. Die von Kahl erwähnte dorsale Längsfurche fand ich nicht bei allen Individuen vor. Protargolpräparate zeigen, dass Mundbucht drei kleine Membranellen stehen, deren Aufbau und Anordnung konstant sind. Diese Organell setzt sich aus nur 1–2 µm langen Cilien zusammen.

Es sei nochmals auf die einzelne cV hingewiesen. Unter ihr befindet sich zwar stets ein weiteres Gebilde, dass bei oberflächlicher Betrachtung mit einer zweiten cV verwechselt werden kann, jedoch pulsiert diese „Vakuole" nicht (Einlagerung von Öl o.ä, Reservestoffen?).

Wenzel (1953), der ebenfalls Exemplare dieser Art beschrieb, beobachtete, dass Formen mit und ohne Furchung auftreten können, und hob deswegen die Art *D. sphagni* Kahl auf, die sich nach Kahl (1931) durch das Fehlen der Furche von *D. revoluta* Penard unterschied. Die von mir beschriebene Form wird regelmässig in den Böden der „savane brûlée" und des Galeriewaldes angetroffen und ernährt sich von Bakterien.

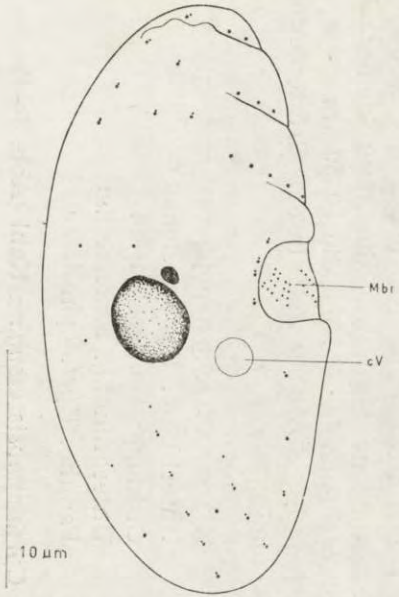

Abb. 6. *Drepanomonas revoluta* Penard, Aspekt der Ventralseite nach Protargolpräparaten

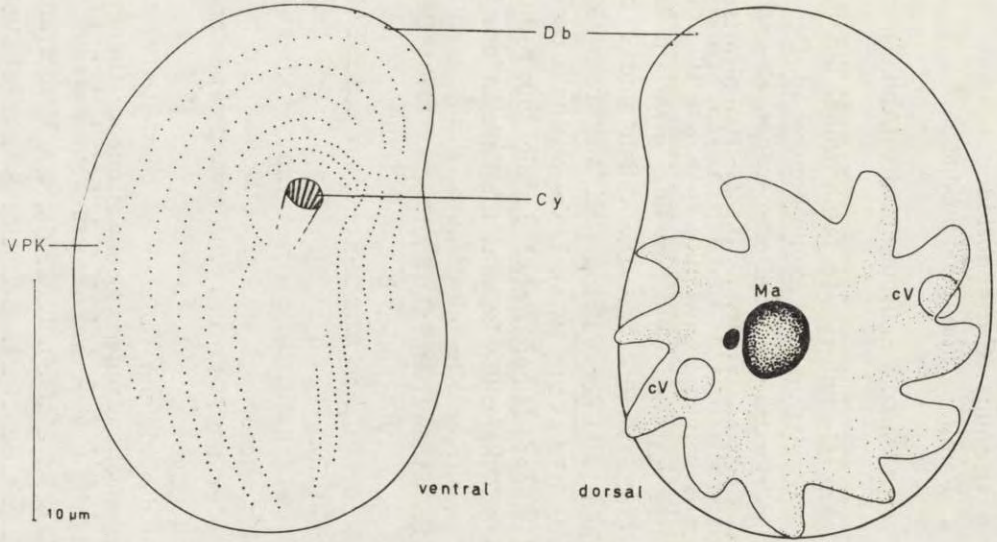

Abb. 7. *Chilodonella gouraudi* Certes, Ventral- und Dorsalseite, nach Protargolimprägnation

Ordnung: *Cyrtophorida*
Famile: *Chlamydodontidae*

Chilodonella gouraudi Certes (Abb. 7)

Grösse: ca. 25 µm, Gestalt relativ breit, wobei die linke Seite eine leichte Depression aufweist und die rechte konvex ist. Der dorsale Körper trägt „zahnradartige" Fortsätze, redial angeordnet. Er enthält einen runden oder ovalen Ma und den Mi. Die Zahl der Radialfortsätze, die unterschiedlich gross sein können, schwankt zwischen 10 und 15. Das von einer geraden Reuse gestützte Cytostom liegt etwa am Ende des ersten Körperdrittels auf der Ventralseite. Die Cilien sind 4 bis 5 µm lang. Neben der typischen Lage der Vakuolen erinnert auch die Bewimperung sehr an *C. uncinata* (Pätsch 1974). Links oberhalb des Cytostoms beginnend ziehen 4 längere Kineten etwa parallel zur rechten Körperseite nach hinten. Die 5. Cilienreihe beginnt im Bereich des Cytostoms, das frontal noch von 3 weiteren Kineten gesäumt wird, 2 circumorale Kineten und einer präoralen Kinete (vgl. Grain et al. 1973). Der ventrale Teil der peripheren Kinete (VPK), der bei der Teilung eine wichtige Rolle spielt, besteht aus nur 3 Cilien (zur Morphogenese der *Chlamydodontidae* siehe: Deroux 1970, Deroux et Dragesco 1968). Links vom Cytostom stehen ebenfalls 5 Cilienreihen, die jedoch kürzer und etwas versetzt zueinander angeordnet sind. Das Dorsalbürstenorganell besteht aus 6 Borsten, die in der Mitte des Vorderrandes beginnend nach links auf die Ventralseite zieht.

Die Bewegung des kleinen Ciliaten ist charakteristisch gleitend. Als Nahrung dienten nach eigenen Beobachtungen Bakterien. Ich fand *C. gouraudi* nie häufig in den drei untersuchten Standorten.

Die Artdiagnose ist einfach durch die am Vorderrand stehende Db und vor allem durch den in Radialfortsätze gegliederten Dorsalkörper, der nach Kahl (1931) offenbar nur bei dieser Art beobachtet worden ist. Die von mir gefundene Form war durchweg kleiner als die von Kahl (1931) beschriebenen Individuen, die 30–50 µm gross sein sollen. Wenzel (1953) fand diese Art ebenfalls in den Grössenordnungen vor, wie Kahl (1931) angibt.

Klasse: *Oligohymenophora*
Unterklasse: *Hymenostomata*
Ordnung: *Scuticociliatida*
Unterordnung: *Philasterina*
Familie: *Cyrtolophosiidae*

Cyrtolophosis elongata Kahl (Abb. 8a–f)

Grösse: 15–35 µm. Gestalt lanzettlich, dorsoventral abgeflacht (Abb. 8 a).

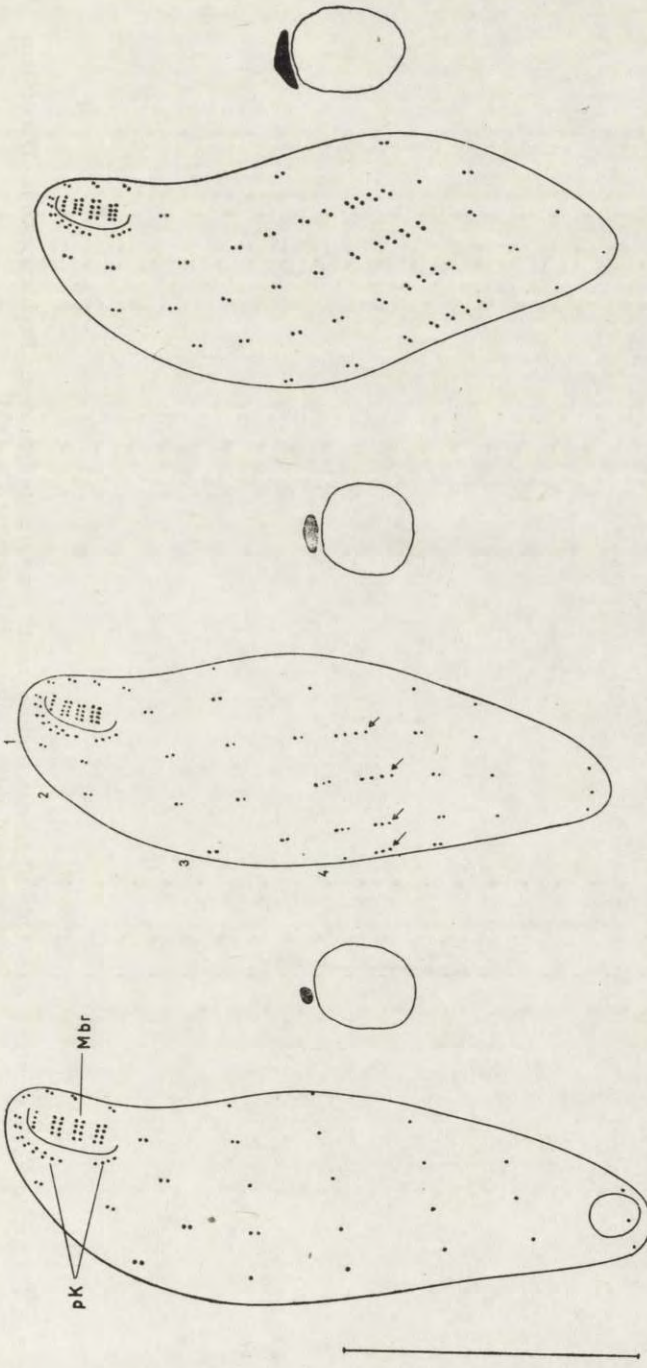

Abb. 8. a–c *Cyrtolophosis elongata* Kahl, Morphogenesestadien nach Protargolpräparaten. Rechts jeweils Darstellung der Zellkerne und ihrer Teilung. Erläuterung im Text

Abb. 8. d–f. *Cyrtolophosis elongata* Kahl, Morphogenesestadien nach Protargolpräparaten. Rechts jeweils Darstellung der Zellkerne und ihrer Teilung. Erläuterung im Text

Der Ciliat ist stark metabolisch. Er baut kein Gehäuse wie *C. mucicola* Stokes. Der runde Ma liegt mit einem Mi zentral, die cV terminal. Die Somaciliatur ist dorsal lockerer als ventral. Im Frontalbereich sind die Cilien in den Kineten paarweise angeordnet. *C. elongata* hat insgesamt 8 Somakineten.

Die Mundbucht wird rechts von einer paroralen Kinete umgeben, die in ihrem frontalen Abschnitt als Doppelkinete ausgebildet ist. Im hinteren Abschnitt stehen die Cilien einzeln. Dieser Teil der paroralen Kinete ist in seinem mittleren Bereich beim ausgewachsenen Tier nicht mit Protargol zu imprägnieren, so dass sich der Aspekt bietet, wie er in Abb. 8 dargestellt ist. Ob hier wirklich Cilien stehen oder nicht, war von mir nicht auszumachen. Während der Zellteilung ist die parorale Kinete jedenfalls bei einigen Stadien in ihrer vollen Länge imprägnierbar.

In der Mundbucht selbst stehen 4 Membranellen. Die erste ist einreihig, die hinteren 3 sind zweireihig. Jede Reihe setzt sich aus 4 Cilien zusammen.

Der Ciliat lässt sich als Bakterienfresser ausgezeichnet kultivieren, allerdings bricht eine Kultur nach etwa einer Woche zusammen, d.h. die Organismen encystieren sich wie viele andere Organismen des Bodens nach einer relativ kurzen Zeit der Entfaltung in den Kulturschälchen. *C. elongata* wurde als Kosmopolit in allen untersuchten Standorten teilweise häufig gefunden. Es ist ausserordentlich schwierig, den Ciliat mit Protargol zu imprägnieren.

Die Morphogenese, die wie die von *C. mucicola* Stokes nach McCoy (1974) zum somatischen Typus gehört, läuft folgendermassen ab:

Zu Anfang findet eine Proliferation von Kinetosomen der Kineten 1–4 hinter der Körpermitte statt (Abb. 8 b, Pfeile), gleichzeitig setzt die Teilung des Mi ein und der Ciliat beginnt sich zu verkürzen. Im weiteren Verlauf verdichten sich die neu gebildeten Kinetosomen, der Verlauf der Kineten wird deutlicher spiralig, der Mi streckt sich und der Zelleib wird breiter (Abb. 8 c). Aus den verdichteten Kinetosomen bilden sich die Anlagen der neuen Membranellen und der paroralen Kinete der hinteren Tochterzelle heraus (Abb. 8 d). Die Anlage der paroralen Kinete besteht aus 7 Kinetosomenpaaren, die der zukünftigen Membranellen aus jeweils zweireihigen Kineten. Die alte parorale Kinete ist im Protargolpräparat nicht mehr durch einen unimprägnierten Bereich geteilt, sondern durchgehend sichtbar. In diesem Stadium hat der sich teilende Ciliat seine grösste Breite und seine stärkste Verkürzung erfahren, während sich der Ma einschnürt und die Teilung des Mi weit vorangeschritten ist. Im nächsten Stadium (Abb. 8 e) ist der Organismus gestreckt und in seiner mittleren Zone unbewimpert. Die Anlage der paroralen Kinete der hinteren Tochterzelle hat ihren Kinetosomenbesatz verdoppelt. Auch die

Membranellen der beiden Tochterzellen haben ein Vermehrung ihrer Kinetosomen erfahren: die jeweils vorderen Membranellen sind zweireihig, die übrigen sind dreireihig geworden (Pfeile: Vermehrung der Kinetosomen). Der Ma teilt sich. Während der Einschnürung der Zelle findet eine Drehung der vorderen zur hinteren Tochterzelle statt (Abb. 8 f). Die Kernteilung ist fast abgeschlossen. Die Kinetenzahl der Membranellen sind nach der Teilung wieder reduziert. Ob dabei die Kinetosomen resorbiert werden oder irgendwelche andere Funktionen übernehmen, konnte ich nicht beobachten. Im übrigen bleibt die alte Mundbewimperung erhalten und wird von der vorderen Tochterzelle übernommen. Damit entspricht die Morphogenese prinzipiell dem Schema, welches McCoy (1974, und pers. Information) für *C. mucicola* Stokes angibt („somatische" Stomatogenese).

Die systematische Stellung der Gattung *Cyrtolophosis* ist noch nicht vollständig geklärt. Ich schliesse mich dem Vorschlag McCoys an, der die Wiedereinrichtung der Familie *Cyrtolophosiidae* mit der einzigen Gattung *Cyrtolophosis* anregt. Eine phylogenetische Verwandtschaft mit den übrigen Vertretern der *Scuticociliatida* scheint gesichert zu sein (vergl. Small 1967, McCoy 1974).

Auffallend ist die sehr grosse Ähnlichkeit des Teilungsmodus mit der Gattung *Platyophrya*, der von Grollière (1975) wiedergegeben wird. *Platyophrya* ist aber durch den Besitz einer kräftigen Reuse phylogenetisch tiefer zu stellen. Auch diese Gattung ist noch von unsicherer systematischer Stellung. Möglicherweise kann eine ultrastrukturelle Bearbeitung der Mundorganellen und der Stomatogenese zur Klärung dieser eigentümlichen Ähnlichkeit der Gattung führen.

Familie: *Cinetochilidae*

Sathrophilus muscorum Kahl (Abb. 9)

Grösse: 25–35 µm. Gestalt oval, dorsoventral abgeflacht. Der runde Ma liegt mit einem Mi zentral, die cV terminal. Das Cytostom befindet sich oberhalb des Äquators.

Insgesamt findet man 15 Kineten vor. Jedoch weisen die tropischen Formen, die meist ca. 5 µm kleiner als die mitteleuropäischen sind, 12 Kineten auf. Im apikalen Bereich aller Kineten stehen die Cilien doppelt, während sie sonst einfach angeordnet sind. Die Cilien sind in der vorderen Region dichter zusammengerückt als in der hinteren. Während die Somacilien etwa 8 µm lang sind, ist die Caudalcilie körperlang.

Die Buccalciliatur setzt sich nach dem typischen Schema für die *Scuticociliatida* (M_1, M_2, M_3 und UM) zusammen. Jede Membranelle ist drei-

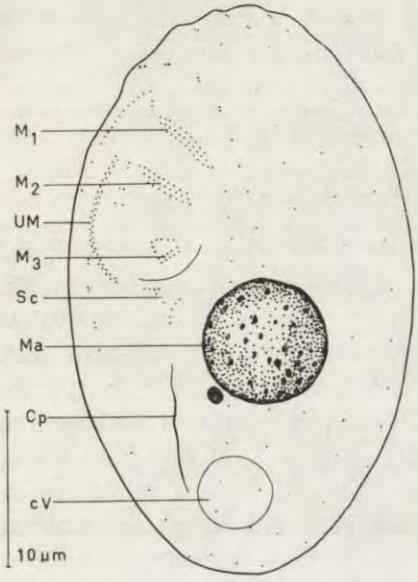

Abb. 9. *Sathrophilus muscorum* Kahl, Infraciliatur der Ventralseite (nach Protargol)

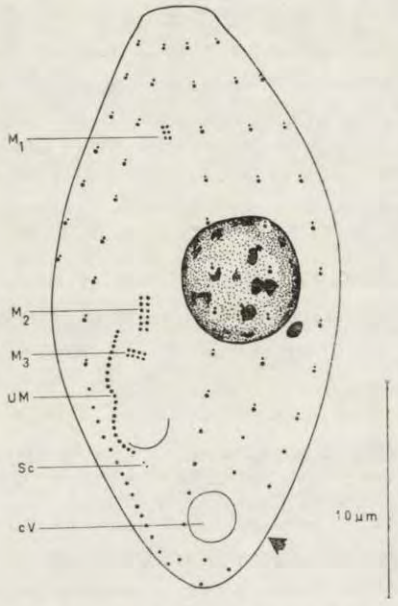

Abb. 10. *Homalogastra setosa* Kahl, Infraciliatur nach Protargolimprägnation. Der Pfeil weist auf die wimperfreie Zone nahe des caudalen Poles

reihig, wobei M_3 etwas abweichend aufgebaut ist. Zwischen der M_2 und der UM findet man stets ausserdem einige einzelne Cilien. Die Wimpern der beiden rechts von der UM stehenden Somakineten sind in ihren Frontalabschnitten so eng aneinandergerückt, dass der Eindruck zusätzlicher Membranen entsteht.

Damit liegen ähnliche Verhältnisse vor, wie bei der Art *Paradexiotricha puytoraci* Grolière, bei der dieses Organell in Einzahl vorliegt und als zusätzliche parorale Membran bezeichnet wird (Grolière 1974–75). Unterhalb der Mundbucht befindet sich im Anschluss an die UM der Scuticus, der bei der Zellteilung eine wichtige Rolle spielt (Small 1967). Zwischen ihm und dem caudalen Pol liegt die Cytopyge.

S. muscorum gehört zum „semi-autonomen" Morphogenesetypus, d.h. an der Neubildung der Mundorgallen sind sowohl Scuticus als auch die rechte postorale Kinete beteiligt (Grolière 1973). Die Morphogenese läuft nach dem Schema ab, das Grolière (1973, 1974–75) für *S. vernalis* Dragesco und Grolière beschrieben hat.

S. muscorum ernährt sich von Bakterien und lässt sich relativ leicht kultivieren. Der Ciliat kommt in der „savane brûlée" und im Galeriewald vor.

Beschreibungen dieser Art wurden von Czapik (1968 a) und Thompson und Cone (1968) veröffentlicht, wobei jedoch morphologische Einzelheiten nicht angegeben wurden, so dass eine Neubeschreibung nach Protargolpräparaten erforderlich war.

Familie: *Uronematidae*

Homalogastra setosa Kahl (Abb. 10)

Grösse: 20–30 µm. Gestalt gestreckt oval, mit einer Depression auf dem letzten Drittel der Ventralseite. Ma in Ein- oder Zweizahl, ein Mi, cV terminal. Das Cytostom liegt deutlich hinter dem Äquator. Der Ciliat weist wie viele *Scuticociliatida* eine unbewimperte Frontalplatte auf. Eine weitere cilienfreie Zone findet man im caudalen Bereich (Pfeil): Sie ist ringförmig und nur auf der Ventralseite durch Cilien unterbrochen. Der Ciliat besitzt eine Caudalwimper, die mit etwa 30 µm körperlang ist. 11–13 Kineten laufen spiralig über den Körper. Vom Frontalbereich bis in die Höhe des Cytostoms stehen die 10 µm langen, spreizbaren Cilien paarig angeordnet, wobei jeweils der frontal liegende Kinetosom kleiner als der hinter ihm liegende ist. Die Somakinete, die rechts von der UM verläuft, unterbricht unterhalb des Cytostoms den wimperfreien Ring (Pfeil) durch einige einzelne Cilien, die in einer relativ dichten Reihe his zum Hinterende ziehen. Auch die links vom Cytostom stehende Kinete unterbricht diesen Ring mit wenigen Cilien.

Die Mundbewimperung setzt sich wie folgt zusammen: es sind drei Membranellen vorhanden, wobei M_1 weit nach frontal mit deutlichen Abstand zu M_2 verlagert ist. M_2 und M_3 liegen dicht beieinander. Rechts von ihnen beginnt die 5 µm hohe UM, die zwei leichte Bögen beschreibend in die ventrale Depression zieht. An ihrem Ende liegt der oft schwer erkennbare Scuticus, der sich aus nur 2 Kinetosomen zusammensetzt.

H. setosa ist ein Bakterienfresser, der in allen untersuchten Standorten in manchmal grossen Zahlen vorkommt.

Diese von Kahl (1931) zuerst beschriebene Form ist einigen *Uronema*-Arten sehr ähnlich. Die grösste Ähnlichkeit weist *H. setosa* mit den Arten *U. marinum* Duj., *U. elegans* Maupas, *U. filificum* Kahl und *U. parva* Czapik (vgl. Borror 1963, Thompson und Kaneshiro 1968, Czapik 1968 b). Als gemeinsame morphologische Merkmale sind folgende zu erwähnen: Frontalplatte, Kinetenanzahlen, ähnlich gestaltete UM, ein grösserer Abstand Zwischen M_1 und M_2 und — wie bei *U. parva* — ein hinter dem Äquator liegendes Cytostom. Von den genannten Formen setzt sich *H. setosa* ab durch die gestreckte ovale Gestalt, der eigentümlich wimperfreien Zone am Hinterende sowie den spiraligen Kinetenverlauf.

Sollte sich der Morphogeneseablauf mit dem der *Uronema*-Arten decken, so wäre es naheliegend, die Gattung *Homalogastra* aufzuheben und in die Gattung *Uronema* einzugliedern.

Klasse: *Polyhymenophora*
Unterklasse: *Spirotricha*
Ordnung: *Heterotrichida*
Unterordnung: *Heterotrichina*
Familie: *Spirostomatidae*

Blepharisma hyalinum Perty (Abb. 11)

Länge: ca. 100 µm; mehr oder weniger spindelförmige Gestalt, schmal, „schiffchenartig". Hinterende zugespitzt, manchmal leicht abgerundet, oft vakuolisiert. Ma oval, Mi einfach (laut K a h l 1932, sind 2–3 Mi

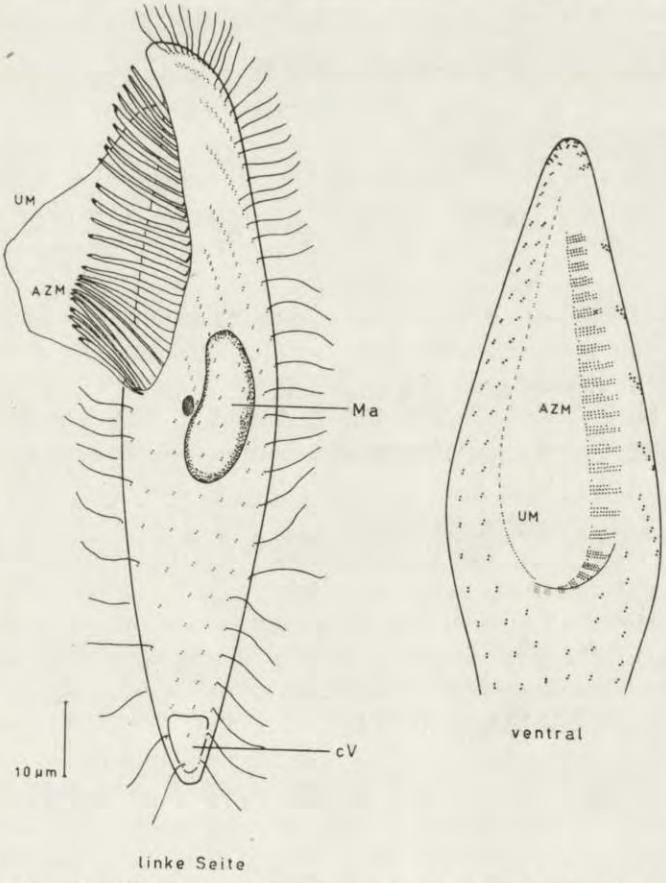

Abb. 11. *Blepharisma hyalinum* Petry, Aspekt der linken Seite (links) und Sicht auf die Peristomregion (rechts) nach Lebendbeobachtungen und Protargolpräparaten

vorhanden). Die cV liegt nicht ganz terminal. Etwa die Hälfte des Körpers wird von dem Peristom eingenommen (Abb. 11 rechts). Hier befinden sich auf der linken Seite ca 25 Membranellen, die einen dreireihigen Aufbau zeigen und etwa 15 µm hoch sind. Sie beginnen etwa vom apikalen Pol abgesetzt und verlaufen unter einer Rechtsdrehung ins Cytostom. Auf der rechten Seite des Peristoms steht die 12 µm hohe UM, die aus Doppelcilien aufgebaut ist. Parallel zu ihr verlaufen die Somakineten der rechten Seite, während sie links nicht parallel zum Peristom angeordnet sind (Abb. 11 rechts). Die 12 µm langen Cilien stehen immer paarweise zusammen, wobei die vordere Körperhälfte dichter bewimpert ist.

B. hyalinum ernährt sich von Bakterien. Ich fand den Ciliat nur in der „savane brûlée", jedoch nie in grösserer Anzahl.

Ordnung: *Hypotrichida*
Unterordnung: *Stichotrichina*
Familie: *Holostichidae*

Holosticha distyla n. spec. (Abb. 12)

Länge: 150–180 µm. Bandförmige Gestalt, biegsam. Die 16 Kernteile des Ma liegen links von der Körperachse. Nur zwei Mi konnten erkannt werden. Die cV liegt links zwischen dem Cytostom und der Körpermitte.

Die AZM, bestehend aus 30–33 Membranellen, ist vergleichsweise lang: sie nimmt etwa ein Drittel der Gesamtlänge ein. Die endorale sowie die parorale Membran sind nur einreihig. 4–5 kräftige Cirren stehen auf dem Frontalfeld. Rechts von ihnen beginnen die beiden Ventralreihen. Sie setzen sich aus 33 bis 34 Cirren zusammen. Vor den 2 Transversalcirren, die 18 µm lang sind, stehen ausserdem noch 2 sehr schwache Ventralcirren. Die Marginalreihen sind durch relativ dicht stehende Cirren gekennzeichnet, die den gleichen Aufbau wie die Ventralcirren besitzen. Die rechte Marginalreihe besteht aus 43, die linke aus 34 Cirren, die ebenso wie die Ventralcirren 12 µm lang sind. Im übrigen stossen die Marginalreihen nicht zusammen, sondern überkreuzen sich. Caudalcirren fehlen völlig. Dorsal verlaufen 4 Reihen von Borsten, wobei jede Reihe 20 Borsten von 5 µm Länge aufweist.

Der Ciliat, der sich von Testaceen und Flagellaten ernährt, wurde nur in der „savane non brûlée" und im Galeriewald gefunden. Diese bisher unbekannte Form unterscheidet sich von den übrigen *Holosticha*-Arten durch den Besitz von konstant 2 kräftigen Transversalcirren. Nach diesem Merkmal wählte ich den Artnamen.

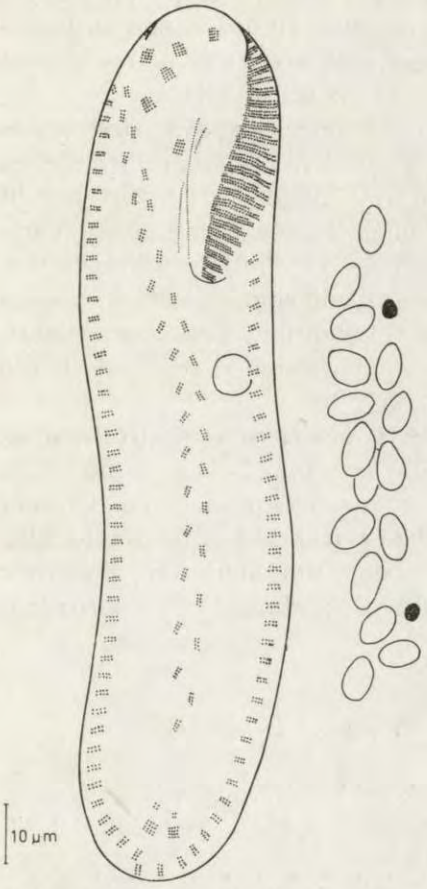

Abb. 12. *Holosticha distyla* spec. n., Kinetom der Ventralseite nach Protargolpräparation. Rechts Darstellung der Kernverhältnisse

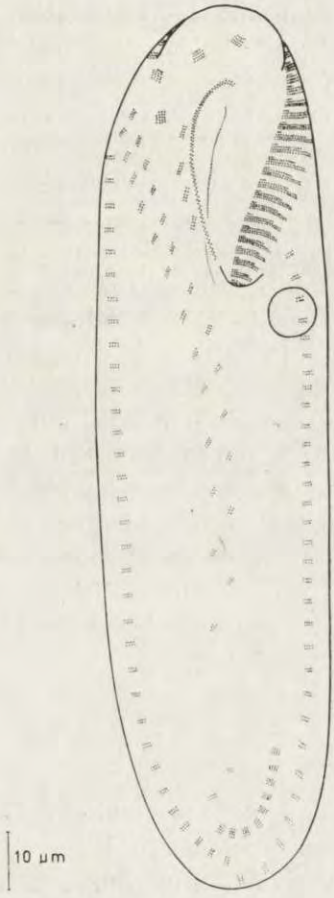

Abb. 13. *Holosticha multistilata* Kahl, Kinetom der Ventralseite (Protargolimprägnation). Kernteile des Ma sehr zahlreich, nicht abgebildet

Holosticha multistilata Kahl (Abb. 13)

Länge: 170–200 µm. Schlank ellipsoide Gestalt. Die Ma-Fragmente sind sehr zahlreich, die Anzahl der Mi konnte ich nicht feststellen. Die cV liegt zwischen dem Cytom und der Körpermitte auf der linken Seite.

Die AZM nimmt weniger als ein Drittel der Körperlänge ein und setzt sich aus 26 Membranellen zusammen. Die parorale Membran ist zweireihig, die endorale einreihig mit sehr dichten Cilien. Das Frontalfeld ist mit 4 kräftigen, 15 µm langen Frontalcirren ausgestattet, ausser-

dem sind 3–4 schwächere Buccalcirren an der Peristomlippe vorhanden. In den beiden Ventralreihen sind 26 Cirren von 10 µm Länge zickzackförmig angeordnet. Dicht vor den Transversalcirren stehen noch zwei zusätzliche Ventralcirren. Die Transversalcirren selbst bilden eine J-förmige Reihe, die sich aus 6–11 Cirren zusammensetzt. Sie sind etwas stärker als die Ventral- und Marginalcirren. In den Marginalreihen stehen rechts 35–55, links 30–52 Cirren von 10 µm Länge. Es ist also sowohl im Transversalcirren- als auch im Marginalcirrenbesatz eine grosse Variabilität zu registrieren, die von der Grösse der Organismen abhängig ist. Dorsal stehen 4 Reihen von Borsten, die 4 µm lang sind.

Der Ciliat ist ein Räuber, der sich von Ciliaten und Testaceen ernährt. Er wurde im Mischwald und im Galeriewald gefunden, tritt jedoch nie in grösserer Anzahl auf.

H. multistilata wurde von Kahl (1932) als *Keronopsis* (*Holosticha*) *multistilata* beschrieben. Gleichzeitig gibt er eine Beschreibung eines offensichtlich identischen Infusors: *Keronopsis muscorum* Kahl. Obwohl er für beide Formen grössere Masse abgibt (ca. 250 µm), dürfte hier ebenfalls die gleiche Art vorliegen. In der Revision von Borror (1972 a) werden beide Formen unter der Bezeichnung *H. multistilata* aufgeführt.

Lamtostyla lamottei n. gen. n. spec.[3] (Abb. 14)

Länge: um 100 µm; Gestalt ellipsoid, kurz bandförmig mit gleichartig gerundetem Vorder- und Hinterende. Es sind 2 Ma — Teile und 3 Mi vorhanden, cV links vor der Körpermitte.

Die AZM ist relativ kurz und setzt sich aus 19 Membranellen von 12 µm Länge zusammen. Die endorale und die parorale Membran sind einfach, wobei die endorale aus feineren Cilien aufgebaut ist. Auf dem Frontalfeld findet man neben drei verstärkten Griffeln 4 weitere Cirren, von denen einer an der Peristomlippe als Buccalcirrus steht. Eine stark verkürzte Ventralreihe, bestehend aus 10 Cirren, beginnt rechts vom Frontalfeld und endet kurz hinter dem Cytostom. Weitere postorale Ventralcirren fehlen. Kurz vor dem caudalen Pol inserieren 2 Transversalcirren, die 16 µm lang sind. Die Cirrenanzahl in den Marginalreihen schwankt je nach Grösse des Individuums: rechts findet man 28–35, links 24–32 Cirren von einer Länge vom 10 µm. Eigenartigerweise ist auch eine Veränderung des Cirrenaufbaus in den Marginalreihen feststellbar.

[3] Benannt nach Prof. Dr. Maxime Lamotte, Paris, Leiter von Lamto währed des IBP.

Oberhalb der Mitte findet man einen Cirrenaufbau von meist 3 × 2 Cilien, unterhalb einen Aufbau von 2 × 2 Cilien vor. Diese Reduzierung der Cirrenstärke von vorn nach hinten war stets zu beobachten. Im übrigen ist der Cirrengrundriss allgemein variabel.

Caudalcirren sind nicht vorhanden. Auf der Dorsalseite stehen 4 Reihen von 3 µm langen Borsten.

Der Ciliat, der vereinzelt im Boden der „savane brûlée" vorkommt, ist ein Infusorienräuber, frisst aber auch Diatomeen.

Obwohl ihr Teilungsmodus nicht erkannt werden konnte, vermute ich, dass die Gattung *Lamtostyla* zu den *Holostichidae* gehört, wobei die verkürzte Ventralreihe als Gattungsmerkmal anzusehen wäre.

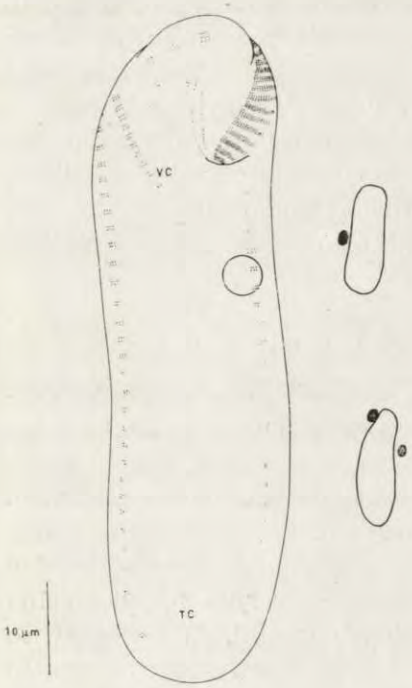

Abb. 14. *Lamtostyla lamottei* gen. n. spec. n., Kinetom der Ventralseite nach Protargolimprägnation. Die Kernverhältnisse sind rechts dargestellt

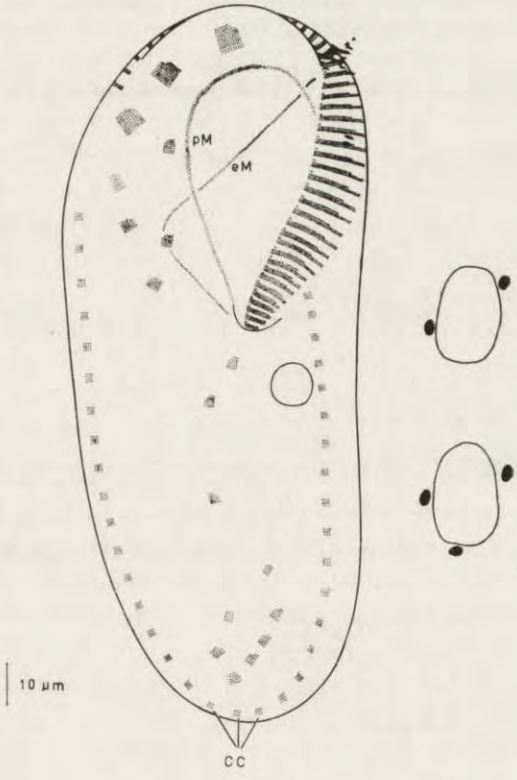

Abb. 15. *Oxytricha platystoma* Ehrb., Kinetom der Ventralseite nach Protargolpräparaten. Kerne rechts abgebildet

Unterordnung: *Sporadotrichina*
Familie: *Oxytrichidae*

Oxytricha platystoma Ehrb. (Abb. 15)
(= *Steinia platystoma* Ehrb. — Stein)

Grösse: 200–250 µm. Ovoide Gestalt, vorn breiter gerundet als hinten. Die beiden Ma-Teile sind von 5 Mi umgehen, cV in der Mitte links.

Die AZM zieht fast bis zur Körpermitte und wird von ca. 45 Membranellen gebildet. Die parorale Membran verläuft in grossem Bogen und stösst an die AZM, die endorale weist einen rechtwinkeligen Knick auf und zieht ebenfalls zur AZM. Beide Membranen sind zweireihig, jedoch ist die parorale Membran in ihrem mittleren Bereich aus drei Reihen aufgebaut.

Die Stellung der Cirren entspricht dem *Oxytricha*-Schema, sie sind aber teilweise ungewöhnlich kräftig (Frontalgriffel aus 10 × 12 Cilien). Die rechte Marginalreihe besteht aus 17, die linke aus 16 Cirren. Die Cirren sind etwa 35 µm lang. Die Borsten der Dorsalseite verlaufen in 5 Reihen und sind 4 µm hoch. Die Teilung des Tieres setzt ein mit einer Proliferation von Kinetosomen des ersten Transversalcirrus, entsprechend den Befunden von Grolière (1969) bei einigen *Oxytricha*-Arten.

Der Ciliat frisst Algen und Flagellaten und wurde nur im Boden der „savane non brûlée" angetroffen, wo er regelmässig auftritt. Ähnliche, aber nicht völlig übereinstimmende Formen fanden in neurer Zeit Dragesco (1970) in Kamerun und Wilbert (pers. Information) im Grossen Heiligen Meer (bei Rheine/Westfalen).

Oxytricha setigera Stokes (Abb. 16)

Grösse: 40–50 µm. Gestalt gestreckt oval, sehr biegsamer Vorderkörper. Der Ma ist zweiteilig, dazwischen findet sich ein einzelner Mi. Die cV liegt links in der Körpermitte.

Die AZM ist aus 12–13 Membranellen aufgebaut, von denen die ersten drei isoliert vom übrigen Strudelorganell stehen. Es ist nur eine undulierende Membran vorhanden, die sich aus einfachen, locker stehenden Cilien zusammensetzt. Die Cirrenausstattung ist zwar typisch, jedoch sind die Frontoventralcirren weiter als gewöhnlich nach hinten verlagert, so dass Frontalcirren auch noch im postoralen Feld angetroffen werden. In den Marginalreihen stehen jeweils 5 Cirren sehr weit auseinander. Die Cirren werden aus 4 Cilien gebildet, worin nur die drei vordersten Frontalsowie Transversalcirren eine Ausnahme darstellen (2 × 3 Cilien). Die Marginal- und Ventralcirren sind 10 µm, die Transversalcirren 20 µm lang. Die Dorsalborsten, die in 4 Reihen angeordnet sind, sind 10 (!) µm lang.

O. setigera frisst Bakterien und wurde häufiger in der „savane brûlée" angetroffen.

O. setigera wird von Borror (1972a) mit *Tachysoma pellionella* O.F.M. synonymisiert, jedoch sicherlich nicht zu Recht. Die auffälligen Caudalcirren sprechen für eine Beibehaltung der früheren systematischen Stellung, da bei *Tachysoma* keine Caudalcirren vorhanden sind.

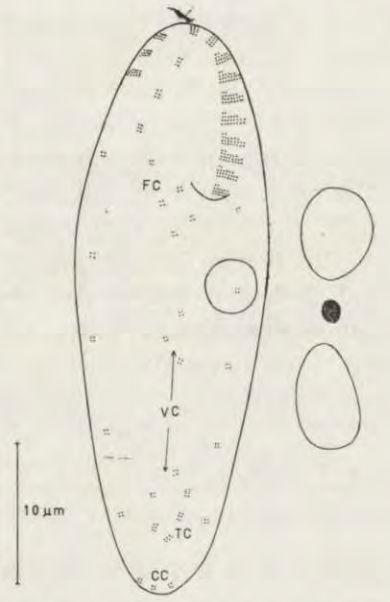

Abb. 16. *Oxytricha setigera* Stokes, Kinetom der Ventralseite nach Protargolpräparation. Kerne rechts abgebildet

Oxytricha tricirrata n. spec. (Abb. 17)

Grösse: 80 µm. Gestalt oval, wenig biegsam. Ma und Mi in Zweizahl vorhanden, cV in der Mitte links. Die AZM besteht aus 23–25 Membranellen und reicht nicht ganz bis zur Körpermitte. Die parorale und die endorale Membran sind einfach und locker aufgebaut. Die Stellung der Cirren ist für *Oxytricha*-Arten typisch, allerdings sind stets nur 3 Transversalcirren vorhanden. Die rechte Marginalreihe setzt sich aus 11–13, die linke aus 10—13 Cirren zusammen. Die Cirren sind 10 µm lang. Dorsal stehen die 2 µm langen Borsten in 5 Reihen.

O. tricirrata frisst kleinste Infusorien und Amöben. Ich fand diese Art nur im Boden der „savane brûlée".

Gewisse Ähnlichkeit mit dieser Art zeigt die Form *O. fallax* Stein. *O. tricirrata* unterscheidet sich von ihr hauptsächlich durch den Besitz von nur 3 Transversalcirren, einfachen Membranen an der Peristomlippe, wenigen Marginalcirren sowie durch eine geringere Grösse.

Histriculus similis (Quenn.) Corliss, forma *tricirratus* (Abb. 18)

Grösse: 120 µm. Gestalt oval, seitlich nicht biegsam, dorsoventral stärker abgeflacht als andere Oxytrichiden. Der Ma ist vierteilig und in Kettenform links von der Körperachse gelegen; 4 Mi sind vorhanden. Die cV liegt links in der Körpermitte.

Die AZM besteht aus 22–23 Membranellen, die parorale und die feinere endorale Membran sind als Doppelreihen ausgebildet. Ausser der für

die Oxytrichiden typischen Cirrenanordnung findet sich noch ein sehr kleiner Cirrus zwischen dem 2. und 3. Frontalcirrus (Pfeil). Die rechte Marginalreihe besteht aus 20, die linke aus 17 Cirren. Die Teilung der Zelle beginnt hier ebenfalls mit einer Proliferation des ersten der drei Transversalcirren. Im allgemeinen sind die Cirren etwa 15 µm lang. Die Dorsalborsten sind 3 µm lang und in 6 Reihen angeordnet.

H. similis, der sich von Blaualgen und Bakterien ernährt, fand sich nur im Boden der „savane non brûlée".

Durch den Besitz von konstant nur 3 Transversalcirren weicht die hier beschriebene Form vom Grundtyp leicht ab, besitzt aber wie dieser den vierteiligen Ma (vgl. K a h l 1932, R e u t e r 1963, B o r r o r 1972 b).

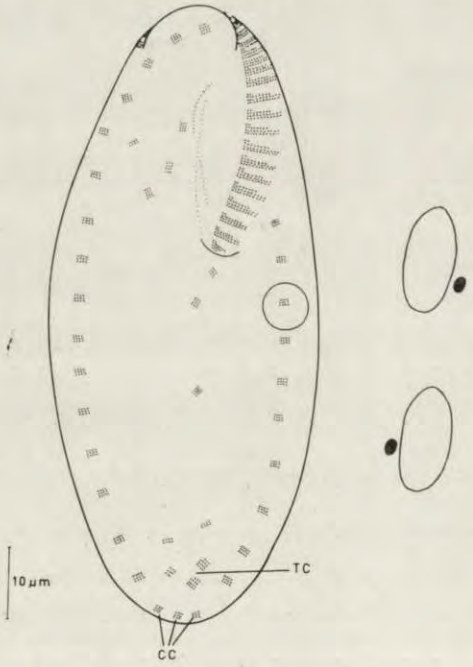

Abb. 17. *Oxytricha tricirrata* n. spec., Kinetom der Ventralseite nach Protargolimprägnation. Rechts Darstellung der Kernverhältnisse

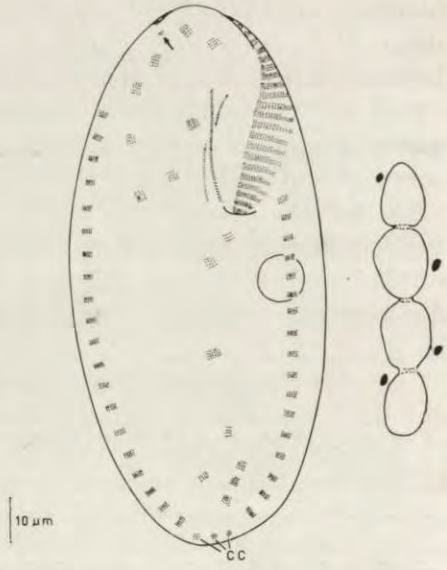

Abb. 18. *Histriculus similis* (Quenn.) Corliss, forma *tricirratus*; Kinetom der Ventralseite nach Protargolimprägnation. Die Kernverhältnisse sind rechts dargestellt. Der Pfeil weist auf den kleinen Cirrus zwischen dem 2. und 3. FC

RÉSUMÉ

On a recherché la composition des espèces des Ciliés du sol trouvés dans trois emplacements africains („savane brûlée", „foret-galerie" près des Lamto, Côte d'Ivoire). Entre les 40 espèces on a trouvé quatre formes nouvelles (*Holosticha distyla* spec. n., *Lamtostyla lamottei* gen. n. spec. n., *Oxytricha tricirrata* n. spec.,

Spathidium bonneti n. spec.). 18 espèces sont démontrées par des dessins; on décrit la morphogénèse de *Cyrtolophosis elongata* Kahl. Toutes les descriptions données sont principalement fondées sur la technique d'imprégnation au Protargol.

LITERATUR

Bodian D. 1936: A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat. Rec., 65, 89–97.

Borror A. C. 1963: Morphology and acology of the benthic ciliated *Protozoa* of Alligator Harbour, Florida. Arch. Prostistenk., 106, 465–534.

Borror A. C. 1972 a: Revision of the order *Hypotrichida* (*Ciliophora, Protozoa*). J. Protozool., 19, 1–23.

Borror A. C. 1972 b: Tidal marsh ciliates (*Protozoa*): morphology, ecology, systematics. Acta Protozool., 10, 29–71.

Buitkamp U. 1976: Ökologische und taxonomische Untersuchungen an Ciliaten ausgewählter Bodentypen. Dissertation der Mathem.-Naturwiss. Fakultät, Universität Bonn.

Corliss J. O. 1961: The Ciliated Protozoa. Characterization, classification, and guide to the literature. Pergamon Press, Oxford–London–New-York–Paris.

Corliss J. O. 1974 a: The changing world of ciliate systematics: historical analysis of past effort and a newly proposed phylogenetic scheme of classification for the protistan phylum *Ciliophora*. Syst. Zool., 23, 91–138.

Corliss J. O. 1974 b: Remarks on the composition of the large ciliate class *Kinetophragmophora* de Puytorac et al., 1974, and recognition of several new taxa therein, with emphasis on the primitive order *Primociliatida* n. ord. J. Protozool., 21, 207–220.

Corliss J. O. 1975: Taxonomic characterization of the suprafamilial groups in a revision of recently proposed schemes of classification for the phylum *Ciliophora*. Trans. Amer. micros. Soc., 94, 207–220.

Czapik A. 1968 a: La famille Tetrahymenidae et son importance dans la systématique et l'évolution des ciliés. Acta Protozool., 5, 315–357.

Czapik A. 1968 b: La morphologie de *Uronema elegans* Maupas et de *Uronema parva* n. sp. Acta Protozool., 5, 225–228.

Czapik A. 1971: Les observations sur *Playophrya spumacola* Kahl. Acta Protozool., 8, 363–366.

Deroux G. 1970: La série „chlamydonellienne" chez les *Chlamydodontidae* (holotriches, *Cyrtophorina* Faure-Fremiet). Protistologica, 6, 155–182.

Deroux G. und Dragesco J. 1968: Nouvelles données sur quelques cilies holotriches cyrtophores à ciliature ventrale. Protistologica, 4, 365–403.

Dragesco J. 1962: L'orientation actuelle de la systématique des ciliés et la technique d'imprégnation au proteinate d'argent. Bull. Microsc. Appl., 11, 49–58.

Dragesco J. 1970: Ciliés libres du Cameroun. Ann. Fac. Sci. Yaoundé, Yaoundé.

Gellért J. 1954: Die Ciliaten des sich unter der Flechte *Parmelia saxatilis* Mass. gebildeten Humus, Acta Biol. Acad. Sci. Hung., 6, 77–111.

Gellért J. 1955: Ciliaten des sich unter dem Moosrasen auf Felsen gebildeten Humus. Acta Biol. Acad. Sci. Hung., 6, 337–359.

Grain J., Puytorac P. de, et Bohatier J. 1973: Essai de systématique des ciliés gymnostomes fondée sur les characteristiques de l'infraciliature circumorale. C. r. Acad. Sci. Paris, 277, serie D, 69–72.

Grolière C. A. 1969: Etude comparée de la morphogenèse au cours de la bipartition, chez plusieurs espèces de ciliés hypotriches. Ann. Stat. Biol. Besse-en-Chandesse, 4, 335–365.

Glorière C. A. 1973: Description de quelques espèces de ciliés hyménostomes des genres *Sathrophilus* Corliss, 1960, *Cyclidium* O. F. Müller, 1786, *Histiobalantium* Stokes, 1886. J. Protozool., 20, 369–376.

Grolière C. A. 1974–75: Etude de quelques ciliés hyménostomes des eaux acides de la région de Besse-en-Chandesse. Ann. Stat. Biol. Besse-en-Chandesse, 9, 79–109.

Grolière C. A. 1975: La stomatogenèse du cilié *Platyophrya spumacola* Kahl, 1927; son inérét por la comprehension de la diversification buissonnante des *Kinetophragmophora* de Puytorac et coll. C. r. Acad. Sci. Paris, 280, série D, 861–864.

Jankowski A. W. 1967: A new system of ciliate *Protozoa* (*Ciliophora*). Akad. Nauk SSSR, Trudy Zool. Inst., 43, 3–54.

Jankowski A. W. 1973. Taxonomic revision of subphylum *Ciliophora* Doflein, 1901. Zool. Zh., 52, 165–175.

Kahl A. 1930–35: Wimpertiere oder Ciliate (*Infusoria*). In: Die Tierwelt Deutschlands, (ed. Dahl F.) Teile 18, 21, 25, 30, Jena.

McCoy J. W. 1974: Biology and systematics of the ciliate genus *Cyrtolophosis* Stokes, 1885. Acta Protozool., 13, 41–52.

Pätsch B. 1974: Die Aufwuchsciliaten des Naturlehrparks Haus Wildenrath. Monographische Bearbeitung der Morphologie und Ökologie. Arbeiten aus dem Institut für Landwirtschaftliche Zoologie und Bienenkunde Bonn, Nr. 1.

Puytorac P. de, Batisse A., Bohatier J., Corliss J. O., Deroux G., Didier P., Dragesco J., Fryd-Versavel G., Grain J., Grolière C. A., Horvasse R., Iftode F., Laval M., Roque M., Savoie A. et Tuffrau M. 1974: Proposition d'une classification du phylum *Ciliophora* Doflein, 1901. C. r. Acad. Sci. Paris, 278, 2799–2802.

Reuter J. 1963: Some ciliates from western Norway. Sarsia, 10, 1–8.

Small E. B. 1967: The *Scuticociliatida*, a new order of the class *Ciliatea* (phylum *Protozoa*, subphylum *Ciliophora*). Trans. Am. Microsc. Soc., 86, 345–370.

Thompson J. C. und Cone M. V. 1968: A derescription of the hymenostome ciliate *Sathrophilus* (*Saprophilus*) *muscorum* (Kahl, 1931) Corliss, 1960, with particular attention to the buccal apparatus. VA J. Sci., 14, 16–22.

Thompson J. C. und Kaneshiro E. S. 1968: Redescription of *Uronema filificum* and *U. elegans*. J. Protozool., 15, 141–144.

Tuffrau M. 1967: Perfectionnements et pratique de la technique d'impregnation au Protargol des Infusoires ciliés. Protistologica, 3, 91–98.

Wenzel F. 1953: Die Ciliaten der Moosrasen trockener Standorte. Arch. Protistenk., 99, 70–141.

