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Wallackia schiffmanni nov. gen., nov. spec. (*Ciliophora, Hypotrichida*) ein alpiner hypotricher Ciliat

Synopsis. Es wird ein neuer hypotricher Ciliat, *Wallackia schiffmanni* nov. gen., nov. spec., aus den österreichischen Alpen beschrieben. *W. schiffmanni* ist durch die Art der ventralen Bewimperung eine Übergangsform zwischen den Genera *Gonostomum* und *Trachelochaeta*. Die verwandtschaftlichen Beziehungen zwischen den Genera *Wallackia, Gonostomum* und *Trachelochaeta* werden diskutiert.

In der vor kurzem veröffentlichten Revision der hypotrichen Ciliaten teilte Borror (1972) die insgesamt 51 Genera in 6 Familien auf und gab für jedes Genus eine exakte Diagnose. Bei meinen, im Rahmen des österreichischen MAB-6 Programmes der UNESCO durchgeführten Studien über Hochgebirgs — Ciliaten fand ich ein interessantes hypotriches Infusor, für das auf Grund der speziellen Bewimperung der Ventralseite ein neues Genus errichtet werden muß. In der folgenden Beschreibung dieses neuen Ciliaten wird die von Borror (1972) vorgeschlagene Terminologie verwendet.

Untersuchungsmethoden

Der Großteil der Kenntnisse über die Cytologie und über die Anordnung der ventralen und dorsalen Bewimperung von *Wallackia schiffmanni* wurde durch sorgfältige Lebendbeobachtungen im Phasenkontrastmikroskop erhalten. Zur Darstellung der argyrophilen Strukturen wurde eine trockene Versilberungsmethode verwendet (Foissner 1968, 1976), die bei dieser Species allerdings nur mittelmäßige Resultate ergab. Die Kernfärbung erfolgte mit Orcein – Essigsäure.

Beschreibung von Wallackia schiffmanni nov. spec. (Genotyp.) [1]

Wallackia schiffmanni (Abb. 1) wird außer den unten angeführten Genusmerkmalen noch durch folgende Species-Merkmale charakterisiert, die sich als sehr wenig variabel erwiesen: Das Infusor ist 85–100 µm lang, 25–33 µm breit und in der vorderen Hälfte etwas schmäler als am gleichmäßig abgerundeten Hinterende. Es ist mäßig abgeflacht und in der Mitte etwa 7–10 µm dick. Die Tiere waren ausnahmslos sehr undurchsichtig, da das Cytoplasma dicht mit stark lichtbrechender Granula unbekannter Natur gefüllt war. Diese Granula sind farblos, 2–4 µm im Durchmesser und unregelmäßig rund. Außerdem liegen im Ektoplasma viele, ziemlich regelmäßig angeordnete stäbchenartige Strukturen (Abb. 2), die bei stärkerem Deckglasdruck undeutlich werden. Möglicherweise sind es Protrichocysten. Die Tiere enthielten stets nur wenige kleine, mit Bakterien gefüllte Nahrungsvakuolen (Abb. 2).

Das Silberliniensystem (Abb. 3) ist ein typisches Engmaschengitter, mit unregelmäßig polygonalen Maschen von 1–5 µm Größe. Es gleicht also dem der meisten anderen hypotrichen Ciliaten.

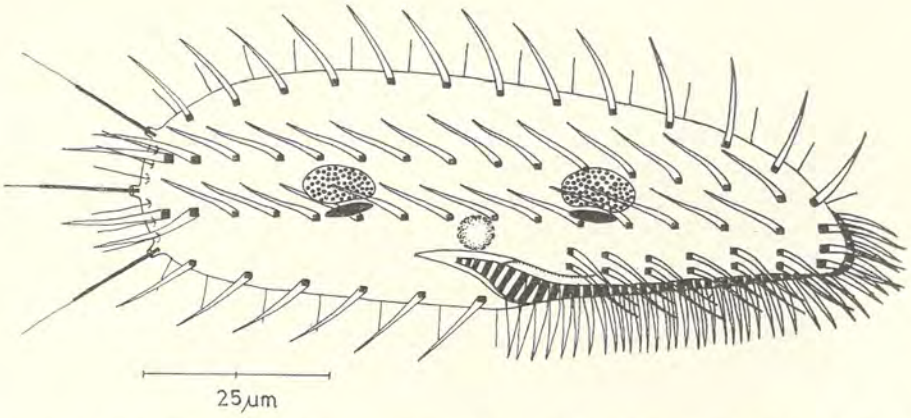


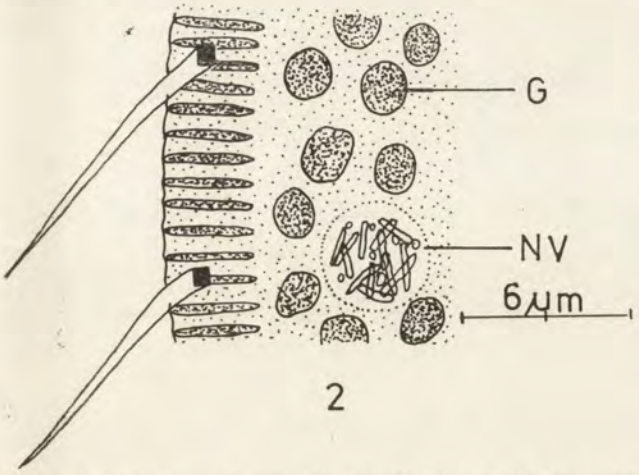
Abb. 1. *Wallackia schiffmanni* nov. spec. (Typ.). Gezeichnet nach Lebendbeobachtungen im Phasenkontrastmikroskop

Der zweiteilige, unregelmäßig nierenförmige Makronucleus ist etwa 10 µm lang und fein granuliert. Jeder Teil besitzt einen, stets links liegenden, etwa 7 µm großen, sehr kompakten spindelförmigen Mikronucleus.

Die Bewegung von *W. schiffmanni* ist *Trachelochaeta* — ähnlich. Es

[1] Diese Art widme ich meinem langjährigen Freund und ständigen Förderer meiner wissenschaftlichen Arbeiten, Herrn Hubert Schiffmann.

kriecht auf seinen Cirren um verschiedene Detrituspartikelchen herum, fährt dann hastig hin und her und steht nur kurz still, wobei die Cirren abgespreizt werden. Die Bewegung ist daher mehr oder weniger ausgeprägt ruckartig, nie typisch gleitend.



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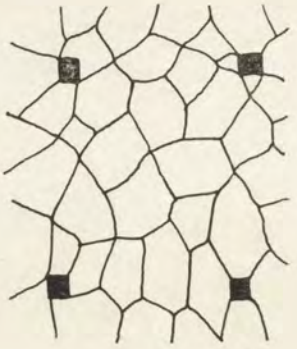

3

Abb. 2. Körperrand von *W. schiffmanni* bei starker Vergrößerung. Der Stäbchensaum (Protrichocysten ?), zwei Cirren, eine Nahrungsvakuole (NV) und die stark lichtbrechende Granula (G) sind erkennbar
Abb. 3. Teil des ventralen Silberliniensystems mit vier Cirren

Die adorale Membranellenzone beginnt an der Stirnseite und endet ziemlich genau in der Mitte des Tieres. Sie ist schmal und ganz an den linken Körperrand verschoben und mit dichten, verhältnismäßig kurzen Membranellen ausgestattet. Diese münden in den gut sichtbaren Pharynx, der keine Schlundfasern erkennen läßt. Die undulierende Membran ist klein und aus mehreren Reihen von verklebten Wimpern aufgebaut. Die kontraktile Vakuole mündet auf der rechten Seite in den Pharynx. Diese für die Oxytrichidae ungewöhnliche Lage der kontraktilen Vakuole sollte noch einmal überprüft werden; in meinen Skizzen habe ich sie jedenfalls stets in der beschriebenen Lage gezeichnet.

Die ventrale und dorsale Bewimperung weist eine Reihe von sehr charakteristischen Besonderheiten auf. Außergewöhnlich kompliziert ist auch das caudale Ende dieses Ciliaten gestaltet. Alle Cirren sind sehr fein, etwa 12–15 µm lang und können abgespreizt werden. Die verhältnismäßig weit voneinander entfernt stehenden Cirren der Marginalreihen sind ganz dem Körperrand genähert. Die linke Reihe beginnt bei der Einmündung der adoralen Zone in den Pharynx und endet so wie die rechte Reihe mit dem Beginn der caudalen Rundung. Die rechte Marginalreihe geht ohne deutliche Unterbrechung in die drei leicht verlängerten, verstärkten, griffelartigen Frontalcirren über, die häufig fühlerartig vorge-

streckt werden. Diese drei Frontalcirren sind von den übrigen Fronto-Ventralcirren erkennbar abgesondert. Ganz der adoralen Zone genähert verlaufen zwei Reihen regelmäßig angeordneter Frontalcirren, von denen jede aus 5–7 Cirren besteht. Ziemlich genau in der Mitte des Tieres befinden sich zwei Reihen von dichter stehenden Ventralcirren, die caudad ohne deutliche Abgrenzung in die nur wenig verstärkten Transversalcirren übergehen. Dadurch ergibt sich eine rechteckige Anordnung der vier Transversalcirren. Da sie so wenig verstärkt sind, habe ich sie zuerst für Ventral — bzw. Marginalcirren gehalten. Sie unterscheiden sich von diesen aber auch insofern, als sie häufig leicht nach innen gekrümmt sind und sich bei einer Schädigung in kräftigere Cilien auflösen als die Cirren der Ventral- und Marginalreihen. Die drei Caudalcirren sind etwa 20 µm lang und entspringen in der Mitte der Dicke des Tieres in rohrartigen Verlängerungen des Ektoplasmas. An der Basis sind sie kräftig entwickelt, werden aber auf der halben Länge plötzlich ganz dünn. Diese feinen Fortsätze sind sehr empfindlich und leicht zu übersehen.

Die Dorsalborsten sind etwa 5 µm lang und stehen zwischen den Cirren der Marginalreihen. Die Anzahl der dorsalen Borstenreihen beträgt 4–6. Am caudalen Ende findet sich eine Gruppe deutlich verlängerter (etwa 10 µm) Caudalborsten, die aus grubenartigen Vertiefungen des Ektoplasmas hervorgehen. Je drei solcher Borsten liegen rechts und links des mittleren Caudalcirrus.

Locus typicus: *Wallackia schiffmanni* fand ich zwei Tage nach der Aufsammlung der Probe in vereinzelten Exemplaren in einem Aufguß von Heuresten eines kleinen Schneewassertümpels in der Nähe des Hochtores (Österreichische Alpen, Großglockner-Hochalpenstraße, 2575 m ü. d. M.). Mit zunehmender Fäulnis des Aufgusses und der damit verbundenen starken Vermehrung der Bakterien, trat auch eine starke Vermehrung von *W. schiffmanni* auf. Nach zwei Wochen gingen aber alle Tiere zugrunde. *W. schiffmanni* dürfte ein β bis α-mesosaprober Ciliat sein.

Diagnose von *Wallackia* nov. gen. [2]

Oxytrichidae mit je einer vollständigen rechten und einer in der Höhe des Cytostoms beginnenden linken Reihe von Marginalcirren, die caudad nicht geschlossen sind. Zwei gerade verlaufende, durchgehende Reihen von Ventralcirren, die ohne deutliche Unterbrechung in die leicht verstärkten Transversalcirren übergehen. Rechts der adoralen Zone noch zwei regelmäßig angeordnete Reihen von Frontalcirren, von denen die

[2] Das neue Genus wird zum Andenken des Herrn Ing. Wallack, dem Erbauer der Großglockner-Hochalpenstrasse, benannt.

vorderen leicht verstärkt und griffelartig ausgebildet sind. Die Caudalcirren sind lang und entspringen in rohrartigen Verlängerungen des Ektoplasmas. Der Körper ist schlank — oval und mäßig abgeflacht. Der Makronucleus ist zweiteilig und besitzt je einen Mikronucleus.

Diskussion

Das Genus *Wallackia* weist eine sehr enge Verwandtschaft mit den Genera *Gonostomum* Sterki, 1878 und *Trachelochaeta* Šramek-Husek, 1954 auf. Vergleicht man die in Tabelle 1 zusammengestellten Angaben über die ventrale Bewimperung dieser drei Genera, so nimmt das Genus *Wallackia* eine Mittelstellung ein. Einerseits besitzt es die rechteckig angeordneten Transversalcirren und die durchgehende rechte Marginalreihe des Genus *Gonostomum*, andererseits sind aber die zwei regelmäßig angeordneten Reihen von Frontalcirren und die zwei geraden — wenn auch durchgehenden — Ventralreihen für das Genus *Trachelochaeta* charakteristisch.

Tabelle 1

Vergleich der ventralen Bewimperung der Genera *Gonostomum*, *Wallackia* und *Trachelochaeta*

Gonostomum	*Wallackia*	*Trachelochaeta*
Rechte Marginalreihe bei den Frontalcirren beginnend und in der Höhe der Transversalcirren endend.	Rechte Marginalreihe bei den Frontalcirren beginnend und in der Höhe der Transversalcirren endend.	Rechte Marginalreihe in der Höhe des Pharynx beginnend und in der Höhe der Transversalcirren endend.
Zwei schräg verlaufende, nicht durchgehende Reihen von Ventralcirren. Rechts der adoralen Zone nicht reihenförmig angeordnete Frontalcirren.	Zwei gerade verlaufende, durchgehende Reihen von Ventralcirren. Rechts der adoralen Zone zwei regelmäßig angeordnete Reihen von Frontalcirren.	Zwei gerade verlaufende, in der Tiermitte beginnende Reihen von Ventralcirren. Rechts der adoralen Zone zwei regelmäßig angeordnete Reihen von Frontalcirren.
Kein abgegrenztes Feld mit drei Frontalcirren.	Abgegrenztes Feld mit drei Frontalcirren.	Abgegrenztes Feld mit drei Frontalcirren.
Die vier Transversalcirren sind quadratisch angeordnet.	Die vier Transversalcirren sind rechteckig angeordnet.	Die fünf Transversalcirren sind schräg angeordnet.
Caudalcirren vorhanden oder fehlend.	Lange Caudalcirren vorhanden.	Lange Caudalcirren vorhanden.

Wallackia schiffmanni kann daher als eine Übergangsform zwischen den Genera *Gonostomum* und *Trachelochaeta* aufgefaßt werden. In Aner-

kennung des von Precht (1935) formulierten Satzes, "daß es sicherlich einem natürlichen System widersprechen würde, wollte man alle Reduktionsformen oder auch Übergangsformen der Gattungen zusammenfassen", scheint es gerechtfertigt, diese Art in ein neues Genus zu stellen. Dies umsomehr, als auch viele andere Genera der hypotrichen Ciliaten nur durch sehr geringe Unterschiede der ventralen Bewimperung getrennt werden (s. Borror 1972). Die abschließende Klärung der systematischen Stellung der Genera *Gonostomum, Trachelochaeta* und *Wallackia* kann nur durch das Studium ihrer Morphogenese erfolgen, über die derzeit aber keine Daten verfügbar sind.

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Mit dankenswerter Unterstützung des österreichischen MAB-6 Programmes der UNESCO, des Fonds zur Förderung der wissenschaftlichen Forschung (Projekt 1838 und N 39), der Jubiläumsstiftung der österreichischen Nationalbank, der Gesellschaft zur Förderung der Hochschule für Bodenkultur und der Naturkundlichen Station der Stadt Linz.

SUMMARY

A new hypotrichous ciliate, *Wallackia schiffmanni* nov. gen., nov. spec., occurring in the Austrian Alps is described. Its ventral ciliature shows that *W. schiffmanni* is a transition form of the genera *Gonostomum* and *Trachelochaeta*. The relationships amongst the genera *Wallackia*, *Gonostomum*, and *Trachelochaeta* are discussed.

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C. K. SINHA and A. K. MANDAL

Trypanosoma enhydris sp. n. from a Fresh Water Snake *Enhydris enhydris* (Schneider)

Synopsis. The description of a new species of Trypanosome, *Trypanosoma enhydris* sp. n. (*Trypanosomatidae*) from a fresh water snake, *Enhydris enhydris* collected from West Bengal, India is incorporated in the paper. Its affinities with the known species of the genus and differences to consider it as new species are also incorporated.

This is the first instalment of the series deals with the haemoflagellates of some Indian reptiles. It includes the description of a new species of trypanosome from a fresh water snake *Enhydris enhydris* (Schneider). The snakes were captured from Chakdah, Nadia, West Bengal, India during the month of March to June 1975, and brought to the laboratory for examination. Out of the 50 snakes examined 30 were found positive for trypanosome. Blood films were obtained by clipping the tail and stained with Giemsa's, Leishman's stain and Wright stain. Tissues fixed in Bouins Duboscq brasil's fluid for studying endogenous stage. No tissue phase is seen in the preparation. However, observation to note down the tissue phase, intermediate vector along with some cross transmission experiments are being carried out in the laboratory. The results of which will be published in due course. Drawings were made with the help of a camera lucida with the uniform magnification 1600 ×. Measurements were made from camera lucida drawing along a line drawn from the anterior to the posterior end through the middle of the parasite. Estimated parasitemia 2–5 trypanosomes per cubic mm of blood films. The slides will be deposited to the National collection of the Zoological Survey of India.

Description

Trypanosoma enhydris sp. n.

Holotype: Z. S. I. Registration No 1835
Type Host: *Enhydris enhydris* (Schneider)
Type Locality: Chakdah, Nadia, West Bengal, India.

The trypanosoma is polymorphic. Three distinct forms viz. small with free flagellum of considerable length, intermediate with a long free flagellum and a large form with a short flagellum are seen in the peripheral blood. Some undergoing division along with the epimastigote forms have also been encountered.

In the citrate solution, they were found sluggish and sometimes form a knot. Measurements of the organism are given in Table 1.

Table 1

Measurements of *Trypanosoma enhydris* sp. n. (in microns)

Form	Small	Intermediate	Large
Length from posterior end to the kinetoplast	8.5 (7.5–9)*	16 (15.5–17.5)	39 (38–49)
Length from kinetoplast to the nucleus	2.15 (2–3.5)	2.5 (2–3)	1.5 (1–2)
Length of the nucleus	1.75 (1.50–2.50)	2 (2–2.5)	6 (5–7)
Length from nucleus to anterior end of body	15 (14–16.5)	18.5 (17–19.5)	60 (55–62)
Length of the free flagellum	13 (12–14)	15 (14.5–17.5)	10 (9–11)
Length of the kinetoplast	0.86 (0.5–1)	0.85 (55–1.5)	1.5 (1–2)
Width of the kinetoplast	0.45 (0.40–0.86)	0.65 (55–0.86)	0.40 (0.40–0.42)
Width of cell body	2 (1.5–2.4)	5 (4–5.5)	27 (24–30)

* Mean and range (in parantheses).

Small form

Slender, elongated and sharply pointed at both ends (Fig. 1). Their number varies from 5 to 1″ in the films. Cytoplasm homogenous with scattered small vacuoles. It is deeply stained with Giemsa and more towards the border opposite to undulating membrane. Cytoplasm towards periphery stains faintly and become almost without any stain at the posterior extremity. No myonemes or granular arrangements are found on the cytoplasm.

Nucleus exhibits a round or oval shape situated almost at the middle. The chromatin granules are arranged towards the distinct nuclear membrane sometimes leaving a decided gap at the centre.

Kinetoplast almost subspherical situated slightly posterior to the nucleus and appeared as a black dot with a halo around, hardly conical form with deep stain is noticed in the preparation.

Flagellum originates from the base of the kinetoplast, trails anteriorly bordering the undulating membrane and extends beyond the body as free flagellum.

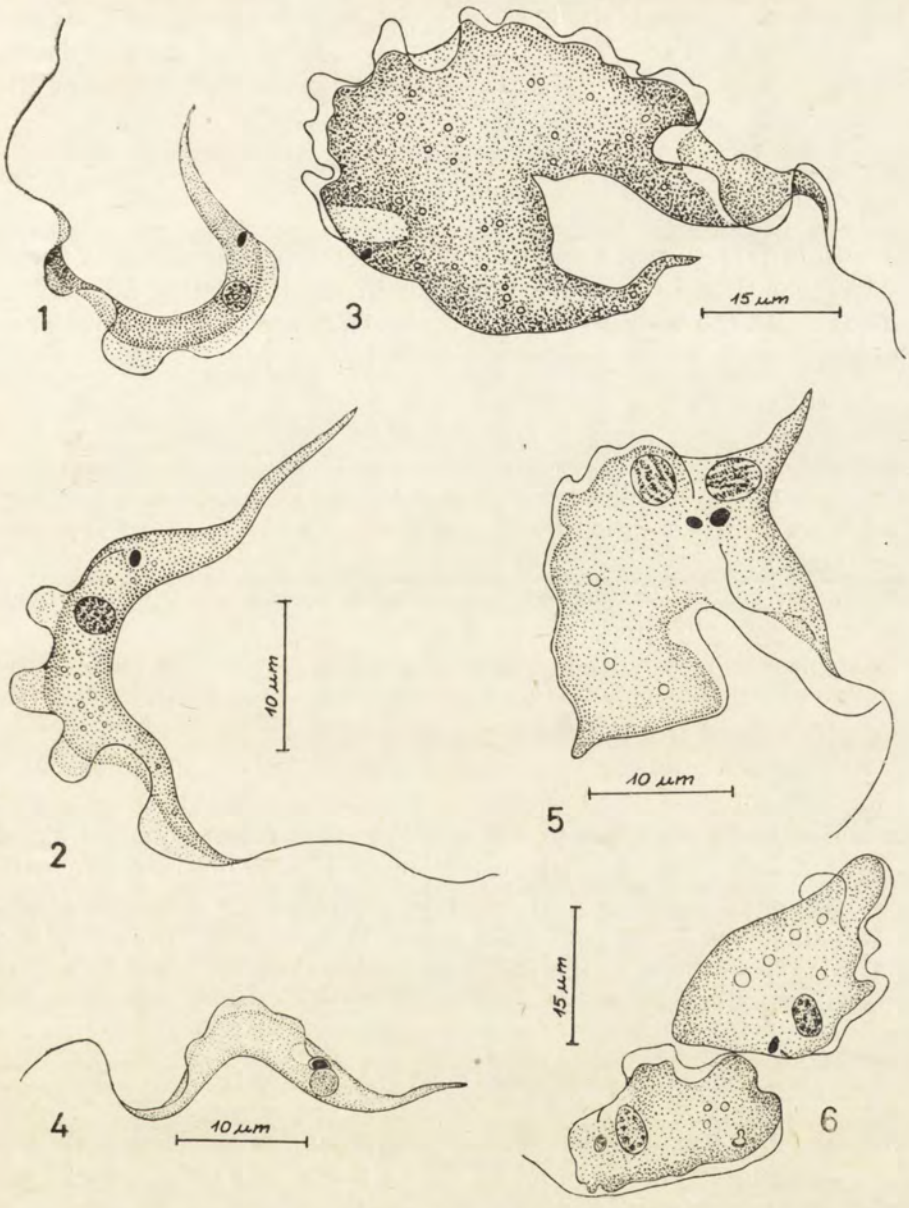

Fig. 1-6. *Trypanosoma enhydris* sp. n. 1 — Small form, 2 — Intermediate form, 3 — Large form of *T. enhydris* in peripheral blood, 4 — Epimastigote form of *T. enhydris* in the peripheral blood, 5 — Trypanosome (divisional stage) with two nuclei and two kinetoplast, 6 — Two daughter individual after division. (Figs. 1, 2, 4, 5 are in one, and Figs 3, 6 in other magnification)

The undulating membrane is conspicuous with three or four folds and stains light red with Giemsa. The body margin bordering the undulating membrane is evenly curved and does not follow the undulations of the membrane.

Intermediate form

This form (Fig. 2) resembles the small one morphologically but varies in size (Table 1) and the number of folds in the undulating membrane. The folds touch the body five or six times. They are in great abundance in the peripheral blood approximately 15–20 in each film.

Large form

They are few in number sometimes about 3 to 5 in one film; heavily stained body with a narrow undulating membrane and a short free flagellum (Fig. 3).

Cytoplasm coarse, vacuolated and takes a deep stain. Nucleus oval, stains very light with faintly granular chromatin materials.

Kinetoplast adheres to the nucleus and stains deeply. The folds of the undulating membrane waved 12 to 14 times bordered by the thick flagellum with a short free portion at the anterior end.

In addition to these, some epimastigote forms (Fig. 4) are also encountered in the peripheral blood where a dark blue kinetoplast is located at the anterior end very close to the nucleus. This form measures about 23 μm to 27 μm (mean 25 μm) by 3.5 μm to 4.5 μm (mean 4 μm) with a free flagellum 9 μ to 10 μm in length. Moreover, some divisional stages with two kinetoplasts and two nuclei almost divided with their cytoplasm (Fig. 5) have been found in the peripheral blood along with some daughter trypanosomes (Fig. 6).

Diagnosis of *Trypanosoma enhydris* sp. n.

The described haemoflagellate is polymorphic; small, intermediate and large; measuring 40 μm by 2 μm, 54 μm by 5 μm, and 116 μm by 27 μm respectively in total length including the free flagellum.

Cytoplasm homogeneous with few vacuoles and without any volutin granules. Kinetoplast always away from posterior end, nucleus sub-central and situated at the middle of the cytoplasm; undulating membrane distinct, prominently bordered by the thick flagellum with considerable free end. The body cytoplasm does not participate in forming the folds of undulations.

Discussion

Walliker (1965) reviewed the raptilian trypanosomes and listed fifteen snake trypanosomes of which three were reported as *Trypanosoma* sp.

The species under report resembles *Trypanosoma primetti* Mathis et Legger, 1909 (Host: *Torpidonotus*=*Natrix piscator*) due to body configuration and shape, but the former is polymorphic and the latter is dimorphic.

Moreover, the total length of the polymorphic forms including the free flagellum, measure about 40 µm by 2 µm, 54 µm by 5 µm, and 116 µm by 27 µm, of the species encountered; whereas in *Trypanosoma primetti* the dimorphic forms measure 57 µm by 7 µm and 105 µm by 14 µm.

Another species of trypanosome from *Natrix piscator* was reported in Pakistani Science congress by Haq and Mohiuddin (1956), without giving any detail account. So the specimen dealt herewith could not be possible to compare with that species. However, efforts were made to inoculate the present trypanosome to *Natrix piscator* in the laboratory but were unsuccessful. Simultaneously the present trypanosome was inoculated to infection free *Enhydris enhydris*, the natural host and found to be positive after 3 to 5 days.

These experiments led us to conclude that the present trypanosome is quite different from those of the species occurring in *Natrix piscator*. In addition, the present species does not approaches any other known trypanosomes hence it is described as new and designated as *Trypanosoma enhydris* sp. n. The specific name of the parasite was given after the specific name of the host.

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RÉSUMÉ

L'article s'agit de la description d'une nouvelle espèce, *Trypanosoma enhydris* (*Trypanosomatidae*), recueillie d'un serpent, *Enhydris enhydris* (Schneider), qui vit dan l'eau douce du Bengale occidental de l'Inde. Son affinité avec les espéces connues du genre et ses caractéres trés distingués sont aussi mentiònés.

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ACTA PROTOZOOLOGICA

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Three New Coccidian Species (*Coccidia*: *Eimeriidae*) Found in Wild Birds from Bulgaria

Synopsis. The present report describes three new coccidian species belonging to the genera *Eimeria* and *Isospora*, found in wild birds from Bulgaria: *Eimeria turturi* n. sp. (Host *Streptopelia turtur turtur* (L.)), *Eimeria ridjakovi* n. sp. (Host *Perdix perdix perdix* (L.)) and *Isospora nankinovi* n. sp. (Host *Garrulus glandarius graecus* Kleiner). A morphometric characteristics of the oocysts was made of the newly described taxons and date have been given concerning the sporulation time, the prevalence, location in the host and localities where the investigated birds have been collected.

Eimeria turturi n. sp. Pl. I 1, 2 Fig. 1

Description: The oocysts are colourless, ellipsoidal or broadly oval in shape. Micropyle absent. Oocyst wall smooth, double layered, 1.5 μm thick. The endocystic layer is thicker and darker in colour. The size of 57 of the measured oocysts ranges from 22.8 to 29.2 μm in length and from 17.8 to 25.4 μm in breadth, the average being 26 × 21.6 μm.

The cytoplasmic body in unsporulated oocysts is round and usually eccentrically situated. Its average diameter being 17.5 μm. The sporulation time is about 48 h at room temperature ($t° = 22°C \pm 1°C$). Oocyst residuum and polar granules have not been observed in the sporulated oocysts.

The sporocysts are elongate-ellipsoidal and tapering at the one end. Stiedae body absent. Sporocyst size ranges from 11.5 to 13 μm in length and 6.0 to 7.5 μm in breadth. The sporocyst residuum dispersed among the sporozoites.

Type host: turtle-dove (*Streptopelia turtur turtur* (L.))

Location: the oocysts were found in the large intestine and the faeces of the investigated birds.

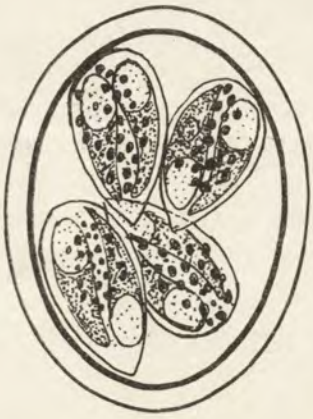

Fig. 1. *Eimeria turturi* n. sp. × 2000

Prevalance: 12.12% out of 33 turtle-douve investigated. Locality: county of Mihailovgrad, Nord-West of Bulgaria (17.VIII. and 23.VIII. 1975).

Discussion: Out of the known species of genus *Streptopelia* (17 species according to Kartachev 1974) only the species *Eimeria choudari*, found in *Streptopelia decaocto* from India has been so far described (Bhatia et al. 1972). *E. choudari's* oocysts are subspherical to broadly ellipsoidal in shape and considerably smaller in size: 16.9–22.1 μm in length and 13.0–18,2 μm in breadth (the average being 19.24 × 16.6 μm). Besides the difference in shape and size of the oocysts, a comparatively large polar granule in the sporulated oocysts and the presence of Stiedae body in the sporocysts have been observed in *E. choudari* that are missing in *E. turturi* n. sp.

Pellerdy (1974) dealing with the specificity of the coccidian species in douves states that "It still remains to be clarified, however, whether or not the genera *Columba* and *Streptopelia* are host specific". Proceeding from the above statement, the comparison we made between *E. turturi* n. sp. and known coccidian species of the genus *Columba* shows a certain morphological resemblance of the oocysts of the reported new species from turtle-doves to the species *E. kapotei* Chatterjei and Ray, 1969 from *Columba livia intermedia* and *E. labbeana* (Labbe, 1896) Pinto, 1928 from *C. domestica*, *C. livia livia* and *Streptopelia orientalis mecna*. *E. turturi* n. sp. differs from *E. kapotei* in the lack of micropyle of the oocysts and of Stiedae body of the sporocysts. The newly described species differs from *E. labbeana* as well in its larger sized and colorless oocysts and the lack of Stiedae body in the sporocysts.

Eimeria ridjakovi n. sp. Pl. I 3, 4, Fig. 2

Description: The oocysts are elongate oval or ellipsoidal and rarely egg-shaped. They are light yellow. The oocystic wall is about 2.4 μm thick. The ectocystic wall is lighter in colour and slightly punctate. The endocystic wall is smooth and darker in colour. The size of 46 of the measured oocysts ranges from 24.1 to 30.5 μm in length and from 16.5 to 20.3 μm in breadth, the average being 27.3 × 18.4 μm.

The sporulation time of the oocysts is about 48–56 h at $t° = 24°C \pm \pm 1°C$. One — two or sometimes more polar granules are observed in

the sporulated oocysts. In some cases, when their number is greater, the polar granules are distributed in groups of 2–4 among the sporocysts.

The sporocysts are elongate oval and tapered at the one end. The tapered pole is full of hyaline substance, lighter in colour. The sporocysts are 12.6–17.8 × 8.9–11.0 μm in size, the average being 15.2 × 11.2 μm. The sporocyst residuum is dispersed among the sporozoites.

Type host: patridge (*Perdix perdix perdix* (L.)).
Location: small intestine.
Prevalence: 8.6% out of 36 examined specimens.
Locality: counties of Vidin and Mihailovgrad, Nord-West of Bulgaria (2.XI. and 8.XI.1975).

Discussion: There have been so far described two coccidian species from the patridge belonging to the genus *Eimeria: E. kofoidi* Yakimoff et Matikashwili, 1936 and *E. procera* Haase, 1939. *E. ridjakovi* n. sp. differs considerably from *E. kofoidi* namely in the shape and the size of the oocysts. *E. kofoidi* has oval or round oocysts, which are smaller in size: 16–25 × 14–20 μm (the average being 20 × 17.6 μm) (cit. according to Pellerdy 1974).

Fig. 2. *Eimeria ridjakovi* n. sp. × 2000

E. ridjakovi n. sp. is similar in shape and size to the oocysts of *E. procera,* described by Haase (1939) from Germany. According to Haase the oocysts of *E. procera* are elongate ellipsoidal in shape and are 28.8–31.2 × 16.4–17.2 μm in size. The oocystic wall is smooth, about 1.5 μm thick, greenish in colour. Besides, the author points out that the oocysts are slightly flattened laterally and have a micropyle, which is readily observed after sporulation. The light yellow colour of oocysts, the lack of micropyle, the more oval shape of the oocysts and especially the punctate structure of the ectocystic wall are typical taxonomic features of *E. ridjakovi* n. sp. which distingish this species from *E. procera,* found also in patridges from Bulgaria by Golemansky (1976 in press).

Isospora nankinovi n. sp. Pl. II 5–7, Fig. 3

Description: The oocysts are oval and colourless. The oocystic wall is comparatively thin (about 1.2–1.5 μm). The endocystic wall is darker and thicker. The size of the oocysts ranges from 30 to 35 μm in

length and from 22.5 to 28.5 μm in breadth. The average being 32.5 × × 25.5 μm.

The sporulation time of the oocysts is about 36–42 h at $t° = 25°C \pm \pm 1°C$.

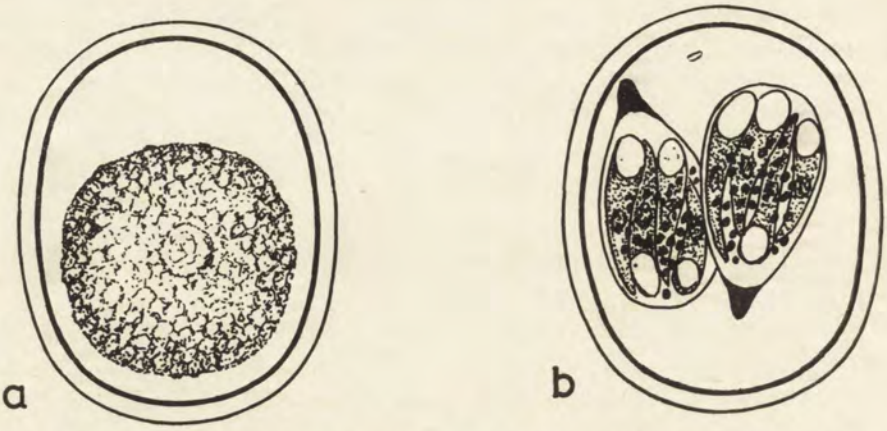

Fig. 3. *Isospora nankinovi* n. sp. × 2000, a — unsporulated oocyst, b — sporulated oocyst

Oocyst residuum absent in the sporulated oocysts, but a polar granule could be observed. Sporocysts pear-shaped with a conspicuous Stiedae body. The size of the sporocysts ranges from 18 to 21 μm in length and from 10 to 11.5 μm in breadth. The sporocyst residuum dispersed among the sporozoites.

Type host: jay (*Garrulus glandarius graecus* Kleiner).

Location: small intestine.

Prevalence: 12.5% out of 8 examined jays.

Intensity of invasion: *I. nankinovi* n. sp. has been observed in one case with a very high intensity of invasion: 32 oocysts on the average in the range of vision of the microscope with 10 × eye-piece lens and 16 × objective. (Pl. II, 5).

Locality: county of Mihailovgrad, Nord-West of Bulgaria (25. VII. 1975).

Discussion: Ray et al. (1952) describe for the first time the species *E. garrulusae* from the jays of the Himalayan region (*Garrulus glandarius bispecularis*). This species has oval or subspherical oocysts with a greenish tinge and considerably smaller size: 25–27.5 × 20–25 μm (the average being 25.5 × 21.2 μm). Besides, a micropyle has been observed in the oocysts of *I. garrulusae*, which is not present in the newly described species. In shape and size the oocysts and sporocysts of *I. nankinovi* n. sp. differs considerably as well as from the rest of the known up to now coccidia of genus *Isospora* in the birds from the family *Corvidae*.

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I take the opportunity to acknowledge my gratitude and thanks to my colleagues Dr. D. Nankinov from the Institute of Zoology in Sofia and Eng. N. Ridjakov from the town of Berkovitza for the kindly offered materials from wild birds for parasitological investigation.

RÉSUMÉ

Dans le présent travail sont decrites trois nouvelles espèces de Coccidies des genres *Eimeria* et *Isospora*, parasites des oiseaux sauvages en Bulgarie, a savoir: *Eimeria turturi* n. sp. (Hôte *Streptopelia turtur turtur* (L.)), *Eimeria ridjakovi* n. sp. (Hôte *Perdix perdix perdix* (L.)) et *Isospora nankinovi* n. sp. (Hôte *Garrulus glandarius graecus* Kleiner). Ils sont indiqués aussi les caractères morphométriques des ookystes des nouveaux taxons, le temp de la sporulation, l'extensité de l'invasion, la localisation dans l'hôte et les localités, d'ou ils sont recoltés les oiseaux explorés.

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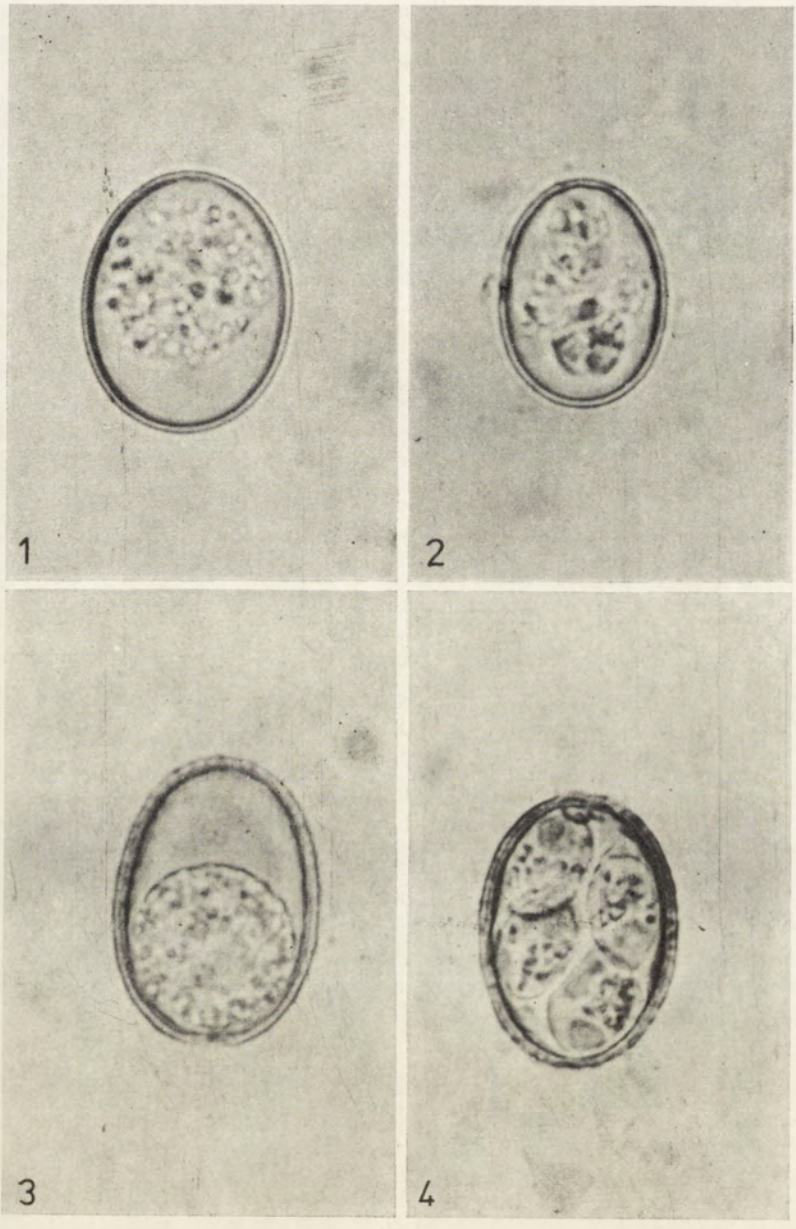
EXPLANATION OF PLATES I–II

1 : *Eimeria turturi* n. sp., unsporulated oocyst. × 1350
2 : Id., sporulated oocyst. × 1350
3 : *Eimeria ridjakovi* n. sp., unsporulated oocyst. × 1350
4 : Id., sporulated oocyst. × 1350
5 : Oocyst of *Isospora nankinovi* n. sp. × 160
6 and 7 : *Isospora nankinovi* n. sp. × 700. The arrow points out the polar granule



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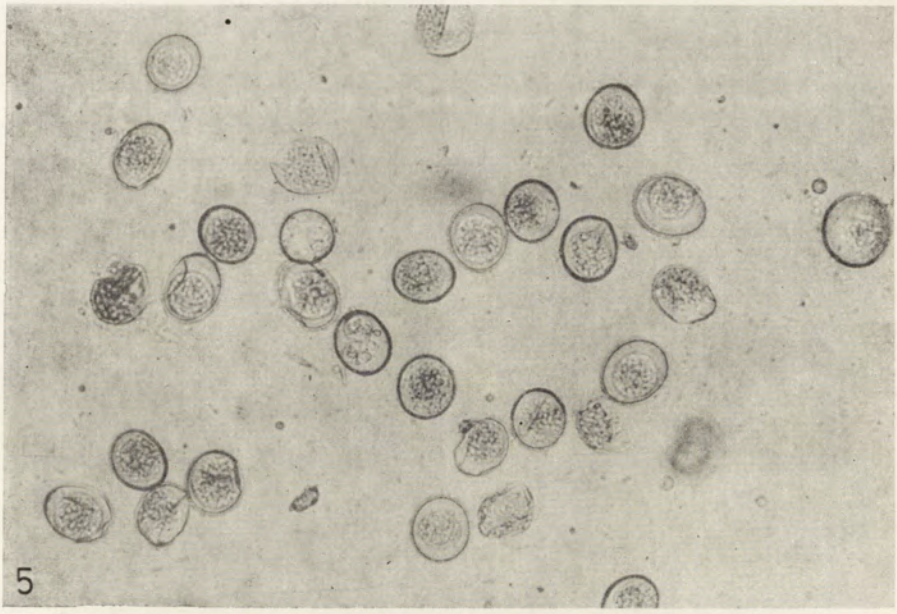

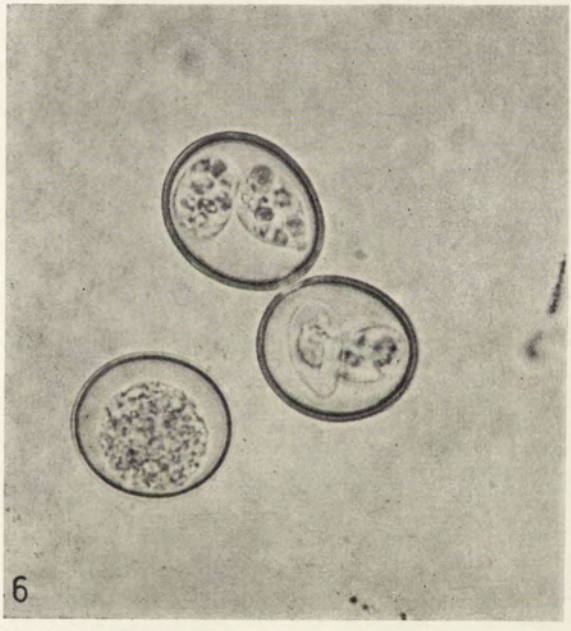

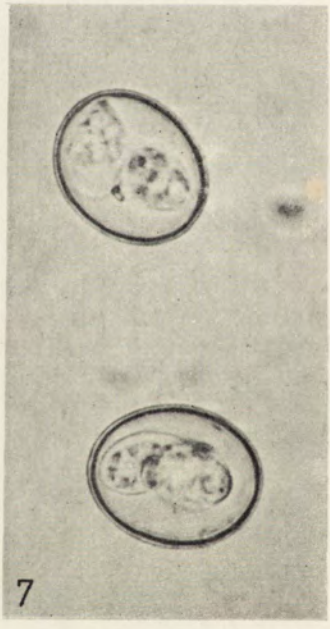

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ACTA PROTOZOOLOGICA

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Caryospora bengalensis sp. n. and *Eimeria fibrilosa* sp. n., New Coccidia (*Protozoa: Eucoccida*) from a Fresh-water Snake, *Enhydris enhydris* (Schneider)

Synopsis. Observations on the sporulations and sporulated oocysts of two new coccidia viz. *Caryospora bengalensis* sp. n. and *Eimeria fibrilosa* sp. n. (*Eimeriidae, Eucoccida*) from an Indian fresh water snake, *Enhydris enhydris* (Schneider) have been made. They have been compared with the allied species.

During the course of investigation of the coccidian parasites of Indian snakes the present author has examined 50 specimens of a fresh-water snake, *E. enhydris*, from Chakdah Nadia Dist., West Bengal. Of which, 30 examples are found to be infected with two species of coccidia one is tetrasporocystid dizoic belonging to the genus *Eimeria* and the other is monosporocystid octozoic falls under the genus *Caryospora*. In almost all instances simultaneous infections of both the species are noticed except in few, where single infection has also been encountered.

Four species of *Eimeria* from Indian snakes was described by Ray and Dasgupta (1936, 1938) (*E. piscatori* and *E. najae* and *E. stolatae*) and Bhatia (1936) (*E. gupti*). A. K. Mandal (in press) in his work on coccidia of Indian vertebrates described in detail the above mentioned species. Only one Caryosporan species from India has so far been described by Chakravorty and Kar (1947), that too from a wall lizard, *Gecko gecko*. The type slides will be deposited to the National Collection of the Zoological survey of India.

Material and Methods

The snakes were brought to the laboratory and faecal samples were directly taken out from the rectum and examined under microscope. In case of positivity, the smears from different regions of the intestine were also examined. The faecal

samples were kept in 2.5% Potassium dichromate solution. The sporulation and morphological details of oocyst were studied under oil immersion objective of Olympus phase contrast microscope with 10 × eye-piece lens. Sketches were drawn with the aid of a Spencer's camera lucida. Different tissues have been preserved in Bouin's, Duboscq fluid for endogenous study and the results of this will be communicated elsewhere.

Description

Caryospora bengalensis sp. n.

The oocysts (Fig. 1) are spherical in shape, and 50 of them measuring 20 μm–22.5 μm in diameter with a mean 21.5 μm. The shape index is 1.07. The oocystic wall consists of double layers, appears very thin and with slight pressure it ruptures. A micropyle is visible on the wall of the oocyst but devoid of any oocystic residuum. The cytoplasm of the oocyst is centrally placed and appears like a globular mass. Only one double-layered pear-shaped sporocyst (Fig. 2) measuring 18 μm 20.5 μm in length with a mean 19 μm and 12 μm–14 μm width with a mean 13.5 μm is seen inside the oocyst after development. The shape index of the sporocyst

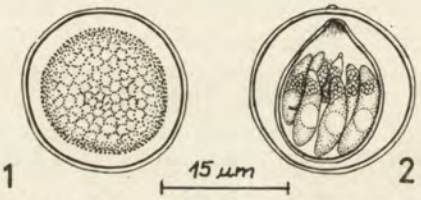

Fig. 1–2. *Caryospora bengalensis* sp. n. 1 — Unsporulated oocyst. 2 — Sporulated oocyst

is 1.4. At the pointed end of the sporocyst a steida-body with a shiny plug is found in the preparation. Sporocystic residuum is globular with many variable sized refractile granules scattered throughout. Eight bean-shaped sporozoites measuring 10 μm–12 μm in length with a mean 11.00 μm and 2.5 μm in broadest width are encountered with centrally placed nucleus. The smear of the intestine is also shown a good number of unsporulated oocyst.

Sporulation time 24 to 38 h at room temperature 30°C–32°C.

Diagnosis: Oocysts spherical, measuring 20.00 μm to 22.5 μm in diameter with a mean 21.5 μm, double layered wall with a micropyle. Sporocyst pear-shaped, measuring 18 μm–20 μm in length with a mean 19 μm and 12 μm to 14 μm in width with a mean 13.5 μm. It is provided with a steida-body, sporozoites bean-shaped measuring 10 μm-12 μm in length with a mean 11 μm, and 2.5 μm in broadest width; sporocystic residuum present as scattered refractile granules.

Type-host — *Enhydris enhydris* (Schneider)
Seat of infection: Intestine
Type locality: Chakdah, Nadia dist., West Bengal, India
Holotype: Z. S. I. Registration No 1842

Remarks: According to Pellerdy (1969), nineteen species of *Caryospora* have so far been reported from different group of vertebrates. Out of which eleven species have been described from ophidian host. The present species resembles *S. brasiliensis* Carini, 1932 due to shape and size of the oocyst, and possession of double layers in oocyst and sporocyst. But the species dealt with differs from *C. brasiliensis* due to presence of micropyle and absence of the same on sporocystic wall. The sporozoite of the former is oval-shaped but that of the latter is bean-shaped. Steida-body with a shiny plug in the sporocyst encountered is also not found in *C. brasiliensis*.

It also comes close to *C. japonicum* Matsubayashi, 1937 in shape of the oocyst, bilayered oocystic wall, steida-body in sporocyst and banana-shaped sporozoites but differs due to presence of micropyle on oocyst wall and double-layered sporocystic wall. The oocyst also varies in size (18.6 μm vs. 21.5 μm).

Hence it is evident that the present one does not resemble to any known species described so far and designated as new and named as *Caryospora bengalensis* sp. n.

Eimeria fibrilosa sp. n.

Both unsporulated and sporulated oocysts are encountered in the preparations. They are ellipsoidal (Fig. 3) in shape and fifty of them measuring 24 μm–27 μm length with a mean 25.5 μm and 13.5 μm to 22.5 μm in width with a mean 18 μm. The length-width ratio is 1.36. It is provided with double-layered wall of 0.75 μm thickness, the outer being thinner than the inner one. No micropyle is visible on the oocystic wall. The cytoplasm of the oocyst is coarsely granular and almost spherical in shape measuring 9 μm–10 μm in diameter. During the course of development, four rounded sporoblasts of 5 μm–6 μm in diameter are seen in the preparation. A cytoplasmic prolongation from both sides of the sporoblast comes out with a definite wall to form the naviculoid sporocyst. (Figs. 5, 6). A clear shiny steida-body at both ends of the sporocyst is clearly visible and appears like a glume of the paddy. On careful examination, fine fibril of 6 μm in length is observed attached to each end of sporocyst which comes out from the side of the sporocyst (Fig. 7).

The oocystic residuum appears as refractile globular mass. Sporocyst measures 10 μm–15 μm in length with a mean 12.5 μm and 5 μm–6 μm

in width with a mean 5.5 μm. The shape index 2.09. The cytoplasm of sporocyst appears as beaded refractile mass. Two bean-shaped sporozoites along with refractile bodies situated at both ends of each sporozoites are clearly visible in the preparation. The sporozoite measures 10.5 μm–14.5 μm × 7.5 μm–8.5 μm with a mean 12.5 μm × 8 μm. The length width ratio being 1.56. After sporulation the sporocystic residuum appears like a beaded mass and takes the position at both ends of the sporocyst. On the examination of the oocyst after few days from 2.5% Potassium dichromate solution, it is seen that four refractile globules of the sporozoite become very prominent.

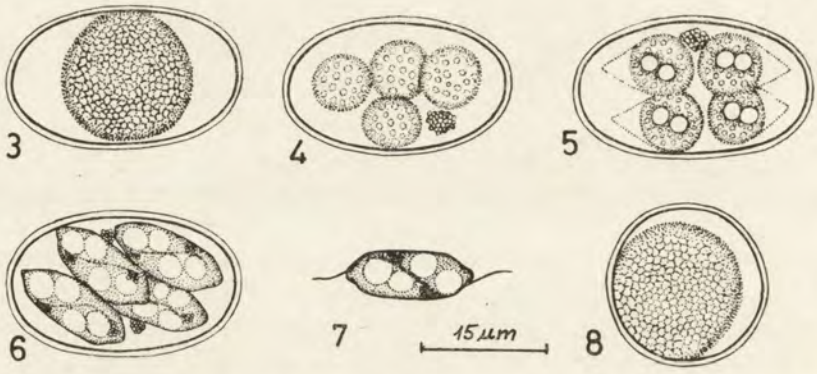

Fig. 3–8. *Eimeria fibrilosa* sp. n. 3 — Unsporulated elliptical oocyst. 4 — Oocyst with four sporoblast. 5 — Sporoblast with cytoplasmic prolongation (showing in one side only) during sporulation. 6 — Four naviculoid sporocysts after complete sporulation. 7 — Sporocyst of *E. fibrilosa* with fibril at each end. 8 — Unsporulated spherical oocyst of *E. fibrilosa*

Though the elliptical form of the oocyst is very common in the preparation, simultaneously some sub-spherical to round (Fig. 8) forms of 13.5 μm to 19.5 μm in diameter with a mean 16.5 μm are also been encountered in the preparation.

Sporulation time 50–72 h at room temperature 30°C–35°C.

D i a g n o s i s: Oocysts are elliptical measuring 24 μm-27 μm × 13.5 μm–25.5 μm with a mean 25.5 μm × 18 μm. Sporocysts are naviculoid in shape, measuring 12.5 μm × 5.5 μm. Both ends of the sporocyst are provided with shiny-areas with a free fine fibril of 6 μm in length in each end. Two sporozoites, bean-shaped measuring 12.5 μm × 8 μm, contain two refractile globules.

Type host: Common water snake, *Enhydris enhydris* (Schneider)
Seat of infection: Intestine
Type locality: Chakdah, Nadia, West Bengal, India
Holotype: Z. S. I. Regd. No 1841

Remarks: Twenty seven species of *Eimeria* have so far been described from ophidian host Pellerdy (1969). Of which only four are from Indian snake. The present species resembles *E. amarali* Pinto, 1928 due to the shape of the oocyst and absence of micropyle but differs in size of the oocyst 24 μm–27 μm × 13 μm–22 μm vs. 27.2 μm–34 μm × 17 μm–18.7 μm, in the shape of the sporocyst, round to elongated one vs. naviculoid and the presence of oocystic residuum. Moreover, the oocyst of the present one is double layered in lieu of three layered-wall in *E. amarali.*

It also comes close to *E. cerastis* (Chatton, 1912) Phisalix, 1921, due to the ellipsoidal shape of the oocyst but differs in having oocystic residuum and naviculoid shape of sporocyst. Moreover, the former is reported from the gall-bladder of a viper, where as the latter inhabits in the small intestine of a fresh water snake. The size of the oocyst of *E. najae* Ray et Das Gupta, 1936 also approaches the present form but the shape of the oocyst of the former is oval where as that of the latter is ellipsoidal. The present one also resembles to *E. zamenis* Phisalix, 1921 due to the shape of the oocyst but the species dealt with is smaller in size with an oocystic residuum and oval sporocyst instead of naviculoid sporocyst of the latter. In addition, *E. zamenis* inhabits in the gall bladder where as the present form occurs in the small intestine. The presence of fibrilar appendage in the sporocyst of the species dealt with is unique but almost similar structure has also been noted by Setna and Banna (1935) while describing *E. harpodoni* from a fish *Harpodon nehereus* (Ham-Buch).

So, the present one is unique in its peculiar characteristics such as the naviculoid sporocyst with shiny-areas at both ends along with free fine fibril, therefore, it is considered as new and named as *Eimeria fibrilosa* sp. n.

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RÉSUMÉ

L'auteur s'essaie de presenter à l'article ses observations sur le phénomène de sporulation et sur la conduite des oocystes surgis dans deux nouvelles espèces

des *Coccidia*, viz., *Caryospora bengalensis* et *Eimeria fibrilosa* (*Eimeriidae, Eucoccida*), d'un serpent indien *Enhydris enhydris* (Schneider), vivant dans l'eau douee. Elles ont été comparées à leurs alliées.

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Four New Species of Microsporidians from Termites[1]

Synopsis. The morphology and life-history of four new species of microsporidians, *Gurleya spraguei* n. sp. infecting the adipose tissue of *Macrotermes estherae* (Desn), *Pleistophora weiseri* n. sp. from the gut of *Coptotermes heimi* (Wasm), *Pleistophora ganapatii* n. sp. from the gut of *Odontotermes horni* (Desn) and *Stempellia odontotermi* n. sp. from the gut of *Odontotermes* sp. are described.

The seasonal distribution of one of the microsporidians, *Gurleya spraguei* n. sp. showed the maximum percentage infection in February and the minimum in the months of May, June and July.

Hypertrophy of the host cells has been observed in *Gurleya spraguei*. The absence of fat globules and mucoidal strands in the infected cells suggest that the parasites may utilize these cell inclusions during growth and differentiation.

Isopterans, popularly known as termites are a complex group of social insects causing considerable damage to plant life and timber. Although there is voluminous literature on the biology of termites and a fair amount of information on the symbiotic hypermastigine flagellates in the alimentary tract of these termites very little is known regarding the microsporidian parasites infecting the various organs of the termites.

A perusal of the literature on the microsporidia of insects shows that the order *Isoptera* has received scanty attention and only three species, *Pleistophora* sp. from *Reticulotermes lucifugus* (Georgevitch, 1930), *Duboscqia legeri* from *Termes lucifugus* (Kudo, 1941, 1942) and *Nosema termitis* from *Reticulotermes flavipes* (Kudo, 1938, 1943) have so far been reported. The present contribution makes an addition of four new species of microsporidians from termite hosts.

Material and Methods

Termites belonging to different genera were obtained from different habitats from the University Campus at Waltair (Andhra Pradesh) and surrounding areas. Some of the termites showed external indications of infection while others had to

[1] Part of the Thesis approved for the award of the degree of Doctor of Philosophy of the Andhra University, Waltair.

be dissected and the various parts of the body examined before infection could be detected.

Observations on the parasite in the fresh condition were made using a light microscope, phase contrast and dark field illumination. Spores were kept in 2.5% aqueous solution of potassium dichromate and examined at regular intervals. Smears were either fixed in methyl alcohol and stained in Giemsa's stain or fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin. Initial hydrolysis of the smears in 1 N HCl for 10 min at 60°C prior to staining gave better results. Material for sectioning was fixed in alcoholic Bouin's fluid for 1 h at 60°C and for 24 h at room temperature. Sections were cut at 8.0 μm thickness and stained either with Heidenhain's iron haematoxylin or according to Feulgen's technique. Various conventional methods were used for the release of the polar filaments. They involved the use of mechanical pressure or treatment with body fluid, saline, 1% KOH, ammonia, saturated aqueous urea, hydrogen peroxide, 1 N HCl and paraldehyde. The most effective method for the release of the polar filaments for a particular species is given at the proper place.

Observations

Gurleya spraguei n. sp.

Host species: *Macrotermes estherae* (Desm)
Host tissue involved: Adipose tissue of workers.
Locality: Andhra University Campus, Waltair

Vegetative stages: Meronts with variable number of nuclei measuring about 2.8–20.0 × 1.6–15.0 μm were found in the lightly infected cells.

Sporogony: Each pansporoblast gave rise to four binucleate sporoblasts, each of which develops into a spore.

Spore: Spores are oval in shape measuring 4.0–4.5 × 2.0–2.5 μm. The spore wall was refractive and thick. There is a trilobed PAS positive polar cap at the anterior end. The extruded polar filament is uniformly thin and measured about 75.0–80.0 μm in length.

Description: Fresh smears from the infected tissue revealed a large number of pansporoblasts measuring about 8.0–10.0 × 2.0–2.5 μm. There are four spores in each pansporoblast characteristically arranged as shown in the Figure (Fig. 1 A). Fresh spores were uniform in size and shape. They were ovoid and refractive. An anterior polaroplast and a posterior vacuole were clearly seen in fresh spores (Fig. 1 B).

Spores stained according to the PAS technique reveal the presence of a PAS positive polar cap at the anterior end. This is trilobed with a central spherical and two lateral oval lobes. The polar filament which is faintly stained with PAS is seen along the margines (Fig. 1 C). Spores stained according to Feulgen's technique revealed the presence of two feulgen positive granules which were connected by two thin strands (Fig. 1 D).

Spores stained with Giemsa's stain showed the sporoplasm in the form of a band occupying a space between the polaroplast and the posterior vacuole (Fig. 1 E, F). The sporoplasm stained pale blue and contained one or two deeply stained nuclei.

Addition of saturated aqueous urea extruded the polar filaments in 50% of the spores. The polar filament measured 75.0–85.0 μm in length and was uniformly thin (Fig. 1 G).

Seasonal incidence

The incidence of parasitism varied from 0%–7.0% in 1967–1968 and 0%–8.0% in 1968–1969. The maximum percentage of infection in both the years is in February which was towards the end of winter. The minimum percentage of infection in both the years was in the months of

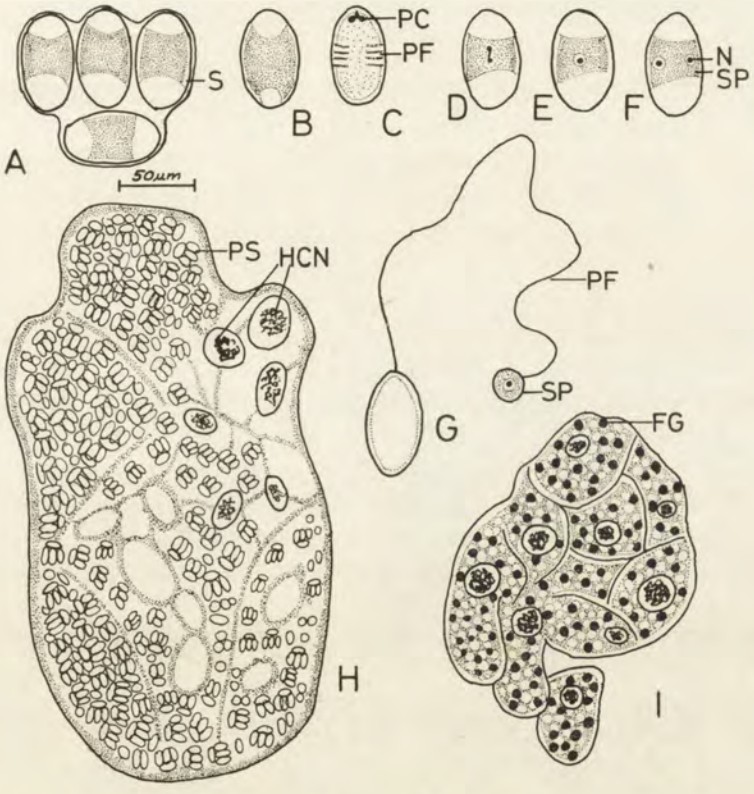

Fig. 1. *Gurleya spraguei* n. sp. A — Pansporoblast (Fresh), B — Fresh spore, C — Spore stained according to PAS technique, D — Spore stained according to Feulgen's technique, E, F — Spores stained with Giemsa's stain, G — Spores with extruded polar filament, H — Infected adipose tissue, I — Uninfected adipose tissue. Key to Lettering (1–4) FG: Fat globules, HCN: Host cell nucleus, PC: Polar cap, N: Nucleus, PF: Polar filament, PS: Pansporoblast, S: Spores, SP: Sporoplasm

Table 1

Availability of *Macrotermes estherae* (Desm) and the Rate of Incidence of *Gurleya spraguei* n. sp. during 1967–1968 and 1968–1969

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
1967–1968 Number of termites examined	198	334	435	447	363	336	274	211	209	162	155	146
Number of termites infected	4	8	10	12	15	15	17	15	5	3	0	0
Percentage of infection	2.0	2.5	2.5	2.5	4.5	6.0	6.5	7.0	2.5	2.0	0	0
1968–1969 Number of termites examined	365	400	425	485	470	470	452	326	390	286	120	99
Number of termites infected	7	8	12	14	21	27	29	26	9	6	0	0
Percentage of infection	1.8	2.0	2.5	3.0	4.7	5.7	6.4	8.4	2.3	2.0	0	0

May, June and July which were summer months. The maximum availability of the hosts, however, was immediately after the monsoon months which was the September–October period (Table 1).

An examination of infected termites during the different months of the year revealed that those collected during July–September period showed 70% plasmodial stages and 30% spores. Those observed in October period showed 30% plasmodial stages and 70% spores while those examined in December showed 80% spores and 20% plasmodial stages. Finally termites examined during January–April period showed only spores and no plasmodial stages. Infected termites had not been observed in May–June period (Table 2).

Histopathology of Infection

The infected termites could easily be identified by the presence of whitish patches on the body at the junction of the head and thorax.

Table 2

Annual Pattern of Occurrence of *Gurleya spraguei* n. sp. in the Adipose Tissue of *Macrotermes estherae* (Desm) during 1967–1968 and 1968–1969

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
1967–1968 Percentage of early stages	100	75	70	40	30	20	—	—	—	—	—	—
Percentage of spores	—	25	30	60	70	80	100	100	100	100	—	—
1968–1969 Percentage of early stages	100	75.9	71.6	41.5	31.0	18.0	5.00	2.0	—	—	—	—
Percentage of spores	—	24.1	28.4	58.5	69.0	82.0	95.0	98.0	100	100	—	—

Unlike the uninfected individuals which are mottled brown and transluscent the infected forms were opaque white. A cereful examination of the different organs of the infected forms showed that the infection was restricted to the adipose tissue. The infected adipose tissue appeared milky white in contrast to the transluscent appearance of the healthy ones. This was probably because of the large number of spores which occupied the adipose tissue cells. In heavy infections the cells ruptured and the spores were discharged into the body cavity.

A comparison of the sections of the infected and uninfected cells stained by the several histological and histochemical methods revealed that there was hypertrophy of the infected cells and they were almost twice the size of the uninfected cells (Fig. 1 H, I). The wall of the infected cells was hardly visible and the nucleus of the host cell increased in volume. The nucleus which was stained homogenously deep in healthy cells stained lightly in the infected cells.

The uninfected cells contained a large number of spherical fat globules filling up the entire space in the cell. The globules were PAS positive and Sudan Black B positive and were digested with pyridine. There were a few strands of mucoidal material running between the fat globules. The strands were positive to PAS, Alcian blue, Toluidin blue and dialysed iron. Neither the fat globules nor the mucoidal strands were observed in heavily infected cells. In cells with light infection vacuoles were seen developing in the cytoplasm with simultaneous reduction in the number of fat globules and mucoidal strands. Both the reserve materials were apparently utilized by the microsporidian parasite which grows at the expense of the host cell.

Discussion

The genus *Gurleya* Doflein, 1892 is characterized by the formation of four spores in each pansporoblast. So far four species of *Gurleya* have been reported from insect hosts.

The present report is the first record of a species belonging to the genus *Gurleya* from a termite host. A comparison of the measurements shows that the present form does not resemble any of the other described species in all its features. Since it occurs in a new host and differs considerably from others it is considered new to science for which the name *Gurleya spraguei* n. sp. is suggested in honor of Prof. Victor Sprague.

Stempellia odontotermi n. sp.

Host species: *Odontotermes* sp.
Host tissue involved: Epithelial cells of the foregut of workers.
Locality: Engineering College Campus, Waltair.

Vegetative stages: Meronts irregular in shape measuring 6.0–12.0 × 3.5–10.0 μm containing a maximum number of 32 nuclei were seen in the lightly infected cells. In mature meronts the nuclei were arranged in pairs (Fig. 2 A, B, C). They divide and finally form 4–16 binucleate bodies. The two nuclei in the binucleate bodies enlarged and finally became closely associated finally fusing to form a single sporont mother cell.

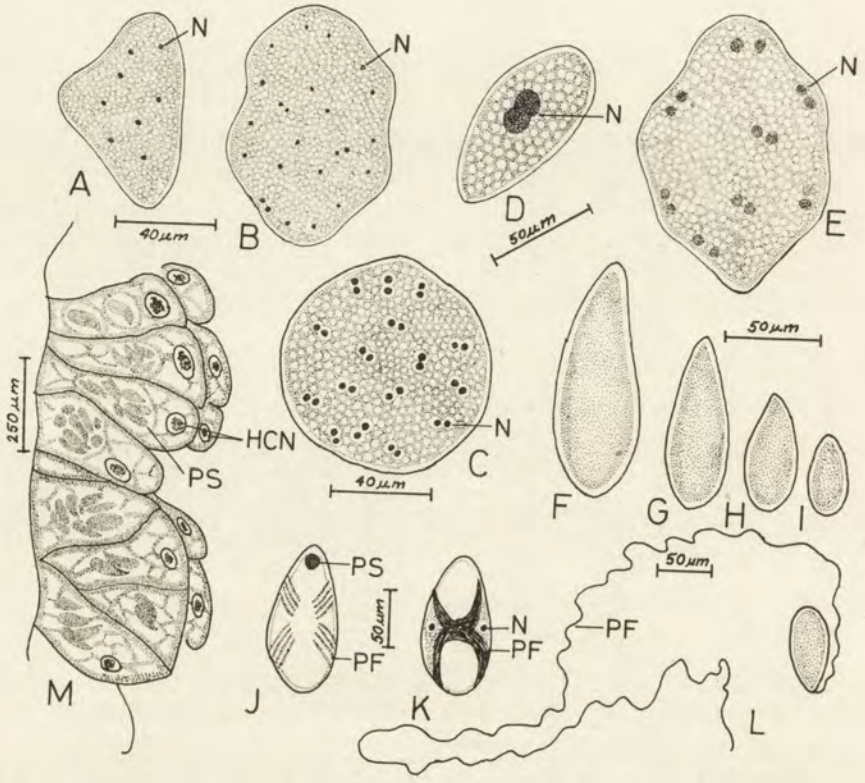

Fig. 2. *Stempellia odontotermi* n. sp. A, B, C — Different stages in merogony, D, E — Different stages in sporogony, F–I — Spores showing size variation, J — Spore stained according to PAS technique, K — Spore stained with Heidenhain's iron haematoxylin, L — Spore with extruded polar filament, M — Section of termite gut showing pansporoblast

Sporogony: The uninucleate sporont mother cell was oval in shape and measured about 8.0 × 4.0 μm. The chromatin was in the form of deeply stained fine granules dispersed over a wide area unlike in the meront. The nuclear membrane was not clearly visible (Fig. 2 D). The cytoplasm was coarsely alveolated and lightly stained. The sporont

mother cell undergoes nuclear division resulting in the formation of sporogonial plasmodia with 4, 8 or 16 nuclei. This was followed by cytoplasmic division resulting in the formation of 2, 4 or 8 spores (Fig. 2 E, M).

Spores: The spores were ovoidal with a slightly pointed anterior end. A PAS positive spherical polar cap could be seen at the anterior end. The polar filament was uniformly thin and measured 300.0–380.0 μm in length when fully extruded.

Description: In sections stained with Heidenhain's iron haematoxylin pansporoblasts with 1, 2, 4 or 8 spores could be seen clearly. Spores were usually binucleate but uninucleate ones were also encountered occasionally. The spores show considerable variation in size (Fig. 2 F–I). When only a single spore was formed from each pansporoblast it measured 12.6 × 4.0 μm but if it is one of the 8 spores formed it measured 4.2 × 3.0 μm. The spores are ovoid with one end slightly more pointed than the other which was rounded. A polaroplast was present at the anterior end which was pointed. A vacuole was seen at the other end which was the posterior vacuole. The wall of the spore was thick. A spherical PAS positive polar cap was present at the anterior end (Fig. 2 J). The polar filament was found in the form of "8" in the middle of the spore. Several methods for the extrusion of the polar filament were tried and the best results were obtained by air drying the smears for about 30 min at room temperature and later adding a drop of saline and exerting slight pressure. The sporoplasm formed a girdle extending across in the middle of the spore.

Discussion

The genus *Stempellia* Leger and Hesse is characterized by the formation of 1, 2, 4 or 8 spores from each pansporoblast. So far eight species of *Stempellia* Léger and Hesse have been described from insect hosts.

The present report is the first record of a species belonging to the genus *Stempellia* from a termite host. The size of the spores of the present form approach that of *S. magna* from the mosquito *Culex pipiens* (Kudo, 1925) which was the largest sized spore among the recorded species of *Stempellia*. However, the difference in the host seems sufficient justification for describing the present form as a new species for which the name *Stempellia odontotermi* n. sp. is proposed.

Pleistophora weiseri n. sp.

Host species: *Coptotermes heimi* (Wasm).
Host tissue involved: Epithelium of the fore-gut.
Locality: Andhra University Campus, Waltair.

Vegetative stages: Meronts having two to numerous nuclei were encountered in the epithelial cells of the fore-gut of the workers. Percentage infection varied from 20–25. They were oval to irregular in shape measuring about 7.0–10.0 × 2.0–3.0 μm. In heavy infections as many as five meronts were seen in a single epithelial cell.

Sporogony: Pansporoblasts give rise to variable number of uninucleate sporoblasts each of which gives rise to a single spore.

Spore: Spores are generally elongately oval in shape measuring 5.4–6.0 × 1.8–2,2 μm. No internal structure was seen in fresh spores. There is a dot-like PAS positive polar cap. The polar filament is uniformly thin and measures 70.0–80.0 μm in length.

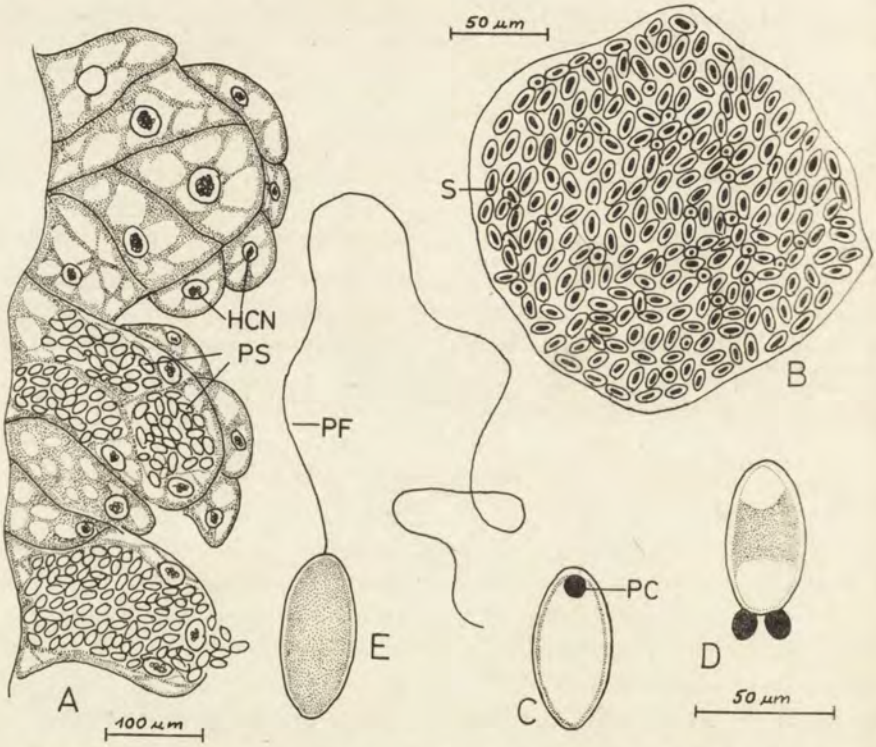

Fig. 3. *Pleistophora weiseri* n. sp. A — Section of the gut showing the pansporoblasts, B — Pansporoblast, C, D — Spores stained according to PAS technique, E — Spore with extruded polar filament

Description: A large number of fragile "cysts" were seen in heavily infected guts (Fig. 3 A) giving the latter an opaque appearance. The cysts are surrounded by a very thin transparent membrane which bursts when it comes in contact with water, saline or when subjected to slight pressure and as such intact "cysts" were rare in smears. The cysts

contained from a minimum of 60 to a maximum of 280 spores. The largest pansporoblast measured was 20.0 μm in diameter (Fig. 3 B).

A fresh spore measures 5.4–6.0 × 1.8–2.2 μm and is elongately oval with rounded ends and thick walls. The polar cap is a single spherical dot lying just beneath the wall at the anterior end (Fig. 3 C). It has not been possible to distinguish the polar filament inside the spore either in fresh or stained material. Sporoblasts subjected to an initial hydrolysis in 1 N HCl for 10 min followed by staining with iron haematoxylin showed the presence of a band-shaped sporoplasm extending between the polaroplast and the posterior vacuole (Fig. 3 D). When air-dried smears were treated with hydrogen peroxide and allowed to act for 10–15 min the polar filaments were released in 50–60% of the spores. The fully extruded polar filaments measure 70.0–80.0 μm in length and are uniformly thin (Fig. 3 E).

Pleistophora ganapatii n. sp.

Host species: *Odontotermes horni* (Desm).
Host tissue involved: Epithelial cells of the fore-gut.
Locality: Waltair Uplands, Waltair.

Vegetative stages: Only workers were infected. The percentage infection was 15–20. Meronts measuring 3.0–4.0 μm with two deeply stained nuclei are seen in the epithelial cells. The largest meront encountered was about 12.0 μm in diameter and contained 40–45 nuclei. There are pairing nuclei in the latter stages (Fig. 4 A).

Sporogony: Sporogonial plasmodia with varying number of nuclei could be seen clearly in Giemsa stained material. 16–48 spores were formed from each pansporoblast (Fig. 4 B).

Spores: The spores were oval in shape and there were micro- and macrospores. There is a strongly PAS positive polar cap which is bilobed at the anterior end. The polar filament is uniformly thin and measures about 170.0–180.0 μm in length.

Description: Numerous fragile cysts were observed when the fore gut was examined under a compound microscope. They were spherical in shape measuring 18.0–32.0 μm in diameter and contained 16–48 spores. The spores could easily be released by exerting a slight pressure on the cover slip.

The spores are oval in shape with an outer thin wall. They measure 8.0–9.0 × 5.0–5.4 μm (Fig. 4 C). Abnormal spores measuring about 10.0–11.0 × 6.0–7.0 μm were also occasionally seen (Fig. 4 D). These are probably the macrospores. The structure of the macro- and microspores seemed to be essentially the same. In the fresh condition the spores

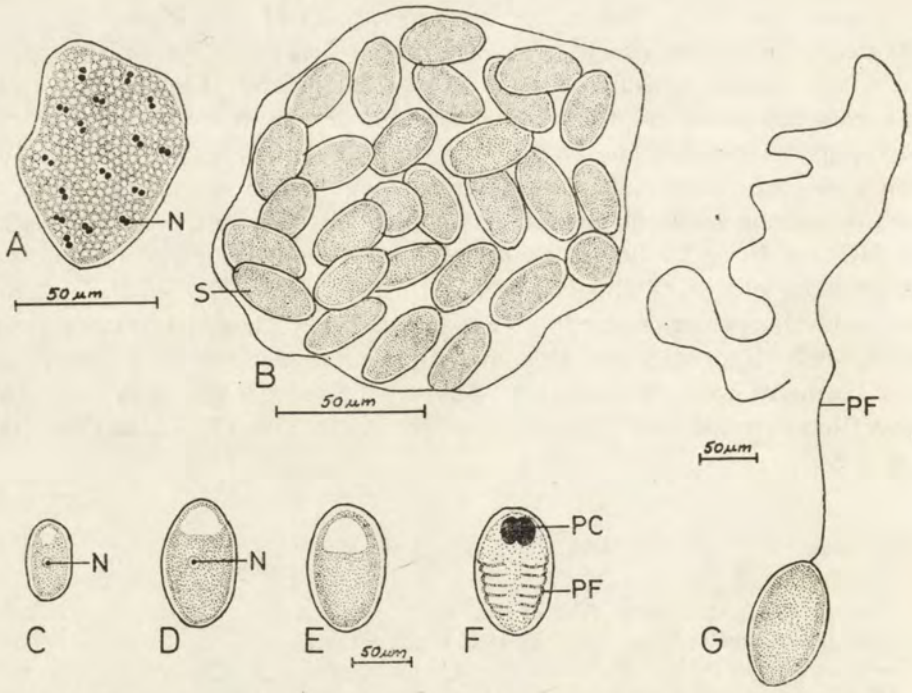

Fig. 4. *Pleistophora ganapatii* n. sp. A — Mature meront showing paired nuclei, B — Pansporoblast, C — Microspore, D — Macrospore, E — Fresh spore, F — Spores stained according to the PAS technique. Note the PAS positive polar cap and polar filament, G — Spore with extruded polar filament

showed the polaroplast at the anterior end (Fig. 4 E). A posterior vacuole was not observed. In spores stained in Giemsa's solution the sporoplasm showed either one or two nuclei lying below the polaroplast. The polar filament could not be seen in fresh preparations but in material stained by the PAS technique a coiled polar filament was seen along the walls in the region of the sporoplasm. A deeply stained bilobed polar cap was present in front of the polaroplast (Fig. 4 F). The polar filament was extruded in 90% of the spores by allowing the fresh spores to dry up at room temperature for 1 h and later adding a drop of saline and exerting slight pressure. They are uniformly thin and measured 170.0–180.0 µm in length (Fig. 4 G).

Discussion

The genus *Pleistophora* Gurley is characterized by the production of more than 16 spores from each pansporoblast. 32 species of *Pleistophora*

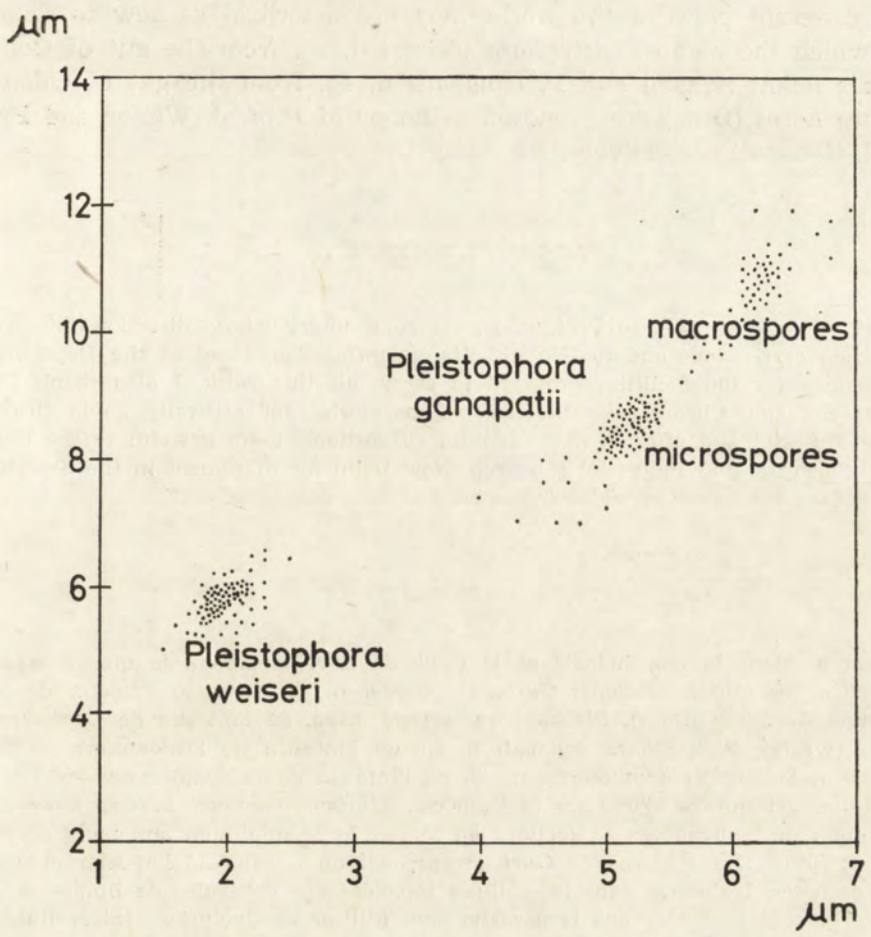

Fig. 5. Scattergram showing the size variation of spores of *Pleistophora ganapatii* and *P. weiseri*

have so far been described from insect hosts. The only previous record of *Pleistophora* sp. (Georgevitch, 1930) from the termite host is from the malpighian tubules of *Reticulotermes lucifugus* which has been incompletely described. The two species now described are from the gut of *Coptotermes heimi* (Wasm) and *Odontotermes horni* (Desn). In *Pleistophora weiseri* n. sp. the spores are all of the same type and measure 5.4–6.0 µm × 1.8–2.2 µm and more than 60 (maximum of 280) spores are formed in each pansporoblast. In *P. ganapatii* n. sp. the spores occur in two different sizes, the microspores measuring 8.0–9.0 × 5.0–5.4 µm and the macrospores measuring about 10.0–12.0 × 6.0–7.0 µm (Fig. 5) and 16–40 spores are formed in each pansporoblast.

In view of the fact that the present forms are from different hosts

and different parts of the world they are described as new to science for which the name *Pleistophora weiseri* n. sp. from the gut of *Coptotermes heimi* (Wasm) and *P. ganapatii* n. sp. from the gut of *Odontotermes horni* (Desn) are proposed in honor of Prof. J. Weiser and Prof. P. N. Ganapati respectively.

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RÉSUMÉ

On a décrit la morphologie et le cycle de développement de quatre espèces nouvelles des microsporidiens: *Gurleya spraguei* n. sp. du tissu adipeux de *Macrotermes estherae* (Desn), *Pleistophora weiseri* n. sp. de l'intestin de *Coptotermes heimi* (Wasm), *Pleistophora ganapati* n. sp. de l'intestin de l'*Odontotermes horni* (Desn), et *Stempellia odontotermi* n. sp. de l'intestin de l'*Odontotermes* sp. La distribution saisonnière d'une de ces espèces, *Gurleya spraguei* n. sp., montre le maximum du pourcentage d'infections en février et le minimum aux mors de mai, juin et juillet. Dans le cas de *Gurleya spraguei* on a constaté l'hypertrophie des cellules hôtes. L'absence dans les cellules infectées des sphérules de lipides et des depôts mucoïdes suggère que le parasite peut utiliser ces inclusions intracellulaires pour sa croissance et différentiation.

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Les ciliés psammophiles de la mer Baltique aux environs de Gdańsk

Synopsis. On étudiait les ciliés vivants le sable submergé des eaux saumâtres aux environs de Gdańsk où la salinité oscille entre 3–7‰. On a trouvé 53 espéces dont deux nouvelles. On a examiné avec une attention particulière la morphologie de *Plagiopogon loricatus* qui se distingue par une structure peu commune de la pellicule. On a constaté que la salinité diminuante sélectionne les cilies de sorte qu'un nombre limité seulement d'espèces marines pénètrent dans les eaux saumâtres.

Pendant deux saisons d'été consecutives nous avons étudié les ciliés psammophiles vivant dans le fond sablonneux de la mer Baltique aux environs de Gdańsk [1]. On prenait des prélevements de sable submergé aux endroits suivants: dans le golf de Puck entre les villages Chałupy et Kuźnica, dans le lit mort de la Vistule et dans le lac saumâtre sur l'île Bonzak.

La salinité de l'eau est plus haute dans le golf de Puck, où elle s'éleve à 7‰ et plus basse dans le lac saumâtre et Vistule morte où elle baisse jusqu'à environ 3‰.

Le golf de Puck est fort pollué surtout en été par les eaux résiduaires de la ville. Pendant les périodes calmes, sans vent on peut y observer sur la surface du sable submergé des taches rosatres formées par les bactéries sulfuriques. Si l'on examine ces taches de plus prés on trouve sous une couche de sable la vase noire qui sent fort l'hydrogéne sulfurique. De tels endroits fourmillent de ciliés. Les grosses pierres qui fortifient le bord du village sont couvertes d'épaisses couches grises formées aussi par les bactéries.

Le lac saumâtre atteint vers l'ouest la Vistule morte dont il n'est séparé que par une mince digue; au nord il est bordé par la plage qui

[1] Nous exprimons notre reconnaissance au Directeur de la Station de l'Université de Gdańsk à Górki Wschodnie pour l'hospitalité qu'il a bien voulu nous accorder pendant notre travail.

le sépare de la mer. La plage, en général large et bordée par les dunes devient à son extrémité ouest plate et étroite. Ici, pendant les tempêtes les vagues rompent le rivage et l'eau marine coule dans le lac. L'eau du lac et son rivage sont fort eutrophisés à cause de nombreux oiseaux aquatiques qui nichent dans le jonc.

Le sable émergé au bord du lac contient juste au-dessous de sa surface une couche verte remplie d'algues, surtout de différentes espèces de cyanophycés qui représentent la nourriture de base pour plusieurs ciliés.

Les échantillons étaient pris d'une façons très simple: on enlevait la couche superficielle (1–1.5 cm) du sable avec de l'eau à l'aide d'une cuillère à soupe. Au laboratoire on mettait des prélevements dans les bocaux. Pour examiner la microfaune on mettait un peu de sable dans les petites boites de Petrie et sous une loupe on pêchait les ciliés à l'aide d'une pipette fine. On examinait d'abord chaque espèce in vivo et puis, si leur nombre le permettait on faisait des préparations imprégnées ä l'argent d'après la méthode de Chatton modifiée par Corliss (1953) et d'aprés celle de Bodian modifiée par Tuffrau (1967). Afin de mettre en évidence le noyau on utilisait le vert de methyl et la méthode de Feulgen.

On a trouvé en somme 53 espèces. Sur la liste ci-dessous on donne la déscription détaillée seulement d'espèces nouvelles ou celles qui ont été décrites d'une façon insuffisante. Pour tous les autres on ne donne que quelques remarques en se réferrant aux déscriptions originales.

La liste des espèces trouvées

Urotricha baltica n. sp.

Dans le sable pollué du golf de Puck se trouvaient en asez grand nombre des ciliés appartenant sans doute au genre *Urotricha* mais il était impossible d'identifier avec une espèce décrite jusqu'à présent. Le corps de ce cilié est largement ovoide, relativement grand: 65–80 μm de long (Pl. I 1–4). Autour de la bouche la ciliature somatique est différenciée d'une façon typique pour le genre; les extremités antérieures des cinéties se terminent par deux cercles de cinétosomes doubles. Puis il y a un cercle constitué d'environ 25 grains d'où sortent les stries argentophiles radiaires. Au-dessous de la bouche se trouve "la brosse" composée de trois courtes cinéties; la première est constituée de 6, la deuxième de 5 et la troisième de 3 cinétosomes. Les cinéties au nombre de 32–36 après avoir parcouru trois quarts du corps, se dissolvent en cinétosomes isolés, épars qui couvrent le dernier quart du corps. La structure de la pellicule est typique; elle présente un reseau de mailles rectangulaires.

Les vacuoles digestives de ce cilié étaient foncées, remplies probablement de bactéries.

Urotricha armata Kahl, 1922

Cette espèce a été redécrite recemment par Dragesco et al. (1974). Nous n'avons rien à ajouter à cette déscription (Pl. I 5–7).

Prorodon discolor Ehrb. Blochm. Schew.

Ce cilié omnivore a été redécrit recemment par Jordan (1974). Il vit dans l'eau douce et dans l'eau marine; Dragesco (1965) l'a trouvé sur la côte africaine.

Prorodon raabei Czapik, 1965

Ce gymnostome histophage trouvé sur la côte polonaise (Czapik 1965), où il est très frequent, n'a pas été encore retrouvé ailleurs. Sa brosse dorsale distincte ainsi que la forme du noyau sont bien caracteristiques.

Placus striatus Cohn, 1866

Ce cilié, très caracteristique à cause de la forte spiralisation de ses cinéties (Fig. 1) est plutôt rare sur la côte polonaise. Cité par plusieurs auteurs aux différentes endroits par exemple par Dragesco (1963 a), Agamaliev (1968) et Kahl (1930).

Lacrymaria coronata Clap. et Lachm., 1858

C'est une espèce très commune, signalée dans les mers à pleine salinité (Dragesco 1960, 1965) ainsi que dans les eaux saumâtres (Kahl 1930). Elle se nourrit de petits ciliés (Fig. 2 A–B).

Lacrymaria salinarum Kahl, 1928

Cette petite espèce (Fig. 2 D) est d'après Kahl (1928) répandue dans la mer Baltique. Burkowski (1970 b) l'a trouvé aussi dans la mer Blanche.

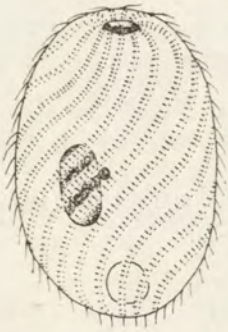

Fig. 1. *Placus striatus* Cohn

Lacrymaria marina Kahl 1933

Kahl a décrit cette espèce comme *Lacrymaria olor* var. *marina*. Dragesco (1963 a) qui a trouvé cette forme dans le mesopsammon à Roscoff précise différences qui la distinguent de *Lacrymaria olor* (le corps plus èlacé, le cou moins allongé, le taille moyenne plus petite — environ 250 μm) et la classe au rang d'espèce (Fig. 2 C).

Enchelys marina Meunier, 1907

Cette espèce a été trouvé uniquement par Meunier (1907), dans le plankton conservé de l'ocean Glacial. La déscription cité par Kahl (1930) est trop sommaire pour permettre une identification sure. Quoiqu'il en soit le cilié trouvé dans la mer Baltique correspond assez bien à l'espèce décrite par Meunier. On n'a pêché que quelques individus et les observations étaitent faites uniquement sur le vivant. De longueur entre 150–200 μm, largeur de 60 μm, le cilié nage lentement. La bouche est apicale, circulaire, entouré par des trichites très courtes. Le cinétome

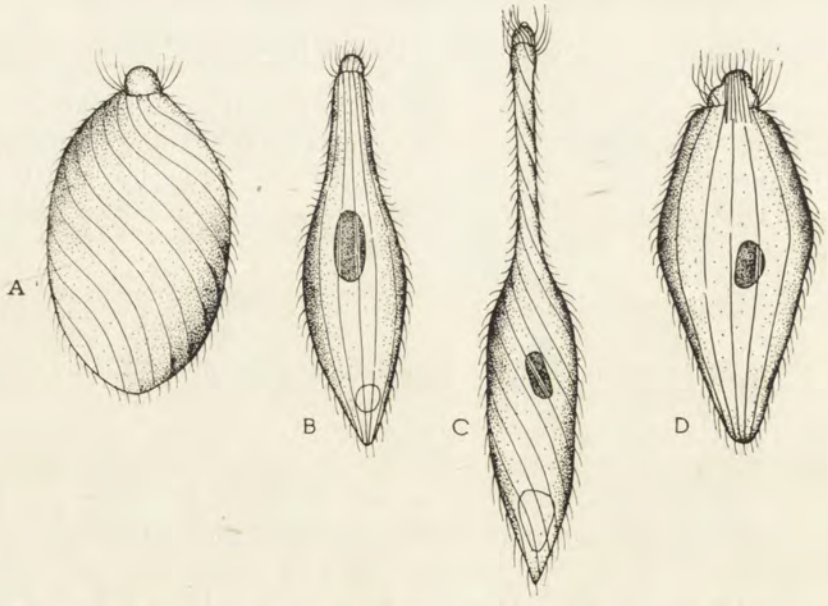

Fig. 2. A–B — *Lacrymaria coronata* Clap. et Lachm. (individus et contracté), C — *Lacrymaria marina* Kahl, D — *Lacrymaria salinarum* Kahl

est constitué par 36 cinéties méridiennes bipolaires. Les cils d'une longueur moyenne de 4–5 μm, deviennent plus longs et plus nombreux au voisinage de la bouche. La vacuole contractile est terminale. L'apareil nucléaire est constitué par un macronucleus en ruban, courbé dans sa partie terminale. On n'a pas observé de micronucléi. Le cytoplasme est transparent et claire (Fig. 3 A).

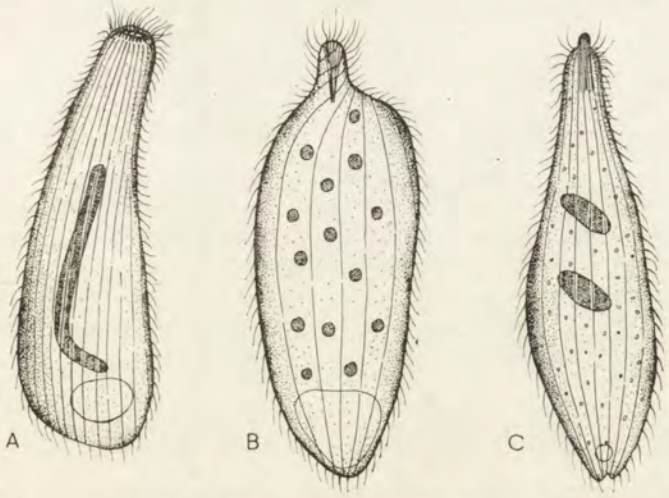

Fig. 3. A — *Enchelys marina* Meunier, B — *Chaenea teres* Dujardin, C — *Trachelophyllum apiculatum* Perty

Plagiopogon loricatus Kahl, 1933

Ce petit cilié a été retrouvé par quelques autors (Bock 1960, Burkowski 1970 a, Fenchel 1969) dans la mer Baltique et dans la mer Blanche. Comme la déscription originelle de Kahl (1935) ainsi que celle plus recente de Burkowski (1970 a) et Hartwig (1973 a) ne s'accordent pas en tous les details avec nos propres observations nous avons étudié cette espèce très attentivement *in vivo* et sur les préparations imprégnées à l'argent. Les individus trouvés dans le golf de Puck avaent 67–70 μm de long. Le corps est ovalaire, le cytostome apical, sur le pôle posterieur il y a un cil caudal. Le caractère le plus important de ce cilié est la structure fine de son surface (Fig. 4). Les individus que nous avons trouvés avaient 14 cinéties (d'après Kahl 18–20, d'après Burkowski 12–16, d'après Hartwig 12). Chaque cinétie est accompagnée d'une rangée de 22 plaques eetoplasmiques. Burkowski (1970a) les réprésente comme rectangulaires. En effet si l'on étudie les préparation imprégnées on a l'impression de voir une structure rectangulaire (Pl. II 8-9). Si l'on observe cependant l'animal vivant sous l'immersion l'image

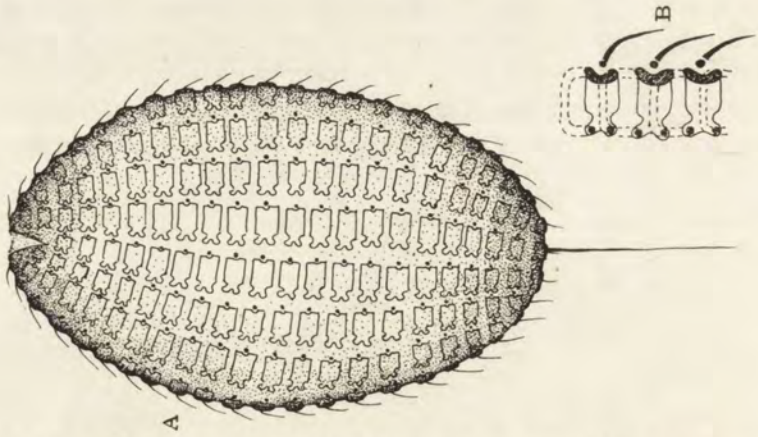

Fig. 4. A — *Plagiopogon loricatus* Kahl, B — La construction des plaques

devient beaucoup plus compliqué. Il se montre que les plaques ont une forme bizarre, qui ressemble au petit verre vu de côté. Du coté droit de chaque plaque se trouve un cil. Chaque plaque est implantée sur le corps à l'aide de deux boucles qui s'enfoncent dans l'ectoplasme. Cette structure correspond beaucoup mieux à la déscription de Kahl (1935) qu'au dessin de Burkowski.

Les vacuoles digestives de ces ciliés étaient remplies de petites algues vertes.

Chaenea teres Dujardin, 1841

Cette forme microphage découverte par Dujardin (1841) dans le sapropel d'eau douce et ensuite trouvé par Kahl (1930) dans l'eau saumâtre a été rédécrite recemment par Borror (1963) qui l'a trouvé sur la côte de Floride. Il parait que c'est une espéce non seulement cosmopolite mais euryhaline, indifférente à la concentration de sels. Les individus que nous avons trouvé dans le sapropel du golf de Puck correspondent parfaitement aux déscriptions de Kahl et Borror (Fig. 3 B). Une varieté do cette espèce a été trouvé aussi dans le sapropel de la mer Noire par Vuxanovic (1963).