Wilbert N. 1975: Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos, 6, 171–179.

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Some Ultrastructural Variations of the Nucleus in Dinoflagellates throughout the Life Cycle

Synopsis. Nuclear division is the main determinant of the ultrastructural variation considered in this note. In the studied material, direct division is more often observed than mitosis, and the near cause for this is supposed to be the great increase in chromatic material observed in the early stage of division. Direct nuclear division, which may be unequal, and the development of the "small form" are quite dependent on the two characters of the Dinoflagellate nucleus: polyteny and aneuploidy. Some other nuclear ultrastructural variations are related to the ageing of the cell, itself depending on the ageing of the culture.

We have been studying nuclear division in about 20 species of Dinoflagellates and the related ultrastructural variation of the nucleus throughout the life cycle in some of them. Beyond mitosis, studied by several authors (Dodge 1964, Leadbeater and Dodge 1967) we believe that direct division, described by earlier workers (Apstein 1911, Borgert 1910), is a common way of multiplication in those microorganisms and must be revived and searched with the recent techniques for electron microscopy. Since our observations under the light microscope have been described and discussed before (Silva 1969, 1971), we are now considering the great variability of the nuclear fine structure seen in some 1974 a, b, c) those "fragments" could be the chromosomal units, but also to the ageing of the cell.

Material and Methods

Among the species cultured and observed in the electron microscope, we have given more attention to the following: *Gymnodinium splendens* Leb., *Gyrodinium instriatum* Freud. and Lee, *Peridinium trochoideum* (Stein) Lemm., *Gonyaulax tamarensis* Leb., *G. spinifera* (Clap. and Lach.) Dies. and *G. polyedra* Stein. These species are cultured in synthetic media by Provasoli (1963): ASP_7, ASP_2, ASP_1 and different mixtures of them. The cells were fixed in 5% glutar-

aldehyde solution in caccodilate or phosphate buffers; and postfixed in 2% O_sO_4 solution in the same buffer of the fixative solution used before. Sections were stained with uranyl acetate (different solutions in water and alcohol) and Reynold's Pb cytrate. Micrographs were obtained in a Philips 301 E.M.

Results

We can see under the light microscope the direct dividing nucleus very much enlarged mainly in one direction and showing the typical individualized chromatic elements, always present in Dinoflagellates (Pl. I 6, 7, 15). Afterwards that nucleus is generally strangulated and fragmented into two daughters by invagination of the nuclear envelope, the whole of the chromatic elements being divided into two portions apparently without any particular arrangement.

Related to the great enlargement of the nucleus at the beginning of direct division, there is a great increase in the number of the chromatic elements, first observed under light microscope in material treated by the Feulgen reaction (Silva 1965, 1971). The electron microscope observations quite appear to confirm that interpretation. In the earlier stages of direct division the nuclear structure reveals the chromatic elements in a clear multiple fragmentation which may occur on different planes, frequently budding or even multiple budding (Pl. II 19, 20, 21). The small "fragments" of chromatic material appear to be growing during this phase of nuclear direct division, and even, perhaps, throughout the entire process. If we consider the dinoflagellate chromosomes as polytenic (Grassé and Dragesco 1957, Soyer and Haapala 1974 a, b, c) those "fragments" could be the chromosomal units, but they can also be parts of the chromosome which is replicating in small portions (Kihlman 1971). The direct dividing nucleus has dense chromatic elements presenting often small nodules of yet denser and still fibrillar structure (Pl. II 19, 20). We think that these nodules of dense chromatic material may be the origin of some buds on the chromosome surface. The buds or small segments of chromosomes may originate either in a partial split (Pl. II 21) of the same or in the "growth" of a dense chromatic nodule formed on its surface (Pl. II, 20). Also, some DNA fibrils permeated from the chromatic elements would be able to "grow" and become small chromatic elements. Beyond the more or less intensive enlargement of the nucleus, a dense distribution of the chromatic elements is generally observed. In the same nuclei, some chromatic material (DNA fibrils, small fragments of chromatin or even typical chromatic elements) is often seen inside the nucleolus (Pl. III 23, 24); besides, DNA fibrils were found in other organelles, mainly plasts and

dictyosomes (Pl. IV 28, Pl. V 30). All this behaviour of the nucleus at the beginning of direct division corresponds to a period of intensive DNA synthesis within the cell.

The two daughter nuclei are generally separated by invagination of the nuclear envelope (Pl. V 32); however, the whole of the chromatic elements is sometimes divided into two groups by distribution and concentration in opposite zones inside the nuclear envelope (which often reveals interruptions) and before any invagination takes place (Pl. IV 27). This aspect was observed in *G. tamarensis* and *G. spinifera*.

The nuclear envelope generally remains during direct division and can present two different aspects: (1) undulated surface related to an intensive enlargement which precedes the increase in volume of the nucleus (Pl. III 22, Pl. V 32); (2) numerous interruptions, beyond the normal "pori", seem to result from the intensive increase of the chromatic material, preceding then the enlargement of the nuclear envelope (Pl. IV 27). Although rarely seen the nuclear envelope may be reabsorbed for large regions.

We sometimes noted in the dividing nucleus, a clear relationship between some elements and the nuclear envelope; this seems to confirm the important role it can play on the movements of the chromatic material during nuclear division such as it has been interpreted by some authors (K u b a i and R i s 1969). A few times bundles of microtubules were seen within invaginations of the nuclear envelope, and more often bundles of microtubules and (or) irregular fibrils, in the enlarged space between the two membranes (Pl. III 25, 26).

The nucleolus is generally fragmented into two or three, or many, small portions and these dividing aspects differ from species to species; for instance, in *G. tamarensis* the nucleolus can divide into two or three very different fragments; in *Gym. splendens* it is generally divided into many small portions distributed in groups.

The differences between nuclei dividing by mitosis and those dividing by direct division are quite clear under the light microscope (Pl. I), mainly in the early stages and when the daughters are separated. The number of the chromosomes in free dinoflagellates is very high and more or less fixed for each species when the nucleus begins mitosis (D o d g e 1963). They are longer, denser and generally in less number than the chromatic elements in a resting nucleus. The chromosome split seems to begin from the axial line, but does not always occur all along the chromosome. We have seen different aspects of chromatid separation; it is not rare to find incompletely splitting chromosomes, others showing a short split on the extremity resulting in some bud, and also, more rarely, some double splitting (Pl. II 17, 18, 21). In all these aspects of

chromosome splitting, the separation of the two chromatids is morphologically evident, being complete or not. Sometimes, after anaphase, the two daughter nuclei may show splitting chromosomes without our being able to say whether the two chromatids have passed to the same daughter or if a new mitosis is beginning. Direct nuclear division is characterized by the apparently irregular fragmentation of the chromatic elements and the growing of the resulted fragments. When the daughter nuclei are separated in anaphase, one can see the typical aspects under the light microscope (Pl. I 11), but this is not so easily observed in E. M., and we need to obtain some good sections or better serial sections. During direct division the whole of the chromatic elements is generally divided into two more or less identical portions. Sometimes the distribution of the chromatic material may be unequal, thus resulting two (or more) differently sized nuclei (Pl. I 2, 8, 12) and, later on, two differently sized cells (Pl. I 14). A small fragment separated from the mother nucleus appears to retain great synthetic activity of the chromatic material that had begun at an early stage of direct division, as well as intensive metabolism, quick development of the small cells resulting from these two factors. The "small form" studied before (Silva 1965, 1971) was observed in 16 of the 23 species of Dinoflagellates already cultured in our laboratory, and also in a few others from the sea, always caught during blooms (Silva 1968, 1974).

The frequence of each nuclear division type appears to be related to some external factors. Most of the studied species from culture have direct division more often than mitosis. In material from natural blooms, we have also found mainly cells showing direct dividing nucleus.

Very often the nucleus can present an intermediate behaviour between the two nuclear division types: a rather intensive increase of the chromatic elements, typical of direct division, and some long chromosomes, even splitting ones, as in early mitosis. In those cases the whole chromatic material is generally divided into two portion by simple fragmentation. Nuclear division in Dinoflagellates can thus vary in a complete gamut from mitosis to direct division, there being always individualized chromatic elements. All these variations in the behaviour of the nucleus can be observed in the same species, and often in cells of the same population.

It is when the nucleus divides by direct division or by same intermediate way that a wider morphological variability can be seen. The rather well known fine structure of the chromatic elements may be somehow modified through the cell cycle. More or less density of the typical arrangement of the DNA fibrils is generally due to the greater

or smaller closeness of their spiral turns. In the early stages of direct division dense chromatic elements showing very close spiral turns in the arrangement of their DNA fibrils are common. Another ultrastructural variation in the dividing nucleus, referred to above, is related to the splitting of the chromosomes during mitosis or to the irregular and multiple section of the chromatic elements during early direct division (Pl. II).

Beyond the ultrastructural variation depending on the nuclear division, some other different aspects could be observed, unless one can relate them with certainty to some stage of the life cycle. They represent more or less deep changes in the nuclear fine structure and have been found in cells from cultures of different ages. In crowded cultures, beyond the log phase of growth, we have often found cells with the nucleus presenting small dense nonfibrillar masses throughout the nucleoplasm often with a particular and regular distribution around the chromatic elements. According to Soyer and Haapala who observed the same in *Gyrodinium cohnii* (1974 b), they are removed by pronase. Sometimes those nuclei, during direct division, present their chromatic elements distributed for two groups, bringing into their circuit the dense nonfibrillar masses, all the remaining nucleoplasm inside the nuclear envelope being without any of them (Pl. IV 27).

In crowded cultures some cells may present the nucleus, their chromatic elements showing both lighter density to the electron beam and closer distribution. As the culture grows older this nuclear structure can be observed more often in many cells, showing successive modifications interpreted as following: the chromatic elements are joined here and there by narrow strands, and their structure is less and less condensed; later on, they show anastomosis and at last become melted (Pl. VI 34, 35, 36). In nucleus with a reduced nucleoplasm can be then observed a net of chromatic material with a very light fibrillar structure, the typical helicoidal arrangement of the DNA fibrils having almost disappeared. In young cultures a few cells at an early stage of that ultrastructural modification were observed. All this morphological variability (or at least its first steps) in fine structure of the nucleus occurs perhaps at any time of the growing up of a culture, and the rarity of those cells in young cultures may be due to the quick development of the same cells within their life cycle, in such environmental conditions. Synchronous cultures could not yet be obtained, and in crowded ones a large ultrastructural variation has thus been observed; also, renewed cells side by side with "old" cells can be found in growing old cultures.

Conclusions and Discussion

Some earlier authors (Borgert 1910, Apstein 1911) had already considered nuclear direct division or amitosis in Dinoflagellates. Later on, Grell (1964) doubted that nuclear division is essentially mitotic both in Euglenoids and Dinoflagellates. Leedale (1959) described amitosis in 3 species of *Euglena*, and Kubai and Ris (1969) concluded after their study in *Gyrodinium cohnii* that "...the nuclear division in Dinoflagellates differs fundamentally from typical mitosis and must be considered a new type of nuclear division". As for ourselves (Silva 1969, 1971) we have been observing in these microorganisms both mitosis and direct division although any of the two nuclear division types has not been found like the classical known modes. Also, not rarely intermediate ways corresponding to a complete gamut of variation between those two division types could be seen.

The near cause for a nuclear direct division in Dinoflagellates appears to be the intensive chromatic increase which is always followed both by an enlargement of the nucleus and by a denser distribution of its chromatic elements. Leadbeater and Dodge (1967) have described, after an electron microscopical study, an increase of the chromosomes, their condensation and budding at an early stage of nuclear division in *Wollosynzskia*. Our own search on the fine structure of the nucleus in the species referred to above, led us to interpret the origin of that increase in the number of chromatic elements as being both the great and apparently irregular division of the pre-existing ones, their budding or multiple budding, and an intensive DNA synthetic activity. To the "fragments" resulting from those sections or budding are to be added the small chromatic masses originated either in dense DNA nodules formed in the chromosomes or in fibrils permeated throughout the nucleoplasm (Pl. II 19, 20). Zingmark (1970) and Soyer and Haapala (1974 b, c) have described those fibrils, and the latter authors after their experiments with pronase have supported the hypothesis of "genetic activity" for those extrachromosomal looped fibrils. We have seen quite distinctly those nodules becoming free from the chromosomes. All those chromatic small portions, in cells from young cultures, appear to have a rapid growth which would be due to the full synthetic activity of the nucleus and supported by an intensive metabolism of the cell. After some autoradiographic studies, Dodge (1965) considered that DNA synthesis in Dinoflagellates takes place probably in a continuous way; although our data are still very scanty we think that DNA synthesis may not occur always with the same intensity all over the life cycle, and of course under different ambient

conditions. Kihlmann (1971) concluded, after recent works, that DNA is synthesized at first in small portions which quickly join to form segments, these being united in the final DNA product. Also, Okazaly et al. (after Kihlmann 1971) observed that the DNA replication occurs in a discontinuous manner. These arguments can help in the interpretation of the great and apparently irregular increase of the chromatic material in the nucleus of Dinoflagellates during direct nuclear division.

Grassé and Dragesco (1957) were the first workers to consider the nucleus of Dinoflagellates as polytenic. Britten and Kohn (1968) referred to the repeated DNA sequences in *Gyrodinium cohnii*. Recently Haapala and Soyer (1973, 1974 b), after their study on several species, discussed and adopted the hypothesis of polytenic structure for the nucleus of those microorganisms. We have also considered this in a communication on the same subject to an international meeting (1975), and the observations partly described above reinforce that idea.

For Haapala and Soyer (1974 a) "the equal distribution of chromatids into daughter chromosomes" during mitosis is assured by the attachments of their ring-shaped ends to the nuclear envelope. However, in species with a very high number of chromosomes no particular arrangement of them could be seen in a way that revealed those attachments for all the chromosomes, even when there are many tubular invaginations of the nuclear envelope. Also, after their polytenic structure and being frequent the partial splitting of some chromosomes, we believe that the distribution of the chromatids into daughters is not necessarily equal during dinomitosis. The attachment of chromosomes to the nuclear envelope can also be observed in direct dividing nucleus, although it appears much less common. Then, the division of the chromatic material in two (or more) daughter nuclei is not depending on the same mechanism, although it may occur.

On the other hand, the polyploidy or, to be more precise, the aneuploidy has revived for the Dinoflagellates. According to Dodge (1963) the discontinuities observed in counts of chromosomes suggest the presence of polyploid series. DuPraw (1972) has referred to heteroploid cells, which generally show an abnormal variability in the number and form of the chromosomes, and considered that as being a consequence of fusions and fissions of the chromosomes in abnormal mitosis (seen in cells of Mammalians). The direct dividing nucleus of Dinoflagellates, showing a great variability in the size and form of the chromatic elements, could be compared to abnormal mitosis of other microorganisms or even of cells of the higher organisms, without being considered as abnormal themselves. This is true especially because

normal cells always result from the different nuclear division types described above. Considering the aneuploidy in this way, we think that there are several complete genomes within the nucleus. However, the typical nucleus (non-dividing nucleus) in each species would not have the chromatic arrangement that would allow the separation of 2 (or more) genomes. If the old hypothesis of homologous chromosomes for these microorganisms must be ruled out, there may be in fact homologous groups of chromosomes, the genomes, we think. Starting from polytenic chromosomes and aneuploid nucleus, both the multiple section of the chromatic elements and, at the same time, the increase of the whole chromatic material are necessary to obtain the conditions for separation of two (or more) genomes by simple fragmentation.

Raikov and Ammermann (1963) think that the macronucleus in *Stylonychia* and *Euplotes* has a certain number of groups of chromosomes the sub-nuclei — each one being a complete genome. Kubai and Ris (1969) said about *Gyrodinium cohnii* "...many complete genomes are contained within the nucleus as in Ciliate macronucleus". Roberts et al. (1974), after chemical mutagenesis experiments in the same species, argue against both interpretations of the Dinoflagellate nucleus, polyteny and polyploidy. They consider it to be a haploid nucleus, the only one consistent with the frequence of mutation obtained. In so vast a group of microorganisms with so great a morphological and physiological variability as the Dinoflagellates, it is at least premature to generalize from what has been observed in one species. Also, some other authors working on Dinoflagellates do not accept the polyploidy (or aneuploidy): Haapala and Soyer (1973) considered the hypothesis of the diploid-polytenic nucleus; Van Stosch (1964, 1973) described the life cycle of five freshwater species and one from the sea and concluded that they are haploid, the first division after gametes fusion being meiotic.

We believe that, in the studied species, the nucleus is both polytenic and aneuploid. These two characters allow, in good environmental conditions, of the dominance of nuclear direct division which may often be unequal. For this behaviour of the nucleus very good nutritional conditions and others are needed. The ecological factors that may allow of the starting of a natural bloom and the appearing of the "small form" were treated and discussed before (Silva 1974). The interpretation of the origin and development of the "small form" is also supported by the polytenic and aneuploid characters of the Dinoflagellate nucleus. Those small cells have a very large nucleus in relation to their small size, having not the specific relationship N/C. Also, they present a fine structure corresponding to an intensive chromatic

activity like the mother nucleus; this nuclear behaviour and an intensive cellular metabolism lead to their rapid development, reaching quickly the typical form, through intermediate forms (Silva 1971) (Pl. V 30, 31). Van Stosch (1973), after his study of the sexual life cycle of some species, considers the gametes originated by "depauperating mitosis", the meiosis occurring in the zigote, and does not admit the budding process or the amitosis. He interprets Borgert's description of unequal amitosis as "anisogamic sexual fusion read in the inverse order". We can not accept this interpretation for the process that we have described above for our material: we believe in the occurrence of unequal nuclear direct division, from which very differently sized cells are originated. Both the nucleus of the "small form" and the nucleus of the mother cell having the same dense structure, and given the very different size of each one, they really contain a very different number of chromatic elements and, therefore, a very different quantity of DNA. Among all the studied material, we could observe that the nuclear structure is much more developed in the "small form" than that one of the small fragment just after its separation from the mother nucleus. This aspect, already described and documented before (1965, 1971), supports mainly the hypothesis of direct unequal nuclear division rather than the anisogamic fusion.

Although we have not seen clearly the sexual cycle in the studied species, some cytological aspects were found that could be interpreted as corresponding to a part played in a sexual process (Silva 1961 and recent unpublished data). Meiosis could not be detected yet by our observations.

Cachon et Cachon-Enjumet (1974) described in *Syndinium* and *Oodinium* (parasitic dinoflagellates) some nuclei with ramified chromosomes which even communicated here and there, with irregular arrangement of the DNA fibrils. They found this ultrastructural situation in nucleus between two division. In our material the same ultrastructural aspects were often observed in cells from aged cultures and rarely in a few from young ones. While in the latter case the cells seem to be quickly recovered, given their rarety, identical cells from crowded cultures and especially from old ones appear to take more time for recovery and so they are rather common in them. According to Soyer and Haapala (1973), the lighter organization of the chromosomes with DNA permeating throughout the nucleoplasm would correspond to the DNA fibrils duplication period. All along the cell cycle there appears to be a chromosomal structure variation that occurs in two opposite senses: one of them tending to the section or division of the chromatic elements, the other to the fusion or melting of the same. In cells from

young cultures, in good nutritional (ecological in general) conditions, the first tendency is more evident what may be related to the frequence of cell division and so to a very intensive chromatic activity; the second tendency, better detected in cells from crowded and aged cultures, corresponds to a lesser frequence of cell division and to a slowed and more prolongated cellular differentiation. Dodge and Crawford (1971) think that some chromosomal variations may result "either of the physiological conditions in culture or of the different phases in chromosome cycle".

The observations on Dinoflagellates in the last 15 years lead us to believe that these microorganisms cannot be interpreted according to rigid and classical conceptions considering their life cycle. All the knowledge obtained about this group of Flagellates, not only by recent authors but also by earlier workers, shows that the Dinoflagellates may present a behaviour not delimited in a well characterized line but varying over a wide area of their physiology and morphology, which are still far from being well known.

RÉSUMÉ

Le motif le plus important qui détermine les variations ultrastructurales nucléaires, considerées dans cette note, c'est la division. Dans le matériel étudié la division directe est plus fréquente que la mitose, étant l'enorme augmentation du matériel chromatique, observée dans une phase précoce de la division, la plus proche raison de ce comportement. La division nucléaire directe, qui peut être inégale, et le dévelopement de la „petite forme" sont très dépendants des deux characteristiques du noyeau des Dinoflagellés: la polytenie et la aneuploidie.

Quelques autres variations de l'ultrastructure nucléaire sont rélationées avec l'âge de la cellule, dependante celle-ci de l'âge de la culture.

REFERENCES

Apstein C. 1911: Biologische Studie über *Ceratium tripos* var. *subsalsa* Ost. Wiss. Meeressunters. Abt. Kiel., 12, 137–162.

Borgert C. 1910: Kern und zellteilung bei merinen *Ceratium* — Arten. Arch. Protistenk., 20, 1–46.

Britten R. J. and Kohne D. E. 1968: Repeated sequences in DNA. Science, 161, 539–540.

Cachon J. et Cachon-Enjumet M. 1974: Comparaison de la mitose des Péridiniens libres et parasites a propos de celle des *Oodinium*. C. r. Acad. Sci., Paris, 278, 1735.

Dodge J. D. 1963: Chromosome numbers in some marine Dinoflagellates. Botanica mar., 5, 121–127.

Dodge J. D. 1964: Nuclear division in the Dinoflagellate *Gonyaulax tamarensis*. J. gen. Microbiol., 36, 269–276.

Dodge J. D. 1965: Chromosome structure in the Dinoflagellates and the problem of the mesocaryotic cell. Progress in Protozoology, Abstr. Second Int. Conf. Protozool., London 1965.

Dodge J. D. 1971: Fine structure of the *Pyrrophyta*. Bot. Rev., 37, 481–508.
Dodge J. D. and Crawford R. 1971: Fine structure of the Dinoflagellate *Oxyrrhis marina*. I. The general structure of the cell. Protistologica, 7.
DuPraw E. J. 1972: Advances in cell and molecular biology. Academic Press.
Grassé P. P. et Dragesco J. 1957: L'ultrastructure du chromosome des Peridiniens et ses consequences genetiques. C. r. Acad. Sci., 245, 2447–2452.
Grell K. G. 1964: The protozoan nucleus. In: The Cell, (ed. Brachet and Mirsky), Academic Press, 6, 1–79.
Haapala O. K. and Soyer M. O. 1973: Structure of Dinoflagellate chromosomes. Nat. New Biol., 244, 195–197.
Haapala O. K. and Soyer M. O. 1974 a: Electron microscopy of whole-mounted chromosomes of the Dinoflagellate *Gyrodinium cohnii* Hereditas, 78, 146–150.
Haapala O. K. and Soyer M. O. 1974 b: Effect of alkali-urea treatment on Dinoflagellate chromosomes. Hereditas, 78, 295–298.
Kihlman B. A. 1971: Molecular mechanisms of chromosome breakage and rejoining, (ed. DuPraw); Advances in Cell and Molecular Biology. Academic Press, 1, 59–107.
Kubai D. F. and Ris H. 1969: Division in Dinoflagellate *Gyrodinium cohnii* Sch. J. Cell Biol., 40, 508–528.
Leadbeater B. and Dodge J. D. 1967: An electron microscope study of nuclear and cell division in a Dinoflagellate. Arch. Mikrobiol., 57, 238.
Leedale G. F. 1959: Amitosis in three species of *Euglena*. Cytologia, 24, 213.
Provasoli L. 1963: Growing marine seaweeds. Proc. 4th Int. Seaweed Symp., Biarritz (1961), 9–15.
Roberts T. M., Turtle R. C., Allen J. R., Loeblich A. R. III. and Klotz L. C. 1974: New genetic and physicochemical data on the structure of dinoflagellate chromosomes. Nature, 248, 446–447.
Silva E. S. 1961: Some observations on marine Dinoflagellate cultures. II. *Glenodinium foliaceum* Steini and *Gonyaulax diacantha* (Meun.) Schil. Botanica mar., 3, 75–100.
Silva E. S. 1965: Note on some cytophysiological aspects in *Prorocentrum micans* Ehr. and *Goniodoma pseudogonyaulax* Biech. from cultures. Notas Estudos. I.B.M., 30, 1–30.
Silva E. S. 1968: Plancton da Lagoa de Óbidos (III). Abundância, variações sazonais e grandes "blooms". Notas Estudos I.B.M., 34, 1–79.
Silva E. S. 1969: Cytological aspects on multiplication of *Goniodoma* sp. Botanica mar., 12, 140–152.
Silva E. S. 1971: The "small form" in the life cycle of Dinoflagellates and its cytological interpretation. Proc. II Plankt. Conf., Roma (1970) 1137–1167.
Silva E. S. 1974: Ecological factors responsible for intensive growth in Dinoflagellates and their expression in the life cycle phases. Progress in Protozoology, Abstr. Fourth Int. Congr. Protozool., 224, Clermont-Ferrand 1973.
Soyer M. O. 1971: Structure du noyau des *Blastodinium* (Dinoflagellé parasite). Division et condensation chromosomique. Chromosoma, 33, 70–114.
Soyer M. O. and Haapala O. K. 1973: Filaments extra-chromosomiques: variation et relation avec l'envelope nucleaire pendant la division chez les Dinoflagellés. J. Microsc., 18, 267–270.
Soyer M. O. and Haapala O. K. 1974 a: Division and function of Dinoflagellate chromosomes. J. Microsc., 19, 137–146.
Soyer M. O. and Haapala O. K. 1974 b: Structural changes of Dinoflagellate chromosomes by pronase and ribonuclease. Chromosoma, 47, 179–192.
Soyer M. O. and Haapala O. K. 1974 c: Electron microscopy of RNA in Dinoflagellate chromosomes. Histochemistry, 42, 239–246.
Stosch A. von 1964: Zum Problem der sexuellen Fortpflanzung im der Peridineengattung *Ceratium*. Holgolander Wiss. Meeresunters., 10, 140.
Stosch A. von 1973: Observations on vegetative and sexual life cycles of two freshwater Dinoflagellates, *Gymnodinium pseudopalustris* Sch. and *Wolloszynskia apiculata* sp. nov. Br. Phycol. J. 8, 105–134.
Zingmark R. G. 1970: Ultrastructural studies on two kinds of mesocariotic Dinoflagellate nuclei. Am. J. Bct., 57, 586–592.

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EXPLANATION OF PLATES I–VI

Light micrographs (Pl. I), Feulgen-light green (1–4, 16: 1800 ×; 5–15: 2100 ×)
1–4: *Gyrodinium instriatum*, cell with nucleus in anaphase and another cell in three planes, showing nucleus dividing by unequal direct division
5–8: *Gonyaulax polyedra*, 4 different nuclei, anaphase (5), direct nuclear division (6), nucleus fragmented in three portions (7), budding nucleus (8)
9, 10: *Gonyaulax spinifera*, two cells showing nuclei dividing by direct division and mitosis respectively
11–13: *Gonyaulax* sp., nuclear unequal direct division in three specimens
14, 15: *Peridinium trochoideum*, two cells showing nuclei dividing by mitosis and direct division respectively
16: *Gymnodinium splendens*, nucleus dividing by unequal direct division

Electron micrographs of sections (Pl II–VI)

17: *Gyrodinium instriatum*, splitting chromosomes during mitosis, 16 000 ×
18: Idem, chromosome showing two splitting planes, 45 000 ×
19: Idem, many small chromatic fragments freed from the chromosomes during direct division; one dense fibrillar mass still united (←) 50 000 ×
20: Idem, dense fibrillar mass getting free from the chromosome (←) 50 000 ×
21: *Peridinium trochoideum*, multiple division of the chromatic elements. 50 000 ×
22: *Gymnodinium splendens*, nucleus in early direct division showing fragmented nucleoli 3000 ×
23: *Gonyaulax tamarensis*, chromatic material inside the nucleolus 45 000 ×
24: Idem, small chromatic elements included in nucleolus or related to (←) 55 000 ×
25: Idem, DNA fibrils (←) in the enlarged space between the membranes of the nuclear envelope 90 000 ×
26: *Peridinium trochoideum*, tubules within an invagination of the nuclear envelope 60 000 ×
27: *Gonyaulax tamarensis*, the whole of the chromatic elements divided in two different groups, inside nuclear envelope which is interrupted in several zones (←) 14 000 ×
28: *Peridinium trochoideum*, small fragment separated from the nucleus; DNA fibrils inside the plast (←) 12 000 ×
29: *Gonyaulax tamarensis*, non dividing nucleus 2000 ×
30: Idem, "small form", nucleus showing some dividing chromatic elements by several planes; DNA fibrils inside the plast (←) 6000 ×
31: Idem, intermediate form showing the nucleus with an intensive chromatic increase 5000 ×
32: *Gyrodinium instriatum*, invaginations of the nuclear envelope delimiting different fractions of the nucleus 5000 ×
33: *Peridinium trochoideum*, DNA fibrils permeated from the chromosomes 90 000 ×
34: Idem, part of the nucleus showing close chromatic elements, some of them communicating through DNA fibrils (←) 70 000 ×
35: *Gonyaulax tamarensis*, dense distribution of the chromatic elements, communicating on several points (←) 50 000 ×
36: *Gyrodinium instriatum*, chromatic elements melted in many zones (←) 60 000 ×

Abbreviations used: chr — chromosome, nu — nucleolus, np — nucleoplasm, ne — nuclear envelope, mi — mitochondria, pl — plast.


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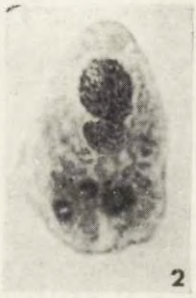
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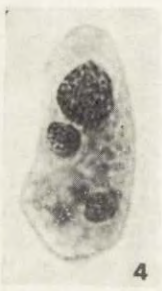
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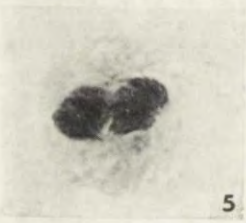
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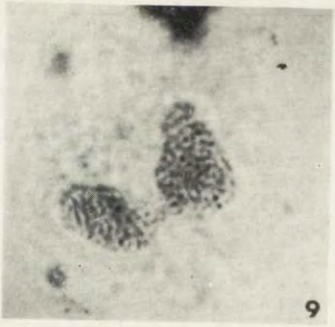
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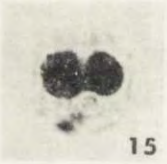
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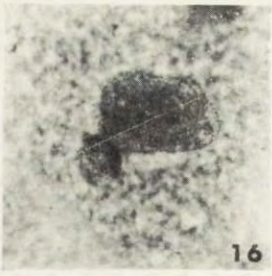
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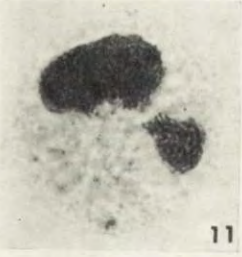
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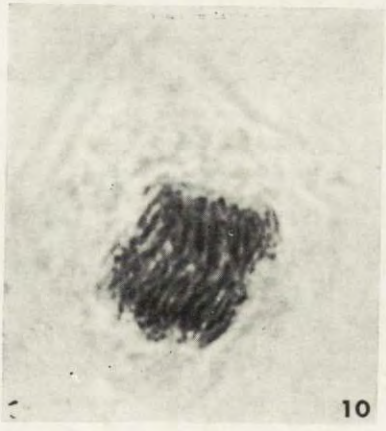
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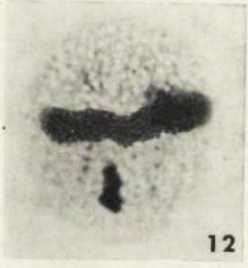
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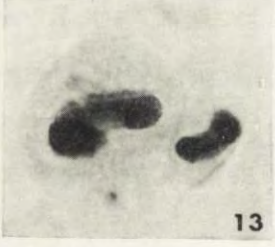
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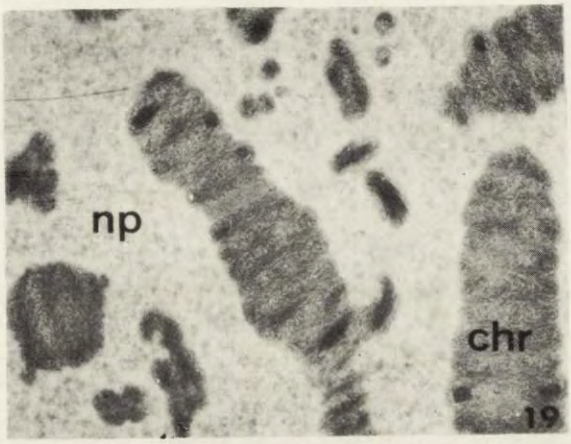

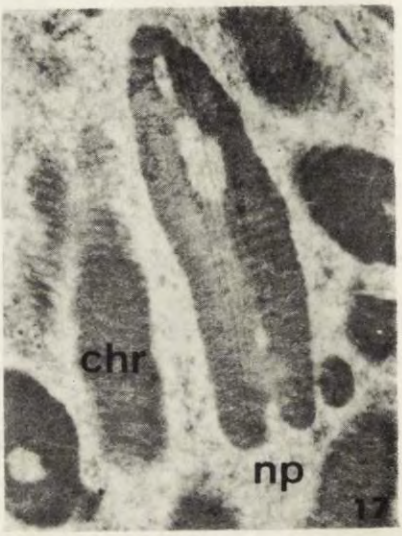

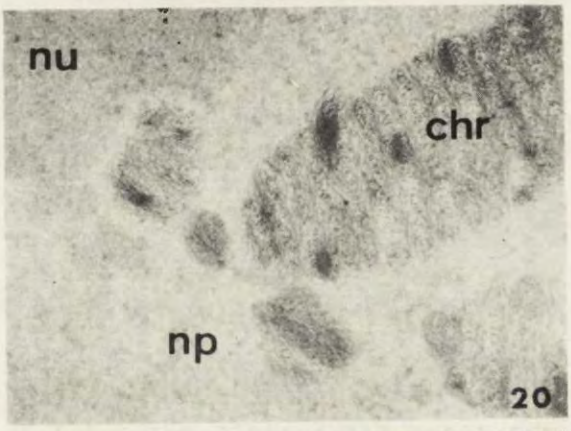

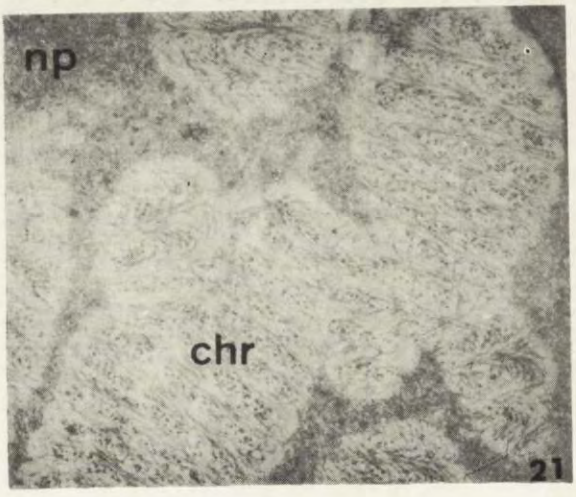

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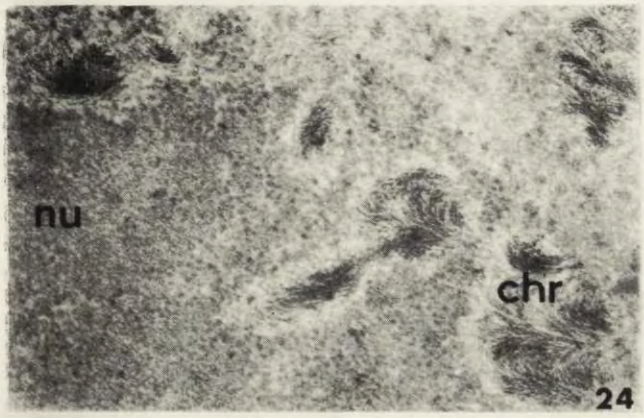

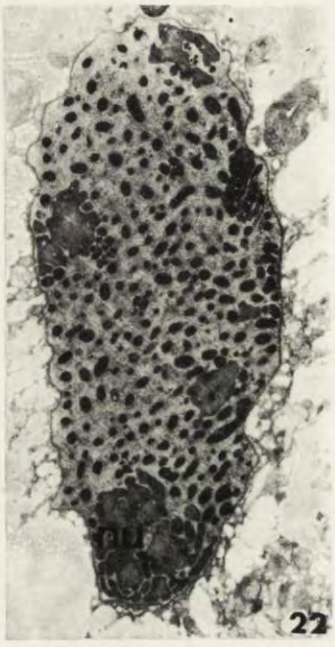

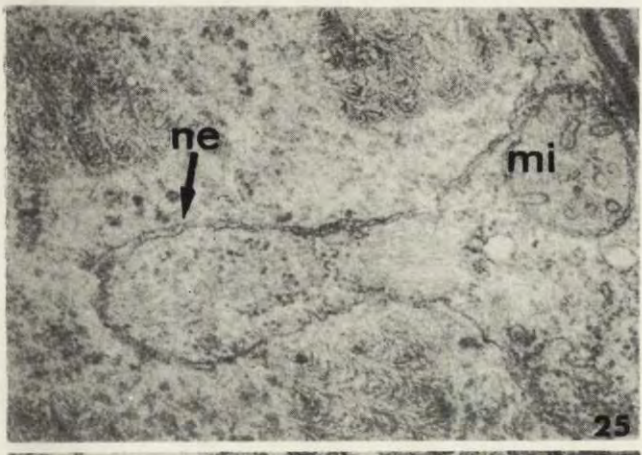

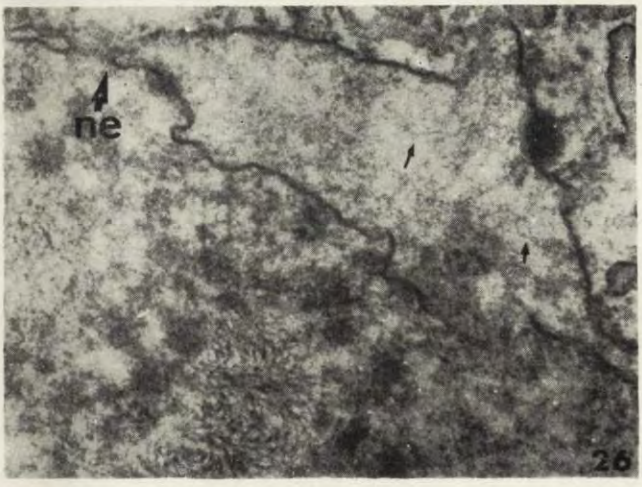

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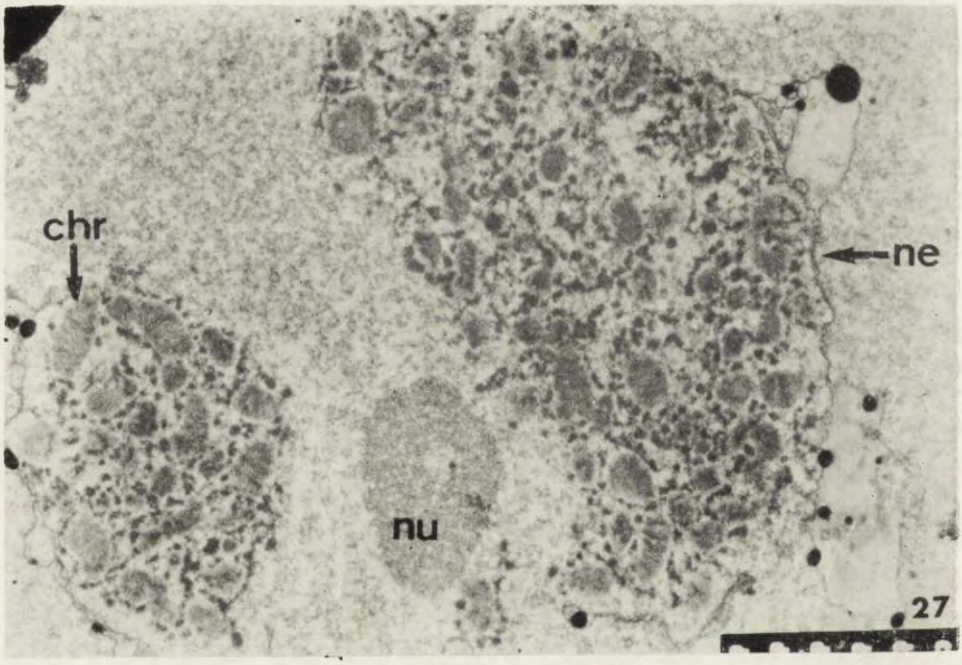

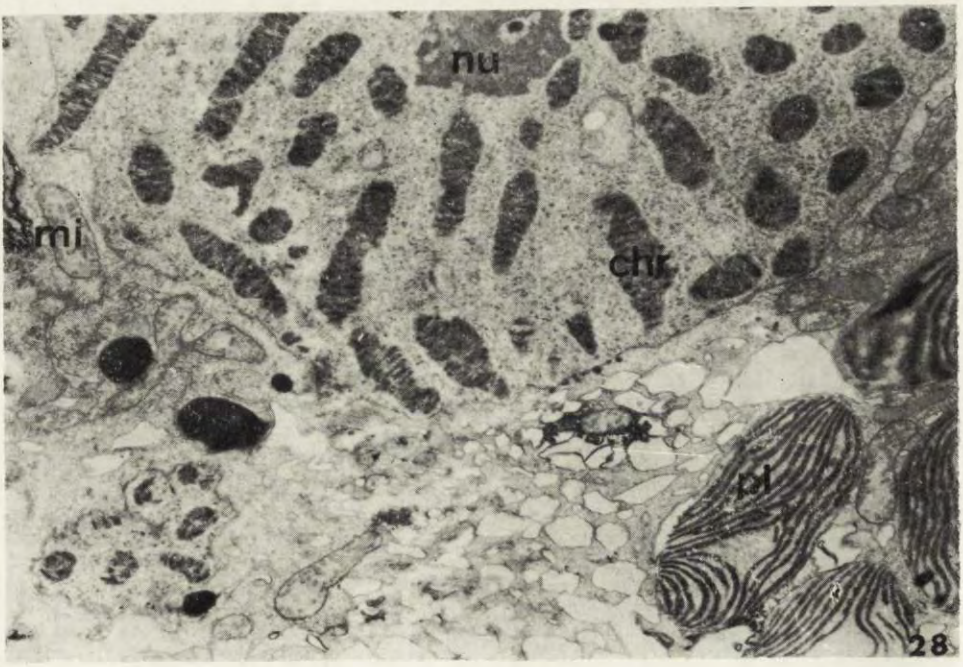

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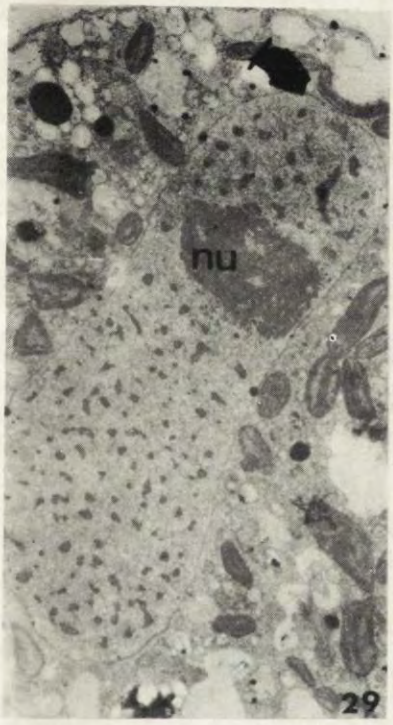

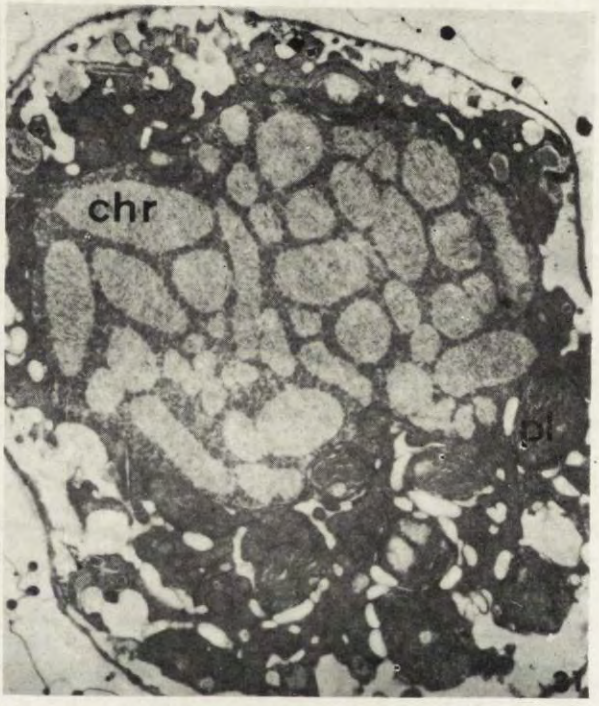

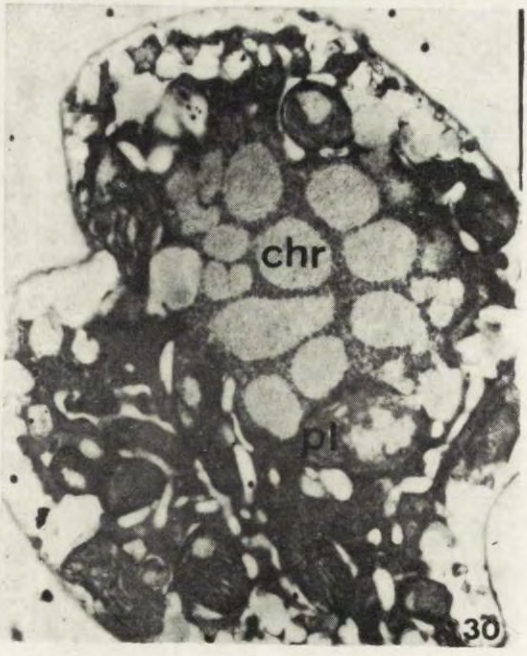

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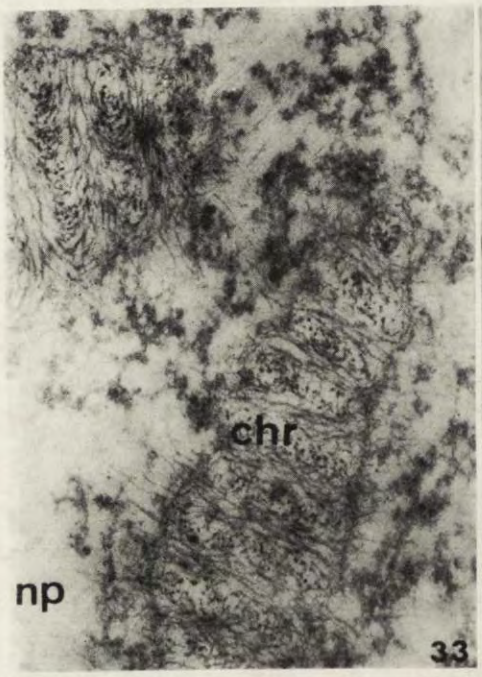

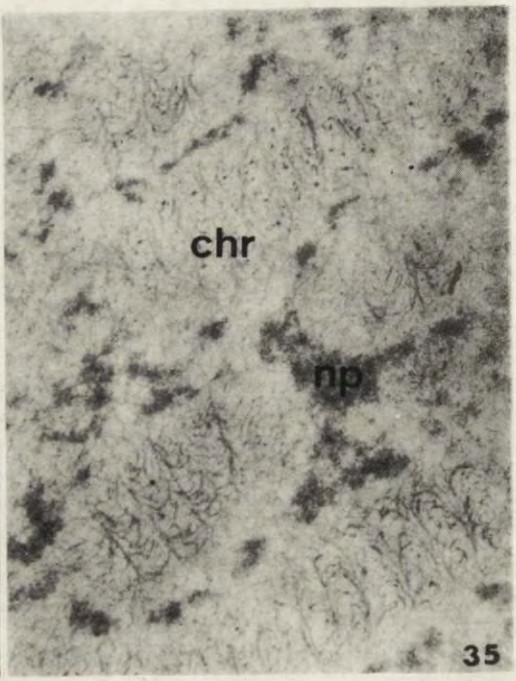

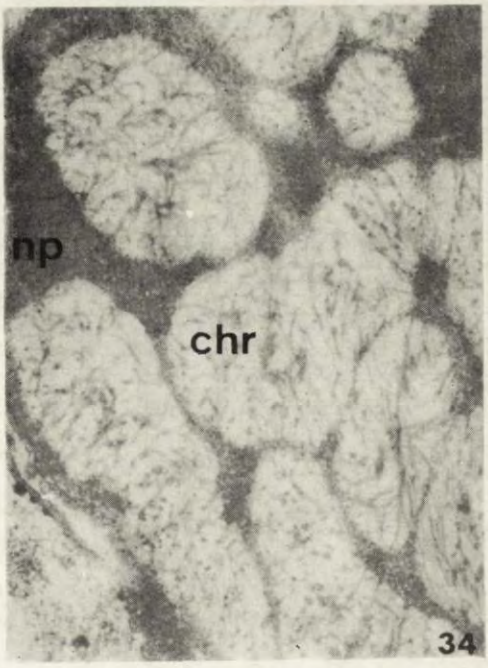

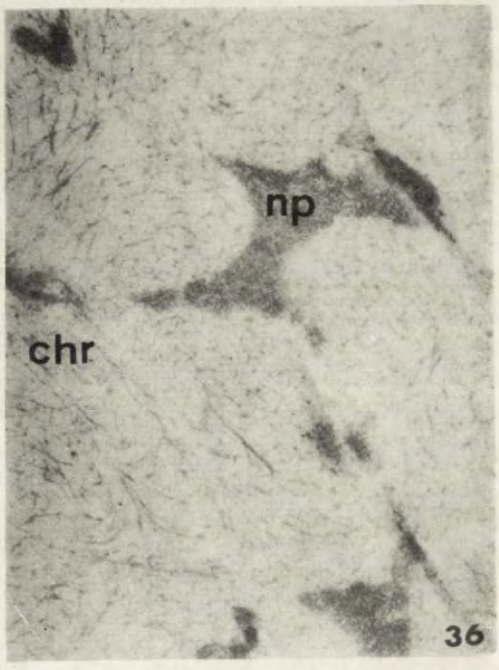

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ACTA PROTOZOOLOGICA

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Электронномикроскопическое исследование ранних стадий заражения *Paramecium caudatum* симбионтами макронуклеуса (йота-бактериями)

Electron Microscope Examination of Early Stages of Infection of *Paramecium caudatum* by Bacterial Symbionts of the Macronucleus (Jota-bacteria)

Синопсис. Изучен характер изменений ультраструктур клетки *Paramecium caudatum* и высокоспециализированных, в отношении локализации, симбиотических бактерий на ранних стадиях инфекции и при проникновении симбионтов в макронуклеус (МА) инфузорий. В процессе фагоцитоза парамеций инфекционные формы бактерий попадают из внешней среды в пищеварительные вакуоли, где происходит созревание и выход эуинфекционной стадии из оболочек „споры". Симбионт проникает в цитоплазму парамеции, инвагинируя мембрану пищеварительной вакуоли. В цитоплазме вокруг симбионта образуется мембранный комплекс (МК), состоящий из 4–5 слоев сдвоенных элементарных мембран уплощенных цистерн, связанных своим происхождением с эндоплазматическим ретикулумом. Предполагается, что избирательный транспорт бактерий к МА связан с особенностями организации МК. Процесс проникновения симбионта через оболочку МА можно сравнить со шлюзованием бактерии мембранами разного происхождения, благодаря чему ядерное содержимое ни на одном этапе не вступает в прямой контакт с цитоплазмой парамеций. Симбионты обнаруживаются в МА уже через 1 час от момента добавления споровых форм в культуральную среду к „чистым" клеткам. Кажется вероятным, что направленный транспорт симбиотических бактерий в ядро определенного типа (МА) обеспечивается оригинальным механизмом эстафетной смены разных мембран клетки-хозяина, которые окружают симбионта, лишенного органоидов движения и способности к самостоятельному активному движению, на всех последовательных этапах проникновения.

Инфузории как модельные объекты обладают рядом очевидных преимуществ при анализе цитофизиологических и молекулярных механизмов, посред-

ством которых в ряду клеточных агамных поколений сохраняются крайне различные структурные и функциональные состояния ядер со сходной генетической информацией (Gorovsky 1973). Для изучения функциональных различий между макронуклеусом (МА) и микронуклеусом (МИ) ранее нами был предложен "симбиотический" метод, основанный на использовании разнообразных эндонуклеарных симбиотических микроорганизмов инфузорий (Осипов и др. 1976).

Инфузории, содержащие симбионтов разных типов, способны к делениям, что позволяет получать клеточные линии инфузорий, в которых симбионты поддерживаются стабильно. Важной особенностью каждого из этих видов бактерий является строгая специфичность локализации в определенном структурном элементе ядерного аппарата инфузорий. Например, у инфузории *Paramecium caudatum* симбиотические бактерии омега-частицы (*Holospora undulata*) локализуются только в МИ, а йота-частицы (*H. obtusa*) — только в МА. Для некоторых видов бактерий установлена возможность заражения "чистых" клеток и, напротив, освобождения инфицированных инфузорий от симбионтов.