Trachelophyllum apiculatum Perty, 1852

Avec corps allongé, très extensible, au bout pointu, ce cilïé était plutôt recontré dans l'eau douce. Les auteurs précédentes ont décrit les diverses formes de cette espèce. L'animal trouvé par nous avait les mêmes dimensions que rapporte Kahl (1930) (130—150 μm). La partie entérieure du

corps s'allonge dans un "cou" qui se termine par un appendice arrondi contenant les trichites de l'appareil buccal (Fig. 3 C). Penard (1922) a observé des individus avec un large bouton en forme d'un cône détourné. Sur le cou on a observé une ligne de soies fines décrites déjà par

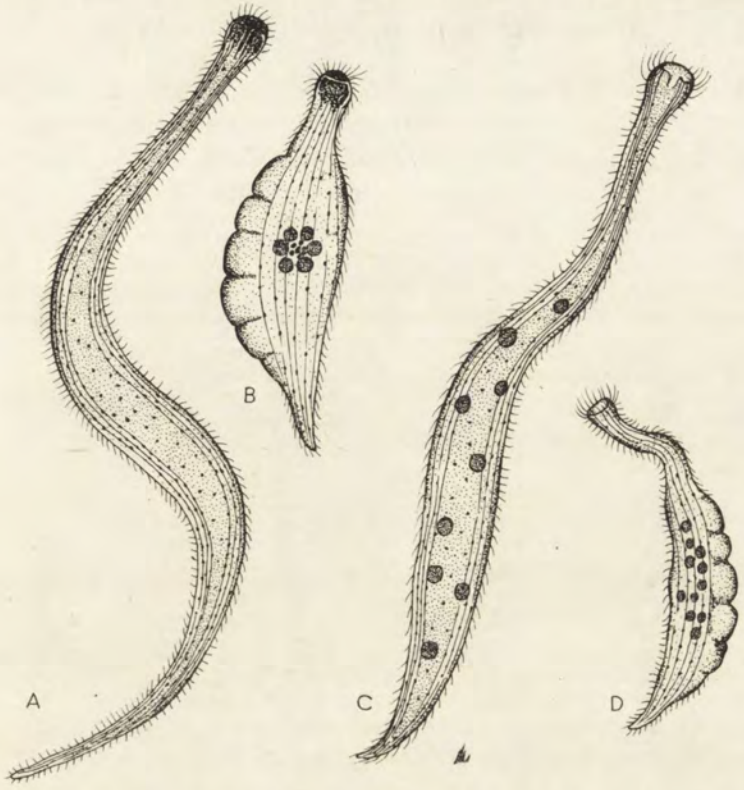

Fig. 5. A–B — *Tracheloraphis phoenicopterus* (Cohn), C–D — *Tracheloraphis margaritatus* (Kahl) (individus nageant et contractés)

Penard. Les cinéties au nombre de 16–18 sont bipolaires, les cils longs (8–9 μm) et fins. L'appareil nucléaire se compose de deux macronuclei ovalaires. La vacuole pulsatile se vide par un petit canal tout près du pôle posterieur. Le cytoplasme est transparent et clair. On n'a pêché que quelques exemplaires alors toutes les observations étaient faites *in vivo*. Cette espèce a été trouvé dans l'eau douce et dans leau saumâtre (Penard 1922).

Tracheloraphis Dragesco, 1960

Les espèces appartenantes au genre *Tracheloraphis* sont cosmopolites, signalés des mers du monde entier. Dragesco (1960, 1963 a, 1965) et

surtout Raikov (1962), Raikov et Kovaleva (1968) ont donné d'excellentes déscriptions, qu'il serait inutile de répéter. Nous rappellons alors seulement les caractères les plus essentiels.

Tracheloraphis phenicopterus (Cohn, 1866)

La "tête" plus épaisse que le "cou", le corps incolore, un seul noyau composé (Fig. 5). Les individus trouvés contenaient dans leur vacuoles digestives les Peridiniens et les Diatomées. Cette espéce était très nombreuse dans le golf ainsi que dans le lac saumâtre.

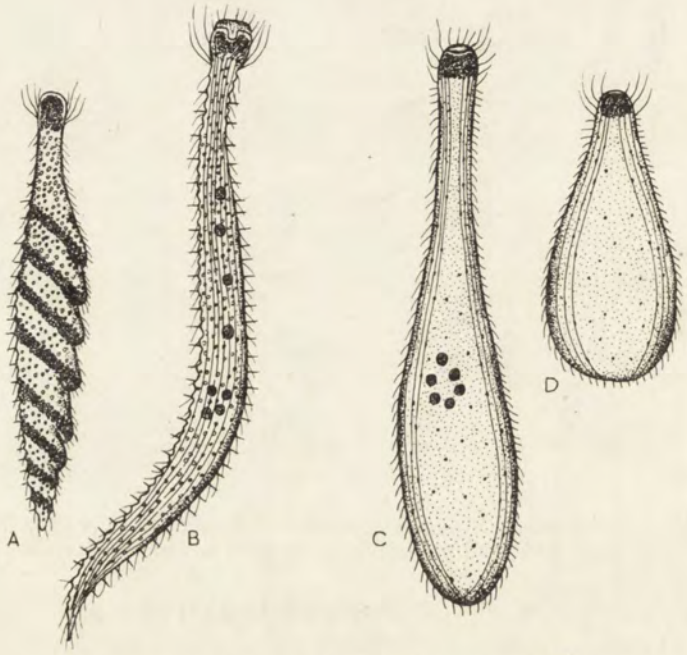

Fig. 6. A–B — *Trachelonema oligostriata* Raikov, C–D — *Tracheloraphis incaudatus* (Kahl) (individus nageant et contractés)

Tracheloraphis margaritatus (Kahl, 1930)

Le corps brun, la tête peu indiquée. L'appareil nucléaire est composé de 9–35 macronuclei dispersés dans le cytoplasme (Fig. 5 C–D). Les individus trouvés se nourrissaient de Peridiniens. Cette espèce était aussi nombreuse que la précédente.

Tracheloraphis kahli Raikov, 1962

Cette espèce ressemble fort par sa morphologie extérieure à *Tracheloraphis phenicopterus*. La différence principale s'exprime dans la structure du noyau qui consiste ici en plusieurs (3–11) noyaux composés (PL. II 10).

Tracheloraphis incaudatus (Kahl, 1930)

Le corps brun est arrondi au bout. L'appareil nucléaire consiste en 6 macronuclei ramassés en groupe. C'est une espèce très fragile difficille à observer (Fig. 6 C–D).

Tracheloraphis drachi Dragesco, 1960

Le corps brun, grand, à la région caudale pointu. Le caractére le plus important est l'appareil nucléaire qui contient 12 macronuclei enfermés dans une capsule.

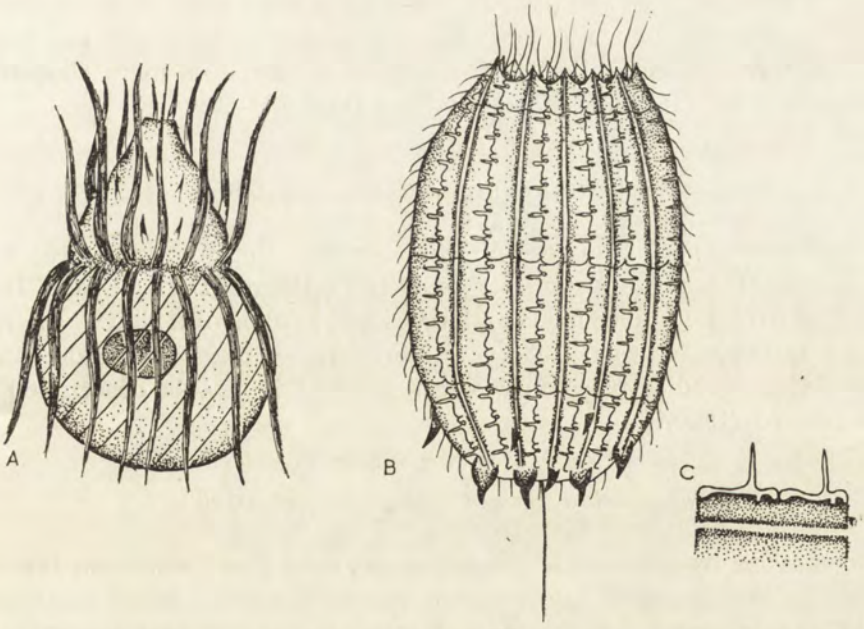

Fig. 7. A — *Mesodinium pulex* Clap. et Lachm., B–C — *Coleps similis* Kahl (l'animal entier et fragment des plaques)

Trachelonema oligostriata Raikov, 1962

Cette espèce a été trouvéé et décrite par Raikov (1962) dans la mer Blanche et plus tard retrouvés par Dragesco (1963 a) en La Manche. Le corps incolore et aplati dans le sens dorso-ventral est cilié seulement sur le côté ventral (8 cinéties). Il y a 8–18 macronuclei. Pendant la contraction le corps forme des plis obliques visibles surtout sur les bords (Fig. 6 A–B). Cette espèce était très nombreuse dans le lac saumâtre. Les individus trouvés avaient environ 400 μm de long.

Mesodinium pulex Clap. et Lachm., 1858

Cette espèce était fréquent surtout dans le lac saumâtre (Fig. 7A) les individus trouvés avaient une belle couleur bleu-verte à cause de cyanophycés avalées. Ce cilié cosmopolite est cité par plusieurs auteurs des eaux douces ainsi que des mers (Agamaliev 1972, Borror 1963, Dragesco 1960, Kahl 1930).

Coleps similis Kahl, 1933

Il y a une déscription parfaite de cette espèce dans l'ouvrage de Hartwig (1973 a). *Coleps similis* trouvé par nous dans tous les prélevement était seulement un peu plus petit (30-45 μm) que celui étudié par Hartwig (53–76 μm). Ce cilié a été trouvé aussi dans les mers Caspienne et Blanche ainsi que dans la Mediterrané (Fig. 7 B–C).

Paraspathidium fuscum (Kahl, 1928)

Paraspathidium fuscum est un cilié cosmopolite, omnivore, très bien connu (Fig. 8). Il a été trouvé par plusieurs auteurs (Dragesco 1960, 1963 a, Fenchel 1968 a, Burkowski 1970 b, Raikov et Kovaleva 1968, Petran 1968 et autres) dans les mers du monde entier. Les individus étudiés par nous correspondaient bien à la déscription de Dragesco (1960).

Paraspathidium obliquum Dragesco, 1963

Nous avons trouvé ce cilié une seule fois avec *Paraspathidium fuscum*. Il était moins abondant que l'espèce précédente mais aussi nombreuse que l'espèce suivante. Les individus trouvés correspondaient parfaitement à la déscription de Dragesco avec une seule différence à savoir leur

trichites buccales étaient courts comme chez *Paraspathidium fuscum*, tandis que ceux découverts par Dragesco (1963 a) avaient un faisceau de trichites longues (Fig. 8).

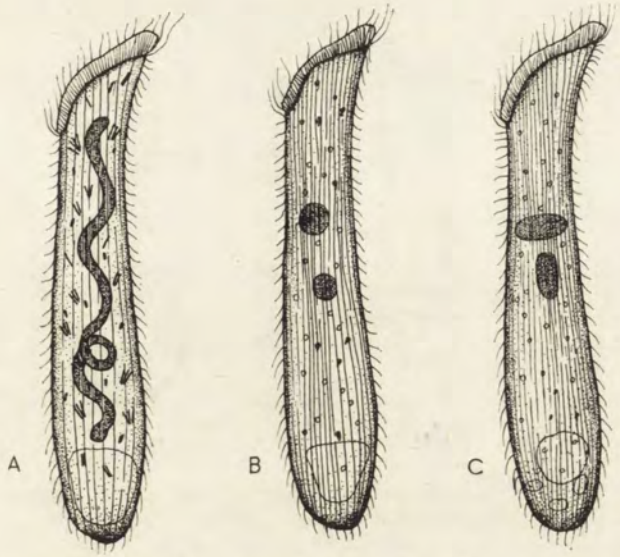

Fig. 8. A — *Paraspathidium longinucleatum* n. sp., B — *Paraspathidium fuscum* (Kahl), C — *Paraspathidium obliquum* Dragesco

Paraspathidium longinucleatum n. sp.

Cette forme est la plus grande de tous les représentants du genre *Paraspathidium*. Par sa morphologie extérieure elle ressemble fort à *Paraspathidium fuscum* (Fig. 8). Le corps a environ 450–530 μm de long et 75–95 μm de large. Les cinéties sont peu nombreuses (24) par rapport à *Paraspathidium fuscum* (44). Elles sont écartés de 7 μm. L'appareil buccal a la même construction que celui de *Paraspathidium fuscum* (Pl. II, 11). La différence essentielle est dans le macronucleus qui a la forme de ruban très long, un peu embrouillé. Il y a environ 10 micronuclei. Le cytoplasme est opaque, bourré d'inclusions réfringeantes. Dans l'ectoplasme on peut remarquer les trichites dispersées, isolées ou réunies en faisceaux de trois ou quatre. Le cilié est très souple et assez contractile. Les vacuoles digestives étaient remplies de bactéries et de petites algues.

Litonotus anguilla Kahl, 1930

La forme trouvé par nous correspond bien aux déscriptions précedentes (Fig. 9). Il y a une seule différence à savoir les individus observés

sont plus petits que ceux pêchés par Kahl (1931) et Dragesco (1960): 100–130 μm contre 170–300 μm. C'est un cilié cosmopolite, cité par plusieurs auteurs qui se distingue par une grande variabilité de la forme du corps ainsi que par sa souplesse (Fig. 9).

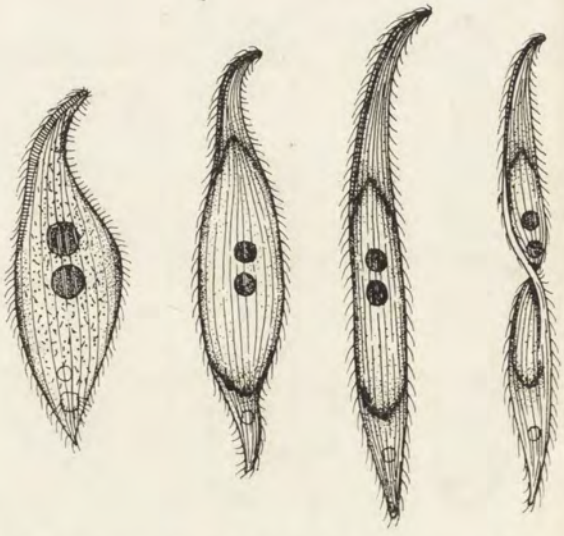

Fig. 9. *Litonotus anguilla* Kahl — différentes formes du corps

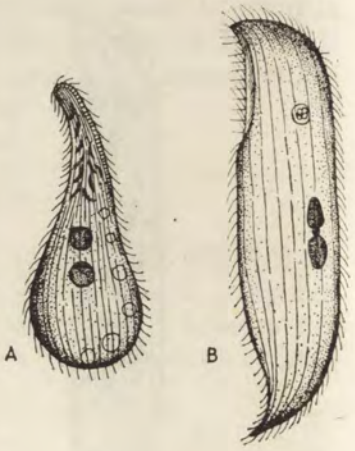

Fig. 10. A — *Litonotus marina* (Kahl), B — *Remanella unicorpusculata* Kahl

Litonotus (Hemiophrys) marina (Kahl, 1931)

C'est un cilié plutôt rare. Il était rencontré une seule fois par Borror (1963) sur la côte de Floride depuis la découverte de Kahl (1931). Nous avons trouvé cet animal dans le golf de Puck seulement une fois. (Fig. 10). La structure en général correspond bien à la déscription de Borror. Nous avons pourtant constaté de petites différences. Des exemplaires trouvés par nous ne dépassaient jamais 130–160 μm, tandis que Kahl et Borror ont observé les ciliés plus grands: 150–300 μm. Il y a aussi une divergence dans le nombre de cinéties: *Litonotus marina* étudié par Borror en a 27–28, le nôtre 25–27.

Loxophyllum multinucleatum Kahl, 1928

Nous avons trouvé ce cilié seulement une fois dans le golf de Puck à l'endroit pollué (Fig. 11 B). Nos observations confirment et précisent la déscription de Kahl (1930). Les dimensions de l'animal sont confor-

mes à celles rapportées par Kahl: 150–180 μm. Le corps est large et plat, pointu dans sa partie postérieure, sur sa surface on oberve 5 plis dont un est plus fort que les autres. Auprès de celui-ci dans l'endoplasme sont situés les macronuclei en nombre 22. Le cinétome consiste en 14 cinéties contre 10 décrites par Kahl. Les trichocystes bordant tout le corps de l'animal sont disposés régulièrement. Nous n'avons pas observé les papilles péribuccales.

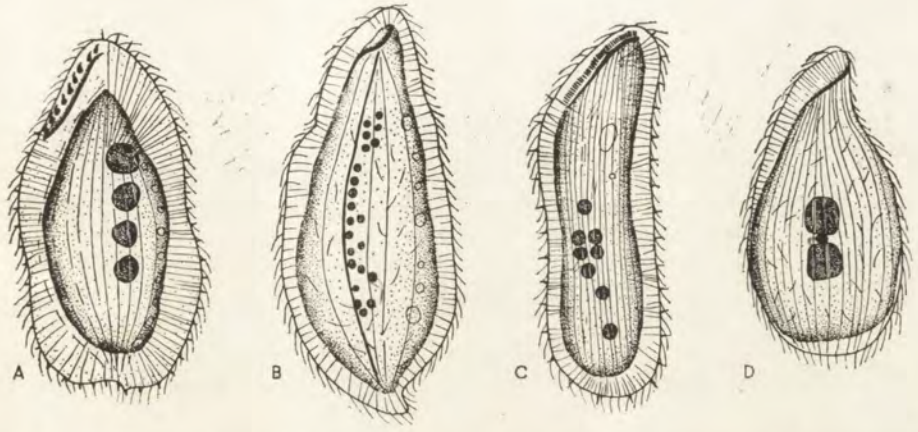

Fig. 11. A — *Loxophyllum setigerum* Quennerstedt, B — *Loxophyllum multinucleatum* Kahl, C — *Loxophyllum verrucosum* (Stokes), D — *Loxophyllum serratum* Kahl

Loxophyllum verrucosum (Stokes, 1893)

Ce cilié trouvé par nous dans le lac saumâtre sur l'île Bonzak ressemble bien an *Loxophyllum verrucosum* étudié par Dragesco (1965) en Afrique. Il a 6–8 macronuclei, 35–40 cinéties et environ 50 papilles buccales (Fig. 11 C). Les soies sont plus puissantes que chez *Loxophyllum setigerum*. Il y a seulement une différence par rapport aux déscriptions précédentes à savoir, les individus de la côte polonaise avaient toujours une ou deux vacuoles contractiles contre "plusieurs" chez Kahl (1931) et "généralement multiples" chez Dragesco.

Loxophyllum setigerum Quennerstedt, 1867

C'est un cilié très fréquent (Fig. 11 A). Sa taille et sa forme sont très variables mais les individus que nous avons rencontré correspond bien aux déscription de Kahl (1930) et de Dragesco (1960).

Loxophyllum serratum Kahl, 1933

Nous avons trouvé ce cilié pour la première fois depuis la déscription de Kahl (1933). Nos individus ne ressemblaient pas en tous les détails à l'animal étudié par l'auteur allemand (Fig. 11 D). Pourtant l'appareil nucléaire et la couleur du cytoplasme nous ont décité à des identifier avec *Loxophyllum serratum*. La forme générale du cilié n'est pas aussi variable que chez le espèces voisines. Le contour du corps et à peu près pyriforme. L'animal mesure environ 100–150 μm de long et 50–75 μm de large. Sa bouche ressemble bien à celle de *Loxophyllum multinucleatum*. Dans le cytoplasme il y a des trichocystes isolés; sur le côté ventral ils sont groupés dans la partie antérieure du cilié. Le côté droit porte 16–18 cinéties. La vacuole pulsatile est volumineuse, terminale. L'appareil nucléaire se compose de deux macronuclei unis par un micronucleus. Le cytoplasme a une couleur rosâtre, ce qu'est une rareté parmi les espéces du genre *Loxophyllum*.

Remanella unicorpusculata (Kahl, 1933)

L'espèce *Remanella unicorpusculata* est répandu dans les mers du monde entier et cité par de nombreux chercheurs (Dragesco 1960, 1965, Hartwig 1973 a, b, Raikov et Kovaleva 1968). Elle se caractérise par un seul corps de Müller et est commune dans la mer Baltique (Fig. 10 B). Nous avons trouvé quelques individus dans le golf de Puck; ils harmonisaient parfaitement avec la déscription de Dragesco (1965).

Kentrophoros fasciolatus Sauerbrey, 1928

Comme la forme précédente, ce bizzare cilié en forme de ruban vit dans les mers du monde entier (Fig. 12) le détail le plus eurieux de sa morphologie, sont les bactéries symbiotiques décrites par Fauré-Fremiet (1950 b). Nous avons trouvé quelques individus de cette espèce dans le golf de Puck.

Plagiopyla ovata Kahl, 1931

Cette espèce est typique pour le sapropel marin où elle se nourrit de bactéries sulfuriques (Kahl 1931, Fenchel 1968 a, b, Burkowski 1070 a, b). On l'a rencontrée dans le golf de Puck aux endroits fort pollués en présence de H_2S (Pl. II 12). Les individus trouvés étaient plus

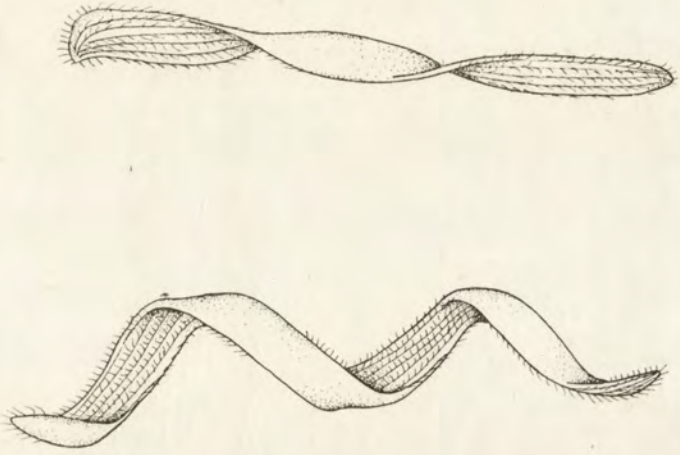

Fig. 12. *Kentrophoros fasciolatus* Sauerbrey

petits que ceux de Kahl (1931); ils mesuraient 70–95 μm de long. Les individus trouvés par Burkowski dans la mer Blanche correspondaient aussi à la description originelle seulement ils étaient plus grands (110–130 μm).

Sonderia sinuata Kahl, 1930

De même que la précedente, cette espèce vit dans le sapropel marin (Pl. III 13–14). D'après Kahl (1931) et Borror (1933) cette espèce se nourrit de cyanophycés et diatomés. Fenchel (1968 a) a constaté la nourriture variée: à part des algues, aussi flagellés, bactéries sulfuriques et ciliés. Les individus trouvés dans le golf de Puck vivaient aux mêmes endroits que *Plagiopyla ovata.* Ils rampaient parmi les filaments de *Beggiatoa* dont ils se nourrissaient. Les dimensions des individus trouvés par nous n'étaient pas si grandes que le rapporte Kahl (240 μm); ils mesuraient en moyen 140–150 μm, correspondant ainsi parfaitement aux dimensions donnés par Borror.

Cardiostomatella vermiforme Kahl, 1928

Ce cilié dont la morphologie correspond parfaitement à la déscription de Dragesco (1963 a) est asez fréquent dans le golf de Puck (Pl. III 15). Il appartient aux ciliés mésopsammiques cosmopolites et a été trouvé dans différentes mers.

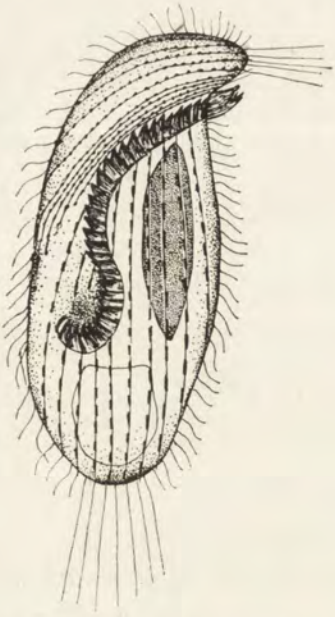

Fig. 13. *Metopus contortus* Quennerstedt

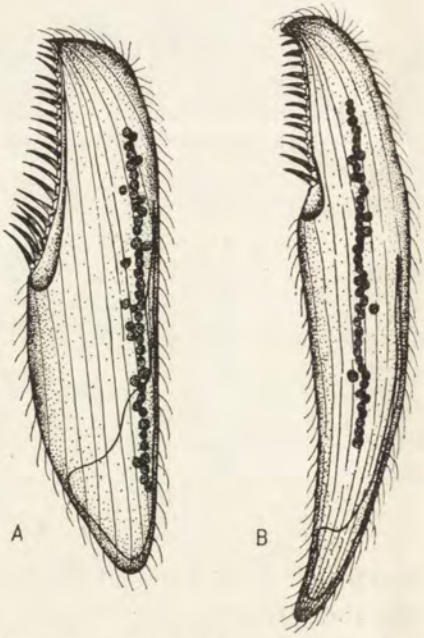

Fig. 14. Deux formes d'*Anigsteinia clarissimum* (Anigstein) (explication dans le texte)

Frontonia marina Fabre Domergue, 1891
Pleuronema coronatum Kent, 1881

Toutes les deux espèces sont communes dans les mers du monde entier et citées par nombreux chercheurs. Elles étaient fréquentes dans tous les endroits où nous prenions les échantillons. (Pl. III 16–17).

Metopus contortus Quennerstedt, 1867

Nous avons recontré ce cilié pendant tout l'été dans les endroits pollués du golf de Puck (Fig. 13). On le trouvait dans tous les prélevements du sable avec de la vase noire. *Metopus contortus* est un cilié saprobe, très abondant dans les endroits riches en bactéries sulfuriques. Il a été cité par les auteurs des mers du monde entier (Borror 1963, Fenchel 1968 a, Agamaliev 1974). L'animal étudié par nous correspond parfaitement à la déscription de Borror (1963) et de Jankowski (1964). Les individus trouvés avaient 100–110 μm de long. Ils avaient 30–34 cinéties et un macronucleus volumineux. Nous pouvons ajuter un détail aux déscriptions précédentes: les préparations faites à l'aide

de protéinate d'argent ont révélé la présence d'un faisceau de fibrilles qui commence au cytostome et s'enfonce profondement dans le cytoplasme (Pl. IV 18–20).

Spirostomum minus Roux, 1901
Spirostomum teres Clap. et Lachm., 1859

Tous les deux ciliés appartiennent aux espèces les plus communes et abondantes surtout dans l'eau douce. Les formes trouvées étaient conformes à la déscription de Kahl (1932) et de Repak et Isquith (1973).

Anigsteinia clarissimum (Anigstein, 1912)

Les individus identifiés par nous comme *Anigsteinia clarissimum* ont montré des differences en taille et en nombre de macronuclei en comparaison avec la déscription de Isquith et Repak (1974). Nous avons trouvé dans le golf de Puck deux types d'*Anigsteinia clarissimum* (Fig. 14,

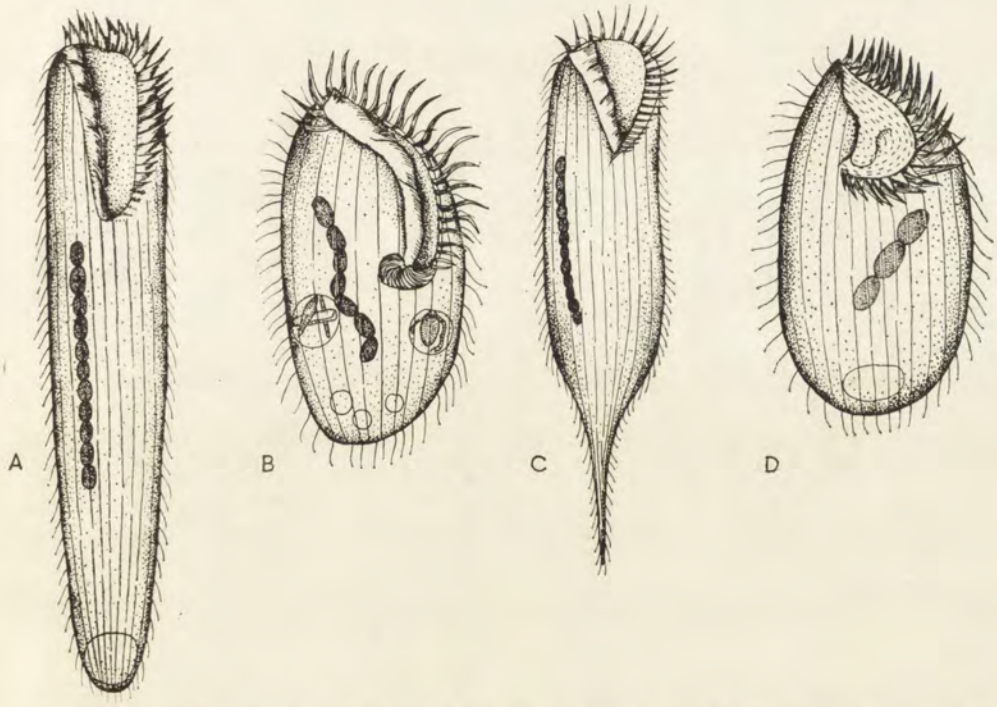

Fig. 15. A — *Condylostoma patulum* Clap. et Lachm., B — *Condylostoma remanei* Spiegel, D — *Condylostoma tardum* Penard

Pl. IV 21) Le premier correspondait bien à *Anigsteinia clarissimum* varietas *arenicola*, décrite par Kahl (1932) et Dragesco (1960); l'autre ressemblait plutôt par sa grande taille à *Anigsteinia longissima* (500–600 μm) mais à cause du nombre des macronuclei (44) il fallait la classifier aussi comme *Anigsteinia clarissimum*.

Climacostomum virens (Ehrenberg, 1833) forma *salinarum*

Seulement une fois nous avons rencontré quelques exemplaires de ce cilié. Il ressemblait bien à la forme salinarum décrite par Kahl (1932). Biernacka (1962) l'a trouvé aussi dans le golf de Gdańsk.

Genus *Condylostoma* Bory, 1924

Les ciliés du genre *Condylostoma* sont très frequents et abondants dans les sables marins. Quelques espèces semblent même être spécifiques pour ce biotope. Nous avons rencontré dans la mer Baltique quatre espèces: *Condylostoma patulum* Clap. et Lachm. 1858 *C. tardum* Penard, 1922,

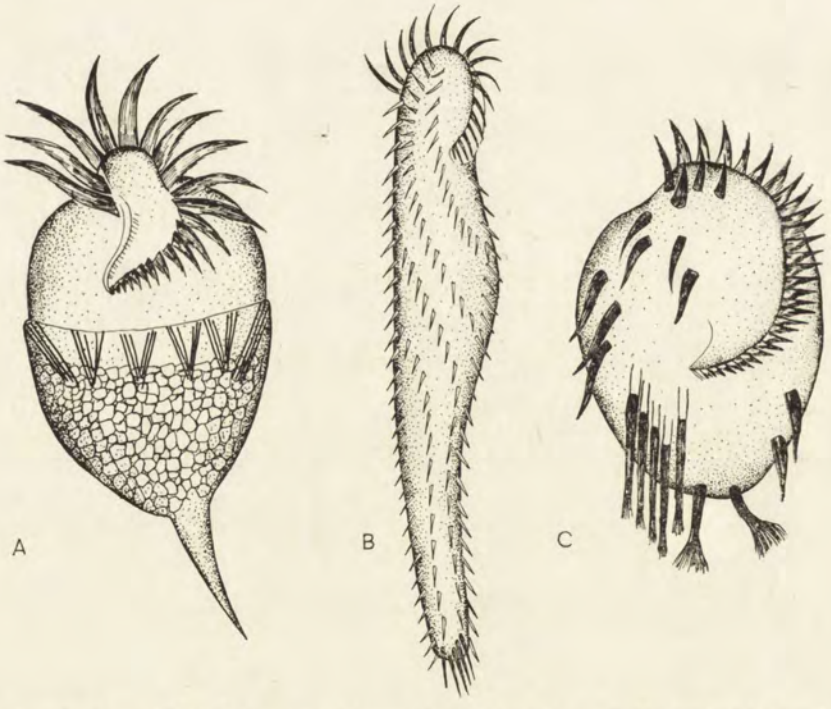

Fig. 16. A — *Strombidium styliferum* Levander, B — *Epiclintes ambiguus* (O. F. Müller), C — *Euplotes harpa* Stein

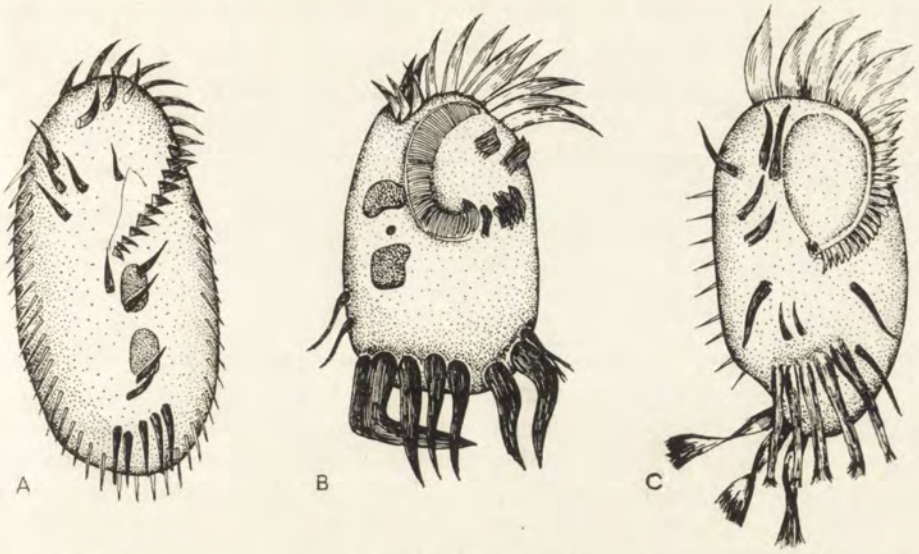

Fig. 17. A — *Oxytricha halophila* Kahl, B — *Diophrys appendiculata* (Ehrenberg), C — *Uronychia transfuga* (O. F. Müller)

C. remanei Spiegel, 1926, *C. minima* Dragesco, 1960 (Fig. 15). Dragesco (1963 b) qui a étudié toutes ces espèces en a donné d'excellentes déscriptions auquelles nous n'avons rien à ajouter.

Strombidium sauerbreyae Kahl, 1932

Ce cilié appartient aux espèces communes et cosmopolites. Cité par de nombreux auteurs. Les individus trouvés correspondaient exactement aux excellentes déscriptions de Kahl (1932) et Fauré-Fremiet (1950 a).

Strombidium styliferum Levander, 1894

Cette espèce est beaucoup moins répandue que la précédente. Très caractéristique à cause de son épine sur le pôle postérieur elle a été redécrite par Kahl (1932) et récemment par Borror (1972). Elle était assez nombreuse dans le golf de Puck (Fig. 16).

Hypotricha

Quelques espèces appartenant à cet ordre que nous avons rencontré dans la mer Baltique sont communes dans les mers du monde entier (Fig. 16, 17, Pl. IV 22). Elles ont été décrites par plusieurs auteurs. Nous

croyons qu'il est inutile de répéter leur déscriptions, donc nous présentons simplement la liste des espèces:

Epiclintes ambiguus (O. F. Müller, 1787)
Trachelostyla pediculiformis (Cohn, 1866)
Oxytricha halophila Kahl, 1932
Euplotes harpa Stein, 1859
Uronychia transfuga (O. F. Müller, 1786)
Diophrys appendiculata (Ehrenberg, 1838)

Discussion

Les ciliés vivants dans les sables marins éveillent depuis plusieurs années l'interêt des chercheurs à cause de leurs curieuses adaptations morphologiques et physiologiques ainsi que de l'importance qu'ils ont grâce à la densité de leurs populations dans la circulation de la matière dans les fonds sablonneux. Parmi les nombreuses publications faut avant tout citer d'excellentes monographies de Dragesco (1960) et Raikov (1962) décrivant la morphologie des espèces découvertes ainsi que la remarquable étude écologique de Fenchel (1968 a, b, 1969) qui révèle le rôle de ces ciliés dans les chaines nutritives.

Fauré-Fremiet qui, déjà en 1950 a reconnu les ciliés mésopsammiques comme un ensemble spécifique, après avoir comparé les résultats de ses recherches sur le fond sablonneux en Angleterre (Fauré-Fremiet, 1951) avec les recherches de Kahl (1928, 1933) aux environs de Kiel a conclu que les espèces psammophiles sont cosmopolites. Ce fait a été confirmé par plusieurs chercheurs comme Agamaliev, Borror, Burkowski, Hartwig, Petran, Raikov, qui ont trouvé les mêmes espèces psammophiles de ciliés dans differentes mers et océans.

Il reste à savoir jusqu'a quel dégré ces ciliés pénètrent dans les eaux saumâtres c'est à dire quelle est la quantité minimale de sels suffisant à leur existence. La mer Baltique présente des conditions très favorables à cette recherche parce qu'on y trouve tous les dégrés de salinité, depuis la haute concentration aux environs de Kiel jusqu'à pratiquement l'eau douce sur la côte finnoise. La salinité de la mer Baltique aux environs de Gdańsk oscille autour de 7‰, dans le lac saumâtre sur l'ile Bonzak elle descend jusqu'au 3‰.

Biernacka (1962, 1963) a publié une liste de protozoaires trouvés dans le golf de Gdańsk sans étudier pourtant leur morphologie de plus près. De même Czapik (1962) a publié une liste préliminaire des ciliés trouvés dans le sable du lac saumâtre sur l'île Bonzak.

La liste d'espèces trouvées présentée dans ce travail est probablement loin d'être complète. Elle contient pourtant 53 espèces marines

psammophiles. La plupart d'entre eux vivent non seulement dans le golf de Puck, où la concentration du sel atteint environ 7‰ mais aussi dans le lac saumâtre dont la salinité est très basse: elle ne depasse pas 3‰. Parmi les espèces typiquement marines supportantes la salinité tellement basse on peut citer par exemple: *Tracheloraphis margaritatus, Tracheloraphis phoenicopterus, Trachelonema oligostriata, Condylostoma remanei.* D'autre part on n'a jamais trouvé dans le lac certaines espèces comme *Kentrophoros fasciolatus* ou *Remanella rugosa.* Enfin on n'a jamais rencontré aux environs de Gdańsk un représentant du genre *Geleia.* Il en résulte que la quantité du sel selectionne les ciliés marins, bien que cette selection soit moins fort exprimée que chez les Metazoaires. Il parait aussi qu'il n'y a pas d'espèces qui vivent exclusivement dans les eaux saumâtres; tous les ciliés rencontrées dans les eaux saumâtres sont des espèces marins. Donc parmi les ciliés marins il y en a qui exigent une haute (océanique) concentration du sel (sténohalines) tandis que les autres pevent vivre aussi dans l'eau d'une minimale salinité, (euryhalines). La seule exception est peut-être le cilié *Prorodon raabei* qui est fréquent sur la côte polonaise mais qui jusqu'ici n'a pas été retrouvé dans les autres régions.

SUMMARY

A study has been made on ciliates living in submerged sable of brackish water in the Gdańsk Bay (Baltic Sea) where salt concentration attains 3–7‰. There were found 53 species of ciliates, two of them appeared to be new. Special attention has been paid to *Plagiopogon loricatus,* distinguishing by a peculiar structure of its pellicule. It was found that lowered salt concentration restrict the number of marine species penetrating brackish water.

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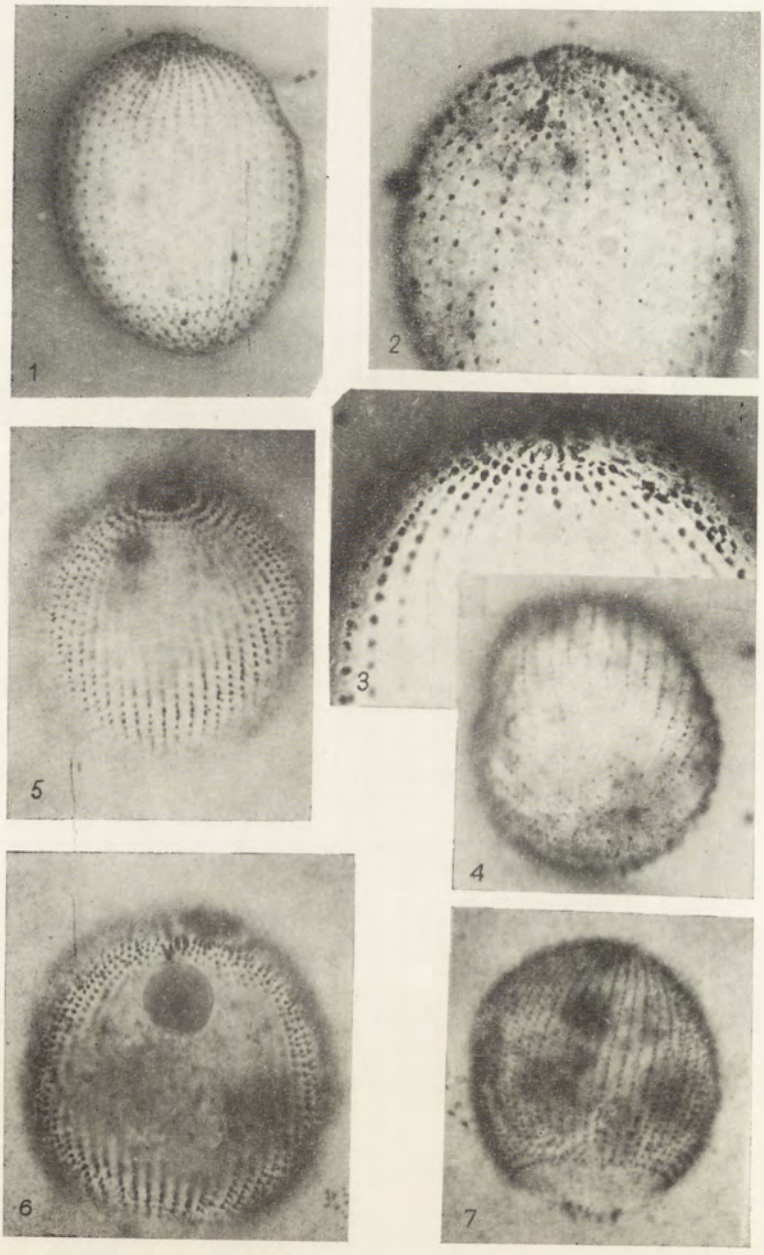
EXPLICATIONS DE PLANCHES I–IV

1–4: *Urotricha baltica* n. sp., 1 — vue générale de l'animal, 2 — entourage de la bouche et la "brosse", 3 — les cercles de cinétosomes doubles autour de la bouche, 4: pôle posterieur
5–7: *Urotricha armata* Kahl, 5 — vue générale de l'animal, 6 — "brosse", 7 — pôle posterieur
8–9: *Plagiopogon loricatus* Kahl
10: *Tracheloraphis kahli* Raikov
11: *Paraspathidium longinucleatum* n. sp. — la bouche
12: *Plagiopyla ovata* Kahl
13–14: *Sonderia sinuata* Kahl, 13 — vue générale de l'animal, 14 — fragment de la surface du corps
15: *Cardiostomatella vermiformis* (Kahl)
16: *Frontonia marina* Fabre Domerque
17: *Pleuronema coronatum* Kent
18–20: *Metopus contortus* Quennerstedt, 18 — face ventrale du corps, 19 — face dorsale, 20 — le faisceau de fibrils commençant au cytostome
21: *Anigsteinia clarissimum* (Anigstein)
22: *Euplotes harpa* Stein



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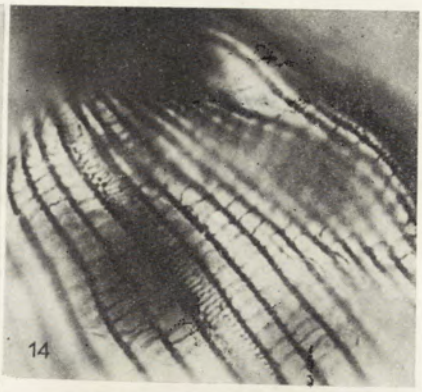

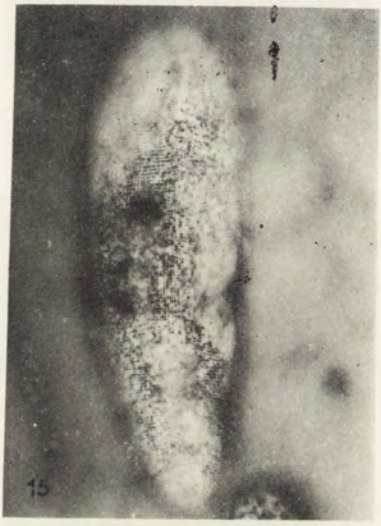

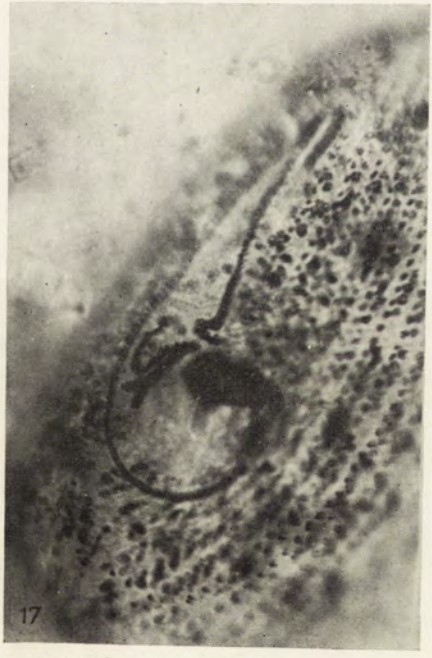

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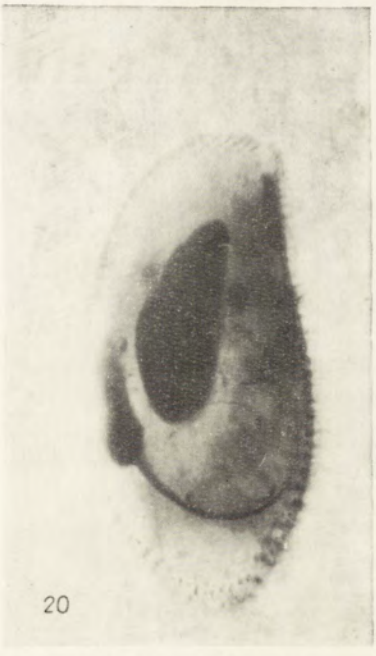

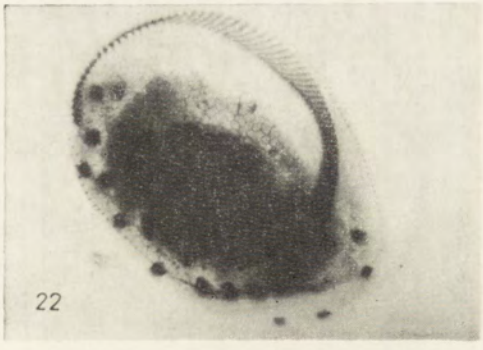

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Паразитические инфузории (*Peritricha, Urceolariidae*) рыб Белого моря

Parasitic Ciliates (*Peritricha, Urceolariidae*) of Fishes of the White Sea

Синопсис. На основании стандартной методики импрегнации азотнокислым серебром проведена ревизия урцеоляриид, паразитирующих на рыбах Белого моря. У 24 видов морских и солоноватоводных рыб обнаружено 12 видов урцеоляриид: *T. cottidarum* Dogiel, 1948; *T. murmanica* Poljansky, 1955; *T. elegini* Schulman-Albova, 1950; *T. galyae* Lom et Laird, 1969; *T. puytoraci* subsp. *marisalbi* G. Stein subsp. n.; *T. raabei* Lom, 1962; *T. oviducti* Poljansky, 1955; *T. borealis* (Dogiel, 1940); *Trichodina* sp. I (на жабрах *Platessa platessa*); *T. domerguei* subsp. *domerguei* Haider, 1964; *T. tenuidens* Fauré-Fremiet, 1943; *Trichodina* sp. 2 (из мочевого пузыря *Coregonus albula*).

В своей предыдущей работе, посвященной урцеоляриидам с некоторых рыб Баренцова моря (Штейн 1973), мы уже ставили вопрос о необходимости ревизии старых данных по урцеоляриидам, наряду со сбором новых материалов, на основе стандартной методики импрегнации азотнокислым серебром. Настоящая работа представляет собой очередной этап монографического описания урцеоляриид, обитающих в водоемах Советского Союза.

В работе использованы материалы, собранные в июле–августе 1971 г. на базе Беломорской биологической станции Зоологического института АН СССР в Чупинской губе Кандалакшского залива (район мыса Картеж), а также наши сборы на острове Ряжков и в его окрестностях (также Кандалакшский залив), относящиеся к 1959 г. Список исследованных и зараженных рыб приведен в Таблице 1.

Сухие мазки импрегнированы азотнокислым серебром по Клейну. Все измерения сделаны на фотографиях, выполненных с узкопленочной фотона-

Таблица 1 — Table 1

Список вскрытых и зараженных рыб Белого моря — List of Fish Species from White Sea Examined for Trichodinids

Виды рыб — Fish Species	Вскрыто экз. Examined	Заражено Infected	Виды урцеоляриид — Species of Trichodinids
(1) *Raja radiata* — скат звездчатый	1	1	*Trichodina oviducti*
(2) *Clupea harengus pallasi* n. *marisalbi* — сельдь беломорская	6	3	*Urceolariidae* sp.
(3) *Salmo trutta* — кумжа	4	1	*Urceolariidae* sp.
(4) *Coregonus lavaretus pidschian* n. *pidschianoides* — сиг беломорский проходной	8	—	—
(5) *Coregonus albula* — ряпушка[1]	1	1	*Trichodina* sp. 2
(6) *Gadus morhua marisalbi* — треска беломорская прибрежная	12	8	*Trichodina murmanica*
(7) *Eleginus navaga* — навага	11	10	*T. elegini*; *T. puytoraci* subsp. *marisalbi*; *T. galyae*
(8) *Gasterosteus aculeatus* — колюшка 3-иглая[1,2]	34	28	*T. domerguei* subsp. *domerguei*; *T. tenuidens*
(9) *Pungitius pungitius* — коюшка 9-иглая[2]	38	24	*T. domerguei* subsp. *domerguei*; *T. tenuidens*
(10) *Anarichas lupus* — зубатка	7	—	—
(11) *Pholis gunnelus* — маслюк	9	—	—
(12) *Lumpenus fabricii* — лумпенус	5	—	—
(13) *Zoarces viviparus* — бельдюга	2	—	—
(14) *Ammodites hexapterus* — песчанка	2	—	—
(15) *Myoxocephalus scorpius* — керчак	16	15	*T. murmanica*; *T. cottidarum*
(16) *Gymnocanthus tricuspis* — гимнокантус	4	—	—
(17) *Cyclopterus lumpus* — пинагор	10	6	*T. murmanica*
(18) *Leptagonus decagonus*	3	—	—
(19) *Liparis liparis*	1	—	—
(20) *Limanda limanda* — лиманда	8	5	*T. raabei*
(21) *Platessa platessa* — камбала морская	4	4	*Trichodina* sp. 1
(22) *Pleuronectes flesus bogdanovi* — камбала беломорская речная	6	3	*T. raabei*; *T. borealis*
(23) *Liopsetta glacialis* — камбала полярная	2	2	*Urceolariidae* sp.
(24) *Perca fluviatilis* — окунь[1]	5	—	—
	199	112	

[1] Рыбы из пресноводного озера Кривого на Мысе Картеж
[1] Fishes from fresh-water lake Krivoe situatad on Karteš peninsula.
[2] Сборы 1959
[2] Collected in 1959.