Существование бактерий в ядерном аппарате клетки накладывает отпечаток на особенности организации симбионтов, их жизненный цикл и на характер взаимоотношений организмов, составляющих симбиотическую систему. В жизненном цикле симбионтов имеет место смена двух морфологических форм: коротких веретеновидных, делящихся поперечными перетяжками и удлиненных спороподобных, деление которых никогда не наблюдается. Первые выполняют функцию размножения симбионта (вегетативная стадия), вторые обеспечивают распространение бактерий и заражение „чистых" парамеций (инфекционная стадия). Однако до сих пор оставалось неизвестным, какие механизмы обеспечивают проникновение частиц в МА инфузорий.

Настоящая работа посвящена электронномикроскопическому исследованию изменений ультраструктур клетки парамеций и симбиотических йота-бактерий на ранних стадиях инфекции и при проникновении в МА инфузорий. Ранее уже отмечалась крайне высокая специфичность локализации бактерий в элементах ядерного аппарата парамеций. Йота-частицы заражают только МА, никогда не были отмечены в МИ инфузорий (Ossipov et al. 1975, Громов и др. 1976, Осипов и др. 1976). Следовательно, можно полагать, что процесс проникновения симбионтов в свой клеточный компартмент строго направлен. Всестороннее изучение механизмов, обеспечивающих проникновение инородных частиц (бактерий) в ядро клетки, когда не происходит нарушений клеточных функций и структурной организации, несомненно, могло бы представлять общебиологическое значение для понимания форм и путей ядерно-цитоплазматических взаимоотношений. Среди последних основное внимание исследователей, естественно, привлекает противоток макромолекул (РНК, белков, коферментов, АТФ и др.) и транспорт ионов между

ядром и цитоплазмой. Несравненно в меньшей степени изучены иные формы обмена, обнаруженные у некоторых одноклеточных и специализированных клеток высших животных, связанные с локальным разрушением ядерной мембраны и отшнуровыванием в цитоплазму небольших фрагментов лопастных ядер или целых ядрышек и глыбок хроматина. Функциональное значение такого рода аномального поведения ядерных структур остается неясным (Ченцов и Поляков 1974, Goldstein 1974, Franke and Scheer 1974). Инфекционный внутриядерный симбиоз бактерий отчасти сравним с такого рода явлениями и связан с переносом (транспортом) инородных тел (симбиотических бактерий) из цитоплазмы в ядро. Однако нам не известно ни одного исследования ультраструктурных изменений клетки, посвященных проникновению симбиотических микроорганизмов в ядро.

С общебиологических позиций широкое экспериментальное изучение механизмов адаптации бактерий к внутриядерному симбиозу может послужить одним из важных отправных пунктов в разработке теоретических основ происхождения и организации эукариотной клетки. Вопрос о происхождении эукариотной клетки с ее несколькими взаимодействующими генетическими системами в своей основной части совпадает с вопросом становления первичных механизмов интеграции эволюционных предшественников органелл или их аналогов. Предварительное сообщение по материалам данной работы опубликовано ранее (Осипов и Подлипаев 1976).

Материал и методика

Работа проведена на инфузориях *Paramecium caudatum* клона М-339, которых инфицировали йота-частицами из клона парамеций М-339-йота. Использованные методики ведения культур и заражения "чистых" клеток йота-бактериями из неочищенного гомогената парамеций были аналогичны ранее описанным (Sonneborn 1970, Ossipov et al. 1975). Опыты проводили при 25°С.

После добавления гомогената клеток М-339-йота к парамециям клона М-339 клетки фиксировали через 1, 2, 4, 8 часов и 2, 7 суток. Фиксацию производили 2% OsO_4 на какодилатном буфере (pH 7.4), материал заключали в аралдит. Срезы изготовлены на ультрамикротоме LKB-III, дополнительно отконтрастированы уранил-ацетатом и цитратом свинца. Изучение срезов проводили с помощью электронных микроскопов: Hitachi HU-11E (при ускоряющем напряжении 75 кв) и Tesla BS-500 (60 кв) в лаборатории электронной микроскопии Биологического института ЛГУ (рук. лаборатории Е. Р. Гагинская).

Одновременно с электронномикроскопическим исследованием над экспериментально зараженными парамециями проводились светооптические наблюдения: на живых парамециях с помощью фазово-контрастного устройства, а на фиксированных при окраске по Фельгену или по Dippell (1954) с увеличенным содержанием прочного зеленого, что позволяло наблюдать за перемещением йота-частиц в клетке на последовательных стадиях инфекции.

Спустя 1 час от начала заражения клетки отмывались от остатков гомогената М-339-йота в нескольких порциях неинкубированной культуральной среды, с тем чтобы исключить возможность попадания новых йота-бактерий в уже инфицированные клетки. Этим достигалась относительная синхронность развития последующих стадий инфекции.

Результаты

Для того, чтобы выявить какие изменения происходят в клетке в связи с заражением МА "чистых" *P. caudatum* йота-бактериями, нами сравнивались особенности ультраструктурной организации "чистых" и инфицированных парамеций на последовательных этапах опыта. На Табл. I 1, 2 представлены участки цитоплазмы и ядерного аппарата (МА и МИ) "чистых" клеток *P. caudatum*.

Первый этап проникновения симбиотических бактерий в "чистые" неинфицированные парамеции осуществляется током жидкости, который вызывается биением соматической околоротовой цилиатуры в процессе нормального питания инфузорий. В результате работы специализированной ротовой цилиатуры захваченные пищевые объекты (обычно бактерии *Aerobacter aerogenes*) и другие (близкие по размерам) частицы, содержащиеся в культуральной среде, оказываются на дне глотки, где через цитостом попадают в формирующуюся пищеварительную вакуоль (ПВ). Светооптические наблюдения позволяют обнаружить йота-частицы в некоторых из первых образовавшихся ПВ уже через несколько минут после добавления в культуру "чистых" парамеций гомогената М-339-йота. Иногда в одну ПВ попадает по 2–3 "споры" йота-бактерий. Сформировавшиеся ПВ вовлекаются током цитоплазмы в обычный цикл движения по клетке: сначала в задний конец парамеции, затем мимо МА в передний конец и, наконец, направляются снова к заднему концу клетки, где непереваренное содержимое ПВ выводится из клетки через порошицу. Итак, несмотря на свои крупные размеры (длина "споры" до 15–18 μm), йота-частицы из внешней среды попадают в ПВ, в которой происходит их дальнейшее развитие.

Гомогенат М-339-йота содержит популяцию симбионтов, где наряду со споровыми инфекционными частицами имеются также частицы вегетативной и промежуточной стадий, отличающиеся не только размерами клетки, но и особенностями организации бактериальной стенки (Громов и др. 1974). Поэтому в ПВ парамеций помимо "спор" попадали с током жидкости и йота-бактерии других стадий.

Фазу пищеварительного процесса можно легко установить по характеру изменения пищевых частиц (*Aerobacter aerogenes*), находящихся в ПВ, и кроме того на основании ультраструктурных особенностей внутриклеточного пищеварения парамеций, довольно детально изученного рядом авторов (Jurand and Selman 1969, Esteve 1970, Vivier 1974). У йота-частиц вегетативной стадии и незрелых "спор" клеточная оболочка и протоплазма не претерпевает никаких существенных изменений. Такие бактерии выглядят интактными даже на заключительных этапах цикла ПВ. Дальнейшая их судьба в парамециях остается неизвестной. По-видимому, они выводятся из клетки через порошицу с остатками непереваренных объектов.

Цитоплазма зараженных парамеций, как и в норме, содержит многочисленные лизосомы, нередко лежащие в тесном контакте с мембраной ПВ. Обнаруженные нами картины изменений пищевых объектов в ПВ, содержащих одновременно и йота-"споры", позволяют предполагать, что у этих вакуолей имеет место сплавление мембраны ПВ с мембраной лизосомы, в результате чего происходит впрыскивание гидролитических ферментов в фагосому и создаются необходимые условия для нормального переваривания. Дальнейший материал будет посвящен описанию изменений "спор", попавших в ПВ, поскольку именно они обеспечивают процесс инфекции ядер.

В отличие от пищевых объектов, которые вскоре начинают перевариваться, "споры" в первые минуты пребывания в ПВ (Табл. II 3–6) имеют точно такую же структуру, как и в МА парамеций (Табл. XIV 30), находящихся на стадии стабильного заражения (Громов и др. 1976). Диаметр палочковидной бактерии составляет 0.7 μm. Её оболочка имеет сложное строение: снаружи она покрыта клеточной мембраной толщиной 8 nm, под ней лежит электроннoплотный слой толщиной 10 nm. Далее между клеточной мембраной и собственно протоплазматическим телом, окруженным протоплазматической мембраной, залегает периплазматический слой сравнительно низкой электронной плотности. Последний имеет разную степень выраженности по длине "споры": толщина его всего около 20–25 nm в части "споры", занятой протоплазматическим телом (длина которого достигает 6–8 μm), тогда как содержимое другой части клетки, составляющей 9–10 μm, фактически, представляет собой гипертрофированный периплазматический слой. Протоплазматическая мембрана образует хорошо выраженную систему инвагинаций к центру клетки и коротких выростов. Протоплазма "споры" содержит крупнозернистый материал очень высокой плотности для электронов. С ней связаны прозрачные для электронов овальные участки клетки размером около 40 nm неизвестной природы.

В ПВ йота-"споры" претерпевают ряд последовательных изменений, заканчивающихся выходом протоплазматического тела из оболочки (Табл. III 7–10). Оболочка споры, имеющая вначале ровные очертания, становится все более волнистой, клеточная мембрана споры и подлежащий электроннoплотный слой отслаиваются, последний постепенно деградирует (Табл. III 8, 9); на поперечных срезах протоплазматическое тело становится округлым. Сам момент выхода протоплазматического тела из оболочек "споры" на срезах наблюдать не удалось, вероятно, он происходит очень быстро. Протоплазматическое тело, вышедшее из оболочки "споры", имеет организацию, существенно отличную от клеток симбионта на всех других стадиях его жизненного цикла (Табл. III 10). Поскольку именно эти частицы претерпевают серию характерных изменений и осуществляют проникновение в МА инфузорий, мы будем в дальнейшем обозначать их термином эуинфекционная стадия.

Также как и на всех других этапах жизненного цикла, бактерии этой стадии

лишены органоидов движения. Длина их достигает 8 μm и диаметр 0.5 μm, протоплазма заполнена крупнозернистым электроннoплотным содержимым обнаруживающим на периферии клетки рыхлую сеть (Табл. III 10). Так как применявшийся нами фиксатор не считается лучшим при изучении оболочек бактерий, то мы затрудняемся однозначно расшифровать строение поверхностных структур эуинфекционных частиц. По-видимому, внешний рыхлый слой представляет собой клеточную мембрану, имеющую волнистые очертания. Обычный для вегетативной и споровой стадий подлежащий электроннoплотный слой не выражен. Цитоплазматическая мембрана плохо идентифицируется. Прозрачность протоплазмы для электронов быстро увеличивается, содержимое становится мелкозернистым с одиночными крупными глыбками неправильной формы. Интересной особенностью протоплазмы эуинфекционных частиц является отсутствие светлых зон, содержащих фибриллярный материал и соответствующих диффузному нуклеоиду, которые ранее были отмечены у йота-бактерий вегетативной стадии (Громов и др. 1976).

Частицы эуинфекционной стадии осуществляют выход из ПВ в цитоплазму парамеций. В месте контакта бактерии с мембраной ПВ, имеющей толщину 8 nm, образуется глубокое выпячивание (Табл. III 11, V 14). В этом месте мембрана ПВ плотно облегает тело бактерии. Прогрессирующее выпячивание мембраны включает все большую и большую часть клетки бактерии (Табл. IV 12, 13). Какова судьба мембраны ПВ, окружающей симбионта в момент его проникновения в цитоплазму, остается неустановленным. Можно думать, что она вскоре разрушается, поскольку на последующих ближайших стадиях продвижения йота в цитоплазме клетки мы никогда не наблюдали, чтобы симбионт был окружен одиночной элементарной мембраной, которую можно было бы считать остатком (производным) мембраны ПВ.

Итак, йота-частица попадает в цитоплазму парамеций. Существенной особенностью следующего этапа проникновения симбионта к своему специфическому месту локализации является то, что вокруг бактерии образуется сложная система цистерн, являющихся, скорее всего, производными эндоплазматического ретикулума (Табл. V 15, VI 16a, b). Пузырки ретикулума диаметром 35–50 nm сливаются между собой в цистерны, которые постепенно уплощаются и располагаются вокруг симбионта. В результате клетка симбионта, находящегося в цитоплазме, всегда оказывается окруженной особым образом организованной замкнутой мембранной структурой, которую мы будем обозначать как мембранный комплекс (МК). В цитоплазме парамеций наряду с образованием МК вокруг симбионтов, вышедших из ПВ, наблюдается возникновение обширных зон, резко обедненных или даже лишенных ретикулума. Создается впечатление, что в формировании МК участвуют элементы эндоплазматической сети, которые перед агрегацией каким-то образом перемещаются на довольно большое расстояние по направлению к симбионту. На поверхности мембран МК не обнаруживаются рибосомы. В момент обра-

зования многослойного МК некоторые его участки состоят из параллельно лежащих небольших цистерн шириной 18–23 nm и мелких пузырьков (Табл. VI 16a), чем напоминают описанные у целого ряда инфузорий комплексы Гольджи (Dutta 1974).

Сформированный МК имеет во всех наблюдавшихся нами случаях определенный план строения и четко выраженную полярность (Табл. VII 17). Вдоль симбионта лежит до 4–5 плотно прилегающих друг к другу параллельных слоев мембран уплощенных цистерн, создающих вокруг бактерии своеобразный многослойный чехол, толщина которого достигает 85–90 nm. Уплощенные цистерны образуются за счет сближения внутренних поверхностей двух элементарных мембран (каждая из которых имеет толщину 8 nm) и представляют собой протяженные электроннoплотные пласты толщиной 17 nm. Интересно отметить, что последние могут образовываться и в результате сближения наружных поверхностей мембран, принадлежащих двум соседним параллельно лежащим цистернам (Табл. VI 16 b). Иногда в образовании двух параллельно лежащих сдвоенных мембран принимают участие, за счет сближения их наружных поверхностей, три слоя цистерн (Табл. VI 16 c).

На расширенном конце МК (условно будем называть его "задним") регулярность слоев нарушается (Табл. VIII 20). Здесь образуется сложная хаотичная система извитых мембран, цистерн, вакуолей и островков цитоплазмы. На противоположном ("переднем") конце МК количество правильно ориентированных слоев уменьшается, и наблюдаются картины отпочковывания мелких пузырьков от внутренних уплощенных цистерн МК и встраивание их в наружный слой мембран (Табл. VIII 18, 19). Отмеченные выше отличительные особенности организации МК: отсутствие рибосом на поверхности мембран; наличие в формировании стадии, напоминающей комплекс Гольджи; многослойное параллельное расположение цистерн; необычное сближение двух мембран уплощенных цистерн; сближение мембран двух соседних цистерн — позволяют считать, что мембраны МК и эндоплазматического ретикулума значительно отличаются по своим свойствам и функциям.

Другая особенность МК состоит в том, что он закономерно распадается вблизи МА или, возможно, даже при непосредственном контакте с ядерной оболочкой МА. Важно отметить, что около МИ йота-частицы на срезах никогда не наблюдались, т.е. транспорт симбионта в клетке-хозяине является строго направленным — в данном случае только к ядерной оболочке МА, но не к МИ. Отмеченные особенности организации МК позволяют предположить, что эта эфемерная ультраструктура цитоплазмы парамеций осуществляет направленный транспорт симбионта, лишенного органоидов движения, к МА (см. обсуждение).

Обращают на себя внимание определенные отличия в толщине цистерн в разных зонах сформированных МК. Если в условно заднем конце МК толщина цистерн не менее 30 nm (Табл. VIII 20) то в передней и средней частях

МК, непосредственно окружающих эуинфекционную йота-частицу, большинство цистерн сильно уплощено и их мембраны лежат строго параллельно и предельно сближены так, что уплощенная цистерна представляет собой, фактически, пласт толщиной 17 nm, состоящий из двух элементарных мембран (Табл. VIII 18, 19). Важно подчеркнуть, что такие уплощенные цистерны не отмечены ни в каких других органеллах нормальных парамеций и являются характерной чертой только МК. На Табл. VIII 18, 19 можно видеть, что такие уплощенные цистерны способны к динамическим перестройкам — от их краев в передней части МК образуются многочисленные мелкие пузырьки диаметром 30–35 nm, тогда как в средней части МК две мембраны цистерны могут отходить друг от друга, образуя обычный просвет цистерн около 23 nm. Как будет показано ниже, прилегающий к бактерии слой уплощенных цистерн распадающегося МК способен образовать контакт и сливаться с выростами наружной мембраны ядерной оболочки МА (Табл. XI 24). Уплощенные цистерны сохраняются в остатках распадающегося МК, лежащих около МА, после того, как симбионт покидает МК (Табл. X 23 XII 26). По-видимому, уплощенные цистерны вскоре перестраиваются в обычные элементы эндоплазматической сети, т.к. на последующих стадиях инфекции сдвоенные мембраны не обнаруживаются ни свободно лежащими в цитоплазме парамеций, ни в автофагических вакуолях.

Некоторые симбионты, заключенные в МК, не достигают МА и перевариваются клеткой-хозяином. На срезах йота-бактерий, лежащих в автофагических вакуолях, особенно четко проявляется сложная структура МК, образованная концентрически закрученными складками уплощенных цистерн (Табл. XV 31).

Таким образом, йота-бактерии, окруженные МК, оказываются лежащими около и, как правило, вдоль ядерной оболочки МА. В непосредственной близости от МА происходит перестройка "переднего" конца МК. Количество окружающих симбионта мембранных слоев уменьшается, уплощенные цистерны перестраиваются в трубочки диаметром 24–31 nm, пузырьки и короткие цистерны (Табл. IX 21). Далее вокруг участка йота-частицы, прилегающего к МА, остается лишь один слой плотно облегающих симбионта отдельных фрагментов уплощенных цистерн (Табл. X 22). В ряде случаев удается видеть рядом с МА миелиноподобную фигуру, состоящую из параллельно лежащих сдвоенных мембран толщиной 17 nm, которую уверенно можно считать остатками пустого МК (Табл. XII 26) судя по сдвоенным мембранам, вероятно, средней его части. Последний факт может, по-видимому, свидетельствовать о том, что бактерия эуинфекционной стадии каким-то образом перемещается внутри МК.

Итак, эуинфекционная частица, покинув ПВ и преодолев участок цитоплазмы, оказывается в непосредственном контакте с вегетативным ядром (МА) инфузорий. Прежде чем рассматривать процесс собственно проникно-

вения симбионта через ядерную оболочку внутрь МА, остановимся на некоторых особенностях строения оболочки ядра, которые, как нам кажется, могут способствовать проникновению йота-частиц в МА. Конечно, некоторые из отмеченных ниже особенностей организации поверхности МА инфицируемых клеток описаны в литературе и для нормальных парамеций. К таким чертам, вероятно, прежде всего следует отнести большую изрезанность оболочки МА изучавшихся нами парамеций. Ядро уже через один час от момента заражения принимает весьма причудливую форму. Наряду с выпячиваниями и инвагинациями крупных участков (лопастей) МА, имеет место значительное число небольших выростов ядерных мембран. Такие выпячивания могут быть образованы как обеими ядерными мембранами, так и, более многочисленные, только наружной ядерной мембраной (Табл. XI 24). Вокруг йота-частиц, лежащих в непосредственной близости около МК, можно наблюдать небольшие трубочки 24–31 nm, некоторые из них, по-видимому, можно трактовать как перерезанные поперек выросты наружной ядерной мембраны, хотя их и бывает трудно отличить от трубочек распавшегося МК (Табл. X 22). В ряде случаев ядерная оболочка образует более сложные структуры, представляющие значительные расширения перинуклеарного пространства, причем наружная мембрана дополнительно образует вдающиеся внутрь инвагинации. Создается впечатление, что по крайней мере некоторые изменения ядерной оболочки, а именно, тонкие выросты наружной ядерной мембраны, преимущественно связаны с находящимися у МА йота-частицами.

Последующие этапы процесса инфекции завершают проникновение симбионта через оболочку ядра. Прежде всего следует отметить, что отрезок ядерной оболочки, непосредственно прилегающий к бактериальной клетке, существенно изменен по сравнению с близлежащими участками (Табл. XI 24, 25). Обращают на себя внимание две особенности: во-первых, отсутствие ядерных пор на участке длиной до 1–1.5 μm, в то время как рядом обычные поры диаметром 55–60 nm хорошо заметны; во-вторых, значительное расширение перинуклеарного пространства до 70–85 nm, по сравнению с обычным — около 25 nm. Об изменениях организации мембран возможно говорит и то, что на прилежащем к йоте-чатице участке ядерной оболочки трехслойное строение мембраны, имеющей толщину 8 nm, разрешается существенно лучше, чем на неизмененных участках.

На основании изучения срезов дальнейший ход проникновения симбионтов внутрь МА можно реконструировать следующим образом (см. Рис. 1–6). Часть МК, обращенная к ядерной оболочке, распадается на отдельные фрагменты так, что со стороны ядра МК оказывается не замкнутым. Мембраны фрагментов уплощенных цистерн МК, окружающие симбионта с латеральных и дистального краев (по отношению к ядру), сливаются с выростами наружной мембраны ядерной оболочки (Табл. XI 24). Последние одновременно с этим сливаются между собой, образуя плоскую цистерну, которая охва-

тывает симбионта с проксимального края. В результате завершения этого процесса симбионт оказывается заключенным в камеру, образованную сдвоенными мембранами, которые имеют разное происхождение: прилегающие к ядру — производные наружной ядерной мембраны и собственно ядерная оболочка МА, тогда как дистальная от ядра часть — фрагмент уплощенной цистерны МК (Табл. XI 25). Далее происходит уже отмеченное частично выше изменение ядерной оболочки МА в зоне непосредственного контакта с симбионтом: перинуклеарное пространство резко расширяется, после чего мембраны фрагментируются и замыкаются в отдельные пузырьки (Табл. XI 25). Одновременно от разомкнутых краев ядерной оболочки образуются выросты мембран (строго их нельзя считать внешними или внутренними мембранами ядерной оболочки, т.к. они не замкнуты), которые охватывают симбионта. В заключении, по-видимому, происходит смыкание этих выростов ядерной оболочки над симбионтом, чем завершается реорганизация ядерной оболочки МА и весь многоэтапный процесс проникновения симбионта в МА.

Таким образом, процесс проникновения йота-частицы через оболочку ядра можно сравнить с последовательным шлюзованием симбиотической бактерии мембранами разного происхождения. Проникновение осуществляется таким образом, что ядерное содержимое ни на одном этапе не вступает в прямой контакт с цитоплазмой. Вторая существенная черта этого процесса — его строгая пространственная ограниченность, свидетельствующая, по-видимому, о том, что симбионт воздействует на участок ядерной оболочки клетки-хозяина весьма специфично.

Среди изменений ультраструктурной организации МА, имеющих место начиная со стадии I часа от момента инфекции, следует отметить появление овальных зон размером до 0.8 μm, в которых отсутствуют хроматиновые тела (0.05–0.1 μm), характерные для нормальных МА парамеций. Кариоплазма этих зон заполнена фибриллярным материалом диаметром около 11 nm. Отмеченные зоны диспергированного хроматина сохраняются и на последующих стадиях инфекции МА йота-частицами (Табл. XIII 28).

Йота-частицы обнаруживаются в МА уже спустя 1 час от момента добавления гомогената М-339-йота к „чистым" клеткам (Табл. XII 27) в принятых нами условиях проведения эксперимента (см. Материал и методика). На некоторых срезах в одном МА удается обнаружить до 3 — 4 йота-частиц. Симбионты, только что попавшие в МА, отличаются по своей организации от бактерий вегетативной стадии, подробно описанной ранее (Громов и др. 1976). В протоплазме еще присутствуют отдельные крупные глыбки и участки электронноплотного вещества, тогда как, однако, диффузный нуклеоид и структуры, которые соответствовали бы скоплениям рибосом, не обнаруживаются; электронноплотный слой в оболочке бактерии слабо выражен (Табл. XII 27). Интересной особенностью бактерий этой стадии является образование вокруг них электронноплотного чехла толщиной до 30–50 nm, вероятно, хрома-

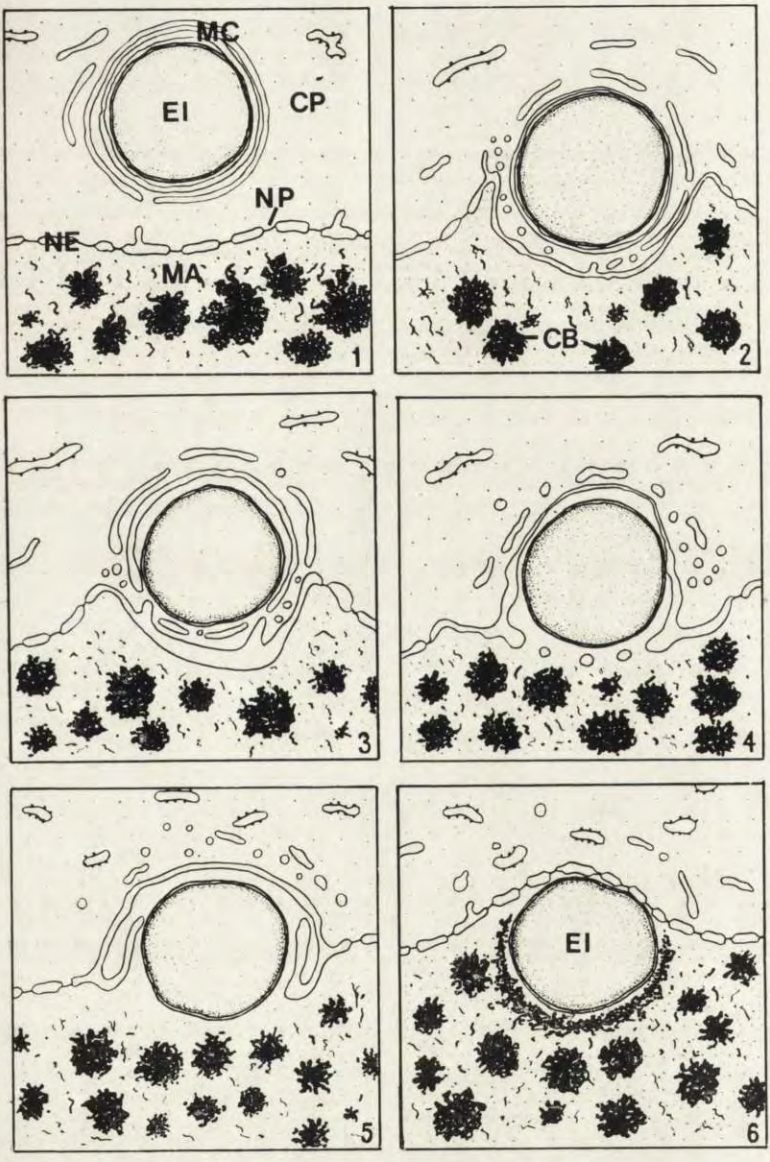

Рис. 1–6. Последовательные этапы проникновения симбиотических йота-бактерий через оболочку макронуклеуса инфузории *Paramecium caudatum*. (Модель "шлюзования" симбионта). *1*— Ближайшая к МА часть многослойного МК начинает распадаться. Симбионта окружают 1–2 слоя уплощенных цистерн эндоплазматического ретикулума. Контур ядерной оболочки МА сравнительно ровный, выросты наружной мембраны редки, поры многочисленны. *2* — Продолжающий распадаться участок МК сближается с ядерной оболочкой МА. Выросты лопастей МА начинают с двух сторон охватывать симбионта. *3*— В зоне контакта ядерной оболочки с МК возникают многочисленные выросты наружной мембраны ядерной оболочки, ядерные поры исчезают, перинуклеарное пространство заметно расширяется. *4*— Выросты наружной мембраны ядерной оболочки сливаются с уплощенными цистернами МК, образуя вокруг бактерии замкнутую камеру. Ядерная оболочка МА в непосредственной зоне контакта с симбионтом начинает фрагментироваться. *5*— Процесс фрагментации ядерной оболочки МА в зоне контакта с симбионтом завершается. Образовавшиеся дополнительные выросты ядерной оболочки между стенками камеры и симбионтом постепенно охватывают симбионта. *6*— Дополнительные выросты ядерной оболочки сливаются над симбионтом. МК распадается. В МА вокруг симбионта образуется электронноплотный чехол

Fig. 1–6. The successive stages of penetration of the symbiotical iota-bacteria through the macronuclear envelope in *Paramecium caudatum*: The "symbiont-sluicing" model. *1* — A part of multilayered membrane complex (MC) nearest to the macronucleus (MA) begins to desintegrate. The symbiont is surrounded by 1–2 layers of flattened cisterns of the endoplasmic reticulum. The outline of MA nuclear envelope is considerably even, elaborations of the external membrane are rare, pores are numerous. *2* — A part of MC proceeding to desintegrate approaches the MA nuclear envelope. Elaborations of MA begin to embrace the symbiont from two sides. *3* — Numerous elaborations of the external membrane of nuclear envelope begin to form in the area of MC and nuclear envelope contact; nuclear pores disappear, perinuclear space widens noticeably. *4* — Elaborations of the external membrane of the nuclear envelope fuse with flattened MC cisterns, forming the closed camera around the bacterium. The fragmentation of the MA nuclear envelope in the zone of the contact with the symbiont begins to take place. *5* — The process of MA nuclear envelope fragmentation in the zone of the contact with the symbiont is completed. Additional elaborations of the nuclear envelope formed between the camera walls and the symbiont gradually embrace the symbiont. *6* — Additional elaborations of the nuclear envelope fuse over the symbiont. MC desintegrates. Electrone-dense cover around the symbiont is forming in MA

тиновой природы. Чехол имеет фибриллярную структуру, причем размер тяжей близок по величине к обычному диаметру (10–11nm) фибрилл хроматина МА *P. caudatum*. Подобное образование отмечено ранее и вокруг некоторых вегетативных йота-частиц в стабильно зараженных МА (Громов и др. 1976).

На стадии 4–8 часов от начала инфекции цитоплазма парамеций совершенно не содержит остатков МК и по своей организации не отличается от нормы. Йота-бактерии обнаруживаются только в МА, но не отмечены в ПВ, цитоплазме, автофагических вакуолях или МИ. Следовательно, процесс транспорта симбионта к своему специфическому внутриклеточному компартменту — МА осуществляется направленно и за короткий срок.

Начиная со вторых суток инфекции йота-частицы, обнаруживаемые в МА, ничем не отличаются по своей организации от типичных вегетативных форм. Среди них обнаруживаются делящиеся клетки, тогда как "споровые" формы симбионтов еще не представлены. Тот же состав популяции симбионтов в МА и на 4-е сутки инфекции, однако, количество "спор" в ядре становится значительно большим. Следовательно, в течение первых суток после заражения "чистого" МА вегетативные формы бактерий активно делятся и количество симбионтов в ядре возрастает. Только на 7-е сутки в МА инфицированных парамеций начинают обнаруживаться споровые формы йота-бактерии (Табл. XIV 29, 30). Таким образом завершается жизненный цикл симбиотических бактерий МА.

Обсуждение

В настоящее время имеются сравнительно полные сведения относительно особенностей ультраструктурной организации нескольких форм эндонуклеосимбиотических бактерий: альфа-частиц из МА *Paramecium aurelia* (Preer et al. 1974), омега- и йота-частиц из *P. caudatum* (Осипов и др. 1976) и фрагментарные данные о ряде других внутриядерных симбионтов нескольких видов инфузорий (см. обзор Осипов и др. 1976). Однако до сих пор оставались совершенно вне поля зрения вопросы, связанные с тем, как осуществляется проникновение симбионтов в специфические компартменты ядерного аппарата. Отметим, что такая же картина, к сожалению, имеет место и для всех многочисленных симбиотических бактерий цитоплазмы инфузорий (Ball 1969, Preer et al. 1974), некоторые из которых довольно детально изучены во многих аспектах.

В плане обсуждения вопросов, рассматриваемых в настоящей работе, следует отметить, что для простейших встречаются довольно многочисленные данные о внутриклеточной локализации облигатных внутриклеточных паразитов: риккетсий, бактерий, грибов и простейших (Kirby 1941, Ball

1969). Однако о механизмах проникновения микропаразитов в ядра клеток-хозяев имеются весьма отрывочные соображения, носящие скорее спекулятивный характер. Так, например, для внутриклеточных паразитов *Toxoplasma gondii* и *Besnoitia jellisoni*, культивируемых в клетках культуры ткани, Akinchina and Zasukhin (1971) допускают два возможных механизма проникновения паразитов в ядро. Во-первых, за счет активного проникновения благодаря секреции парных органелл паразита и, во-вторых, в результате фагоцитарной функции ядра (кариофагии). К сожалению, на приведенных в указанной работе электронограммах трудно однозначно трактовать особенности ультраструктурных перестроек мембран цитоплазматических органелл и ядер клетки-хозяина. Кроме того, возникает сомнение в корректности использования термина фагоцитоз (кариофагия) по отношению к ядру.

Представленные в настоящей работе данные являются первой попыткой изучения механизмов проникновения симбиотических бактерий в ядерный аппарат клетки-хозяина. Каковы же основные ультраструктурные особенности процесса проникновения симбиотических йота-бактерий в МА инфузории *P. caudatum*?

Несмотря на то, что бактерии ни на одной из стадий жизненного цикла симбионта не имеют органоидов движения и лишены способности к самостоятельному активному движению, заражение МА происходит благодаря морфо-функциональным адаптациям, которые обеспечивают последовательный транспорт симбионтов из внешней среды в пищеварительную вакуоль, цитоплазму и, наконец, в МА.

Кажется вероятным, что направленный транспорт неподвижных симбиотических бактерий в ядро определенного типа обеспечивается оригинальным механизмом эстафетной смены разных мембран клетки-хозяина: пищеварительной вакуоли, эндоплазматического ретикулума, ядерной оболочки МА, которые окружают симбионта на последовательных этапах его проникновения в МА. Процесс инфекции йота-частицами "чистых" парамеций состоит из строго последовательной совокупности этапов: изменение "споровой" формы в пищеварительной вакуоли, проникновение эуинфекционной частицы через мембрану вакуоли, образование и распад МК, сложные изменения ядерной оболочки МА. Весьма важными чертами транспорта йота-частиц в ядро являются следующие. Бактериальная клетка на всех этапах оказывается практически изолированной от непосредственного контакта с цитоплазмой клетки хозяина. Выход симбионта из пищеварительной вакуоли происходит таким образом, что не возникает разрывов мембран вакуоли, и, следовательно, исключается действие ферментов ПВ на цитоплазму инфузорий. При проникновении симбионта в МА ядерная оболочка реорганизуется таким образом, что кариолимфа не вступает в прямой контакт с цитоплазмой парамеций (Рис. 1–6).

Можно видеть, что симбионт оказывает определенное воздействие только

на весьма ограниченный участок ядерной оболочки МА, изменяя ее строение: размер модифицированного участка сравним с диаметром бактерии эуинфекционной стадии. Наличие выраженной локальности ультраструктурных изменений ядерной оболочки в момент проникновения симбионта позволяет предполагать выделение симбионтом специфического индуктора этих изменений. Если это предположение верно, то следует допустить, что этот гипотетический индуктор должен либо обладать весьма малой скоростью диффузии в цитоплазме, либо очень быстро распадаться.

Внутриядерные симбионты на последовательных этапах инфекции используют временну́ю и пространственную организацию нескольких функциональных процессов клетки-хозяина: фагоцитоз пищевых частиц, внутриклеточное пищеварение, циклоз цитоплазмы, динамический характер перестроек мембран эндоплазматического ретикулума и ядерной оболочки. К этому следует добавить, что один из этапов жизненного цикла йота-частиц — выход споровых форм из стабильно зараженной клетки в окружающую среду — обеспечивается еще одним процессом, а именно, делением инфицированного МА (Осипов и др. 1976), в результате которого часть "спор" популяции симбионтов попадает в остаточное тело ахроматинового аппарата ядра и из него через порошицу выводится во внешнюю среду. Вегетативные формы пассивно отходят вместе с хроматиновыми элементами в дочерние ядра делящихся парамеций. Таким образом обеспечивается как сохранение симбионтов в клеточных поколениях, так и создаются условия для инфекции "чистых" парамеций. Инфекционные "споровые" формы симбионтов снабжены более плотными оболочками, благодаря чему "споры" могут находиться вне парамеций, не теряя инфекционной способности, по крайней мере, в течение нескольких суток.

Остается не совсем ясным, насколько скорость и направленность отмеченных процессов в клетке могут подвергаться изменениям от нормы в период транспорта инфекционных частиц. Например известно, что скорость фагоцитоза, продолжительность циркуляции и путь в цитоплазме пищеварительных вакуолей у парамеций зависит от размеров пищевых частиц (Kuźnicki and Sikora 1972, Раилкин 1975). Согласно этим автором у *Paramecium caudatum* оптимальная для заглатывания величина пищевых частиц составляет около 1–5μm, тогда как частицы размером 15–20μm заглатываются парамециями в исключительных случаях. Кроме того отмечено, что более крупные частицы резко снижают скорость фагоцитоза. В этой связи напомним, что длина споровой частицы йота-бактерии достигает 15–18μm.

Поскольку йота-бактерии попадают в место своей локализации через пищеварительную вакуоль, возникает вопрос, чем достигается устойчивость симбионтов к гидролитическим ферментам парамеций? Известно например, что устойчивость внутриклеточных паразитов и симбионтов к перевариванию в клетках-хозяина может быть обеспечена самыми различными механизмами: предотвращением контакта микроорганизмов с клеточной стенкой хозяина,

предотвращением захвата в фагоцитарную вакуоль, индукцией лизиса фагоцитарной вакуоли, предотвращением слияния лизосом с фагосомой, ингибированием гидролитических ферментов, устойчивостью клеточной поверхности к гидролитическим ферментам (см. например Jones 1974; Muscatine et al. 1975).

Данные, полученные нами в настоящей работе, убеждают в том, что у инфицируемых парамеций не нарушена функция слияния мембраны лизосом с мембраной первичной фагосомы, т.к. обычные пищевые частицы (*Aerobacter aerogenes*) нормально перевариваются в вакуолях, содержащих одновременно и йота-бактерии. Скорее, устойчивость йота-бактерий обеспечивается особенностями организации клеточной стенки симбионта "споровой" формы. Более того, можно думать, что именно нормальный цикл развития пищеварительной вакуоли обеспечивает созревание "споровых" форм и выход из их оболочки эуинфекционных частиц. Ранее Громовым и др. (1974) было показано, что в растворах 0.05–0.25 М HCl наблюдается мгновенный выход из оболочки йота-бактерии в окружающую среду тела, очевидно соответствующего протоплазматическому содержимому "споры" — эуинфекционной стадии; вегетативные клетки бактерий никакой реакции на HCl не обнаруживают. В период транспорта эуинфекционной частицы в цитоплазме парамеций симбионт окружен многослойным МК, состоящим из уплощенных цистерн, у которых, по-видимому, отсутствует сродство к слиянию с лизосомами.

Другой существенной стороной проблемы направленного перемещения йота-частиц в клетке-хозяина является образование мембранного комплекса (МК) и механизм избирательной доставки симбионта к МА. Волна структурных реорганизаций мембран эндоплазматического ретикулума у инфицированных парамеций инициируется, по-видимому, локальным процессом — выходом бактерии эуинфекционной стадии из пищеварительной вакуоли. В цитологической литературе известно много примеров регуляции клеточных функций путем кооперативных конформационных переходов мембран, затрагивающих большую часть объема клетки (Конев и др. 1970), при этом вслед за структурными изменениями мембран, как правило, почти мгновенно имеют место скачкообразные изменения их функции. Весь процесс образования и последующий распад МК в цитоплазме парамеций напоминает генерализованные кооперативные структурные перестройки, для которых характерно два основных признака: во-первых, обратимые дискретные структурные состояния, во-вторых, скачкообразные изменения биологических функций при переходе от одного из них к другому.

При описании динамики образования МК вокруг симбионта мы уже отмечали, что МК возникает скорее всего за счет перестройки существующих элементов эндоплазматического ретикулума. Поскольку все мембраны МК не содержат рибосом, то следует признать, что сначала происходит преобразование шероховатого ретикулума в гладкий, после чего, возможно через

промежуточные стадии, последний дает МК. Наличие в цитоплазме парамеций наряду с МК обширных зон, лишенных мембран, говорит в пользу такой точки зрения. С другой стороны, в процессе образования МК, помимо принципа "мембрана от мембраны", нельзя исключить и возможности агрегации предшественников мембран путем самосборки. Однако без специальных исследований с использованием соответствующих радиоактивных меток мембран высказанные соображения о биогенезе мембран и механизме возникновения МК вокруг симбиотической бактерии остаются не более чем предположениями.

Независимо от того, как образуется МК, особенности организации этой эфемерной структуры цитоплазмы клетки-хозяина позволяют высказать предположение, что она каким-то образом участвует в направленном транспорте симбионта к МА. С одной стороны, МК, окружающий симбионта, может как и другие включения цитоплазмы, пассивно перемещаться на значительные расстояния благодаря циклозу цитоплазмы, и за счет чисто случайных событий иногда сталкиваться с МА. Конечно, одним только случайным столкновением не объяснить избирательности контакта МК именно с МА, но не с МИ парамеций. Возможно, особенности организации МК как самостоятельной структуры цитоплазмы (его заряд, гидрофобность сдвоенных мембран, какие-то специфические белки на поверхности уплощенных цистерн) определяют сродство только с ядерной оболочкой МА, но не МИ; тем самым увеличивается вероятность контактов мембран МК с ядерной оболочкой МА.

Хорошо известно, что ядерная оболочка обладает механизмом, осуществляющим активный транспорт макромолекул и надмолекулярных комплексов между ядром и цитоплазмой и функцией регуляции активного транспорта цитоплазматических и ядерных продуктов (Goldstein 1974, Franke and Scheer 1974). Следует признать, что йота-частицы каким-то образом так изменяют механизм регуляции транспорта через ядерную оболочку МА *P. caudatum*, что становится возможным проникновение в ядро „макрообъектов" — симбиотических бактерий.

По мнению Осипова и др. (1976) изучение взаимодействия симбиотических бактерий с ядерным аппаратом инфузорий можно рассматривать как своеобразный методический подход к выяснению ряда сторон дифференцировки ядер. Очевидно, что "симбиотический" метод окажется тем более эффективным, чем больше разнообразных форм симбионтов будет использоваться в исследованиях, и чем детальнее будут разработаны приемы работы с ними. Данные настоящей работы убеждают в том, что возможности "симбиотического" метода могут оказаться гораздо шире, поскольку позволяют исследовать широкий круг вопросов ядерно-цитоплазматических отношений, направленность динамических преобразований мембранных структур клетки, проблемы избирательного транспорта, перемещения надмолекулярных комплексов, цитоплазматических включений и органелл.

SUMMARY

In ciliates the symbiotic gram-negative bacteria are found. These denote usually by the letters of greek alphabet. Some of them infect the nuclear apparatus of ciliates, the infection being rigorously specific: either somatic nucleus (MA) or generative one (MI) is infected and that seems to deal with the profound morpho-functional differences between these two kinds of nuclei.

In *Paramecium caudatum* the symbionts of MA — *Holospora obtusa* (iota) and of MI — *H. undulata* (omega) are found (Ossipov et al. 1976). Both of them have the life cycle with reproductive and spore-like forms. During this cycle the unusual for another bacteria cyto-differentiation takes place. None of the stages of bacterial life cycle is characterised by the capacity to active movement. By each division of stable infected paramecium a number of "spores" remains outside the daughter nuclei and shortly often they are led out to the environment.

The data presented are the first known attempt to study the penetrative mechanism of symbiotic bacteria into nuclear apparatus of the host cell. Ultrastructural changes of *Paramecium caudatum* cell and iota-bacteria in the early stages of infection and in the stage of penetration into MA are studied. During the phagocytosis of paramecium the "spore" bacterial forms get from environment into digestive vacuoles, where the ripening and hatching of euinfective stage from the "spore" envelopes take place. Symbiont penetrates into cytoplasme of paramecium invaginating the membrane of digestive vacuole. In the cytoplasme arround the symbiont the membrane complex (MC), consisting of 4–5 layers of paired elementary membranes of flattened cisterns, is formed. The origin of cisterns are related with endoplasmatic reticulum. Due to peculiarities of MC the directed transport of symbiont to MA takes place. Symbionts penetration through the MA nuclear envelope may be compared with sluicing, owing to which the nuclear content never comes into contact with the cytoplasme of paramecium (Fig. 1–6). The symbionts are found in MA 1 h after the moment of addition of "spore" forms into cultural medium with "pure" cells. Its seems reasonable that the directed transport of symbiotic bacteria into the definite type of nucleus (MA) is provided by the original mechanism of "ralay--race" change of various host-cell's membranes, which surround the self-immovable symbiont in different stages of penetration.

ЛИТЕРАТУРА

Akinchina G. T. and Zasukhin D. N. 1971: Intranuclear localization of *Toxoplasma gondii* and *Besnoitia jellisoni* in conditions of tissue culture and some remarks on the intranuclear parasitism. Acta Protozool., 8, 341–348.

Ball G. H. 1969: Organisms living on and in *Protozoa*. In: Research in Protozoology, (ed. T. T. Chen), Pergamon Press, Oxford — New York, vol. 3, 565–718.

Ченцов Ю. С. и Поляков В. Ю. 1974: Ультраструктура клеточного ядра. Изд. Наука, Москва, 174.

Dippell R. V. 1955: A temporary stain for *Paramecium* and other ciliated *Protozoa*. Stain technol., 30, 69–71.

Dutta G. P. 1974: Recent advances in cytochemistry and ultrastructure of cytoplasmic inclusions in *Ciliophora* (*Protozoa*). In: International Review of Cytology, vol. 39, 285–343.

Esteve J. C. 1970: Distribution of acid phosphatase in *Paramecium caudatum*: Its relations with the process of digestion. J. Protozool., 17, 24–35.

Franke W. and Scheer U. 1974: Structure and functions of the nuclear envelope. In: The Cell Nucleus, (ed. H. Busch), Academic Press, New York and London, vol. 2, 219–347.

Goldstein L. 1974: Movement of molecules between nucleus and cytoplasm. In: The Cell Nucleus, (ed. H. Busch), Academic Press, New York and London, vol. 2, 387–439.

Gorovsky M. A. 1973: Macro- and micronuclei of *Tetrahymena pyriformis*: A model system for studing the structure and function of eukaryotic nuclei. J. Protozool., 20, 19–25.

Громов Б. В., Осипов Д. В., Мамкаева К. А. 1974: Особенности реакции на экстремальные значения pH удлиненных клеток — "спор", симбиотических бактерий макронуклеуса *Paramecium caudatum*. ДАН СССР, 217, 712–714.

Громов Б. В., Мамкаева К. А., Осипов Д. В. 1976: Ультраструктура йота-частиц — симбиотических бактерий макронуклеуса *Paramecium caudatum* (*Protozoa, Ciliata*). Изв. АН СССР, сер. биол., 3, 399–409.

Jones, T. C. 1974: Macrophages and intracellular parasitism. J. Reticuloendothel. Soc., 15, 439–450.

Jurand A. and Selman G. G. 1969: The anatomy of *Paramecium aurelia*. Macmillan, London and St. Martin's Press, New York, 218.

Kirby H. 1941: Organisms living on and in *Protozoa*. In: *Protozoa* in biological research, (ed. G. N. Calkins and F. M. Summers), Columbia University Press, New York, 1009–1113.

Конев С. В., Аксенцев С. Л., Черницкий Е. А. 1970: Кооперативные переходы белков в клетке. Изд. Наука и техника, Минск, 204.

Kuźnicki L. and Sikora J. 1972: The hypothesis of inverse relation between ciliary activity and cyclosis in *Paramecium*. Acta Protozool., 11, 243–250.

Muscatine L., Pool R. R. and Trench R. K. 1975: Symbiosis of algae and invertebrates: aspects and the host-symbiont interface. Trans. Amer. Micros. Soc., 94, 450–469.

Ossipov D. V., Skoblo I. I. and Rautian M. S. 1975: Iota-particles, macronuclear symbiotic bacteria of Ciliate *Paramecium caudatum* clone M-115. Acta Protozool., 14, 263–280.

Осипов Д. В., Громов Б. В., Мамкаева К. А., Раутиан М. С., Скобло И. И. и Борхсениус О. Н. 1976: Использование симбиотических бактерий для анализа структуры и функции ядерного аппарата инфузорий. В сб.: Кариология и генетика простейших. Изд. Наука. Ленинград, 101–139.

Осипов Д. В. и Подлипаев С. А. 1976: Электронномикроскопическое исследование ранних стадий заражения *Paramecium caudatum* симбионтами макронуклеуса — йота-бактериями. Тезисы докладов X Всесоюзной конференции по электронной микроскопии. 5–8 октября 1976 г. Ташкент. Москва, т. 2, 108–109.

Preer J. R., Preer L. B. and Jurand A. 1974: Kappa and other endosymbionts in *Paramecium aurelia*. Bacteriol. Rev., 38, 113–163.

Раилкин А. И. 1975: Фагоцитоз и выбор пищи у инфузорий-седиментаторов. Автореф. на соискание уч. степени канд. биол. наук. Ленинград.

Sonneborn T. M. 1970: Methods in *Paramecium* research. In: Methods in Cell Physiology, (ed. D. M. Prescott), Academic Press, New York, vol. 4, 241–339.

Vivier E. 1974: Morphology taxonomy and general biology of the genus *Paramecium*. In: *Paramecium*. A Current Survey. (ed. W. J. van Wagtendonk), Elsevier Scientific Publishing Company, Amsterdam, London, New York, 1–89.