садкой "Praktica" к микроскопу "Lumipan-Zeiss" при объективе 90× и компенсационном окуляре 3.2×.

Наряду с обычными показателями, такими как диаметр прикрепительного диска, диаметр венчика и т. п., для более полной характеристики видов мы вводим ряд новых показателей, представляющих собой отношения отдельных элементов прикрепительного диска, выраженные в процентах. Мы надеемся, что эти показатели могут оказаться полезными при разделении близких видов.

В сборе и обработке материала принимала участие Н. В. Винникова, которой автор выражает свою глубокую благодарность.

Trichodina cottidarum Dogiel, 1948

Обнаружены на жабрах у 15 из 16 вскрытых бычков *Myoxocephalus scorpius* из разных участков Чупинской губы (районы острова Феттах, Левин-наволока, Красного мыса, Кив-губы). Встречается одновременно с *T. murmanica* Poljansky, 1955. По строению прикрепительного диска *T. cottidarum* с жабер беломорских рыб не отличаются от представителей этого вида, описанных с бычков *Myoxocephalus scorpius* в Баренцовом море (Штейн 1973) и с *M. scorpius* и *M. octodecemspinosus* в Северной Атлантике у побережья Канады (Lom and Laird 1969). Сравнение результатов измерений элементов прикрепительного диска и некоторых их соотношений (Таблица 2) еще раз наглядно демонстрирует наличие широкой внутривидовой изменчивости у представителей этого вида. Изменчивость ярко проявляется при сравнении инфузорий с разных экземпляров хозяев, выловленных в разных участках (графы 1–5: в каждой представлены результаты измерений инфузорий с одного экземпляра рыбы). Сопоставляя суммарные данные измерений беломорских *T. cottidarum* с представителями этого вида из других водоемов, следует отметить некоторое уменьшение размерных показателей у беломорских триходин, по сравнению с баренцовоморскими (Штейн 1973) и северо-атлантическими (Lom and Laird 1969).

Trichodina murmanica Poljansky, 1955 (Табл. I 1–3)

(Син. вероятно частично *Trichodina cottidarum* f. *marisalbi* Schulman et Schulman-Albova, 1953; *T. cottidarum* f. *cyclopteri* Poljansky, 1955; *T. domerguei* subsp. *saintjohnsi* Lom et Laird, 1969)

Хозяева: *Cyclopterus lumpus*, *Myoxocephalus scorpius*, *Gadus morhua marisalbi*, *Pholis gunellus*, *Triglops murrayi*, вероятно также *Melanogrammus aeglefinus* и *Pollachius virens*. Локали-

Таблица 2

Table 2

Сравнение *Trichodina cottidarum* Dogiel, 1948 с жабер *Myoxocephalus scorpius* из разных мест обитания

Comparison of *Trichodina cottidarum* Dogiel, 1948 from the Gills of *Myoxocephalus scorpius* Originating from Various Localities

	Остров Феттах Ostrov Fettah	Красный мыс Krasnyj Mys	Кив-губа Kiv-Guba	Левин-наволок I Levin-navolok I	Левин-наволок II Levin-navolok II	Суммарно по всем станциям Summarized data from all localaties
Диаметр прикрепительного диска (a)	26.2–39.6[1]	26.2–37.0	28.8–38.7	24.9–40.4	27.9–36.1	24.9–40.4
Diameter of adhesive disc (a)	32.0±0.25(136)[2]	32.6±0.61(30)	33.3±0.55(29)	34.0±0.64(30)	31.3±1.11(9)	32.6±0.21(234)
Наружный диаметр венчика (b)	22.5–32.9	21.9–33.5	24.5–34.7	24.5–32.3	24.5–32.3	21.9–33.5
Outer diameter of denticulate ring (b)	28.0±0.21(139)	28.6±0.50(31)	29.4±0.49(30)	28.6±0.39(27)	28.9±0.92(9)	28.4±0.16(236)
Внутренний диаметр венчика (c)	14.2–26.1	15.1–20.6	16.7–22.1	15.1–21.5	16.3–22.4	14.2–26.1
Inner diameter of denticulae ring (c)	18.5±0.15(139)	18.6±0.30(29)	19.5±0.29(30)	19.3±0.23(33)	19.2±0.73(9)	18.8±0.12(240)
Длина наружного отростка (d)	3.9–6.3	3.4–5.9	3.4–6.7	3.9–6.3	3.9–5.9	3.4–6.3
Length of outer part of denticle (d)	4.9±0.02(627)	5.0±0.05(133)	5.0±0.05(144)	4.9±0.04(155)	4.8±0.07(50)	4.9±0.20(1109)
Длина внутреннего отростка (e)	3.2–6.3	1.7–5.9	3.6–6.3	3.0–5.9	3.4–5.9	1.7; 3.0–6.3
Length of inner part of denticle (e)	4.8±0.02(584)	4.7±0.06(138)	5.0±0.05(144)	4.7±0.05(144)	4.7±0.09(49)	4.8±0.02(1050)

Число зубцов	20–28	19–26	20–28	22–26	22–27	19–28
Number of denticles	22.9±0.12(127)	23.0±0.18(78)	23.1±0.20(51)	23.7±0.10(83)	24.1±0.49(11)	23.0±0.07(393)
Число полос	5–10	5–10	6–10	7–10	7–10	5;6–10
Number of rays	8.2±0.04(373)	8.0±0.10(91)	8.2±0.08(93)	8.0±0.06(125)	8.0±0.14(29)	8.1±0.03(711)
Ширина краевой мембраны	2.6–4.9	2.6–5.1	2.2–4.5	2.6–3.9	2.6–4.3	2.2–5.1
Width of border membrane	3.4±0.04(122)	3.6±0.10(33)	3.1±0.12(25)	3.1±0.08(22)	3.4±0.17(8)	3.4±0.04(210)
Соотношение c : b	57.6–81.1	60.4–69.5	60.0–83.0	57.4–70.0	63.2–69.4	57.4–83.0
Coefficient of c : b	66.0±0.29(133)	64.6±0.40(29)	66.2±0.78(30)	65.9±0.54(29)	66.2±0.60(10)	65.9±0.22(231)
Соотношение c : a	48.6–70.6	52.5–69.0	53.2–71.0	48.5–64.8	56.2–63.7	48.5–71.0
Coefficient of c : a	58.1±0.32(134)	57.0±0.58(30)	58.6±0.78(29)	56.4±0.76(30)	59.9±0.87(9)	57.8±0.25(232)
Соотношение d : c	19.8–35.3	22.0–39.1	20.2–35.4	21.2–31.2	22.8–28.8	19.8–39.1
Coefficient of d : c	26.3±0.10(599)	27.4±0.29(144)	25.7±0.26(145)	24.7±0.19(155)	25.6±0.25(50)	26.1±0.08(1093)
Соотношение e : c	16.6–36.2	19.6–31.8	16.3–32.6	16.6–33.8	20.8–36.6	16.3–36.6
Coefficient of e : c	25.5±0.12(606)	25.4±0.22(144)	25.7±0.23(145)	24.4±0.26(151)	25.4±0.43(50)	25.3±0.09(1096)
Соотношение d : e	69.5–164.0	70.0–276.0	66.1–175.0	73.0–185.0	79.7–137.0	66.1–276.0
Coefficient of d : e	105.6±0.72(603)	108.7±1.78(149)	103.6±1.73(142)	106.9±1.63(147)	103.7±2.01(49)	106.5±0.56(1077)

В Таблицах 2-7 все измерения сделаны в μм, а соотношения умножены на 100; [1] размах вариаций, [2] среднеяя ощибка — среднего — число промеренных экземпляров

In Tables 2-7 all dimensions made in μм, and coefficients × 100: [1] range, [2] mean — mean error — number of examined specimens.

зация: жабры и плавники. Местообитание: бассейн Атлантического океана — побережье Канады, Баренцово, Белое моря.

Основанием для рассмотрения *T. domerguei* subsp. *saintjohnsi* в качестве синонима *T. murmanica* Poljansky, 1955 послужили общий вид прикрепительного диска, форма отростков, особенно наружных (см. Рис. 397–398 в Определителе паразитов пресноводных рыб СССР, 1962), цифровые характеристики, независящие от особенностей фиксации (диаметр прикрепительного диска, внутренний диаметр венчика, число зубцов в венчике), и, наконец, сравнительно небольшая широта изменчивости (Таблица 3).

Описание *T. murmanica* приведено в нашей предыдущей работе (Штейн 1973), и поэтому мы считаем возможным ограничиться лишь результатами измерений в разных водоемах (Таблица 4). Как следует из приведенных данных, *T. murmanica* из Белого моря, по сравнению с баренцовоморскими и северо-атлантическими, отличаются более мелкими размерами. У беломорских инфузорий (в частности с *Cyclopterus lumpus*) вершины наружных отростков более закругленные, чем у баренцовоморских триходин с бычков, наружные отростки более резко отходят от центральных конусов (баренцовоморские *T. murmanica* ближе к типичным экземплярам из работы Лома и Лэрда 1969), и у большинства экземпляров менее массивные внутренние отростки. Однако, эти различия, по-видимому, не выходят за рамки внутривидовой изменчивости. Выделение *T. murmanica* в качестве самостоятельного вида подтверждается его широким распространением в бассейне Атлантического океана.

Триходины с жабер *Gadus morhua marisalbi* (остров Ряжков, 1959 г.) судя по биометрическим данным, представляют собой, по-видимому, смешанную популяцию *T. murmanica* с каким-то другим видом (измерения сделаны на неимпрегнированных препаратах, зафиксированных формалином).

Trichodina elegini Schulman-Albova, 1950 (Табл. I 4–5)

На жабрах *Eleginus navaga*.

Довольно крупные инфузории. На импрегнированных препаратах центр прикрепительного диска светлый. Края светлого центра изрезаны в разной степени, аргентофильные включения различны по форме и варьируют в числе. Наружные отростки в виде широких лопастей с почти параллельными краями, выпуклым передним и прямым или слабо вогнутым задним краем. Вершина лопасти закруглена или слегка заострена. У части экземпляров хорошо заметен направленный вперед вырост перед внутренним отростком. Внутренние отростки прямые, равномерно палочковидные или слегка сужающиеся к концам, не доходят до центральной светлой зоны (у "аберрантных" форм с меньшим числом зубцов внутренние отростки почти доходят до светлой зоны). По сравнению с наружными внутренние отростки сдвинуты назад,

Таблица 3

Table 3

Сравнение *Trichodina murmanica* Poljansky, 1955 с *Myoxocephalus scorpius* из разных мест обитания

Comparison of *Trichodina murmanica* Poljansky, 1955 from the Gills of *Myoxocephalus scorpius* Originating from Various Localities

	Остров Феттах Ostrov Fettah	Красный мыс Krasnyj Mys	Кив-губа Kiv-Guba	Суммарно по всем станциям Summarized data from all localities
Диаметр прикрепительного диска (a) Diameter of adhesive disc (a)	34.0–46.4 40.1±0.46(54)	34.8–44.3 39.6±0.88(14)	34.8–41.9 38.4±0.83(11)	34.0–46.4 39.8±0.36(80)
Наружный диаметр венчика (b) Outer diameter of denticulate ring (b)	29.2–41.9 36.7±0.42(57)	31.8–39.2 35.8±0.62(14)	31.0–39.2 35.1±0.80(12)	29.2–41.9 36.3±0.32(84)
Внутренний диаметр венчика (c) Inner diameter of denticulate ring (c)	18.5–31.5 26.5±0.36(60)	22.4–28.3 25.4±0.48(14)	21.5–27.5 24.9±0.61(12)	18.5–31.5 26.0±0.28(88)
Длина наружного отростка (d) Length of outer part of denticle (d)	4.3–6.3 5.1±0.03(277)	4.3–5.9 5.1±0.03(70)	3.9–6.3 5.1±0.08(53)	4.3–6.3 5.1±0.02(405)
Длина внутреннего отростка (e) Length of inner part of denticle (e)	2.6–6.3 4.8±0.03(274)	3.9–5.5 4.9±0.04(70)	3.6–6.3 4.8±0.07(60)	2.6–6.3 4.8±0.03(409)
Число зубцов Number of denticles	25–29 27.0±0.11(89)	25–28 26.6±0.29(16)	24–29 27.3±0.43(16)	24–29 27.0±0.11(122)
Число полос Number of rays	6–12 9.0±0.07(146)	5–10 8.6±0.20(36)	6–11 8.8±0.24(22)	5–12 9.0±0.06(219)
Ширина краевой мембраны Width of border membrane	4.1–5.9 4.8±0.10(25)	4.3–5.1 4.7±0.11(8)	3.0–4.9 3.9±0.28(6)	3.0–5.9 4.6±0.09(39)
Соотношение c : b Coefficient of c : b	50.0–84.0 72.0±0.56(56)	67.5–74.5 70.8±0.45(14)	68.4–73.9 71.3±0.53(12)	50.0–84.0 71.7±0.40(82)
Соотношение c : a Coefficient of c : a	44.8–75.8 65.6±0.49(53)	60.3–65.6 63.5±0.45(13)	61.9–67.5 64.3±0.53(11)	44.8–75.8 65.1±0.36(78)
Соотношение d : c Coefficient of d : c	13.2–27.6 19.4±0.13(270)	17.9–24.1 20.3±0.19(68)	15.7–24.0 20.4±0.25(60)	13.2–27.6 19.9±0.12(404)
Соотношение e : c Coefficient of e : c	13.0–25.4 18.7±0.13(250)	16.6–22.8 19.4±0.16(62)	13.1–24.0 19.3±0.29(60)	13.0–25.4 18.9±0.10 (377)
Соотношение d : e Coefficient of d : e	90.7–195.1 109.6±0.74(279)	85.5–131.0 105.0±0.82(69)	67.8–150.0 107.9±1.77(60)	67.8–195.1 108.5±0.59(413)

Таблица 4

Table 4

Сравнение *Trichodina murmanica* Poljansky, 1955 с разных хозяев и из разных водоемов

Comparison of *Trichodina murmanica* Poljansky, 1955 from Various Fish Species and Seas

	Белое море White sea			Баренцово море Barents Sea		Северная Атлантика North Atlantic		
	Cyclopterus lumpus	*Gadus morhua marisalbi*	*Myoxocephalus scorpius*	*Myoxocephalus scorpius*	*Pholis gunnelus*	*Cyclopterus lumpus*	*Myoxocephalus scorpius*	*Triglops murrayi*
Диаметр прикрепительного диска (a) Diameter of adhesive disc (a)	30.7–44.0 38.1±0.53(45)	34.6–48.0 42.7±1.1(13)	34.0–46.4 39.8±0.36(80)	35.5–52.6	40.9–55.8	36.0–54.0	43.0–52.0	33.0–50.0
Наружный диаметр венчика (b) Outer diameter of denticulate ring (b)	25.9–39.4 33.8±0.38(80)	30.7–46.6 38.9±0.92(18)	29.2–41.9 36.3±0.32(84)	29.2–48.1	33.7–49.5	—	—	—
Внутренний диаметр венчика (c) Inner diameter of denticulate ring (c)	17.8–28.5 23.2±0.27(82)	21.1–33.6 27.8±0.73(19)	18.5–31.5 26.0±0.28(88)	20.7–36.0	23.4–36.4	21.0–31.0 25.0	25.0–33.0 29.0	20.0–32.0 25.0
Длина наружного отростка (d) Length of outer part of denticle (d)	3.6–7.0 5.1±0.03(395)	4.3–6.7 5.5±0.03(169)	4.3–6.3 5.1±0.02(405)	4.5–6.7	4.5–7.2	4.5–5.0	5.0	5.0
Длина внутреннего отростка (e) Length of inner part of denticle (e)	2.7–6.3 4.9±0.03(383)	3.8–7.2 5.4±0.05(175)	2.6–6.3 4.8±0.03(409)	4.0–6.3	4.5–7.2	3.5–5.0	5.0	5.0

Число зубцов Number of denticles	21–29 25.0±0.15(77)	25–28;32 26.8±0.47(15)	24–29 27.0±0.11(122)	25–29	26–29	23–31 26	25–30 28	25–30 28
Число полос Number of rays	7–12 8.7±0.14(97)	8–12 9.7±0.16(34)	5–12 9.0±0.06(219)	7–12	8–10	9–10	8–9	6–7
Ширина краевой мембраны Width of border membrane	2.4–4.9 3.9±0.09(43)	2.9–3.8 3.4±0.14(7)	3.0–5.9 4.6±0.09(39)	4.5–5.8	4.5–6.7	4.0–5.0	5.0	5.0
Соотношение c : b Coefficient of c : b	63.5–81.8 69.1±0.30(79)	68.8–75.0 72.1±0.42(19)	50.0–84.0 71.7±0.40(82)	—	—	—	—	—
Соотношение c : a Coefficient of c : a	59.0–70.5 63.7±0.32(45)	61.0±69.0 64.1±0.66(13)	44.8±75.8 65.1±0.36(78)	—	—	—	—	—
Соотношение d : c Coefficient of d : c	16.3–31.0 22.1±0.11(393)	14.7–24.0 19.7±0.13(162)	13.2–27.6 19.9±0.12(404)	—	—	—	—	—
Соотношение e : c Coefficient of e : c	15.2–28.5 21.2±0.11(378)	14.0–24.0 19.5±0.15(157)	13.0–25.4 18.9±0.10(377)	—	—	—	—	—
Соотношение d : e Coefficient of d : e	73.0–180.0 105.6±0.61(385)	70.0–137.0 104.8±0.92(165)	67.8–195.1 108.5±0.59(413)	—	—	—	—	—

Таблица 5

Table 5

Сравнение урцеоляриид, обитающих на жабрах *Eleginus navaga*
Comparison of Trichodins from Gills of *Eleginus navaga*

	Trichodina elegini	*Trichodina galyae*	*Trichodina puytoraci marisalbi*
Диаметр прикрепительного диска (a) Diameter of adhesive disc (a)	38.2–53.8 47.2±1.09(14)	48.1	27.8–44.8 35.7±0.81(25)
Наружный диаметр венчика (b) Outer diameter of denticulate ring (b)	35.0–49.0 42.4±0.73(22)	42.1–43.2	26.4–38.3 31.9±0.55(39)
Внутренний диаметр венчика (c) Inner diameter of denticulate ring (c)	24.5–36.5 30.9±0.57(25)	28.1–29.7	17.3–25.9 21.8±0.43(39)
Длина наружного отростка (d) Length of outer part of denticle (d)	4.3–7.7 6.0±0.06(136)	6.5–8.1	4.3–7.0 5.1±0.04(197)
Длина внутреннего отростка (e) Lenght of inner part of denticle (e)	3.8–7.7 6.0±0.07(131)	45.–8.6	3.4–7.6 5.6±0.06(190)
Число зубцов Number of denticles	28–34 30.6±1.99(20)	24	24–27 25.4±0.16(34)
Число полос Number of rays	9–12 10.2±0.16(26)	7–8	6.8–11 9.2±0.22(25)
Ширина краевой мембраны Width of border membrane	2.9–4.8 4.3±0.28(7)	4.3	2.4–3.8 3.1±0.12(17)
Соотношение c : b Coefficient of c : b	69.8–79.0 72.4±0.44(22)	65.0–70.5	63.0–72.0 68.1±0.33(39)
Соотношение c : a Coefficient of c : a	61.5–69.2 64.8±0.49(14)	57.7	53.5–67.2 61.3±0.70(25)
Соотношение d : c Coefficient of d : c	11.8–22.2 19.2±0.18(122)	23.0–27.8	16.8–28.3 23.7±0.16(192)
Соотношение e : c Coefficient of e : c	11.8–24.8 19.1±0.23(121)	19.2–29.0	18.6–34.2 25.7±0.22(181)
Соотношение d : e Coefficient of d : e	81.8–140.0 101.1±1.03(129)	86.6–147.0	69.2–128.5 92.7±0.94(184)

однако, в пределах одного венчика степень сдвига может быть различной. Центральные части зубцов широкие.

Учитывая результаты измерений (Таблица 5), форму зубцов в венчике (См. Определитель паразитов пресноводных рыб СССР, Рис. 399–401), мы сочли возможным идентифицировать описываемых инфузорий с триходинами, обнаруженными Шульман-Альбовой у беломорской наваги. Основанием для такого сближения послужили следующие признаки: (1) совпадающие довольно крупные размеры; (2) число зубцов (как правило превышающее 30);

(3) число полос (чаще всего 10); (4) совпадение центральной оси внутреннего отростка с наиболее плотной частью лопасти (на одной вертикали); (5) общность хозяев. *T. elegini*, в понимании Шульман и Шульман-Альбовой (1953) и Штейн (1967), по-видимому, представляют собой смешанную популяцию, включающую как собственно *T. elegini*, так и два следующих вида, обитающих одновременно с *T. elegini*.

Trichodina galyae Lom et Laird, 1969 (Табл. I 6)

Этот вид, описанный Ломом и Лэрдом (Lom and Laird 1969) с жабер *Cyclopterus lumpus* у побережья Канады, впервые регистрируется нами в водоемах Советского Союза на жабрах *Eleginus navaga*.

Два экземпляра, из которых один плохо сохранился, обнаружены нами на жабрах *Eleginus navaga* одновременно с двумя другими видами. Зубцы венчика с массивной центральной конусовидной частью и широким аппертурным отверстием. Широкие наружные отростки серповидно изогнуты (наиболее широкой является нижняя треть отростка, так что форма некоторых из них близка к треугольной). Внутренние отростки палочковидные, тонкие, прямые или слабо изогнутые, сдвинуты назад, так что центральная ось внутреннего отростка, перпендикулярная кольцу венчика, проходит около вершины наружной лопасти или даже позади нее. На импрегнированных препаратах центральная часть диска темная. Измерения приведены в Таблице 5.

Идентичность описываемых инфузорий с *T. galyae* не вызывает особых сомнений, хотя следует отметить, что в работе Лома и Лэрда число полос в прикрепительном диске *T. galyae* равно 10–12, тогда как нам удалось насчитать лишь 7–8, правда сохранность прикрепительных дисков была очень плохой.

Trichodina puytoraci subsp. *marisalbi* subsp. n. (Табл. II 7–9)

Также на жабрах *Eleginus navaga*.

Инфузории среднего размера (Таблица 5). Венчик из тесно сближенных зубцов с тонкими центральными конусами. Наружные отростки широкие лопастевидные с закругленными вершинами. Передний край выпуклый, задний — прямой или почти прямой. Внутренние отростки палочковидные, прямые или слабо изогнутые, иногда слегка расширенные на концах, сдвинуты назад и расположены на уровне заднего края наружной лопасти. Вырост перед внутренним отростком нечеткий. На импрегнированных препаратах центральная часть диска темная или содержит несколько небольших светлых включений.

От *T. elegini* эти триходины отличаются по своим биометрическим показателям, по числу и форме зубцов, по строению центральной зоны прикре-

пительного диска. От *T. jarmilae* Lom et Laird, 1969, которую *T. puytoraci* subsp. *marisalbi* напоминает по строению наружных отростков, эта инфузория отличается биометрическими показателями и особенно формой внутренних отростков. Наибольшее сходство триходины с наваги обнаруживают с *T. puytoraci* Lom, 1962, описанной в Черном море, в оз. Палеостоми и в Керченском проливе на жабрах кефалей *Mugil saliens*, *M. cephalus*, *M. auratus*, а также пузанка азовского *Alosa caspia tanaica* (Lom 1962, Штейн 1976), особенно с популяцией *T. puytoraci* с жабер *M. saliens* из района Севастополя. Сходство это заключается в близком совпадении биометрических показателей, в строении зубцов венчика и центральной части прикрепительного диска у *T. puytoraci* и некоторых инфузорий из беломорской популяции. Вместе с тем, если мелкие аргентофильные включения, напоминающие зерна риса, характерные для *T. puytoraci*, присутствуют в центре прикрепительного диска почти у всех особей этого вида, у описываемых инфузорий они не являются постоянным компонентом и имеют вид небольших округлых светлых пятен. Второе отличие касается формы наружных лопастей. У *T. puytoraci*, как правило, наиболее расширена концевая треть лопасти, а ближе к основанию лопасть слегка сужается. У инфузорий с наваги лопасти либо одинаковой ширины на всем протяжении, либо заметно расширены вблизи основания. Однако, как нам кажется, эти различия не настолько существенны, чтобы относить инфузорий с наваги к новому виду. Весьма вероятно, что различия обусловлены обитанием в разных водоемах и на разных хозяевах. Поэтому мы считаем возможным отнести инфузорий с наваги к отдельному подвиду вида *T. puytoraci* Lom, 1962 — *T. puytoraci* subsp. *marisalbi* subsp. n. Типовой препарат хранится в Лаборатории цитологии одноклеточных организмов Института цитологии АН СССР.

Trichodina raabei Lom, 1962 (Табл. II 10–11)

Обнаружены на жабрах *Limanda limanda* и *Pleuronectes flesus bogdanovi*. Впервые этот вид был описан Ломом (Lom 1962) в Черном море на жабрах *Platichthys flesus luscus*, затем на жабрах *Pleuronectes platessa* в Ламанше (побережье Бретани, Франция) (Lom 1970).

Сравнительно мелкие инфузории. Наружные отростки в виде довольно широких и сравнительно слабо изогнутых лопастей, чаще всего вершины их закруглены. Внутренние отростки шиповидные, сужающиеся к концам, реже палочковидные. Наружный и внутренний отростки расположены примерно на одной вертикали. При сравнении с типовым экземпляром, фотография которого приведена в работе Лома, зубцы кажутся менее массивными. На импрегнированных препаратах центральная часть диска темная. Измерения *T. raabei* приведены в Таблице 6.

Таблица 6

Table 6

Сравнение *Trichodina raabei* Lom, 1962 с разных хозяев

Comparison of *Trichodina raabei* Lom, 1962 from Various Hosts

	Белое море White Sea		Черное море Black Sea
	Limanda limanda	*Pleuronectes flesus*	*Platichthys flesus luscus*
Диаметр прикрепительного диска (a) Diameter of adhesive disc (a)	22.8–34.6 26.8±0.96(11)	23.9–32.8 28.0±0.96(9)	26.0–32.0 28.0
Наружный диаметр венчика (b) Outer diameter of denticulate ring (b)	20.5–28.9 24.0±0.65(11)	21.0–31.5 25.2±0.37(34)	—
Внутренный диаметр венчика (c) Inner diameter of denticulate ring (c)	14.4–20.5 17.1±0.44(14)	15.1–23.1 18.4±0.24(43)	14.0–18.0 16.0
Длина наружного отростка (d) Length of outer part of denticle (d)	3.0–4.2 3.7±0.04(103)	2.5–5.0 3.6±0.02(228)	3.0
Длина внутреннего отростка (e) Length of inner part of denticle (e)	2.7–5.7 4.9±0.06(110)	2.5–5.5 4.3±0.05(189)	2.7
Число зубцов Number of denticles	26–30 28.1±0.41(10)	24–31 28.1±0.30(35)	21–27 25
Число полос Number of rays	6–8 7.5±0.33(13)	6–8 7.3±0.25(8)	—
Ширина краевой мембраны Width of border membrane	2.3–2.7 2.4±0.06(10)	2.1±2.9 2.5±0.09(11)	2.5–3.0
Соотношение c : b Coefficient of c : b	67.2–73.7 70.8±0.63(11)	69.0±75.4 72.8±0.30(33)	—
Соотношение c : a Coefficient of c : a	59.2–69.4 65.2±0.93(10)	61.0–72.0 67.3±1.27(9)	—
Соотношение d : c Coefficient of d : c	17.8–28.0 21.7±0.27(108)	13.9–25.0 19.7±0.14(204)	—
Соотношение e : c Coefficient of e : c	16.7–39.2 26.1±0.45(107)	14.3–33.4 23.2±0.33(162)	—
Соотношение d : e Coefficient of d : e	61.6–100.0; 143 87.7±1.45(89)	60.0–140.3 89.7±1.24(178)	—

Trichodina oviducti Poljansky, 1955

Крупные инфузории из клоаки и яйцеводов ската *Raja radiata*. Впервые были обнаружены и описаны по гематоксилиновым препаратам из скатов Баренцова моря (Полянский 1955). Затем *T. oviducti* обнаружены еще у трех видов ската (*Raja magellanica*, *R. scabina*, *R. brachirops*) в районе Патагонского шельфа в юго-западной Атлантике (Евдокимова и др. 1969). По морфоло-

гическим и биометрическим признакам принадлежность беломорских инфузорий к *T. oviducti* не вызывает сомнений. Зубцы тесно сближены. Наружные отростки с параллельными или почти параллельными краями и закругленными вершинами. Тонкие длинные внутренние отростки прямые или слабо изогнуты, заметно сдвинуты назад, по сравнению с наружными, равны или длиннее их. На импрегнированных препаратах центральная часть диска темная. Измерения инфузорий из скатов разных видов приведены в Таблице 7. Как видно из таблицы, *T. oviducti* из беломорских скатов не имеют существенных отличий от инфузорий из других водоемов. Обращает на себя внимание незначительная длина наружных и внутренних отростков, по данным Ю. И. Полянского (графа 3), по сравнению с другими авторами. Это отклонение объясняется тем обстоятельством, что измерение в графах 1, 4–5 сделаны на основании импрегнированных препаратов, в графе 2 — после фиксации формалином, хорошо сохраняющим форму и размеры прикрепительного диска, тогда как в графе 3 измерения выполнены на препаратах, окрашенных гематоксилином. В последнем случае при дифференцировке краска может быть оттянута от менее плотных частей, так что подлинные размеры зубцов не выявляются.

Trichodina borealis (Dogiel, 1940) (Табл. II 12)

(Син. *T. domerguei* f. *borealis* Dogiel, 1940)

Два экземпляра этих инфузорий обнаружены на жабрах *Pleuronectes flesus bogdanovi*. Инфузории среднего размера. Наружный диаметр венчика 38.2, внутренний 27.3 — 29.4 μm. Длина наружного отростка 5.0–6.3, внутреннего 5.5–5.9 μm, зубцов 24 и 26. Отношения: внутреннего и наружного диаметров венчиков 71.6; наружного отростка к внутреннему диаметру венчика 18.4–21.4; внутреннего отростка к внутреннему диаметру венчика 18.6–20.0; наружного отростка к внутреннему 105.0–114.5. На импрегнированных препаратах центральная часть прикрепительного диска светлая, с аргентофильными включениями, сливающимися в крупные пятна.

При сравнении с инфузориями, паразитирующими на камбалах и также имеющими светлый центр прикрепительного диска, триходины с *Pleuronectes flesus* при большом морфологическом сходстве оказались значительно более крупными, чем *T. borealis* (Dogiel, 1940) с черноморских камбал *Solea lascaris*, *Platychthys flesus luscus*, *Scophthalmus maeoticus torosus* (Штейн 1976). С описаниями Догеля (1940), Stryjecka-Trembaczowska (1953) и Штейн (1967) сравнивать довольно трудно, так как во всех этих работах, выполненных на гематоксилиновых препаратах, авторы явно имели дело со смешанными популяциями, о чем свидетельствует большая изменчивость приведенных данных.

Значительное морфологическое сходство и близкие размеры у наших инфузорий с *Trichodina* cf. *borealis* Schulman et Schulman-Albova, 1953 с мор-

Таблица 7

Table 7

Сравнение *Trichodina oviducti* Poljansky, 1955 с разных хозяев и из разных водоемов

Comparison of *Trichodina oviducti* Poljansky, 1955 from Various Fish Species and Seas

	Белое море White Sea	Баренцово море Barents Sea		Юго-западная Атлантика South-west Atlantic	
		Raja radiata			
	Raja radiata	Наши данные Our data	Полянский, 1955 Poljansky, 1955	*Raja scabina*	*Raja brachirops*
Диаметр прикрепительного диска (a) Diameter of adhesive disc (a)	86.9–97.7 92.8±2.45(4)	118.0–166.0	145.0	102.0–123.0	93.0
Наружный диаметр венчика (b) Outer diameter of denticulate ring (b)	66.2–92.6 78.9±1.93(13)	74.0–120.0	65.0–95.0	88.5–102.0	84.0
Внутренний диаметр венчика (c) Inner diameter of denticulate ring (c)	47.3–66.2 58.1±1.38(13)	—	—	60.0–72.0	—
Длина наружного отростка (d) Length of outer part of denticle (d)	8.2–13.9 11.0–0.16(67)	8.0–14.0	5.4–7.2	12.0–13.5	12.0
Длина внутреннего отростка (e) Length of inner part of denticle (e)	8.2–15.8 13.1±0.22(72)	10.0–14.0	3.6–5.4	13.5–15.0	15.0
Число зубцов Number of denticles	48–54 50.9±0.54(14)	44,46–56 50–51	45–57 51–54	46–55 49–51	50
Число полос Number of rays	—	10–16	12	10–12	12(?)
Ширина краевой мембраны Width of border membrane	5.7–6.3(3)	—	—	—	—
Соотношение c : b Coefficient of c : b	69.6–83.0 73.9±1.08(11)	—	—	—	—
Соотношение c : a Coefficient of c : a	62.5–71.0 64.9±2.02(4)	—	—	—	—
Соотношение d : c Coefficient of d : c	12.7–22.4 18.2±0.36(47)	—	—	—	—
Соотношение e : c Coefficient of e : c	11.9–28.2 21.2±0.67(41)	—	—	—	—
Соотношение d : e Coefficient of d : c	60.9–113.1 85.2±1.14(71)	71.0–117.0	—	75.0–100.0	80.0

ских собачек *Istioblennius zebra* с острова Оаху (Гавайские острова, Тихий океан) (Lom 1970). По-видимому, окончательное решение о характеристике вида *T. borealis* (Dogiel, 1940) мы сможем принять после того, как будет обработан весь материал по морским урцеоляриидам с камбаловых рыб, находящийся в нашем распоряжении.

Trichodina sp. I (Табл. II 13)

Единственный экземпляр обнаружен на препаратах с жабер *Platessa platessa*. Сравнительно крупная триходина с наружным диаметром венчика 59.9 и внутренним — 41.6 μm. Зубцы массивные с широкой центральной частью. Широкий серповидный наружный отросток сужается к вершине и выдвинут вперед по отношению к аппертурному отверстию. Его длина, как и длина внутреннего отростка 8.8–9.5 μm. Последний-прямой шиповидный, сдвинут назад, по сравнению с наружным, так что продолжение его центральной оси проходит через вершину или через верхнюю треть наружного отростка. Перед внутренним отростком имеется направленный вперед вырост. В венчике 23 зубца. Отношения: внутреннего диаметра венчика к наружному — 69.8; наружного и внутреннего отростков к внутреннему диаметру венчика — 21.2–22.7; отношение наружного и внутреннего отростков — 100.0. Характер центральной части прикрепительного диска неясен, четко оформленной светлой зоны на этом экземпляре нет.

Сравнение описываемой триходины с другими урцеоляриидами не позволяет ее идентифицировать с уже известными видами. От *T. raabei* с *Limanda limanda* и *Pleuronectes flesus* она отличается как по размерам, так и по строению зубцов. От *T. borealis* — по строению зубцов, центральной зоны прикрепительного диска и также по размерам. Поэтому мы обозначаем инфузорий с жабер *Platessa platessa* как *Trichodina* sp.

Одновременно со сборами морских урцеоляриид мы обследовали рыб из небольшого озера Кривого, расположенного на территории Беломорской биологической станции на мысе Картеж. На *Gasterosteus aculeatus* и в мочевом пузыре ряпушки *Coregonus albula* были обнаружены урцеоляриды. Окуни *Pelca fluviatlis* в наших сборах оказались незараженными. На жабрах *Gasterosteus aculeatus* одновременно обитали два вида триходин — *T. domerguei* subsp. *domerguei* и *T. tenuidens*.

Trichodina domerguei subsp. *domerguei* Haider, 1964

(Син. *T. domerguei* f. *latispina* Dogiel, 1940; *T. latispina* (Dogiel, 1940) Schulman et Schulman-Albova, 1953)

По своей морфологии триходины, обнаруженные нами, не отличались

от типичных представителей этого вида, описанных из других водоемов (Lom and Stein 1966). Поэтому приведем лишь результаты измерений.

Диаметр прикрепительного диска 30.5–50.3, наружный диаметр венчика 29.2–46.3, внутренний — 19.4–31.5 μm. Длина наружного отростка 4.7–7.8, внутреннего 3.3–7.4 μm. Число зубцов варьирует от 17 до 23, наиболее часто 20–22, число полос 6–11, чаще всего 10. Ширина краевой мембраны 2.2–6.5 μm. Отношения: внутреннего диаметра венчика к наружному 61.8–71.2; к диаметру прикрепительного диска — 55.1–73.7; наружного отростка к внутреннему диаметру венчика 19.7–32.3; внутреннего отростка к внутреннему диаметру венчика — 14.8–35.0; отношение наружных отростков к внутренним — 100.0–199.0, преобладание в интервале 100.0–109.9.

Trichodina tenuidens Fauré-Fremiet, 1943

(Син. *T. gracilis* Poljansky, 1955)

По морфологии и результатам измерений не отличаются от представителей этого вида, описанных из других водоемов (Lom and Stein 1966). Приводим лишь результаты измерений. Диаметр прикрепительного диска 36.3–62.7; наружный диаметр венчика 34.2–57.2, внутренний — 24.1–41.3 μm. Длина наружного отростка 4.7–8.5, внутреннего — 4.1–9.9 μm. Число зубцов в венчике варьирует от 24 до 32 с наибольшей частотой 27–29 зубцов, число полос 6–13, преимущественно 10. Ширина краевой мембраны 3.7–7.5 μm. Отношения: внутреннего диаметра венчика к наружному 51.1, 61.5–82.5; внутреннего диаметра венчика к диаметру прикрепительного диска 54.5–79.4; наружного отростка к внутреннему диаметру венчика 13.9–29.0; внутреннего отростка к внутреннему диаметру венчика 13.1–31.8; наружного отростка к внутреннему 56.5–158.5 с преобладанием в интервале 100.0–109.9.

Поскольку *T. domerguei* subsp. *domerguei* и *T. tenuidens* встречаются одновременно очень важно различать эти два вида в смешанных популяциях. Для полностью сформированных вегетативных особей может быть составлена табличка. представлена на стр. 464.

Опираясь на характеристики обоих видов с 3-иглой колюшки, полученные на импрегнированных препаратах, мы пересмотрели свои сборы 1959 г. из района Кандалакши и острова Ряжков. На гематоксилиновом материале с 3- и 9-иглой колюшки мы также различаем эти два вида, однако, не всегда можем дать точное определение из-за совпадающих (заходящих) признаков, тогда как такой существенный критерий как строение центральной части прикрепительного диска на гематоксилиновых препаратах отсутствует.

T. tenuidens	*T. domerguei* subsp. *domerguei*
(1) Светлая центральная часть прикрепительного диска содержит большое число аргентофильных включений или раздроблена на отдельные участки.	(1) Центральная часть прикрепительного диска в виде единственного светлого пятна с немногочисленными включениями.
(2) Задний край наружного отростка почти прямой или очень слабо изогнут.	(2) Задний край наружного отростка резко серповидно изогнут.
(3) Число зубцов в венчике варьирует от 24 до 32 (наибольшая частота в интервале 27-29).	(3) Число зубцов в венчике варьирует от 17 до 23 (наибольшая частота в интервале 20--22); в других популяциях максимальное число зубцов в венчике может быть больше.
(4) Преимущественно на жабрах.	(4) Преимущественно на плавниках.

Trichodina domerguei subsp. *domerguei* была обнаружена на поверхности тела, плавниках и жабрах *Pungitius pungitius* из реки Нива, впадающей в Кандалакшский залив, из пресного озера на острове Лодейный и в заливе в районе Девичьей Луды. *T. tenuidens*: с кожи, плавников и жабер *Pungitius pungitius* в тех же местах и с жабер *Gasterosteus aculeatus* в заливе в районе острова Ряжков. Таким образом, еще раз было показано, что *T. domerguei* subsp. *domerguei* и *T. tenuidens*, как и их хозяева *Pungitius pungitius* и *Gasterosteus aculeatus* являются эвригалинными видами.

В мочевом пузыре ряпушки *Coregonus albula* были обнаружены несколько экземпляров небольших инфузорий, плохо сохранившихся на препаратах. Мы их обозначили как *Trichodina* sp.

Trichodina sp. 2 (Табл. II 14)

Частично промерены два экземпляра. Сравнительно узкие наружные отростки с закругленными или слегка заостренными вершинами. Передний край наружного отростка выпуклый, внутренний прямой или слабо вогнутый. Внутренние отростки палочковидные, прямые или слабо изогнутые, более длинные, чем наружные, слегка сдвинуты назад. Внутренний диаметр венчика 18.9; длина наружного отростка 4.2–4.6, внутреннего 5.0–5.9 μm; зубцов 23–24, полос 8–9, ширина краевой мембраны 2.5 μm. Отношение наружного отростка к внутреннему 71.5–92.0.

По форме зубцов триходины из мочевого пузыря ряпушки напоминают *T. platyformis* Davis, 1947 (Wellborn 1967). Отличаются от этого вида меньшими размерами зубцов, меньшим числом зубцов, полос. С *T. discoidea* Davis, 1947, по-видимому, близки по размерам, числу зубцов и полос, отличаясь взаиморасположением наружного и внутреннего отростков: у *T. discoidea* наружный и внутренний отростки прикрепляются на одной вертикали и, в отличие от триходин из ряпушки, наружная лопасть заметно расширена на конце.

В настоящее время из мочевого пузыря пресноводных рыб известны следующие виды: *Vauchomia nephritica* Mueller, 1938; *V. renicloea* Mueller, 1938; *Trichodina urinaria* Dogiel, 1940; *T. alburni* Vojtek, 1957; *T. polycirra* Lom, 1960; *T. schizothoraci* Aschurova et Stein, 1972; *Tripartiella* (*Paratrichodina*) *phoxini* Lom, 1963; *Trichodina abramidis* Osmanow, 1963; *Urceolaria stammeri* Haider, 1964.

Сразу же при сравнении следует отбросить виды с большим числом зубцов в венчике, такие как *Vauchomia nephritica*, *V. renicola*, *Trichodina urinaria*, *T. abramidis*. *T. polycirra*, видимо, отличается по строению зубцов и центральной зоны прикрепительного диска, так как у триходин с ряпушки в центре диска отсутствуют светлые включения. *T. schizothoraci* и *Tripartiella phoxini*, хотя и характеризуются наличием темной центральной зоны диска, имеют совершенно иную форму наружных отростков. У *Urceolaria stammeri*, судя по ее родовой принадлежности, вообще отсутствую внутренние отростки.

Что касается *Trichodina alburni* Vojtek, 1957, то этот вид из мочевого пузыря уклеи *Alburnus alburnus* был описан по гематоксилиновым препаратам. Эргенс и Лом (Ergens and Lom 1970) приводят фотографию урцеоляриды, которую они идентифицируют с *T. alburni* Vojtek, 1957 и считают ее синонимом *Tripartiella* (*Paratrichodina*) *phoxini* Lom, 1963. Пожалуй больше всего триходины из ряпушки напоминают *Trichodina* sp. I, описанную Кандиловым (1964) из мочевого пузыря жереха *Aspius aspius* в бассейне Куры. Сравнивая нашу находку с рисунком Кандилова, мы можем говорить о сходстве в строении зубцов, но триходина из жереха имела 34–36 зубцов в венчике, тогда как у инфузорий из ряпушки зубцов всего 23–24.

Таким образом, просмотрев все известные нам данные об урцеолярииды из мочевых путей пресноводных рыб, мы не смогли отнести триходин из ряпушки к какому-либо определенному виду и обозначили их как *Trichodina* sp. 2.

Опираясь на результаты нашего исследования, можно подвести некоторые итоги изучения фауны урцеолярииды Белого моря. По нашим данным, у 24 видов рыб, включая и обитателей пресных и солоноватых вод, обнаружено 12 видов урцеолярииды (Список видов смотри ниже). По сравнению с монографией Шульмана и Шульман-Альбовой (1953), в которой были описаны 7 видов урцеолярииды, число видов увеличилось почти вдвое. Впервые для Белого моря отмечены такие виды как *T. galyae*, *T. puytoraci* subsp. *marisalbi*, *T. raabei*, *T. oviducti*. *T. murmanica* и *T. tenuidens* уже отмечались нами ранее (Штейн 1962). Вид *T. cottidarum*, в понимании Шульмана и Шульман-Альбовой, оказался смешанным и включает в свой состав собственно *T. cottidarum* и *T. murmanica*. Прежний вид *T. elegini* распался на три вида: собственно *T. elegini*, *T. galyae* и *T. puytoraci* subsp. *marisalbi*. По частоте встречаемости на первом месте стоит *T. puytoraci*, затем *T. elegini*. *T. galyae* была встречена всего несколько раз. Изменились и наши представления о трихо-

динах с камбал. Начиная с работы Догеля (1948), считалось, что на камбалах обитает один вид — *T. borealis*. По нашим наблюдениям, массовым видом на камбалах оказался *T. raabei* a, *T. borealis* встречены всего несколько раз.

К сожалению, из-за отсутствия материала мы не могли идентифицировать на импрегнированных препаратах *T. californica* Davis, 1947 с жабер *Salmo salar*, *Trichodina* sp. из мочевого пузыря *Myoxocephalus scorpius*, *Trichodina* sp. с жабер *Boreogadus saida*, *Lumpenus fabricii* и *Salmo trutta*, описанных Шульманом и Шульман-Альбовой. Весьма вероятно, что эти виды или их синонимы в дальнейшем пополнят список урцеоляриид Белого моря. Как мы предполагаем, в этот список в дальнейшем, вероятно, будут включены *T. liparisi* Zhukov, 1964 с *Liparis liparis* и *Trichodina* sp. с *Zoarces vivipalus*, описанные в Баренцовом море. Хозяева этих инфузорий в нашем материале были вскрыты в недостаточном количестве (соответственно 1 и 2 экземпляра). Отсутствовала у нас и пикша *Melanogrammus aeglefinus*, вследствие чего мы не могли провести ревизию *Tripartiella melanogrammi* Stein, 1961, также описанной в Баренцовом море.

В конечном итоге список урцеоляриид, обитающих на рыбах в Белом море, представляется нам таким:

(1) *T. cottidarum* Dogiel, 1948 — *Myoxocephalus scorpius*.

(2) *T. murmanica* Poljansky, 1955 — *Cyclopterus lumpus*, *Myoxocephalus scorpius*, *Gadus morhua marisalbi*, предположительно *Pholis gunellus*, *Melanogrammus aeglefinus*, *Pollachius virens*.

(3) *T. elegini* Schulman-Albova, 1950 — *Eleginus navaga*.

(4) *T. galyae* Lom et Laird, 1969 — *Eleginus navaga*.

(5) *T. puytoraci* subsp. *marisalbi* subsp. n. — *Eleginus navaga*.

(6) *T. raabei* Lom, 1962 — *Limanda limanda*, *Pleuronectes flesus bogdanovi*.

(7) *T. oviducti* Poljansky, 1955 — *Raja clavata*.

(8) *T. borealis* (Dogiel, 1940) — *Pleuronectes flesus bogdanovi*.

(9) *T. domerguei* subsp. *domerguei* Haider, 1964 — *Gasterosteus aculeatus*, *Pungitius pungitius*.

(10) *T. tenuidens* Faurè-Fremiet, 1943 — *Gasterosteus aculeatus*, *Pungitius pungitius*.

(11) *Trichodina* sp. 1 — *Platessa platessa*.

(12) *Trichodina* sp. 2 — *Coregonus albula*.

(13) *T. liparisi* Zhukov, 1964 — *Liparis liparis* (по аналогии с Баренцовым морем).

(14) *Trichodina* sp. Stein, 1973 — *Zoarces viviparus* (по аналогии с Баренцовым морем),

(15) *T. californica* Davis, 1947 — *Salmo salar* (нуждается в проверке на импрегнированных препаратах).

(16) *Trichodina* sp. 1 Schulman et Schulman-Albova, 1953 — *Myoxocephalus scorpius* (нуждается в проверке на импрегнированных препаратах).

(17) *Trichodina* sp. 2 Schulman et Schulman-Albova, 1953 — *Boreogadus saida, Lumpenus fabricii, Salmo trutta* (нуждается в проверке на импрегнированных препаратах).

(18) *Tripartiella melanogrammi* Stein, 1961 — *Melanogrammus aeglefinus* (нуждается в проверке на импрегнированных препаратах).

Препараты хранятся в коллекции Лаборатории цитологии одноклеточных организмов Института цитологии АН СССР.

В заключение отметим некоторые особенности фауны урцеолярид Белого моря. Прежде всего бросается в глаза, что все виды относятся к одному роду — роду *Trichodina*. Единственное исключение — *Tripartiella melanogrammi*. Но в этом случае необходима проверка на импрегнированных препаратах, в результате которой может измениться как видовая, так и родовая принадлежность. Обращает на себя внимание некоторое уменьшение размеров беломорских триходин, по сравнению с баренцовоморскими и с инфузориями из Северной Атлантики (см. таблицы с измерениями *T. cottidarum* и *T. murmanica*).

Любопытно, что наряду с видами, характерными для Баренцова моря и Северной Атлантики (*T. cottidarum, T. murmanica, T. galyae*) были обнаружены *T. puytoraci* и *T. raabei*, впервые описанные в Черном море. Можно надеется, что дальнейшие исследования, в частности исследования урцеолярид из морей Дальнего Востока, позволят полнее охарактеризовать и сравнить между собой урцеолярид крупных морских бассейнов — Атлантического, Тихоокеанского и Средиземноморского.

SUMMARY

In the smears taken from 24 species of fishes the presence of 12 ciliate species was ascertained: ***Trichodina cottidarum*** Dogiel, 1948; ***T. murmanica*** Poljansky, 1955; ***T. elegini*** Schulman-Albova, 1950; ***T. galyae*** Lom et Laird, 1969; ***T. puytoraci marisalbi*** G. Stein subsp. n.; ***T. raabei*** Lom, 1962; ***T. oviducti*** Poljansky, 1955; ***T. borealis*** (Dogiel, 1940); ***Trichodina*** sp. 1 (on the gills of ***Platessa platessa***); ***T. domerguei*** subsp. ***domerguei*** Haider, 1964; ***T. tenuidens*** Fauré-Fremiet, 1943; ***Trichodina*** sp. 2 (from the urinary bladder of ***Coregonus albula***). The revision of the fauna of ***Urceolariidae*** of fishes of the White Sea made on the basis of the standard silver impregnation method.