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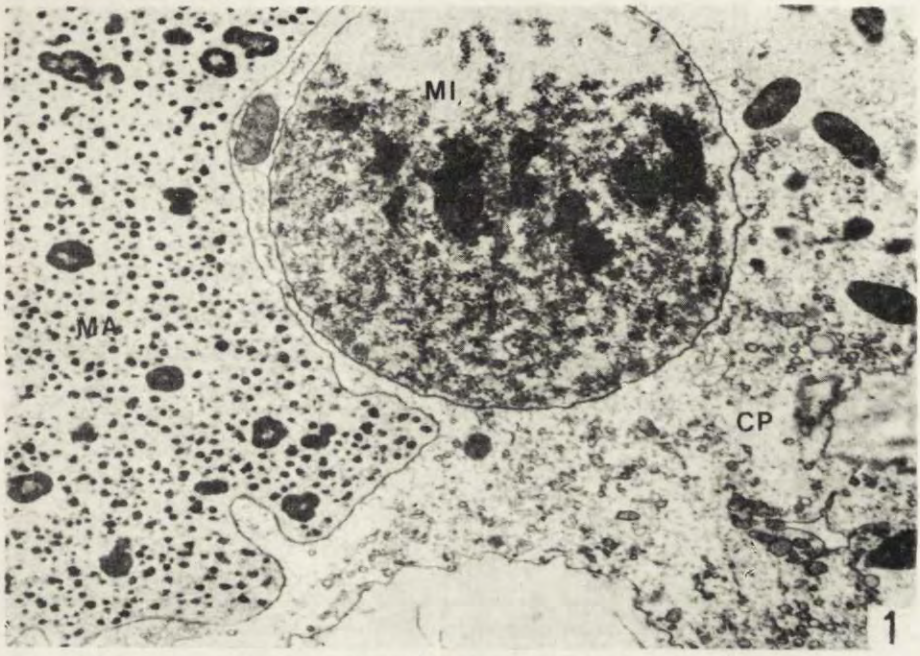
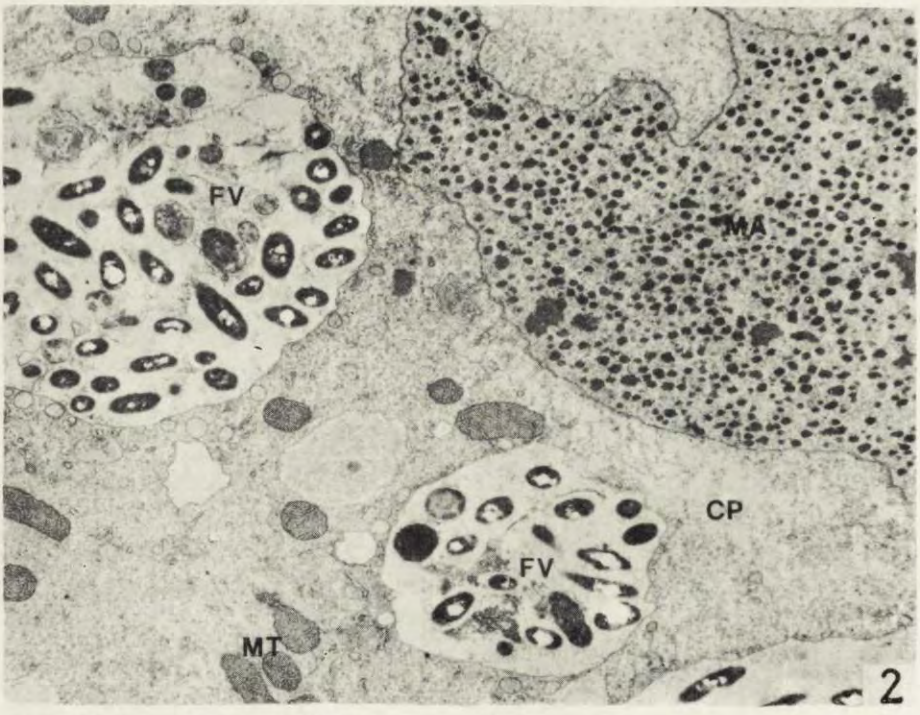
Обозначения: CB — хроматиновые тела макронуклеуса, CM — клеточная мембрана йота-частицы, CP — цитоплазма парамеций, CR — цистерна ретикулума, EC — электронноплотный хроматиновый чехол вокруг йота-частицы, E — йота-частица эуинфекционной стадии, EL — электронноплотный слой оболочки "споры", FC — уплощенная цистерна ретикулума, FP — пищевые частицы в пищеварительной вакуоли парамеции, FV — пищеварительная вакуоль парамеции, IS — йота-частица споровой стадии, IV — йота-частица вегетативной стадии, L — лизосома парамеций, MA — макронуклеус, MC — мембранный комплекс, MI — микронуклеус, MT — митохондрия парамеции, MV — мембрана пищеварительной вакуоли, NE — ядерная оболочка макронуклеуса, NL — нуклеола макронуклеуса, NP — ядерная пора оболочки макронуклеуса, PL — периплазматический слой "споры" йота-частицы, PM — плазматическая мембрана йота-частицы, PN — перинуклеарное пространство оболочки макронуклеуса, TR — трубочки ретикулума, V — пузырьки ретикулума,





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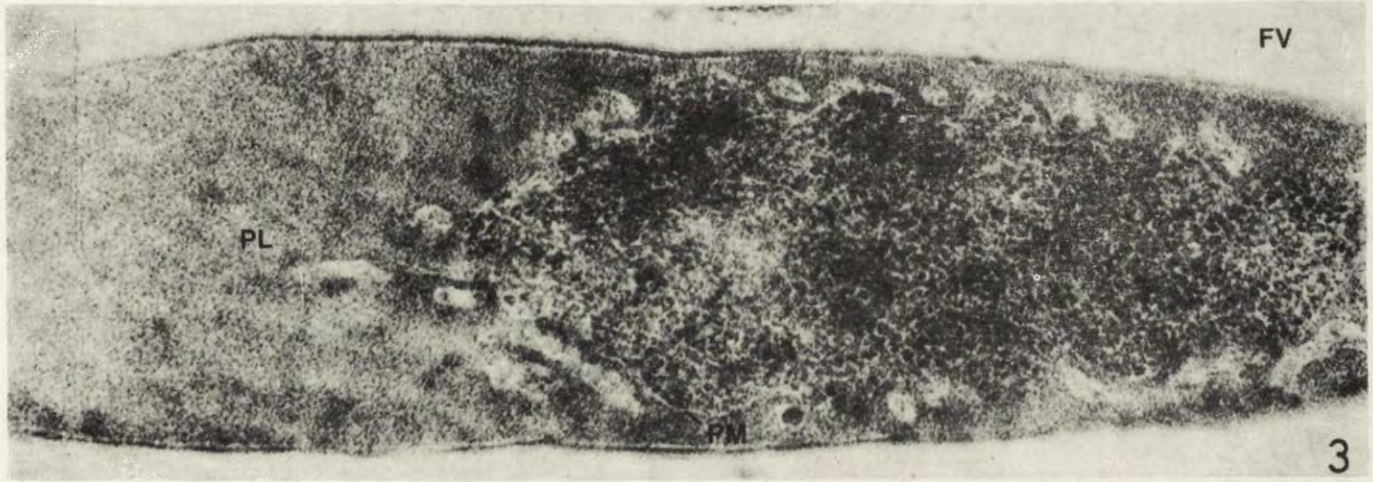

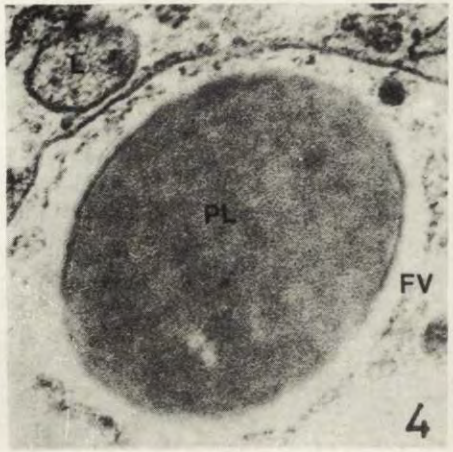

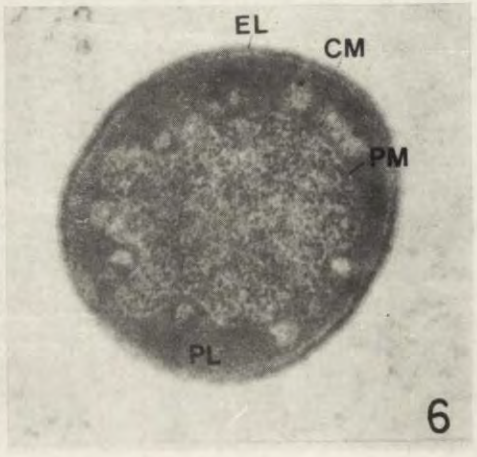

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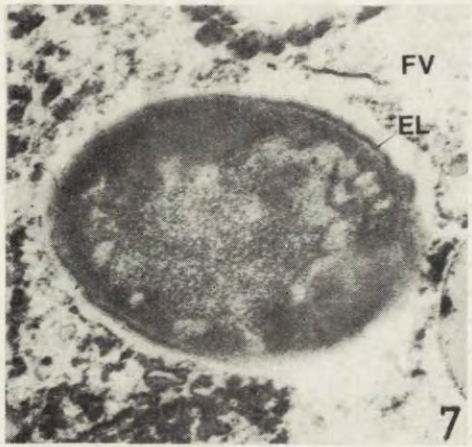

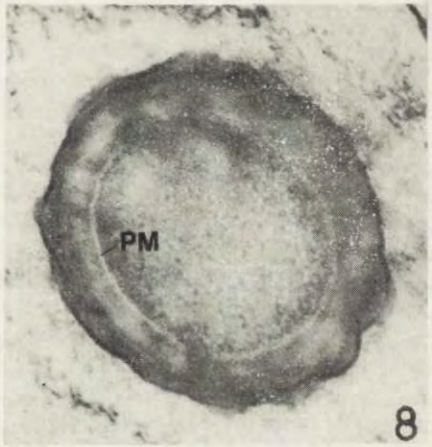

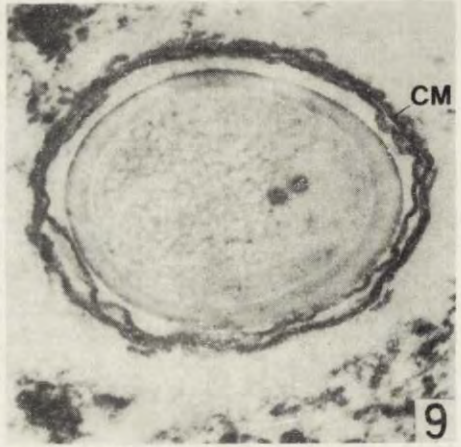

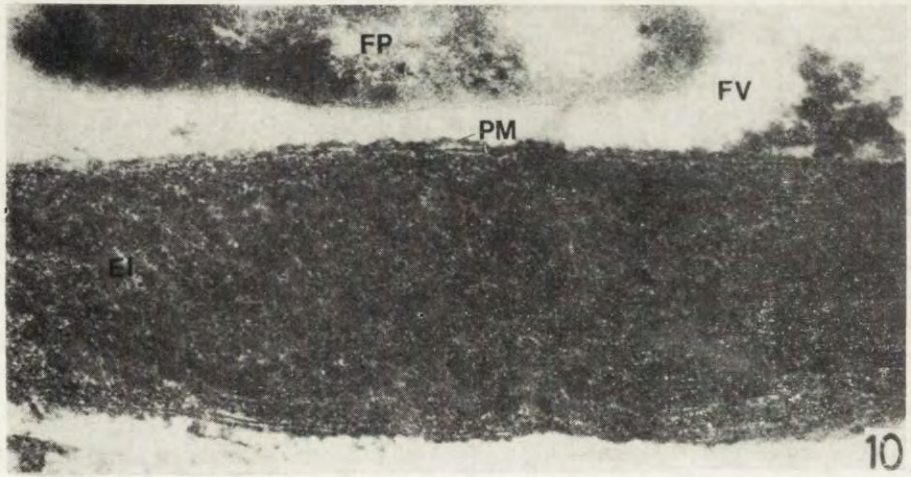

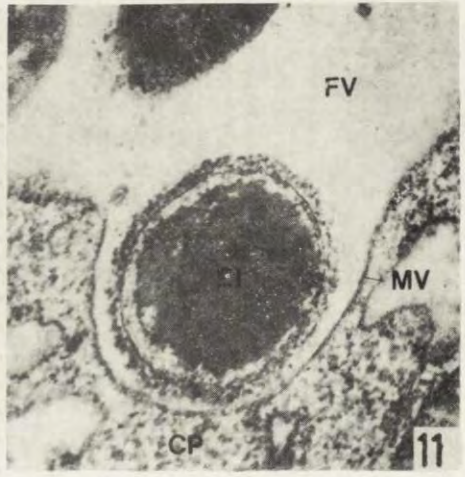

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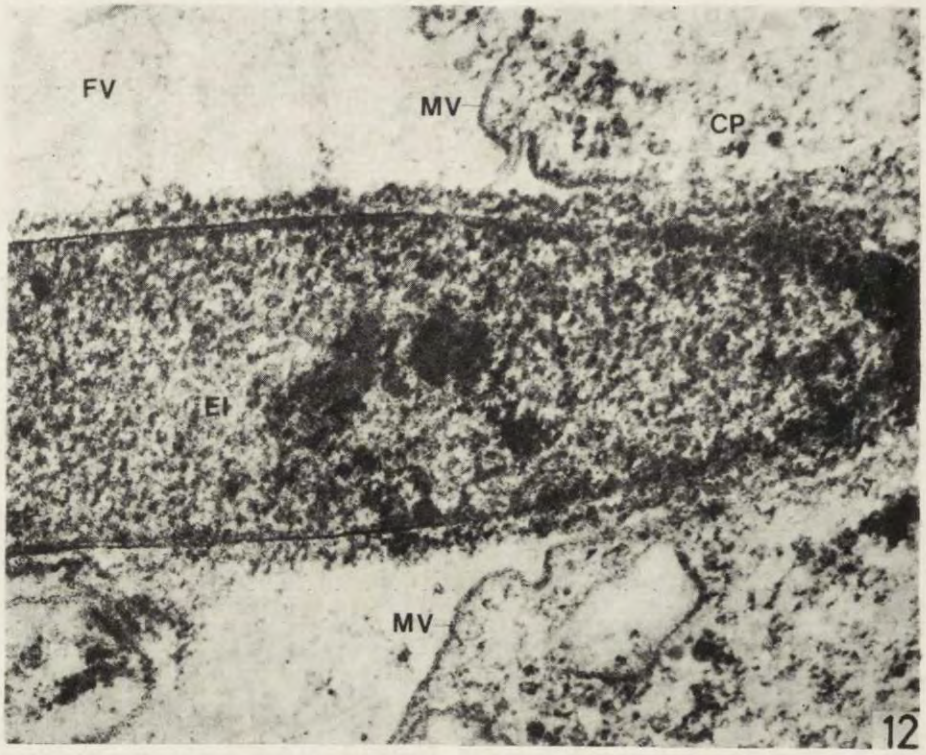

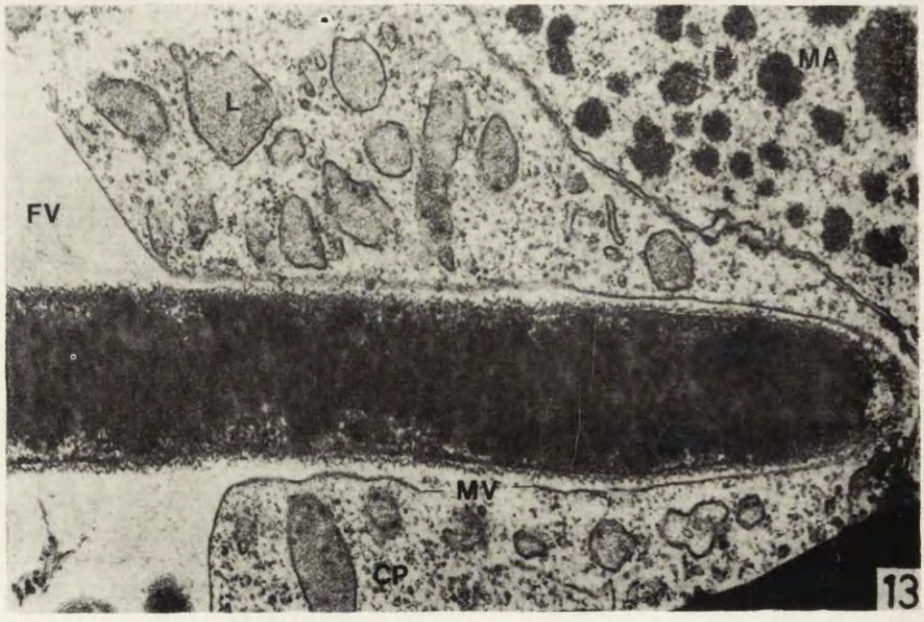

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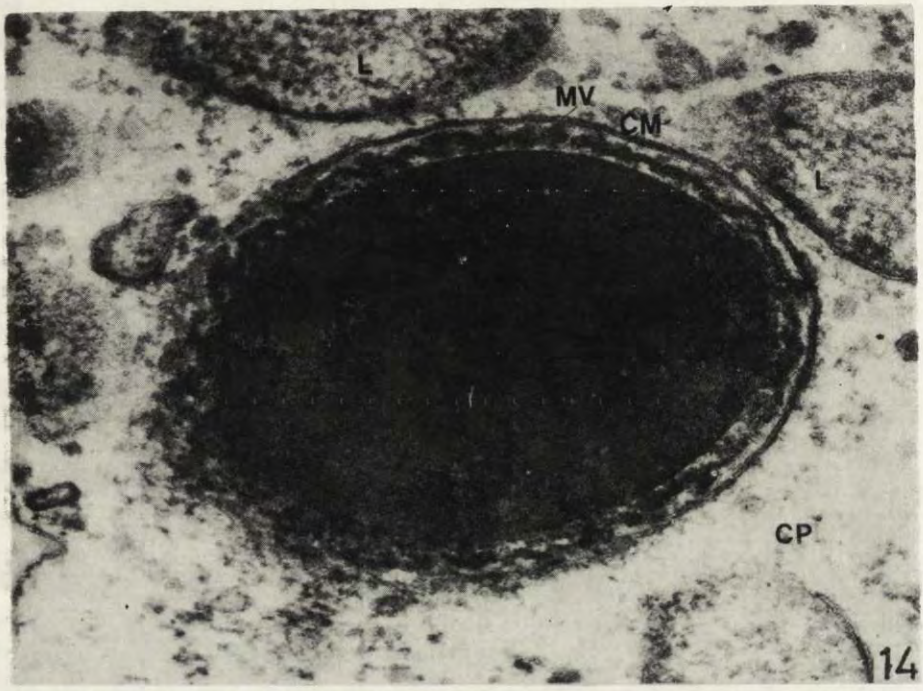

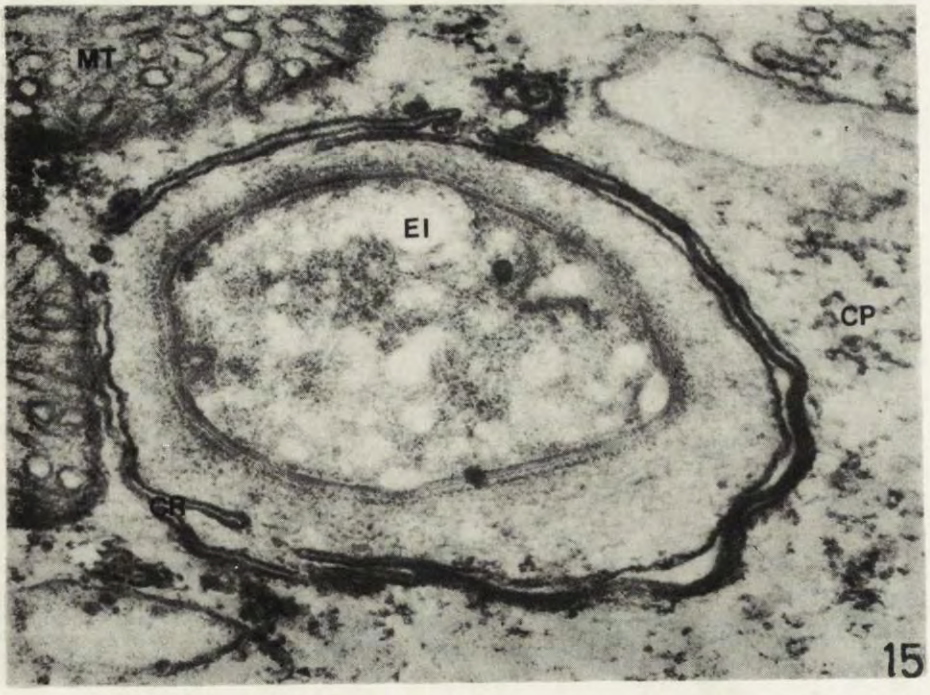

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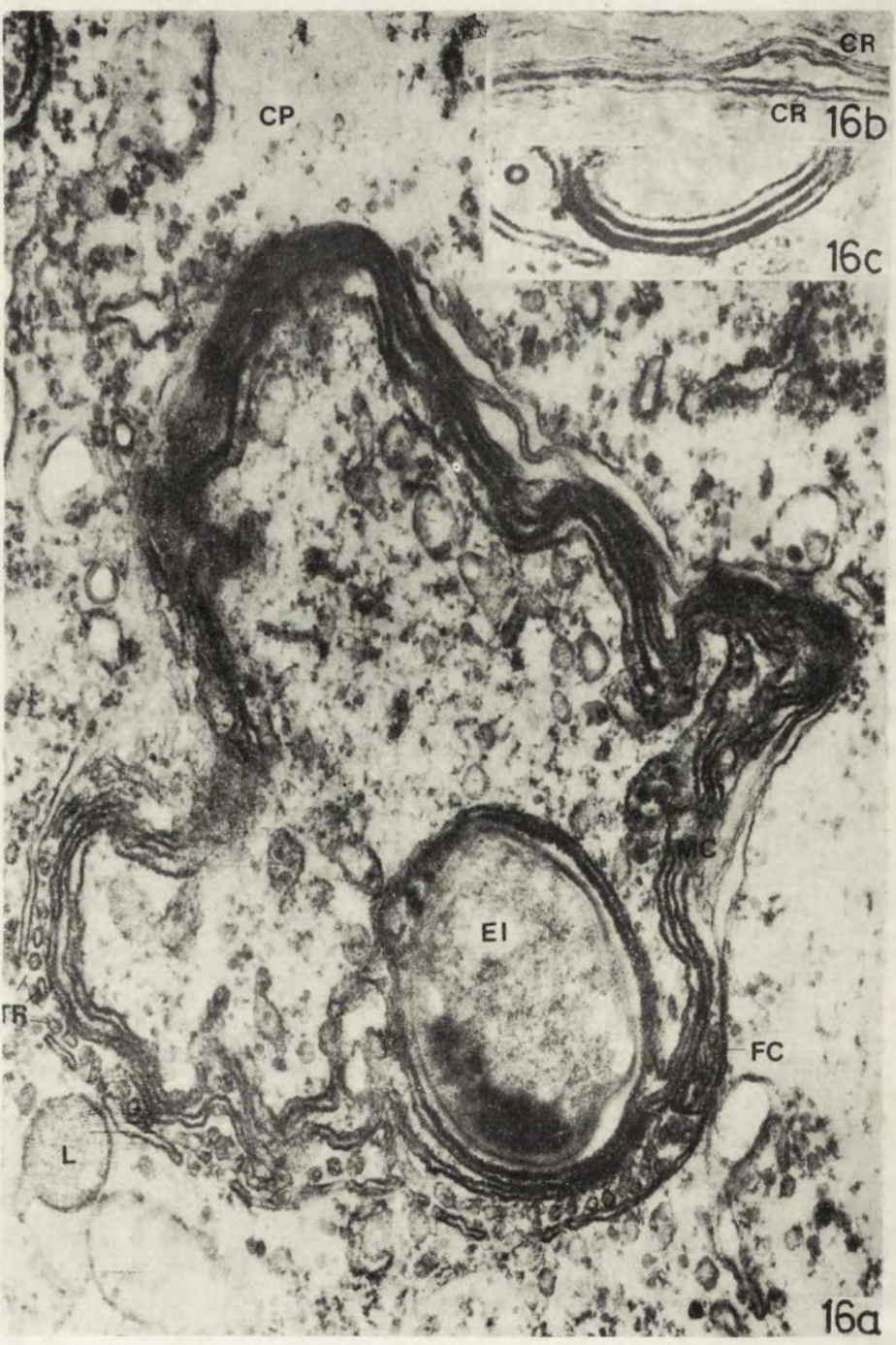

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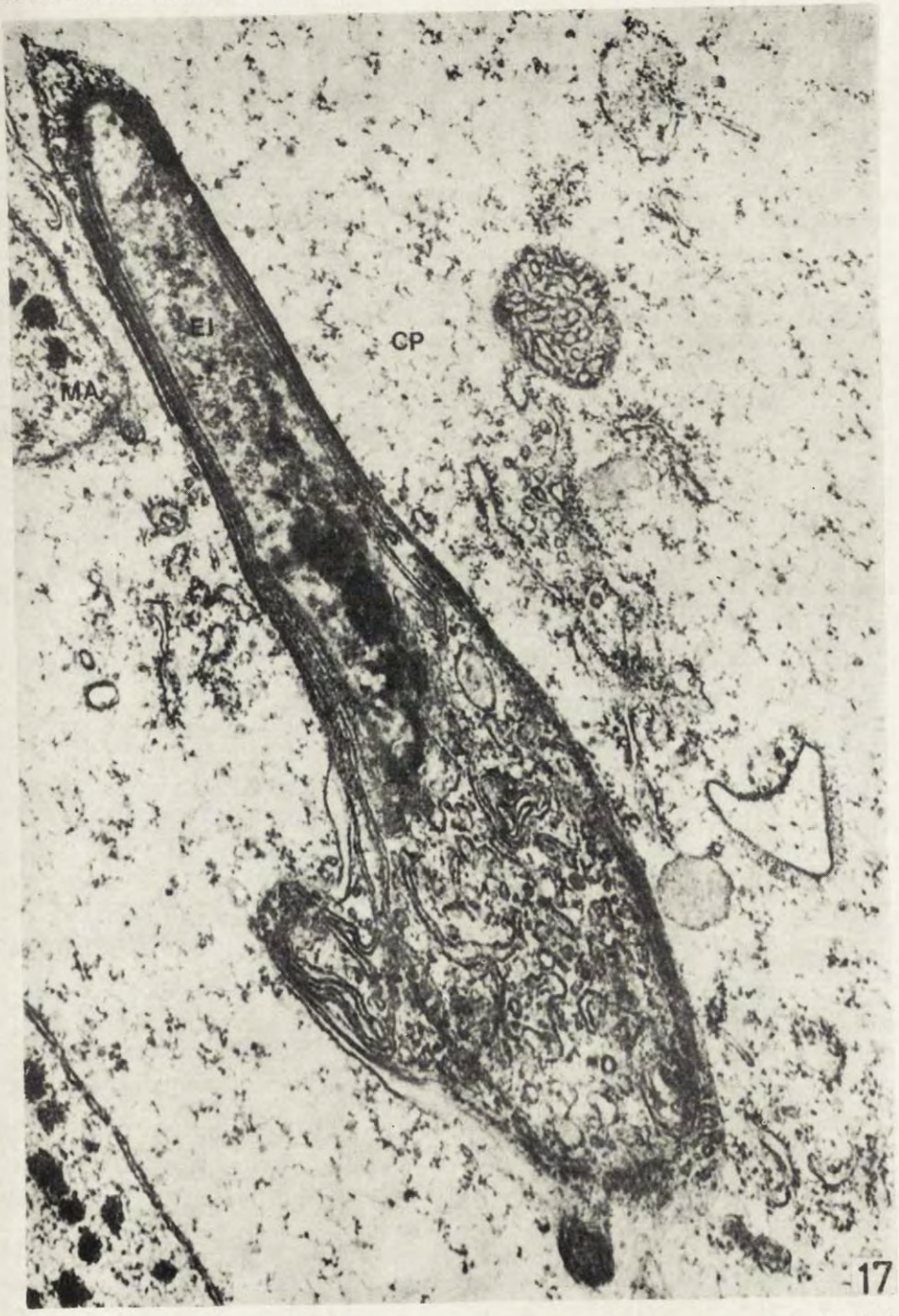

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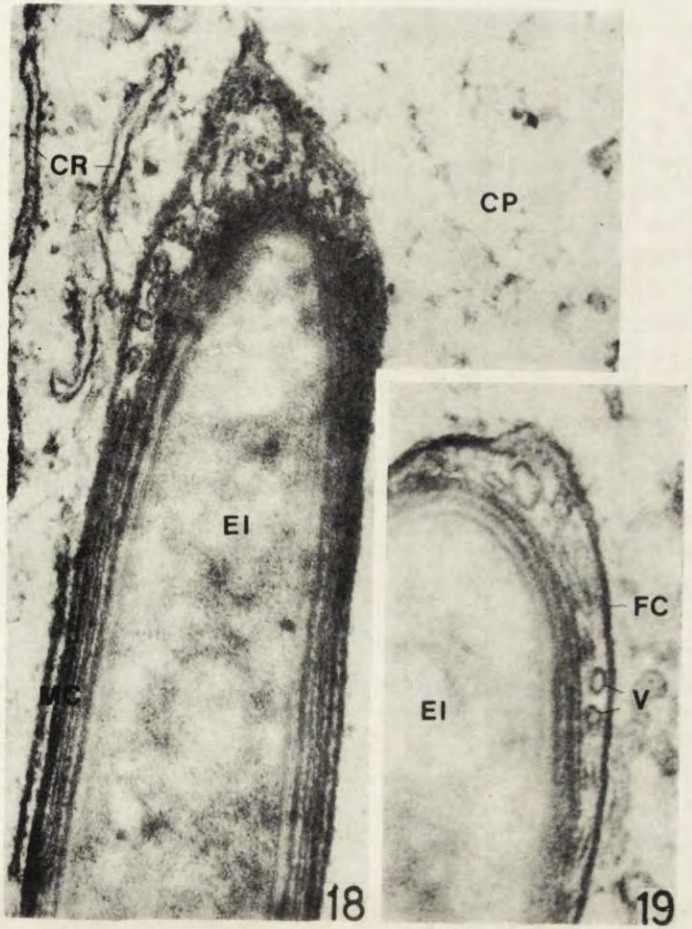

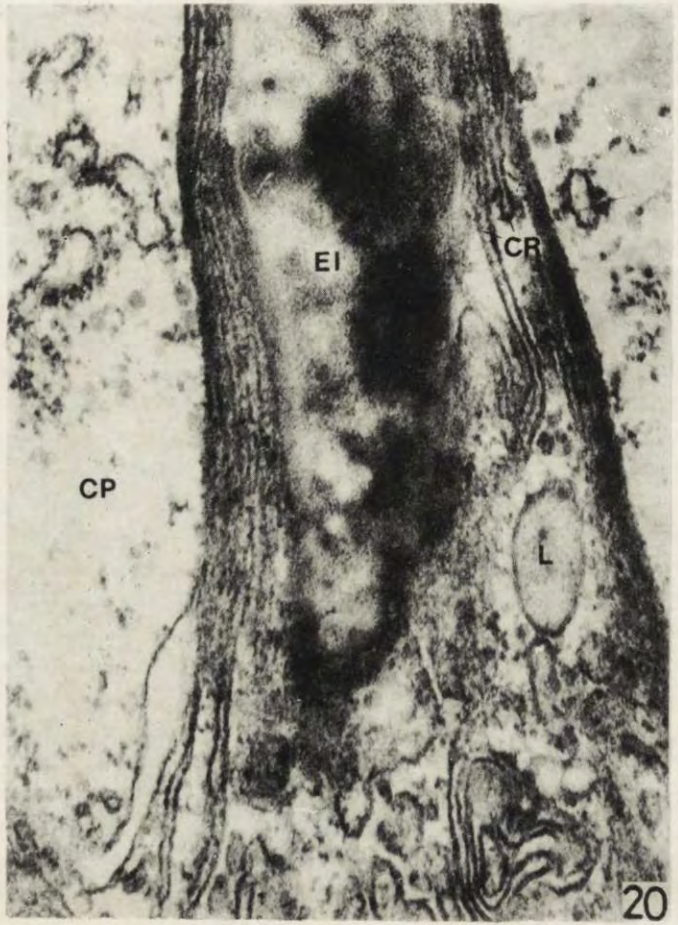

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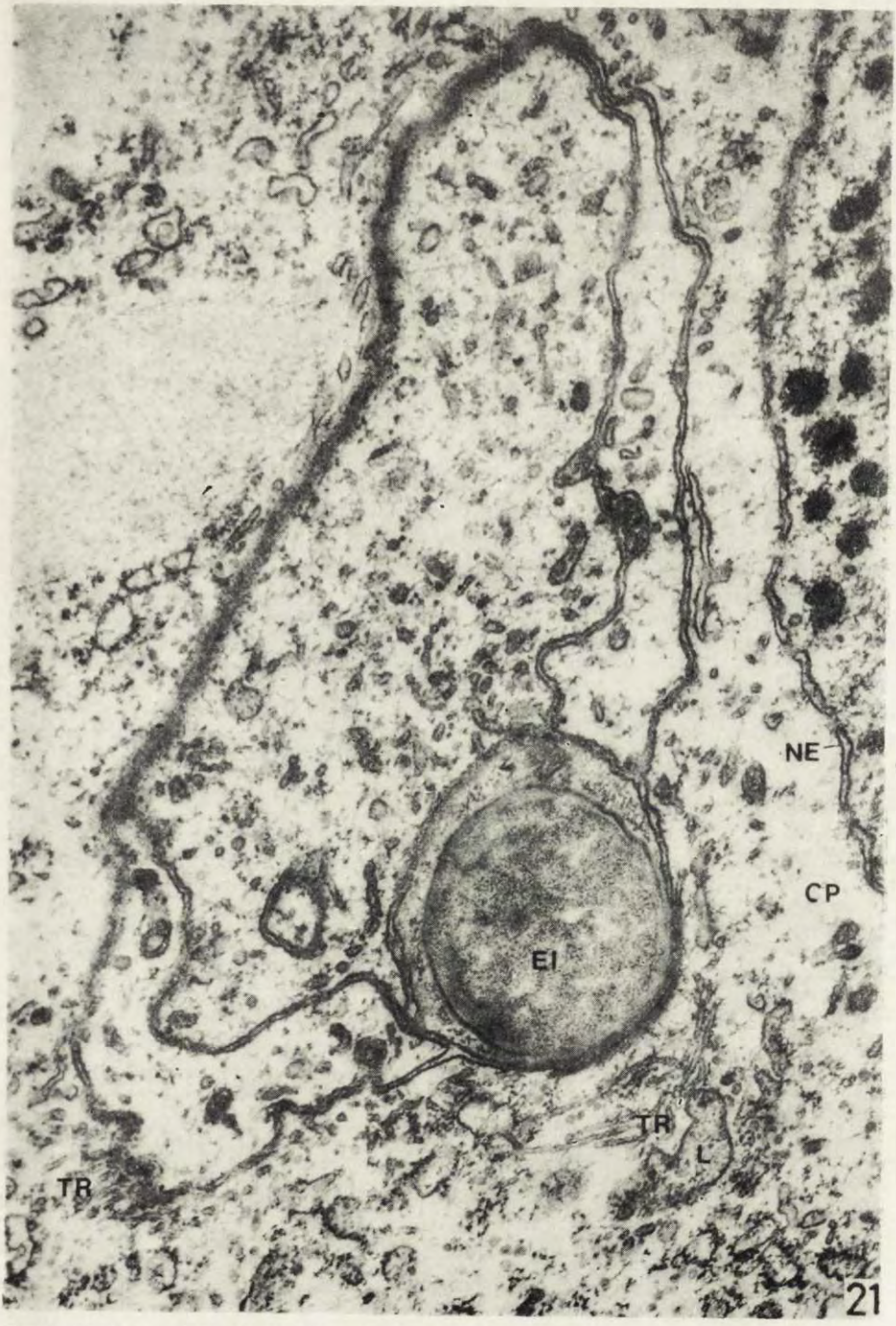

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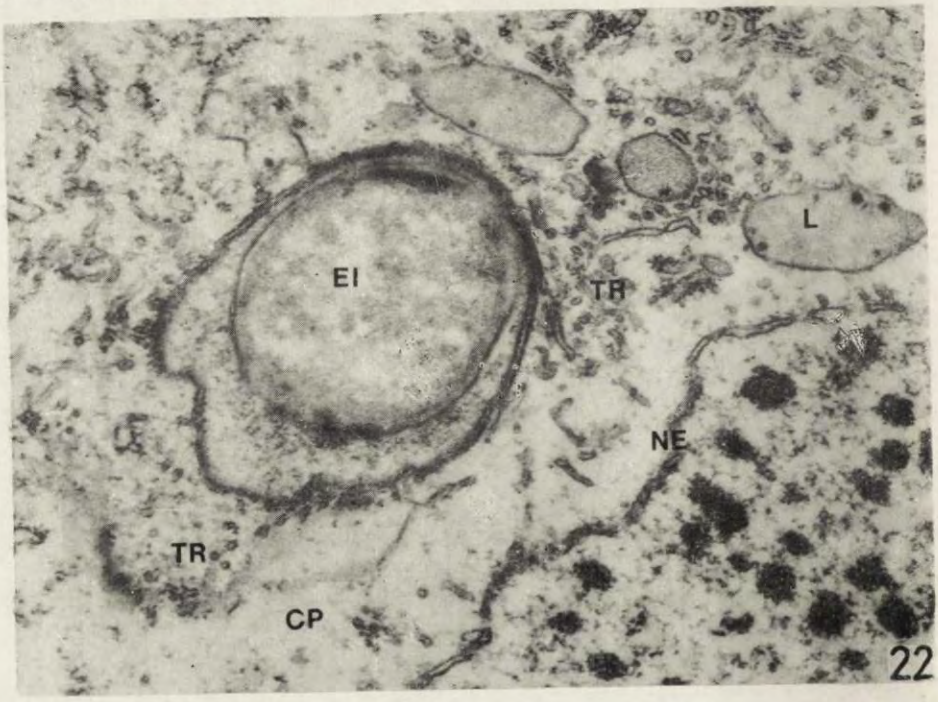

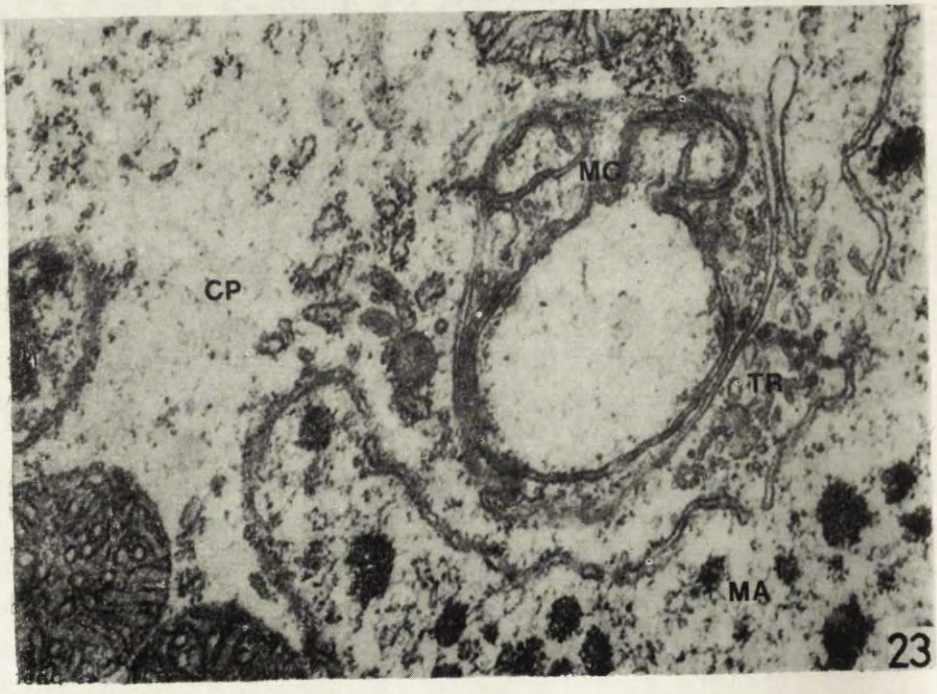

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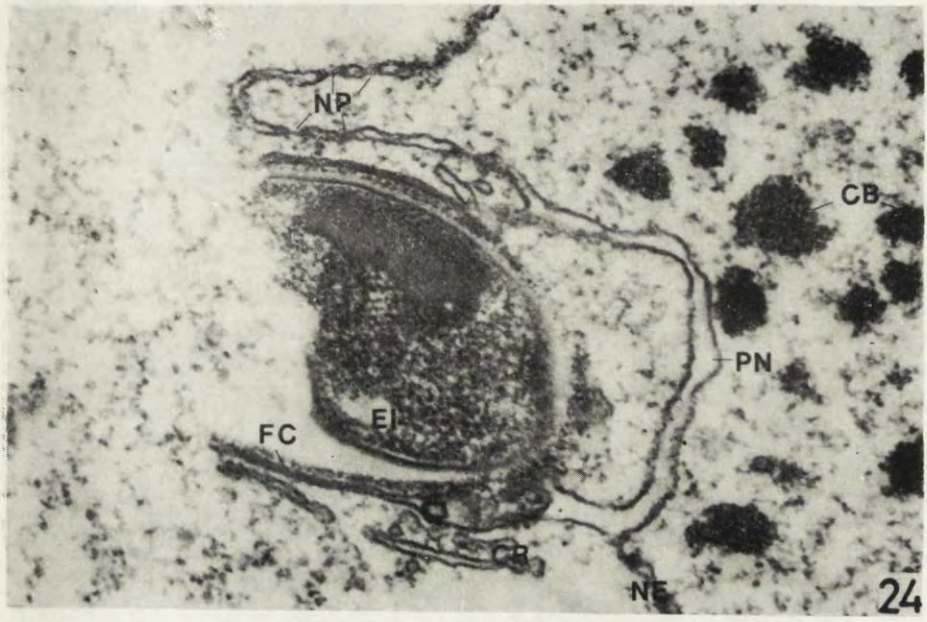

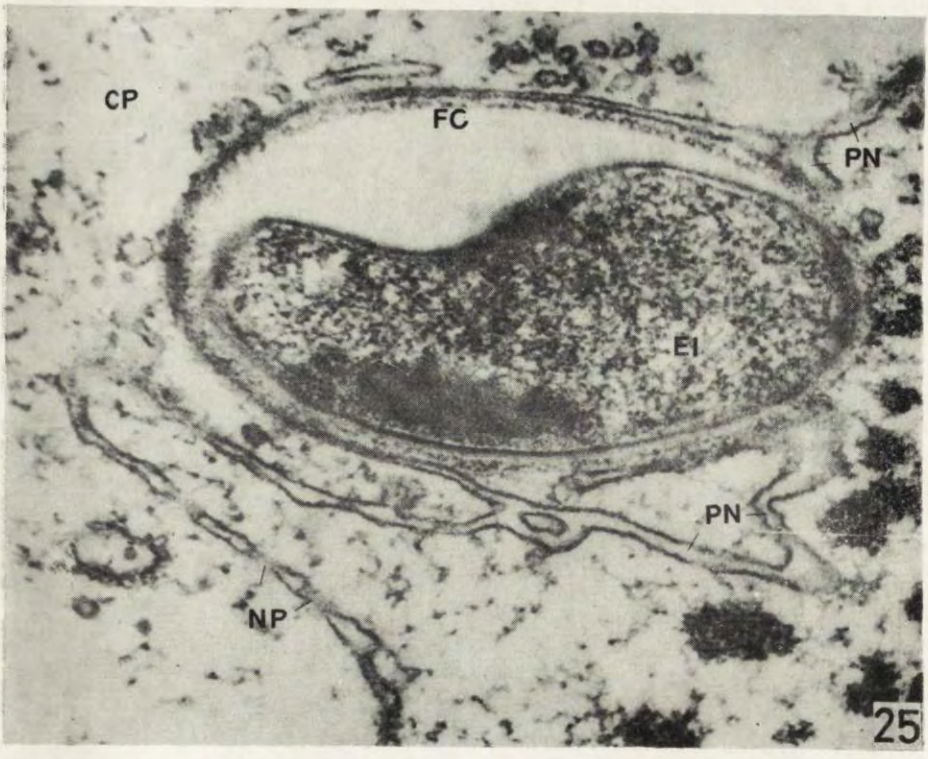

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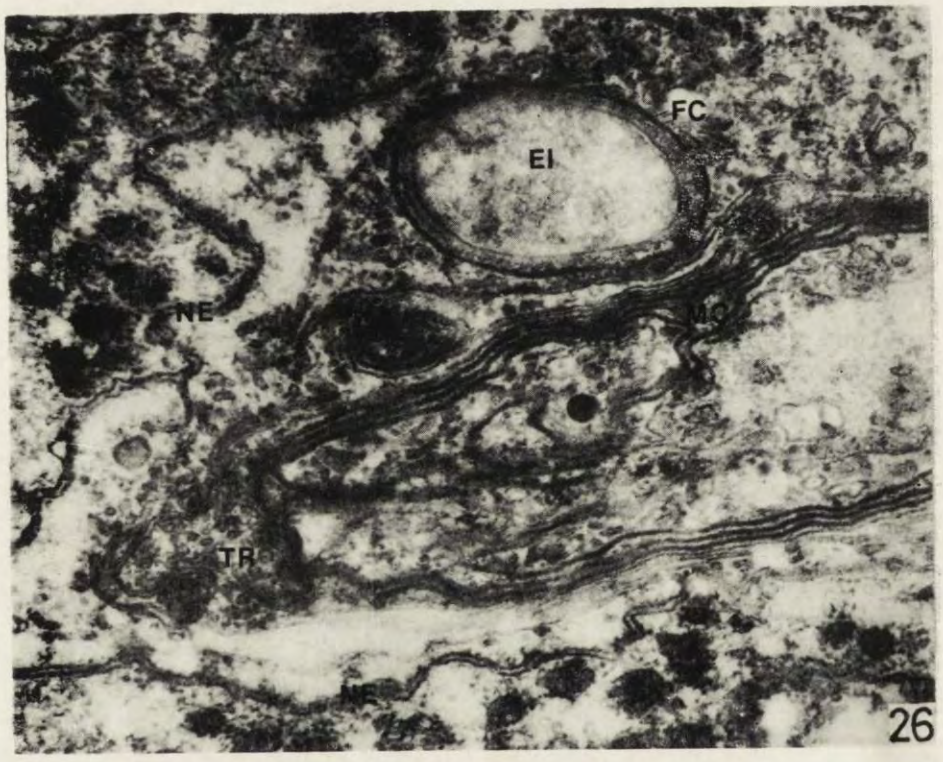

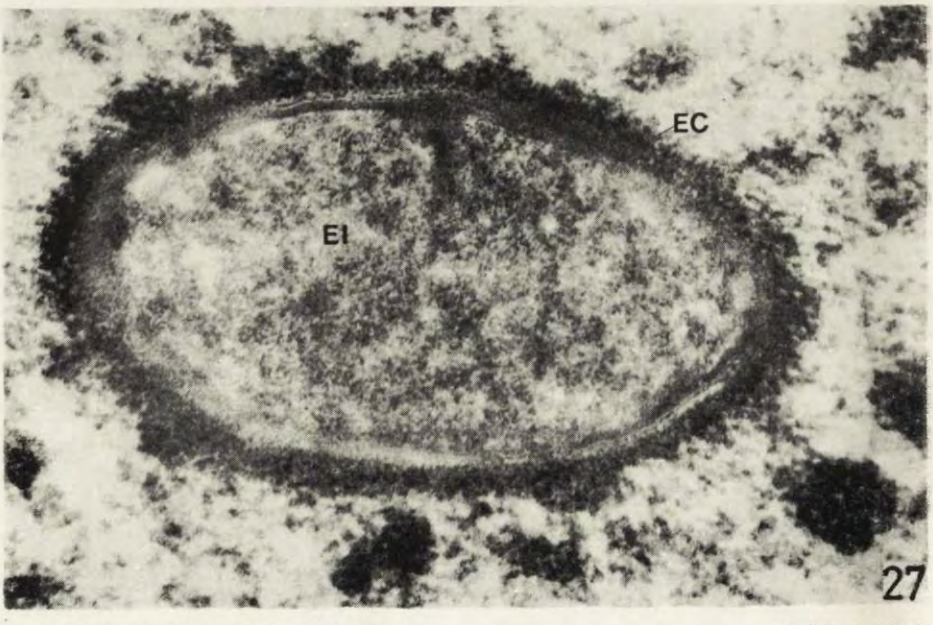

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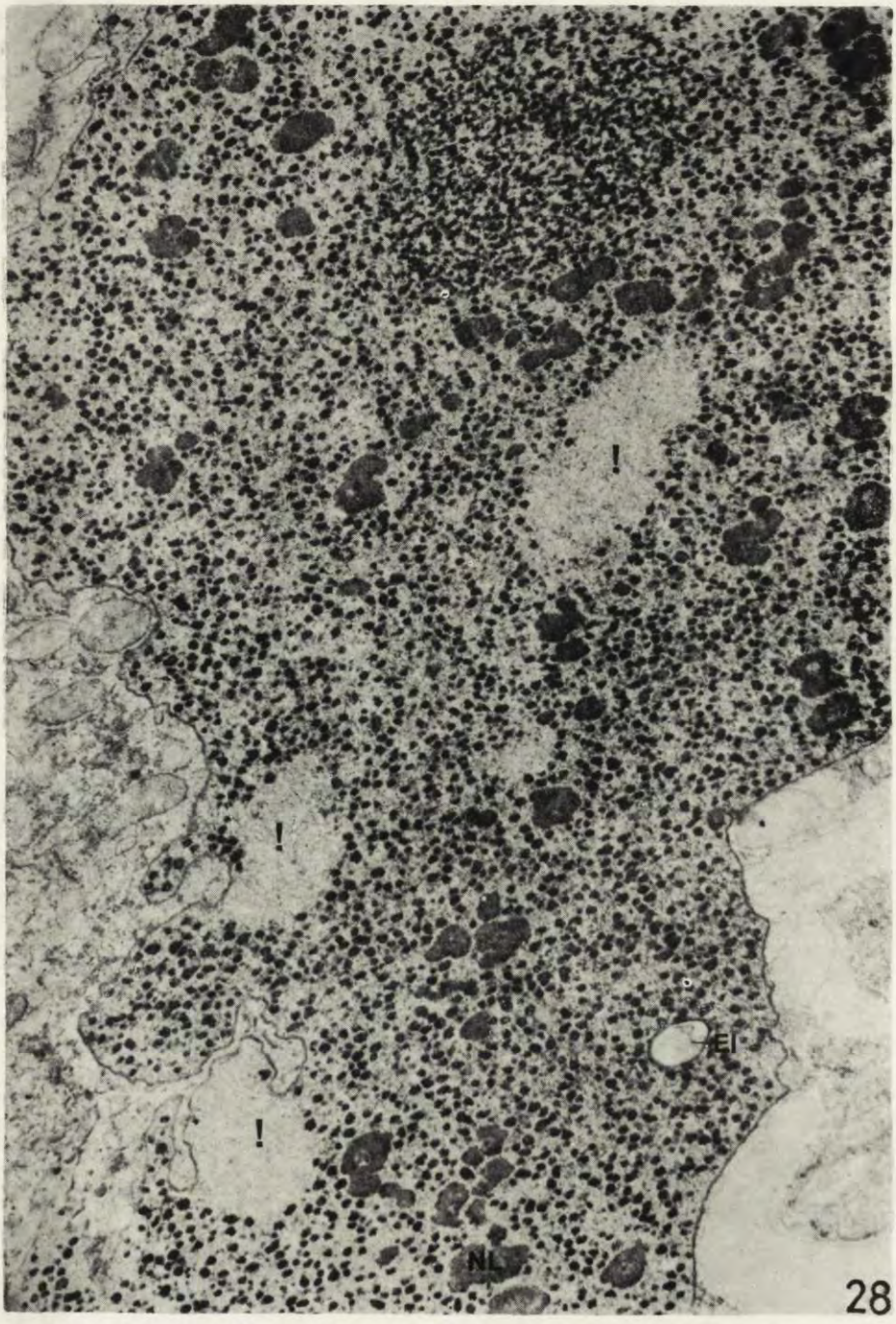

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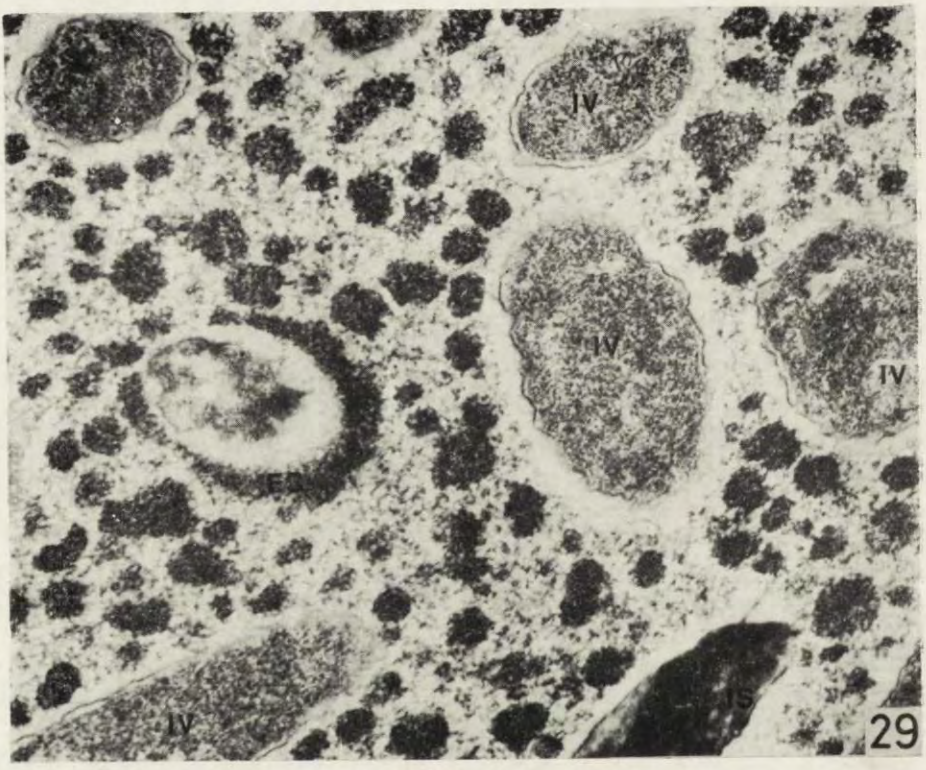

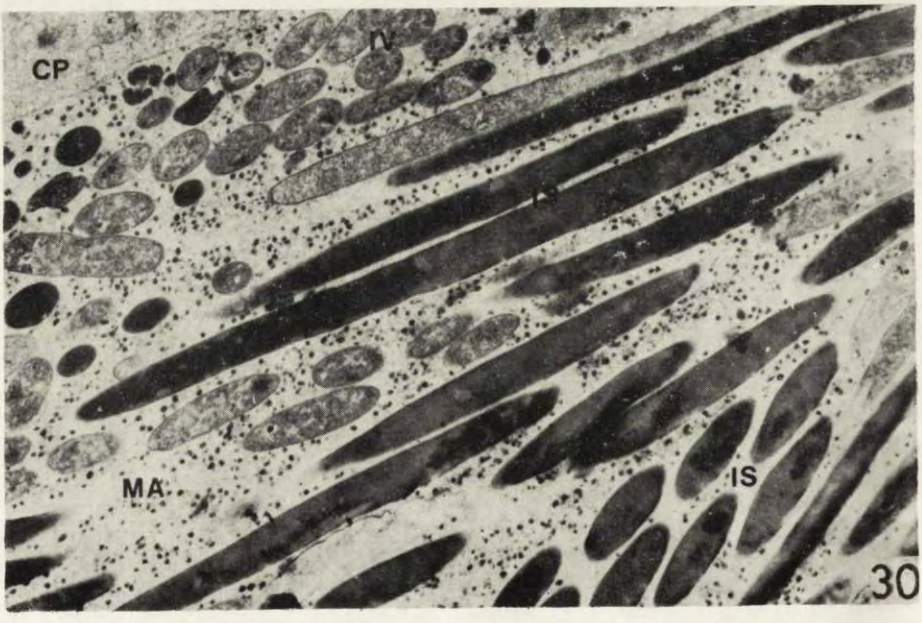

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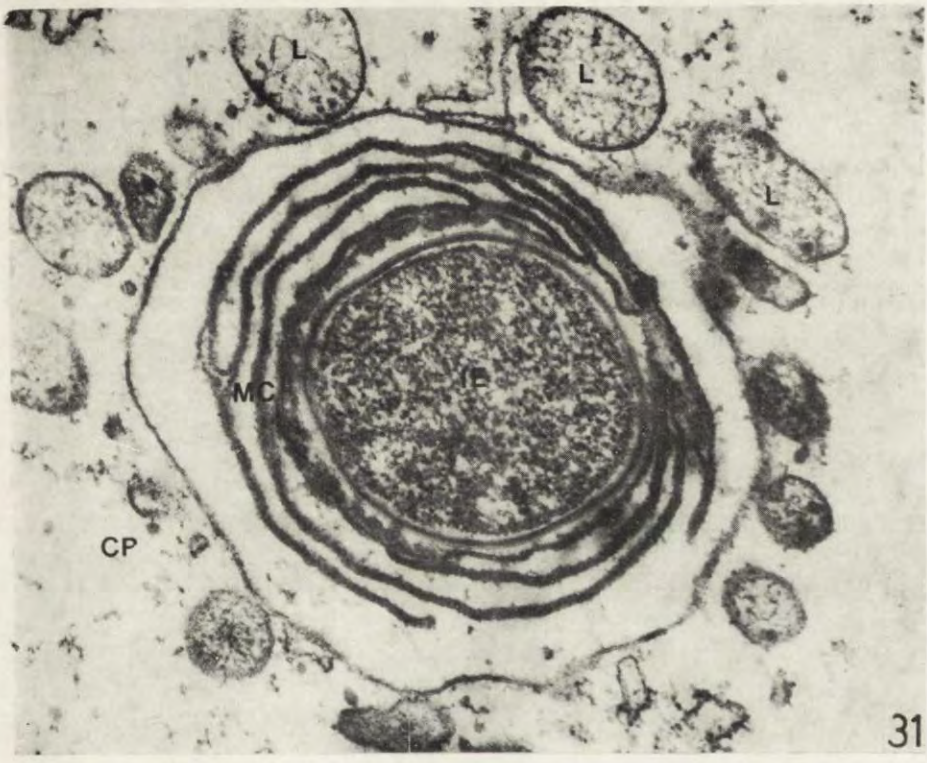

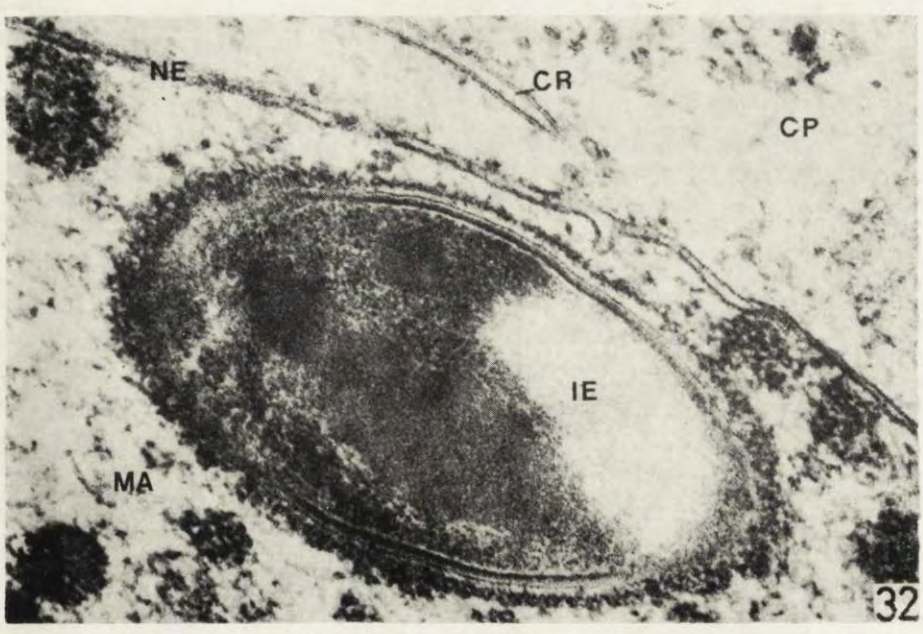

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L. N. SERAVIN and E. E. ORLOVSKAJA

Feeding Behaviour of Unicellular Animals. I. The Main Role of Chemoreception in the Food Choice of Carnivorous Protozoa

Synopsis. This study deals with selection of food in carnivorous Protozoa (*Didinium nasutum, Dileptus anser, Lacrymaria olor, Coleptus hirtus*), amoebae (*Amoeba proteus*) and in flagellates (*Peranema trichophorum*). Chemoreception has been shown to be the main mechanism responsible for food selection. Using chemical food models the present authors have identified a number of chemical inducers of feeding response — capture and ingestion of formerly indifferent items — specific to particular species of Protozoa under study. The combinations of such induced vary from species to species. Physical properties of the prey such as size, surface structure, ability to discharge trichocysts are not essential for identification of prey by the predator. These peculiarities, however, may sometimes be a barrier to realization of the predator's feeding response, interferring with the capture and ingestion of food.