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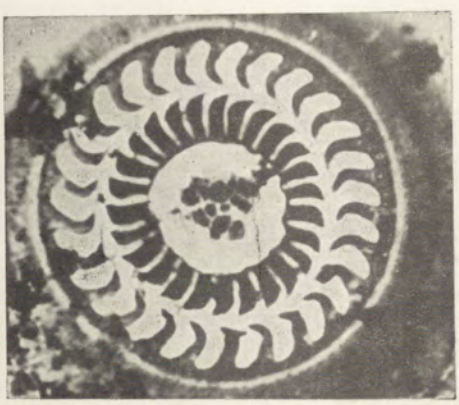
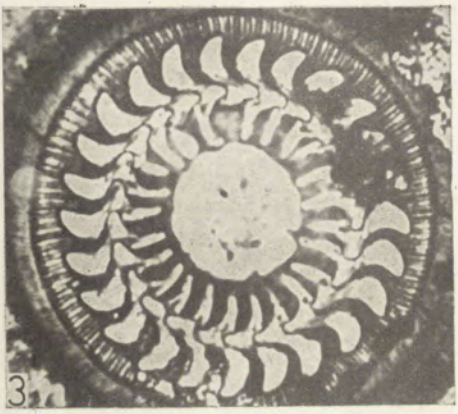
ПОДПИСИ К ТАБЛИЦАМ I—II

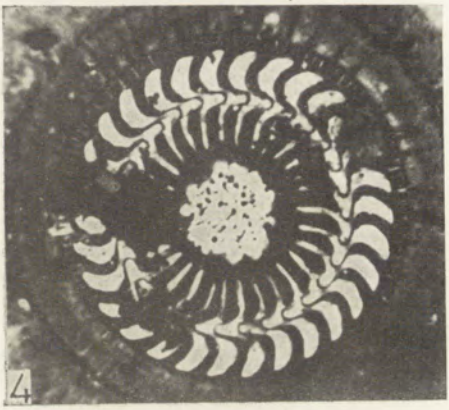
1: *Trichodina murmanica* Poljansky, 1955 с *Cyclopterus lumpus*
2: *Trichodina murmanica* Poljansky, 1955 с *Myoxocephalus scorpius*
3: *Trichodina murmanica* Poljansky, 1955 с *Gadus morhua*
4–5: *Trichodina elegini* Schulman-Albova, 1950 с *Eleginus navaga*
6: *Trichodina galyae* Lom et Laird, 1969 с *Eleginus navaga*
7–9: *Trichodina puytoraci marisalbi* subsp. n. с *Eleginus navaga*
10–11: *Trichodina raabei* Lom, 1962 с *Limanda limanda*
12: *Trichodina borealis* (Dogiel, 1940) с *Pleuronectes flesus*
13: *Trichodina* sp. 1 с *Platessa platessa*
14: *Trichodina* sp. 2 с мочевого пузыря *Coregonus albula*

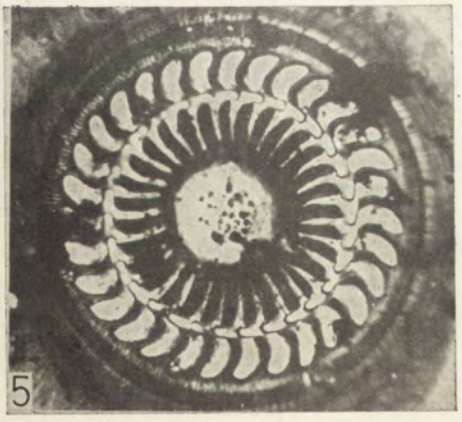
EXPLANATION OF PLATES I–II

1: *Trichodina murmanica* Poljansky, 1955 from *Cyclopterus lumpus*
2: *Trichodina murmanica* Poljansky, 1955 from *Myxocephalus scorpius*
3: *Trichodina murmanica* Poljansky, 1955 from *Gadus morhua*
4–5: *Trichodina elegini* Schulman-Albova, 1950 from *Eleginus navaga*
6: *Trichodina galyae* Lom et Laird, 1969 from *Eleginus navaga*
7–9: *Trichodina puytoraci marisalbi* subsp. n. from *Eleginus navaga*
10–11: *Trichodina raabei* Lom, 1962 from *Limanda limanda*
12: *Trichodina borealis* (Dogiel, 1940) from *Pleuronectes flesus*
13: *Trichodina* sp. 1 from *Platessa platessa*
14: *Trichodina* sp. 2 from urinary bladder *Coregonus albula*









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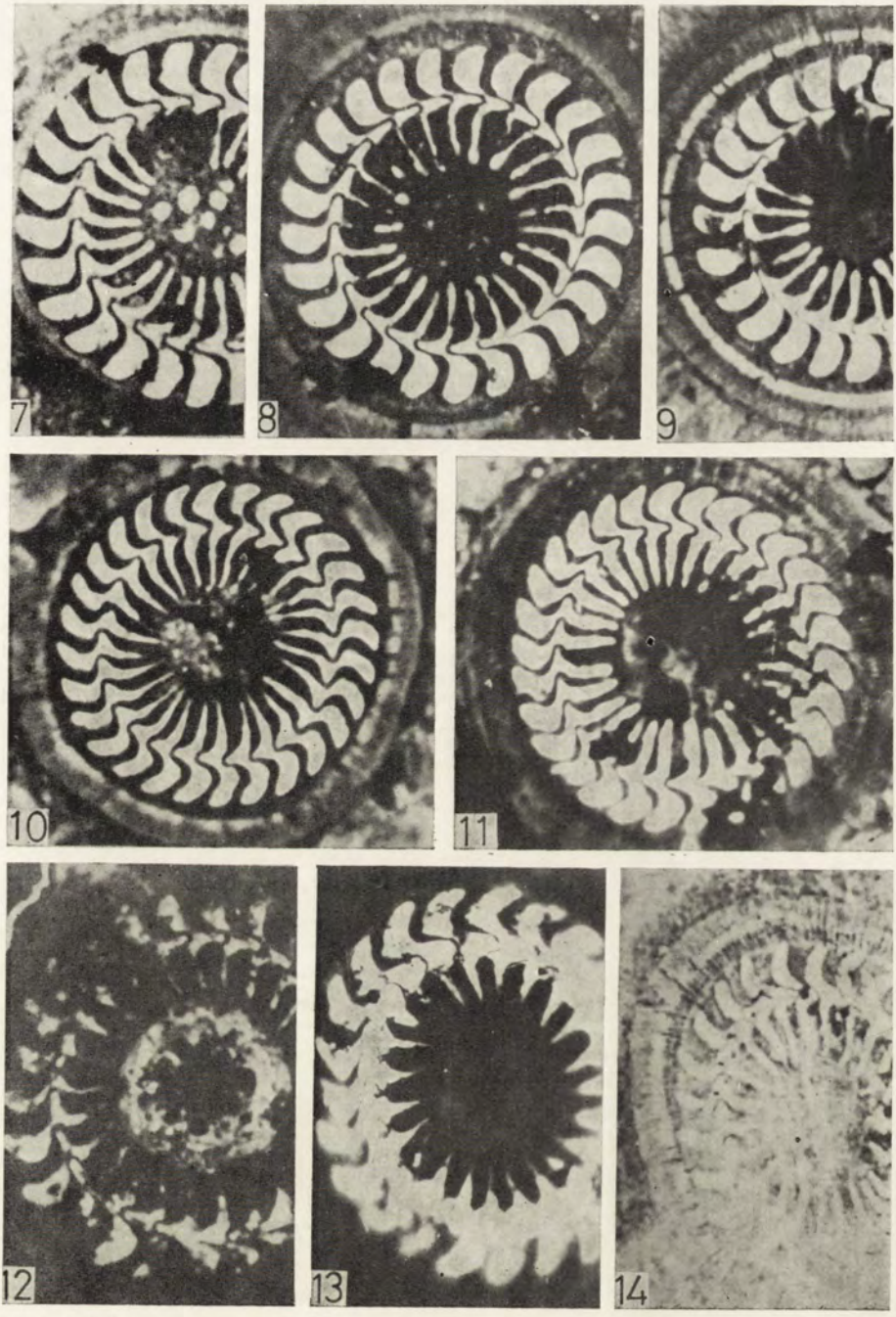

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Cadmium: Quantitative Methodology and Study of its Effect upon the Locomotor Rate of *Tetrahymena pyriformis*

Synopsis. A change in ciliate motility is among the first observable modifications to occur after an organism in exposed to a toxicant. Quantitative determination of the locomotor-rate-changes effected by varying concentrations of cadmium (Cd^{++}) upon *Tetrahymena pyriformis*, provides a means of assessing the toxicity threshold of the metal. An expanded materials and methods section describes the usage of standard laboratory apparatus for the locomotor rate studies. The methodology described for cadmium may apply to the quantitative assay of other physiologically significant substances, whether present singly or in multiples.

The growing realization, that heavy metals present a serious toxicological problem to life systems, prompts needed research in quantitative measurements of their effects. Although this problem has been studied using diverse species, these methods tend generally to require considerable amounts of both time and equipment. Thorough toxological studies must include both acute and chronic effects produced by a pollutant (Apostol 1972). It is not only of academic, but also potential practical interest, that the physiological effects of the heavy metals be thoroughly understood.

Recent publications devoted exclusively to the biological effects of cadmium (e.g., Friberg et al. 1974) demonstrate the justifiable concern and study regarding the metal's effects on humans and other large animals. The effects of cadmium on aquatic organisms have also received attention, e.g., blue-gills (Sparks et al., 1972), and largemouth bass (Crearley and Coleman 1974). Additionally, unicellular species have been studied relative to the effects of the metal, e.g., algae (Bartlett and Rabe 1974), yeast (Lindegren and Lindegren 1973) and bacteria (Doyle et al. 1975). However, understanding the molecu-

lar and ecological significance of the metal must also include studies on aquatic protozoans. The present study deals with the decline in ciliate locomotor rate effected by exogenous cadmium in the ubiquitous and well-known freshwater protozoan, *Tetrahymena pyriformis*.

Laboratory experimentation with various ciliates has established the effects of common parameters such as pH (Dryl 1961), antagonistic cations (Naitoh and Kaneko 1972), and temperature (Tawada and Oosawa 1972) upon motility. The known toxic effects of heavy metals on ciliates has also received some attention, e.g., mercury (Tingle et al. 1973), uranium (Shaw et al. 1949) and nickel (Andrivon 1972, Organ 1972 and de Puytorac et al. 1963). Such previous studies provide the background for quantitative study of physiologically detrimental substances, especially heavy metals, as they modify ciliate locomotor rates. The present paper deals with results produced by cadmium, however, it also contains an expanded materials and methods section, since the modified techniques employed here have not previously been described.

Description of the Method

Tetrahymena pyriformis, strain HSM, were grown axenically in media containing 2% proteose peptone (Difco), 0.1% sodium acetate (as $CH_3COONa \cdot 3H_2O$) and 0.1% sodium phosphate (Na_2HPO_4) (Sims 1968). The initial pH of this media is 6.9. Other growth media may also be used; however, the pH of the media should approximate that at which the organisms will be tested.

Prior to actual use of the test-organisms, the test-solution is prepared and the pH adjusted to 7.0 with NaOH and/or HCL. Freshly prepared metal-containing solutions have proven best. A non-buffered Chalkley's solution (Chalkley 1930) is used to reduce potential interaction of the metal being studied and any buffering anions (e.g., CO_3^{2-}, PO_4^{3-}). This, however, necessitates that the solution be repeatedly monitored and readjusted as the pH shifts downward 0.3–0.5 pH units. The pH is maintained near 7.0 since minor fluctuations in hydrogen-ion concentration have a reduced effect upon ciliate locomotor rate in this concentration region (Dryl 1961). The sensitivity to fluctuations in hydrogen-ion interaction with other cations is also lessened around pH 7.0 (Grębecki and Kuźnicki 1963). The methodology was established using a control-solution (Chalkley's) and Chalkley's solution containing 0.1, 0.5, 0.75, 1, 5, or 10 ppm Cd^{++} ion, added as $CdCl_2$.

After the test-medium is prepared, approximately 10 ml of a 1–2 day old (early to middle log-phase) culture of *Tetrahymena* is spun in a clinical centrifuge at 1550 rpm for 1.5 min. The supernatant is discarded

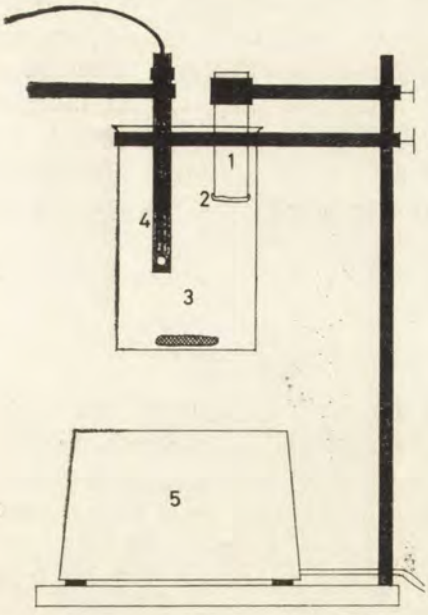

Fig. 1. The cells are placed into the holding chamber which is suspended in ≃ 200 ml of the solution being tested. The solution is held ≃ 5 cm above the magnetic stirring motor to reduce heat transfer. 1 — Holding chamber, 2 — Whatman No. 5 filter paper, 3 — Test solution, 4 — pH probe, 5 — Magnetic stirrer

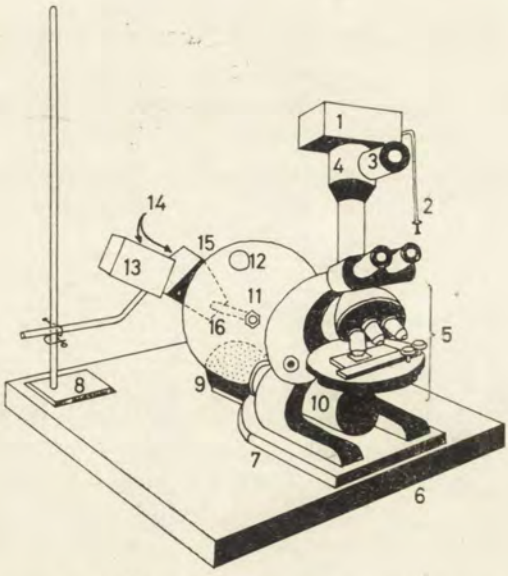

Fig. 2. A standard laboratory microscope and rotating disc provide the multiple image photographics. 1 — 35 mm camera, 2 — Time exposure release, 3 — Viewer, 4 — Light beam splitter, 5 — Phase contrast microscope, 6 — Laboratory bench, 7 — Plywood support, 8 — Ring stand, 9 — Lamp, 10 — Prism, 11 — Rotating disc, 12 — Opening, 13 — Motor, 14 — Electric stirrer, 15 — Reduction gear housing, 16 — Shaft

and the cells rinsed in Chalkley's solution. They are again centrifuged as before and the supernatant discarded. The test-solution is then added and that time designated as time zero for each test. All solutions and subsequent photography are at room temperature (21°C).

Immediately after the addition of the test-medium to the pellet of cells, the organisms, now in test-solution, are transferred by pipette to a holding chamber. This chamber (an open-ended plastic cylinder) is suspended in a relatively large volume (200 ml) of the test-solution (Fig. 1). The bottom of the holding-chamber is covered by a piece of Whatman No. 5 filter paper to restrict the *Tetrahymena,* while allowing circulation of fluid. The test-solutions are continuously stirred in addition to periodic manual transfer of the test solution, from the beaker to the open end of the test chamber, using a pipette. The cells in the chamber are therefore uniformly exposed to the ions being tested. Stirring is done by a magnetic stirring-motor and bar. The beaker of solution is suspended ≈ 5 cm above the magnetic motor to minimize heat-transfer (Fig. 1).

Since the test-solution is non-buffered, its pH will shift slowly downward with time due to the uptake of atmospheric CO_2 and subsequent H_2CO_3 formation and dissociation. In order to minimize any effects due to pH, it is repeatedly monitored by a pH probe (Fig. 1) and the pH readjusted to 7.0 whenever it drifts 0.3–0.5 pH units below 7.0.

Measuring time from the designated zero-time, a sample of cells is removed from the holding chamber at an appropriate interval and placed onto a clean, glass, microscope slide. A different sample is removed for each period of observation (e.g., 1 min, 5 min). The organisms are left uncovered while they are viewed and photographed.

Photographic Apparatus

The present photographic method is a modification of various stroboscopic methods previously employed by several investigators. Gray (1930) was among the early investigators using stroboscopic photomicrography. Later Wingo and Browning (1951) also used stroboscopic photomicrography to study protozoans. More recently, Ferguson (1957), Sears and Elveback (1961) and Gittleson and Noble (1973) have employed similar techniques to study protozoan locomotor rates. Two useful variations of the basic stroboscopic techniques are used in this present method.

The first useful adaptation is the utilization of a standard laboratory microscope, rather than specially developed optical systems (Fig. 2). These studies were done on a Zeiss Standard R A Microscope equipped with polarized interference-system dark-field. However, other types of microscopes and optics may be used if they permit intermittent breaking of the illuminating beam by a rotating disc (will be described later) or other means. Whereas dark-field optics are desired so that maximum image contrast will be obtained, transmitted-light photomicrography may also be used with proper intensity-adjustments.

Relatively low-power magnification (63 ×) was used throughout the study in order to provide a broader field over which to observe and measure the swimming organisms.

The second variation from that found reported elsewhere, was the use of a variable speed, perforated, rotating disc to provide pulsed illumination (Fig. 2). This apparatus is simply constructed as it consists of a medium-gauge circular piece of sheet-aluminum into which holes have been drilled at regular intervals. The apparatus can be tailor-made for the desired purpose. This apparatus was a 10 inch diameter piece of aluminum with two 1.25 inch diameter holes near the edge of the disc placed 180° from each other. The number of holes can be varied as desired. One of the two holes was sometimes masked when longer inter-flash intervals

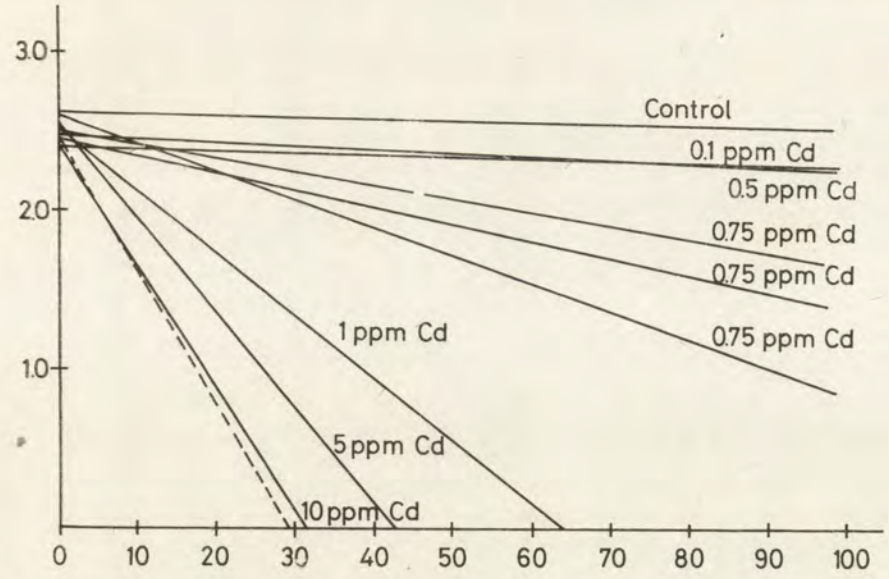

Fig. 3. The locomotor rates of the *Tetrahymena* decline steadily relative to time and concentration of the pollutant (cadmium). Lines are based on total data sample at each concentration (——) and in one case an arbitrary point omission was made (- - - -). *Abscissa*: Time after cells were placed in the test solution (min), *Ordinate*: Log locomotor rate (mm/s)

were desired. A short bolt is attached through the center of the disc and this is then inserted into the chuck of a variable-speed laboratory stirring-motor. I used a constant-speed motor which was equipped with a conical-shaped friction gear and powered by a controlled-voltage line. The constant-speed motor provided relative stability, and the reduction-gear allowed adjustment of the strobe-rate. The time intervals between flashes at the various speeds can be determined by repeated visual counting of flashed during a known time interval, determined by a standard lab timer. This stroboscopic apparatus is accurate to 0.01 s, which proves accurate for these studies. By adjusting the strobe-rate, images of the organism can be distributed on the film to maximize the distance and increase the accuracy of measurement.

Both Kodak Plus-X and Tri-X films have been used to photograph the organism. All photography was done at constant light-intensity, the magnitude of which was determined during preliminary trials of the apparatus. Each group of photographs (taken at an appropriate time interval after zero-time), was taken during a 1 min time-period; the center of this designated period corresponds with the stated time for that time-period (e.g., 1 min, 5 min). After shooting, the multiple exposure films was developed in Kodak Microdol-X.

Calculation of Relative Swimming Rates

The multiple-image photographic negatives (a print of such is shown in Pl I) are projected onto a translucent screen for measurement. Magnification of the actual distance moved by the organisms is thereby increased 500 times. Metric units (mm) are used to measure the distances between successive images and the locomotor rates are then determined using the previously calibrated time intervals between images.

In the multiple image photographs, rate determinations are based upon that portion of the path which exhibits the maximal rate of movement (Pl. I). The minor additional pathway length covered as the organisms followed their normal helical pathway was not considered. This method records only the forward or linear change exhibited in the pathways. Rapidly moving organisms produce an easily measured nearly linear pattern of movement. Slower moving organisms are depicted as traveling more nearly circular paths. Calculation of the estimated non-linear paths was not necessary to make a statistically valid mathematical comparative study of the swimming rates. It was therefore omitted.

Since large numbers of zero values will cause non-normality of distribution in a standard bell-shaped curve, data recording was terminated when metal-induced., as opposed to normal behaviorally-induced, non-motility was observed. Metal-induced non-motility can ben recognized by three criteria. The cells generally become swollen and rounded in appearance (there may be some variability depending upon the metal involved). Metal-induced non-motility requires a period of time, the length of which is proportional to the concentration of the metal being used, before it is observed. In addition, those cells which still are moving will be either spinning in place or swimming in tightly circular paths.

Locomotor rates for each of the measured times are transformed into logarithmic values. Variances are then compared by the F_{max} test or other suitable tests (Sokal and Rohlf 1969). A linear regression is then established relating the decline in locomotor rate with time for each concentration of the test solution. Figure 3 provides an example of such results using cadmium. The equations for the lines are also shown in Table I. The slopes of these lines may be further compared relative to their respective metal concentrations; such comparison produces a sigmoid curve for cadmium (Fig. 4). Even if the data does not meet all the requirements for statistical analysis, or such treatment is not desired, relative comparisons of the slopes can be made by visual plotting of the mean locomotor rate values at each time period. Further comparisons can be made by using the axis-intercept values as obtained in regression.

Table 1

A Regression Equation Describes the Decline in Locomotor Rate at each Concentration of the Pollutant (cadmium)

Descriptive Equations For Log Locomotor Rate vs. Time	
form of the equation, $\log Y = a + bX$	degree of statistical significance for linear equation in which deviations from regression are not significant
Control	
$\log Y = 2.658 + (-0.001) X$	significant at the 0.05 level
0.1 ppm Cd	
$\log Y = 2.432 + (-0.00123) X$	significant at the 0.001 level
0.5 ppm Cd	
$\log Y = 2.512 + (-0.00235) X$	significant at the 0.01 level
0.75 ppm Cd	
$\log Y = 2.557 + (-0.00838) X$	not significant
$\log (Y+1) = 2.526 + (-0.01078) X$*	not significant
$\log Y = 2.668 + (-0.01765) X$	not significant
1 ppm Cd	
$\log (Y+1) = 2.549 + (-0.039) X$*	significant at the 0.05 level
5 ppm Cd	
$\log Y = 2.575 + (-0.059) X$	significant at the 0.001 level
10 ppm Cd	
$\log Y = 2.440 + (-0.076) X$	not significant
$\log Y = 2.485 + (-0.084) X$	significant at the 0.01 level, point omitted

* In some cases a few 0 values were obtained with the collected locomotor rates. In such cases it is necessary to code the values (+1) thereby permitting the log transformation.

Results

Figure 3 clearly demonstrates the relationship between log locomotor rate and time at each concentration of the metal tested. In one instance the line shown (- -) is based upon data wherein one data point was arbitrarily omitted for experimental reasons. Logarithmic transformation of the locomotor rate values produces a linear and statistically significant regression. Table 1 presents the regression equation and level of statistical significance, when found significant ($P < 0.05$), for each of the lines shown in Fig. 3. Comparison of the equation values describing the slope of each line, readily demonstrates the increasing magnitude of response with increasing concentration of cadmium.

Figure 4 is a graphical comparison of these slopes with varying concentrations of cadmium. A sigmoid curve is apparent, demonstrating a concentration threshold (approximaly 0.75 ppm Cd^{++}) for the locomotor rate demise effected by cadmium under these conditions. Slopes for the

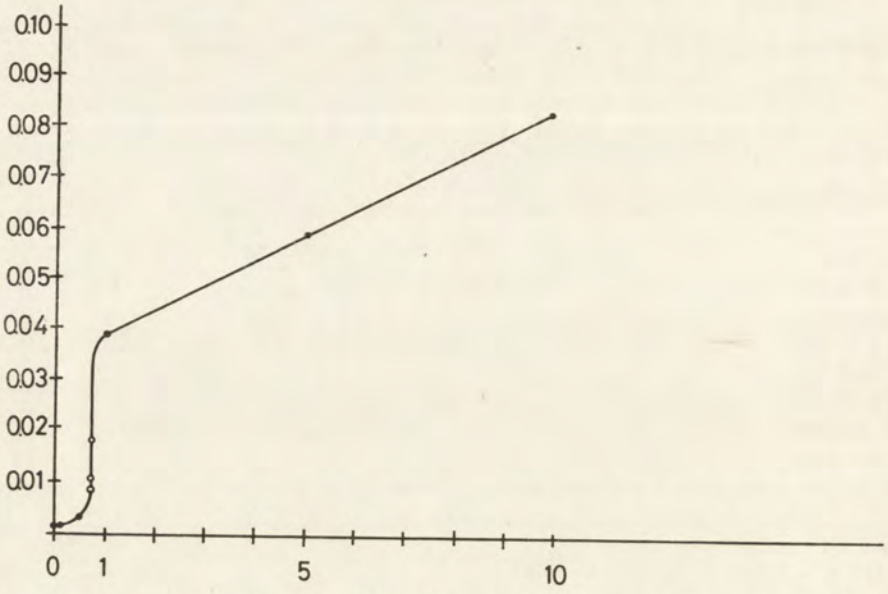

Fig. 4. A graphical comparison of the pollutant (cadmium) concentration and the decline in locomotor rate (slope) clearly demonstrates a toxicity threshold for the effect of the pollutant (cadmium) on locomotor activity. Slopes for the effect of 0.75 ppm cadmium were not statistically significant, $P > 0.05$ (○). Slopes for the effects of the other test solutions were found statistically significant, $P < 0.05$ (●). *Abscissa*: Cadmium concentration (ppm), *Ordinate*: Change (slope) of the locomotor rate

effects of cadmium at 0.75 ppm were not found statistically significant ($P > 0.05$, (○)), whereas those for other concentrations of the metal and the control were found significant ($P < 0.05$, (●)).

Discussion

Assets

The greatest single asset of this methodology is the large sample sizes which may be obtained. This in itself rectifies many problems, especially those associated with random variability and deviations from regression in smaller samples. From a practical point of view the relatively low cost of supplies and equipment and minimal time, expended by one person, are its principal assets. Since no special equipment other than that used in standard culture work and standard photomicrography is required, the method is relatively inexpensive. Since data can be rapidly accumulated, it consumes less time than other assay methods.

Methodology

Although the described methodology is useful as a research tool in the study of cadmium or other pollutant toxicology, it is not without its technical problems. These problems are such that they may somewhat influence statistical results when such confirmative validity is desired.

High concentrations of a pollutant may produce such a rapid decline in locomotor rate that it becomes technically difficult to record data at enough time intervals. It also becomes apparent that the actual locomotor rate for any one organism may change significantly during the 1 min period over which measurements are taken. This method has made the assumption that the relative rates of the protozoans is constant during the 1 min measuring window. This becomes increasingly less true as pullutant-concentrations are increased. This may be partially rectified by decreasing the measured time period, but this will reduce the net sample size and increase statistical problems.

In practice, a compromise must be reached between the validity of the assumption (constant speed over the individual measurement period) and the need for large sample sizes. Lower concentrations of a pollutant do not present these problems. However, another problem may develop if the period of time over which repeated sampling and measurement occurs is unduly extended. Since the pH of the non-buffered test-solution must be repeatedly readjusted, a base (e.g., NaOH) must be added. Too much time will therefore require the addition of too many cations (Na^+). This is only a problem if the cation interferes with the action of the pollutant being tested. Na^+ has been tested and found to have minimal interaction with cadmium, but this at best should be reduced.

Previous investigations by Sears and Elveback (1961) and Gittleson and Noble (1973) have employed a period of habituation after the organisms were introduced into the test chamber. Whereas Sears and Elveback allowed a 200 min habituation period before recording locomotor rates in *Paramecium*, such a long period was not possible using this methodology. Cadmium and other pollutants may exhibit such rapid effects that essentially no time can be allowed for habituation, especially at higher concentrations. At lower concentrations of the pollutants, early time-period data, which do exhibit a lower mean locomotor rate, may be eliminated. Habituation is believed to be involved in producing this effect.

Certain metals, e.g., lead, do not lend themselves to testing by this method as they may form insoluble carbonates at this pH. Even so, lesser concentrations of lead have been tried and found to inhibit locomotor rates.

When a toxicity-threshold is found, as shown in Fig. 4, the regressions of those concentrations within this region may not be found statistically significant (Table 1). Slight changes in cadmium-concentration may produce major shifts in locomotor rate response in this region. Other variables of indirect influence may also more easily modify locomotor rates in this region.

It is desirable to collect all locomotor rate data during one test run. However, careful technique will minimize variability between runs.

Ideally a somewhat bell-shaped distribution curve should describe the locomotor rate values for a population of organisms at a given time interval. In order to provide a sample of values in the median region of such a distribution curve, it is necessary to make some subjective judgments and eliminate those cells exhibiting "normal" behavioral non--motility. Criteria for differentation between behavioral and metal-induced non-motility have already been discussed.

It is also apparent, from the stroboscopically recorded photographs, that the rate of locomotion of any single organism may vary within the period of the multiple-image photograph. If a organism had been still and had just begun moving, it would logically require some time to accelerate up to maximum or "normal" speed. Therefore, the locomotor rate data are taken from that portion of the photograph indicating maximal rates of movement (Pl. I).

In this study on cadmium, the cation was added as the chloride salt, $CdCl_2$. Experimentation with other metals has also involved the chlorine-containing salts since this anion is generally not toxic at low concentrations as compared to its cation (Dunham 1964). Other compounds (e.g., sulfates) may also serve as a means of cationic addition. However, the results may vary from the chlorides when salts of different anionic composition are used, especially at higher concentration.

Effects of Cadmium

A recent review by Vallee and Ulmer (1972) describes cadmium as enhancing 25 and inhibiting 35 different enzymes. It is therefore impossible to define cadmium's effects succinctly. Nevertheless it is possible to draw a few conclusions regarding its mode of action.

The similarity in size and divalent charge relates cadmium to calcium providing a basis for competitive interaction between the two ions (Baes 1973). Not only will Cd^{++} interfere with those sites normally involving Ca^{++}, but it will also behave antagonistically with other similar cations (Hill and Matrone 1970). Accumulative metals such as cadmium also have the possibility of interaction with other essential metals which are dissimilar in structure (Schroeder and Nason 1974).

The visible swelling of cadmium-exposed cells, which results in cell lysis at those Cd^{++} concentrations definitively affecting cellular motility ($Cd^{++} \geqslant 1$ ppm), may provide a partial explanation for the demise in locomotor rate. Distortion of the normal morphology may modify normal cilia-media interactions, thus contributing to modified locomotor activity.

The decline in locomotor activity is also undoubtedly a product of decreased energy release and/or availability of the energy yielding substrate ATP. This explanation is supported by the observation that starved ciliates also exhibit a decreasing locomotor rate due to decreased ATP availability (Bovee 1974). The demonstrated reduction in the ATPase activity, produced by cadmium, also supports this contention (Specht and Robinson 1973).

One additional line of evidence supporting cadmium's effect on motility via interference with ATP availability, is the demonstrated action of cadmium on mitochondrial integrity. For example cadmium-treated yeast cells exhibit morphologically modified mitochondria and a respiratory deficiency (Lindegren and Lindegren 1973). Thurberg et al. (1973) have also demonstrated the *in vivo* reduction of O_2 consumption in crabs exposed to cadmium. And more directly to the point is cadmium's ability to effect reduced oxidative phosphorylation (Fluharty and Sanadi 1962 and Brierley 1967).

Whereas the definitive effects of cadmium upon ciliates is of academic interest, it is also important ecologically, especially if physiologically significant quantities of the metal are introduced into aquatic environments. The results and methodology herein described for cadmium, support a need for further study regarding other heavy metals and multiionic systems capable of modifying aquatic communities.

ZUSAMMENFASSUNG

Unter den ersten Veränderungen, die zu beobachten sind, ist die Veränderung der Bewegungsfähigkeit der Ciliaten nach dem Ausgesetztsein dem Organismus mit Giftstoff. Quantative Bestimmung von der Veränderungen der Fortbewegungsgeschwindigkeit, die durch abwechselnde Konzentratione vom Cadmium (Cd^{++}) auf *Tetrahymena pyriformis* bewirkt sind, besorgt ein Bestimmungsmittel für den toxischen Schwelle des Metalls. Der umfassende Absatz über Methodik beschreibt die Benutzung einer normalen Laborapparat für die Untersuchungen über die Fortbewegungsgeschwindigkeit. Die Methodenlehre, die für Cadmium beschrieben werden, mögen sich auf dem quantitativen Analyse von anderen physiologisch-bedeutsamen Stoffe anwenden lassen, ob sie einzig oder vielfach da sind.

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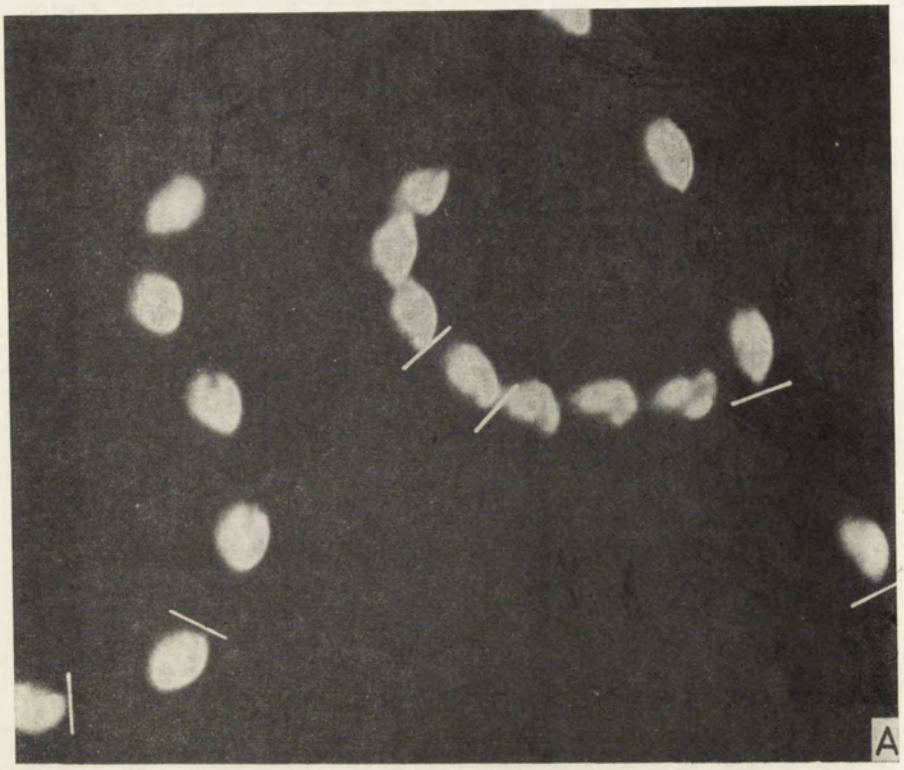
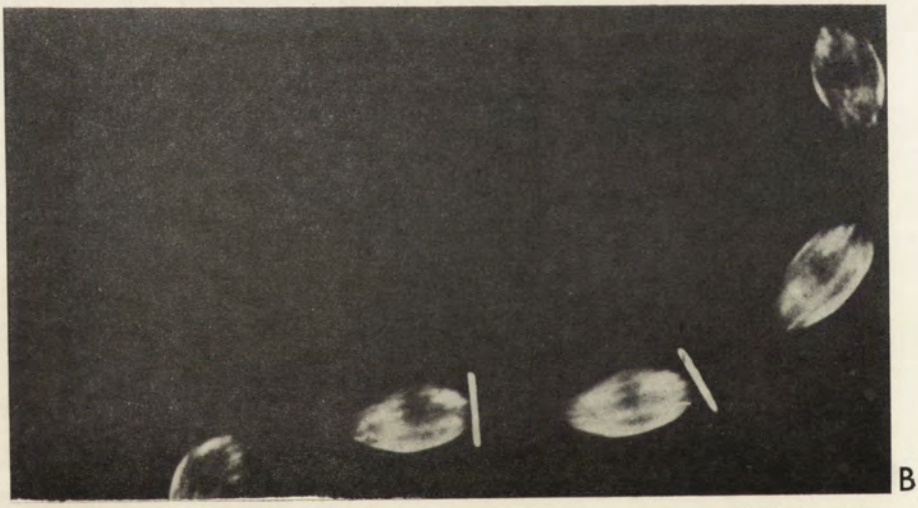
EXPLANATION OF PLATE I

A — This print from one of the experimental negatives demonstrates the measured distances from one image to the comparable position in a successive image (spaces between the white lines). The maximal distances (rates) in the photograph are measured for each of the three organisms. (approx. 250 ×)
B — The maximal rate of movement is often found in the more nearly linear portion of a curvilinear path. (approx. 400 ×)





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Course of Glycerination of *Amoeba proteus* and Contraction of Glycerinated Models

Synopsis. Glycerination of *Amoeba proteus* and contraction of resultant models were observed under interference and holographic microscopes. The process of glycerination can be divided into three parts: (a) shock reaction, (b) rapid dehydration and cell death and (c) expansion of the model. Shock reaction is the main factor deforming the cell during glycerination. Expansion of the model is a complex phenomenon consisting of expansion of cortical layer and partial redistribution of internal material. The method of glycerination and of washing the model influences strongly its contractility. Contractility is demonstrated to be mainly of cortical origin. The contractile behaviour of glycerinated *Amoeba* models is thought to be the reflection of the contractile properties of the intact cell.

The technique of glycerol extraction developed in late fourties (Szent-Györgyi 1949) is one of the most widely used to obtain contractile models. Glycerinated fibers of striated muscle are the only one known to meet the requirements of the ideal contractile model, which can be characterized as follows:

a) the great part of noncontractile material is removed,

b) the cell membrane is no longer selective and excitable, and

c) the contractile material is not touched by the extraction process.

Due to such properties the unmasked structure and function of the contractile machinery can be easily studied. Lack of membrane selectivity provides the possibility of studying the direct effects of physical and chemical agents upon the contractile proteins and their function (Arronet 1973, Hasselbach and Weber 1955).

To investigate an amoeba glycerinated model it is necessary to know the morphological deformations evoked by the glycerol treatment in order

to compare its contraction with the observed motion of the living cell. Rapid warm or cold glycerol extraction is known to preserve cell shape and integrity and distribution of actin filaments (Chang and Goldman 1973, Goldman 1975, Rinaldi et al. 1975).

The present investigation deals with the influence of glycerination on cell morphology and possible correlations between the living cell and the model.

Material and Methods

Organisms

Amoeba proteus (S strain) cultured in Chalkley's solution and fed with *Colpidium* was used exclusively. No attempts were made to determine the stage of cell cycle or the starvation state of amoebae during experimentation.

Solutions

Extraction solution: KCl 0.05 M, EDTA 0.005 M, Tris 0.01 M and glycerol 50% (v/v). pH adjusted (by 0.01 M Mes) to 7.2.

Washing solutions: 40%, 30% and 25% (v/v) glycerol with KCl 0.05 M and Tris 0.01 M. pH adjusted (by 0.01 M Mes) to 7.2.

Contraction solution: ATP 0.0025 M, $CaCl_2$ 0.0025 M, $MgCl_2$ 0.0025 M, KCl 0.03 M and glycerol 25% (v/v). pH adjusted (by 0.01 M Tris) to 7.2 or 5.5.

Conventional microscopy

MPI 5 (PZO) interference microscope was used. Dark field illumination was achieved with KFZ (PZO) condenser, which gives a uniform dark field for 10 × objective.

Holographic microscopy

The holographic microscope developed in Central Optical Lab. (Warsaw) (Pawluczyk and Pluta 1975), working with LG 600 He–Ne laser was used. Wavelength of light emitted was 6328 Å, laser power was 5 mW. Coherent noises were suppressed by means of the built in coherent noise eliminator (Pawluczyk 1973). The holographic microscope was used mainly as a real time interferometer. In this method the object-disturbed wave is superimposed on the same, non-disturbed wave reconstructed from the hologram. Interference of these waves gives in result the interference pattern indicating phase distribution in object. Depending on the angle of imposition of object and reference wave the observations can be performed in the fringe or uniform interference field. In the uniform field optical paths differences result in appearance of closed contour lines which are optical path levels, whereas in the fringe field optical paths differences result in fringe displacements. Double exposure method consists in recording images of two (or more) different states of object on the one holographic plate. Illumination of developed holographic plate with reference beam (called reconstruction beam) brings about reconstruction of the two images which are superimposed resulting in the interference image of object changes. General description of the holomicrointerferometric methods and their application to biology and medicine was given by

Pawluczyk (1970) and Cox (1971, 1974) and the application of uniform field holomicrointerferometry was given by Baranowski (1976).

Experimental procedure

Amoebae were placed on the slide with cover slip fixed in position with vaseline. Two opposite edges of the cover slip were not sealed with the vaseline to allow perfusion with the experimental solution. Glycerination was according to Chang and Goldman (1973). However, the application of glycerol solution was slower, since it was applied under the cover slip. In this case the shock reaction of amoebae could sometimes seriously change their "amoeboid" shape. During glycerination, at the point of maximal compactness amoebae were slightly compressed with the cover slip, so during subsequent expansion they filled the space between the slide and the cover slip. Some of the models were obtained according to Rinaldi et al. (1975). No attempt was made to estimate the absolute refractive index value of subjects. The description of interferometric images are given in terms of changes of optical path (refractive index of object × geometrical path of beam) or of relative changes of refractive index (when geometrical path of beam through object was unknown but constant).

Cine and photo recording

The process of glycerination was recorded by 35 mm 1 KSR-1M cine camera at 6 fr/s before and 1 fr/3 min after glycerinated amoebae reached the point of maximal compactness. All the photomicrographs were accomplished by Zeiss-Jena microscopical photo camera. ORWO NP3 (microcinematography) and Kodak Plus X (photomicrography) negatives were used. Holograms were recorded on Agfa-Gaevert 10 E75 plates.

Results

The process of glycerination of *Amoeba proteus* can be described as a sequence of events which may be divided into three groups: (a) response of living cell to increasing glycerol concentration in the medium, (b) events connected with cell death and, finally, (c) expansion of dead cell. Glycerination of polypodial and monopodial amoebae proceeds in the same way but some of the responses to glycerol are more pronounced in the latter. The final shape of models is less altered than those obtained by gradual glycerination (Simard-Duquesne and Couillard 1962) (Fig. 1 A), but more altered than the shape of models obtained by method of Rinaldi et al. (1975) (Pl. I 1 B).

The first contact of moving amoeba with glycerol solution results in a transitory cessation of movement and small, but rapid decrease of cell volume. After a few seconds, despite increasing glycerol concentration, amoeboid locomotion starts again. Amoeba is able to form new pseudopods and retract old ones, does not lose contact with the substratum and retains surface morphology details (Pl. II 3 B). Further increase of glycerol concentration in the medium stops locomotive movement but does not stop cytoplasmic streaming what may lead to change of shape of po-

lypodial amoebae by retraction and formation of pseudopods (Pl. II 3, III 4). In monopodial amoebae it is often at this stage of glycerination that cytoplasm contracts into rings and sometimes completely plugs these rings, thus leading to cell compartmentalization (Pl. V–VII). Rings of contracted cytoplasm are sometimes formed in polypodial amoebae (Pl. II 3 D. Formation of these rings or plugs is connected with expulsion of water in the region of ring formation (Pl. VII 8 C). With increasing glycerol concentration cell volume decreases, due to dehydration, but from microscopical observations it may be concluded that this process is not continuous but stepwise. During cell diminution the granules are being clustered and at later stages of glycerination the amoebae are completely filled with tightly packed granules (Pl. II 3 C, III 4 C, IV 5 C, D, V 6 C, VI 7 B, VII 8 C). Clustering of granules causes an almost entire disappearance of the fringe image in the holographic microscope (Pl. III 4 C, IV 5 C, D, VI 7 B, VII 8 C). Note, that in monopodial amoebae the hyaline cap is at all times easily observed (Pl. V–VII). The last step of dehydration is always recognizable due to rapid and eruptive water expulsion (Pl. IV 5 D). Probably this is connected with cell death. A few seconds later the cell reaches point of maximal compactness (Pl. II 3 D, III 4 D, IV 5 E, V 6 D, VI 7 C, VII 8 D) in which it is completely filled with tightly packed granules (with the exception of monopodial amoebae in which the hyaline cap area is strongly reduced but transparent) and its volume is minimal.

Behaviour of an amoeba exposed to increasing glycerol concentration in the medium up to the moment of cell death will be further called the shock reaction. Duration of shock reaction depends upon the speed of glycerol concentration increase and lasts between one and three minutes. The last expulsion of water and reaching the point of maximal compactness lasts between ten and thirty seconds.

A few minutes after reaching the point of maximal compactness the first signs of expansion can be observed. Cortical layer is the first to expand and its expansion is the fastest in those parts of model from which cytoplasmic streams originated (prior to death of amoeba), i.e., uroid (Pl. II 3 E, III 4 E, F, IV 5 F, V 6 F, VII 8 E), or pseudopods being retracted (Pl. IV 5 F). The term "cortical layer" is reserved for the cell membrane with its mucous coat and associated meshwork of filaments on the inner membrane side. During shock reaction in some monopodial amoebae the reversal of cytoplasmic streaming direction can be observed, i.e., endoplasm is streaming from the hyaline cap to the uroid. In such a case the cortical layer surrounding the hyaline cap expands as fast or even faster than that of the uroid (Pl. VI 7 D). During expansion of cortical layers the centripetal gradient of refractive index can be observed

(Pl. III 4 E, F, IV 5 F, G). This gradient disappears after termination of model expansion (Pl. III 4 H, IV 5 H). Cytoplasmic granules tightly packed in the maximally compact amoeba are expanding simultaneously with cortical layer. Extent of this phenomenon is different in each cell or even in different parts of the cell. Granules, it is thought, are dragged to the cell periphery by expanding cortical layer (Pl. II 3 H). Three types of models can be distinguished depending on distribution of granules after expansion: (a) with almost uniform granules distribution due to uniform granules expansion (Pl. I 1 B, II 3 H); (b) with large, clear areas; these areas free from granules can be formed in two ways. One posibility is that during expansion of the cortical layer (especially in parts of the cell in which a membrane surplus was accumulated) granules are left in original position, thus leading to formation of large, optically empty areas (Pl. V 6 H, VI 7 H, VII 8 H, IX 10 A, X 11 A). The other is, that the granules are so tightly bound to the cortical layer that during expansion almost all the granules are dragged toward the cell periphery, leaving optically empty area in the cell center (Pl. III 4 H); (c) with few clustered granules — their expansion is slightly marked (Pl. IV 5 H, VIII 9 B, XI 12 A, XII 14 A).

The existence of nearly "empty" models or models with uniformly dispersed granules both with undistinguishable phase differences (Pl. III 4 H, IV 5 H, VIII 9 B, XI 12 A, XII 13 A, 14 A) acress their area indicates that their amoeboid shape is sustained by the cortical layer. Further support to this is given by the recognizability of hyaline blebs, formed by the living amoebae during shock reaction in expanded glycerinated models (Pl. VII 8).

Rings of contracted cytoplasm are fairly stable structures until expansion of adjacent cortical layer. After this the ring can be recognized due to granular arrangement only (Pl. I 2, II 3 H, V 6 H). Notheworthy is, that the rings of contracted cytoplasm are the more stable the closer they are to the uroid (Pl. V 6 F, H).

In spite of cell dehydration and disappearance of phase differences existing between components of living amoeba, plasmagel sheet — if was present in living amoeba prior to the treatment — can be easily recognized during the entire process of glycerination (Pl. I 2, V 6 A–H, VI 7 A–F, VII 8 A–H).

As mentioned above, the degree of cortical layer expansion is not uniform over the model area. The first to expand are parts of model which were, in living amoeba, origins of cytoplasmic streaming, and in most cases their expansion is the most pronounced (Pl. IV 5 F–H, 6 F–H, VI 7 D–F, VII E–H). The time of expansion is between one and two hours.

Origin of streaming in the amoeba prior to death may determine not only the site of the most extensive cortical layer expansion but the site of initiation of contraction as well. The best example is a monopodial amoeba with streaming reversed in shock reaction. In this case (Pl. IX 10) contraction starts, and is stronger at the point of streaming origin i.e., in the region of the pseudopodial tip. The contraction of the uroid is slightly marked — its full contraction was terminated about half an hour later. Contraction in the region of cytoplasmic ring is also visible (Pl. IX 10, double arrows).

To demonstrate the role of the cortical layer in contraction it is essential to apply mild washing procedure to the models, since it is reported (Eisenberg and Eisenberg 1968, Nayler and Merrillees 1964) that the washing procedure, not the glycerination itself destroys cell structure most. Models shown in Pl. X 11, XI 12, XII 14 were obtained according to Rinaldi et al. (1975), but washed gradually and slowly to 25% glycerol solution only. Models shown is Pl. IX 10 and Pl. XII 13 were obtained by the glycerination under the microscope and washed in the above way. To visualize the model contents during contraction pH of the contraction solution applied to the model shown in Pl. X 11 was lowered to 5.5. Visualization is, in this case, due to the increased light scattering properties of the model contents. After application of contraction solution the volume decrease ot the model (Pl. X 11 B) and subseguently, eruptive outflow of the model contents can be seen (Pl. X 11 C). Eruptive outflows of model contents during contraction can be observed in models washed according to procedure described above but it is a rare phenomenon. Contraction in 25% glycerol solution does not differ from that in washing solution or 5% glycerol except that it is longer and sometimes weaker. Observations of such contractions in the fringe or uniform field of the holographic microscope indicate that the area of optical thickness changes is limited to the model periphery (Pl. XI 12, XII 13). This means that during contraction at the periphery refractive index, model geometry or both are changing. In favourable cases, it can be demonstrated that the contraction of the cortical layer of the model is anisodimensional (lateral) but the contraction of model contents (clustered granules) is isodimensional (Pl. XI 12 A, B). Predominantly lateral contraction of almost entirely optically empty models is shown in Pl. XII 13, 14.

Discussion

Prolonged immersion of cell or tissue in 50% glycerol solution results in damage of cell membranes (Hasselbach and Weber 1955, Ha-

yashi 1973, Ishikawa et al. 1969, Nayler and Merrillees 1964, Weber 1955) and loss of electrical activity (Chichibu 1961, Hasselbach and Weber 1955). Even weak solution (about 3%) of glycerol breaks the excitation-contraction coupling by the functional disruption of tubular system and changes electrical properties of muscle cell membrane (Castel and Papir 1975, Dulhunty and Gage 1973, Eisenberg and Eisenberg 1968, Nakjima et al. 1973). Enzymatic activity is also reduced, and can be further limited by the double, glycerol-detergent treatment (Abbott and Chaplain 1966). In glycerol extracted muscle cell the contractile system remains in the same form as in the living cell (Hanson and Huxley 1955, Huxley 1957, Szent-Györgyi 1953, Weber 1955).