Protozoa are believed to have chiefly stereotype hereditary behaviour understood as a number of subsequent stimulant trigger reactions. Each of them arises due to the interaction of certain external stimuli and internal states of unicellular organisms (Dethier and Stellar 1964). But it is far from being clear what particular chemical and physical factors of the environment induce feeding, sex or any other form of protozoan behaviour. Nor is it well known what internal mechanisms governing their behaviour get triggered by these inducers. This makes further investigations essential.

We have chosen feeding behaviour as a model for studying external and internal factors governing the behaviour of Protozoa. The feeding behaviour is a complex multistage process, but we are mostly concerned with its two final stages, namely, capture and ingestion of prey that will be further referred to jointly as "feeding response".

Chauvin (1969) justly considers that in every case of studying animal behaviour it is important to single out that key and specific

direction of the organism's activity which is pivotal for the given type of behaviour. He believes that behaviour should be studied as a function of the chosen direction of activity, but in doing so details should not hide the general picture. Essential for the feeding behaviour of Protozoa is their activity aimed at selection of food among "non-edible" objects.

Ecological observations and direct experimental investigations suggest that all unicellular animals have a physiological mechanism allowing food selection i.e. discrimination between "food" and "non-food" objects (Schaeffer 1910, 1914, 1917, Lund 1914, Dembowski 1922 a, b, Visscher 1923, Losina-Losinsky 1929, Sandon 1932, Mast 1947, Dragesco 1962, Jeon and Bell 1965, Fenchel 1968, Seravin 1968, Pavlovskaya 1970, Seravin and Orlovskaya 1972, 1973 and others). But this ability to selecting food varies from species to species so that all Protozoa studied hitherto can be arranged in some sort of a series. At one end of this series there will be species with a relatively poor ability to distinguish between digestible and indigestible objects. Animals of those species can ingest not only food but also particles of carbon, glass, iron etc. The other end of the series will be occupied by predatory stenophags that are highly selective even while chosing among living prey showing preference to strictly limited food species. All the remaining Protozoa will fit in between the two extremes.

Many attempts were made in order to learn what kind of sensitivity, mechanoreceptory or chemoreceptory, guides the animal in its recognition of the food object. An unambiguous answer to this question is not known.

Some authors engaged in studying the food selection in ciliate filter feeders consider this choice to be always guided by tactile sensitivity enabling Protozoa to perceive the shape, weight, size and surface structure of the particles captured (Schaeffer 1910, Bozler 1924, Sandon 1932, Webb 1956, Fenchel 1968). Nonetheless other workers present data in favour of chemoreception as the only factor that decides the choice of food in filter feeder ciliates (Lund 1914, Losina-Losinsky 1929, Nelson 1933, Bragg 1939 a, b). Those two mutually exclusive points of view have not arisen just because different investigators used different species of ciliates in their experiments. Both Bozler (1924) and Losina-Losinsky (1929) studied the same *Paramecium caudatum* but achieved contradictory results.

This holds true for studying the feeding behaviour and food selection in carnivorous Protozoa. There is no accord even among the authors investigating the same species of predatory Protozoa such as *Didinium nasutum*, *Spathidium spathula*, *Amoeba proteus* and *Chaos chaos*. Some insist on the animals being guided by mechanoreception in the choice

of food, while the others give preference to chemoreception (Mast 1909, Calkins 1915, Schaeffer 1917, Woodruff and Spenser 1922, Mast and Hahnert 1935, Bragg 1939 a, b, Jeon and Bell 1965, Christiansen and Marshall 1965, Schwartz 1965, Wenzel and Baltes 1967, Seravin 1968, Wessenberg and Antipa 1970, etc.).

The evidence, however, clearly shows that chemoreception is of vital importance for feeding behaviour, at least in some protozoan species. In a few cases specific chemical agents inducing a feeding response in unicellular animals have been discovered (Schaeffer 1917, Nelson 1933, Jeon and Bell 1965, Seravin 1968, Rickets 1972, Chapman-Andresen 1958, 1962).

It would be a difficult task to try to sum up all that has been known about the food selection mechanisms in Protozoa. The time has come to give this problem a new insight, using unified methods for studying a wide range of specimens with a view to understand the role of chemical and physical factors in inducing feeding response and thus, the role of chemo- and mechanoreception in the feeding of Protozoa.

Material and Methods

The experiments were carried out on the following *Protozoa:*

Class *Sarcodina* — *Amoeba proteus* (Berkley stok) [1],

Class *Infusoria* — *Didinium nasutum* (Peterhof population) [2], *Dileptus anser* (Leningrad State University and EK-1 stocks) [1], *Lacrymaria olor* (Astrakhan population) [1], *Coleps hirtus* (Peterhof population) [2],

Class *Mastigophora* — *Peranema trichophorum* (Peterhof population) [2].

The choice of the animals was accounted for by several reasons. *Didinium* and *Lacrymaria* have a preference for feeding on a limited number of protozoan species only, thus being stenophags (Mast 1909, 1911, Wessenberg and Antipa 1970). *Amoebae* and *Dileptus* are wider range polyphags (Gibbs and Dellinger 1908, Mast and Hahnert 1935, Dragesco 1962) feeding chiefly on living prey. *Dileptus* is able to take up large-size prey, while amoebae can prey on bacteria. *Coleps* is known as an optional predator, though under natural conditions it normally behaves as a histophag feeding on dead decaying cells and tissues (Dragesco 1962). *Peranema* has a very wide feeding spectrum, acting as a predator in the presence of living prey (for instance, *Euglena*) but also feeding on dead objects and even taking up such indigestible substances as particles of ink and carmine (Chen

[1] Sincere thanks are due to dr A. L. Judin and dr M. V. Tavrovskaya of the Institute of Cytology at the Academy of Sciences of the USSR (Leningrad) for making the material available to the present authors.

[2] The above populations were obtained from the Museum of Unicellular Organisms at the Laboratory of Invertebrate Animals of the Biological Research Institute, Leningrad State University.

1950). So, the Protozoa chosen for the experiment belong to quite different sectors of the above conventional series covering it almost throughout. At the same time all Protozoa under study can feed as predators which facilitates the use of similar methods for the investigation of their feeding behaviour.

The protozoans were cultivated in Prescott's medium (Prescott and James 1955) at a temperature of 20°C. *Amoebae* and *Peranema* were kept in Petri dishes, and the ciliates — in test-tubes with a capacity of 12 ml. In the dishes with the amoebae Prescott's medium was changed every day, while the ciliates were transferred into fresh medium at 5–6 day intervals. *Peranema* were moved into clean Petri dishes once every 2 or 3 months. All Protozoa except *Peranema* were fed on the ciliates *Tetrahymena pyriformis* every second day. Granules of boiled yolk of an egg were used as food for *Peranema*.

Prior to the experiment all animals except *Peranema* were starved for 48 h. Then predators of each species under study together with their prey were introduced into a testing glass microcontainer filled with no more than 0.5 ml of Prescott's medium. Specific methods used in particular sets of experiments will be discussed in due course.

Results

Set I: Choice of Food in Carnivorous *Protozoa* Feeding on Living Prey

Experiments were restricted to carnivorous ciliates *Didinium, Lacrymaria, Dileptus, Coleps*. They were offered as prey a sequence of living ciliate species: *Paramecium caudatum, P. aurelia, Tetrahymena pyriformis, Spirostomum ambiguum, Blepharisma japonicum, Coleps hirtus, Lacrymaria olor, Dileptus anser* and *Didinium nasutum*. All prey organisms were labelled in one of the two ways described below. For the purpose of labelling the filter feeder ciliates were kept in a drop of ink suspension for 10 min to render their food vacuoles black and conspicuous. Upon rinsing the labelled organisms were introduced into the microtanks with "hunter"-ciliates. As to the carnivorous ciliates used as prey for other predators, they were introduced into the testing container after being fed on *Tetrahymena* with darkened vacuoles.

The percentage of predatory ciliates that ingested the offered prey was counted one hour after the beginning of the experiment. Each experiment was repeated 3–5 times. The data obtained are shown in Table 1.

Most of the Protozoa were selected as prey by no more than one or two carnivorous ciliates. *Coleps hirtus*, however, was ingested by a greater variety of predators, namely, *Dileptus, Didinium* and *Lacrymaria*, while the only ciliate consumed by all predators under study was *Tetrahymena pyriformis*. The latter was also used as food species for cultivating all the above Protozoa.

Table 1

Feeding Response of Carnivorous Ciliates to Living Prey

Living ciliates introduced as food	The share of phagocytizing carnivorous ciliates (M±m %)			
	Dileptus anser	*Didinium nasutum*	*Lacrymaria olor*	*Coleps hirtus*
Tetrahymena pyriformis	$n = 150$ 100	$n = 150$ 100	$n = 150$ 100	$n = 150$ 100
Paramecium caudatum	$n = 137$ 39.6±4.2	$n = 70$ 100	$n = 38$ 0	$n = 120$ 0
Paramecium aurelia	$n = 258$ 12.0±2.0	$n = 129$ 87.6±3.9	$n = 67$ 0	$n = 200$ 0
Spirostomum ambiguum	$n = 161$ 36.3 ± 3.8	$n = 101$ 0	$n = 52$ 0	$n = 79$ 0
Spirostomum ambiguum, cut into bits, 30–50 μm	$n = 58$ 55.2±6.5	$n = 57$ 31.6±1.9	$n = 63$ 93.7±2.8	$n = 52$ 71.2±6.3
Blepharisma japonicum	$n = 179$ 70.4±3.4	$n = 62$ 0	$n = 48$ 0	$n = 180$ 14.4±2.6
Dileptus anser	$n = 100$ 0	$n = 100$ 0	$n = 100$ 0	$n = 100$ 0
Coleps hirtus	$n = 244$ 33.6±3.0	$n = 157$ 79.6±3.2	$n = 49$ 30.6±6.6	$n = 100$ 0
Didinium nasutum	$n = 548$ 15.8±1.6	$n = 100$ 0	$n = 100$ 0	$n = 146$ 0
Lacrymaria olor	$n = 72$ 9.7±3.5	$n = 50$ 4.0±2.8	$n = 100$ 0	$n = 100$ 0

n — the number of carnivorous ciliates used in the experiments.

Dileptus anser was the only animal whose feeding spectrum embraced every food species offered (except *Dileptus* of the same clone). But there were obvious preferences in its choice of living prey, with *Lacrymaria* and *Didinium* accounting for no more than 9.7 and 15.8 per cent of the ingested food respectively. Besides *Tetrahymena*, *Didinium nasutum* preys on *Paramecia* and *Coleps* (rarely, on *Lacrymaria*), while *Blepharisma* is the only food for *Coleps hirtus* which in turn is the choice of *Lacrymaria*.

It is not safe to assert, however, that predatory ciliates avoid certain protozoans in their food selection because they do not identify those animals as food items. On the contrary, observations on the behaviour of *Didinium nasutum* clearly show that this ciliate eagerly attacks even such Protozoa which have never been recorded as its prey. So, it attacks

Spirostomum, *Blepharisma* and *Dileptus*, but each attack results in escape reaction of *Didinium* (back movement). Moreover, upon the contact with a potential prey some of the didinia lose temporarily their mobility and fall on to the bottom of the container. That means that the attacked animals frighten and sometimes even injure the predators with their trychocysts. This saves them from becoming the prey for *Didinium*.

Spirostomum ambiguum are so big that no ciliate other than *Dileptus* can feed on them. *Dileptus* partially cytolyses the prey with powerful discharges of its trychocysts, tears away small portions of the injured body and ingests them. It should be noted, however, that all the other ciliates under study actively consume *Spirostomum* when cut into movable bits 30–50 μm big. In this case the share of phagocytizing dilepti increases by 20 per cent (as compared with experiments on uncut *Spirostomum*), while in *Coleps* and *Lacrymaria* the proportion rises from 0 to 71 and 94 per cent respectively. The intact spirostoma survive most probably due to their defence device — organelles — and high mobility (Table 1).

None of the carnivorous ciliates displayed cannibalism, no were there cases of attacking individuals of their own species.

The data obtained from this set of experiments show that a certain degree of food specialization is indicative of almost every predatory ciliate under study. But, as can be seen from the experiment on *Didinium*, this, at least in some instances, may be due not to the prey being identified as a "non-edible" object, but to the predator's inability to capture the potential prey provided with a mechanism of defence.

Set II. Food Selection in Protozoa Feeding on Dead Prey

As we know from the experiments quoted earlier, the defensive mechanism of a living organism can be an obstacle interfering with the predator's attempt to capture the prey even when identified as a food object. To get a true idea of food selectivity in predators, we must devoid the prey of its defensive characters. In order to eliminate such factors as the prey's locomotor activity and its ability to active defence with the help of trychocysts, all Protozoa were introduced as food for predatory ciliates killed by heat (3 to 4 min, $t = 60°$). The selection of prey species in the second set of experiments was exactly the same as in set I. Every experiment was reproduced 3 to 5 times with the results given in Table 2.

It turned out that all the predatory ciliates were capable of feeding on all the protozoans offered them without any exceptions even for individuals of their own species. So, *Coleps* and *Lacrymaria* come to feed

Table 2

Feeding Response of Carnivorous Ciliates to the Prey Killed by Heat

	Dead ciliates introduced as food	The share of phagocytizing carnivorous ciliates (M±m %)			
		Dileptus anser	*Didinium nasutum*	*Lacrymaria olor*	*Coleps hirtus*
Experiment	*Tetrahymena pyriformis*	$n = 63$ 98.4±1.6	$n = 156$ 76.9±3.4	$n = 80$ 31.3±5.1	$n = 132$ 96.1±1.6
	Paramecium caudatum	$n = 102$ 99.0±0.9	$n = 50$ 6.0±3.4	$n = 62$ 40.3±6.2	$n = 143$ 76.9±3.5
	Paramecium aurelia	$n = 76$ 96.0±2.2	$n = 129$ 82.8±3.3	$n = 82$ 82.8±4.2	$n = 82$ 75.6±4.7
	Spirostomum ambiguum	$n = 151$ 24.5±3.7	$n = 85$ 21.5±4.5	$n = 53$ 67.0±5.4	$n = 126$ 76.1±3.8
	Blepharisma japonicum	$n = 227$ 89.0±2.1	$n = 96$ 34.4±4.8	$n = 68$ 94.1±2.9	$n = 200$ 100
	Dileptus anser	$n = 51$ 17.6±5.3	$n = 155$ 7.1±1.9	$n = 63$ 41.3±6.2	$n = 106$ 31.7±4.3
	Coleps hirtus	$n = 50$ 82.0±5.4	$n = 49$ 51.9±7.1	$n = 61$ 32.8±6.0	$n = 140$ 97.9±1.2
	Didinium nasutum	$n = 107$ 18.7±3.8	$n = 119$ 15.1±2.8	$n = 44$ 34.1±7.1	$n = 118$ 92.4±2.4
	Lacrymaria olor	$n = 91$ 40.8±5.2	$n = 96$ 16.7±3.8	$n = 54$ 57.4±6.7	$n = 148$ 9.5±2.4
Control	*Tetrahymena pyriformis* living	$n = 150$ 100	$n = 150$ 100	$n = 150$ 100	$n = 150$ 100

n — the number of carnivorous ciliates used in the experiments.

on *Paramecium caudatum*, *P. aurelia*, *Spirostomum ambiguum* and *Blepharisma japonicum*, which they would avoid when alive. Dilepti display a great increase in the percentage of individuals capturing *Paramecia*, *Coleps* and *Lacrymaria* as compared with Set I of experiments.

Dileptus, *Didinium*, *Lacrymaria* and *Coleps* seem to perceive the dead immovable protozoa at a distance of 200–300 μm. For instance, when *Didinium nasutum* enters the zone of a food object it changes abruptly from circular seeking movements into moving in a straight line towards the dead ciliate and attacks its prey. The predator can be attracted to the prey from a distance in no other way but by some chemicals released by the dead ciliate. Since they bring about a positive chemotaxis in ciliates, these substances may be considered as attractants. At the same time they induce capture and ingestion of the prey acting thus as

initiants and stimulants of phagocytosis in predatory ciliates (feeding response inducers).

Not all dead ciliates are equally attractive to a given predator, while the same species of dead Protozoa induces different feeding reaction in different carnivores (Table 2). So, the share of phagocytizing didinia and dilepti is very low (7 to 28 per cent) when fed on dead animals of their own species. The dead dilepti and didinia killed by heat might give off not only attractants but also repellants reducing their attraction to the predators.

It is of interest to compare the results of experiments of set I and set II on *Didinium nasutum*. These carnivores are capable of feeding on every species of dead ciliates among those offered them. But in most cases the share of individuals that have captured and ingested a dead prey is not very high. This is true even for feeding on such prey species which are readily consumed by didinia when alive. So, living *Paramecium caudatum* induces a feeding response in 100 per cent of *Didinium nasutum*, and *Coleps hirtus* — in 80 per cent, while the share of phagocytizing didinia is reduced to 6 and 52 per cent respectively when the above food species are killed by heat. The difference in the results of the experiments is quite reliable ($P > 99.9$). It may be due to the above prey species attracting *Didinium* not by their chemical properties alone but also by their locomotor activity and the type of movements.

Set III. Feeding of Predatory Ciliates on Paramecia Killed by Heat and Soaked in Prescott's Solution

Paramecia (*P. aurelia*) killed by heat during 3 to 5 min at 60°C were soaked in cooled Prescott's solution. The medium was changed regularly, at 20–30 min intervals. As a result of this treatment the feeding response of the predators to the Paramecia first weakened and then (upon 4 to 5 rinsings) disappeared completely. The experiment was reproduced three times with the results presented in Table 3.

The predators failed to be attracted by the rinsed paramecia even when made to look as if they were moving by introducing a glass needle or by shaking the tank.

But leaving those rinsed Paramecia for 30 min in a water extract of chicken liver homogenate was sufficient to have their ability as feeding response inducers fully recovered (Table 3).

To make the chicken liver homogenate 2–3 grams of finely chopped chicken liver were covered with Prescott's medium in a 1 gr/ml ratio. The mixture was centrifuged during 10 to 15 min at 1000–1500 rotates

Table 3

Feeding of Carnivorous Ciliates with *Paramecium aurelia* Killed by Heat and Soaked in Prescott's Medium

Animals introduced as food	The number of phagocytizing specimens (M±m %)		
	Dileptus anser	*Didinium nasutum*	*Coleps hirtus*
P. aurelia killed by heat	$n = 27$ 92.6±5.0	$n = 35$ 68.6±7.8	$n = 52$ 100
P. aurelia killed by heat and washed twice (during 30 min)	$n = 32$ 9.4±8.1	$n = 28$ 3.6±3.5	$n = 28$ 28.6±8.6
P. aurelia killed by heat and washed four times (during 90 min)	$n = 25$ 0	$n = 37$ 0	$n = 29$ 0
P. aurelia killed by heat, washed four times and soaked in liver extract	$n = 34$ 94.1±4.0	$n = 37$ 54.0±8.2	$n = 50$ 96.0±8.7

n — the number of carnivorous ciliates used in the experiments.

per minute and the deposit used into the experiment. For the sake of brevity the water extract of the chicken liver homogenate will be further referred to as liver extract.

Addition of small amounts of this extract to the medium arouses intense locomotor activity in all ciliates associated with mouth opening in dilepti, the last response having been described in literature as the stage of preparation for attack ("hunting position", Dragesco 1962).

The above experiments clearly show that all ciliates under study can be induced to attack, capture and ingest recently destroyed Protozoa under the influence of chemicals given off by them. Thus, the choice of food in predatory ciliates can be governed by chemotaxis alone. If this observation is correct, the carnivores should ingest particles of indifferent "non-edible" objects given the "scent of food". This type of experiments were conducted in set IV.

Set IV. Feeding of Predators on Chemical Food Models

Food models were prepared out of starch granules and activated carbon particles (20–100 μm). These substances were chosen for being good adsorbents of a wide range of chemicals and for inducing no feeding

response in the predatory Protozoa under study (either in ciliates, or *Amoeba proteus*, or *Peranema trichophorum*) that are normally quite indifferent to starch and carbon ingesting their particles but rarely.

Our purpose was to endow the above adsorbents with the power to induce feeding response in predators. To achieve this, the carbon and starch particles were soaked for 30 min in liver extract, rinsed three times in Prescott's medium and introduced into the microcontainer with predatory Protozoa. Each experiment was reproduced from 5 to 15 times.

The carbon and starch particles having absorbed molecules of chemical agents from the liver extract induced a distinct positive chemotaxis followed by a feeding response in the predators. Some 10–20 min after the food was introduced the bulk of the predators has particles of adsorbents in their cytoplasm (Pl. I 1–4, Table 4).

Table 4

Induction of Feeding Response in Carnivorous Protozoa with the Help of Food Models

Substances offered as food	The share of phagocytizing specimens in the experiment					
	Dileptus anser	*Lacrymaria olor*	*Didinium nasutum*	*Coleps hirtus*	*Amoeba proteus*	*Peranema trichophorum*
pure carbon particles	$n = 250$ 0	$n = 120$ 0	$n = 150$ 0	$n = 440$ 2.6 ± 0.1	$n = 50$ 0.54 ± 0.02	$n = 183$ 1.4 ± 0.5
pure starch	$n = 230$ 0	$n = 135$ 0	$n = 135$ 0	$n = 95$ 6.5 ± 1.7	$n = 120$ 0	$n = 150$ 0
carbon particles soaked in liver extract	$n = 292$ 82.4 ± 2.2	$n = 248$ 88.1 ± 1.9	$n = 45$ 52.2 ± 7.5	$n = 153$ 56.1 ± 5.7	$n = 297$ 94.9 ± 3.6	$n = 210$ 75.4 ± 3.7
starch soaked in liver extract	$n = 253$ 89.7 ± 1.9	$n = 112$ 72.8 ± 4.2	$n = 41$ 71.8 ± 6.8	$n = 230$ 53.0 ± 3.3	$n = 65$ 70.5 ± 3.6	$n = 120$ 0
starch soaked in culture medium of *Tetrahymena*	$n = 75$ 73.0 ± 5.1	$n = 0$ no experiment	$n = 52$ 23.0 ± 5.8	$n = 148$ 14.0 ± 2.9	$n = 0$ no experiment	$n = 0$ no experiment

n — the number of carnivorous Protozoa used in the experiments.

Dileptus and *Lacrymaria* can take up from 10 to 15 starch granules that makes their bodies swell and lose shape looking sometimes like a bunch of grapes (Pl. I 1). *Didinium* and *Coleps* would rather swallow only one very big starch granule comparable in size to the animal itself. In that case the cytoplasm envelopes the ingested particle in a very thin film reproducing the shape of the grain (Pl. I 3). There are instances

when didinia ingest 2 or 3 smaller starch granules. The size of caught adsorbent particles (within the range of 20–100 μm) is of no special importance for predatory ciliates. As to amoebae, a great number of their food cups are formed around small particles of carbon, 40–80 μm. Carbon particles of a still smaller size, 5–15 μm, do not induce the food cup formation in *A. proteus*. Nevertheless those particles — soaked in the liver extract — would stick to the surface of the protozoan plasmalemma and "sucked" inside the body of amoebae. That process is similar or identical with pinocytosis.

Flagellates (*Peranema trichophorum*) actively feed on small coal particles, 5–15 μm, soaked with liver extract. Thirty minutes of feeding are enough for the cytoplasm of Protozoa to be filled with black vacuoles. The starch particles used in our experiments were too big, over 30 μm in length, and *Peranema* never ingested them.

Some experiments made use of other adsorbents alongside carbon and starch particles. An attempt was made to introduce particles of kaolin or small bits of filtering paper. Inducing normally no response in predators those new adsorbents when soaked in liver extract are readily taken up by dilepti.

Didinia actively attack granules of boiled yolk of hen's egg, sticking to them sometimes by means of discharged pexicysts, but to our knowledge never ingest them. Soaking with the liver extract, however, endows those granules with the power to induce a characteristic feeding response in *Didinium*.

The same ability of inducing predators to swallow food models is observed in extracts withdrawn from livers of a frog, a humster and a rat, as well as from chicken muscles. Starch and coal particles soaked in a water extract from *Tetrahymena* and *Paramecium* homogenate would be readily caught and ingested by dilepti and coleps.

It is obvious that chemical food models and ciliates killed by heat and having their surface membrane injured readily release various chemicals, including macromolecules, which living cells usually keep within their cytoplasm. In view of this fact, are we not dealing with an artefact of food response in Protozoa that is not to be found outside of above mentioned experiments? This important problem cannot be solved without proving that living Protozoa also give off various chemicals that may act as food response inducers for predators.

It has been proved recently that *Tetrahymena pyriformis* (the main stock of food for the predatory Protozoa under study) continuously gives off all kinds of substances, including enzymes (Müller 1972). Whether or not some of these substances act as food response inducers was to be solved by the following experiments. *Tetrahymena pyriformis* cultivated

in yeast water was harvested by centrifugation, throughly rinsed in Prescott's medium — 3 to 4 times — and left there for 6 h. Another centrifugation, 3 to 4 min long, followed at 1.5–2 thous rot./min, and the deposit was used in experiments on food response induction. Two control tests were carried out parallely when the ciliates were offered both pure starch and that soaked in liver extract. The results obtained allow for a conclusion that the products of the prey metabolism released into the environment can also induce feeding response in Protozoa.

The above evidence shows that to induce phagocytosis in the carnivorous protozoan species under study one can successfully replace real living prey with a chemical food model. Such a model, though immovable or differing in size and shape from the living food object, will not fail to induce a distinct feeding response in carnivorous unicellular Protozoa if it has certain chemical properties typical of a prey. The experiments with chemical food models provide further support for the view that the choice of food in Protozoa is chiefly governed by their chemosensitivity. Metabolic products of the prey organisms may act as agents capable to attract predatory unicellular animals and induce them to capture and ingest the above prey. A further step in our experiments is the search for particular chemicals that induce the feeding response in Protozoa.

Set V. Search for Chemical Inducers of Food Response in Protozoa [3]

We did not aim to discriminate every possible inducer of feeding response in Protozoa under study. Our main task was to find out whether each predatory species would react to only one specific chemical inducer or to a whole range of them. The experiments made use of different classes of substances in the form of solution among them proteins, amino acids, phospholipids, sugars, nucleic acids, tweens and so on. The choice of particular substances and concentrations was primarily based on the data of earlier similar experiments on *Protozoa* and *Metazoa* (L e n g o f f 1968, L i n d s t e d t 1971).

The method of food models was the main technique used in the search for feeding response inducers. Particles of adsorbents — starch or activated carbon — were introduced into solutions of substances of different nature to be kept there for 30 min (all solutions were prepared with Prescott's medium). Then the particles were washed three times

[3] Sincere thanks are due to M. S. Burenkov, a student, who actively participated in the work described in this section.

in Prescott's medium and transferred to the microcontainer with the test animals. The count of amoebae having ingested particles of adsorbent followed 10 min later, while in the case of ciliates and *Peranema* the time before the count was a full half-hour. A shorter duration of the experiments on amoebae is accounted for by their ability to remove the ingested food models — particulate carbon soaked in the inducer — fairly quickly. This suggests that amoebae must have an internal food choice mechanism enabling them to expell the indigestible object from their cytoplasm.

Experiments on *Peranema* and amoebae made using activated carbon, particles of starch were used as food models for ciliates.

Two control experiments were always run parallely to the main ones. In the first case the protozoa were offered particles of starch or pure activated carbon, while for the second control similar particles had been soaked in liver extract.

Prior to being used in the experiments in question all protozoa were starved during two days, *Peranema* was the only exception.

The results obtained are given below.

(a) Inducers of Feeding Response in *Amoeba proteus*

Using the method of chemical models we have tested a number of organic substances for the ability to induce a feeding response in *A. proteus*. Activated carbon, 30–40 μm, served as an adsorbent for these chemicals. The results of these experiments are shown in Table 5.

Amoebae used as controls ingested pure activated carbon very rarely (about 0.5 per cent of the specimens), while the carbon particles soaked in liver extract induced the bulk of the animals (77.5 per cent) to form food cups and later — food vacuoles. Replicated tests displayed little variation in the percentage of phagocytizing specimens.

Some of the proteins turned out to be good inducers of feeding response in amoebae. Among them are trypsin, RNA-ase, DNA-ase, cytochrome C, as well as pepsin and hyalourinidase. An active phagocytosis of carbon particles may be caused by peptone, phospholipids (cephalin and lecithin), tweens (40, 60 and 80), cysteine and reduced glutathione.

Amino acids, such as lysine, glutamine, proline and valine, do not induce phagocytosis of carbon in amoebae (the observed variations between the experiments and controls are not reliable: $P < 95$ per cent).

The above results confirm the earlier information stating that many proteins as well as tweens and peptone act as inducers of feeding response in amoebae (Schaeffer 1914, 1917, Chapman-Andresen 1962, Jeon and Bell 1965, Seravin 1968). Besides we have discriminated a number of other substances also capable of inducing phago-

Table 5

Feeding Response Induction in *Amoeba proteus*
(Carbon particles used as adsorbents for making food models)

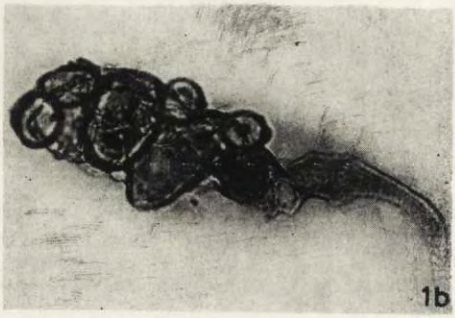
Item number	Substances used as inducers	Concentration	Number of experiments	Average number of specimens per experiment	Average number of phagocytizing specimens (M±m %)
(1)	pure carbon particles		18	50	0.54±0.02
(2)	liver extract		12	65	77.5 ±3.6
(3)	trypsin	2%	3	79	80.7 ±1.2
(4)	pepsin	2%	4	42	27.5 ±2.0
(5)	hyalouranidase	2%	5	75	27.2 ±1.3
(6)	DNA-ase	2%	6	23	64.4 ±1.5
(7)	RNA-ase	1%	6	50	87.3 ±1.2
(8)	cytochrome C	2%	3	50	81.0 ±1.2
(9)	peptone	2%	3	73	83.0 ±0.8
(10)	reduced glutathione	10^{-2} M	5	85	10.2 ±1.6
		10^{-4} M	9	51	26.3 ±4.3
		10^{-6} M	8	28	31.6 ±4.6
(11)	cysteine	10^{-2} M	5	63	23.4 ±4.1
		10^{-4} M	7	70	26.4 ±4.3
		10^{-6} M	7	35	24.8 ±4.9
(12)	lysine	10^{-2} M	3	50	2.0 ±1.1
(13)	proline	10^{-2} M	3	63	0.6 ±0.6
(14)	valine	10^{-2} M	3	59	1.5 ±0.9
(15)	glutamine	10^{-3} M	2	50	0
(16)	cephalin	2%	6	66	66.3 ±2.3
(17)	lecithin	2%	6	68	81.2 ±4.1
(18)	tween 40	5%	2	50	30.0 ±4.6
(19)	tween 60	5%	2	50	40.0 ±4.9
(20)	tween 80	5%	2	50	30.0 ±4.6

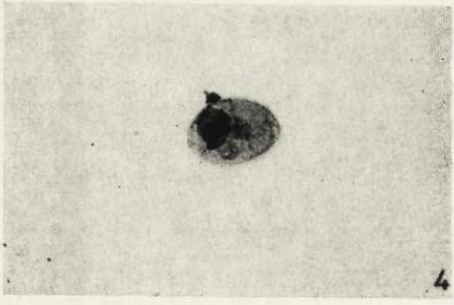
cytosis in *A. proteus*, namely, lecithin, cephalin, cysteine and reduced glutathione.

(b) Inducers of Feeding Response in *Peranema trichophorum*

Peranema of the control group hardly ever phagocytize particles of pure carbon — 1 per cent of the specimens only. On the other hand, the carbon particles soaked in liver extract are readily consumed by them (Table 6).

Lecithin is the best inducer of feeding response in *Peranema*. Practically all flagellates studied would ingest particles of carbon soaked in





L. N. Seravin et E. E. Orlovskaja auctores phot.

Table 6

Feeding Response Induction in *Peranema trichophorum*
(Carbon used as adsorbent for making food models)

Item number	Substances used as inducers	Concentration	Number of experiments	Average number of specimens per experiment	Average number of phagocytizing specimens (M±m%)
(1)	pure carbon particles		60	31	1.4±0.5
(2)	liver extract		50	42	75.4±3.7
(3)	trypsin	0.2%	6	27	8.0±1.8
(4)	trypsin	2.0%	6	37	5.9±1.7
(5)	pepsin	0.2%	6	17	0
(6)	pepsin	2.0%	6	18	0
(7)	peptone	0.2%	6	26	1.0±0.7
(8)	peptone	2.0%	3	42	1.7±0.9
(9)	bactopeptone	0.2%	3	36	16.2±1.6
(10)	baktopeptone	2.0%	6	41	10.3±1.9
(11)	mixture of amino acids		6	32	1.2±0.8
(12)	reduced glutathione	10^{-2} M	9	28	0
(13)	cysteine	10^{-3} M	3	26	0
(14)	serine	10^{-3} M	3	29	1.0±1.0
(15)	glutamic acid	$5 . 10^{-4}$ M	3	28	1.1±1.1
(16)	tween 40	2.0%	6	53	4.8±1.1
(17)	tween 60	2.0%	6	39	5.1±1.3
(18)	tween 80	2.0%	6	50	6.5±1.6
(19)	lecithin	2.0%	6	73	91.1±0.9
(20)	cephalin	2.0%	9	52	45.9±3.1

this substance (91.1 per cent). Another phospholipid, cephalin, has a much weaker effect accounting for phagocytosis in no more than 45.9 per cent of the animals. The difference in the lecithin and cephalin effects is statistically reliable ($P > 99.9$ per cent).

Only a small part of *Peranema trichophorum* would be induced to have a feeding response by such organic substances as trypsin, bactopeptone and tweens. The experimental results show reliable variation from the data produced by controls with pure carbon ($P > 95$ per cent for trypsin and tweens, $P > 99.9$ per cent in case of bactopeptone).

The rest of the substances investigated have induced no feeding response in flagellates.

(c) Inducers of Feeding Response in *Coleps hirtus* (Table 7)

Table 7

Feeding Response Induction in *Coleps hirtus*
(Starch used as adsorbent for making food models)

Item number	Substances used as inducers	Concentration	Number of experiments	Average number of specimens per experiment	Average number of phagocytizing specimens (M±m%)
(1)	pure starch		65	95	6.5±1.7
(2)	liver extract		66	153	56.1±5.7
(3)	reduced glutathione	$1 \cdot 10^{-3}$ M	18	166	44.9±2.4
(4)	reduced glutathione	$1 \cdot 10^{-2}$ M	3	229	52.0±3.3
(5)	oxidized glutathione	$5 \cdot 10^{-4}$ M	13	151	35.2±6.1
(6)	cysteine	$1 \cdot 10^{-3}$ M	9	182	46.4±5.2
(7)	cysteine	$5 \cdot 10^{-3}$ M	3	85	40.9±7.9
(8)	glycine	$1 \cdot 10^{-3}$ M	2	57	36.8±6.3
(9)	glutamic acid	$1 \cdot 10^{-3}$ M	2	36	1.4±1.2
(10)	proline	10^{-2} %	6	101	7.6±4.2
(11)	cysteine	10^{-2} %	4	301	4.1±1.4
(12)	glycine-leucine	$2 \cdot 10^{-2}$ %	6	75	2.6±0.1
(13)	trypsin	2%	2	51	25.5±6.0
(14)	urea	1%	13	176	14.3±3.2
(15)	thiourea	1%	14	161	32.5±4.2
(16)	coenzyme A	10^{-2} %	5	216	0.2±0.2
(17)	coenzyme A	10^{-3} %	6	247	0.7±0.1
(18)	coenzyme A	10^{-4} %	5	202	0.4±0.2
(19)	cephalin	10^{-1} %	2	26	0
(20)	lecithin	10^{-1} %	2	30	0

Just as in the above sets of experiments, *Coleps hirtus* again exhibits a fairly high activity in capturing granules of pure starch (6.5 per cent of the animals). The more carefully are the ciliates washed off the culture medium, the less readily they ingest the granules. Since in washing the animals we intentionally avoided centrifugation as a factor influencing the feeding behavior of *Coleps hirtus*, 6.5 per cent was the lowest value of phagocytizing animals we menaged to obtain. The part of specimens taking up the starch granules soaked in liver extract ranged from 50 to 70 per cent, averaging 56.1 per cent.

Two substances stand out as the best inducers of feeding response in *Coleps hirtus*, that is the tripeptide reduced glutathione and the amino acid cysteine. Since cystin induces no feeding response in *Coleps*, the inducive activity of glutathione could be ascribed to the effect of cysteine

as its component. But the experiments indicate that oxidized glutathione, whose cysteine has been oxidized to cystein, also induces a feeding response in *Coleps*. This made us investigate two other amino acid components of glutathione: glycine and glutamic acid. Starch granules soaked in the latter left *Coleps* indifferent, whereas those soaked in glycine were ingested as readily as in the experiments with oxidized glutathione. So, we have the right to conclude that two of the three components building up glutathione, namely, cysteine and glycine, act as feeding response inducers.

Phagocytosis in *Peranema* was induced by starch granules soaked in trypsin, urea and thiourea — 25.5, 14.3 and 32.5 per cent of the individuals respectively.

In contrast to amoebae and *Peranema*, *Coleps* had no feeding response to phospholipids — cephalin and lecithin.

(d) Inducers of Feeding Response in *Dileptus anser* [4]

The same method of chemical food models was applied to study 53 different substances as inducers of phagocytosis in *Dileptus*. These were a number of proteins, many amino acids, sugars, polysaccharids, vitamins, fatty acids, tweens, glutathione, ATP, ADP, and thiourea. The agents that can induce the feeding response in dilepti are given in Table 8.

As has been shown above, liver extract can induce a distinct feeding response in *Dileptus anser*. So we have made an attempt to segregate

Table 8

Feeding Response Induction in *Dileptus anser* EK-I strain
(Starch used as adsorbent for making food models)

Item number	Substances used as inducers	Concentration	Number of experiments	Average number of specimens per experiment	Average number of phagocytizing specimens (M±m%)
(1)	pure starch		7	34	0.4±0.4
(2)	homogenate of chicken liver		7	41	94.0±1.4
(3)	tween 40	5%	7	38	56.1±3.0
(4)	cysteine	10^{-2} M	7	33	83.5±1.7
(5)	cephalin	2%	7	43	42.0±2.0
(6)	trypsin	2%	4	72	1.7±1.3

[4] A paper on feeding behaviour in *Dileptus* is in press at the moment.

those fractions of the extract which are actually responsible for stimulating the feeding behaviour in *Dileptus*. Gel-filtration on Sephadex was used for the purpose. We succeeded in separating four such fractions containing substances of polypeptide nature with a molecular weight of 40 000, 11 000, 4000 and 2000 respectively.

Experiments using gel-filtrations clearly show that the list of feeding response inducers discovered is far from being complete.

Among the agents that fail to induce in full the feeding behaviour in dilepti a special notice is due to cholesterol, malic and succinic acids. They cause a positive chemotaxis in dilepti that accumulate in big numbers and for extended time around the starch granules soaked in these agents.

Discussion of Results

The evidence obtained indicates that the chemical properties of food are of particular importance for feeding behaviour of carnivorous Protozoa. An "edible" object devoid of its "food odour" due to soaking leaves predators fully indifferent though the size, surface structure and other physical characteristics (except mobility) of the object remain as they were. On the contrary, the predatory Protozoa would ingest completely alien, indigestible particles of carbon and starch provided they have adsorbed chemical inducers of feeding response.

A chemical control of the main stages of feeding behaviour is a widespread phenomenon observed in organisms at different levels of biological organization. Lindstedt (1971) made an attempt to analyze and systematically arrange the major literary data on the significance of chemoreception in the feeding response of a great variety of organisms beginning with bacteria. She believes that the feeding behaviour of animals involves a sequence of responses, that is, orientation and movement towards a food object, the beginning of feeding, continuation of feeding, the end of feeding.

According to Lindstedt (1971) every stage of feeding behaviour is triggered by a specific chemical inducer (attractant, initiant or stimulant). Negative (adverse) chemical stimuli released by a food object — repellents, suppressors and deterrents — may change or interrupt the feeding behaviour at any particular stage causing rejection of food or even avoidance of it by the animal. These processes are the best from all studied in some insects (Hosoi 1958, Lipsitz and Broun 1964, Norris 1965, Acree et al. 1968, Hodson 1968).

As to the reactions in lower organisms, however, there are such

inducers which can easily trigger not just only one stage but the whole sequence of responses involved in the feeding behaviour, from being attracted to the prey to capturing and ingesting it. Such is the effect of glutathione and some amino acids (or their derivatives) that induce a complete cycle of feeding behaviour in the absence of food in coelenterates, annelids and molluscs (Lindstedt 1971).

A similar picture can be observed in the feeding behaviour of carnivorous Protozoa.

Nevertheless, it would be premature to discard Lindstedt's scheme (1971) completely with respect to protozoa. In the course of the experiments some substances would invariably attract the carnivorous Protozoa into the zone with a potential prey, without inducing a feeding response in them — these substances can be called attractants. Cholesterol, malic and succinic acids act as such attractants for *Dileptus anser*. Granules of boiled yolk attract didinia and induce a discharge of their toxicysts and pexicysts, which is not followed, however, by ingestion of the granules captured. The feeding behaviour remains incomplete due to the lack of some chemical initiants in the yolk of an egg. Thus, it is also possible that under natural conditions there are instances when the subsequent stages of feeding behaviour get triggered in Protozoa due to the change of chemical inducers such as attractants, initiants and stimulants.

Very little is known about chemical repellents, suppressors and deterrents that hinder particular stages of feeding behaviour in Protozoa. Our experiments suggest that there are cases when Protozoa serving as potential prey for the predator give off repelling chemical agents (repellents). Without admitting this, it would be very difficult to interpret some of data obtained.

First of all, the predatory ciliates under study do not feed on individuals of their own species. Moreover, they never attack each other, nor discharge their trichicysts at accidental collisions. At the same time, the carnivores are capable of preying, to a certain extent, on the animals of their own species when killed by heat. But even in this last case didinia and dilepti ingest only very few individuals of their species — 15 and 17 per cent, respectively. Dilepti and didinia, even dead, must give off some repellents, scaring off their relatives.

Incidentally, some clones of *Dileptus anser* grown at the Institute of Cytology (Leningrad) display cannibalism towards the clones of other syngenes of that species (M. V. Tavrovskaya, personal communication). The prey clones must be lacking certain repellents precluding cannibalism.

A suggestion that chemical agents suppress cannibalism in *Lacrymaria* and *Coleps* is less grounded as it involves further assumptions. In

the experiments of set II those animals ingested a very big share of specimens of their own species killed by heat (Table 2), while never attacking them when alive. Thus, be it really so that *Lacrymaria* and *Coleps* can produce agents causing aversion in the animals of their own species, such repellents must be non-heat-resistant, loosing their activity in the Protozoa killed by heat?

Some Protozoa may be capable of giving off repellents specific to a particular potential predator. In this case, whether a protozoan is edible or not should be decided by the interrelation of attractants and repellents given off into the environment. This hypothesis, however, requires a serious experimental testing.

The ciliates being potential prey for predatory Protozoa often have some physical means of defence. These are powerful discharges of trychocysts capable of scaring off or even injuring an attacking predator. As can be seen from the experiments on *Spirostomum ambiguum* (when alive or cut into bits), the big size of the prey and its active locomotor responses can also serve as defence devices.

So, some physical characters of a potential prey can be limiting factors, preventing the predator from catching it.

Identification of an organism as a food object is chiefly due to its chemical properties, but whether it will be captured and swallowed by the predator still depends largely on the limiting physical characters of the object. This is the case, when, speaking figuratively, the prey selects a predator of its own choice.

Thus, neither the size of prey, nor its shape, nor surface structure can by themselves induce a feeding response in carnivorous Protozoa. This means that these characters are not utilized by the predators in identification of a food object, and, hence, cannot serve as a basis for food choice. It is due to this reason that in experiments living prey can be successfully substituted by chemical food models having physically nothing in common with the prey, but sharing one faculty with it — the ability to release into the environment a chemical inducer triggering the feeding response in carnivorous unicellular animals.

The chemical food models do not only show very clearly the leading role of chemoreception in the choice of food by carnivorous Protozoa, but also alow to discriminate particular substances specific to the given predator as inducing its feeding response, that is the response which accounts for food selection.

Table 9 shows the feeding response inducers discriminated for *Amoeba proteus*, *Peranema trichophorum*, *Coleps hirtus* and *Dileptus anser*. Though without doubt we could not have discovered the whole range of such inducers for the species under study, the available evidence

Table 9

Inducers of Feeding Response in Some Protozoa

Inducers	*Amoeba proteus*	*Peranema trichophorum*	*Coleps hirtus*	*Dileptus anser*
Proteins:				
hyalouronidase	+			
cytochrome C	+			
pepsin	+			
trypsin	+	±	±	±
RNA-ase	+			
DNA-ase	+			
Peptone	+			
Bactopeptone		±		
Reduced glutathione	±		+	
Oxidized glutathione			±	
Glycine			±	
Cysteine	±			+
Cephalin	+	+		+
Lecithin	+	+		
Thiourea			+	
Tweens: 40, 60, 80	+	±		+

The symbol + denotes those substances which induce a phagocytic reaction in over 50 per cent of individuals.

The symbol ± stands for the substances that induce a feeding response in less than half of individuals used for the experiments.

enables us to draw at least two conclusions. Firstly, each carnivorous protozoan under study was found to give a feeding response to a number of chemical agents of different nature. Consequently, a protozoan must possess either one chemoreceptor of a general type or several types of individual cell chemoreceptors. Secondly, different species of carnivorous Protozoa have different combinations of feeding response inducers.

It should be noted that not all inducers are equally effective. Some of them, due to unknown reasons, induce a feeding response in a part of the population only. So, while phospholipids — cephalin and lecithin — are the major inducers for the flagellate *Peranema*, some of the *Peranema* population also respond to trypsin, bactopeptone and tweens. This may be due to heterogeneity of *Peranema* population used in the experiments.

Establishment of particular chemical inducers of feeding response in some Protozoa makes them particularly suitable for studying the activity of cell chemoreceptors.

Stressing the great importance of chemical inducers and chemo-

reception in the feeding behaviour of carnivorous ciliates we should not overlook one point that has not been thoroughly verified so far. To eliminate the influence of physical factors on the experimental results preference was given to motionless food models. Meanwhile, the data of experiments on *Didinium nasutum* indicate that at least this species of predatory ciliates has fairly complex relationships with living prey. In any case, this predator much more readily ingests *Paramecium* and *Coleps* when they are alive and moveable than when rendered motionless — killed by heat. Besides, it is well-established fact that a feeding response of *Amoeba dubia* can be induced not only by chemicals but also due to mechanical irritation (S e r a v i n 1968). All these facts do not deny the leading role of chemoreception in feeding behaviour of predatory Protozoa, but call for a more careful study of the food object mobility as a factor influencing the feeding behaviour of Protozoa. This problem will be the subject of our next paper.

РЕЗЮМЕ

Исследован выбор пищи у хищных простейших (*Didinium nasutum*, *Dileptus anser*, *Lacrymaria olor*, *Coleps hirtus*), амебы (*Amoeba proteus*) и жгутиконосца (*Peranema trichophorum*). Показано, что он осуществляется на основе, главным образом, хеморецепции. Используя метод химических пищевых моделей, авторы выявили для ряда изученных видов конкретные химические индукторы, которые способны вызывать у простейших пищевую реакцию (заглатывание индифферентных, ранее несъедобных объектов). Такими индукторами служат для *Peranema trichophorum* фосфолипиды кефалин и лецитин (и в меньшей мере трипсин и твины); для *Amoeba proteus* — многие белки (трипсин, пепсин, цитохром С, РНК-аза, ДНК-аза и др.), пептон, кефалин, лецитин, твины (и в меньшей мере цистеин и восстановленный глютатион); для *Coleps hirtus* — восстановленный глютатион и тиомочевина (в меньшей мере трипсин и бактопептон), а для *Dileptus anser* — цистеин, твины (и в меньшей мере трипсин и кефалин). Таким образом для каждого вида существует несколько индукторов, причем набор индукторов различен для разных форм простейших. Физические особенности жертвы, такие как размеры, строение поверхности, способность давать залпы трихоцист и т.п., не играют особой роли в идентификации добычи хищником. Однако эти особенности в ряде случаев могут препятствовать осуществлению у хищников пищевой реакции (мешая захвату и заглатыванию пищи).

REFERENCES

A c r e e F., T u r n e r R. B., G o u c k H. R., B e r o z a M. and S m i t h N. 1963: L-lactic acid: a mosquito attractant isolated from human. Science, 161, 1346–1347.

B o z l e r E. 1924: Über die Morphologie der Ernährungsorganellen und die Physiologie der Nahrungsaufnahme von *Paramecium caudatum* Ehrg. Arch. Protistenk., 49, 163–215.

Bragg A. N. 1939 a: Selection of food by Protozoa. Turtox News, 17, 19–25.
Bragg A. N. 1939 b: Selection of food by Protozoa. Turtox News, 17, 41–44.
Calkins G. N. 1915: *Didinium nasutum*. I. The life history. J. exp. Zool., 19, 225–239.
Chapman-Andresen C. 1958: Pinocytosis of inorganic salts by *Amoeba proteus*. C. r. Trav. Lab. Carlsberg, Sér. Chim., 31, 77–92.
Chapman-Andresen C. 1962: Studies on pinocytosis in amoebae. C. r. Trav. Lab. Carlsberg, 33, 73–384.
Chauvin R. 1969: Le comportement animal. Paris.
Chen J. T. 1950: Investigations of the biology of *Perenema trichophorum* (*Euglenineaceae*). Q. J. microsc. Sci., 91, 279–308.
Christiansen R. G. and Marschall I. M. 1965: A study of phagocytosis in ameba *Chaos chaos*. J. Cell. Biol., 25, 443–458.
Dembowski J. 1922 a: Über die Nahrungswahl und die sogennanden Gedächtniserscheinungen bei *Paramecium caudatum*. Trav. Lab. Biol. Gen. Nencki (Varsovie), 1, 1–37.
Dembowski J. 1922 b: Weitere Studien über die Nahrungswahl bei *Paramecium caudatum*. Trav. Lab. Biol. Gen. Nencki (Varsovie), 2, 1–16.
Dethier V. G. and Stellar E. 1964: Animal behavior. Its evolutionary and neurological basis. Englewood Cliffs, N. Y. Prentice-Hall, Inc. Pennsylvania.
Dragesco J. 1962: Capture et ingestion des proies chez les infusoires cilies. Bull Biol. Fr. Belg., 96, 123–166.
Fenchel T. 1968: The ecology of marine microbentos. II. The food of marine benthic ciliates. Ophelia, 5, 73–121.
Gibbs D. and Dellinger A. P. 1908: The daily life of *Amoeba proteus*. Am. J. Psychol., 19, 230–241.
Hodgson E. S. 1968: Taste receptors of arthropods. In: Invertebrate receptors. Symp. Zool. Soc. London, 23, 269–277.
Hosoi T. 1958: Adenosine-5 -phosphate as the stimulating agent in blood for inducing gorging of the mosquito. Nature, London, 181, 1665–1666.
Jeon K. W. and Bell L. G. 1965: Chemotaxis in large free-living amoeba. Expl. Cell Res., 38, 536–555.
Lenhoff H. M. 1968: Chemical perspectives of the feeding response, digestion and nutrition of selected coelenterates. In: Chemical Zoology 2, 158–217.
Lindstedt K. J. 1971: Chemical control of feeding behavior. Comp. Biochem. Physiol., 39, 553–581.
Lipsitz E. I. and Brown A. W. A. 1964: Studies on the responses of the female *Aedes mosquito*. IX. The mode of activeness of lysine and other amino acids. Bull. Entomol Res., 54. 675–687.
Losina-Losinsky L. 1929: Le phenomenes du chemotactisme en rapport avec le choix de la nourriture chez les infusoires. C. r. Acad. Sci. l'URSS, 17, 403–408.
Lund E. J. 1914: The relation of *Bursaria* to food. I. Selection in feeding and in extrusion. J. exp. Zool., 16, 2–52.
Mast S. O. 1909: The reactions of *Didinium nasutum* (Stein) with special reference to the feeding habits and the function of trichocysts. Biol. Bull, 16, 91–118.
Mast S. O. 1911: Habits and reactions of the ciliate *Lacrymaria*. J. Anim. Behav., 1, 229–243.
Mast S. O. 1947: The food vacuole in *Paramecium*. Biol. Bull., 92, 31–72.
Mast S. O. and Hahnert W. F. 1935: Feeding, digestion and starvation in *Amoeba proteus* (Leidy). Physiol. Zool., 8, 255–272.
Muller M. 1972: Secretion of acid hydrolases and its intracellular source in *Tetrahymena pyriformis*. J. Cell Biol., 52, 478–487.
Nelson E. C. 1933: The feeding reaction of *Balantidium coli* from the chimpanzee and the pig. Am. J. Hyg., 18, 185–201.
Norris K. R. 1965: The bionomics of blowflies. An. Rev. Ent., 10, 47–68.
Pavlovskaja T. W. 1970: Pitanie infusorij Uroleptus viridis diatomovymi vodorosljami. Zool. Zh., 49, 1775–1779.
Prescott D. M. and James T. W. 1955: Culturing of *Amoeba proteus*. Expl. Cell Res., 8, 256–258.
Ricketts T. R. 1972: The inducation of endocystosis in starved *Tetrahymena pyriformis*. J. Protozool., 19, 373–375.
Sandon H. 1932: The food of Protozoa. Publ. fac. Sci. Egyptian Univ. Cairo, 1.

Schaeffer A. A. 1910: Selection of food in *Stentor coeruleus* (Ebs.). J. exp. Zool., 8, 75–135.
Schaeffer A. A. 1914: Feeding habits of amoeba. Science, 39, 1004, 272.
Schaeffer A. A. 1917 a: On the reactions of *Amoeba* to isolated and compound protein. J. exp. Zool., 22, 53–86.
Schaeffer A. A. 1917 b: Choice of food in *Amoeba*. J. Anim. Behav., 7, 220–258.
Schwartz V. 1965: Versuche über Regeneration und Kerndimorphismus bei *Stentor coeruleus* Ehrbg. Arch. Protistenk., 85, 100–139.
Seravin L. N. 1968: Rol mehaničeskih i himičeskih razdražitelej v inducirovanii fagocitarnoj reakcji u *Amoeba proteus* i *A. dubia*. Acta Protozool., 6, 97–107.
Seravin L. N. and Orlovskaja E. E. 1972: Vybor pišči u prostejših. Vestnik Leningr. Gosud. Univ., 15, 7–19.
Seravin L. N. and Orlovskaja E. E. 1973: Factors responsible for food selection in Protozoa. In: Progress in Protozoology, Abstr. Fourth int. Congress Protozool., Clermont-Ferrand 1973, 371.
Visscher I. P. 1923: Feeding reactions in the Ciliate. *Dileptus gigas* with special reference to the function of the trichocysts. Biol. Bull., 45, 113–143.
Webb M. G. 1956: An ecological study of brackish water ciliate. J. Anim. Ecol., 25, 148–175.
Wenzel F. and Baltes W. 1967: Untersuchungen über eine chemotaktische Reaktion von *Spathidium stammeri* (*Ciliata, Holotricha*). Zool. Anz., 178, 151–154.
Wessenberg H. and Antipa G. 1970: Capture and ingestion of *Paramecium* by *Didinium nasutum*. J. Protozool., 17, 250–270.
Woodruff L. L. and Spencer H. 1922: Studies on *Spathidium spathula*. I. The structure and behavior of *Spathidium* with reference to the capture and ingestion of its prey. J. exp. Zool., 35, 189–202.

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EXPLANATION OF PLATE I

Induction of feeding reaction by food models soaked with liver extract

1: *Dileptus anser*, swallowing carbon (a) and starch granules (b)
2: *Lacrymaria olor*, swallowing carbon (a) and starch granules (b)
3: *Didinium*, swallowing a big starch granula
4: *Coleps hirtus*, swallowing carbon particles

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Feeding Behaviour of Unicellular Animals. II. The Role of Prey Mobility in the Feeding Behaviour of Protozoa

Synopsis. The method of magnetic moving models (MMM) proved that the feeding behaviour of Protozoa always depends on certain degree of the mobility of the prey. Bacteria-feeding ciliates (*Paramecium caudatum*, *Stylonychia mytilus* and *Spirostomum ambiguum*) react to the mobility of prey as to a limiting factor reducing the probability of food particles ingestion. Hydrodynamic disturbances of the medium produced by oscillations of MMM-type models serve as a source of stimuli inducing a positive taxis in *Amoeba proteus* by, directing its movements towards potential prey. *Dileptus anser* and *Didinium nasutum* both attack and capture MMM-models without ingesting them. Only in *Amoeba dubia* the mechanical stimulation evoked by prey mobility induces a complete feeding response including the ingestion of oscillating particles.

In their earlier publication Seravin and Orlovskaja (1977) have shown a great importance of chemical inducers and chemoreception for the feeding behaviour of various Protozoa. It was found at the same time that the physical properties of a food item such as size, shape or surface structure had a minor influence as factors merely limiting phagocytosis of the predator. This was established, however, in the course of experiments restricting the choice of food for carnivorous ciliates to those killed by heat or to chemical food immobile models. Mobility of the prey, this very important factor in the feeding behaviour of unicellular animals, was actually outside of the scope of our investigations. Our experiments, however, yielded some data suggestive of a fairly complex predator-prey relationship in the ciliate *Didinium nasutum* (Seravin and Orlovskaja 1977). In any case *Didinium* gives living *Paramecium* and *Coleps* an apparent preference as food over animals of the same species killed by heat and, hence, deprived of motion.

According to Wessenberg and Antipa (1970) *Didinium* would not feed on anything but only on living prey, taking it up as long as it retains at least some of its mobility.

Besides, there is a well-established fact of mechanoreception being as important as chemoreception for the feeding behaviour of Protozoa. That is the case of *Amoeba dubia* (Seravin 1968), when a vibrating end of a capillary was proved to induce the formation of a food cup and later a food vacuole.

We should also keep in mind the evidence of Schaeffer (1916) stating that polyploidal amoebae can take up indigestible particles (for instance, glass) when set into motion by the investigator.

On the basis of experimental data of Christiansen and Marshall (1965) describing the feeding response (capture and ingestion of prey) in *Chaos chaos* as due to the predator's repeated mechanical contacts with a food item. There is an old belief that polypodial amoebae feed on living organisms alone (Gibbs and Dellinger 1908, Mast and Hahnert 1935). Many of other authors share the view that the mobility of the prey may play a certain role in the food choice of various species of Protozoa (Visscher 1923, Sandon 1932, Chen 1950, Bovee 1960, Pavlovskaya 1970, and others).

It should be stressed from the beginning, however, that the experimental conditions in all earlier investigations studying the relationships between the prey's mobility and the predator's feeding behaviour did not allow to eliminate a potential effect of other physical or, in some cases, chemical characteristics of the food item. That is why we set ourselves a task of elaborating a method which would permit to study the significance of the prey mobility for the feeding behaviour of Protozoa as an independent phenomenon with no other factors involved. In our efforts we have elaborated a method of magnetic mechanical models which will be discussed in the next chapter.

Material and Methods

Several species of amoebae (*Amoeba proteus, A. dubia*), carnivorous ciliates (*Didinium nasutum, Dileptus anser*) and bacteria-eating ciliates (*Paramecium caudatum, Spirostomum ambiguum* and *Stylonychia mytilus*) were used in this study.

Paramecium and *Spirostomum* were cultivated on lettuce infusion by the widely accepted method of Sonneborn, (1950, 1970). *Aerobacter aerogenes* serving as food for them. The remaining protozoa were grown on Prescott's medium (Prescott and James 1955) with *Tetrahymena pyriformis* provided as the food species. The carnivorous Protozoa were kept unfed for two days before transferring them into the experimental container, while the bacteria-eating ciliates were starved for no more than 24 h.

The natural food was replaced with magnetic moving models (MMM). The MMM-method consists in the following. A ferromagnetic body placed in a magnetic field is forced to move towards the pole of a magnet or a solenoid. By adjusting the parameters of the magnetic field such as intensity, frequency and gradient together with some characteristics of particles like: magnetic penetrability, size, shape, etc. — one can obtain all kinds of movements of the body in question. The "behaviour" of particle in the magnetic field with regard to gravity and medium resistance is described by the following set of differential equations in the form (Izmodenov 1972):

$$m\frac{dv}{dt} = F_C + F_T + F_M \pm F_K,$$

$$\frac{dl}{dt} = v,$$

where dl — is particle path segment; v — particle velocity; t — time; F_M — resultant of the force due to the particle magnetic charge and the ponderomotive force; F_T — gravity force (that is the sum of the particle weight and the boyancy force); F_C — the force of resistance to particle movement; F_K — the particle interaction force in the magnetic field.

Thus, there is every possibility to use minute ferromagnetic particles as moving food models simulating the movement of the natural prey of Protozoa.

The present experiments made use the ferrite particles type $\Phi = 600$ as magnetic moving models (MMM). Bits of ferrite were grinded in a porcelain jar and separated according to the particle size by fractional precipitation in water. In some cases models were made from the pre-magnetized ferrite.

The magnetic field was produced by a system consisting of an audiofrequency sine wave generator and a solenoid with a core. The generator type ZG-2A was chosen for this purpose, with the lower limit of frequency set down to 5 Hz. The solenoid winding consisted of 5000 coils of the PELŠO-0.12 copper wire. The winding had a resistance of 270 Ω. The intensity of the magnetic field controlling the MMM movements equaled 5–22 e.

The above set of equations shows that the interaction of particle with the magnetic field is due to the member F_M which is the resultant of two forces. If the particle is not magnetized F_M is determined by the value of the ponderomotive force. When the model is magnetized, F_M will chiefly depend on the force due to the particle magnetic charge. So, we can obtain two different types of models, MMM-1 and MMM-2 varying in the nature of their movement.

The MMM-1-type models are produced from non-magnetized ferrite particles. When influenced by the magnetic field set up in the solenoid, such particles would reproduce oscillations in a vertical plane with the frequency of the applied field. Models of MMM-2-type are produced from magnetized ferrite particles. They would exhibit the same type of vertical movement as in MMM-1 at low frequences — up to 50 Hz at the output. But when the frequency of the magnetic field ranges from 125 to 500 Hz, the MMM-2 display an abrupt change in their movement. By adjusting the frequency within the above range we can make the MMM-2 models to crawl over the bottom of the microcontainer filled with a fluid to move in any direction to rotate or deflect from a straight path as well as to change the velocity of their movements. Thus, these models can reproduce almost every type of movement of the living prey.

The dynamics of the ferromagnetic particles is much too complex interaction involving a number of elementary processes which cannot induce in all the models the uniform movements. So, the input data and parameters of the MMM simulating system had to be selected empirically to ensure the type of particle movement required for the given experiment.

The ferrite particles prepared for the experiments were soaked in ethyl alcohol (96%) washed with alcohol 3–4 times during 3 to 4 days and than rinsed in bidistilled water. Immediately before each experiment the particles were put into freshly prepared medium used for cultivating protozoa chosen for the experiment.

A cylindrical microcontainer, 8 × 10 mm, was fixed in a special seat at the butt end of the solenoid installed vertically. A small portion of the chosen protozoan culture was pipetted onto the bottom of the container forming a 5 mm liquid layer, where some ferrite particles were also added. The experiments with carnivorous Protozoa did not take into account the ferrite particles concentration, since it was enough to learn whether those unicellular animals would at all respond to the moving particles and if they did — what type of the particle movement was the most effective.

Results

Ferrite particles, 20–40 μm introduced into the microcontainer with starved *Amoeba dubia*, are ignored by the animals during the entire course of the experiment — 20 min. The amoebae would either avoid the motionless particles or crawl over them. They never would ingest such particles (Table 1). The animals exhibit an entirely different behaviour

Table 1

Response of *Amoeba dubia* and *Amoeba proteus* to MMM-1* Type Models

Species	Model characteristics	Number of animals per experiment	Number of animals ingesting the models (M±m per cent)
Amoeba dubia	MMM-1	97	83 (83.6±3.7)
	Motionless ferrite particles (control)	78	0
Amoeba proteus	MMM-1	117	0
	Motionless ferrite particles (control)	110	0

* MMM-1 — Moving magnetic models produced from non-magnetized ferrite particles.

when subject to the controlling effect of a magnetic field varying with a frequency of 6, 8 or 12 Hz. Nonmagnetized ferrite particles (MMM-1) being to oscilate with the frequency of the applied field, causing a positive taxis in amoebae who envelope the MMM-1 with their pseudopodia, form food cups and finally ingest the ferrite particles. So, in the presence of oscilating food models *A. dubia* display all stages of their feeding behaviour.

It is necessary to check, however, whether the feeding response in *A. dubia* is actually induced by the moving food models (MMM-1) and not by the stimulating effect of the magnetic field itself.

Control experiments conducted for this purpose show that magnetic fields having a frequency of 6, 8, 24 and 500 Hz and a maximum intensity of 5.0, 7.7, 8.4 and 15.5 e neither influence the rate of amoebae's capturing the *Tetrahymena* killed by heat, nor stimulate *A. dubia* to capture immovable carbon particles.

Amoeba proteus is likewise indifferent to immovable ferrite particles 40–60 μm in size, but displays a distinct positive taxis when those particles are made to oscilate with a frequency of 6, 8, 12, 20 and 50 cycles per minute. The amoebae form pseudopodia stretched towards the oscilating MMM-1, and began to envelope them without ever reaching the stage of food cup formation. In all instances the animals would pass by or crawl over the particle like over an obstacle.

Small particles that sometimes stick to the plasmalemma slip towards the posterior part of the body and gradually fall off onto the substrate without being swallowed.

Failure to induce a feeding reaction in *A. proteus* during the above experiments may be due to the type of motions performed by the MMM-1 models, for they inadequately simulate the movement of a living prey. In actual fact, the ciliates and flagellates offered as food to amoebae move over the substrate parallel to its surface. Consequently, the mechanical effects of the natural prey on the Protozoa are also oriented within the substrate plane or parallel to it.

Therefore, the next set of experiments was provided with the MMM-1-type models capable to oscillate in the horizontal rather than vertical plane. For this purpose a pole of the permanent magnet was installed 5 cm from the microcontainer, the field intensity being 200 e, and the magnet axis perpendicular to that of the solenoid. This modification of the MMM-1 technique allowed to discover a very interesting phenomenon.

A. proteus responded to the MMM-1 models with a positive taxis trying to envelope them with pseudopodia, but in much the same way as in the earlier experiments no formation of food cups followed. Nevertheless, 40 to 60 min later the ferrite particles involved into the cyto-

plasma stream could be seen inside the bodies of some amoebae. More detailed observations showed in which way the particles got inside the amoebae: the MMM-1 models, stuck to the plasmolemma of *A. proteus*, gradually slipped to the posterior part of the body, where the larger particles began to sink very slowly into the cytoplasm and stayed there motionless for quite a long time. It was not until 40 to 60 min later that they could be found within flowing endoplasm. The experiments of this type were repeated many times. Out of the total number of 105 amoebae 31 contained the ferrite models inside their bodies.

Now let us examine the response of the carnivorous ciliates *Dileptus anser* and *Didinium nasutum* to the moving magnetic models. Preliminary controls displayed a complete indifference of these predators to motionless ferrite particles which they never attack, and don't ingest. A magnetic field with a frequency of 6 to 60 Hz and a maximum intensity of 22 e did not have a pronounced effect on the phagocytosis in didinia and dilepti when preying on natural food *(Tetrahymena)*.

The experiments with MMM-1 oscillating at frequencies of 6, 8, 10, 12, 20 and 50 Hz never showed any reaction of *Didinium* or *Dileptus* to the models. All this allows a tentative conclusion to be made that the ciliates under study do not identify an object as a food item if its motions are limited to oscillations only. Therefore, the MMM-2-type models were introduced in the further sets of experiments. As mentioned above, the applied controlling field with a frequency of 125–500 Hz and a maximum intensity of 15.8–18.0 e makes these models to reproduce a great variety of movements such as crawling over the bottom of the microcontainer or swimming at variable velocities in complex paths associated with rotating around the longitudinal axis.

Truncation of the probosci in dilepti was used as an index of their response to the MMM-2-models. This phenomenon has been described by many authors studying the feeding behaviour in dilepti (Metzner 1933, Dragesco et Métain 1948, Dragesco 1962, Miller 1968). It involves the ability of the predator to strike the prey with trichocysts that are extruded in great numbers on the proboscis. A number of successive discharges of trichocysts results in gradual truncation of the predator's proboscis up to almost complete disappearance.

Experiments with MMM-2 models (30–80 μm in size) exhibited a distinct response of the dilepti to particles which rotated while moving along their paths. When the ciliate gets close to a MMM-2-model, at a distance of 0.5–1.0 body length, it serves from the original path, discharges the trichocysts from the proboscis and attaches them to the model. *Dileptus* keeps the artificial "prey" adhered for some time and then releases it. If there appears another MMM-2 nearby, the predator

immediately dashes at it. Within 6 min from the beginning of the experiment the bulk of the dilepti have their proboscis strongly truncated or even completely lost (Table 2). There was not a single instance of ingestion of MMM-2 models by dilepti, in other words, their feeding response is incomplete.

Table 2

Response of *Dileptus anser* to MMM-2-Type Models*

Experiment number	Number of animals per experiment	Number of animals attached to MMM-2	Number of animals with proboscis after 6 min of experiment	Number of animals ingesting MMM-2
1	25	25	5	0
2	30	18	8	0
3	20	14	5	0
4	40	40	4	0

* MMM-2 — Moving magnetic models produced from magnetized ferrite particles.

Didinia also respond to MMM-2 models (50–80 μm in size), but not towards all particles. By adjusting various parameters of the simulating system such as particle size and concentration, magnetic field frequency and intensity, the authors found that didinia display the most active response to those particles which can move both along their longitudinal axes and in a spiral path. In this case it is already at a distance as great as 5 to 8 lengths of its body that *Didinium* sets out to attack with an increased velocity, choosing the shortest way to the "prey". Due to a discharge of extrusive organelles that follows the attacking movement the particle gets firmly attached to the proboscis of *Didinium* by a compact bundle of slender strands. During 1 or 2 s the animal jerks sharply from all directions carrying the particle around until it looses it. Then it starts a new attack towards another MMM-2 model showing the same type of movement as the first one, but it never happens that *Didinium* would ingest a particle. It should be also noted that not each attack of *Didinium* results in attaching a ferrite particle to the proboscis (Table 3).

Thus, mechanical irritation stimulates initial stages of feeding behaviour, i.e., orientation and movement towards a moving object or even capture of the prey, what is the beginning of a feeding response both in *Dileptus* and in *Didinium*. But the responses is incomplete since ingestion of the prey model never occurs in the absence of chemical inducers.

Table 3

Response of *Didinium nasutum* to MMM-2-Type Models*

Experiment number	Number of animals per experiment	Number of animals attached to MMM-2 during 10 min	Number of animals ingesting MMM-2
1	40	8	0
2	50	12	0
3	35	5	0
4	60	11	0

* MMM-2 — Moving magnetic models produced from magnetized ferrite particles.

Unlike the carnivorous Protozoa, bacteria-feeding ciliates are well known for their ability to swallow all kinds of indigestible substances such as particles of ink, carmine, chalk, glass etc. The ciliates *Paramecium caudatum, Spirostomum ambiguum* and *Stylonychia mytilus* chosen for our experiments can ingest not only all the above substances but also small particles of ferrite between 3 and 10 μm in size.

Preliminary tests showed that none of the animals under study responded to a magnetic field with a frequency of 6, 8, 12, 50 and 500 Hz as far as the rate of ingestion of carbon or carmine particles was concerned.

In the absence of magnetic field the mean number of food vacuoles (f.v) formed in the medium with ferrite particles per individual fed during 10 min was 5.1 ± 0.3, 2.7 ± 0.1 and 1.3 ± 0.1 for *Paramecium caudatum, Spirostomum ambiguum* and *Stylonychia mytilus*, respectively. The intensity of phagocytosis decreases in the presence of MMM-1 models oscillating with a frequency of 12 Hz, the respective mean values for *Paramecium* and *Stylonychia* observed during 10 min falling down to 3.0 ± 0.1 f.v. ($t_d = 6.7$; $P < 0.001$) and 0.41 ± 0.6 f.v. ($t_d = 7.7$; $P < 0.001$). Similar results were obtained in the experiments with the MMM-2-type models. Within 10 min of the experiment the mean number of vacuoles per one animal was 3.1 ± 0.4 for *Paramecium* ($t_d = 4.0$; $P < 0.001$) and 1.1 ± 0.1 in the case of *Spirostomum* ($t_d = 11.3$; $P < 0.001$).

Discussion

The data obtained in our experiments showed that there is no uniformity in the response of various Protozoa to indigestible objects simulating the movements of living animals. The most effective recognition

of food objects due to mechanoreception can be observed in *Amoeba dubia*, which responds to the simplest longitudinal oscillations of ferrite particles with a full cycle of feeding behaviour including capturing and ingestion of particles (MMM-1).

A. proteus shows no feeding response to the MMM-1-type models whether moving in vertical or horizontal plane. Neither would it ingest the MMM-2 models. In other words, a feeding response in *A. proteus* can be induced by specific food substances alone (Seravin and Orlovskaja 1972, 1977). The effect of mechanical stimulation is limited to orientation movement towards the vibrating particle, what is a first stage of feeding behaviour. These data are in good accordance with our earlier experiments on *A. dubia* and *A. proteus* where the capillary method was used (Seravin 1968).

New features were observed in studies on the response of *A. proteus* to small ferrite particles with the horizontal component predominating in their oscillatory motion. Such particles, attached to the plasmalemma, are sinking into the body of amoeba in its posterior zone. This part of the body of moving *A. proteus* is known as the place of continuous pinocytosis and transport of plasmalemma into the cytoplasm (Wohlfarth-Bottermann und Stokem 1966). In the case of induced pinocytosis the amoebae can take up not only liquids but also solid particles (Holter 1965, Chapman-Andresen and Lagunoff 1966). There is every reason to believe that the uptake of MMM-1 in the posterior part of the body is due to pinocytosis which seems to be greatly stimulated by mechanical stimulations resulting from the MMM-1 oscillations.

Dileptus and *Didinium* respond to moving particles only. The MMM-2-type models both attract these animals and induce them to discharge their trichocysts, that is to capture the prey which is the initial stage of the feeding response. But this response cannot reach its final stage of prey ingestion without chemical feeding inducers. *Dileptus* and *Didinium*, however, choose between the MMM-2 attacking only such models that rotate while moving alone their paths. Moreover, *Didinium* discharged its organelles only when attacking a rotating model on a spiral path, while the trichocysts of *Dileptus* are ejected whenever the predator contacts a particle rotating around its axis. This difference in the feeding behaviour of the two predators seems to be essential.

Dileptus anser is a wide-range polyphage feeding on both unicellular (Metzner 1933, Dragesco et Métain 1948, Miller 1968) and multicellular animals (Brown and Jenkins 1962). Thus, among its prey are organisms representing different types of movement, but in most cases those which rotate around their longitudinal axes.

Didinium nasutum is stenophagous, preying chiefly on some species of *Paramecium* (Mast 1909, Calkins 1915, Párducz 1954, Wessenberg and Antipa 1970) that swim in a stretched spiral path while rotating around the longitudinal axis of the body. So, both *Didinium* and *Dileptus* attack those MMM-2-type models which simulate the movements most typical for their natural prey.

Seravin (1970) discovered that motions of cilia in a ciliate rotating around its axis give rise to a regular system of eddy flow. A similar phenomenon can be observed during the movement of an MMM-2. When such models are introduced into an ink suspension they produce a general eddy flow causing specific hydrodynamic disturbances in the medium. These disturbances probably help the predatory Protozoa to recognize moving prey from a distance. In the medium of a limited volume where a great number of prey and predators are moving simultaneously "the odour gradients" can be preserved but locally losing their continuity and varying in direction. This makes recognition of food organisms by their "chemical traces" hardly probable and unreliable. A predator may enter the "food odour" zone only when being in close proximity from or in direct contact with the living prey. Thus, mechanoreception of carnivorous Protozoa serves as a "system of remote detection" of moving prey. *Amoeba dubia* is the only protozoan whose feeding behaviour due to mechanical stimulation alone reaches the final stage of ingesting the prey. In other Protozoa under study similar stimulations either induce no feeding response at all or their reaction remains incomplete unless specific chemical inducers participate in the feeding process.

Though chemoreception is the decisive factor in the food choice of unicellular animals (Seravin and Orlovskaja 1972, 1977), the complete cycle of their feeding behaviour normally depends on a joint effect of mechano- and chemoreception systems. Mechanical stimulations evoked by a food object, even though unable to induce a feeding response in Protozoa — play at least some role in their reaction to food as they can bring down the threshold of sensitivity to chemicals given off by the above object (Seravin 1968).

Quite a different picture is observed in the experiments with bacteria-feeding ciliates.

As has been shown above, mechanical stimulation produced by the MMM-1 and MMM-2-type models invariably inhibits phagocytosis in *Paramecium caudatum*, *Spirostomum ambiguum* and *Stylonychia mytilus*. Under natural conditions these animals mainly feed on objects of low mobility such as bacteria, algae and small unicellular animals, ingestion being due to a water flow caused by peristomal cilia. Therefore, particles moving with a velocity above a certain critical value would not be

taken by the peristomal flow, thus failing to get inside the cytostome. So, with increasing speed of the models the number of particles available to the bacteria-feeding ciliates will be reduced, what in our opinion, accounts for the inhibition of phagocytosis in this group of animals.

РЕЗЮМЕ

С помощью методики подвижных магнитных моделей (МПМ) показано, что подвижность добычи всегда в той или иной мере играет роль в пищевом поведении простейших. Для инфузорий-седиментаторов (*Paramecium caudatum*, *Stylonychia mytilus*, *Spirostomum ambiguum*) подвижность добычи является органичивающим фактором; она уменьшает вероятность заглатывания пищевых объектов. Гидродинамические возмущения среды, вызываемые колебанием МПМ, служат источником сигналов, которые индуцируют положительный таксис у *Amoeba proteus*, тем самым вызывая направленное ее перемещение в сторону потенциальной добычи. *Dileptus anser* и *Didinium nasutum* не только атакуют МПМ, имитирующие движение живых инфузорий, но и захватывают их, хотя никогда не заглатывают. Только у *Amoeba dubia* механические раздражения, вызываемые подвижностью добычи, выступают как индукторы пищевой реакции, завершающейся заглатыванием колеблющихся частиц.

REFERENCES

Bovee E. C. 1960: Studies of feeding behaviour of amoebas. I. Ingestion of thecate rhisopods and flagellates by verrucosed amoebas, particularly *Thecamoeba sphaeronucleolus*. J. Protozool., 7, 55–60.

Brown H. P. and Jenkins M. M. 1962: A protozoan *(Dileptus, Ciliata)* predatory upon *Metazoa*. Science, 196, 710.

Calkins G. W. 1915: *Didinium nasutum*. I. The life history. J. exp. Zool., 19, 225–239.

Chapman-Andersen C. and Lagunoff D. 1966: The distribution of acid phosphate in amoeba *Chaos chaos* L. C. r. Trav. Lab. Carlsberg, 35, 419–436.

Chen Y. T. 1950: Investigation of the biology of *Peranema trichophorum*. Q. J. microsc. Sci., 91, 279–308.

Christiansen R. G. and Marshall J. M. 1965: A study of phagocytosis in amoeba *Chaos chaos*. J. Cell Biol., 25, 443–458.

Dragesco J. 1962: Capture et ingestion des proies chez les infusoires ciliés. Biol. Bull. France et Belgique, 96, 123–166.

Dragesco J. et Métain Ch. 1948: La capture des proies chez *Dileptus gigas* (Cilie Holotriche). Bull. Soc. Zool., France, 73, 62–65.

Gibbs D. and Dellinger A. P. 1908: The daily life of *Amoeba proteus*. Am. J. Physiol., 19, 230–241.

Holter H. 1965: Passage of particles and macromolecules through cell membranes. In: Function and Structure in Micro-organisms. Cambridge, 89–114.

Izmodenov Ju. A. 1972: Magnitnyi metod gozoočistki. Simferopol, "Tawria", 1–112.

Mast S. O. 1909: The reactions of *Didinium nasutum* (Stein) with special reference to the feeding habits and the function of trichocysts. Biol. Bull., 16, 91–118.

Mast S. O. and Hahnert W. 1935: Feeding, digestion and starvation in amoeba. Physiol. Zool., 8, 255–272.

Metzner J. 1933: The feeding reaction of severed proboscides of *Dileptus anser*. Science, 78, 341–342.

Miller S. 1968: The predatory behavior of *Dileptus anser*. J. Protozool., 15, 313–319.
Pavlovskaya T. V. 1970: Pitanie *Uroleptus viridis* diatomovymi vodorosljami. Zool. Zh., 49, 1775–1779.
Párducz B. 1954: Reizphysiologische Untersuchungen an Ziliaten. Acta microbiol., 1, 175–221.
Prescott D. M. and James T. W. 1955: Culturing of *Amoeba proteus*. Expl. Cell Res., 8, 256–258.
Sandon H. 1932: The food of *Protozoa*. Pubs. faculty Sci. Egyptian Univ., 1—187.
Schaeffer A. A. 1916: On the feeding habits of amoeba. J. exp. Zool., 20, 529–584.
Seravin L. N. 1968: Rol' mehaničeskih i himičeskich razdražitelej v inducirovanii fagocitarnoj reakcji u *Amoeba proteus* i *A. dubia*. Acta Protozool., 6, 97—107.
Seravin L. N. 1970: Left and right spiralling round the body axis in ciliate protozoa. Acta Protozool., 7, 313–323.
Seravin L. N. and Orlovskaja E. E. 1972: Vybor pišči u prostejših. Vestnik Leningrad. Gosud. Univ., 15, 7–19.
Seravin L. N. and Orlovskaja E. E. 1977: Feeding behavior of unicellular animals. 1. The main role of chemoreception in food choice of carnivorous *Protozoa*. Acta Protozool., 16, 309–322.
Sonneborn T. M. 1950: Methods in the general biology and genetics of *Paramecium aurelia*. J. exp. Zool., 113, 87–147.
Sonneborn T. M. 1970: Methods in *Paramecium* Research. In: Methods in Cell. Physiology. New York, Academic Press, 4, 242–339.
Visscher J. P. 1923: Feeding reaction in the ciliate, *Dileptus gigas*, with special reference to the function of trichocysts. Biol. Bull., 45, 113–143.
Wessenberg H. and Antipa G. 1970: Capture and ingestion of *Paramecium* by *Didinium nasutum*. J. Protozool., 17, 250–270.
Wohlfarth-Bottermann K. E. und Stokem W. 1966: Pynocytose und Bewegung von Amoboën. II. Mitteilung. Permanente und induzierte Pinocytose bei *Amoeba proteus*. Z. Zellforsch., 73, 444–474.

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Contribution to Studies on Motor Response of *Stylonychia mytilus* to Mechanical and Chemical Stimuli

Synopsis. Stylonychia mytilus pretreated for 30 min to solution 16 mM KCl + 1 mM $CaCl_2$ + 1 mM Tris/HCl (pH 7.3) shows gradual recovery of motor response to mechanical and chemical stimuli during 16–32 min of washing in medium devoid of potassium. It is postulated that exposure of ciliates to appropriate concentrations of potassium and calcium ions inhibits function of both receptor systems, which are localized within cell membrane of ciliate.

Stylonychia mytilus, like other hypotrichs, may respond to chemical and mechanical stimuli with short- or long-lasting reversed beat of AZM (Adoral Zone Membranelles) or cirri and this reaction can be recorded by photomacrographic technique or by direct microscope observations. The reversed beat of AZM and cirri is an analogous phenomenon to well known ciliary reversal in *Paramecium* and it was found that depolarization of the cell membrane is accompanying these motor responses (Kinosita et al. 1964 a, b, c, Machemer 1965). Dryl 1965 reported inhibiting effects of potassium ions on response of *Stylonychia* to mechanical and chemical stimulation, but it was not investigated either normal response reappears soon after replacement of animals to medium devoid of potassium or the recovery is a long-lasting phenomenon like in the case of *Paramecium* (Dryl 1959, 1973, Dryl and Hildebrand 1975).

Material and Methods

Stylonychia mytilus strain used in this study was isolated from a pool at Królikarnia-Warszawa and was cultivated on *Tetrahymena pyriformis* culture in Pringsheim's solution. One hour before starting experimental series, *Stylonychia* mass culture was transferred to 1 mM $CaCl_2$ + 1 mM Tris/HCl solution of pH 7.3. The

transferring procedure was repeated 2–3 times in order to wash the animals from possible contamination with Pringsheim's medium.

One sample of dense portion of ciliates ("experimental sample") was exposed for 30 min to potassium rich medium (16 mM KCl in Ca/Tris/HCl solution) whereas control sample was kept at the same time in potassium poor medium (0.32 mM KCl in Ca/Tris/HCl solution).

After 30 min of exposure to potassium rich medium experimental sample was diluted immediately 50 times with Ca/Tris/HCl solution in order to reach the same low level of potassium ions in external medium. (The preliminary experiments revealed that 0.32 mM KCl does not affect in significant way motor response of *Stylonychia*).

Empting of pipette from medium containing *Stylonychia* and spreading it on the slide played role of mechanical stimulus evoking avoiding reactions (AR-s). Mean number of reactions were recorded in 50 animals during 30 s.

The movement of ciliates was recorded by photomacrographic technique (D r y l 1958), using 30 s long light exposures. Recordings were carried out 1, 2, 4, 8, 16 and 32 min since immersion of potassium-adapted animals in solution with 0.32 mM KCl. The same kind of photo-recording was performed on control animals, not exposed to higher concentrations of potassium ions. Care was taken that photo-macrographs were performed always 30 s after empting the pipette — in order to avoid any differences in treatment of ciliates before recording.

Response of *Stylonychia* to solution with low concentration of barium ions was checked in similar way, except that potassium-adapted and control (non-adapted) ciliates were exposed to solution, 0.25 mM $BaCl_2$ + 0.32 mM KCl + 1 mM Tris–HCl, pH 7.3. Typical response to barium in form of circling backward movement of *Stylonychia* was recorded also by photomacrographic technique, applying 5 s long exposure. Mean numbers (+ S.D.) of reacting animals were calculated in 20 specimens in 15 experimental series.

Results and Discussion

The preliminary observations on behaviour of *Stylonychia* brought evidence that the animals transferred from the pipette on the surface of cover glass show at the beginning (during first 5–10 s) very fast swimming rate with short-lasting AR-s induced by contact of the ciliate with the solid substrate. This relatively strong and vivid normal (so-called "spontaneous") reaction towards mechanical stimulus decreases with time and becomes very weak after 10–15 min, provided that no additional stimulation would be applied. However, it was established that this response was more or less constant, if the movement of animals was recorded after the same time (30 s) since empting the pipette (Pl. I 1).

In medium 0.32 mM KCl + 1 mM $CaCl_2$ + 1 mM Tris-HCl (pH 7.3) the control animals perform approximately 8–8.5 AR-s per 30 s as it is indicated in Fig. 1.

It was interesting to state that experimental animals exposed pre-

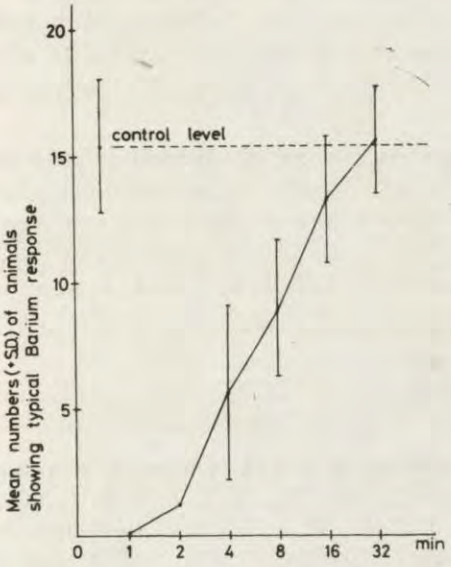

Fig. 1

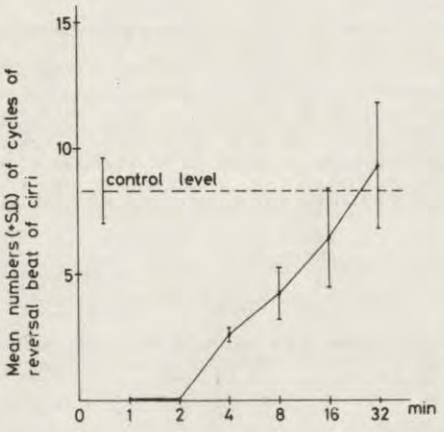

Fig. 2

Fig. 1. Recovery of motor response in *Stylonychia mytilus* towards 0.25 mM $BaCl_2$ stimulation during washing in potassium poor medium after previous 30 min lasting incubation in solution 16 mM KCl + 1 mM $CaCl_2$ + 1 mM Tris/HCl (pH 7.3). Explanation: Abscissa: Time of washing in potassium poor medium (in min). Ordinate: Mean numbers (+ S.D.) of animals showing typical barium response. The data calculated from recordings carried out in 20 animals in 15 experimental series

Fig. 2. Recovery of motor response in *Stylonychia mytilus* towards mechanical stimulation during washing in potassium poor medium after previous 30 min lasting incubation in solution 16 mM KCl + 1 mM $CaCl_2$ + 1 mM Tris/HCl (pH 7.3). Explanation: Abscissa: Time of washing in potassium poor medium (in min). Ordinate: Mean numbers (+ S.D.) of cycles of reversed beat of cirri (AR-s) per animal during 30 s of recording. The data calculated from recordings carried out in 50 animals

viously for 30 min to solution 16 mM KCl showed very slow progressive movement and no avoiding reactions at all during first two minutes of washing in medium with low potassium ions content.

First typical (AR-s) appeared after 4 min while complete recovery of motor response was observed after 32 min since transfer to medium containing low concentration of potassium ions.

Similar series of experiments was performed with potassium-adapted *Stylonychia* exposed afterwards to low concentrations of barium ions. Control animals reacted with short-lasting (2–10 s) ciliary reversal (CR) when exposed to 0.25 mM $BaCl_2$ (diluted with 0.32 mM KCl + 1 mM $CaCl_2$ + 1 mM Tris/HCl, pH 7.3). This kind of motor response was recorded in form of circles, as shown on (Pl. I 3). It was found that all control animals react with CR towards 0.25 mM $BaCl_2$, whereas no reaction was noticed during first two minutes in 16 mM KCl adapted animals, which

were transferred to medium with low concentration of potassium ions (Pl. I 4). The complete recovery of normal response was noticed 16 min after transfer, i.e., earlier than in the case of mechanical stimulus (Fig. 2).

It is suggested that appropriate concentrations of potassium ions (in the presence of calcium) may inhibit CR response towards mechanical and chemical stimuli in very similar way. However, it is still not clear whether both receptor systems are independent or are parts of the same receptor system.

RÉSUMÉ

Stylonychia mytilus qui était exposée pendant 30 min. à la solution contenant 16 mM KCl + 1 mM $CaCl_2$ + 1 mM Tris/HCl (pH 7.3), est capable de restituer graduellement, pendant 16–32 min d'immersion dans le milieu sans potassium, ses réactions motrices aux stimulants mechaniques et chimiques. On suppose que les ions de potassium et de calcium dans certaines concentrations inhibent la fonction de tous les deux systèmes des recepteurs, localisés dans la membrane cellulaire des Ciliés.

REFERENCES

Dryl S. 1958: Photographic registration of movement of *Protozoa*. Bull. Acad. Pol. Sci. Cl. II, 6, 429–430.

Dryl S. 1959: Effects of adaptation to environment on chemotaxis of *Paramecium caudatum*. Acta Biol. Exp., 19, 83–93.

Dryl S. 1965: Motor response of *Stylonychia mytilus* to chemical, electric and mechanical stimuli. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. int. Congr. ser. No. 91, 246.

Dryl S. 1973: Changes of excitability in ciliates during adaptation to external stimuli. Progress in Protozoology, Abstr. Fourth int. Congr. Protozool., Clermont-Ferrand, 1973 (ed. P. de Puytorac and Grain J.), 123.

Dryl S. and Hildebrand E. 1975: Long Lasting Potassium Effect on Excitability of *Paramecium* (*Protozoa, Ciliata*). Fifth int. Biophys. Congr., Copenhagen, Abstr.

Dryl S. and Totwen-Nowakowska I. 1975: Motor response of double animals of *Stylonychia mytilus*. 1. Response to potassium ions in external medium. Acta Protozool., 14, 75–81.

Kinosita H., Dryl S. and Naitoh Y. 1964a: Changes in the membrane potential and the response to stimuli in *Paramecium*. J. Fac. Sci. Tokyo Univ., 10, 291–301.

Kinosita H., Dryl S. and Naitoh Y. 1964b: Relation between the magnitude of membrane potential and ciliary activity in *Paramecium*. J. Fac. Sci. Tokyo Univ., 10, 303–309.

Kinosita H., Dryl S. and Naitoh Y. 1964c: Spontaneous changes in membrane potential of *Paramecium caudatum* induced by barium and calcium ions. Bull. Acad. Pol. Sci. Cl. II, 459–461.

Machemer H. 1965: Analyse kurzzeitlicher Bewegungserscheinungen des Ciliaten *Stylonychia mytilus* Ehrb. Arch. Protistenk., 108, 153–190.

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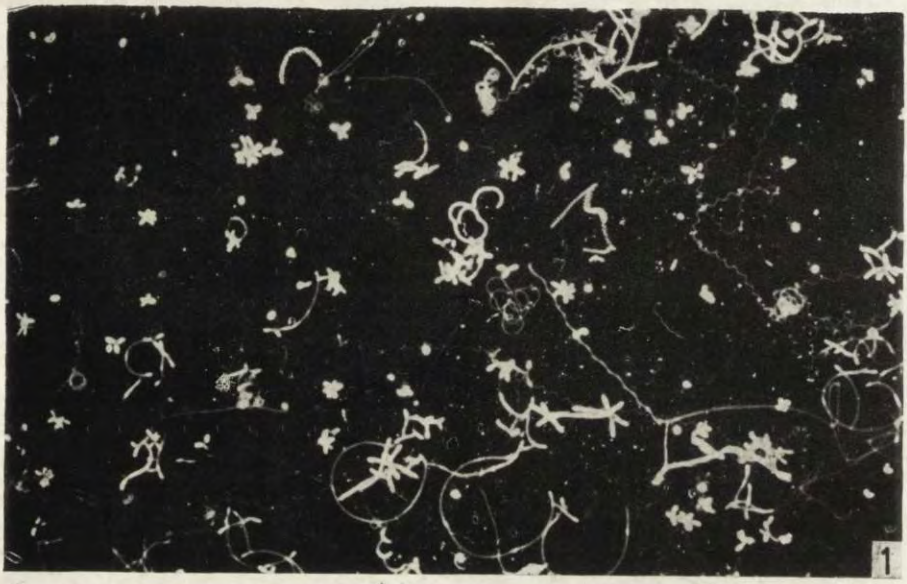
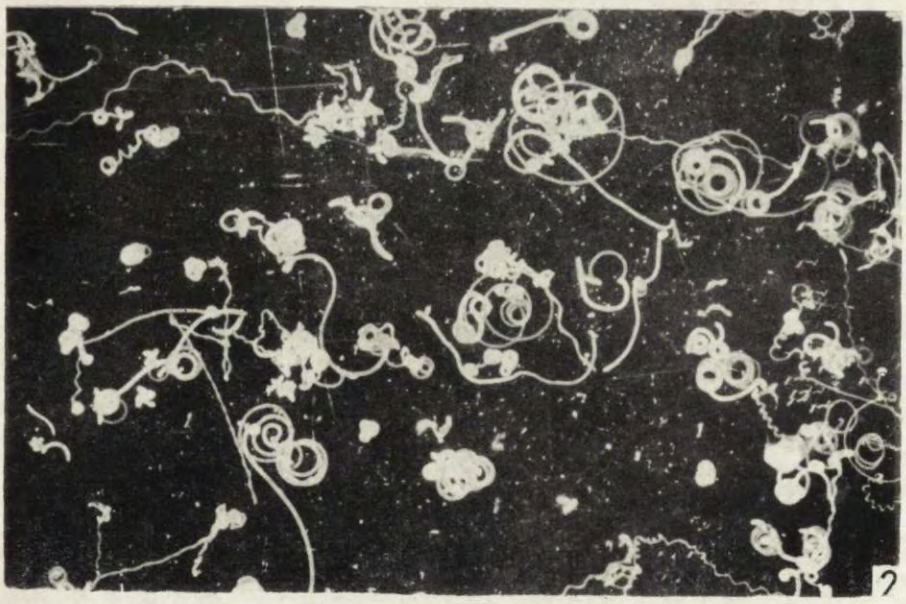
EXPLANATION OF PLATE I

Photomicrographic recording of movement of *Stylonychia mytilus*. Time of exposition — 30 s.

1 — Response of *Stylonychia mytilus towards mechanical stimuli.* Short-lasting reversed beat of cirri in form of zig-zag lines 2 — Response of *Stylonychia mytilus* towards chemical stimulus (0.25 mM $BaCl_2$ in Ca-Tris/HCl buffer solution). Characteristic for barium motor response in form of multiple circles.





S. Dryl et I. Totwen-Nowakowska auctores phot.

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On the Mechanism of Orientation of *Paramecium caudatum* in the Gravity Field. I. Influence of Ciliary Reversal and of External Ca Deficiency on the Geotactic Behaviour

Synopsis. Experimental evidence is provided that physiological models are basically inapplicable to explain geotaxis, because the normal tendency to turn the anterior body end upward is preserved under conditions excluding the physiological mechanisms of orientation: after Ca^{2+} chelation by EDTA and during the continuous ciliary reversal induced by Rb^{+}. It is demonstrated on the other hand, that the present hydrostatic or hydrodynamic models are imperfect because the rate of orientation of actively swimming paramecia is higher than that of the passively sinking ones.

The negative geotaxis of ciliates is known from about 90 years but it did not find yet any satisfactory and generally accepted explanation. In the last decades most authors gave preference to the so called mechanical or hydrodynamic models of this phenomenon. The alternative physiological explanations were rejected, however, only on the basis of pointing out some weakness of the specific physiological models which were earlier proposed, but never by an experimental demonstration that such models are *a priori* inapplicable. Their basic inapplicability could be established by providing evidence that the gravity is not a stimulus in the physiological meaning of this term, i. e., that it does not induce excitation followed by specific motory response.

This point may be decided by testing the ability of paramecia to orient themselves in the gravity field in such conditions under which normal motory responses are made impossible or become strongly modified what leads to disappearence or distorsion of all the types of true taxes, all of them being based exclusively on the ciliary reversal mechanism. It is known that paramecia are insensitive to stimuli in medium strongly decalcified by EDTA (Grębecki 1965), and on the

other hand, it is obvious that they cannot react to stimuli by ciliary reversal when being already maximally excited and swimming continuously backwards, as in the solutions rich in monovalent cations.

Methods

Paramecium caudatum grown in milk fed cultures was used for experiments. The tendency to migrate upwards was every day, prior to experiments, controlled in Pringsheim medium, because sometimes the geotaxis may be in great extent hampered by not yet defined physiological or environmental factors.

The test chambers, made of glass plates, were 100 mm high, 100 mm wide, and 1.7 mm deep. Paramecia were introduced inside them with a syringe, and at the starting moment of experiment they were randomly distributed over the chamber. Their movement was recorded by the macrophotographic method of Dryl (1958). The pictures obtained show the paths followed, during 6 s of exposure time, by all individuals swimming in the field taken by the photo-camera.

The procedures used to introduce paramecia into different test solutions are described further in the text, with the presentation of respective experiments. All the expriments were run in room temperature.

Results

Behaviour in the Pringsheim Medium (Control Solution)

The Pringsheim medium served as the balanced salt solution for control experiments. The geotactic behaviour of paramecia swimming freely in Pringsheim medium, about 1 min after being introduced into the test chamber, is shown in the Pl. I 1. The geotactic aggregation is well seen close under the upper surface, in the lower layers many individuals are swimming straight upwards, many others are just at the moment of assuming the geotactic body orientation and they describe arch-like paths bending upward. The control experiment demonstrates that the adopted method is adequate for observing and recording geotaxis and may serve for analysing the behaviour of paramecia in other test solutions.

Geotaxis of Paramecia Pretreated with EDTA

In the first series of experiments EDTA solutions were used to obtain paramecia swimming forward but not apt to react to external stimuli. It was demonstrated by Grębecki (1965) and confirmed by Dryl and by Hildebrand (personal communications) that, with an appropriate balance between the external concentrations of Ca^{2+} and

of chelator, paramecia do not react even by an avoiding response to stimuli which normally induce a continuous ciliary reversal.

In this study paramecia (5 days after feeding) were geotactically washed in the 0.01 mM/l $CaCl_2$ solution, until the initial medium became diluted one hundred times, and then left for 24 h. The next day they were immersed in the 0.5 mM/l solution of EDTA disodic salt (still in the presence of 0.01 mM/l $CaCl_2$, and neutralized with NaOH to pH 7). Under such conditions paramecia survive and swim normally forwards at least for 5 min, what is sufficient to test their geotactic behaviour. Their insensitivity to external stimuli was checked with RbCl which was unable to provoke any motory response in the final concentration as high as 20 mM/l (normal individuals react to such concentration of Rb ions by continuous ciliary reversal lasting more than 1 min).

Paramecia pretreated with EDTA, when introduced into the vertical chamber, manifest very clear geotaxis as shown in the Pl. I 2. The geotactic aggregation forms soon under the top surface, and the upward orientation of freely swimming individuals appears even more uniform than in the control experiments in Pringsheim medium.

Geotaxis During the Rb-induced Ciliary Reversal

It has been found by K u ź n i c k i (personal communication) that Rb^+ induces a continuous ciliary reversal lasting relatively longer than that produced by other monovalent cations. In our experiments RbCl diluted in Pringsheim medium in the concentration of 30 mM/l, provoked the continuous ciliary reversal lasting about 120 s and followed by other usual motory stages: by the partial ciliary reversal and then by the gradual re-normalization of movement. The Rb-pretreated paramecia survive several hours.

For experiments paramecia were first washed in Pringsheim solution, and then mixed in equal volume ratio with 60 mM/l RbCl solution (diluted also in Pringsheim medium). Immediately after that they were introduced into the vertical chamber and photographically recorded at different stages of behaviour of their ciliary apparatus.