Unlike muscle contractility research, data on nonmuscle contractility involving glycerinated models are limited due to a lack of knowledge concerning arrangement and function of the contractile apparatus. Little is known about deformation introduced by the glycerol treatment to cell morphology.

The first obvious effect of glycerol application to the cell is osmotic shock which seems to be more pronounced in the muscle cell (Dulhunty and Gage 1973) than in *Amoeba*. In the amoeba cell streaming is stopped for a short time, but soon starts again. In the conditions of increased osmotic pressure the cytoplasm tends to divide itself into compartments contracting into rings or plugs. Plug formation may resemble in some manner the reported (Rinaldi et al. 1976) behaviour of amoeba cytoplasm exposed to increased hydrostatic pressure. The simultaneous presence of rings and plugs and the fact that they remain in the model until the expansion of adjacent cortical layer indicates that these structures are formed by contraction of ectoplasm.

It can be concluded that rapid application of glycerol solution is the main factor preventing obvious morphological changes during glycerination as the duration of shock reaction depends on the speed of glycerol concentration increase. During rapid immersion of cell in full strength glycerol solution (50% v/v) the steps of dehydration described in the Results are probably reduced to one, thus preventing streaming and shape changes (Rinaldi et al. 1975). The last step of dehydration, easy to observe due to eruptive water expulsion reflect the breakage of the last osmotic barrier and death of the cell. A few seconds later the shrinking cell becomes filled with tightly paked granules reaching a point of maximal compactness. At this moment the cortical layer is maximally folded.

Extensive folding is probably connected with strong elastic deformation of the cortical layer, so it is not surprising that it expands first. The

most serious deformation of cortical layer is expected to result in the fastest and the most pronounced expansion. It was demonstrated, that the most extensive expansion of cortical layer takes place in these model regions which were (prior to amoeba death) the origins of streaming. This finding supports the folding-unfolding hypothesis of membrane behaviour during amoeba locomotion (Czarska and Grębecki 1966, Haberey et al. 1969, Stockem et al. 1969).

Increasing glycerol concentration in the medium brings about stepwise cell dehydration what in effect blurs the difference between ecto- and endoplasm but, surprisingly, does not annihilate the hyaline cap area in some monopodial amoebae. Since contents of hyaline cap was shown to be approximately 1% protein solution (Allen and Roslansky 1958), and there are no supporting structures in this region, it seems probable that more concentrated protein solution was squeezed into it during shock reaction. Despite dehydration the plasmagel sheet (Mast 1926, Rinaldi 1965) is a fairly stable structure, visible during all the steps of glycerination.

Cortical layer expansion is accompanied by the expansion of clustered granules. It may be stated that the granules are dragged by the expanding cortical layer, because: (a) the extent of granules expansion is sometimes smaller than the extent of cortical layer expansion and (b) sometimes the central cluster of granules is torn up leaving a clear area in center of model. These events are coexistent with the disappearance of centripetal gradient of refractive index, probably reflecting the dragging of the internal material by the expanding cortical layer. Contractility is more pronounced at the model periphery than in the center, so one might suggest that both contractility and elastic deformability are to be attributed to the cortical layer of the model.

It is generally thought that the mechanism of contractility of glycerinated models is the same as in the living cells. Contractility of glycerinated models, assumed to be the reflection of contractile events *in vivo*, was described in striated muscle cell models (Hanson and Huxley 1955, Szent-Györgyi 1949), anaphase and telophase fibroblast models (Hoffmann-Berling 1954 a, b), *Vorticella* stalks (Amos 1971, Levine 1956, Townes and Brown 1965), sperm and protozoan flagella (Hoffmann-Berling 1955), protozoan cilia (Gibbons 1965) and *Paramecium* (Naitoh 1969). In all the above cases contraction can be related to the oriented structures which are believed to be contractile. Description of cytoplasm streaming or amoeboid movements in terms of contractility and, especially, on the basis of glycerinated model contractility is much more difficult, but attempts were made for *Acetabularia* (Takata 1961), leukocytes (Hsu and Becker 1975, Nor-

berg 1970), and *Physarum* (Kamiya 1968, Kamiya and Kuroda 1965).

The application of glycerinated models to the study of movement of large, free-living amoebae was limited by the method of glycerination (Simard-Duquesne and Couillard 1962). Isodimensional contractions of these models under the influence of ATP (Danneel 1964, Holberton and Preston 1970, Schäfer-Danneel 1967, Simard-Duquesne and Couillard 1962) led to the conclusion that cytoplasm of amoeba is probably equipotential in respect to contractile properties. The improved technique of glycerination yields models which retain the amoeboid shape and contract laterally (Rinaldi et al. 1975). Results presented in this paper indicate that contraction of amoeba model starts from and is sometimes limited to its periphery. Moreover, contraction of cortical layer of model is sometimes faster in the region of the streaming origins of the living amoeba, what suggests that this part of model is, in some manner, "better" prepared for contraction. ATP-induced contraction can be, in favourable cases, manifested by the erruptive outflow of contents of the model. The contraction of cortical layer of the model generates hydrostatic pressure inside, what — when the permeability of the membrane is not sufficient to equalize the pressure difference between inside and outside of the model in relatively short time — leads to the membrane rupture and outflow. The interior of the model is, with respect to amoeboid contraction less active, thus the change of amoeboid model shape and dimensions is produced mostly by the contraction of the cortical layer.

At the microscopical level it is hard to state whether the granules are kept together by inherent stickiness or by any binding agent. In the author's opinion the latter is more probable because the clusters of granules possess their own contractility; upon addition of contraction solution isodimensional contraction can be observed. Most likely, granules are "glued" with remmants of cytoplasmic material including the contractile one. Cine recordings analysis of contractions has indicated that the optically empty areas of the model interior are filled with fluid-like substance (Rinaldi and Opas 1976). This fact, together with the presented evidence suggest, that in shape maintenance in amoeba, cortical layer plays a crucial role.

The idea that the cortical layer is a major area of amoeboid contractility (Grębecki 1976, Kalisz and Korohoda 1976, Rinaldi and Opas 1976) receives strong support from electron microscopic research. It has been shown for different amoebae that thick and thin filaments similar to muscle cell filaments are found along the periphery of the cell, being sometimes in arrangement suggesting participation in move-

ment (Bhowmick 1967, Haberey 1973, Komnick and Wohlfarth-Bottermann 1965, Pollard 1973, Pollard and Weihing 1974, Rinaldi and Hrebenda 1975). Filaments are frequently found in association with the plasma membrane of amoebae (Clarke et al. 1975, Korn and Wright 1973, Pollard and Korn 1973 a, b, Spudich 1974). Thick and thin filaments are found also in glycerinated models of amoebae, being distributed similarly (Comly 1973, Holberton and Preston 1970, Pollard et al. 1970, Rinaldi et al. 1975, Schäfer-Danneel 1967). Glycerination does not seem to change the distribution and structure of filaments when compared with nonglycerinated amoebae (Pollard et al. 1970, Schäfer-Danneel 1967).

It seems that the main destruction of cell structure during glycerination is introduced by osmotic shock not by the action of the glycerol as such. In some instances glycerol acts as an antidenaturation agent increasing resistance of proteins of model to physical factors (Arronet 1964. The birefringence of muscle fiber and its glycerinated model is essentially the same (Ströber 1952). Medium consisted of 50% glycerol and 10% DMSO protects the birefringence of isolated mitotic apparatus for a long period (Forer and Zimmerman 1974); weak birefringence can be detected in endo- and ectoplasm of *Physarum* glycerinated models (Nakajima and Allen 1965). To demonstrate the role of cortical layer in the contraction it is necessary to work with models with relatively intact membranes. Rapid washing results in membrane destruction and, probably, injures the model's contractile apparatus. The finding that deglycerination of the model can be more destructive than glycerination is in agreement with earlier data (Eisenberg and Eisenberg 1968, Nayler and Merrillees 1964).

Recently, the contractile activity of amoebae deprived of cell membranes was described (Taylor et al. 1973), being believed to reflect the basis of amoeboid movement. From data discussed above one may draw the conclusion that the cortical layer assumes a main role in cell locomotion. Therefore, contractile events leading to cytoplasmic streaming in "naked cytoplasm" of amoebae (Allen et al. 1960, Gicquaud and Couillard 1970, Taylor et al. 1973) are closer to streaming in "motile" cytoplasmic extracts (Pollard and Ito 1970, Wolpert et al. 1964) than to movement of the living amoeba.

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RÉSUMÉ

Les changements dans la cellule de l'*Amoeba proteus* pendant son traitement à la glycérine, et la contraction des modèls ainsi obtenut, ont été étudiées au microscope à contraste interférentiel et au microscope holographique. La glycérination s'effectue en trois phases: (a) la réaction de shock initiale, (b) la dehydration rapide suivie de mort de la cellule, (c) l'expansion du modèle. La réaction de shock s'avère être le facteur principal de la déformation de la cellule pendant le glycerination. L'expansion du modèle est un phénomène complexe comprenant l'expansion de la zone corticale et une redistribution partielle du matériel intracéllulaire. La procédure de l'administration de la glycérine et de son rinçace ultérieur portent une influence très prononcée sur la contractilité du modèle. On a démontré que sa contraction est principalement d'origine corticale. L'auteur présume que le réponses contractiles d'un modèle glycériné de l'amibe présentent une image probe de capacités de contraction de la cellule vivante.

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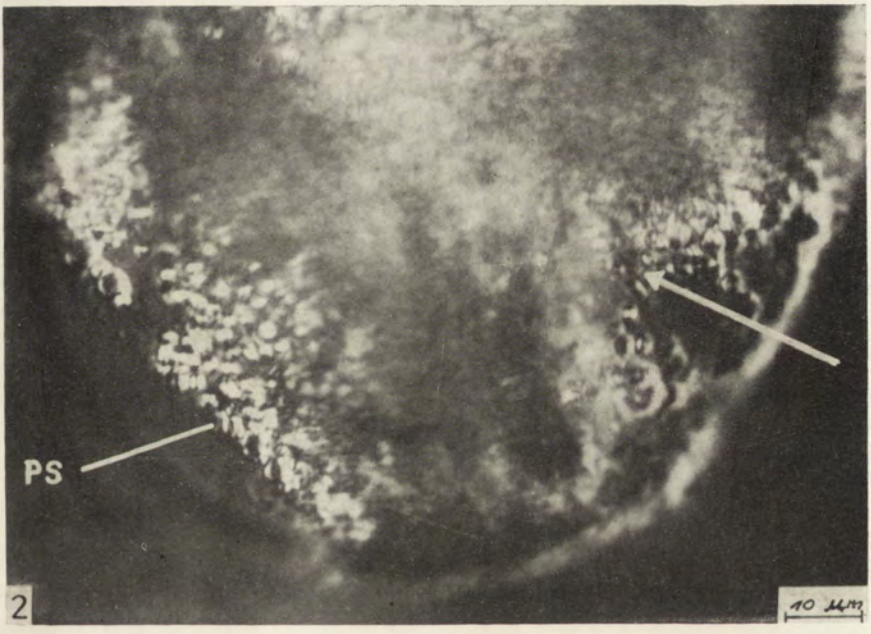

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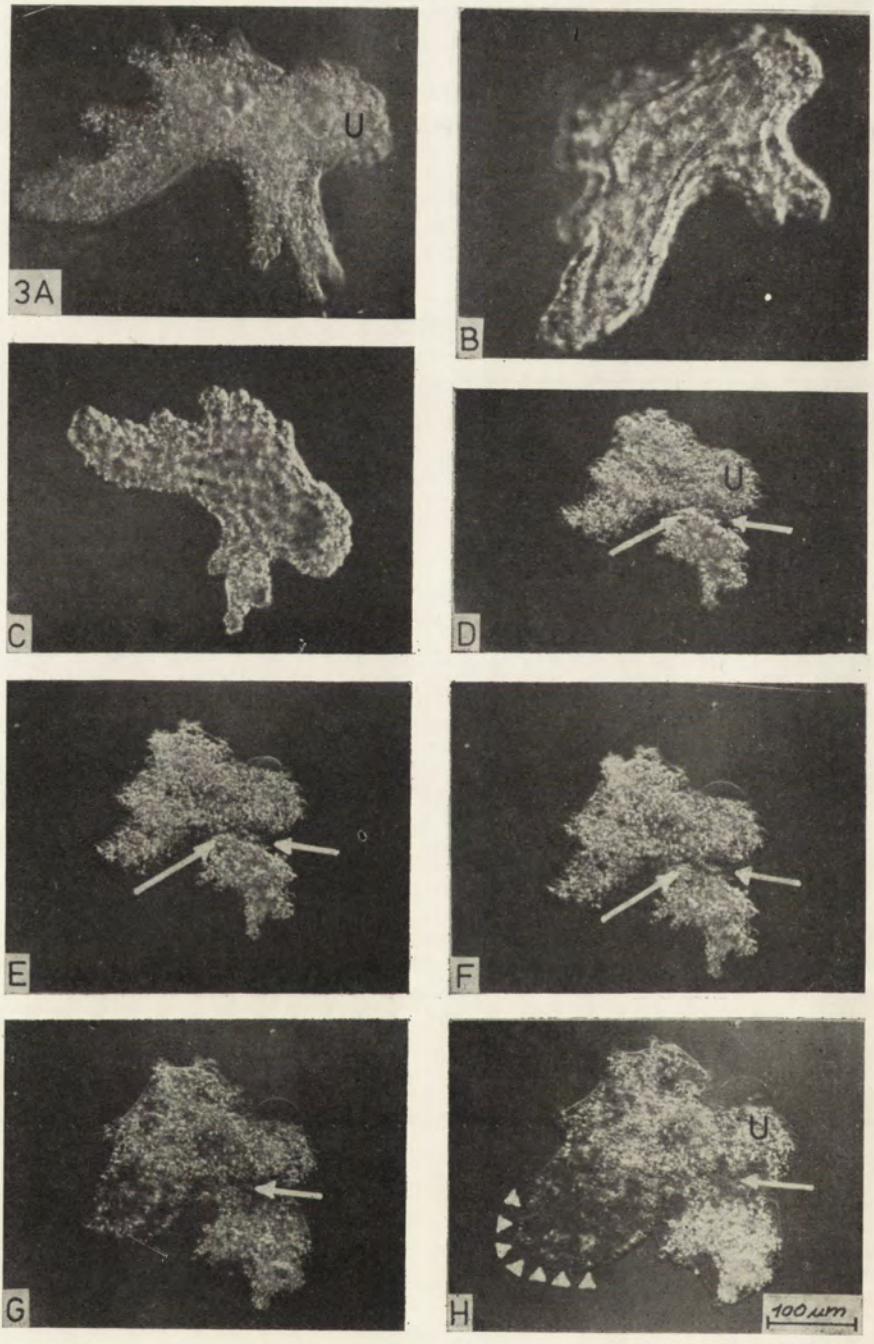

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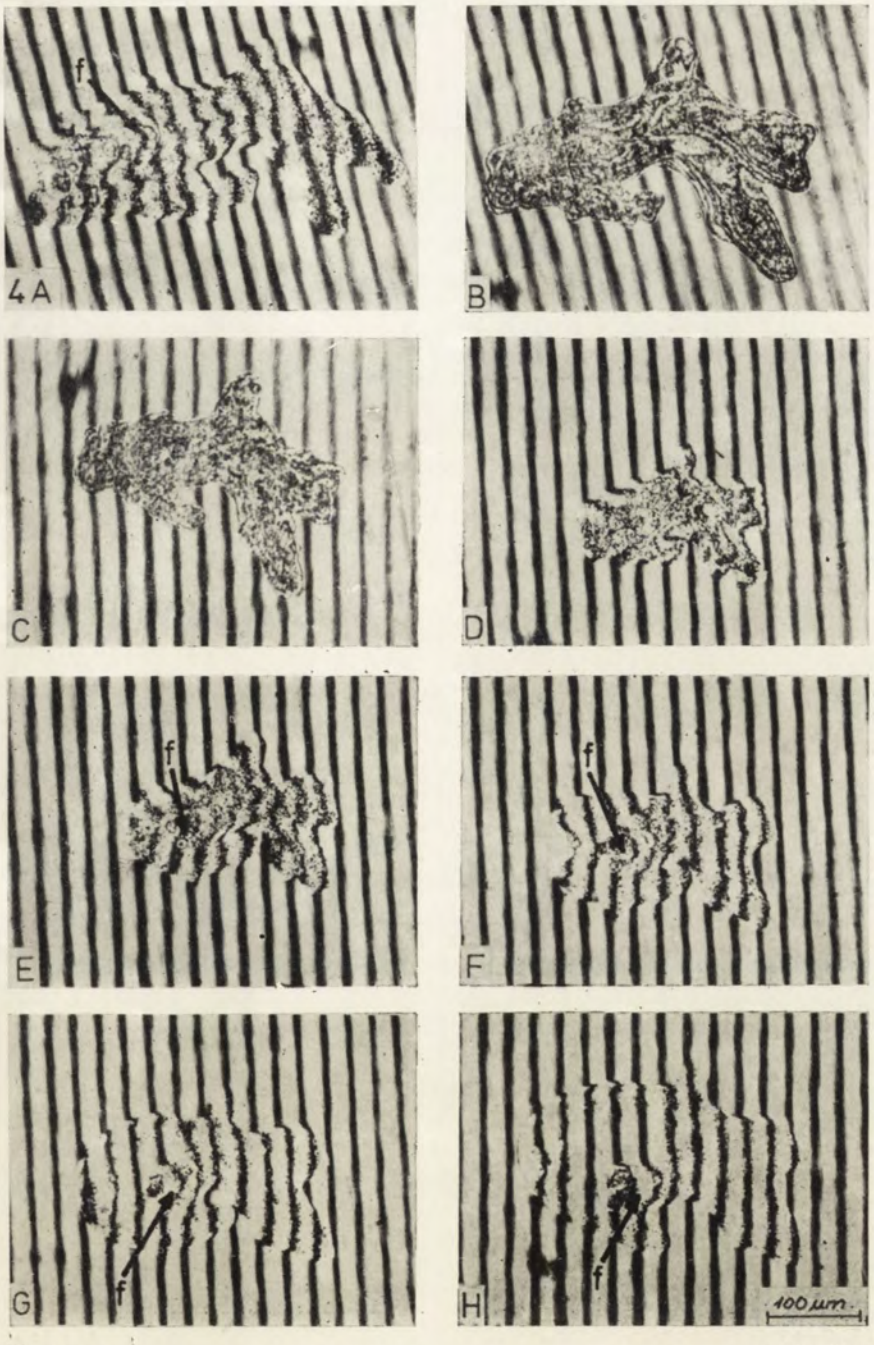

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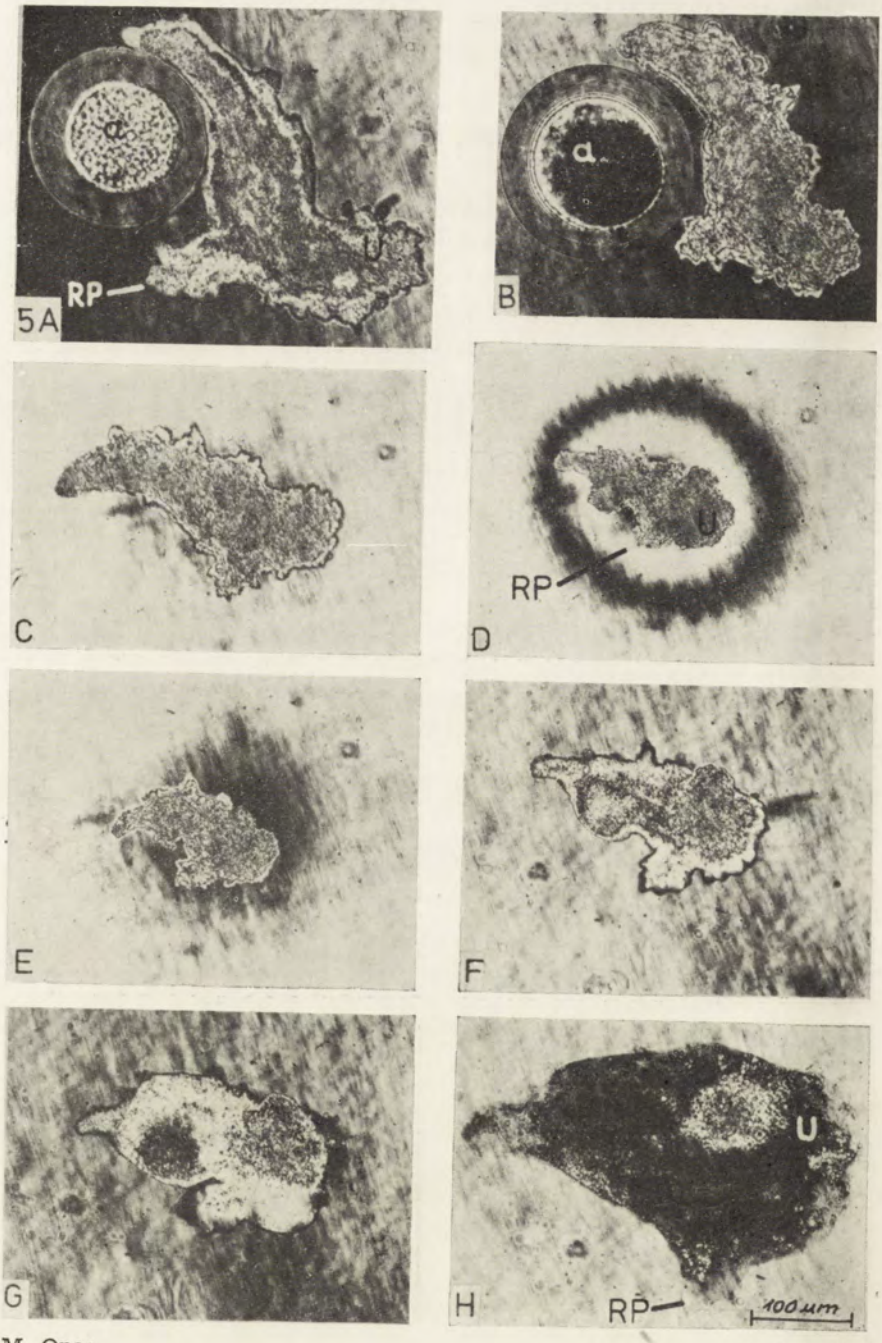

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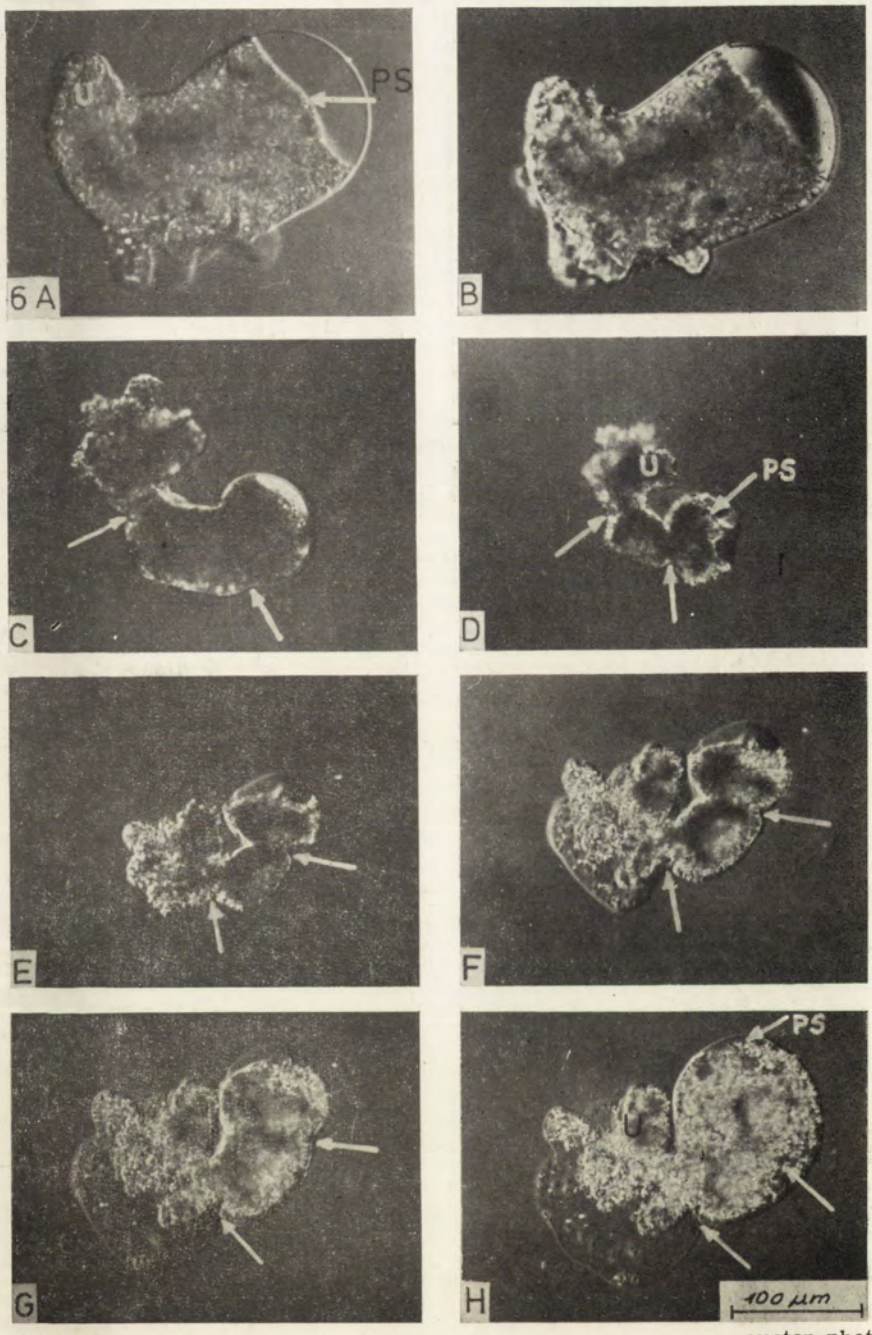

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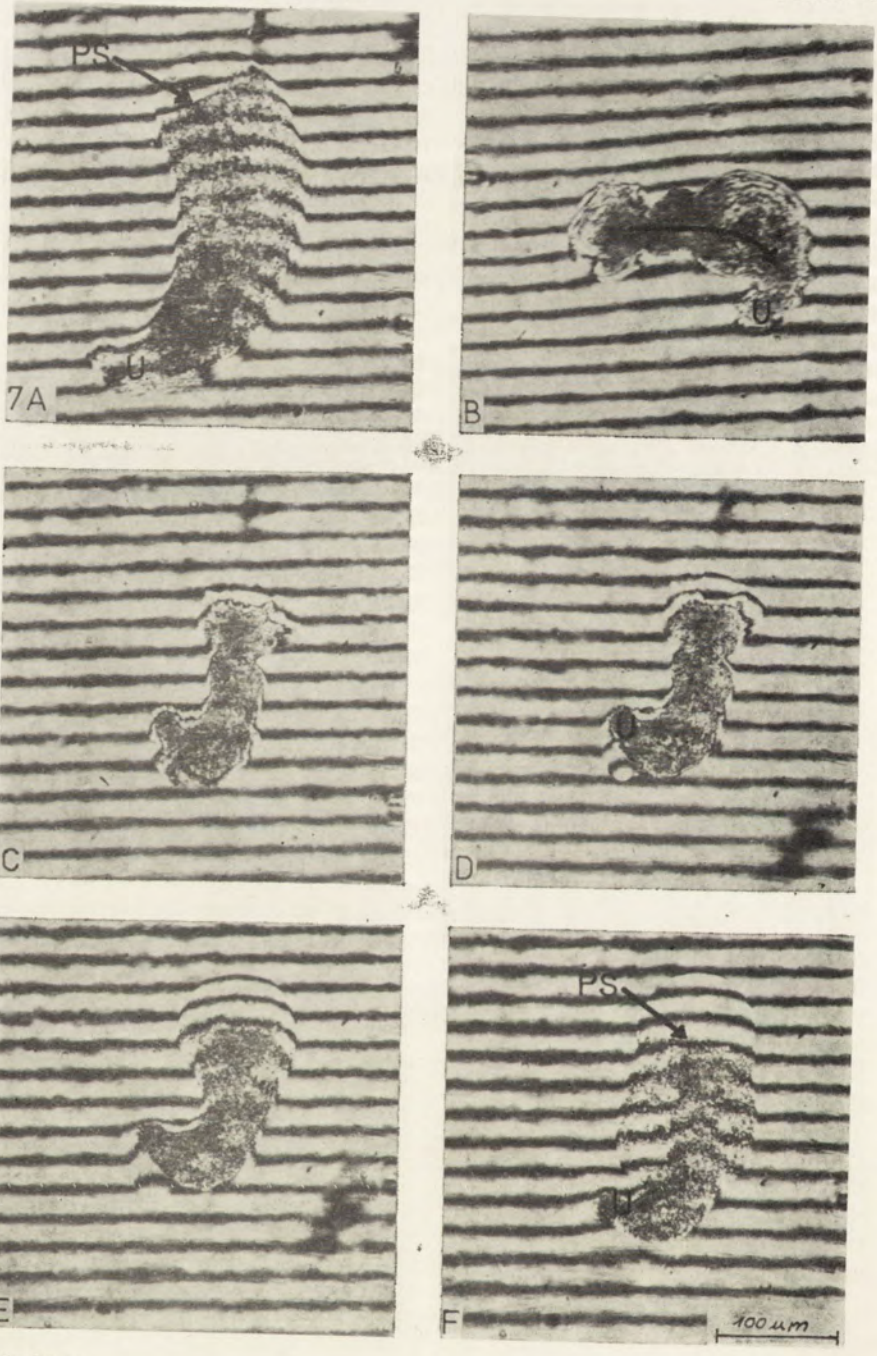

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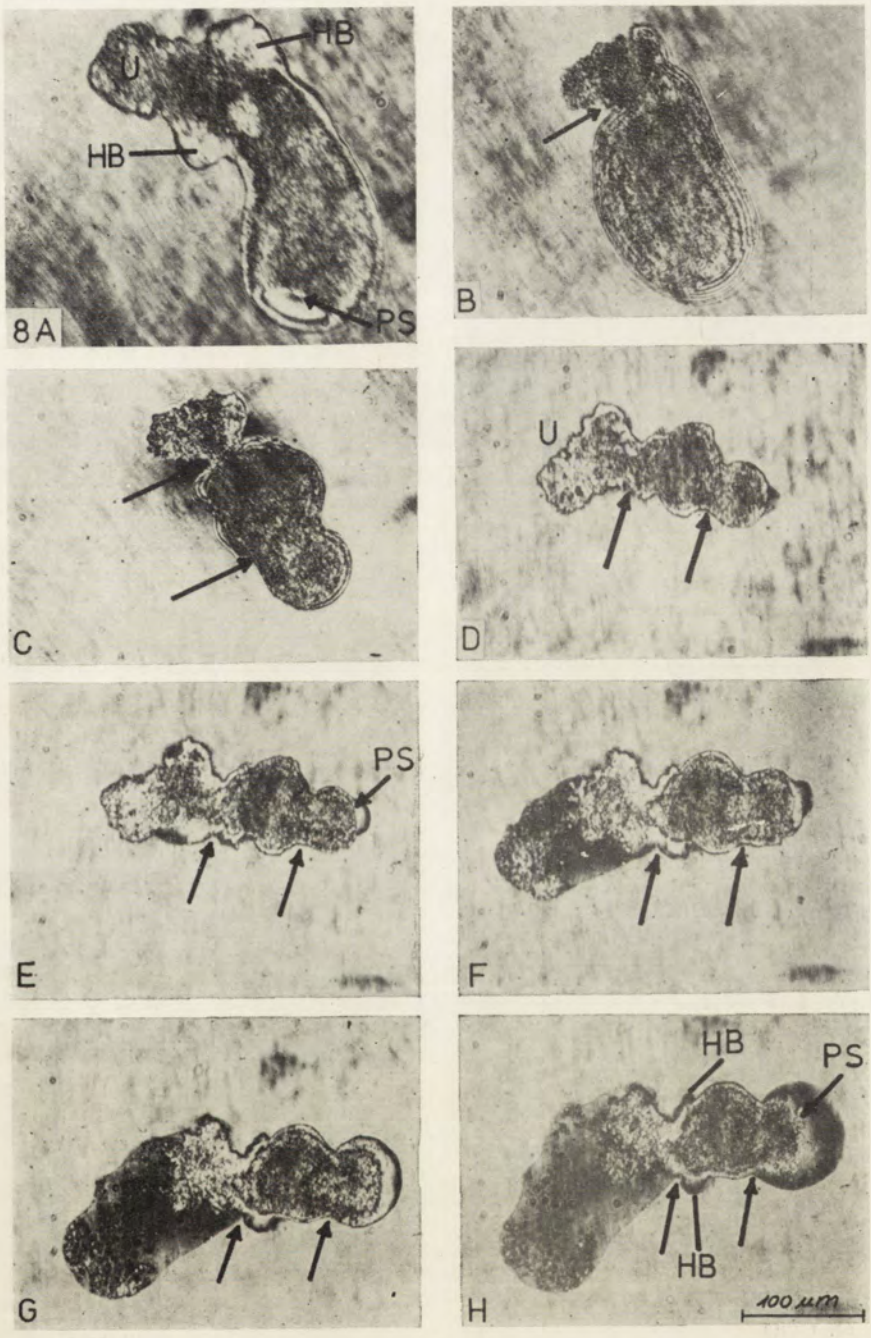

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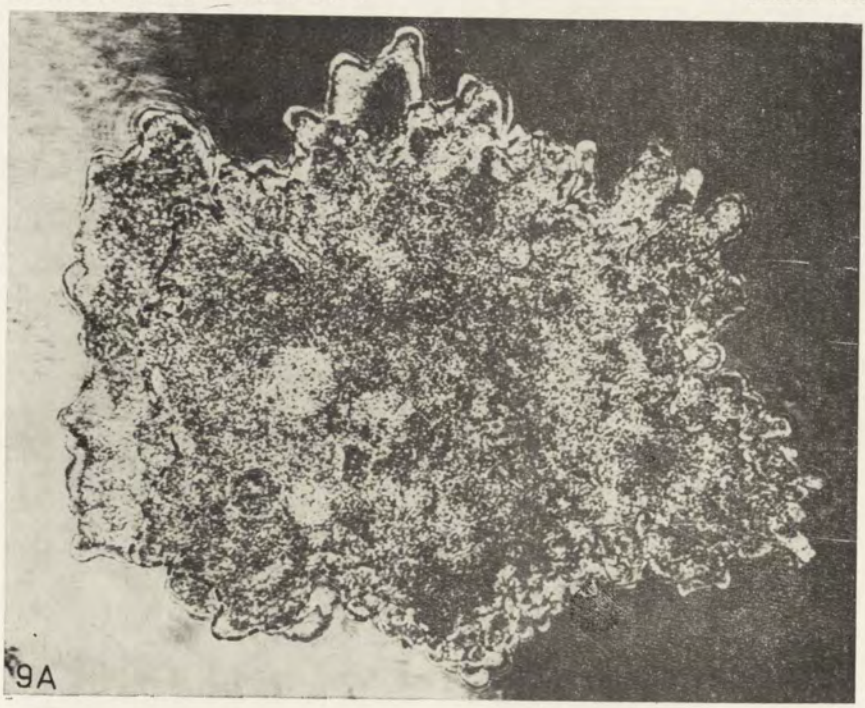

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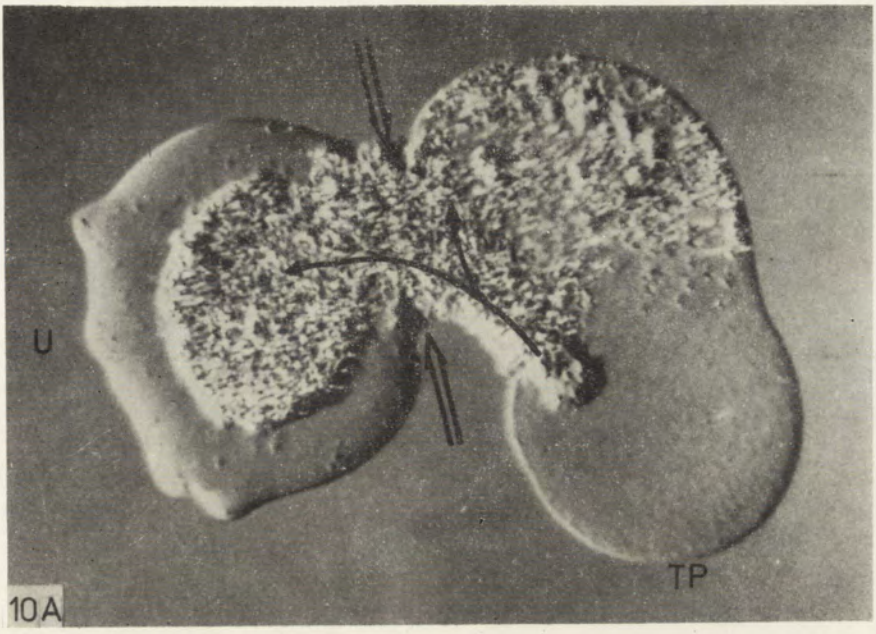

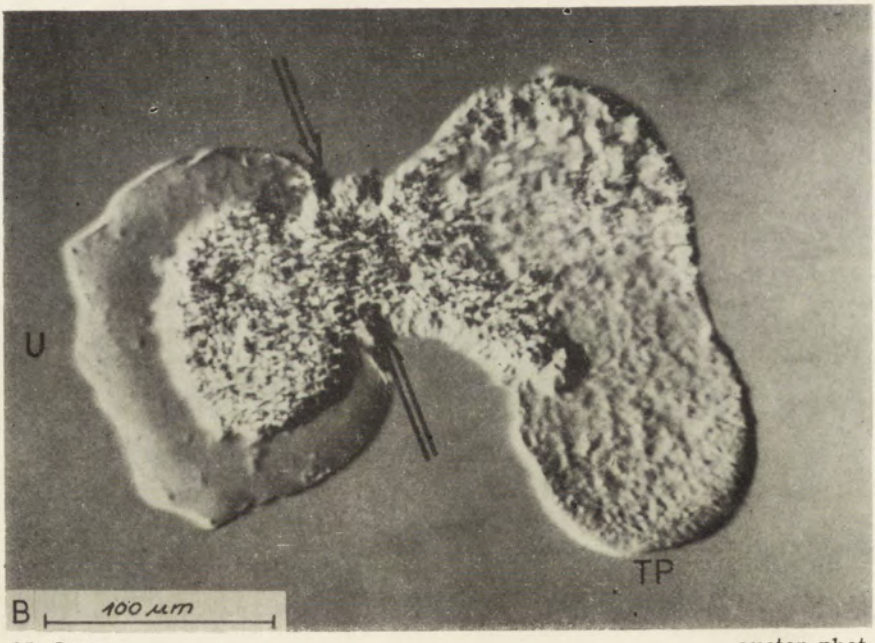

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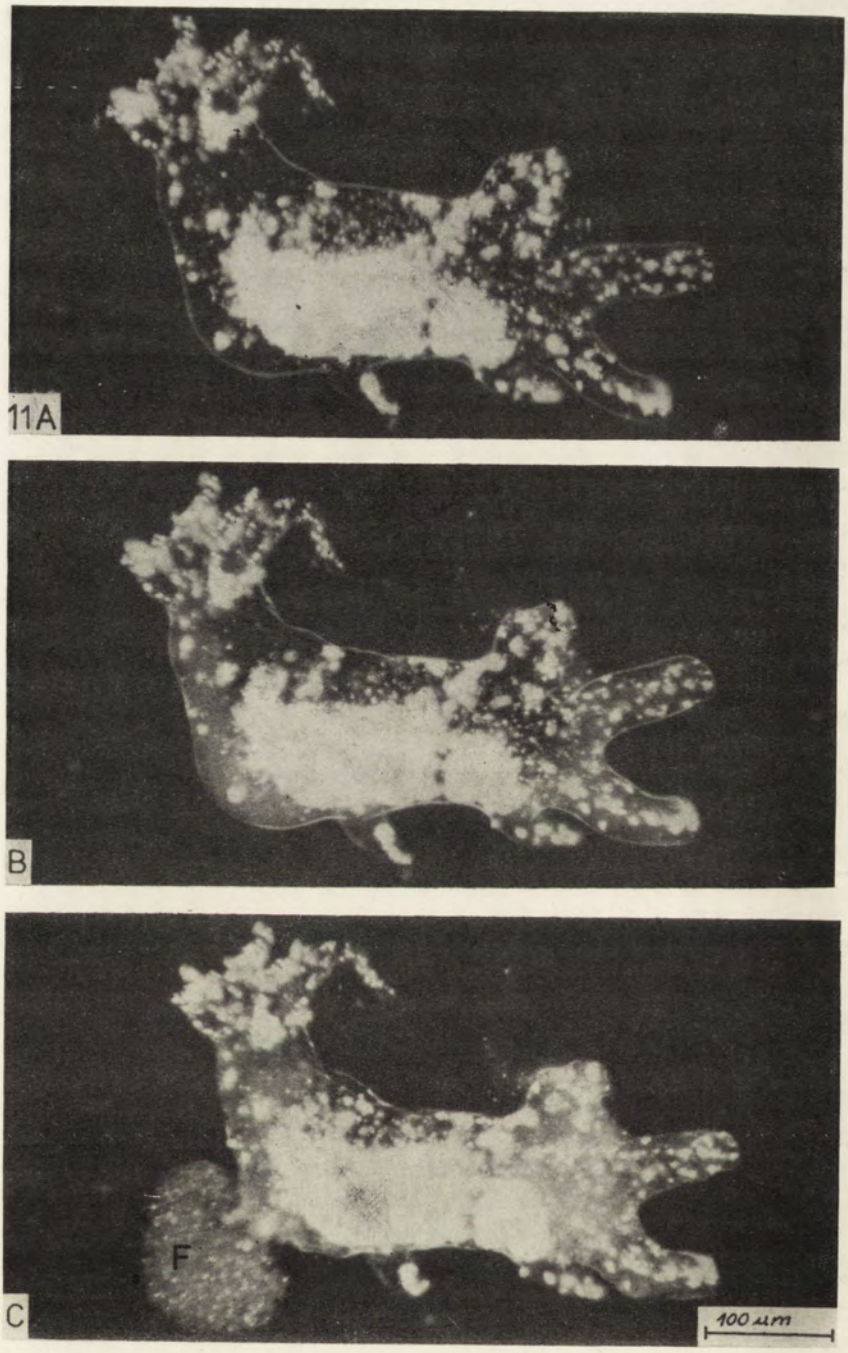

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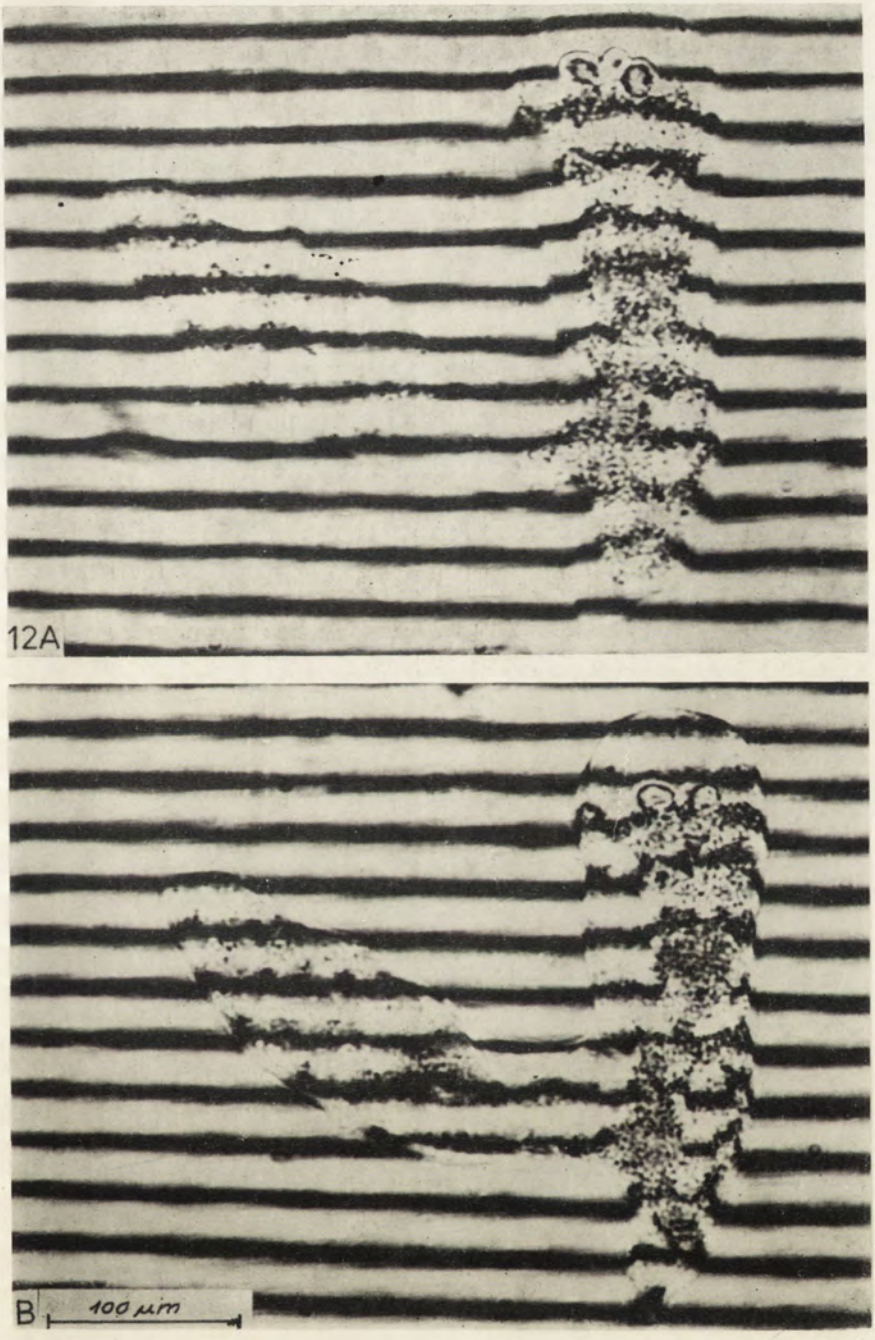

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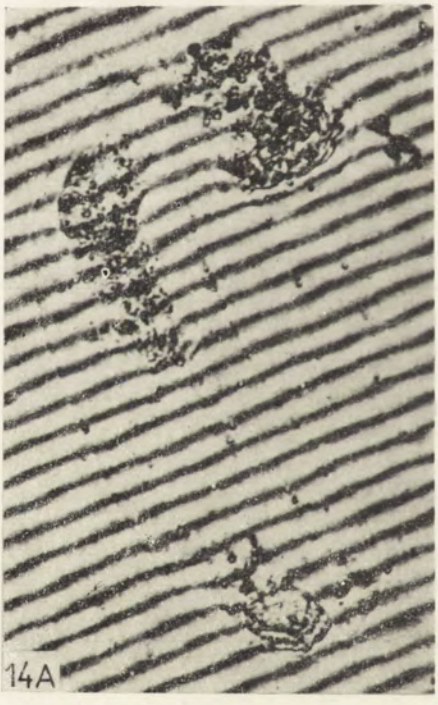

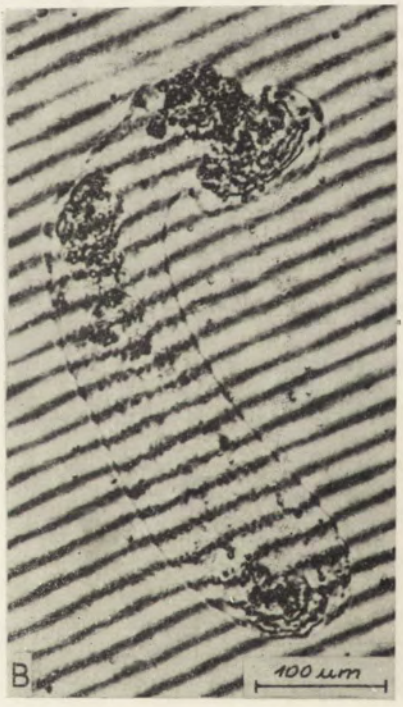

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EXPLANATION OF PLATES I–XII

1: Two types of glycerinated models of *A. proteus*: A — obtained by the method of Simard-Duquesne and Couillard (1962), B — obtained by the method of Rinaldi et al. (1975); U — uroid. Differential interference contrast.
2: The tip of glycerinated model of monopodial *A. proteus* specimen. PS — plasmagel sheet, arrow — contracted ring of cytoplasm. Differential interference contrast.
3: Glycerination of the polypodial specimen of *A. proteus*: A — before addition of glycerol solution; after glycerol addition: B — 10 s (note the structure of amoeba surface) C — 1 min, D — 3 min, amoeba in the point of maximal compactness (PMC); expansion: E — 4th min, F — 6th min, G — 60th min, H — 90th min. U — uroid, arrows — contracted ring of cytoplasm, arrowheads — region in which expanding cortical layer left the granules behind. Differential interference contrast
4: Glycerination of the polypodial specimen of *A. proteus*: A — before addition of glycerol solution; after glycerol addition: B — 10 s, C — 40 s, D — 90 s, PMC; expansion: E — 10th min, F — 20th min, G — 40th min, H — 100th min, f — ingested food placed centrally in F but dragged to the periphery in H. Fringe field, holographic microscope
5: Glycerination of the polypodial specimen of *A. proteus:* A — before addition of glycerol; after glycerol addition: B — 20 s, C — 30 s (dark area near the amoeba reflects expulsion of water), D — 50 s (circular fringes around the amoeba reflect the eruptive expulsion of water), E — 80 s, PMC; expansion: F — 15th min, G — 60th min, H — 120th min, a — air bubble, RP — retracting pseudopod, U — uroid. Uniform field, holographic microscope
6: Glycerination of the monopodial specimen of *A. proteus*: A — before addition of glycerol; after addition of glycerol: B — 30 s, C — 150 s, D — 190 s, PMC; expansion: E — 5th min, F — 20th min, G — 26th min, H — 60th min (note membrane surplus in the uroid). PS — plasmagel sheet, U — uroid, arrows — contracted rings of cytoplasm. Differential interference contrast
7: Glycerination of the monopodial specimen of *A. proteus*: A — before glycerol addition; after glycerol addition: B — 100 s, C — 130 s, PMC; expansion: D — 15th min, E — 40th min, F — 90th min. PS — plasmagel sheet, U — uroid, arrow denotes direction of streaming before cell death. Fringe field, holographic microscope
8: Glycerination of the monopodial specimen of *A. proteus*: after glycerol addition: A — 5 s, B — 60 s, C — 90 s (dark area near the posterior part of amoeba reflects water expulsion), PMC; expansion E — 5th min, F — 20th min, G — 50th min, H — 70th min (note membrane surplus in the uroid). HB — hyaline blebs, PS — plasmagel sheet, U — uroid, arrows — contracted rings of cytoplasm. Uniform field, holographic microscope
9: Interference images of (A) initial stages and (B) later stages (expansion) of glycerination of *A. proteus*. (A) is reconstructed double-exposure hologram showing initial shock evoked by glycerol treatment. Exposure were taken before and 30 s after glycerol addition. The second outline of amoeba is marked by contour line. Fringes are hardly recognizable due to increased light scattering. (B) is reconstructed double-exposure hologram showing different behaviour of clustered granules and cortical layer in expansion. Exposures were taken in 1th and 140th minute of expansion. Note the great expansion of cortical layer and negligible expansion of internal cluster

10: Contraction of the glycerinated model of monopodial *A. proteus* specimen in 25% glycerol. A — before addition of ATP, Ca^{2+}, Mg^{2+}, B — 10 min after addition of ATP, Ca^{2+}, Mg^{2+}. TP — tip of amoeba, U — uroid, arrow — direction of streaming before cell death, double arrows — contracted ring of cytoplasm. Differential interference contrast

11. Contraction of the glycerinated model of polypodial specimen of *A. proteus* in 25% glycerol. A — before addition of ATP, Ca^{2+}, Mg^{2+}, B — and C — after addition of ATP, Ca^{2+}, Mg^{2+}. B and C were taken at 10 s interval. F — erruption of model contents. Dark field

12: Contraction of glycerinated model of "pseudopolypodial" *A. proteus* specimen in 25% glycerol. A — before addition of ATP, Ca^{2+}, Mg^{2+}, B — after addition of ATP, Ca^{2+}, Mg^{2+}. Fringe field, holographic microscope

13: Contraction of the glycerinated model of polypodial *A. proteus* specimen in 25% glycerol. A — before addition of ATP, Ca^{2+}, Mg^{2+}, B — after addition of ATP, Ca^{2+}, Mg^{2+}. Uniform field, holographic microscope

14: Contraction of the glycerinated model of monopodial *A. proteus* specimen in 25% glycerol. A — before addition of ATP, Ca^{2+}, Mg^{2+}, B — after addition of ATP, Ca^{2+}, Mg^{2+}. Fringe field, holographic microscope

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Physiological and Toxic Effects of Detergents on *Paramecium caudatum*

Synopsis. Lethal effects of detergents on *Paramecium caudatum* show approximately linear relation when concentration of detergent and time of exposure of ciliate to detergent solution are presented in form of double logarithmic plot of LD_{50} doses. Six tested detergents could be arranged in following way in dependence on their threshold concentrations of LD_{50} with increasing values from left to right: CTAB, Triton X-100, SDS, Tween 20, Tween 40, Tween 80. Among Tweens, their higher toxic properties were associated with higher HLB and CMC values. All applied detergents — except Tween 80 — induced contraction of ectoplasm which was expressed by characteristic pear-shaped or lemon-shaped deformation of cell body and shortening of longitudinal axis to approximately 65–70% of the initial length in *Paramecium*. The authors suggest that contraction of *Paramecium* induced by detergents results from influx of external calcium throughout injured cell membrane and its direct triggering action on the contractile system within outer subpellicular layer of ectoplasm.

Among the fresh water ciliates, paramecia are exceptionally adequate objects for studies on action of various noxious agents present in external environment.

In spite of an anormous number of papers dealing with physiological and toxic effects of ions, alkaloids, UV- and X-rays irradiation on the fresh water protozoa — there are relatively scarce data available on the effects of surface active substances on unicellular organisms. Toxic and cytopathological action of surfactants on *Tetrahymena pyriformis* was reported by Chaix and Baud (1947) and Bailanger and Troadec (1953). Butzel et al. (1960) noticed decrease of galvanotactic

[1] The study of Mrs Kaukaba Mehr from School of Education, University of Kabul (Afghanistan) has been supported by Research Grant 7/73 from the Polish Academy of Sciences.

threshold in *Paramecium* after exposure to Cetyltrimethylammonium bromide (CTAB) and more recently Dryl and Bujwid-Ćwik (1972 a, b) and Bujwid-Ćwik and Dryl (1975) found significant effect of ionic detergents CTAB and SDS (Sodium dodecylsulphate) on excitability and motor response of *Paramecium caudatum* towards external stimuli. In higher toxic concentrations of CTAB and SDS Dryl and Bujwid-Ćwik noticed very characteristic pear-shaped or lemon-shaped deformation of cell body with distinct shortening of ciliate along its longitudinal body axis. It was suggested that the observed changes of cell shape are due to contraction process within ectoplasm in analogy to similar findings during exposure of *Paramecium* to direct current (Statkewitsch 1907, Jahn 1966, Jones et al. 1966) or to appropriate concentrations of $SrCl_2$ or $CaCl_2$ (Kamada and Kinosita 1945). Shortening of *Paramecium* was also observed after injection of $CaCl_2$ solution into cytoplasm and this experiment brought evidence that calcium ions play an essential role in initiation of contraction phenomena within cytoplasm. Allen (1971) in an outstanding electron microscopy study of *P. caudatum* has described in the outer layer of ectoplasm the bundles of microfibrills with possible contractile function.

The objectives of this paper was study the mechanism of toxic and contraction-inducing action of some ionic and non-ionic detergents on *Paramecium* and to find whether the observed effects are related to their physical and chemical properties.

Material and Methods

Experiments were carried out on *P. caudatum* strain isolated in 1965 from surroundings of Warsaw and continuously grown in laboratories of Department of Cell Biology, Nencki Institute of Experimental Biology in Warsaw. Ciliates were cultivated at temperature 22–24 °C in the lettuce medium inoculated with *Aerobacter aerogenes* according to method of Sonneborn (1950).

Following surfactants were used in the present study: CTAB — Serva, SDS — Serva, Triton X-100 (Polyoxyethylene p.t. octylphenol) — Koch-Light, Tween 20 (PEG 20 sorbitol monolaurate) — Koch-Light, Tween 40 (PEG 20 sorbitol monopalmitate) — Suchard, Tween 80 (PEG 20 sorbitol monooleate) — Koch-Light.

The most important and commonly used physical parameters to characterize detergents include so-called hydrophilic-lipophilic balance (HLB) and critical micelle concentration (CMC). HLB is an arbitrary quantity which is used by biochemists and biophysicists for better understanding of membrane solubilizing power of detergents. The low HLB values (1–10) characterize hydrophobic compounds whereas higher values (> 10) correspond to higher hydrophilic properties of detergent.

CMC represents concentration of detergent at which the first micelles are

Table 1

Physical Parameters of Detergents used in the Study*

Commercial name of detergent	HLB number	CMC	
		mM	mg/l
CTAB		0.92	335
SDS	40**	8.20**	2362
Triton X-100	13.5	0.24	155
Tween 20	16.7	0.05	60
Tween 40	15.6	0.023	29
Tween 80	15.0	0.01	13

* The data collected from paper by Helenius and Simons (1975).

** Based on data from paper by Kagawa (1972).

formed in solution. It should be pointed out that solubilization of membranes occurs usually at detergent concentrations higher than CMC and this is probably associated with splitting of hydrophobic interaction between membrane proteins and lipids. HLB and CMC values of detergents used in the present study are given in Table 1.

Estimation of Toxic Effects of Detergents

Detergents used in the present study have been diluted with following salt solution: 1 mM $CaCl_2$ + 1 mM Tris-HCl, pH 7.3. The 50% survival rate (LD_{50}) was calculated on the basis of ratio between still alive and dead animals in the same solution at different time of exposure to detergent. Before dying, the animals showed a number of pathological symptoms, but arbitrary criterion of cell death was complete stoppage of ciliary movement and lack of any visible activity of contractile and food vacuoles with simultaneous arrest of cyclotic movement within fluid cytoplasm.

Observation of ciliates at LD_{50} concentrations of detergents was based on ten series of experiments, and also ten series were checked with control animals not exposed to detergents. All experiments were carried out at temperature 22–24 °C and the results from ten series of experiments were presented in the diagrams as calculated arithmetic means and standard deviations of observed survival rate values.

It should be added that control paramecia kept for 18–24 h in Ca-Tris-HCl solution showed survival rate higher than 99%, whereas their cell fission rate was lower than 0.01 per day, so that it did not influence significantly the achieved experimental data.

Measurement of Cell Body Length

In studies on the occurrence of ectoplasmic contraction during exposure of *Paramecium* to various detergents, the measurement of the length of animal was taken microphotometrically with light exposure time 4 msec in ten thigmotactic or very slowly moving animals selected at random. Microns microscale was used as a basis for routine calculations. The possible error or measurement arising from eventual motion of ciliates under experimental conditions did not exceed 1% and was neglected in final calculations.

The mean values of cell body length were expressed in μm from micro-photo-negatives in paramecia exposed to various concentrations of surfactants after 15 s, 30 s, 1, 2, 4, 8 and eventually 16 or even 32 min of incubation in experimental medium. For each experimental group the measurements were carried out also with control sample of animals not exposed to detergent.

Special washing procedure was applied to ciliates exposed for rather short period of time (between 15 s to 2 min) to highly toxic concentrations of detergents in order to achieve the recovery of normal shape and cell body length after replacing paramecia to medium devoid of detergent. The dense sample of ciliates was exposed to detergent solution and after desired time of exposure animals were transferred to a large slide covered with thin layer of Ca-Tris-HCl medium (pH 7.3); this simple procedure rendered possible active swimming of affected animals into salt solution without detergent.

In all tests the measurements of cell body length were performed with an accuracy ± 2 μm.

Electron Microscopy of the Cell Surface of *Paramecium caudatum*

Paramecium caudatum from 1- to 4 days-old cultures were concentrated in Laboratory centrifuge (ŚIEĆ) by spinning at 4000 × g for 2 min. After reaching sufficient dense sample of *Paramecium*, the cells were washed twice with steril lettuce medium and finally in 1 mM $CaCl_2$ + 1 mM Tris-HCl buffer solution of pH 7.4.

The medium was rapidly drown off, leaving the ciliates in medium containing:

(1) Ruthenium red staining (L u f t 1971 a):

a — 3.6% glutar aldehyde 0.5 ml,

b — 0.2 M cacodylate buffer, pH 7.3 0.5 ml,

c — RR Stock solution, 1500 PPm in water.

Fixed one hour at 4 °C temperature.

(2) The cells were than washed three times with 0.15 M cacodylate buffer for a period of ten minutes, and post-fixed for three hours at room temperature in solution containing:

a — 5% OSO_4 in distilled water 0.5 ml,

b — 0.2 M cacodylate buffer, pH 7.3 0.5 ml,

c — RR stock solution, 1500 PPm in water 0.5 ml.

Dehydration was carried out in an increasing ethanol series (total time one hour) followed by two 15 min lasting transfers into 100% propylene oxid.

The cells were than embedded in Epon. After a period of time in an evacuated thermostat, the above embedding mixture was polymerized in an 45 °C and 60 °C oven, respectively.

The material was sectioned on a L. K. B. ultramicrotome and mounted unsupported on 200 or 300 mesh copper grids. JEM 100 B Electron microscope operated 80 KV used to examine the cells.

Before starting the experimental procedure, paramecia in test-tubes were exposed for 5 min to 2.7×10^{-5} M SDS, diluted with 1 mM $CaCl_2$ + 1 mM Tris-HCl (pH 7.3) solution. Paramecia from control sample were not exposed to detergent.

Results

Toxic Action of Detergents on *Paramecium caudatum*

The applied detergents showed significant differences in their lethal effects on *Paramecium* as it is shown in Fig. 1.

Six tested detergents could be arranged in the following way in dependence on their threshold molar concentrations of LD_{50} doses with increasing values from left to right: CTAB, Triton X-100, SDS, Tween 20, Tween 40, Tween 80.

It is worth to note that in highly toxic doses all detergents — except Tween 80 — have induced pear-shaped, lemon-shaped or cigar-shaped deformation of cell body due to contraction process within ectoplasm (Pl. I 1, 2). Other cytopathological changes included: vacuolization and darkening of cytoplasm (visible under low or high magnification of the optic microscope), damage of pellicle with "blepses" or demarcation vesicles, sometimes with extrusion of trichocysts and more or less advanced decomposition of cell body with leaking out of cytoplasm from cell interior to external medium (Pl. I 5).

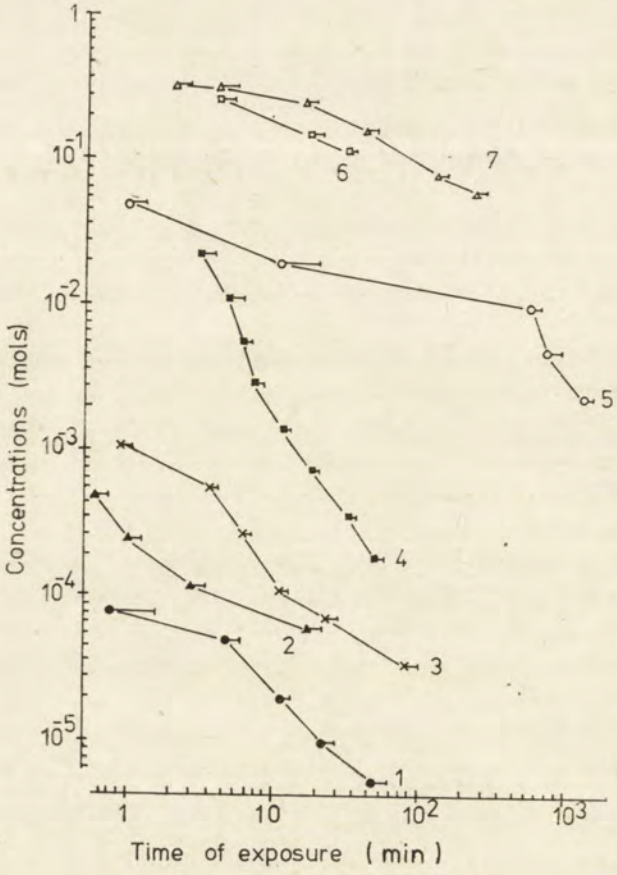

Fig. 1. Toxic effects of various detergents on *Paramecium caudatum* in dependence on their concentration and time of exposure, expressed as double logarthmic plot of LD_{50} doses. Each point represents arithmetic mean (with S.D.) from ten measurements calculated in approximately fifty animals. Paramecia exposed to CTAB (1), paramecia exposed to Triton X-100 (2), paramecia exposed to SDS (3), paramecia exposed to Tween 20 (4), paramecia exposed to Tween 40 (5), paramecia exposed to Saccharose (6), paramecia exposed to Tween 80 (7)

Paramecia exposed to highly toxic concentrations of CTAB showed not only characteristic changes of cell shape but also inhibition of contractile vacuoles activity in the stage of extended vacuoles (Pl. I 4, Pl. II 6).

Lethal-toxic action of detergents increased parallel to longer duration of exposure and higher concentration of substance. The curves from the diagramme (Fig. 1) are very close to straight lines, when both concentration of detergent and time of exposure to detergent are represented as double logarithmic plot of LD_{50} doses. The exception from this rule is the curve for Tween 20 which shows unusually large slope inclination in the concentration range between 0.17×10^{-3} M — 21×10^{-2} M. This may suggest that concentration 0.17×10^{-2} M of Tween 20 is critical for occurrence of some very serious injury of *Paramecium* cell, what is reflected by rather quick destruction of ciliate.

Out from six tested detergents, Tweens appear to be less toxic than CTAB, SDS and Triton X-100. This may depend on extremely low CMC

of Tweens, so that in high concentrations only very small amount of detergent is in the free form in solution while very large part exists in micellar form. It is quite clear from data in Table 1 and Fig. 1, that even the highest LD_{50} concentrations for CTAB, SDS and Triton X-100 are much below corresponding CMC values while in the case of Tweens all LD_{50} doses appear at much higher concentrations than CMC levels.

Among Tweens the compound containing lauryl, i.e., relatively short chain lipophilic group (Tween 20) proved to be significantly more toxic than that containing saturated long chain group — palmityl (Tween 40) or unsaturated long chain group — oleinate (Tween 80).

The mechanism of toxic action of Tween 80 on *Paramecium* seems to be different from other detergents because even in highest lethal concentrations the animals did not show signs of visible damage of pellicle or contraction of ectoplasm. Instead, the animals were extremely thin (Pl. I 3) and the activity of contractile vacuoles was lowered. Although the lethal LD_{50} concentrations of Tween 80 are only slightly below LD_{50} values of classical osmotic-active agents as, e.g., saccharose — the possible osmotic mechanism of its toxic action must be ruled out since:

(a) High concentrations of Tween 80 involve only small amount of compound in the free form whereas large part of compound appears in micellar form (Table 1).

(b) The dying animals never show any signs of pellicle damage which is so typical for osmotic-active substances (Pl. II 7).

Change of Body Shape in *Paramecium caudatum* Exposed to Detergents

In separate series of experiments paramecia were exposed to various concentrations of CTAB, SDS and Triton X-100 and changes of body shape were recorded by means of photomicrography. As it was mentioned before, except Tween 80, all other detergents at higher concentrations induced cigar-, pear- or lemon-like deformation of body with shortening of longitudinal axis and broadening of transversal body axis (Pl. I 2, Pl. II 8).

It is evident from diagramms (Fig. 2, 3, 4) that the degree of shortening of longitudinal body axis is graded, i.e., it depends on the concentration of applied surfactant, being more strongly expressed in their higher than lower concentrations. In the highest concentrations the shortening of *Paramecium* body to approx. 65–70% of initial length appears during first second of exposure to detergents.

The recorded changes of body shape of *Paramecium* were very similar in three tested detergents, so that no specific feature could be described

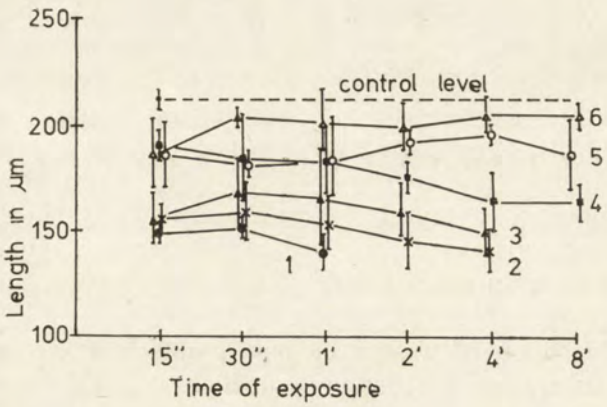

Fig. 2. The change of length of *Paramecium caudatum* exposed to various concentrations of SDS. Paramecia exposed to solution 1.10×10^{-3} M SDS (1), paramecia exposed to solution 0.55×10^{-3} M SDS (2), paramecia exposed to solution 0.27×10^{-3} M SDS (3), paramecia exposed to solution 0.14×10^{-3} M SDS (4), paramecia exposed to solution 0.70×10^{-4} M SDS (5), paramecia exposed to solution 0.35×10^{-4} M SDS (6). Each point of the diagramme corresponds to the mean value (with standard deviation) of measured length in 10 paramecia

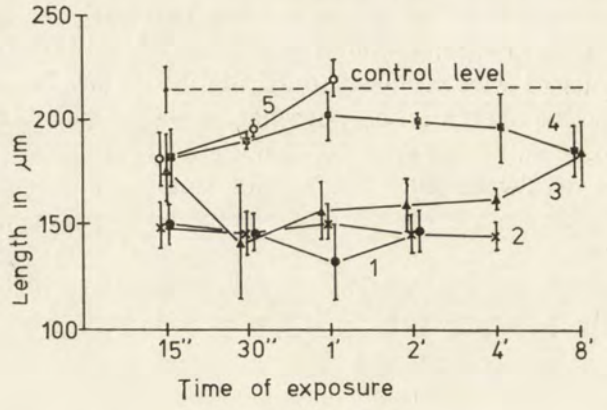

Fig. 3. The change of length of *Paramecium caudatum* exposed to various concentration of CTAB. Paramecia exposed to solution 8×10^{-5} M CTAB (1), paramecia exposed to solution 4×10^{-5} M CTAB (2), paramecia exposed to solution 2×10^{-5} M CTAB (3), paramecia exposed to solution 1×10^{-5} M CTAB (4), paramecia exposed to solution 0.5×10^{-5} M CTAB (5). Each point of the diagramme corresponds to the mean value (with standard deviation) of measured length in 10 Paramecia

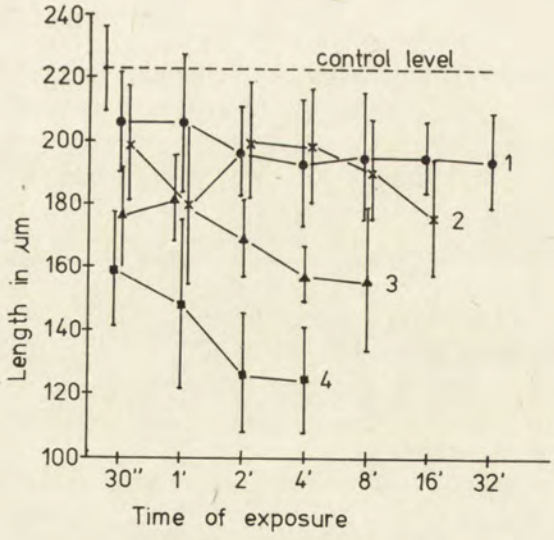

Fig. 4. The change of length of *Paramecium caudatum* exposed to various concentrations of Triton X-100. Paramecia in solution 0.31×10^{-3} M Triton X-100 (1), paramecia in solution 0.62×10^{-3} M Triton X-100 (2), paramecia in solution 1.25×10^{-3} M Triton X-100 (3), paramecia in solution 2.50×10^{-3} M Triton X-100 (4)

for any compound in this respect. It was interesting to find that paramecia may gradually recover normal shape and length when exposed only for short period of time (15 s or 2 min) to high concentrations of CTAB or SDS and washed afterwards for 16–32 min in medium devoid of detergent (Fig. 5).

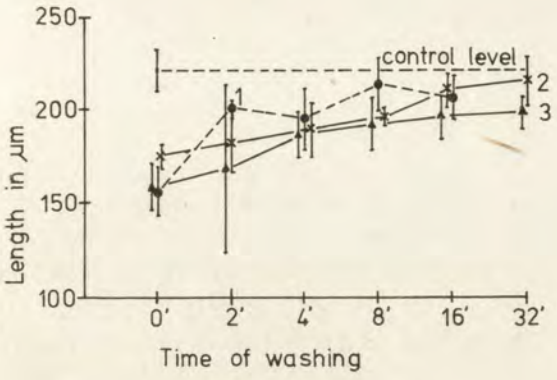

Fig. 5. The recovery of normal length of body in *Paramecium caudatum* exposed to solution of detergent and afterwards washed in the medium devoid of detergent. The arrow indicates the beginning of washing procedure in medium devoid of detergent. Paramecia exposed for 15 s to 0.27×10^{-3} M SDS (1), paramecia exposed for 2 min to 0.14×10^{-3} M SDS (2) before washing, paramecia exposed for 2 min to 2×10^{-5} M CTAB before washing (3)

The observed morphological changes in paramecia exposed to surface active substances and possibility of gradual recovery of normal cell shape suggest strongly that this phenomenon is based on contraction of ectoplasm, as it was described by Kamada and Kinosita (1945) in *Paramecium* exposed to direct current stimulation.

It is suggested that surfactants may induce contraction within ectoplasm of *Paramecium* indirectly by influx of external calcium ions throughout the cell membrane due to its injury by applied detergent. This view is supported by observation of the authors that paramecia exposed to external solution prepared with Ca-EGTA buffer according to method of Portzehl et al. (1964) containing 10^{-8} M Ca^{2+} did not show symptoms of cell body contraction even after addition of highest concentrations of CTAB, SDS or Triton X-100. It is assumed that calcium ions control the process of contraction within epiplasm of *Paramecium*.

Electron Microscopy Study of the Cell Surface in *Paramecium caudatum* by Technique of Ruthenium Red Staining after SDS Treatment

Recent observations of Luft (1971 a, b) showed that Ruthenium Red, a cationic dye, combines with acid mucopolysaccharides and partly with acid phospholipids within cell membrane.

Observations from the present study indicate the occurrence of the

surface coat covering the whole cell of *Paramecium caudatum* as showed on the Pl. II 9 and Pl. III 10.

SDS treatment altered the surface coat to different degree in various regions of *Paramecium* cell surface; the injury of cell membrane of *Paramecium* is shown on Pl. III 11 and Pl. IV 12, 13. It looks like SDS removes mainly the most external fuzzy metrial of *Paramecium* surface coat.

Discussion

It is clear from the data achieved in the present study that both physiological and toxic effect of applied six detergents show large differences which may depend on their chemical structure and also on some of their physical parameters. The strongest toxic action on *Paramecium caudatum* was observed in the case of CTAB, Triton X-100 and SDS. It should be pointed out that Chaix and Baud (1947) noticed highly toxic action of oleinic acid and of some derivates of fatty acids on *Tetrahymena pyriformis* while highly toxic effects of ionic detergents were reported in the same ciliate species by Bailenger and Troadec (1953), Bailenger et al. (1953). Jeon and Bell (1965) and Brewer and Bell (1969) found that cationic detergents were more toxic for *Amoeba proteus* than anionic ones. The authors suggested that cationic detergents react probably directly with anionic groups of the cell surface of *Amoeba* and cause in this way rather rapid disruption of cell membrane, whereas cell lysis by anionic detergents may require higher concentration in order to overcome the repulsive effects of the surface negatively charged groups before penetration into the cell membrane lipid bilayer. This interesting hypothesis finds some support in the present study, since CTAB proved to be much more toxic for *Paramecium caudatum* than anionic detergent SDS (Fig. 1).

Among three non-ionic detergents (Tween 20, Tween 40, Tween 80) the stronger toxic effect on *Paramecium* was associated with higher HLB number and higher CMC. This suggests that deteriorating action of non-ionic detergents on the cell membrane of living animal cell may depend mainly on the action of free, non-micellar form of detergent present in solution. However, in the case of Tweens the chemical structure of lipophilic group seems to play an essential role in toxic action since it was proved in the present study that highly toxic Tween 20 contains lauryl, i.e., short chain (12 C) lipophilic group in contrast to less toxic Tween 40, which has palmityl, a saturated long chain (18 C) group and Tween 80, containing unsaturated long chain (18 C) group — oleinate.

One of puzzling effects of the present study is complete lack of any sings of ectoplasmic contraction in the case of toxic action of Tween 80. Obviously there is no damage of the cell membrane which would be associated with postulated for other surfactants influx of external calcium ions. This observation deserves further detailed studies on the possible mechanism of toxic action of Tween 80 on eucariotic animal cells.

Except Tween 80, all other detergents caused marked contraction of ectoplasm in *Paramecium* and this process was reversible at lower concentrations and short lasting exposure to detergent solution. Contraction of ectoplasm induced by higher concentrations of detergents was due to local or more general damage of the cell membrane which rendered possible influx of external calcium ions to cortical ectoplasmic layer of *Paramecium*. Graded character of observed contraction in lower concentrations of detergents may suggest that limited degree of cell body contraction results from involvement of smaller or larger amount of contractile elements in the above described process. This view seems to be confirmed by the fact that the maximum contraction coresponded always to the shortening of cell body length to approximately 65–70% of initial length, what could correspond to participation of all contractile units (elements) in the cell contraction.

As concern the injury of cell membrane, it is still not clear whether the postulated action of ionic detergents is limited to the "basic" cell membrane portion or it includes also the recently discovered surface coat layer (Wyroba and Przełęcka 1973) in *Paramecium*. It should be pointed out in this connection that the reported in the present study injury effect of SDS on the surface coat in *Paramecium* does not clear this problem since described effect appears at strongly toxic concentrations of applied detergent. In any case, it would be highly desirable to compare in future the effects of ionic and non-ionic detergents on the surface coat in the ciliate protozoa.

RÉSUMÉ

Les effets toxiques des détérgents sur le *Paramecium caudatum* sont caractérises par une rélation presque linéaire entre la concentration efféctive LD_{50} du détérgent et le temps d'exposition de la cellule à son action, si les deux variables sont éxprimées sur l'échelle logarithmique. Les six détérgents étudiés donnent la série suivante de la valeur croissante du seuil de leurs concentrations LD_{50} : CTAB, Triton X-100, SDS, Tween 20, Tween 40, Tween 80. En ce qui concerne les Tweens eux-mêmes, la toxicité augmente avec leur indèxe HLB et CMC. Tous les détérgents utilisés, sauf le Tween 80, provoquent les contractions ectoplasmiques

aboutissant à des déformation typiques du corps cellulaire qui devient soit pyriforme, soit prend la forme d'un citron, et se raccourcie en général de 65–70% en direction de son axe longitudinale. Les auteurs suggèrent que la contraction provoqeé chez le *Paramecium* par les détérgents est due à la pénétration du calcium exterieur à travers la membrane cellulaire endommagée, et par le déclenchement direct par ce ion de la réaction du systéme contractile localisé sous la pellicule dans la couche extérieure d'ectoplasme.

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EXPLANATIONS OF PLATES I–IV

Micrographs of *Paramecium caudatum* exposed to various external conditions. All detergents diluted with solution: 1 mM $CaCl_2$ + 1 mM Tris-HCl, pH 7.3.

1: Paramecia exposed to solution $CaCl_2$-Tris-HCl without detergents. Normal cell shape preserved. × 200

2 : Paramecia exposed to solution 1×10^{-3} M SDS, showing a pear-shaped deformation of cell body with shortening of cell length along longitudinal axis. × 200

3 : Paramecia exposed to solution 3×10^{-3} M Tween 80; marked slenderness of cell body. × 200

4 : Paramecia exposed to solution 5×10^{-5} M CTAB, showing contractile vacuoles in extended stage. × 200

5 : Paramecia exposed to solution 5×10^{-4} M Triton X-100, showing decomposition of cell body with heavy injury of pellicle and formation of demarcation vesicles. × 200

6 : Paramecia exposed to solution 8×10^{-5} CTAB, showing a cigar-shaped deformation of cell body and contractile vacuoles in extended stage. × 250

7 : Paramecia exposed to solution 0.25 M saccharose. Shrinkage of cell with visible demarcation vesicles in spots where pellicle was damaged. × 250

8 : Paramecia exposed to solution 1×10^{-3} M SDS, showing a lemon-shaped deformation of cell body. × 250

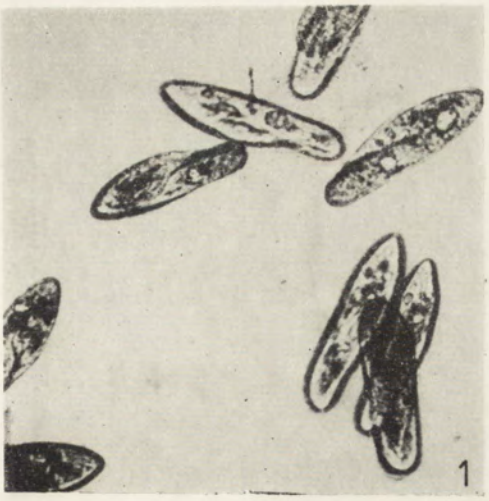
9 : Fragment of the cell surface of *P. caudatum*. Ruthenium red staining. The plasma membrane is covered with distinctly contrasted surface coat (S.C.). RR reaction. No counterstain. × 62 000

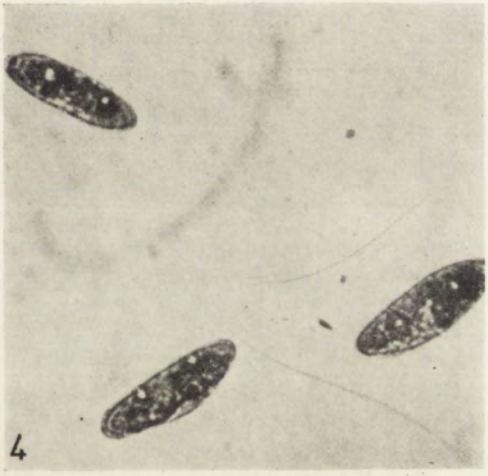
10 : Cilia and cell surface of *P. caudatum* with a layer of coating material. RR reaction. No counterstain. × 38 000

11 : Cell surface of *P. caudatum* after treatment with 2.7×10^{-5} M SDS. The surface coat disarranged to some degree. RR reaction. No counterstain. × 42 000

12 : Cell surface of *P. caudatum* after SDS treatment. Various steps of damaging action of detergent on different fragments of cell surface coat. In some fragments the surface coat is removed. RR reaction. No counterstain. × 100 000

13 : Transverse section of cilia in *P. caudatum* at the level of alveolar spaces after SDS treatment. The structure of surface coat is impaired (arrows). RR reaction. No counterstain. × 30 000





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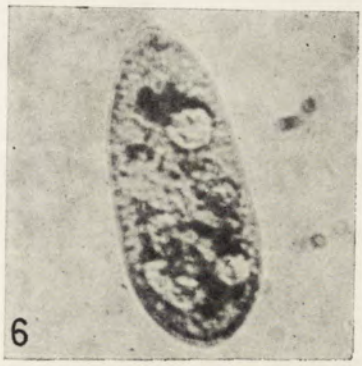

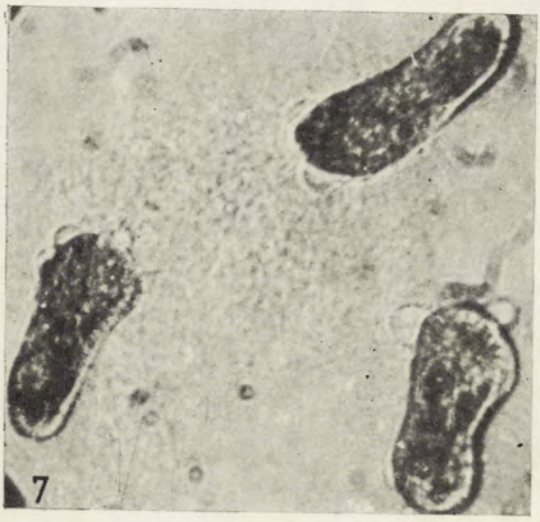

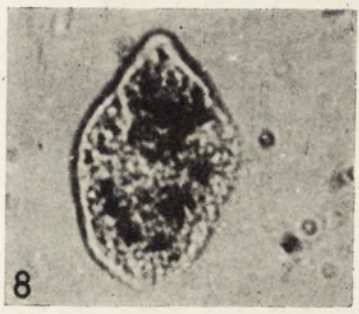

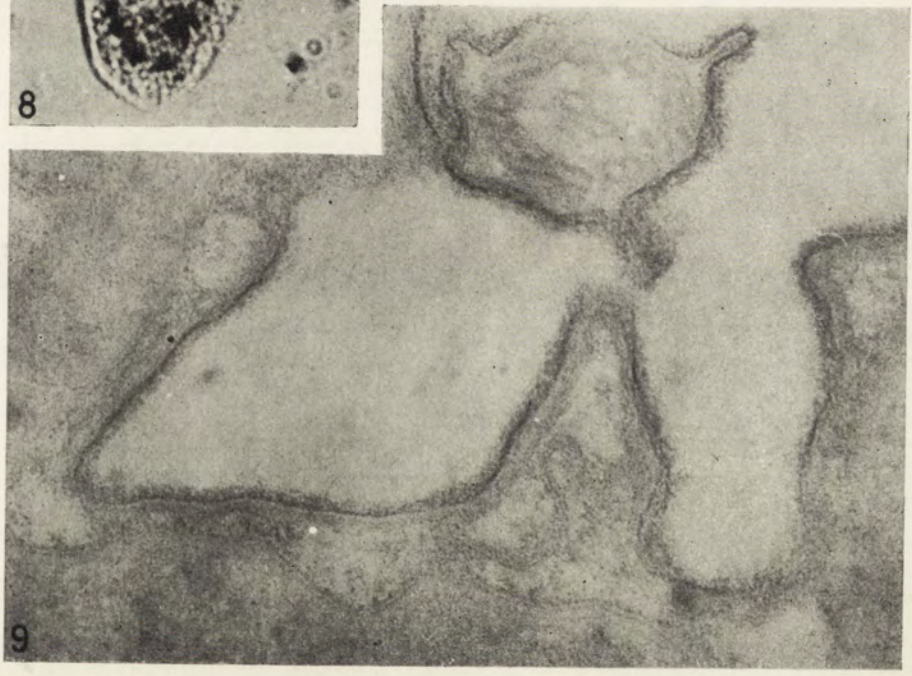

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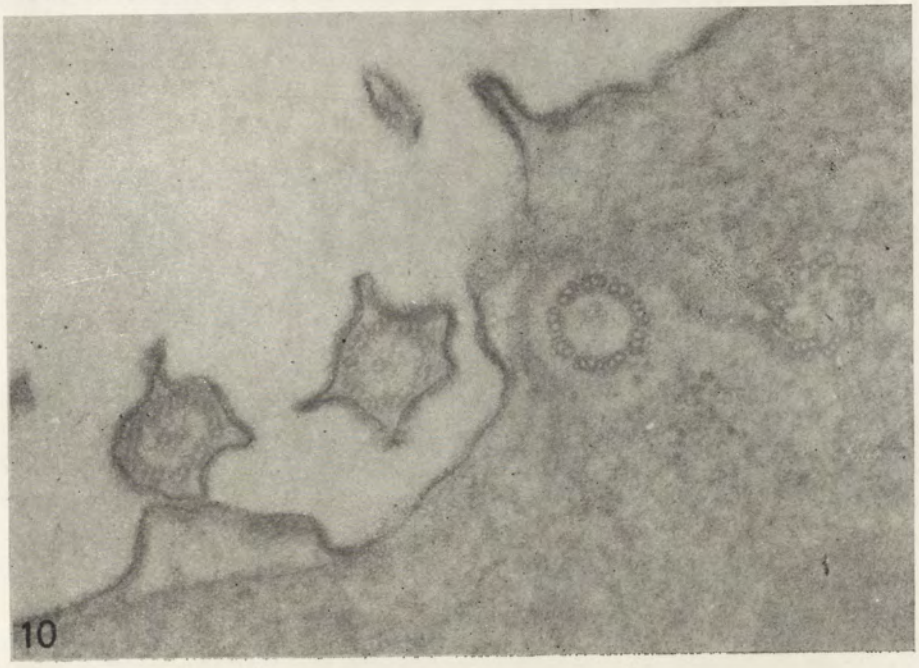

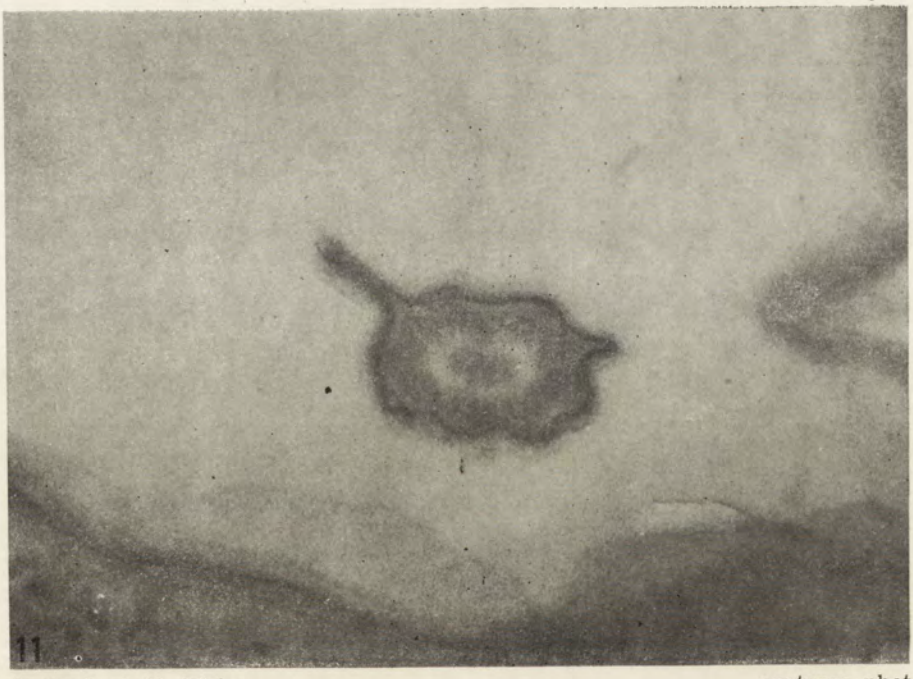

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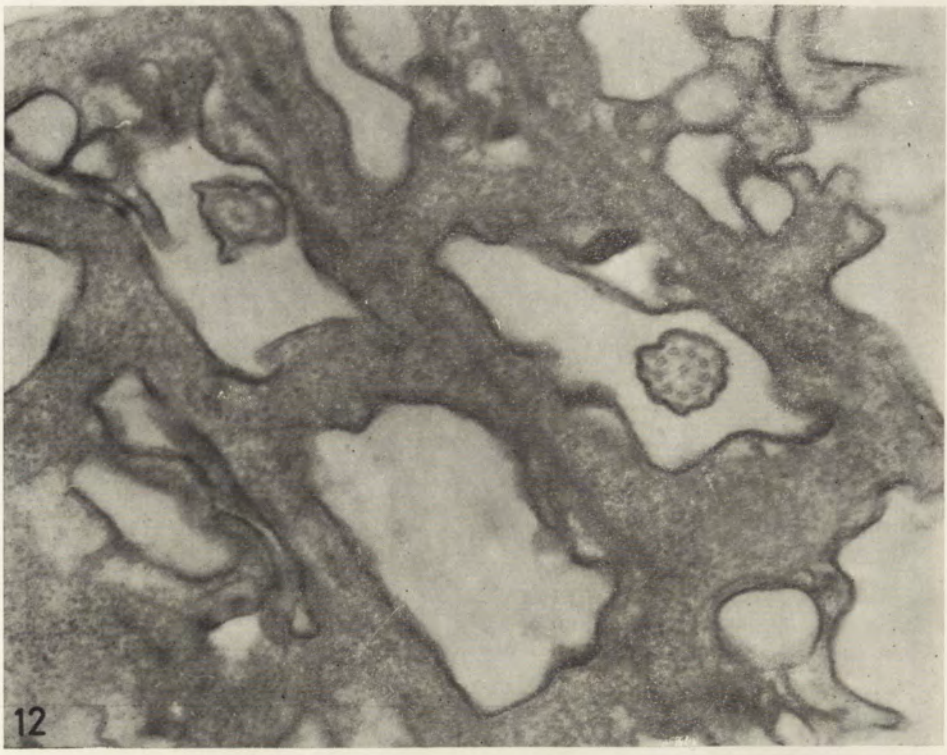

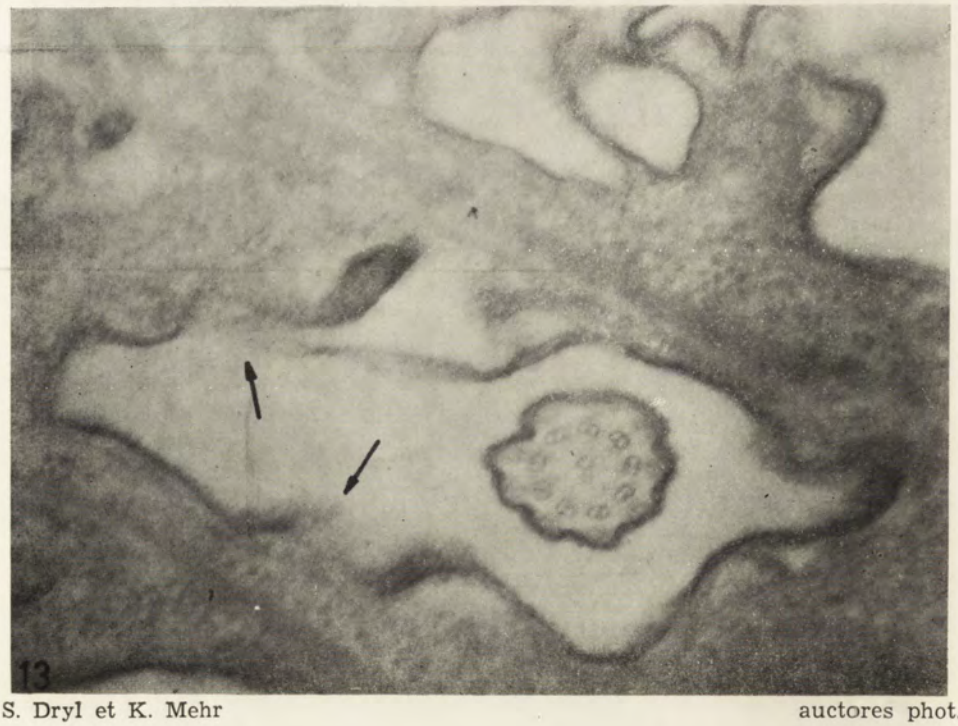

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Иммунологическая дискинетопластия у *Crithidia oncopelti*

Immunological Diskinetoplasty of *Crithidia oncopelti*

Синопсис. Добавление к живой культуре *C. oncopelti* гомологичных иммунных сывороток против целой клетки этого вида, а также против ее кинетопластной и митохондриальной фракций индуцировало появление 8–10% дискинетопластных (ДК) форм. В фазе логарифмического роста этот эффект был более стойким, но менее специфичным.

Появление ДК-форм следовало за агглютинацией клеток и совпадало во времени с диссоциацией агглютинатов и трансформацией простейших в округлые, часто амастиготные формы.

Обсуждается возможный механизм и особенности описанной иммунохимической ДКии и ее роль в объяснении спонтанной ДКии трипаносом в организме позвоночного хозяина.

Отправным пунктом феномена искусственной дискинетопластии (ДКии) трипаносомид является избирательное подавление репликации кинетопластной ДНК, без нарушения не связанных с кинетопластом клеточных структур. Специфичность действия химических агентов на кинетопластную ДНК относительна, так как она ограничена определенными концентрациями агента и зависит от целого ряда других факторов.

Очевидно, что любая специфичность биологического действия может быть наиболее надежно обеспечена антителами против мишени действия. В своих предыдущих иммунохимических работах мы получили иммунные сыворотки против целой клетки *Crithidia oncopelti* и ее отдельных структур: кинетопласта, митохондриальной фракции и биполярных тел (Каллиникова и др. 1973, Каллиникова и др. 1974). Настоящее исследование — попытка использовать эти сыворотки для избирательного нарушения кинетопласта, для получения дискинетопластных (ДК) форм *C. oncopelti* иммунохимическим путем.

Материал и методы

Способы получения и характеристика кроличьих иммунных сывороток против *C. oncopelti* и ее структур были описаны нами в предыдущих работах (Каллиникова и др. 1973, Каллиникова и др. 1974).

Эти сыворотки против целой клетки *C. oncopelti*, ее митохондриальной, кинетопластной и биполярной фракций добавлялись к живой культуре *C. oncopelti* в разные моменты ее развития: перед посевом, на 1-ый день (т.е. в фазе логарифмического роста) или на 3-ий день (т.е. в стационарной фазе) после посева, в различных концентрациях к объему питательной среды ДД-пептон (1 : 5, 1 : 10, 1 : 50). Контролем служили культуры, к которым добавляли нормальную неиммунную кроличью сыворотку или ничего не добавляли.

Через 15–30 минут, 2–5 часов после добавления сывороток и далее ежедневно, иногда вплоть до 10-го дня, культуры контролировались. Просматривалась каждая живая культура, а также морфология клеток на препаратах, окрашенных по Романовскому-Гимза. Учитывались морфология, процент агглютинированных (на 1000), трансформированных, т.е. округлившихся клеток (на 200–800), процент клеток с нарушенным делением (на 300) и ДК-форм (на 300–1000 клеток).

ДК-формами в чистом виде считали клетки с обычным набором органелл, но без видимого в световом микроскопе кинетопласта. Делящиеся клетки с удвоенным набором органелл (с двумя ядрами), но без удвоенного кинетопласта, начавшие или не начавшие цитотомию, считали потенциальными ДК-формами. Приведенные цифры включают в себя и тех и других. Клетки с уменьшенными кинетопластами дискинетопластными не считались.

Результаты

(1) Влияние иммунных сывороток на культуру *C. oncopelti* в стационарной фазе роста

Антикинетопластная сыворотка (АК) с высоким титром специфической активности (1 : 64 – 1 : 1024) вызывала достоверное повышение числа ДК-форм *C. oncopelti*. Уже через сутки после добавления сыворотки число ДК-форм возрастало до 6–10%, позже несколько снижалось, но и через 4 дня составляло около 3% (Рис. 1 *1*), что было выше нормы (Рис. 1 *5*).

Три менее активных АК-сыворотки с титрами 1 : 32–1 : 128 вызывали меньший эффект. Но и в этих случаях процент ДК-форм достоверно превышал норму (достоверность по Стьюденту 99.9%, $p < 0.001$, $t = 4{,}6$), составляя 2–6% на 2–3 дни после добавления сывороток (Рис. 2 *1, 2, 3*).