First picture (Pl. II 4) has been taken during the stage of well manifested quick backward swimming (continuous ciliary reversal). Almost all the individuals swim straight downward, and some others are still at the phase of establishing their vertical orientation and describe arch-like paths bending also downward. Therefore, the animals tend, as usually, to orient their body with the anterior end directed upward, but the inversed swimming direction results in the downward migration. During the second minute of experiment (Pl. II 5) there are only

a few individuals left in the upper layers. At the same period the velocity of backward movement clearly decreases but all the animals strongly keep their vertical orientation. The last record (Pl. II 6) shows the same population at the moment when the majority of individuals recovered the forward swimming direction, and some others were still at the stage of partial ciliary reversal. During the partial ciliary reversal paramecia describe circles or larger loops without any clear movement up or down. After reassuming the forward swimming direction they migrate again upwards and aggregate near the surface.

Behaviour During the Ba-induced Periodic Ciliary Reversal

Dryl (1961) found that paramecia immersed in a balanced mixture of Ba and Ca ions (1 mM/l $BaCl_2$ + 1 mM/l $CaCl_2$), after a brief phase of continuous ciliary reversal, manifest the periodical ciliary reversal and during a practically illimited time they swim alternately forwards and backwards. In this study, paramecia were first transferred by geotactic washing into 1 mM/l $CaCl_2$ solution, then mixed in equal volume ratio with the solution containing 1 mM/l $CaCl_2$ + 2 mM/l $BaCl_2$, and put in the vertical chamber.

As shown by the Pl. I 3, paramecia gradually aggregate in the lower layers of the chamber. At the time of recording some individuals were still swimming backward, but the majority of them already manifested the periodical ciliary reversal. It is known that such individuals, when recorded in a horizontal liquid layer, describe paths in form of asterisks. In the vertical chamber however, the paths in most cases follow the downward direction, indicating that also during the periodical ciliary reversal paramecia keep on the average the vertical position with the anterior body end oscillating about the upward direction. With such average body orientation, the resultant downward direction of migration demonstrates that the distance covered due to the reversal periods and to the sedimentation is longer than this due to the periods of forward movement.

The Rate of Orientation

The photographic technique of time-exposure recording of geotaxis offers the possibility to calculate the rate of orientation of paramecia actively swimming forward or backward, instead of using the passively sinking immobilized specimens (Kuźnicki 1968, Roberts 1970) or artificial models (Dembowski 1929, Roberts 1970).

The arch-like bending paths of specimens just performing a turn

upward (when swimming forwards), or a turn downward (when swimming backwards), as those seen in the Pl. I 1, 2 and Pl. II 4, were redrawn, the tangents to the curve described by the animal were found at the initial and the final positions, and the angle between them was measured. Only the curves which started from a nearly horizontal position ($\pm 20°$) were selected for analysis. The measures were taken for 15 individuals in each solution. It was found that paramecia kept in the Pringsheim medium change their orientation, during 6 s, by $55° \pm 12°$, in the EDTA solution by $34° \pm 12°$, and in the RbCl solution by $43° \pm 12°$.

Discussion

It is a crucial point to discern whether geotaxis is a real physiological motory response, as other types of taxes are, or just a forcible product of interference between the external forces and the forces generated by the swimming animal. It is generally recognized that all active motory responses of paramecium to external stimuli are based on the inversion of ciliary stroke, either appearing locally either generelized all over the ciliary cloth. Therefore, all kinds of taxes sompletely disappear in paramecia in which the inversion of ciliary beating is made impossible by the external Ca defficiency (Grębecki 1965), or their mechanism becomes strongly modified in ciliates which are already manifesting the continuous ciliary reversal (c.f. for example the galvanotactic behaviour of paramecia during the K-induced ciliary reversal, as described by Grębecki 1963).

The results obtained in this study prove that this is not true in the case of geotaxis. Paramecia pretreated with EDTA which failed to respond to chemical stimulation still manifested negative geotaxis, even better pronounced than in the control sample kept in the Pringsheim medium. Ciliates undergoing the continuous ciliary reversal provoked by RbCl also move unidirectionally in the gravity field, but they swim downwards.

The general conclusion should be drawn that as well the untreated paramecia (in Pringsheim medium), as paramecia with suppressed reactivity (in EDTA solutions) and those in the maximal excitation state (in RbCl solutions), tend to keep the same orientation of their body in respect to the vertical direction: the anterior body end is always oriented upward. These three situations are also not very much different as far as the angular speed of orientation is concerned. This means that the basic mechanism of geotaxis remains unaffected, and that the upward or downward migration and aggregation of ciliates is an after-effect

linked only with their forward or backward swimming direction. It may be concluded therefore, that the geotactic orientation is not based on the ciliary steering mechanism, and so, a posibility of explaining it by any physiological model becomes eventually excluded on experimental basis.

Moreover, the ability of normal paramecia to react to external stimuli even hampers their geotaxis. This is proved by the fact that the animals treated by EDTA (Pl. I 2) or by RbCl (Pl. II 4) orient themselves in the gravity field in more uniform way than the control ones (Pl. I 1). This result is consistent with the observation of Jennings (1906) that paramecia during geotactic migration manifest very few avoiding reactions, and with the view of Roberts (1970) that the geotactic aggregation should be better expressed when random local stimuli are more scattered in time.

The fact that paramecia aggregate at the top of a vertical chamber does not result from arresting or slowing down their movement close to the upper surface, as postulated by Dembowski (1929) and Fedecka (1956). Photographic records shown on the Pl. I 1 and 2, demonstrate that as well in the Pringsheim medium, as in the EDTA solution, the swimming velocity in the upper aggregation is similar to that in the lower layers, but the ciliates immediately after rebounding from the air-water interface tend to re-assume their typical geotactic orientation and follow arch-like paths bending up again.

Bending up of paths described by paramecia swimming forwards, and bending down of paths of animals swimming backwards, perfectly well seen in all the layers of vertical chamber immediately after filling it, proves that also the initial geotactic orientation takes place during the active movement, and not at the hypothetical moments of its arresting or slowing down, as suggested by Dembowski (1929).

This last observation seems to be particularly significant because the angular rate of orientation determined in this study for swimming individuals is very much higher than the values obtained for artificial models and for Ni-immobilized paramecia by Kuźnicki (1968) and by Roberts (1970). The mean values obtained here for active ciliates vary from 6° to 9° per second, whereas they were found to amount 0.6°–2.8° per second for immobilized paramecia and 2.3° per second for artificial models. Kuźnicki (1968) was right when presuming that the values he obtained for immobilized specimens were to low to account for the geotactic orientation of active ciliates.

The results obtained in this study indicate, on one side, that the explanation of geotaxis must be based on purely hydrodynamic principles and not on physiological mechanisms of reaction, but on the other side they prove that earlier hydrostatic and more recent hydrodynamic con-

cepts derived from the observation of passive sinking [1] are imperfect. The incoherence of values obtained for passively sinking and actively swimming specimens may be possibly related, either with some difference of hydrodynamic properties of active and inactive ciliated surface, either with the swimming velocity factor neglected in previous concepts.

RÉSUMÉ

On donne les preuves expérimentales que les modèles physiologiques sont par principe inapplicables pour interprêter la géotaxie, parce que la tendance normale chez les paramécies d'orienter vers le haut le bout antérieur du corps, persiste dans les conditions qui excluent la manifestation des mécanismes physiologiques de l'orientation: pendant la chélation du Ca^{2+} extérieur par EDTA, et pendant le rebroussement ciliaire continu provoqué par Rb^{+}. Il a été démontré que de l'autre côté, les modèles hydrostatiques et hydrodynamiques proposés jusqu'à présent ne sont pas satisfaisants, étant donné que la vitesse de l'orientation des paramécies actives est plus grande que dans le cas de leur sédimentation passive.

REFERENCES

Dembowski J. 1929: Vertikalbewegungen von *Paramecium caudatum*. Einfluss einiger Aussenbedingungen. Acta Biol. Exp., 3, 195-240.

Dryl S. 1958: Photographic registration of movement of *Protozoa*. Bull. Acad. Pol. Sci., S. Sci. Biol., 6, 429–431.

Dryl S. 1961: The ciliary reversal in *Paramecium caudatum* induced by simultaneous action of barium and calcium ions. J. Protozool., 8 (Suppl.), Abstr. 55.

Fedecka B. 1956: Badania ilościowe nad geotropizmem *Paramecium caudatum*. Folia Biol., 4, 65–76.

Grębecki A. 1963: Rebroussement ciliaire et galvanotaxie chez *Paramecium caudatum*. Acta Protozool., 1, 99–112.

Grębecki A. 1965: Role of Ca^{2+} ions in the excitability of protozoan cell. Decalcification, recalcification and the ciliary reversal in *Paramecium caudatum*. Acta Protozool., 3, 275–289.

Jahn T. L. and Votta J. J. 1972: Locomotion of protozoa. Ann. Rev. Fluid Mech., 4, 93–116.

Jennings H. S. 1906: Behavior of the Lower Organisms. Columbia University Press, New York.

Kuźnicki L. 1968: Behavior of *Paramecium* in gravity fields. I. Sinking of immobilized specimens. Acta Protozool., 6, 110–117.

Roberts A. M. 1970: Geotaxis in motile micro-organisms. J. Exp. Biol., 53, 687–699.

Winet H. and Jahn T. L. 1974: Geotaxis in Protozoa. A propulsion-gravity model for *Tetrahymena* (Ciliata). J. Theoret. Biol., 46, 449–465.

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[1] This statement does not apply directly to the hypothesis put forward by Jahn and Votta (1972) and developed further by Winet and Jahn (1974). The views expressed by these authors will be discussed in the second part of this study.

EXPLANATION OF PLATES I–II

Time-exposure photograms (magn. 3×) of paths described by paramecia in vertical glass chamber during 6 s

1: Geotaxis in Pringsheim medium, about 1 min after introduction of ciliates into the test chamber (in this picture only the swimming direction has been marked by opening the diafragm at the end of exposure)
2: Geotaxis in the EDTA solution, about 1 min after the beginning of experiment
3: Behaviour of paramecia manifesting the Ba-induced periodical ciliary reversal, at the end of the first minute of experiment
4: Downward migration of paramecia manifesting an intense ciliary reversal at the initial stage of response to Rb ions
5: Downward migration during the second minute of exposure to Rb^+ action, when the velocity of backward swimming is decreasing
6: Upward migration as observed in the same sample a few minutes later, when most individuals have recovered forward movement (some specimens still undergoing the partial ciliary reversal)

A. Grębecki et G. Nowakowska auctores phot.

A. Grębecki et G. Nowakowska auctores phot.

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On the Mechanism of Orientation of *Paramecium caudatum* in the Gravity Field. II. Contributions to a Hydrodynamic Model of Geotaxis

Synopsis. Orientation of paramecium in the gravity field was studied by cinematographic analysis of movement in a vertical glass chamber, under conditions of normal and of reversed ciliary work. Behaviour of paramecia during partial ciliary reversal, when the translational component of movement is negligible, is inconsistent with the postulations of the "mechanical" and of the "propulsion-gravity" models. The instantaneous rate of angular orientation was found to be substantially higher during active swimming than during passive sinking. During non-vertical swimming the gyration axis is deflected upward from the resultant direction of locomotion. It is suggested that due to such position the medium reaction has two components: the drag and the lift, and that the lift might be the main factor contributing to the torque which turns the ciliate towards vertical.

In the precedent paper (Grębecki and Nowakowska 1977) the evidence was given that physiological models are basically inapplicable to explain the geotactic orientation of paramecium. The purpose of this paper is to apply movement analysis for testing the validity of models which consider geotaxis as an effect of interaction of external forces and paramecium body. First type of such models is derived from the classical "mechanical" concept of Verworn (1889) and is based on hydrostatic principles only. Second model to be dealt with is that proposed by Jahn and his co-workers (Jahn and Votta 1972, Jahn and Winet 1973, Winet and Jahn 1974). It will be discussed in its probably most definite form, called the propulsion-gravity model, as it had been presented by Winet and Jahn (1974). Finally, the validity and implications of the hydrodynamic model of Roberts (1970) will be considered.

Methods

General methods to grow paramecia, to prepare them for experiments, to immerge them in test solutions, and to obtain geotaxis in vertical glass chambers, were exactly the same as in the first part of this study (Grębecki and Nowakowska 1977). Some photomacrographic records of ciliates movement were taken again. However, the major part of data collected for the present discussion were provided by the cinematographic method. A low power bright field microscope (objective lens 1.25 ×, eye-piece 5 ×) has been horizontally mounted, and the test chamber put on its stage in vertical position. Bolex H16 Reflex movie camera, driven by electronically stabilized Bolex ESM motor, has been used. Swimming ciliates were filmed at the frequency of 50 frames per second. The films were reviewed and analysed frame-by-frame with the LW optical data analyser.

Data Description

Except the Plate I which shows the trajectories obtained by the photomacrographic technique, other plates show the behaviour of paramecia as it is revealed by graphical analysis of cinematographic records. The graphs were obtained by projecting the film frame-by-frame on tracing paper and redrawing the successive positions of the longitudinal body axis. As a result, the trajectories appear in form of image composed of bundles of lines separated from each other by a distance corresponding to the time interval between the analysed frames. In the case of forward swimming in Pringsheim medium each second frame was redrawn, what means that in the corresponding graphs the successive positions of body axis are marked at the interval of 0.04 s. Graphs showing different stages of behaviour of paramecia exposed to RbCl solution are based on full sequence of frames, and the successive positions of body axis seen on them represent the intervals of 0.02 s.

The analysis of movement in Pringsheim medium puts in evidence a clear difference of character of paths produced by individuals migrating vertically upward (Pl. II) and those described by individuals which at the same time swam still obliquely in the chamber (Pl. III). The helicoidal trajectories of the vertical swimming appear perfectly symmetrical, whereas those followed in different non-vertical directions geometrically differ in their upswing and downswing phases. In the upswing phase the lines representing the successive positions of body axis are tightly superposed one upon another, and in the downswing phase on the contrary, they are clearly spaced and even fail to intersect.

The asymmetrical character of non-vertical trajectories becomes easy to be understood when one separates two basic components of movement,

as shown in the diagram (Fig. 1). When keeping in mind the elements given by the diagram, one can read out directly from the plates the effect produced by the sedimentation factor, which interferes with the forward locomotory component. It is also sufficient to compare visually the positions of body axis in the upswing and the downswing phases with the general direction of the trajectory, to discover that the average position of body axis (and of the axis of gyration) is always deflected upwards from the resultant direction of locomotion.

In the case of ciliates which recovered their normal forward swimming after a period of ciliary reversal phenomena, induced by 30 mM/l RbCl solution (Pl. V), the picture is essentially similar to that found in the Pringsheim medium, but the asymmetricity of non-vertical swimming appears even better expressed because the translational component of movement is reduced and the gyrational one more pronounced.

Also during the phase of continuous ciliary reversal the trajectories become symmetrical in animals oriented vertically (Pl. IV 17), but in the specimens swimming obliquely or horizontally (Pl. IV 18–19) they are asymmetrical. This deformation of non-vertical trajectories in the backward swimming is functionally similar to that established for the forward swimming, although it may appear different from the morphological point of view. In both types of movement the body axis is deflected upwards, in respect to the resultant direction of locomotion, by its leading

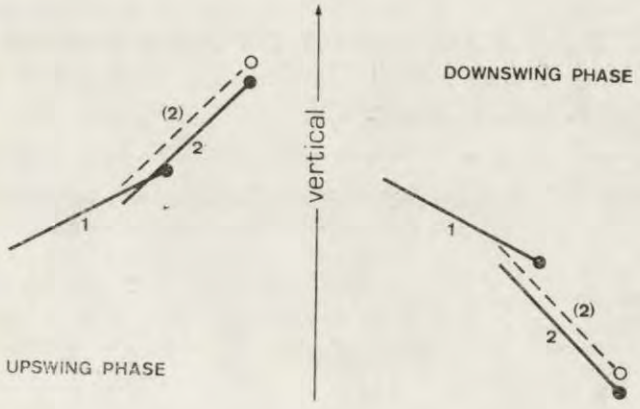

Fig. 1. Origin of the asymmetricity of trajectories in a case of horizontal forward swimming. The upswing and the downswing phases of the same trajectory would be perfectly symmetrical, if they were composed of stages 1 + (2), the stage (2) representing an imaginary shift due to the ciliary propulsion only. In fact, they are asymmetrical because they are composed of stages 1 + 2, the stage 2 expressing the real shift due to the ciliary propulsion plus sedimentation. The longitudinal body axes are marked with circles at their anterior ends

extremity which is morphologically anterior in one case and morphologically posterior in another.

The ciliates exposed to RbCl, after the phase of continuous ciliary reversal and before the effective re-normalization of movement, pass through the stage of partial ciliary reversal. The successive positions of their body axis (Pl. IV 20–22) indicate that only the gyrational component of movement is preserved at this stage, and it is manifested in form of rotation of longitudinal body axis, effected approximately in one plane. Besides the gyrational component, the effects of sedimentation factor are seen, and in some cases the translational movement is just starting again, what results in a tendency to describe loops. The behaviour of individuals which manifested only the gyrational and the sedimentation components, and which longitudinal axis effected rotations in the plane of observation, was subjected to more detailed analysis.

Evidence From Movement without Translational Component

The Angular Velocity of Gyration

Paramecia in the phase of partial ciliary reversal may serve as a good model for checking the validity of some concepts describing the mechanism of geotaxis. Their translational velocity is almost negligible, and therefore they should manifest the behaviour deduced from the theories neglecting the role of swimming velocity in geotactic orientation, if the theories in question are correct. To test this one has to measure some parameters of the gyrational component of movement, in specimens with longitudinal body axis rotating in the plane of observation, i.e., parallel to the walls of vertical chamber.

Table 1

Analysis of Movement of paramecia Manifesting Gyration Without the Translational Motion (Partial Ciliary Reversal) after being Exposed to Rb Ions*

Quarter	45–135° downswing	135–225° horizontal	225–315° upswing	315–45° horizontal
Gyration time (in s)	0.21 ±0.04	0.21 ±0.04	0.21 ±0.04	0.21 ±0.04
Relative distance of gyration axis from the anterior body end	63 ±4	64 ±3	65 ±3	65 ±4

* Data calculated from 25 specimens. The standard deviation values depend mostly on differences between individuals but very little on any irregularities of the individual behaviour.

The velocity of gyration was measured by frame-by-frame analysis, the successive positions of the ciliate being marked at 0.02 s intervals. As it is indicated in the Table 1, the full circle described by gyrating paramecium was divided in four quarters, each of 90°. The position in which the anterior body end is directed vertically up was considered to be 0°. The sequence of quarters was arranged in one or in another direction depending on the apparently clockwise or anticlockwise gyration of the individual recorded. With such a procedure, the quarter assigned as 45–135° always corresponds to the downswing phase of motion of anterior body end, the quarter marked as 225–315° to its upswing motion phase, and the quarters of 315–45° and 135–225° may be both considered as approximately horizontal.

The data given in the Table 1 prove that, between all the quarters, there is no difference of time needed to change the position by 90°. This result makes evident that the torque generating the gyrational movement does not depend on the changing angle between the longitudinal body axis and the gravitation force direction.

In the Fig. 2 the forces acting on the paramecium body are schematically shown as follows: gravitation (G), buoyancy (B), ciliary motory force provoking gyration (M_g). The longitudinal body axis is represented by the segment ap, and O indicates the axis of its gyration. Generally speaking, the points of action of all these forces might be separate and lie out of the gyration axis. Such a hypothetical situation is drawn in the part A of the scheme. In such a case the resultant torque producing gyration would change during the movement, and it should result in a corresponding change of angular velocity, particularly well expressed when the downswing quarter (45–135°) and the upswing quarter (225–315°) are compared.

Since such changes were not detected in the experiment, one should look for another, more specific, distribution of acting forces. The simplest imaginable distribution fulfilling the empirical requirement of constant angular velocity of gyration is presented in the Fig. 2 B. It is assumed that the buoyancy and gravitation act at the same point which coincides with the intersection of the longitudinal body axis and of its axis of gyration (point O). It would mean in other words, that there is no significant difference of density across the paramecium body, and that during the partial ciliary reversal the longitudinal body axis gyrates around the centre of mass.

Implications for the "Mechanical" Model

If the final conclusion of the precedent paragraph was correct, the film records analysed in this study permit to estimate the position of the

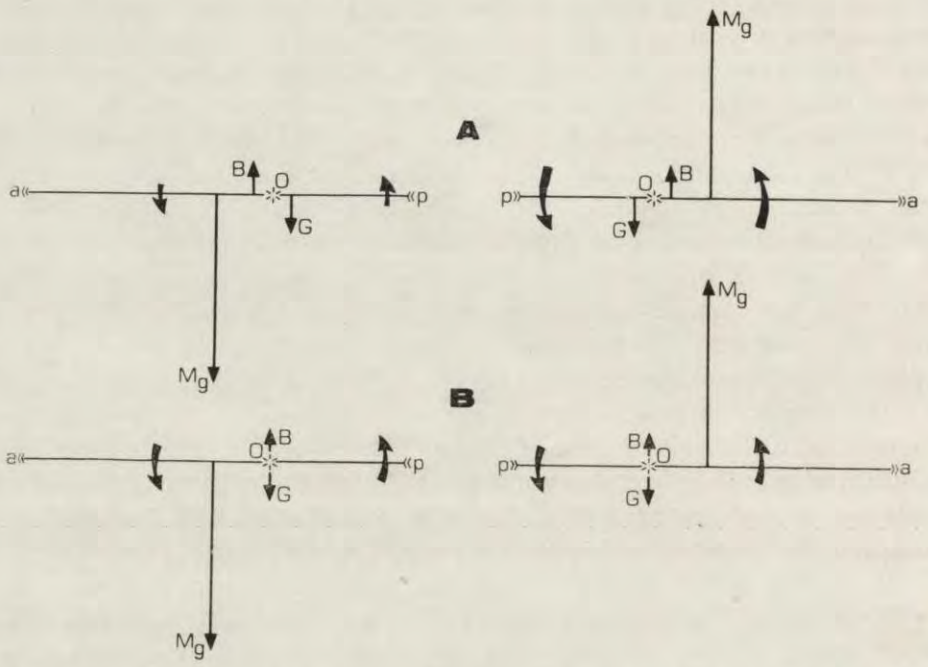

Fig. 2. Distribution of forces acting on paramecium body during the downswing phase (left) and the upswing phase (right) of partial ciliary reversal. A: hypothetical distribution with the centre of mass and the centre of buoyancy separated and lying out of the gyration axis, what leads to cycling changes of the angular velocity of gyration. B: distribution corresponding to a steady gyration velocity, characterized by that the centre of mass and centre of buoyancy coincide at the intersection of body axis and gyration axis. Symbols are explained in the text

centre of mass of paramecium, by detecting the point of intersection of the longitudinal body axis and of the axis of its gyration. In the case of ciliates manifesting partial ciliary reversal, when the translational component is absent, when the gyration is effected in one plane and the gyration axis is normal to the body axis, it is enough to find the point of intersection of two lines representing the successive positions of the longitudinal body axis recorded at any time interval.

The results of such analysis are given in the Table 1. The relative position of the intersection point defined above was determined, for each quarter separately, as its relative distance from the anterior body end (the whole body length being considered to equal 100). The necessary adjustment was made to eliminate the recorded effect of sedimentation. The data prove that, within the limits of error, the gyration axis during partial ciliary reversal keeps a constant position. In average, the intersection point of the longitudinal body axis and the gyration axis (= centre of mass) is distant from the anterior body end by 64% of body length.

The conclusions drawn are consistent with the view of Roberts (1970) that "the variable-density orientation is unlikely to be significant in paramecium". As a matter of fact, the classical hypothesis of Verworn (1889) and the theories developed by later followers of his "mechanical" model, would necessitate a separation of the centre of mass and of the centre of buoyancy, what has been demonstrated here as highly improbable.

Implications for the "Gyration-Propulsion" Model

Paramecium manifesting gyration of its longitudinal axis during partial ciliary reversal may serve as a convenient model to test also the validity of theoretical considerations developed by Winet and Jahn (1974), because the translational component is negligible, and only the gyrational one and the sedimentation factor persist. Under such conditions Winet and Jahn (1974) expect an unbalancing of the gyrational torque and the sedimentation torque "...leading to a reduced ω and a reduced ability to turn downward", and respectively an increase of the angular velocity during the upward phase. However, in our experiments the angular velocity remained strictly unchanged regardless of the angle between the longitudinal body axis and the vertical.

Moreover, Winet and Jahn (1974) suppose that during downswing "...the gyration point is shifted aft" and that "...the gyration point shifts forward... during upswing". The data resumed in the Table 1 demonstrate that, within the limits of error, there is no shifting of the gyration axis forward or backward along the body. It was also found, that the horizontal position of the gyration axis is maintained, during 15 s of recording, without any noticeable tendency to re-orient it towards the vertical.

It should be concluded in general that the experimental testing of the "gyration-propulsion" hypothesis gives negative results.

Evidence from Forward and Backward Swimming

Characteristics of Symmetrical Vertical Trajectories

The analysis of present cinematographic records shows a significant difference between some characteristics of forward and backward motion. It may be seen in the Pl. II 10–12 (quick swimming vertically forward) and Pl. V 26 (slow vertical forward swimming soon after re-normalization) on one side, and on the other side in the Pl. IV 17 (quick backward swimming down). The successive positions of the longitudinal body axis,

in respect to the path recorded upon the plane of picture, differ depending on the forward or backward type of locomotion.

During forward swimming the body axis looks parallel to the general direction of locomotion in its extremal side positions, and in the intermediate positions it is declined in two opposite ways. During continuous ciliary reversal on the contrary, the ciliate is apparently parallel to the general swimming direction when it passes through the intermediate points of the recorded path, whereas at the extremal points its axis assumes the extremal values of deviation.

To understand correctly the difference between the forward and the backward manners of swimming we should, of course, realize that the description given above does not refer to the real position of body axis in space in respect to the real helicoidal trajectory, but only to their flattened images as they are projected upon the plane of recording. Therefore, the actual three-dimensional geometry of forward and backward swimming has to be reconstructed from pictures which reflect but deform the reality.

An essay to do that is shown in the Figs. 3 and 4. The Fig. 3 brings a schematic presentation of the pictures recorded, and it stresses the main differences between the forward and the backward manners of swimming as they appear after being projected upon the plane of observation. In the Fig. 4, four motion stages representing a full gyration cycle are drawn in the three-dimensional co-ordinates system, but the translational movement is omitted in order to make more clear the position changes which depend on gyration.

The diagrams demonstrate that the motion of the centre of mass of paramecium may be decomposed, as well for the forward as for the backward movement, into the same two components: translation with the velocity v along the vertical axis of co-ordinates, and horizontal circling about it at a distance forming the radius r. The posterior location of the centre of mass in paramecium body, as established in the precedent chapter, explains why in all records shown on the Plates, the anterior body end is always exceeding beyond the trajectory line.

The instantaneous gyration axis circulates together with the centre of mass and remains parallel to the translational vector. The gyration angle, which is defined as the angle between the instantaneous gyration axis and the instantaneous longitudinal body axis, remains the same all over the cycle. This means that the inclination change of body axis as observed in planary records, occurs in reference to observer's eye, but in the three-dimensional space paramecium keeps constant its real inclination in reference to the trajectory axis and to the motory vectors.

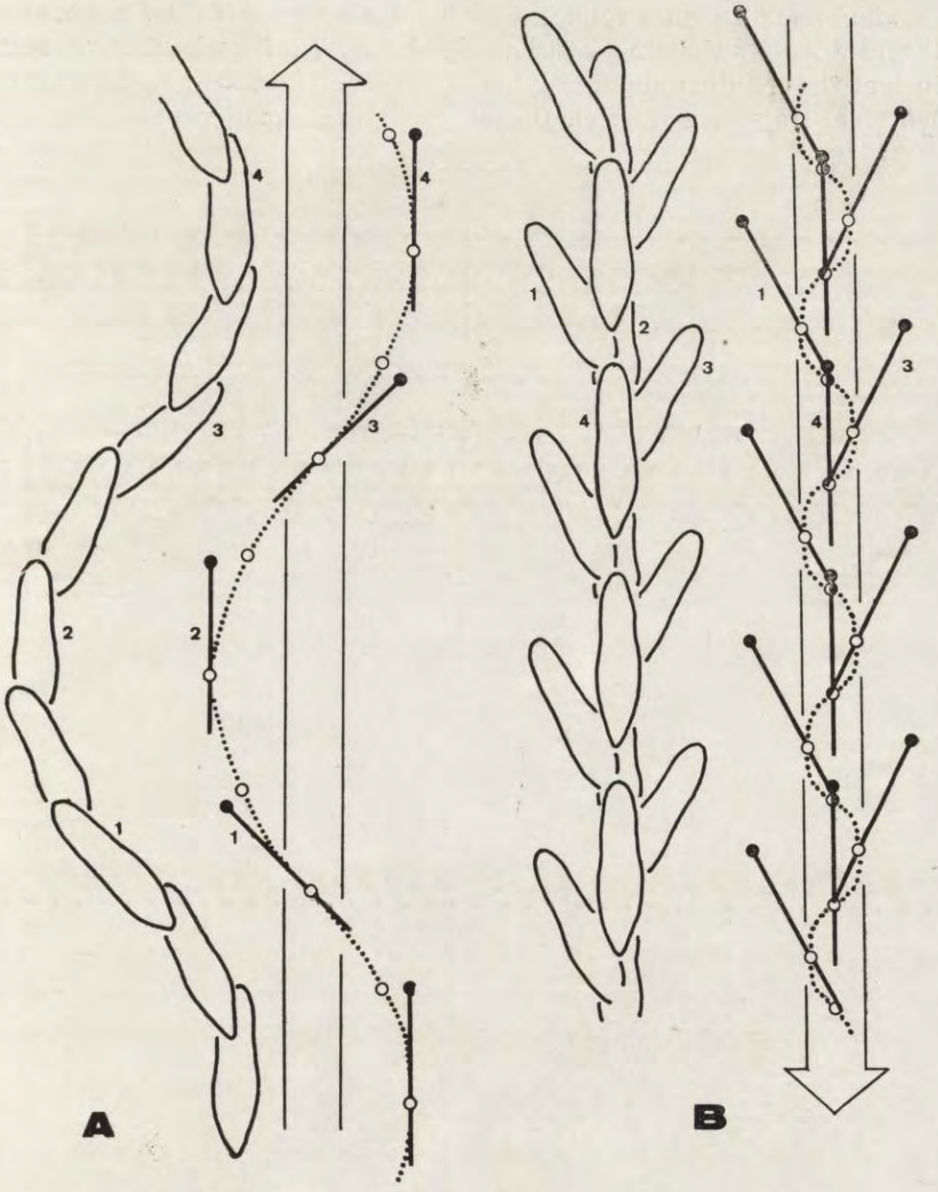

Fig. 3. Diagrammatic presentation of locomotion as it appears on the records in one plane. The successive body positions are marked as contours on the left sides of both pictures, and on their right sides they are redrawn in form of body axes and correspondingly numbered. The dotted curves represent the recorded paths and the empty circles the positions of the centre of mass. A: vertical forward swimming. B: vertical backward swimming

The basic difference reflected in the planary records (Fig. 3), between the modes of swimming respectively forwards of backwards, consists in that the gyration angle is lying in different planes in respect to the helicoidal trajectory (Fig. 4). During forward locomotion paramecium is

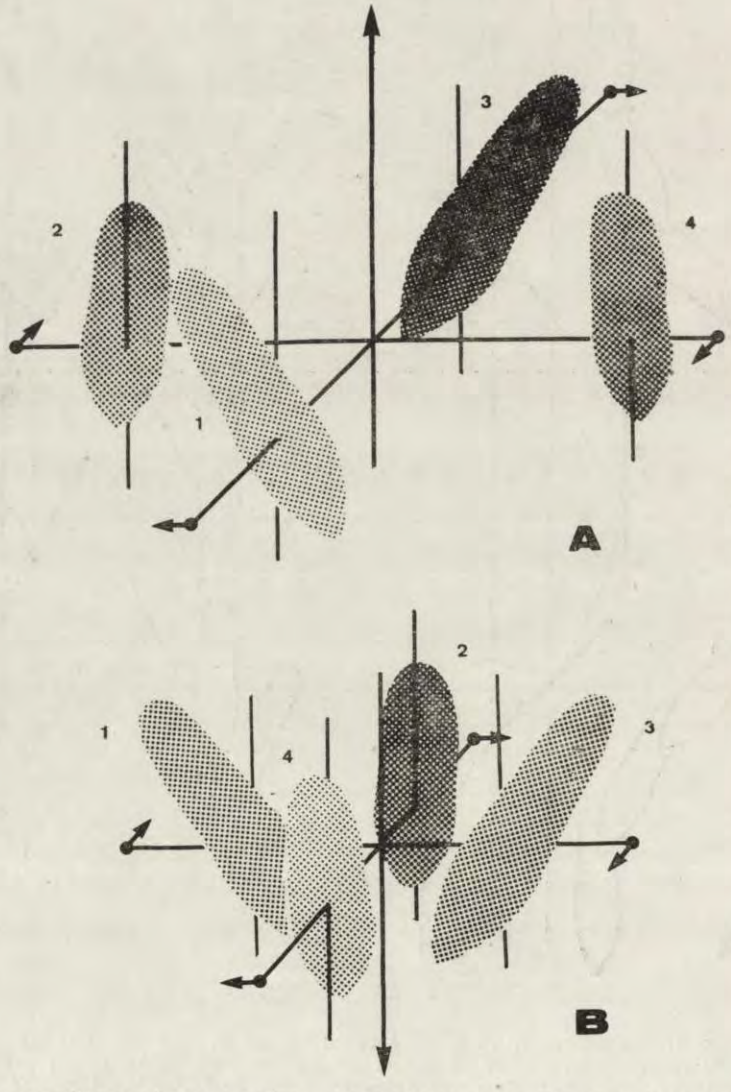

Fig. 4. Four positions of paramecium, selected from the Fig. 3 and correspondingly numbered, shown in a three-dimensional convention. Their translational shift is neglected, and its direction is indicated only by an arrow on the vertical axis of co-ordinates. The circular component of the helicoidal trajectory may be reproduced, starting from any body position, by rotating the whole horizontal system of co-ordinates in the direction indicated. The instantaneous gyration axes are shown for each body position. A: vertical forward swimming. B: vertical backward swimming

inclined in the plane tangential to the helix, but during continuous ciliary reversal its anterior end is centrifugally deflected in the plane which sections the helix and passes through its axis.

Such a difference in the body position means that the instantaneous longitudinal body axis is tangential to the real trajectory during the forward movement, but not during the backward locomotion. As a result paramecium, when swimming forwards, attacks the liquid almost exactly by its anterior end, but when swimming backwards it attacks it by side surface of its rear part.

Asymmetricity of Non-Vertical Trajectories

Two important conclusions drawn in the precedent paragraph, that the gyration axis is kept parallel to the general direction of locomotion, and that paramecium swimming forwards attacks the liquid symmetrically by its anterior end, apply only to the specific case of vertical motion. However, as it has been demonstrated in the first chapter of this study, the non-vertical trajectories present clear deformations which remain to be analysed.

The equations were found to describe trajectories which follow any direction, and are recorded as flat paths in the plane of observation:

$$x(t) = (r \cos \omega t - r) \cos \Theta + vt \sin \Theta,$$
$$y(t) = -(r \cos \omega t - r) \sin \Theta + vt \cos \Theta - ut,$$

where x — horizontal co-ordinates axis, y — vertical co-ordinates axis, t — time, v — translational velocity, u — sedimentation velocity, ω — angular velocity of gyration, Θ — angle between the vertical and the instantaneous trajectory axis.

Parameters r and v are depending on the angular velocity of gyration ω and on the value of the gyration angle β. For the forward movement probably the following correlation exists:

$$r = \frac{v}{\omega} \operatorname{tg} \beta.$$

The angle Θ depends on the Θ_0 value at the starting point and on the medium reaction $R(t)$.

Theoretical curves obtained from the equations given above may be fitted to experimentally recorded trajectories what would allow to find some lacking parameters which cannot be directly measured, for example the sedimentation velocity. Such an exercise has not yet been done by the authors in the quantitative way, but the experimental data presented in this study already permit to demonstrate that the

qualitative pattern of theoretical trajectories corresponds well to the pattern of real trajectories effectively recorded.

The Figure 5 presents theoretical trajectories of forward swimming, effected vertically, obliquely upward and horizontally, when the medium resistance as well as the geotactic changes of orientation are neglected. The translational velocity was supposed to be equal to 433 μm/s, the angular velocity of gyration to 360° per second, and the gyration angle to 30°. The plain curves correspond to the sedimentation velocity $u = 100$ μm/s, and the dotted ones to $u = 0$. It is clearly seen that introduction of the sedimentation factor results in a deformation of trajectories which become asymmetrical in all cases except the vertical swimming. As a matter of fact, such asymmetricity of paths described by paramecia was recorded in specimens swimming obliquely or horizontally, and not in the vertically oriented ones, as shown in the Pl. I 3–9.

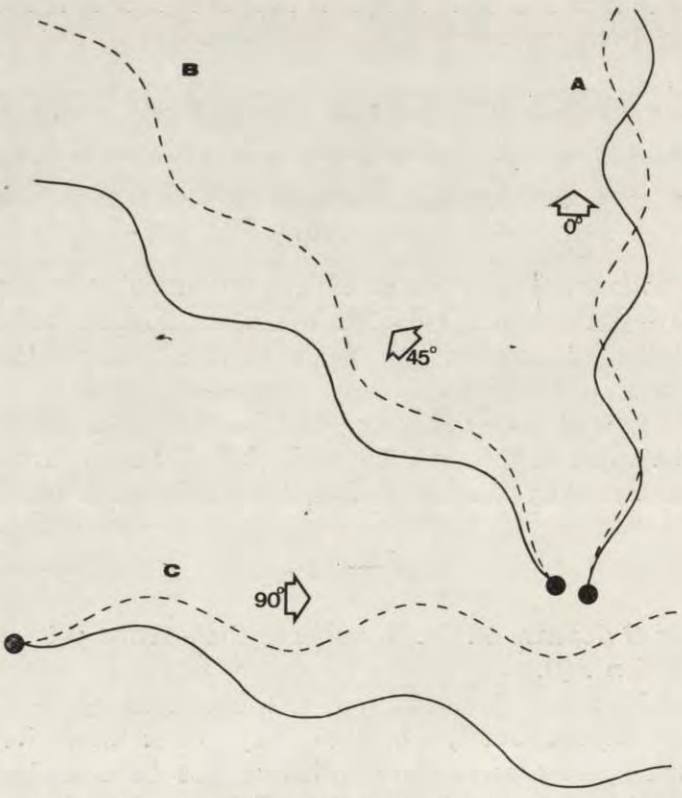

Fig. 5. Theoretical trajectories of forward swimming (at three different angles in respect to the vertical) obtained from equations and parameters given in the text. For the dotted curves the sedimentation factor has been omitted, for the plain ones the ciliary propulsion and sedimentation were both taken into account

Trajectories of individuals swimming in different oblique directions in Pringsheim medium (Pl. III 13–16), and in RbCl solution during the ciliary reversal (Pl. IV 18–19) and re-normalization (Pl. V 24–25), prove that the gyration axis is always deflected from the resultant direction of locomotion. The forces distribution responsible for this phenomenon is shown in the Fig. 6. The vector M_t represents the mean value of the

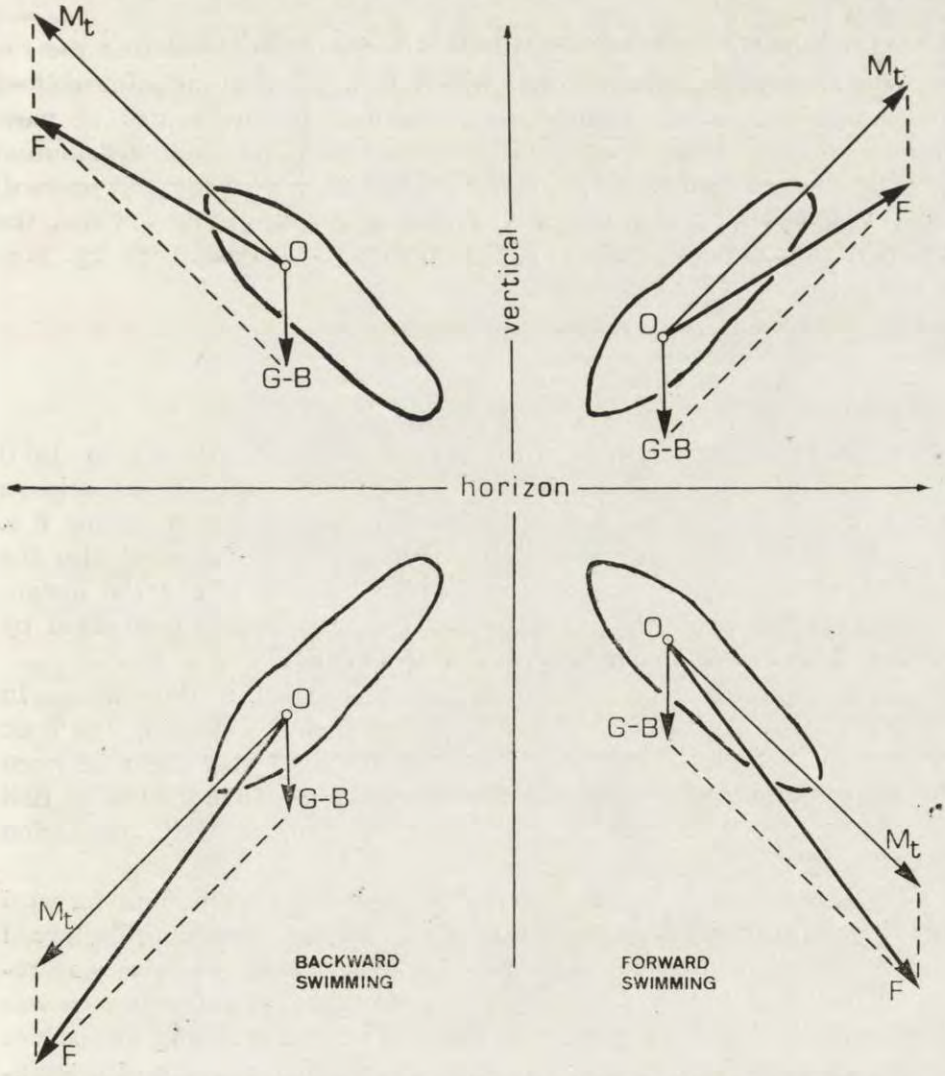

Fig. 6. Diagrammatic presentation of relationships between the ciliary motory force, sedimentation, resultant direction of locomotion, and body position, in some cases of oblique swimming forward or backward. Note that the leading extremity of the body is always deflected upward in respect to the resultant direction of locomotion. Symbols are explained in the text

translational component of the motory forces, average for one full gyration cycle (i.e., its direction is parallel to the direction kept by the gyration axis). Presence of the sedimentation factor (shown as G-B, i.e., gravitation less buoyancy) makes the direction of the resultant total locomotory force F to be deviated downward from the vector M_t. This deviation would remain constant in time, and obviously would have nothing to do with geotactic orientation, unless the medium resistance factor is taken into account.

When supplementing this picture with a vector representing the resultant medium resistance force which is applied at a point shifted forwards along paramecium body in respect to the centre of mass (because of the body shape), it becomes clear that such distribution of acting forces creates a torque turning the anterior body end upward. The observation that the body shape factor is responsible for that the medium resistance creates a turning moment was first made by Roberts (1970).

Angular Rate of Orientation

In the precedent paper (Grębecki and Nowakowska 1977) the values of the angular velocity of orientation were given only as means calculated for the totality of each recording period lasting 6 s. The technique described there permits, however, to calculate also the relation between the instantaneous angular velocity Ω and the instantaneous inclination of the trajectory axis to the vertical (expressed by the angle Θ). The following procedure was adopted:

The trajectories of paramecia turning up or down, as those shown in the Pl. I 1–2 were fitted with the forth order polynom using the least squares method. Derivative of this polynom gives the angle between the horizontal and the tangential to the trajectory. This allowed to find the instantaneous angular velocity as a function of body inclination to vertical.

Three examples of such analysis, for paramecia swimming forward in Pringsheim medium and in EDTA solution, and swimming backward in RbCl solution, are shown in the Fig. 7. The same exercise was repeated for several other specimens and a similar qualitative pattern was obtained. It is well seen in the Fig. 7 that in ciliates orienting themselves upwards during the forward swimming, the angular velocity of orientation is very high when the animal is in a position close to the horizontal: it may reach about 30° per second. When the trajectory approaches the vertical, the angular velocity of orientation decreases regularly (but not

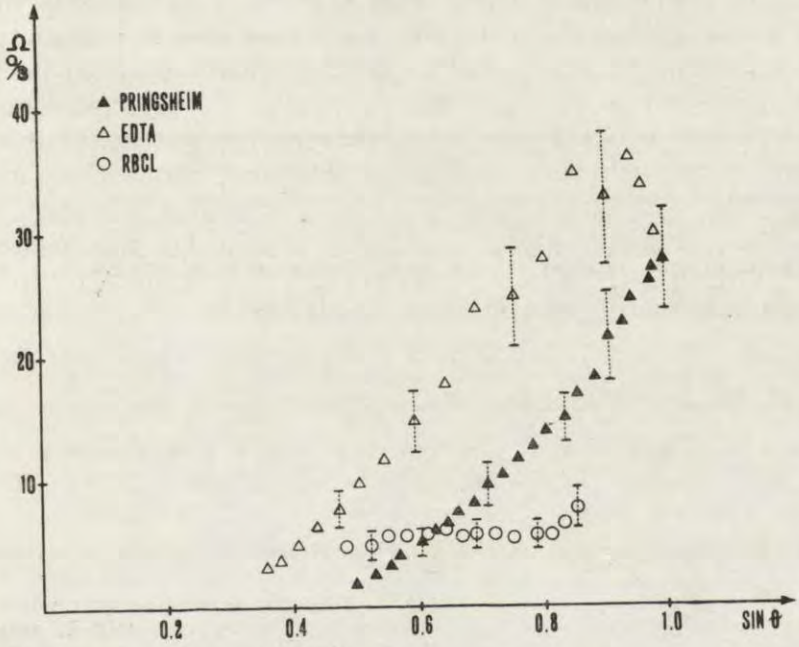

Fig. 7. Relationship between the instantaneous velocity of geotactic orientation Ω and the instantaneous value of the angle Θ expressing the inclination of paramecium body toward vertical

linearly as expected by Roberts 1970). This relationship appears less clear during backward swimming.

It should be concluded that the turning moment produced by medium resistance, although it has a rather low value when developed against the sedimentation only (as in the experiments of Kuźnicki 1963 and Roberts 1970 on immobilized specimens and artificial models), becomes very significant when developed against the active locomotory forces.

Lifting Force Hypothesis

Origin of the substantial difference between the angular rates of orientation in the passively sinking and the actively swimming ciliates, seems to be a key for understanding geotaxis. This difference cannot be explained if the medium reaction is represented only by a vector opposed to locomotion and parallel to it, because in such a case one should adhere to the statement made by Roberts (1970) that "in this approximation the rate of orientation is independent of swimming velocity".

The present authors are convinced that the most probable solution of this problem is related to the fact, well established in this study, that the gyration axis in non-vertical swimming is deflected from the resultant direction of locomotion, or more precisely, the expected solution is thought to depend upon some hydrodynamic implications of this phenomenon. With such body position a swimming paramecium may be considered as symmetrical solid which axis is oblique in respect to the streamtubes. This means that the medium reaction has two components: the drag which is parallel and opposed to the resultant translational movement, and the lift which is normal to it (Fig. 8).

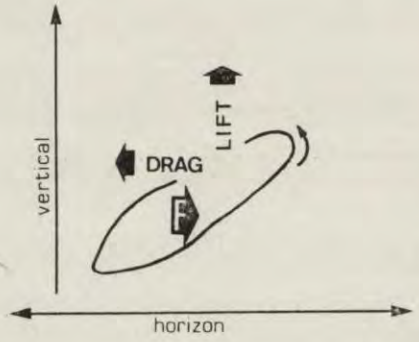

Fig. 8. Diagram explaining that when paramecium is oriented obliquely in respect to the resultant direction of its locomotion (F), the medium reaction is composed of drag and of the lift which may play a major role in generating the turning moment

The lift component is developed during the active swimming, and it may be responsible for high angular rates of orientation. If so, the distribution of forces acting on the ciliate would be in some extent comparable to the distribution of forces on an airfoil, and for the purpose of convenience a similar terminology could be applied.

The angle between the gyration axis and the resultant swimming direction may be considered as the angle of attack, and the anterior body end of an individual swimming forwards as the leading edge. The lifting force would be applied to the centre of pressure, which certainly does not coincide with the centre of mass, and as a consequence, the lift would generate the torque necessary to turn the body towards vertical.

To check the validity of this hypothesis one should calculate and establish empirically many parameters completely unknown for paramecium, as it is done when designing and testing the airfoils: (1) record and analyse the pattern of streamlines around paramecium moving forwards and backwards, (2) calculate the lifting efficiency of paramecium profile, i.e., the lift vs. drag ratio, (3) find out the position of the centre of pressure for the forward and the backward swimming in respect to

the centre of mass, and describe its dependence on the angle of attack, (4) check the relationship between the lift and swimming velocity. The extent and the complexity of such investigations persuaded the authors to present the lifting force concept of geotaxis in a purely hypothetical way.

RÉSUMÉ

L'orientation des paramécies dans le champ de gravitation, pendant le travail normal de la ciliature et pendant le rebroussement ciliaire, était soumise à une analyse cinématographique. Le comportement des paramécies pendant le rebroussement ciliaire partiel, quand la composante translatoire du mouvement est négligeable, est incohérent avec les prévisions basées soit sur le modèle „mécanique" de la géotaxie soit sur le modèle dit „propulsion-gravity". Il a été établi que la vitesse angulaire momentanée de l'orientation est beaucoup plus élévée pendant la locomotion active que pendant la sédimentation passive. Pendant la locomotion horizontale ou oblique l'axe de gyration du corps se trouve toujours incliné vers le haut par rapport à la direction du mouvement résultant. On suggère que, avec une telle position du corps de la paramécie, la réaction du milieu liquide inclue deux composantes: la résistance et la portance. La force de portance peut apporter la contribution majeure à la création du moment qui tourne le cilié vers la direction verticale.

REFERENCES

Grębecki A. and Nowakowska G. 1977: On the mechanism of orientation of *Paramecium caudatum* in the gravity field. I. Influence of ciliary reversal and of external Ca deficiency on the geotactic behaviour. Acta Protozool., 16, 351–358.

Jahn T. L. and Votta J. J. 1972: Locomotion of protozoa. Ann. Rev. Fluid. Mech. 4, 93–116.

Jahn T. L. and Winet H. 1973: Mechanism of negative geotaxis. Progress in Protozoology. Clermont-Ferrand, 197.

Kuźnicki L. 1968: Behavior of *Paramecium* in gravity fields. I. Sinking of immobilized specimens. Acta Protozool., 6, 109–117.

Roberts A. M. 1970: Geotaxis in motile micro-organisms. J. Exp. Biol., 53, 687–699.

Verworn M. 1889: Psycho-Physiologische Protistenstudien. Fischer Verlag. Jena.

Winet H. and Jahn T. L. 1974: Geotaxis in Protozoa. A propulsion-gravity model for *Tetrahymena* (*Ciliata*). J. Theoret. Biol., 46, 449–465.

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EXPLANATION OF PLATES I–V

Photomacrographic records of paths described by paramecia in vertical glass chamber (6 s exposure time)

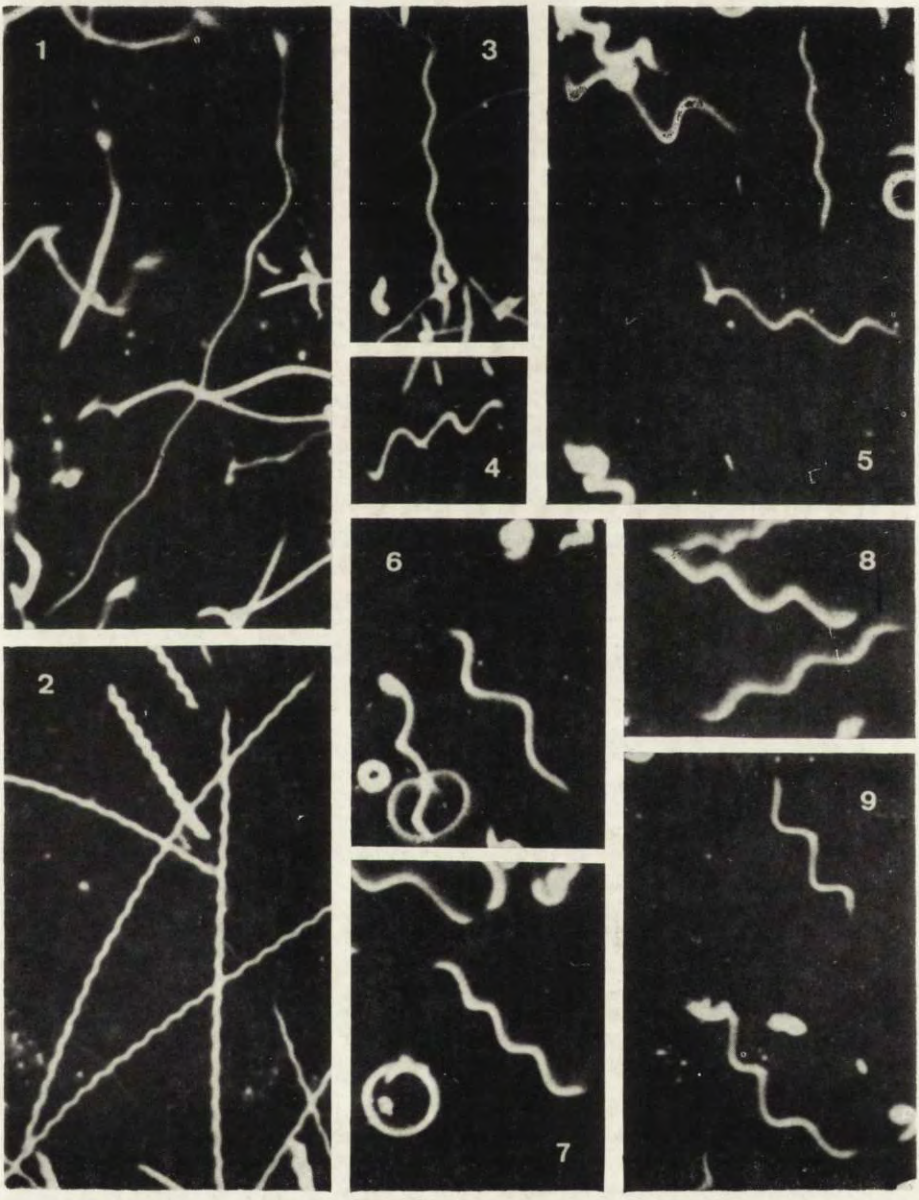
1:Arch-like path bending upwards followed by a specimen which is assuming the vertical orientation when swimming forward in Pringsheim medium
2: Arch-like paths bending downward followed by ciliates which were assuming the vertical orientation when swimming backward in RbCl solution
3–4: Symmetrical vertical path and an asymmetrical oblique path recorded during forward swimming in Pringsheim medium
5–9: Symmetrical vertical path and different asymmetrical non-vertical paths recorded during re-normalization of swimming in RbCl solution

Graphical analysis of the cinematographic records of forward swimming in Pringsheim medium (0.04 s intervals between the successive positions of body axes)

10–12: Symmetrical character of trajectories of paramecia swimming vertically upward
13–16: Asymmetricity of different non-vertical trajectories

Graphical analysis of the cinematographic records of movement of paramecia manifesting different types of motory behaviour induced by Rb ions (0.02 s intervals between the successive positions of body axes)

17: Symmetrical character of trajectories of paramecia swimming vertically downward during the continuous ciliary reversal
18–19: Asymmetricity of oblique and horizontal trajectories during the continuous ciliary reversal
20–22: Gyration without translational movement during the partial ciliary reversal
23: Inclination upward of gyration axis in a specimen which was just recovering its capacity to move forwards
24–25: Asymmetricity of oblique trajectories soon after re-normalization of locomotion
26: Symmetrical character of trajectory of individual which soon after re-normalization of movement assumed the vertical swimming direction



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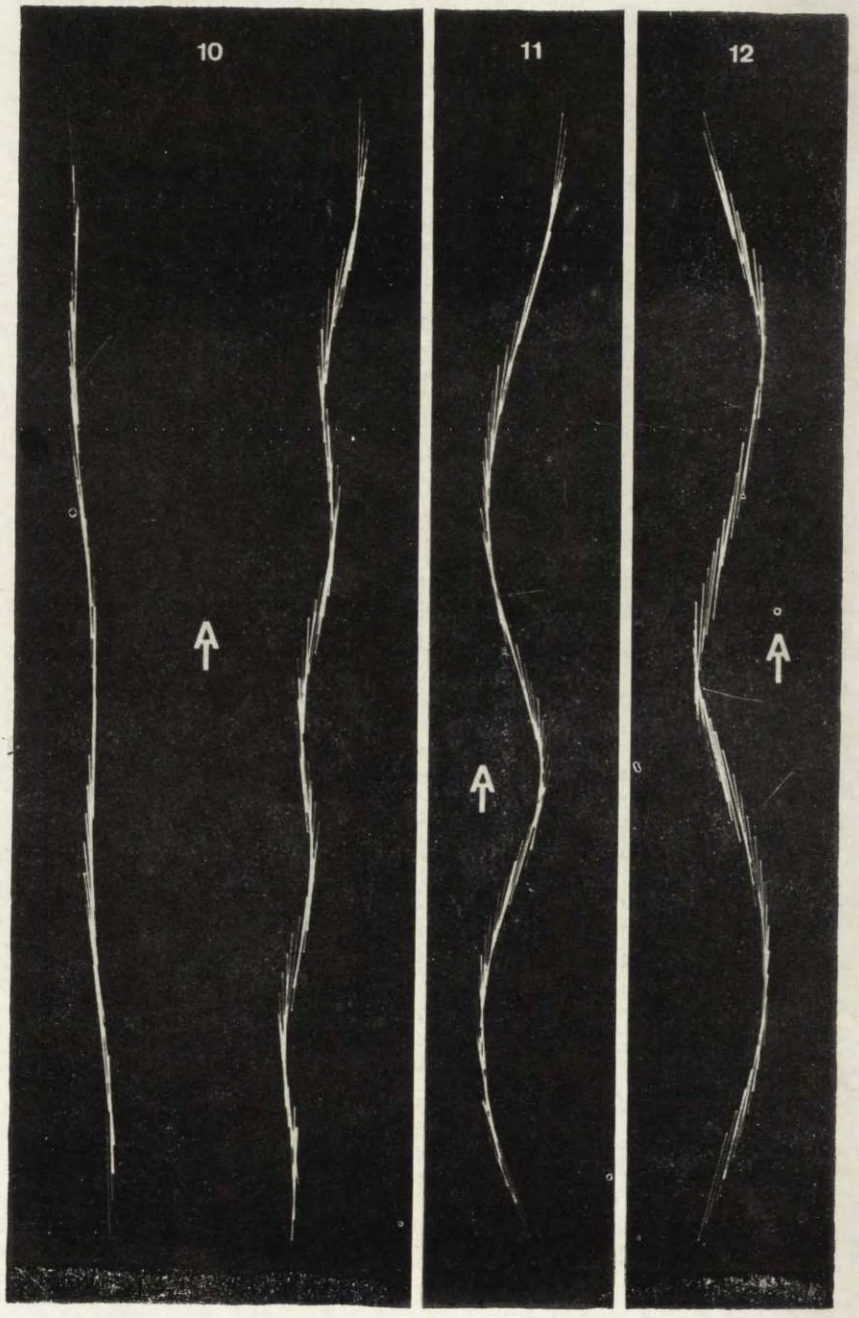

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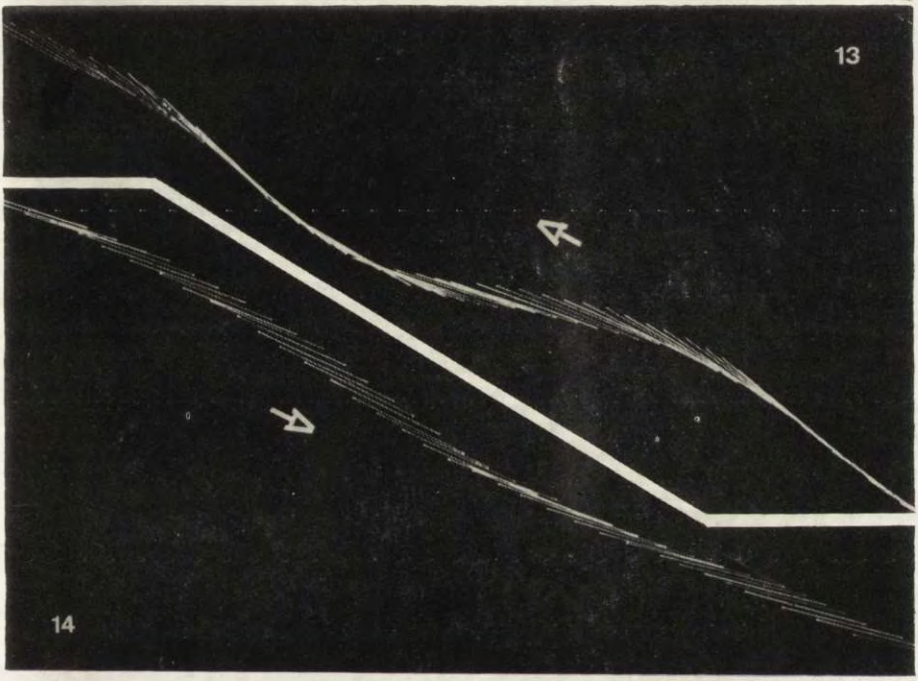

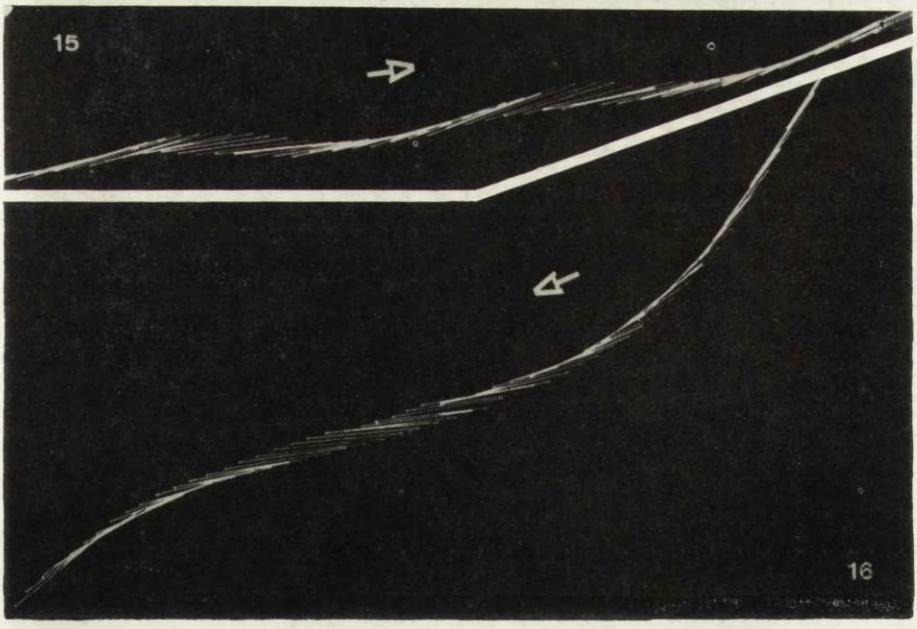

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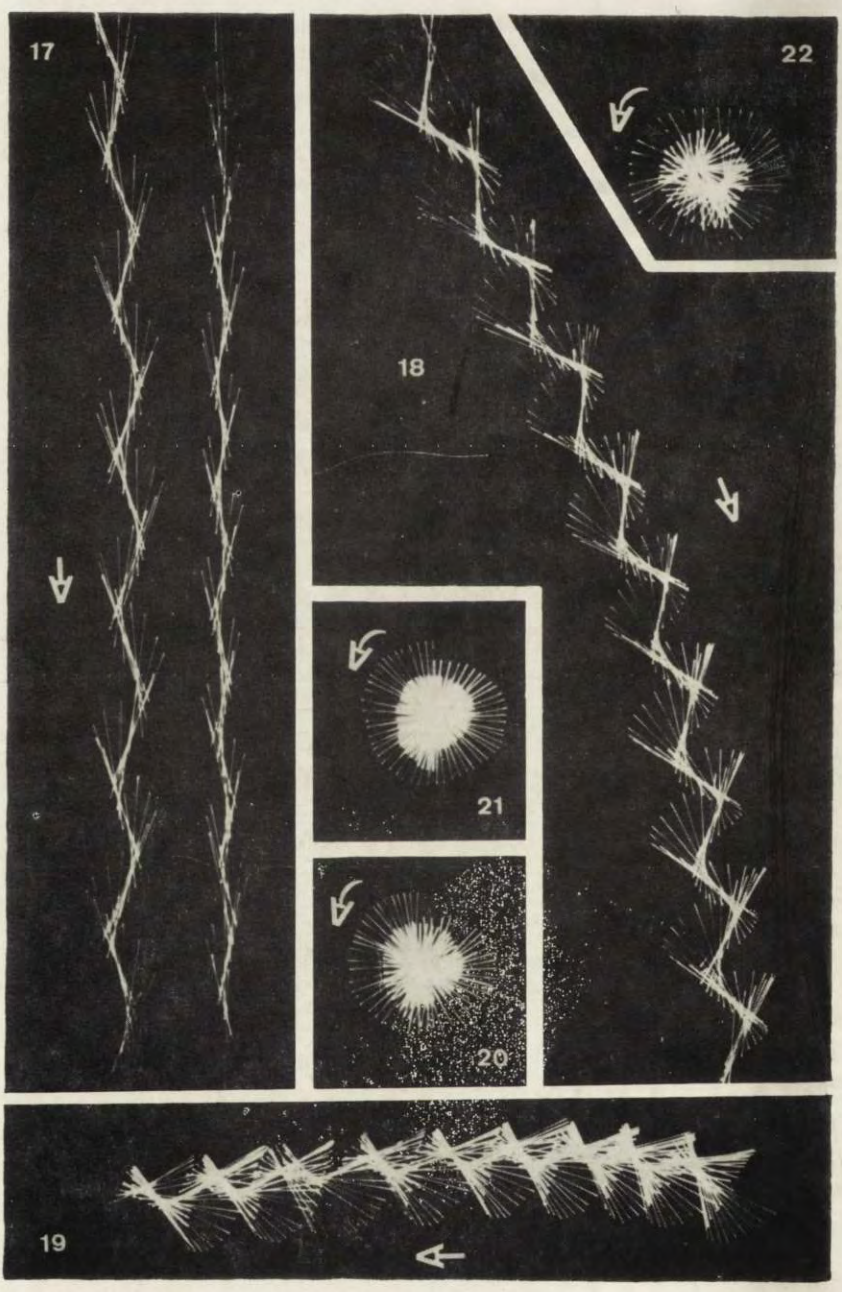

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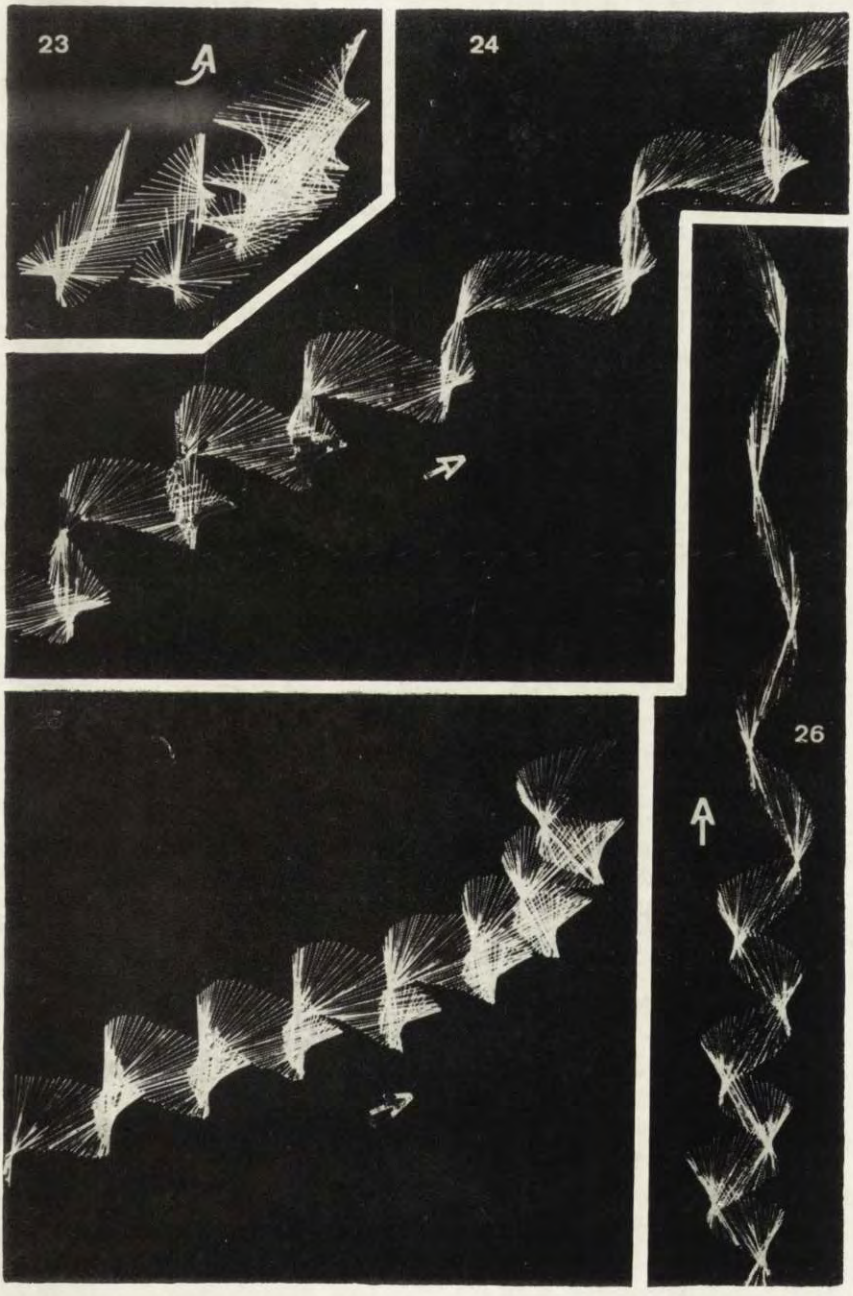

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Reactivation of Tritonated Models of *Opalina*

Synopsis. Tritonated models of *Opalina obtrigonoidea* were reactivated by ATP. Optimal mobility and ciliary beat frequency attained in the reactivation were less than in live cells. The forward swimming velocity of the reactivated models was solely dependent on the reactivated ciliary beat frequency which, in turn, was affected by both the ATP and Mg^{++} concentrations. In the absence of Mg^{++}, reactivation would not take place even at high concentrations of ATP. Chelators (e.g. EDTA) and common divalent cations (e.g. Ca^{++}, Fe^{++}, Mn^{++}, Co^{++}) are not essential and do not figure significantly in the reactivation of tritonated models of *Opalina*.

Ciliary models which can be reactivated to beat when supplied with an energy source (e. g. ATP) have been prepared by several investigators (Cheung 1973, 1975, Gibbons 1965, Naitoh and Kaneko 1972, 1973, Preston 1972). The main goal is a partial or complete disruption of the membrane to allow entry of ATP and other necessary ions and molecules without too much degradation of the internal structures essential for ciliary beating (Goldstein 1974, Naitoh 1972). Two kinds of ciliary models can be obtained with extraction techniques — isolated cilium capable of being reactivated and entire organism with intact cilia as one complete unit capable of being reactivated.

Until recently, glycerol was generally utilized for the extraction process and the cilia isolated as a result could be used for biochemical and motility investigations (Cheung 1973, 1975, Preston 1972). However, it is obvious that glycerol extraction is not an ideal process as the extraction involves some messy experimental procedures and the percentage of reactivation is very low — typically 25–50 % (Cheung 1975, Brokaw and Benedict 1968). Also, glycerol extraction for ciliates succeeds only in the isolation of individual cilium for reactivation. Reactivation of such isolated cilia models can generate interesting data

on the activity of the cilia but will have no significant bearing on the locomotion of the organism with intact cilia as a unit.

Naitoh and Kaneko (1972, 1973) succeeded in extracting ciliary models for reactivation with a non-ionic detergent, Octylphenoxy polyethoxyethanol (commercially known as Triton X-100) and it appears to be the best available reagent to dissolve the membrane (Naitoh 1972, Wallace 1975). Utilization of Triton X-100 on ciliates, at certain concentrations, can provide entire ciliate models with intact cilia for reactivation investigations (Cheung 1975; Naitoh 1972).

Reactivation of isolated cilia (glycerinated) from *Opalina* has been investigated previously (Cheung 1973, 1975). Interesting preliminary data have been obtained but the percentage of reactivation is low (50 %) and the technique has yet to be perfected. Reactivatable (entire) ciliate models with intact cilia cannot be obtained from glycerol extraction for *Opalina*.

This report describes a method for the model extraction of *Opalina* by Triton X-100 and reactivation by ATP. The models obtained will be entire organisms with intact cilia capable of being reactivated. The percentage of successful reactivation and the various ciliary beat characteristics will be investigated and the effects of pH and varying concentrations of ATP, chelators and divalent cations will also be studied. This report represents one of three parts of a research project designed to investigate the energy utilization and viscous effects on the locomotion of *Opalina*. The other two parts are presented in separate reports.

Materials and Methods

Biological Material and Medium

Organisms. *Opalina obtrigonoidea* were obtained from the rectum of common grass frogs purchased from biological supply firms in Southern California. The organisms were isolated out, washed and then resuspended in Naitoh's *Opalina* medium (Naitoh 1964).

Naitoh's *Opalina* Physiological Medium

NaCl	60 mM
KCl	5 mM
$CaCl_2$	0.1 mM
$MgCl_2$	1 mM
Tris buffer	10 mM

with pH adjusted to 7.8.

Wash Solution

KCl	50 mM
Tris buffer	10 mM

with pH adjusted to 7.5.

Extraction Medium

Triton X-100	0.02% (v/v)
KCl	50 mM
EDTA	10 mM
Tris buffer	10 mM

with pH adjusted to 7.5.

Reactivation Medium

ATP	in mM (to be determined)
KCl	50 mM
$MgCl_2$	in mM (to be determined)
EGTA	0.1 mM
Tris buffer	10 mM

with pH adjusted to 7.5.

Methods: (General)

Specimens of *Opalina obtrigonoidea* were washed thoroughly in the wash solution. The suspension was then cooled in an ice bath (crush ice) and hand-centrifuged to form a loose pellet. The pellet was then resuspended in a new wash solution, cooled and recentrifuged to make a new loose pellet. The new pellet was then removed carefully and suspended in a cold extraction medium (0°C) for 45 min. The extracted specimens obtained were individually pipetted into a wash solution and gently washed twice. After removal from the last wash, they were kept at 0°C in wash solution, ready for reactivation.

One hundred cell models which were suspended in a minute amount of wash solution were micro-pipetted into 2 ml of reactivation medium in a depression chamber for observation. The reactivation was performed at 20–21°C (68–70°F). Reactivation normally started in a minute or less as the models could be seen swimming in the medium.

A few of the swimming models were micro-pipetted out and transferred to a microscopic slide with a cover slip ringed for a clearance of about 200 μm. Their activities were observed and later photographed by a Milliken high-speed camera under Zeiss Nomarski differential interference contrast optics at high magnification (The high-speed cinemicrographic set-up was described in detail in previous reports (Cheung 1973, Cheung and Jahn 1975). The high-speed cinemicrographs obtained were later frame-by-frame analyzed for ciliary beat characteristics. Plate I shows two successive frames of a 16 mm high-speed cinemicrograph on the swimming of a reactivated model. Plate II shows two successive frames of another 16 mm high-speed cinemicrograph on the swimming of a normal live *Opalina*.

Methods: (Specific Investigations)

Effect of ATP Concentration

Forward swimming velocity and ciliary beat frequency were used as the criteria to determine the effective concentration range of ATP (0.5–10.0 mM) while other ionic compositions were kept constant (5 mM–$MgCl_2$).

Effect of Mg^{++} Concentration

Forward swimming velocity and ciliary beat frequency were used as the criteria to determine the effective concentration range of Mg^{++} (0.25–10.0 mM) while ATP was kept at a fixed concentration (6 mM).

Effect of Divalent Cations

The effects of Ca^{++}, Mn^{++}, Co^{++} and Fe^{++} were tested with a constant concentration of ATP (6 mM). In various trials, 5 mM concentration of each divalent ion was used in the absence of chelators.

Effect of pH

pH values of from 6.8 to 7.8 were tested with constant ATP and Mg^{++} concentrations (6 mM-ATP; 5 mM-Mg^{++}).

Results and Observations

In a minute or less after the tritonated models were transferred into the reactivation medium, the cilia began to beat (at first slowly, then progressively faster) with near perfect symplectic metachrony and in the continuous helical beat pattern characteristic of live *Opalina*, as shown in Pl. I. The percentage of successful reactivation (under optimal conditions) was about 92–95 % for newly prepared models and the percentage deteriorated in course of storage time for models not used immediately.

The reactivated ciliary beat frequency started slowly at the onset of reactivation; increased quickly in the first minute and reaching a peak in 2 to 3 min. However, the cilia could only maintain the peak frequency for 5 to 7 min as the beat frequency started to slow down in 10 min and finally stopped in an hour (50–60 min). Renewal and addition of reactivation medium did not effect the frequency change nor did it prolong the time of ciliary reactivation.

The optimal mobility and ciliary beat frequency of reactivated models were less than in live cells. Even with an optimal reactivation medium (6 mM–ATP; 5 mM–$MgCl_2$), only a reactivated forward swimming velocity of 80–100 μm/s could be reached with a peak beat frequency of 18–20 Hz, as compared with a velocity of 120–150 μm/s and a beat frequency of 25–30 Hz for live *Opalina*. The metachronal wave velocity of the reactivated models was about 130–150 μm/s, still a bit slower than the velocity of 140–180 μm/s for live *Opalina*.

The forward swimming velocity of the reactivated models was entirely dependent on the beat frequency of the cilia which, in turn, was controlled by both the concentrations of ATP and Mg^{++}. In the absence or at very low concentrations of ATP, reactivation could not take place. The effective concentration of ATP started at around 0.5 mM as reactivation at low beat frequency started to take place. With increasing ATP concentration, beat frequency started to increase progressively,

peaking at 18–20 Hz with an ATP concentration of about 6–8 mM. However, reactivation could not take place in the absence of Mg^{++}, even at high concentrations of ATP. At ideal ATP concentration (6 mM), the effectiveness of reactivation (as indicated by ciliary beat frequency) was dependent on Mg^{++} concentrations. Reactivated ciliary beat frequency (and consequently forward swimming velocity) increased with increasing Mg^{++} concentration, reaching a plateau at 4–6 mM. The experimentally determined ideal combination for ATP and Mg^{++} concentrations in the reactivation medium was an ATP concentration of 6 mM and a Mg^{++} concentration of 5 mM.

In the reactivation of tritonated models of *Opalina* by ATP, the divalent cation Mg^{++} was required. However, other divalent cations tested were not necessary in the reactivation and did not figure significantly in the overall reactivation process.

Reactivations had been obtained at pH values of from 6.8 to 7.8 However, the best results were consistently obtained at pH 7.5. From the experimental data obtained, it was quite obvious that the pH value was not a very significant reactivation factor. As long as the pH values were within the 6.8 to 7.8 limit, reactivation would take place.

Frame-by-frame analyses of the high-speed cinemicrographs revealed that, except for the variations in beat frequency, forward swimming velocity and metachronal wave velocity, the quantitative parameters of the locomotion of live *Opalina* and reactivated models of *Opalina* did not show any appreciable difference.

Discussion

The isolation of cilia from *Opalina* by treatment with glycerol is analogous to the preparation of glycerol-extracted muscle fibers (C h e u n g 1975). In all cases when reactivation is successful, the resulting movement is some form of a sluggish wave bending with a slow forward progression through the medium. The measured forward velocity of locomotion and the rate of wave propagation are extremely low compared with the performance of the cilia of live specimens. The precise effect of glycerol extraction is a breakdown of the membrane of the cilia and the cell but, at the same time, leaving the 9 + 2 axonemes intact — making it easy for ATP to reach the axonemes and initiate ciliary movement. The slow velocity of forward movement for the glycerinated cilia models during reactivation indicates that a partial disruption of the membrane has taken place and ATP can only attain a partial passage for reactivation. For tritonated models, the disruption

of the membrane is more extensive, but still incomplete — such a phenomenon is reflected by the fact that the reactivated forward swimming velocity of the tritonated *Opalina* models and the rate of wave propagation, though much faster than in glycerinated models, are still less than in live cells.

The experimentally-determined optimal reactivation medium consists of 6 mM-ATP, 5 mM-$MgCl_2$, 50 mM-KCl, 0.1 mM-EGTA and 10 mM-Tris buffer (adjusted to pH 7.5). It should be emphasized, however, that within-limit variations of the concentration of the above reagents may have an effect on the quantitative parameters of the reactivated ciliary activities (beat frequency, forward velocity, etc); but do not bear much significance in the overall reactivation process.

Variation of the concentration of ATP in the reactivation medium affected the ciliary movement and forward propagation of the tritonated models in a manner similar to that of glycerinated models (C h e u n g 1975). In the presence of Mg^{++}, ATP is the only essential and available energy source and no reactivated ciliary movement can be attained in the absence of ATP-Mg^{++} or at low concentrations. Utilizing reactivated ciliary beat frequency as a criterion to evaluate the effective concentration of ATP-Mg^{++}, the minimal concentration to render reactivation for both ATP and Mg^{++} is at 0.5 mM, yielding a reactivated beat frequency of 1–2 Hz. Increases in concentration of ATP-Mg^{++} progressively elevates the magnitude of ciliary activities, reaching a peak for ATP at 6 mM and for Mg^{++} at 5 mM. Further increases in ATP-Mg^{++} concentration do not cause any noticeable change in the reactivated ciliary activities. As long as the ATP-Mg^{++} concentration is within the 1 mM to 6 mM range, the percentage of successful reactivation stays within 80–92 %. However, the optimal reactivation medium can give a successful percentage of over 95 %.

Similar to the data obtained for glycerinated cilia models, reactivation of tritonated models can be achieved at pH values from 6.8 to 7.8. However, the best results are obtained at pH 7.5. It is quite obvious that the pH value is not a very significant reactivation factor, as long as it is within the effective range.

To study the biochemistry and kinetics of ciliary activities, it is essential to have a "model" system that can be readily subjected to quantitative experimentation. Reactivation investigations on glycerinated and tritonated models are part of a search for such an ideal "model" system.

Cilia are cellular organelles that are considered to be extensions of the cell body and are distinguished by their specific locomotory function and their physical remoteness from the cell body. Are the locomotory

characteristics self-contained or are they controlled by the cell are the 2 key questions most often asked. Since there is no indication that the basic axonemal structure of motile cilia has any function other than movement, the cilia appear to be completely self-contained organelles specialized for motility (See Goldstein 1974). It is common belief, therefore, that reactivatable isolated cilia can possibly provide a good "model" system for both biochemical and kinetic studies on ciliary movement.

The ATP-induced reactivation of glycerinated cilia (Cheung 1973, 1975, Gibbons 1965, Preston 1972) confirms that the locomotory characteristics of motile cilia are indeed self-contained and it is quite possible that such isolated cilia may serve as a pure "model" system in studying ciliary movement. Further experimentation, however, indicates that such a "model" system, though ideal theoretically, does not serve the purpose — the percentage of successful reactivation is simply too low to make the experimentation practical and the reactivated beat frequency (as well as all other physical beat characteristics) is far too low when compared with the performance of live specimens.

Although ideal biochemical and kinetic analyses generally require complete isolation with a single cilium, many investigations can still be conducted with the cilia attached to the cell body as a reactivatable unit (Goldstein 1974). In fact in certain aspects, such a "model" can serve to generate quantitative data as close to live form as possible and such data cannot be obtained from isolated cilia. It has now been confirmed that tritonated ciliate models do provide cell biologists with near perfect models for biochemical and kinetic studies. The percentage of successful reactivation is extremely high (92–95 %) and the reactivated physical beat parameters (beat frequency, forward swimming velocity, etc.) which are solely ATP-Mg^{++} dependent, can easily be monitored.

Further investigations are now in progress at CalTech, utilizing the availability and versatility of such tritonated ciliate models, to look deeper into the bioenergetics and biomechanics of ciliary movement.

ACKNOWLEDGMENT

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RÉSUMÉ

Les modèles de *Opalina obtrigonoidea* obtenus avec du Triton X-100 étaient réactivés par l'ATP. La motilité optimale des modèles réactivés et la fréquence de leurs battements ciliaires étaient inférieures à celles des cellules vivantes. La vélocite de la locomotion progressive des modèles réactivés ne dépend que de la fréquence de leurs battements ciliaires, qui est influencée à son tour par la concentration de l'ATP ainsi que par la concentration des ions Mg^{++}. En absence du Mg^{++} la réactivation fait défaut même à des concentrations élévées de l'ATP. Les agents chélateurs (comme l'EDTA) et les cations bivalents courants (comme Ca^{++}, Fe^{++}, Mn^{++}, Co^{++}) sont dépourvus d'importance et ils n'exèrent pas d'influence signifiante sur la réactivation des modèles d'*Opalina* obtenus avec du Triton X-100.

REFERENCES

Brokaw C. J. and Benedict B. 1968: Mechanochemical coupling in flagella. I. Movement-dependent dephosphorylation of ATP by glycerinated spermatozoa. Arch. Biochem. Biophys., 125, 770–778.

Cheung A. T. W. 1973: Doctoral Thesis. University of California at Los Angeles, USA.

Cheung A. T. W. 1975: Reactivation of glycerinated cilia from *Opalina*. Acta Protozool., 14, 99–104.

Cheung A. T. W. and Jahn T. L. 1975: Helical nature of the continuous ciliary beat of *Opalina*. Acta Protozool., 14, 219–232.

Gibbons I. R. 1965: Reactivation of glycerinated cilia from *Tetrahymena pyriformis*. J. Cell. Biol. 25, 400–402.

Goldstein S. F. 1974: Isolated, reactivated and laser-irradiated cilia and flagella. Cilia and Flagella (ed. M. A. Sleigh), Academic Press, New York, 111–132.

Naitoh Y. 1972: Personal discussions.

Naitoh Y. 1974: Determination of physiological saline solution for *Opalina*. Zool. Mag. Tokyo, 73, 267.

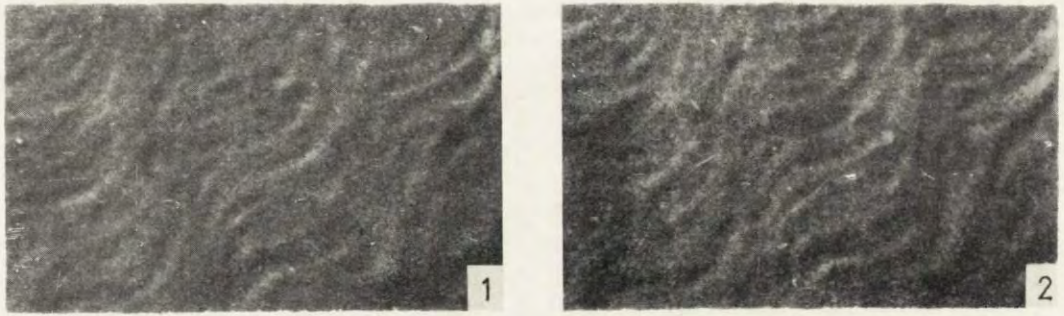
Naitoh Y. and Kaneko H. 1972: Reactivated Triton-extracted models of *Paramecium:* modification of ciliary movement by calcium ions. Science, 176, 523–524.

Naitoh Y. and Kaneko H. 1973: Control of ciliary activities by Adenosinetriphosphate and divalent cations in Triton-extracted models of *Paramecium caudatum*. J. exp. Biol., 58, 657–676.

Preston J. R. 1972: Doctoral Thesis. University of California at Los Angeles, USA.

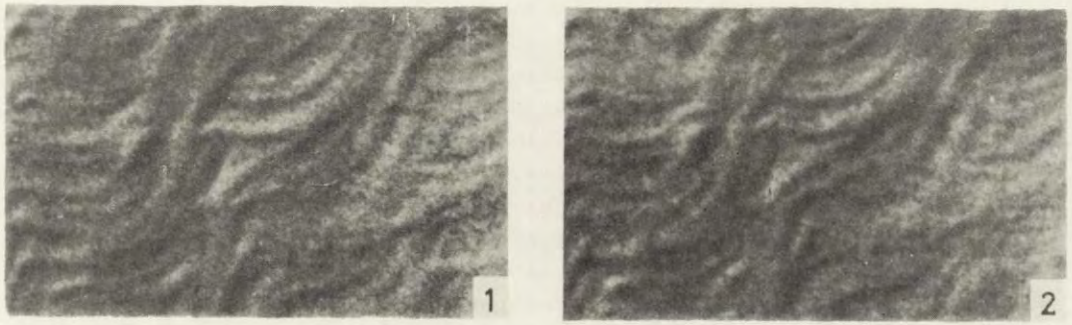
Wallace R. B. 1975: Personal discussions.

Received on 28 January 1976



Two successive frames (cropped) from a selected 16 mm high-speed movie sequence on the surface view of a reactivated (tritonated) *Opalina* model. Reactivation has taken place for 5 minutes and the model is swimming freely in the reactivation medium. Note the existence of the metachronal waves and the continuity in the ciliary beat pattern. The beat frequency is 20 Hz. (Magnification: 645 ×, under Zeiss Nomarski optics).

A. T. W. Cheung auctor phot.



Two successive frames (cropped) from a selected 16 mm high-speed movie sequence on the surface view of a free swimming live *Opalina*. Note the similarity of the metachronal waves and beat pattern to those of the reactivated model. The beat frequency is 25 Hz. (Magnification: 662 ×, under Zeiss Nomarski optics).

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Observations on the Effect of Rotation on *Tetrahymena pyriformis* Aggregations (Pattern Swimming)

Synopsis. The effect of rotational forces on pattern swimming of *Tetrahymena pyriformis* has been investigated. Slow rotational forces (0.1 rpm) do not prevent aggregation but do alter their configuration. These changes seem to be associated with an interaction of convection currents and negative geotactic responses of the organism.

The aggregation of free swimming protozoa and algae is commonly observed in laboratory cultures and often under natural circumstances in lakes, ponds, and ocean e.g. in algal blooms (Gittleson, unpublished observations). This phenomenon has been the object of a number of reports designed to describe the appearance of aggregate patterns formed by free swimming ciliates (Levandowsky et al. 1975, Winet and Jahn 1972 a, b, Loefer and Mefferd 1952) and flagellates (Gittleson and Rogers 1972, Gittleson and Resler 1972, Gittleson et al. 1970, Gittleson and Jahn 1968 a, b, Thiele 1960, Robbins 1952, Wager 1910, 1911). It is generally concluded that these aggregations do not occur by means of cell attachments and that their origin does not depend upon surface tension, magnetic fields, thermal convection, light gradients or carbon dioxide and oxygen gradients (Platt 1961). However, these factors may influence already formed aggregations. Steep gradients of cell populations in the region of the culture surface brought about by negative geotactic movements of individuals appears to underly pattern formation (Winet and Jahn 1972 a, b).

Most studies to date on pattern swimming have been carried out on static cultures. Loefer and Mefferd (1952) did comment on the complex effects of turbulence which they apparently observed by shaking cultures of *T. pyriformis*. In this report we show that rotatory

forces applied uniformly have a significant effect upon the aggregations of *T. pyriformis*. Such forces produce convection currents which alter the response of the organisms to gravitational forces.

Materials and Methods

Tetrahymena pyriformis was grown axenically in 1 l of medium in 2 l Erlenmeyer flasks or in 100 ml of medium in 250 ml Erlenmeyer flasks. The medium consisted of 1.0 g sodium acetate (Fisher), 2.0 g yeast extract (Difco), and 1.0 g tryptone (Difco) per 100 ml glass distilled water.

Experiments with Single Axis Rotation (Tissue Culture Flasks). Aggregating cultures were transferred aseptically to 30 ml tissue culture flasks (Falcon). In order to prevent air bubbles, each flask was filled and a piece of Parafilm was placed over the opening before capping. This preparation was allowed to equilibrate for 15–30 min. Ambient temperature varied from 20–22°C.

Six tissue culture flasks were used for each experiment. As a control, four of these flasks were placed on a stationary vertical board in the 12, 3, 6 and 9 o'clock positions (see Fig. 1). Two experimental flasks were mounted initially in the 12 and 6 o'clock positions on a turntable to be rotated in the vertical plane. Each of the

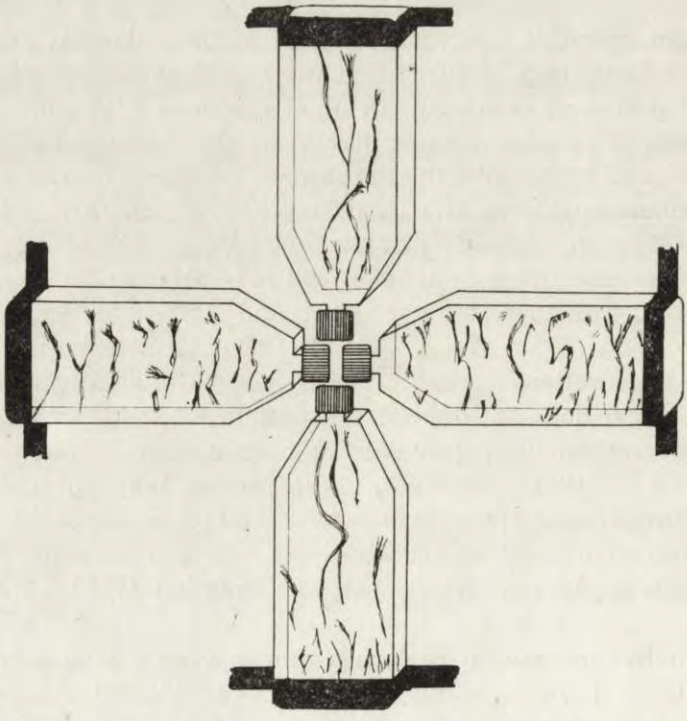

Fig. 1. The appearance of pattern swimming *Tetrahymena pyriformis* under control conditions, viz. stationary

six flasks was used once as an experimental and twice as a control. This eliminated the possibility of an inherent bias. The rotation of the turntable was regulated to approximately 0.1 rpm. Thus, the long axis of the aggregation pattern was changing its position relative to gravity. Just prior to rotation, all of the flasks were agitated in order to disperse the aggregates. The aggregation patterns were then observed continuously for 30 min, during which time, three revolutions were completed.

Identical experiments were run with latex particles of 2.02 μm diameter (Coulter Diagnostics). This allowed an evaluation of the impact of the forces on a non-motile system compared with the motile ciliates.

Experiments with Single Axis Rotation (Petri Dishes). Aggregating cultures of *T. pyriformis* were transfered aseptically to 60 × 15 mm diameter petri dishes (Falcon). Four petri dishes were mounted on the turntable, 24 mm from the center of rotation. The same dishes observed before and after experimentation served as controls. Again Parafilm was used to eliminate the presence of air bubbles in the dish. After equilibration of 15–30 min rotation was begun. Rotation was varied from 0.1–29 rpm. In any given trial, speed of rotation was held constant.

Experiments with Double Axis Rotation (Petri Dishes). In these experiments the petri dishes were mounted on small free swinging boards which in turn were mounted on the turntable. The centers of gravity of the small boards were below their points of attachment to the turntable, therefore, each dish was prevented from rotating on its own axis as the turntable rotated. Cultures were prepared in the petri dishes as described above.

Results

Experiments with Single Axis Rotation (Tissue Culture Flasks). The control cultures of *T. pyriformis* were observed to form typical aggregation patterns in all cases. These patterns formed within 20 s of the cessation of agitation and remained throughout the test period. Figure 1 shows the appearance of one set of controls.

When rotation was begun immediately after agitation pattern formation was not inhibited but configuration of the pattern varied with position of the flask (Fig. 2). Flasks in the vertical position exhibited aggregate patterns similar to the control (positions at 12 and 6 o'clock). As the flask moved from vertical toward horizontal positions (12 to 9 o'clock and 6 to 3 o'clock) aggregate columns appeared to cease forming, the columns became short and they persisted longest in the lower regions of the flask. New faint patterns developed in the horizontal positions (9 and 3 o'clock). As the flasks were approaching the vertical position, just prior to 12 and 6 o'clock, aggregating became more intense; and the columns were oriented parallel to the long axes of the flasks. These aggregate patterns persisted as the flasks reached the vertical position (12 and 6 o'clock).

It was observed that individual *T. pyriformis* moved in a pronounced clockwise direction during the transition from positions 12 to 9 o'clock and 6 to 3 o'clock. These movements appeared to reflect the occurrence of physical currents in the medium. How rotational forces might induce this phenomenon will be discussed below.

Latex particles did not form aggregate patterns either during rotation of under stationary conditions. The movement of latex particles indicated that a fluid current occurred in a clockwise direction i.e., in the direction opposite the rotation of the turntable.

Experiments with Single Axis Rotation (Petri Dishes). Figure 3 shows the appearance of aggregate columns in 12 positions as the turntable rotated at 0.1 rpm through 360°. In the 12 o'clock position before rotation began vertical columns had formed. When rotation commenced the entire pattern shifted about 45°. As rotation continued the following series of events occurred repeatedly from one position to the next: (1) the columns shortened and it was observed

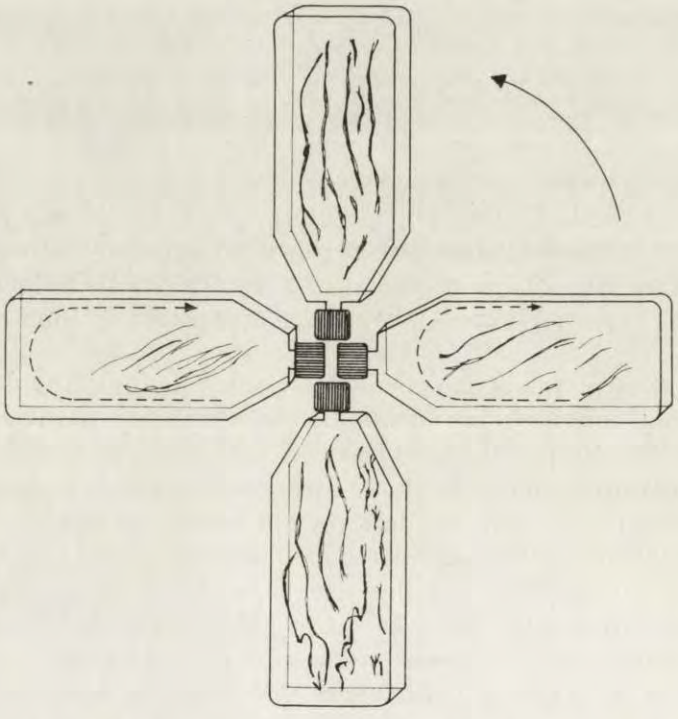

Fig. 2. The appearance of pattern swimming *Tetrahymena pyriformis* under experimental conditions with rotation of 0.1 rpm. (——→ direction of turntable, — — → direction of major convection currents)

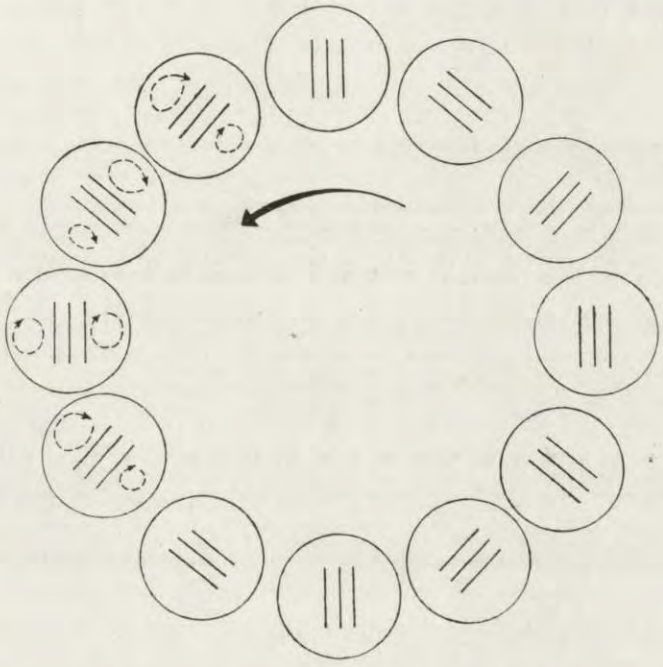

Fig. 3. Appearance of pattern swimming *Tetrahymena pyriformis* in successive stages of rotation (0.1 rpm). The 4 petri dishes on the apparatus were actually 60 mm in diameter and placed 24 mm from the center of rotation. Each of the 4 dishes exhibited the patterns through 360° depicted in this diagram. (——→ direction turntable, — — → direction of convection current)

that input of cells to these columns ceased; (2) columns developed in a different position as the organisms accumulated at a new locus.

When the turntable was rotated at 29 rpm the aggregate columns became spirally shaped. In this configuration the central region was devoid of organisms. Upon abrupt cessation of rotation the cells rapidly moved into the center of the dish and subsequently dispersed. A typical pattern reformed within one min.

Experiments with Double Axis System (Petri Dishes). In these experiments no change in pattern orientation occurred even at a speed of 29 rpm.

Discussion

The aggregate patterns of *T. pyriformis* shift their spatial orientation in response to rotatory forces. These variations in pattern configuration are related to the induction of convection currents and to the geometry

of the culture container. Evidence for the effect of convection currents is based upon observations of the displacement of individual cells and of non-motile latex spheres. Since the non-motile spheres never formed aggregate patterns it can be concluded that rotation modifies the appearance of *T. pyriformis* aggregates without inducing the process. However by causing a shift in the site of accumulation of *T. pyriformis* new pattern orientations develop. Also higher rotation can produce convection currents strong enough to eliminate aggregation by interacting with responses to gravitational forces.

The series of changes in pattern configuration in the petri dishes subjected to low rpm on a single axis are difficult to explain, although the studies of S t o m m e l (1949) on trajectories of small bodies sinking slowly through convection cells have suggested possible mechanisms. The presence of the aggregate columns of *T. pyriformis* physically separates the fluid within the petri dish into two distinct compartments. Rotation induces convection cells within these compartments. The changing configuration of the pattern suggests that a major convection cell occurs originally in the upper left portion of the dish (see notation in dish at 11 o'clock, Fig. 3) and shifs clockwise during rotation.

It is hypothesized that there is a strengthening and weakening of the convection cells caused by the interaction of negative geotaxis with rotational and gravitational forces. In the transition from 11 to 10 to 9 o'clock, the major convection cell which is being shifted clockwise gradually weakens. By the time the dish reaches the 9 o'clock position the original major cell has weakened to become about equivalent to the other convection cell which is now on the left side. This whole cycle is repeated with 90° periodicity.

When the columns are vertical there may occur a strong tendency for organisms leaving the bottom of the column to move into the left upper portion of the dish. That is because of the interaction of negative geotaxis and convection. We interpret the disappearance of the pattern as due to that interval of time when a new locus of accumulation is forming. Generally, only when gravitational forces overcome turbulence can a locus of accumulation of organisms form to yield vertical aggregates.

The influence of container geometry is well demonstrated by the tissue culture flask experiment. Since the shape of the flask produces greater turbulence than observed in the petri dish, distinct aggregation occurs only in the 12 and 6 o'clock positions. Whereas, vertical aggregations occurred in the 3, 6, 9, 12 o'clock positions in the petri dishes. These observations support the random density fluctuation instability model by W i n e t and J a h n (1972 a, b) for *T. pyriformis* aggregations.

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ZUSAMMENFASSUNG

Die Einfluss von Umlauf-Kräften auf den schwimmen Vorbild des *T. pyriformis* hat sich Untersuchen lassen bei uns. Langsam Umlauf-Kräften (0.1 rpm) können ansammlung nicht angehalten sondern konnen ihre gestalten verandern. Es scheinst möglich das diesen Veranderlichkeiten verbünden mit sich einen gegenseitig beeinflussen von konvektional Zirkulation und negative-geotactischen reaktionen bei dem organismus.

REFERENCES

Gittleson S. M. and Jahn T. L. 1968 a: Pattern swimming in *Polytomella agilis*. Am. Nat., 102, 413–425.

Gittleson S. M. and Jahn T. L. 1968 b: Vertical aggregations of *Polytomella agilis*. Expl. Cell. Res., 51, 579–586.

Gittleson S. M. and Resler R. J. 1972: *Polytomella agilis* aggregations at ultra-high population densities. Acta Protozool., 10, 175–178.

Gittleson S. M. and Rogers M. 1972: Effect of viscosity on formation and sedimentation of *Polytomella agilis* vertical aggregates. J. Protozool., 19, 630–632.

Gittleson S. M., Woodruff B. and Hoover R. L. 1970: Mass accumulation of protozoa. Acta Protozool., 8, 225–229.

Loeffer J. B. and Mefferd R. B. 1952: Concerning pattern formation by free swimming microorganisms. Am Nat., 86, 325–329.

Levandowsky M., Childress W. S., Spiegel E. A. and Hutner S. H. 1975: Mathematical model of pattern formation by swimming microorganisms. J. Protozool., 22, 296–306.

Platt J. R. 1961: Bioconvection patterns in cultures of free-swimming organisms. Science, 133, 1766–1767.

Robbins W. J. 1952: Patterns formed by motile *Euglena gracilis* var. *bacillaris*. Bull. Torrey Bot. Club 79, 107–109.

Stommel H. 1949: Trajectories of small bodies sinking slowly through convection cells. J. Mar. Res., 8, 24–29.

Thiele R. 1960: Über Lichtadaptation und Musterbildung bei *Euglena gracilis*. Archiv. Mikrobiol., 37, 379–398.

Wager H. 1910: The effects of gravity upon the movements and aggregations of *Euglena viridis* Erhb. and other microorganisms. Proc. Roy. Soc. London, B, 83, 94–96.

Wager H. 1911: On the effect of gravity upon the movements and aggregations of *Euglena viridis* Ehrb. and other microorganisms. Phil. Trans., B, 201, 333–390.

Winet H. and Jahn T. L. 1972 a: On the origin of bioconvection fluid instabilities in *Tetrahymena* culture systems. Biorheol., 9, 87–104.

Winet H. and Jahn T. L. 1972 b: Effect of CO_2 and NH_3 on bioconvection patterns in *Tetrahymena* culures. Expl. Cell Res., 71, 356–360.

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