Это дискинетопластогенное действие сывороток сопровождалось еще двумя эффектами: агглютинацией клеток (Рис. 1 *6* и Рис. 2 *6, 7, 8*) и их трансформацией в округлые, часто амастиготные формы (Рис. 1 *11* и Рис. 2 *11, 12, 13*). Всепоглощающая агглютинация клеток (80–90%) предшествовала ДКии, т. е. наблюдалась сразу после добавления сывороток, уже через 15 мин. Агглютинаты клеток так велики, что были заметны в пробирке даже не вооруженным глазом. Степень агглютинации, а особенно ее стойкость,

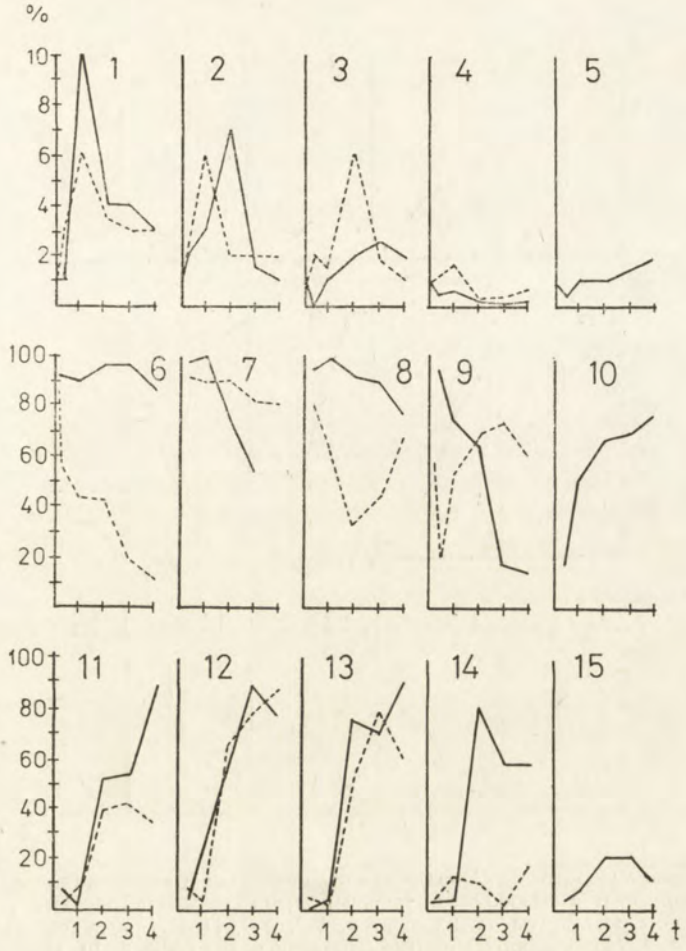

Рис. 1. Действие сывороток на *Crithidia oncopelti* в стационарной фазе роста (добавление к 3-дневной культуре). Опыт с сыворотками высокой специфической активности (титры 1 : 64 — 1 : 1024). 1, 6, 11 — антикинетопластная сыворотка (АК), 2, 7, 12 — антимитохондриальная сыворотка (АМ), 3, 8, 13 — сыворотка против целой клетки (АЦ), 4, 9, 14 — нормальная кроличья сыворотка (контроль), 5, 10, 15 — норма (без добавления сывороток), 1–5 — дискинетопластия, 6–10 — агглютинация, 11–15 — трансформация. По горизонтали — дни после добавления сывороток, По вертикали — % ДК-форм. ——— доза сыворотки 1 : 5, — — — — доза сыворотки 1 : 50. Первая точка графиков (первый день опыта) во всех случаях — среднее из нескольких проб в течение суток

Fig. 1. Influence of antisera on stationary *Crithidia oncopelti* (3-rd day of growth). Experiment with antisera of hight activity (titre 1 : 64 — 1 : 1024). 1, 6, 11 — antikinetoplastic serum, 2, 7, 12 — antimitochondrial serum, 3, 8, 13 — antiserum against whole cells, 4, 9, 14 — normal rabbit serum, control, 5, 10, 15 — without serum, 1–5 — diskinetoplasty, 6–10 — agglutination, 11–15 — transformation. Abscissa — days after addition of sera. Ordinate — % of diskinetoplastic cells. — concentration of serum 1 : 5, - - - - concentration of serum 1 : 50. In all cases initial point of curve (first day of experiment) — mean value from some trials during one day

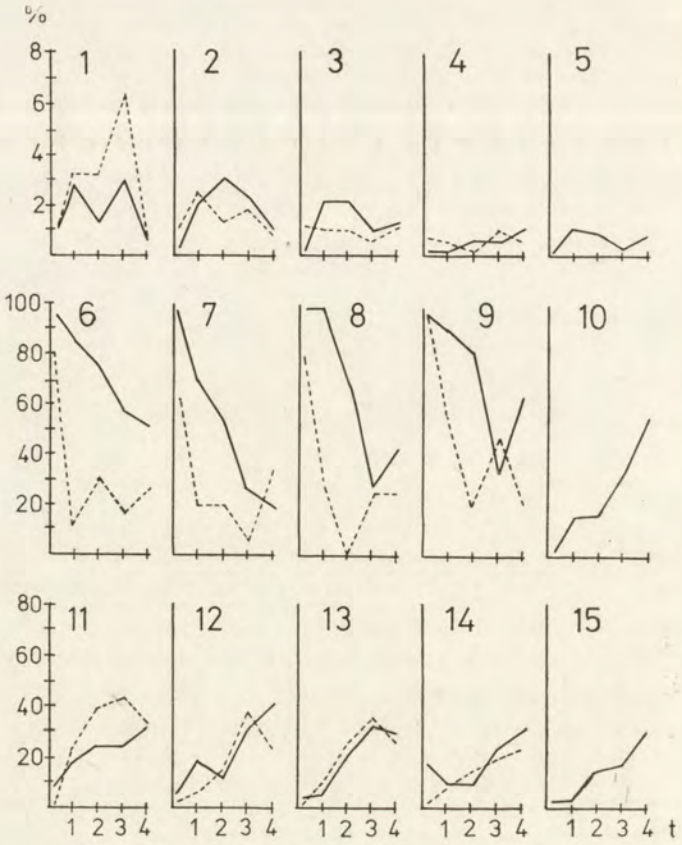

Рис. 2. Действие сывороток на *C. oncopelti* в стационарной фазе роста (добавление к 3-дневной культуре). Опыт с сыворотками меньшей специфической активности (титры 1 : 32 — 1 : 128). Средние результаты из 2-х опытов. 1–3, 6–8, 11–13 — антикинетопластные сыворотки (АК), 4, 9, 14 — сыворотка против биполярных тел (АБ), 5, 10, 15 — норма (без добавления сывороток), 1–5 — дикинетопластия, 6–10 — агглютинация, 11–15 — трансформация. По горизонтали — дни после добавления сывороток, по вертикали — % ДК-форм. ——— доза сыворотки 1 : 5 — — — — доза сыворотки 1 : 50. Первая точка графиков (первый день опыта) во всех случаях — среднее из нескольких проб в течение суток

Fig. 2. Influence of antisera on stationary *Crithidia oncopelti* (3-rd day of growth). Experiment with antisera of low activity (titre 1 : 32 — 1 : 128). Mean results from two experiments. 1–3, 6–8, 11–13 — antikinetoplastic sera, 4, 9, 14 — antibipolar bodies serum, 5, 10, 15 — without serum, 1–5 — diskinetoplasty, 6–10 — agglutination, 11–15 — transformation. Abscissa — days after addition of sera. Ordinate — % of diskinetoplastic cells, ——— concentration of serum 1 : 5, - - - - concentration of serum 1 : 50. In all cases initial point of curve (first day of experiment) — mean value from some trials during one day

длительность положительно коррелировала с дозой сывороток и титрами их активности (сравн. Рис. 1 *6* с Рис. 2 *6*, *7*, *8*). Примерно через 1–2 дня после добавления сывороток, когда происходила диссоциация клеток из агглютинатов, начиналась прогрессирующая трансформация жгутиковых, т. е. их округление, часто с потерей жгута (Рис. 1 *11* и Рис. 2 *11*, *12*, *13*). В зависимости

от активности сывороток число округлившихся клеток увеличивалось до 40–80% против 20–30% в норме (Рис. 1 *15* и Рис. 2 *15*). Степень трансформации мало зависела от дозы сыворотки.

Именно с моментом диссоциации агглютинантов и началом деформации клеток совпадает появление или нарастание числа ДК-форм. Процесс их возникновения легче проследить при несильной агглютинации. В первое время в отдельных, не агглютинированных клетках можно было отметить лишь уменьшение размеров кинетопласта, реже его побледнение, просветление. Позже, когда клетки округляются, становится заметным околожгутиковый резервуар, а в его устье — "пробки" из вещества, окрашивающегося по Романовскому-Гимза в ярко розовый цвет. Такого же цвета "цемент" часто скрепляет небольшие группы из 4–10 клеток. В одних клетках эти пробки кажутся слоистыми (Табл. I 4), в других выглядят очень плотными и как бы заполняют весь околожгутиковый резервуар, доходя до самого кинетопласта (Табл. I 6,7) или соединяясь с ним тяжами (Табл. I 5). Жгут таких клеток окрашен в тот же ярко-розовый цвет и кажется сильно утолщенным, особенно в своем начале, а изредка можно было обнаружить и очень нежную мембрану — "шлейф", сопровождающий жгут на протяжении его трети и истощающийся по мере удаления от тела клетки.

Помимо ДК-форм в чистом виде, т. е. клеток с четким ядром, но без окрашивающегося кинетопласта (Табл. I 12–16), наблюдались потенциальные ДК-формы — клетки с двумя ядрами и одним кинетопластом (Табл. I, 8). Как бы естественным их развитием в истинные ДК-формы были клетки с далеко зашедшей цитотомией, где дочерние особи еще оставались связанными либо маргинально расположенными кинетопластами, либо одним кинетопластом (Табл. I 10). Из всех этих картин очевидно, что одна из дочерних клеток становится дискинетопластной (Табл. I 9, 11).

Под влиянием АК-сывороток часть клеток *C. oncopelti* дегенерирует, иногда приобретая вид мелких гомогенных комочков или просто лизируясь. Число клеток в культуре уменьшалось. Однако при пересеве на свежую среду, не содержащую иммунных сывороток, культура постепенно нормализовалась. Это касалось и ДК-форм, число которых возвращалось к нормальному уровню.

Дискинетопластогенный эффект, но меньшей степени вызывали также антимитохондриальная сыворотка (АМ) и сыворотка против целой клетки (АЦ) *C. oncopelti*. В первом случае число ДК-форм повышалось до 6–7% на 1–2 дни действия агента (Рис. 1 *2*), во втором — до 2–6% на 2–3 дни (Рис. 1 *3*). Это действие АМ- и АЦ-сывороток сопровождали все те явления, которые описаны выше для АК-сывороток. Несмотря на меньшую ДК-генную активность, их агглютинирующие (Рис. 1 *7*, *8*) и трансформирующие (Рис. 1 *12*, *13*) свойства были вполне сравнимы с таковыми АК-сывороток.

В то же время сыворотка против биполярных тел (АБ), также вызывая агглютинацию (Рис. 2 *9*) и трансформацию (Рис. 2 *14*) той же степени,

отличалась от других иммунных сывороток полным отсутствием ДК-эффекта (Рис. 2 *4*). Как правило биполярные тела в клетке тоже сохранялись. Но часто можно было видеть и выпавшие из клетки биполярные тела. Однако такие картины наблюдались и в других опытных вариантах.

Нормальная кроличья сыворотка, не содержащая никаких антител против *C. oncopelti*, не вызывала ДКии (Рис. 1 *4*). В одном из опытов сыворотка одного кролика в максимальной дозе вызвала очень кратковременную аггломерацию клеток (Рис. 1 *9*) с последующей их трансформацией (Рис. 1 *14*). Но во всех других опытах кроличья сыворотка не вызывала ни агглютинации, ни аггломерации, ни трансформации, а даже способствовала более успешному росту культуры. И в этом случае в округлившихся клетках можно было наблюдать "пробки" в устье околожгутикового резервуара. Однако жгуты таких клеток не были утолщены, а клетки, склеенные аналогичным "пробкам" цементом, не встречались.

В стационарной фазе роста, без добавления какой бы то ни было сыворотки культура *C. oncopelti* содержала очень невысокое число ДК-форм, не превышающее 0–2% (Рис. 1 *5* и Рис. 2 *5*). На 4–5 дни после посева начиналась естественная для этого возраста культуры аггломерация клеток (Рис. 1 *10* и Рис. 2 *10*), отличающаяся от агглютинации правильной их ориентацией в виде розеток, охватывающая к концу опыта 50–70% клеток. Днем позже начиналась столь же естественная трансформация стареющей культуры. Число округлившихся клеток не превышало 20–30% (Рис. 1 *15* и Рис. 2 *15*). И в норме, главным образом в округлившихся формах по периферии мазка, можно было наблюдать розовые "пробки" в устье околожгутикового резервуара.

(2) Влияние иммунных сывороток на культуру *C. oncopelti* в фазе логарифмического роста

В интенсивно делящейся культуре *C. oncopelti* АК-сыворотки вызывали более значительную, более стойкую ДКию, чем в стационарной фазе. ДК-формы составляли 8–9% на 2–4 дни после добавления сыворотки и оставались на уровне 4–5%, даже при дозе 1 : 10, иногда на протяжении 4–7 дней опыта (Рис. 3 *1*).

ДК-генным действием обладала также АЦ-сыворотка. Ее эффект также

Fig. 3. Influence of antisera on log *Crithidia oncopelti* (first day of growth). Mean results from two experiments. 1, 6, 11, 16 — antikinetoplastic serum, 2, 7, 12, 17 — antiserum against whole cells, 3, 8, 13, 18 — antibipolar bodies serum, 4, 9, 14, 19 — normal rabbit serum, control, 5, 10, 15, 20 — without serum, 1–5 — diskinetoplasty, 6–10 — agglutination, 11–15 — transformation, 16–20 — disturbance of cell division. Abscissa — days after addition of sera. Ordinate — % of diskinetoplastic cells —— concentration of serum 1 : 5, - - - - concentration of serum 1 : 50. In all cases initial point of curve (first day of experiment) — mean value from some trials during one day

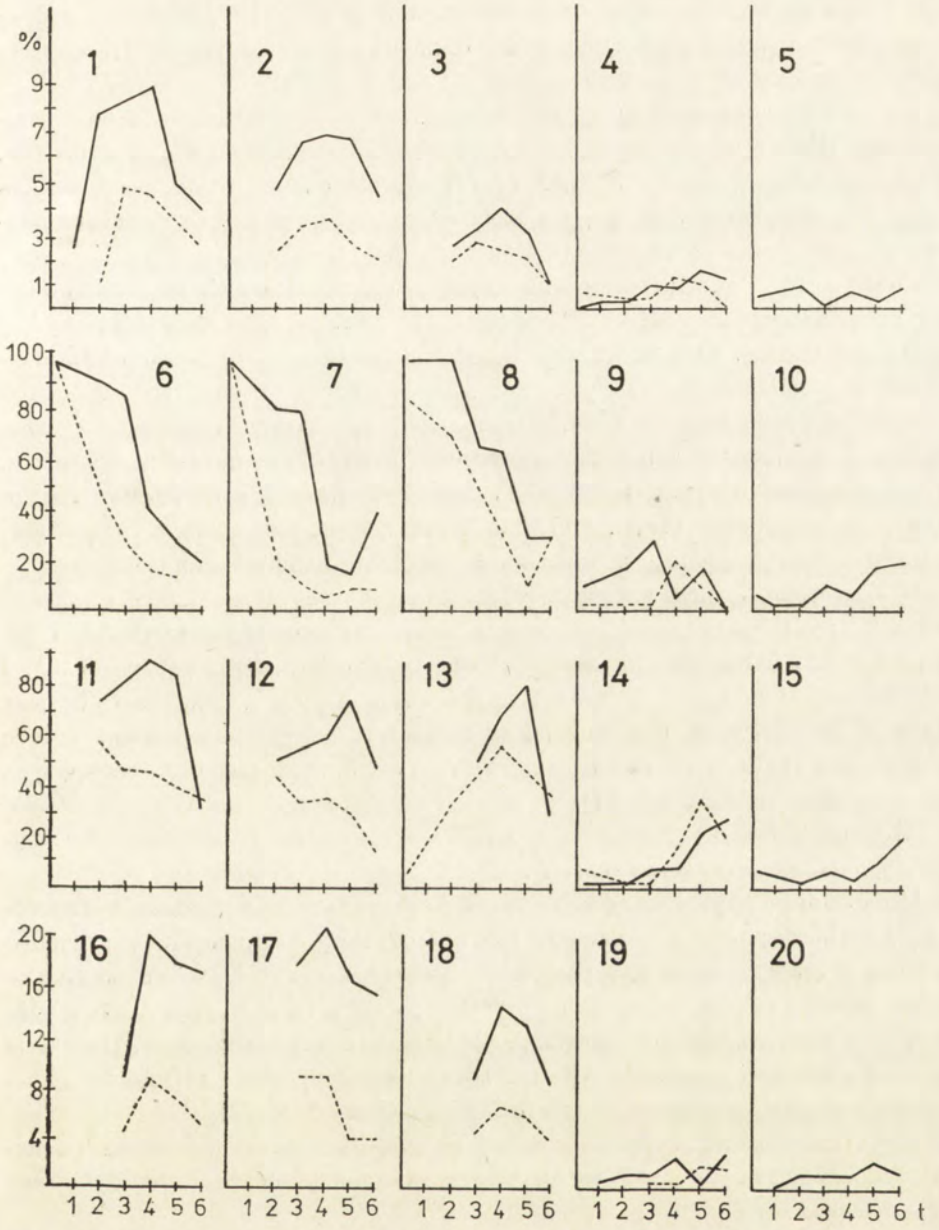

Рис. 3. Действие сывороток на *C. oncopelti* в фазе логарифмического роста (добавление к 1-дневной культуре). Средние результаты из 2-х опытов. 1, 6, 11, 16 — антикинетопластная сыворотка (АК), 2, 7, 12, 17 — сыворотка против целой клетки (АЦ), 3, 8, 13, 18 — сыворотка против биполярных тел (АБ), 4, 9, 14, 19 — нормальная кроличья сыворотка, 5, 10, 15, 20 — норма (без добавления сывороток), 1–5 — дискинетопластия, 6–10 — агглютинация, 11–15 — трансформация, 16–20 — нарушение клеточного деления. По горизонтали — дни после добавления сывороток, по вертикали — % ДК-форм. ——— доза сыворотки 1 : 5, — — — доза сыворотки 1 : 50. Первая точка графиков (первый день опыта) во всех случаях — среднее из нескольких проб в течение суток

был более значительным, чем в стационарной фазе: 5–7% ДК-форм на протяжении нескольких дней (Рис. 3 *2*). В обоих случаях степень ДК-эффекта прямо зависела от дозы сыворотки.

Как и в стационарной фазе, ДКия следовала за зависящей от дозы агглютинацией (Рис. 3 *6*, *7*) и совпадала во времени с диссоциацией агглютинатов, с трансформацией клеток (Рис. 3 *11–12*). Агглютинация была очень велика и при малом накоплении клеток в начале логарифмической фазы всякие подсчеты становились возможными лишь со 2–3 дней опыта.

Отличия от результатов в стационарной фазе заключались не только в более значительном и стойком ДК-эффекте. Принципиально иной была трансформация клеток. Она не только прямо зависела от дозы сыворотки. Интенсивно делящиеся клетки реагировали на действие иммунных сывороток патологическими изменениями. Появлялись гигантские округлые клетки, иногда с настолько гипертрофированным околожгутиковым резервуаром, что цитоплазма с ядром и кинетопластом оттеснялись к периферии клетки в виде узкого ободка (Табл. I 17, 20). Такие формы напоминали сильно увеличенные стадии кольца в жизненном цикле малярийного плазмодия.

В этих увеличенных клетках весьма отчетливыми становились филаментозные структуры, видимо, соответствующие микротрубочкам (Табл. I 18, 19), особенно очевидные на светлом фоне раздувшегося резервуара (Табл. I 20). Часто наблюдался клазматоз цитоплазмы в виде удлиненных, острых (Табл. I 21, 22), реже почкообразных выростов, иногда содержащих в себе кинетопласт (Табл. I 23, 24). Во многих клетках биполярные тела собирались вокруг ядра (Табл. I 14, 24).

ДК-формы часто встречались целыми компаниями, обычно по 2–3 в центре группы из нескольких клеток.

Морфология клеток свидетельствовала не только о нарушениях кинетопласта и гипертрофии жгутикового резервуара, но и о нарушении клеточного деления и прежде всего цитотомии. Во многих клетках было по несколько ядер и кинетопластов, число тех и других не соответствовало друг другу и нормальному ходу клеточного деления, а их размеры были увеличены. Подсчеты показали, что под влиянием АК- и АЦ-сывороток в дозе 1 : 10 до 20% клеточных делений оказываются нарушенными (Рис. 3 *16–17*).

Вероятно, большинство описанных патологических форм являются нежизнеспособными. Гораздо чаще, чем в стационарной фазе, наблюдались следы лизиса клеток.

Все это свидетельствует о том, что в фазе активного деления клеток к ДК-эффекту сывороток примешивается цитопатогенное, общетоксическое действие. Об этом говорят и результаты испытания АБ-сыворотки в этой фазе. В отличие от стационарной фазы, АБ-сыворотка тоже вызывала достоверную ДКию активно делящихся клеток, хотя и несравнимо меньшую, чем АК- и АЦ-сыворотки. Под ее влиянием (доза 1 : 10) число ДК-форм повы-

шалось до 2–4% и держалось на этом уровне на протяжении нескольких дней (Рис. 3 *3*). Как и в выше описанных случаях, это действие АБ-сыворотки в логарифмической фазе сопровождалось агглютинацией клеток (Рис. 3 *8*), их трансформацией (Рис. 3 *13*), появлением патологических форм, нарушением митозов (Рис. 3 *18*), лизисом части клеток. Как и ДК-эффект, нарушение митозов было менее значительным. Степень агглютинации и трансформации была такой же, как от влияния АК- и АЦ-сывороток. Вследствие значительного лизиса клеток внеклеточные биполярные тела были весьма многочисленными. Но это наблюдалось и в других опытных вариантах.

Нормальная кроличья сыворотка и в логарифмической фазе роста культуры не вызывала ни достоверной ДКии (Рис. 3 *4*), ни агглютинации (Рис. 3 *9*), ни трансформации (Рис. 3 *14*), ни нарушения митозов (Рис. 3 *19*), ни появления патологических форм.

В норме, без добавления каких бы то ни было сывороток, культура *C. oncopelti* в фазе логарифмического роста содержала менее 1% ДК-форм (Рис. 3 *5*), число аггломерированных в розетки клеток не превышало 20% на 5–6 дни опыта (Рис. 3 *10*), процент округлившихся клеток колебался около 10 (Рис. 3 *15*), а нарушенные деления составляли 0.3–2% от общего числа делящихся форм (Рис. 3 20).

Попытка добавить АК-сыворотку в питательную среду перед посевом культуры тоже привела к значительной и стойкой ДКии, равной 8–10%, что сопровождалось описанными выше эффектами. Но всякие наблюдения и подсчеты становились возможными лишь на 5 день, когда клеток становилось достаточно, а их агглютинация была не так всепоглащающа.

Краткие итоги и обсуждение

Как свидетельствуют результаты проведенного исследования, гомологичная антикинетопластная сыворотка вызывает ДКию *C. oncopelti*, увеличивая число ДК-форм до 8–10%. ДК-генной активностью обладают также две другие иммунные сыворотки: против целой клетки и против митохондриальной ее фракции.

Реакция живой клетки на действие иммунных сывороток зависит от фазы роста культуры. В логарифмической фазе ДК-эффект более стоек, но менее специфичен, к нему примешивается цитопатогенный эффект, главным образом нарушение цитотомии, лизис клеток. Однако появляющиеся при этом патологические формы весьма сходны с теми, которые наблюдаются при действии на *C. oncopelti* высоких доз трипафлавина — химического индуктора ДКии (Сухарева-Немакова и др. 1971 a, b).

Сыворотка против биполярных тел *C. oncopelti* индуцирует очень незначительную ДКию только в фазе логарифмического роста, когда эффект не-

достаточно специфичен. ДКии тем более не наблюдалось при действии нормальной кроличьей сыворотки.

Действие иммунных сывороток на *C. oncopelti* сопровождается агглютинацией клеток и их трансформацией, что описано многими авторами и для других трипаносомид (Вербицкий 1915, Adler 1958, 1963, Thurston 1958, Cunningham and Vickerman 1962, Dusanic 1968, Хачоян 1970, Wertheim et al. 1970, Strauss 1971).

Агглютинирующий эффект, в отличие от ДКии, был свойственнен всем иммунным сывороткам, как против целой клетки *C. oncopelti*, так и против ее структур, в том числе и в не меньшей степени антибиполярной сыворотке. Эту активность даже "противоорганоидных" сывороток можно объяснить возможной общностью антигенов всех мембран одной и той же клетки, мембран каждой органеллы с клеточной мембраной, как это известно, например, для *Neurospora* (Woodward 1968).

Трансформация клеток, обязательно следует за агглютинацией и даже за аггломерацией, вызванной нормальной сывороткой. В отличие от агглютинации, она больше зависит именно от титра специфических антител, а не от общей дозы сывороточных белков, т. е. более тесно связана с действием специфических антител.

Появление "пробок" в устье околожгутикового резервуара не является специфичным для действия как иммунных, так и неиммунных сывороток.

Новый вид ДКии, индуцированный специфическими иммунными сыворотками, можно назвать иммунологическим.

С точки зрения поставленной в работе задачи, т. е. по содержанию противокинетопластных антител, испытанные сыворотки существенно различаются. Помимо АК-сыворотки, содержащей исключительно эти антитела, их включает в себя сыворотка АЦ, наряду с антителами против всех других структур клетки. Они находятся также в АМ-сыворотке в связи с показанной нами значительной антигенной общностью кинетопластной и митохондриальной фракций (Каллиникова и др. 1973). Из-за отсутствия общих антигенов между кинетопластом и биполярными антителами (Каллиникова и др. 1974) АБ-сыворотка не содержит антител против кинетопласта. Наконец, нормальная кроличья сыворотка не содержит никаких антител против клетки *C. oncopelti*.

Так как ДКию вызывают не любые сыворотки против клетки *C. oncopelti* и ее структур, а лишь те, которые содержат антитела против кинетопласта, можно считать, что ДК-эффект сывороток специфичен, т. е. связан с активностью именно противокинетопластных антител. Это подтверждается зависимостью ДК-эффекта (когда он достаточно специфичен) прежде всего от титра специфических антител, а не от общей дозы сывороточных белков (сравн. Рис. 1 *1–3* и Рис. 2 *1–4*).

Сопровождающие ДКию феномены, хотя и не являются специфическими

аттрибутами только противокинетопластных антител, кажутся весьма важными моментами механизма описанной ДКии. Агглютинация как один из видов меж клеточных контактов может быть фактором, повышающим клеточную проницаемость. Для клеток *Metazoa* известно повышение проницаемости клеточной мембраны во много раз в месте контакта с поверхностью другой клетки (Kano and Loewenstein 1964 a, b, 1966, Penn 1966, Nakas et al. 1966, Loewenstein and Kano 1967, Васильев и Маленков 1968). Трансформация клеток, гипертрофия околожгутикового резервуара, образование в его устье "пробок", утолщение резервуарных микротрубочек — есть гипертрофия аппарата поглощения трипаносомидной клетки, т. е. свидетельство усиленного эндоцитоза.

Все это позволяет предполагать, что специфичность ДК-генного действия использованных сывороток, обусловленная наличием в них противокинетопластных антител, может обеспечиваться проникновением антител в клетку. Это кажется возможным теперь, когда известно, что в клетку вообще могут проникать гораздо более крупные молекулы, чем это казалось раньше. Такую возможность в отношении трипаносом заставляют допустить результаты некоторых иммунохимических исследований (Brown and Williamson 1964). Конечно, проникновение антител в трипаносомидную клетку нуждается в прямом доказательстве, например, с помощью флюоресцирующих антител.

Последовательность описанных явлений во времени показывает, что действие сывороток начинается с агглютинации, т. е. возможного повышения клеточной проницаемости, за которой следует усиленный эндоцитоз и как результат действия противокинетопластных антител — ДКия.

Вряд ли мишенью действия в этих случаях является только кинетопластная ДНК. В сыворотках против фракции целых кинетопластов содержатся антитела и к другим компонентам этой органеллы. ДК-эффект антимитохондриальной сыворотки свидетельствует о том, что в ДК-формах повреждаются те компоненты кинетопласта, которые имеют общие антигены с митохондриальной фракцией клетки.

С этим согласуется и другое своеобразие иммунохимической ДКии. В противоположность химически индуцированной ДКии, возможной только на фоне деления клетки (Werbitzki 1910, Inoki 1957, Mühlpfordt 1959, Cosgrove 1966, Steinert and van Assel 1967 a, b), она в этих условиях менее специфична.

Иммунохимическая ДКия очень невысока: 8–10%. Это более соответствует спонтанной ДКии этого вида в культуре, в норме (4%, по Сухаревой-Немаковой и др. 1970), чем индуцированной акрифлавином (41%, по Guttman and Eisenman 1965) или трипафлавином (до 75%, по Сухаревой-Немаковой и др. 1971). Возможно, лимитирующим моментом здесь является именно трудность проникновения антител в клетку.

Сама возможность иммунохимической ДКии проливает свет на механизм спонтанной ДКии. По крайней мере, в организме позвоночного индукторами ДКии могут быть антитела хозяина.

Автор выражает свою глубокую признательность Н. Г. Федцовой за техническую помощь в работе.

SUMMARY

The homologous sera containing antikinetoplast antibodies had been obtained, among them were antimitochondrial and antikinetoplast ones as well as the sera prepared after injection of the whole *C. oncopelti* cells. All these sera were able to induce diskinetoplasty (DK) in the cultures of *C. oncopelti*.

The effect of the immune sera on the cells depends on the phase of culture growth. At the logarithmic phase DK-effect is more stable but less specific. Cell's agglutination and transformation accompanying the action of the sera may be regarded as the important features of this DK-mechanism.

Thus, the described immunochemical DK being characterized by some peculiarities may explain the spontaneous formation of DK-forms within organism of the vertebrate host.

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ПОДПИСИ К ТАБЛИЦЕ I

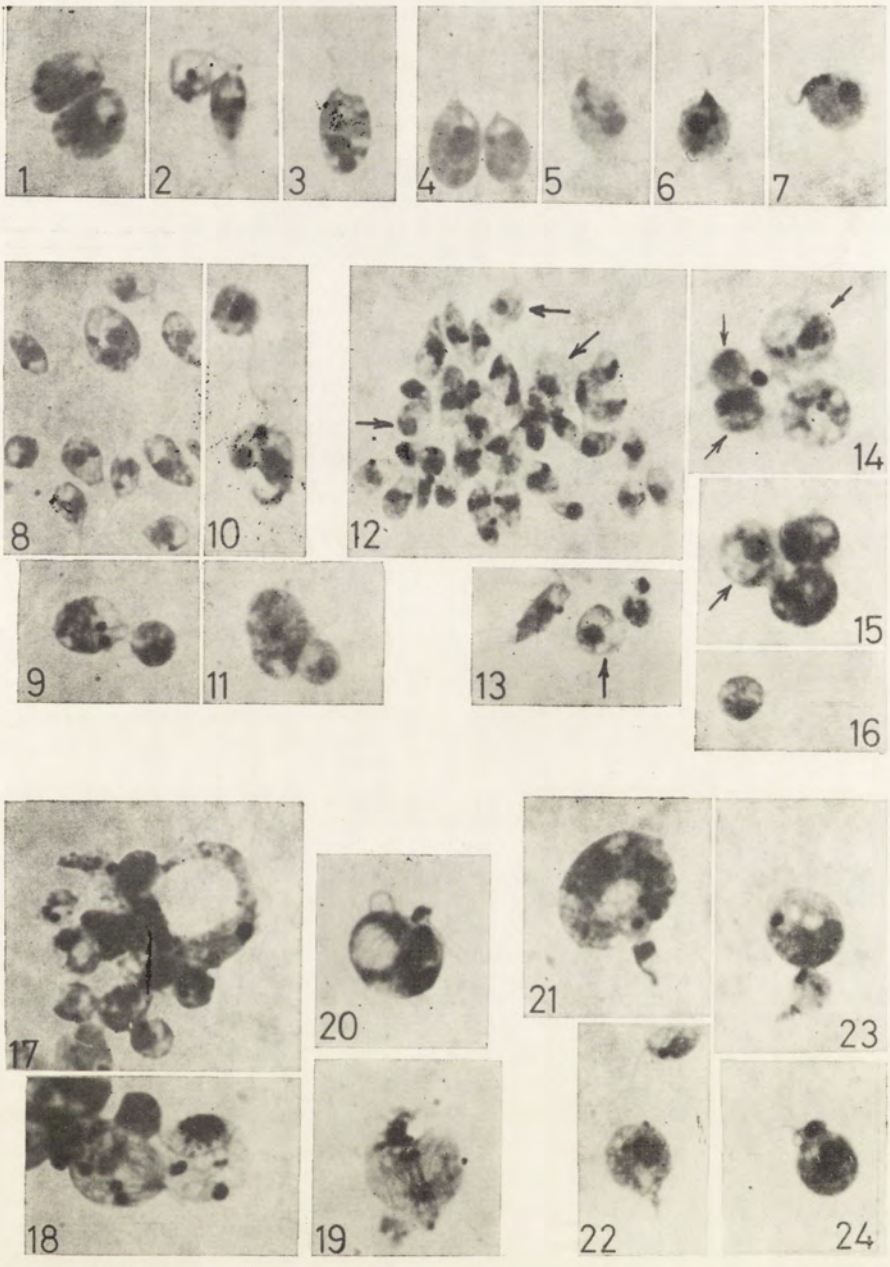
Действие иммунных сывороток на клетки *Crithidia oncopelti*

1–3: нормальные клетки, не подвергшиеся действию иммунных сывороток
4–7: "пробки" в устье околожгутикового резервуара в опытных вариантах
8–11: возникновение ДК-форм под влиянием иммунных сывороток
12–16: дискинетопластные формы (указаны стрелками) наряду с нормальными клетками
17–24: патологические изменения клеток под влиянием иммунных сывороток в фазе логарифмического роста
17–20: гипертрофия околожгутикового резервуара
21–24: клазматоз

EXPLANATION OF PLATE I

Influence of antisera on *Crithidia oncopelti*

1–3 : Normal cells, untreated with antiserum
4–7 : "Stopper" around the flagellar vestibulum
8–11 : Appearance of diskinetoplastic forms after antiserum treatment
12–16 : Diskinetoplatic forms (see arrows) together with normal cells
17–24 : Pathologic changes of the *Crithidia oncopelti* cells after antiserum treatment in log phase
17–20 : Hipertrophy of flagellar
21–24 : Clasmatosis



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Microsporidians Parasitizing the Green Tortrix (*Tortrix viridana* L.) in Poland and their Role in the Collapse of the Tortrix Outbreak in Puszcza Niepołomicka during 1970–1974

Synopsis. The health status of larvae of green tortrix (*Tortrix viridana* L.) during its gradation (1970–1974) in southern Poland was studied. There was a drastic changes in the number of larvae collected on oaks and their mortality during rearing. In 1970 — 1316 larvae were collected on five oaks, in 1971 — 1386 larvae, in 1972 — 3716 larvae, in 1973 — 54 larvae and in 1974 — 72 larvae. The mortality of this larvae during rearing greatly increased during this period: 1970 — 48.5%, 1971 — 49.2%, 1972 — 91.1%. This mortality was caused mainly by four microsporidian species: *Nosema tortricis* Weiser, *Octosporea viridana* Weiser, *Plistophora* sp., *Thelohania weiseri* Günther. All four microsporidians infected 65% of *T. viridana* larvae in 1972 as compared to 12% infection level in 1970. The main pathogen was *Octosporea viridana*.

Under the auspices of the International Biological Program the Institute of Nature Protection of the Polish Academy of Sciences conducted during 1967–1973 the ecological studies in Niepołomicka Primeval Forest (Puszcza Niepołomicka), on the primary production and on the more important groups of consumers. General purpose of these studies has been discussed by Medwecka-Kornaś (1971). The results obtained up to now have indicated, that among phytophagous insects which occur in Niepołomicka Primeval Forest the main species was green-tortrix (*Tortrix viridana* L.) (*Lepidoptera* : *Tortricidae*). According to Medwecka-Kornaś et al. (1974) all recorded consumers consumed about 10% of the general primary green production on the studied area. However, the consumption by larvae of *T. viridana* and of other *Lepidoptera* constitute

up to 80% of the total animal consumption. Therefore, practically these larvae are most important and exclusive consumers in the Niepołomicka Primeval Forest. Therefore, it is obvious that the mass occurrence of *T. viridana* and of other *Lepidoptera* greatly affect the present conditons of oak forest and their economic future. Due to this reason it is necesary to collect information on factors influencing the population dynamics and mass outbreaks of this pest. Knowing this factors it is possible to undertake proper steps with the purpose to prevent the mass occurrence of *T. viridana* or to shorten its gradation.

Schwerdtfeger (1961, 1971) analyzed the role of various factors in the dynamics of *T. viridana* population and emphasized the great role of biotic factors. Weiser (1956) and Günther (1960) discussed the role of microsporidians (*Microsporidia*) in the mortality of *T. viridana*. Franz and Huger (1970) proved that the gradation of *T. viridana* in the German Federal Republic during 1964–1968 was terminated due to high infection of this pest by four microsporidian species.

The aim of this study was to obtain information on the occurrence of microorganisms infecting *T. viridana* in the Niepołomice Primeval Forest and to evaluate their role in the mortality of this pest.

Material and Methods

The larvae of *T. viridana* were collected on common oak (*Quercus robur* L.) in age 80–150 years in the Niepołomice Primeval Forest, Niepołomice Forest Inspectorate, Ispina Forest Range. The experimental area of the Institute of Nature Protection was located there (Medwecka-Kornaś 1971).

Observations on population density of *T. viridana* larvae were conducted on five oak trees having numbers 0, 59, 80, 126 and 151. The larvae for microscopic and macroscopic observations were collected from three levels of tree crown every 7–10 days from the beginning of the occurrence of third instar larvae and this was continued until the majority of larvae pupated. The number of larvae collected in consecutive years 1970–1974 is presented in Table 1, second column.

Basing on the external symptoms and activity the larvae collected at each date were segregated into two groups: healthy and sick. The sick larvae were immediately examined microscopically, the microscopic preparations were prepared stained according to various methods. The healthy larvae were reared with the purpose to obtain pupae and adults. The larvae which died during rearing were always microscopically examined and the cause of death was determined.

The tissue smears of larvae, pupae and adults of *T. viridana* were stained according to various methods in order to check the presence of viruses, bacteria, protozoans and other microorganisms (Lipa 1967, 1975). Spores and developmental stages of microsporidians were fixed in methyl alcohol and then stained in 0.5% Giemsa stain for 24 h.

Results

1. The Health Status of *T. viridana* Larvae in Natural Populations and their Mortality in Rearings

The quantitative results of collection of *T. viridana* larvae during 1970–1974 and results of their microscopical examination are given in Table 1. In 1970–1972 the number of diseased larvae was fairly large. In 1973 and 1974 there was a dramatic decrease in the density of *T. viridana* and only 54 and 72 larvae were collected, respectively.

Although for the further rearing only larvae very active and appearing to be healthy were used the mortality of them in rearing during 1970–1971 was about 50% (Table 1). Among larvae taken for the rearing in 1972 there was an extremely high mortality reaching the level of 91.1% (Table 1). The larvae dying during the rearing showed the same symptoms as those found diseased directly on the trees.

The microscopic studies showed that in 1971 the mortality of *T. viridana* on trees and in rearing was mainly caused by gram-negative bacteria belonging to the families *Enterobacteriaceae* and *Pseudomonadaceae*. The mortality due to *Microsporidia* reached the level 14%.

Table 1

Number of Collected Larvae of *Tortrix viridana* L. and their Health Status under Natural Condition and Mortality during Rearing

Year of study	Number of larvae collected from 5 trees	Number and percent of diseased larvae on trees		Number of larvae taken to rearing and their mortality	
		number	% diseased	number	% mortality
1970	1316	395	30	921	48.5
1971	1386	362	26	1026	49.2
1972	3716	1186	31.8	2530	91.1
1973	54	—	—	—	—
1974	72	—	—	—	—

In 1972, however, *Microsporidia* played the main role in the mortality of *T. viridana* larvae as they killed 65% of larvae.

In 1971 100 adults of *T. viridana* were microscopically examined and 12% of them were infected by *Microsporidia*. The fact that adults of *T. viridana* were infected with *Microsporidia* indicates that these protozoans are transmitted transovarially and therefore they play an important role in the natural reduction of this pest. It seems most probable to con-

sider that due to microsporidian transovarial transmission there was such a high increase of parasitization of *T. viridana* population in 1972, which reached 65%.

Since *T. viridana* larvae were collected on three levels in the tree crown this factor as well as the time of collection were considered when the status of health or parasitization was considered. It was found that during the feeding period the number of diseased larvae changes depending on data of collection and the localization in the tree crown.

In the first period of collecting (about May 15) the lowest number of diseased larvae (on an average 16%) was observed in the high part of the tree crown and the highest — in the middle part of the crown (on an average 44%). In the half-time of the larval feeding period (about May 25) the percentage of diseased larvae among larval groups collected from all crown levels is rather similar — about 30–40%. However, at the and of feeding period (about June 25) the larvae collected in the top and middle crown level were in 100% diseased.

When the disease level is compared with feeding periods it was found that at the beginning of the occurrence of the third instar larvae (about May 20) the percentage of diseased larvae varies from 5.5 to 40%. In the first decade of June the percentage of diseased larvae rapidly increases up to 60% and at the end of the feeding period of fifth instar larvae (about July 20) the parasitization of larvae reached 100%.

2. The Recorded Species of *Microsporidia*

Four microsporidian species in the larvae, pupae and adults of *Tortrix viridana* were recorded: *Nosema tortricis* Weiser, *Octosporea viridana* Weiser, *Thelohania weiseri* Günther and *Plistophora* sp.

In the studied population of *T. viridana* the dominating species was *Octosporea viridana* which was observed in 54% of microsporidian infected larvae; *Nosema tortricis* — in 28%; *Thelohania weiseri* — in 18%; and *Plistophora* sp. — only in 2 larvae.

(1) *Nosema tortricis* Weiser, 1956. This microsporidian infects fat body. Fresh spores measured in water were 3.0–4.5 by 1.0–2.0 μm; fixed and stained spores were 3.0–4.0 by 1.0–1.5 μm (Pl. I 2). Weiser (1956) recorded it only in 3% of larvae while Franz and Huger (1970) found it in 43.6% of larvae in the Federal Republic of Germany. In our studies it infected 15% of larvae (the frequency among microsporidian infections 28%).

(2) *Octosporea viridana* Weiser, 1956. This microsporidian infects fat body. Fresh spores measured in water were 6.0–8.2 by 1.5–2.0 μm; fixed and stained spores were 6.0–7.5 by 2.0–2.2 μm (Pl. I 1). Our observation

confirms the earlier Weiser's (1956) data that this species occurs in the period of density decline of *T. viridana*. As this microsporidian infected 30% of larvae it played an important role in breaking down the tortrix gradation in the Niepołomicka Primeval Forest in 1972. *O. viridana* was recorded in 54% of cases of microsporidian infections.

(3) *Thelohania weiseri* Günther, 1960. This microsporidian infects the fat body. Fresh spores measured in water were 4.0–6.0 by 1.8–2.5 μm; fixed and stained spores were 4.0–6.0 by 2.0–2.5 μm (Pl. I 3). During our studies this microsporidian was observed in 10% of larvae (the frequency among microsporidian infections 18%).

(4) *Plistophora* sp. Franz and Huger, 1970. This microsporidian infects midgut epithelium. Fresh spores measured in water were 1.5–3.0 × 0.8–1.2 μm and fixed and stained spores were 1.7–2.5 × 0.8–1.0 μm (Pl. I 4). This microsporidian was recorded by Franz and Huger (1970) in 0.4% of larvae and in our studies in the Niepołomice Primeval Forest it was found only in 2 larvae.

The size of spores of recorded microsporidians and their frequency distribution is given in Table 2. The clear differences in the size spores make easy to identify and differentiate microsporidian species infecting *T. viridana*.

Discussion

From the data presented by Medwecka-Kornaś et al. (1974) it is seen that the green tortrix (*Tortrix viridana*) and other lepidopterans are the most important consumers of the primary production in the Niepołomicka Primeval Forest as they consume about 80% of oak leaves. Therefore the reduction of population density of *T. viridana* and preventing the mass outbreaks of this pest have great practical significance.

Together with *T. viridana* other species of *Lepidoptera* fed on oak trees. Among them were *Noctuidae* — *Amphipyra pyramidea* L. and *Cosmia trapezina* L., *Tortricidae* — *Archips podana* Scop., *Archips crataegana* Hb. and *Pandemis ribeana* Hbn.; and *Lycaenidae* — *Theda quercus* L. In 1968 number of larvae of all above mentioned species was equal only to about 50% of *T. viridana* number.

In the natural reduction of all phytophagous insects main role played pathogens; birds and mammals destroyed only 3.5% of larvae. The detailed analysis of mortality causes in lepidopteran populations was given by Witkowski (1975).

Witkowski and Langer (1976) analyzed in detail relationship

Table 2

Comparison of Measurements of Spores of Four Microsporidian Species Recorded in *Tortrix viridana* L. and Frequency Distribution of Spore Sizes

Microsporidian species	Spore size in μm														
	1.1–1.5	1.6–2.0	2.1–2.6	2.6–3.0	3.1 3.5	3.6–4.0	4.1–4.5	4.6–5.0	5.1–5.5	5.6–6.0	6.1–6.5	6.6–7.0	7.1–7.5	7.6–8.0	8.1–8.5
Nosema tortricis Weiser				10	22	16	2								
fresh spores: stained spores:				17	16	17									
Octosporea viridana Weiser															
fresh spores:										2	9	8	11	15	2
stained spores:										7	25	14	6		
Plistophora sp.															
fresh spores:	2	16	29	3											
stained spores:		39	11												
Thelohania weiseri Günther															
fresh spores:						1	15	7	13	10					
stained spores:						8	10	7	16	9					

between the feeding intensity of *T. viridana* larvae and their density during 1970–1974 that is during the mass occurrence of *T. viridana*. The feeding intensity in 1971 was estimated as degree IV. In 1972 the density of larvae was higher than in 1971 but the feeding intensity was weaker (degree III). In 1973 there was a distinct decrease in population density of *T. viridana* and its feeding intensity (degree II). In 1974 the feeding intensity was very low (degree I) and in 1975 showed further decrease.

According to Egorov et al. (1961), Satchell (1973) and Witkowski and Langer (1976) the young oak forests are weakly infested with larvae of *T. viridana* while older forests are infested heavily.

Our observations were conducted in old oak stands and the data obtained on mortality of *T. viridana* are in agreement with observations of Witkowski and Langer (1976). We proved that the increase in larval mortality during consecutive years of the mass outbreak of *T. viridana* was due to the increase of parasitization by microsporidians which caused the destruction of pest population.

The fact that among many biotic factors, causing the natural reduction in *T. viridana* populations, a great and sometimes the main role is played by microsporidians was known earlier. However, this was not so well proven as in our studies.

Weiser (1956, 1966) observed that *Octosporea viridana* infected 4%, *Nosema tortricis* infected 3% and *Thelohania weiseri* infected 14% of *Tortrix viridana* population. In populations examined by Franz and Huger (1970) *Nosema tortricis* was the dominating species which infected 43.6% of larvae.

During our studies in Niepołomicka Primeval Forest the population of *T. viridana* was infected by microsporidians in 1970 in 12% and in 1972 in 65%. When we shall consider the number of *T. viridana* larvae infected by microsporidians as 100% then *Octosporea viridana* was observed in 54% of cases, *Nosema tortricis* — in 28% of cases, and *Thelohania weiseri* — in 18% of cases.

The role of microsporidian parasites in the decreasing of density population of *T. viridana* is not only due to the killing effect. More important from the population standpoint is lowering the fecundity of females of *T. viridana* caused by microsporidian infections. Microsporidians infect not only larvae but also pupae and moths e.g., in 1971 among 100 microscopically examined moths 12% of them were infected by various microsporidians. Franz and Huger (1970) reported that the mean fecundity of infected *T. viridana* moths was 23–29 eggs comparing with 50 eggs laid by normal moths (Gregor 1960).

Franz and Huger (1970) indicated that the collapse of the outbreak of *T. viridana* during 1968–1970 in the Federal Republic of Germany was due to an epizootic caused by some microsporidian species. Our studies, too, showed that the microsporidians were responsible for the collapse of the *T. viridana* outbreak during 1970–1974 in the Niepołomicka Primeval Forest in Southern Poland. This was indicated by the high increase of parasitization of *T. viridana* population by microsporidians starting from 1971 which led to the extremely high mortality of larvae in 1972 estimated as 91.1%. Such high mortality caused the drastic collapse of an outbreak of *T. viridana*. In 1973 only 54 larvae and in 1974 only 72 larvae were collected on five oak trees which during 1970–1972 from 1500 to 3000 larvae could be easily found.

ZUSSAMENFASSUNG

Untersucht wurden Larven des Eichenwicklers (*Tortrix viridana* L.) in Hinsicht ihrer Parasitierung während ihres Auftreten in Süd-Polen in den Jahren 1970–1974. Es wurden grosse Unterchiede in der Anzahl der Larven auf den Eichen und in der Larvenmortalität in Laborzuchten festgestellt. Die Anzahl der gefundenen Larven betrug von 5 Eichen im Jahre 1970 — 1913 Stück, 1971 — 1386 Stück, 1972 — 3716 Stück, 1973 — 54 Stück, und im Jahre 1974 — 72 Stück. Die Mortalität der Larven wuchs in dieser Zeit in Laborzuchten auffallend: 1970 — 48.5%, 1971 — 49.2% und 1972 — 91.1%.

Diese Larvenmortalität wurde von folgenden 4 Mikrosporidienarten verursacht: *Nosema tortricis* Weiser, *Octosporea viridana* Weiser, *Plistophora* sp., *Thelohania weiseri* Günther. Im Jahre 1970 betrug die Parasitierung der Eichenwicklerlarven etwa 12%, dagegen im Jahre 1972 — 65%. Als Hauptparasit wurde *Octosporea viridana* festgestellt.

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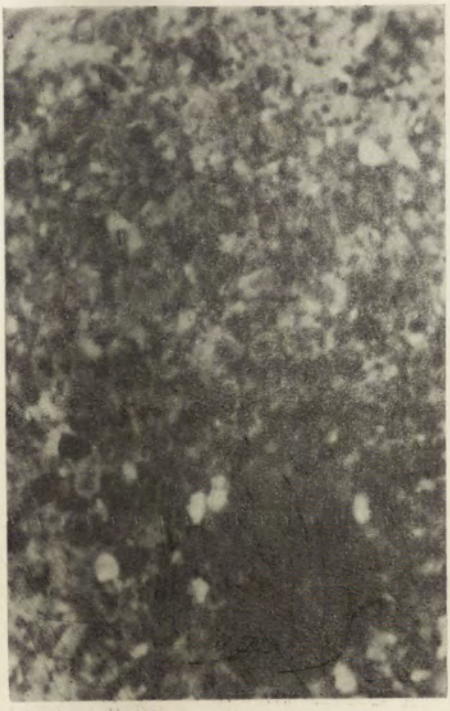
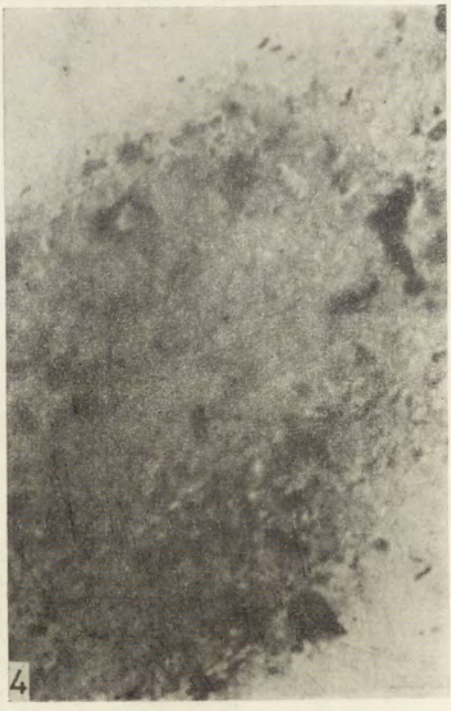
EXPLANATION OF PLATE I

1 : Spores of *Octosporea viridana* Weiser stained with Giemsa
2 : Spores of *Nosema tortricis* Weiser in smeared preparation of fat body stained with Giemsa
3 : Spores of *Thelohania weiseri* Günther and blood cells stained with Giemsa
4 : Spores of *Plistophora sp.* in smeared preparation stained with Giemsa





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