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ACTA PROTOZOOLOGICA

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Archiastomata adami nov. gen., nov. spec. (*Archiastomatidae* nov. fam.), ein freilebender astomater Ciliat

Synopsis. Es wird ein freilebender astomater Ciliat aus einem stark eutrophierten Viehweidetümpel der Hohen Tauern beschrieben. *Archiastomata adami* nov. spec. repräsentiert sich durch seine Mundlosigkeit und eine riesige Zentralvakuole, die sich in einem etwa ½ — stündigen Rhythmus entleert, als Vertreter einer neuen Familie, *Archiastomatidae* nov. fam., die provisorisch in die Ordnung *Astomatida* eingereiht wird. Dieser Schritt erscheint durch eine Anzahl von morphologischen Gemeinsamkeiten (z. B. Mundlosigkeit, "Système sécant", zahlreiche kontraktile Vakuolen) mit einigen Familien der *Astomatida* gerechtfertigt. *Archiastomata adami* bietet aber auch die Möglichkeit, die Entstehung von astomaten Ciliaten aus prostomen *Gymnostomata* zu verstehen. Ein Stammbaum für diesen Entwicklungsweg wird diskuriert.

Unter den freilebenden Ciliaten sind bisher nur wenige Arten bekannt geworden, die vermutlich mundlos sind und daher im Cytoplasma keine typischen Nahrungsvakuolen erkennen lassen. Diese Arten, *Sphaerobactrum warduae* Schmidt, 1920 und *Cyclotrichium gigas* Faure-Fremiet, (1924) weichen aber in der übrigen Körperorganisation so sehr von den in der Ordnung *Astomatida* Schewiakoff, 1896 vereinigten mundlosen Ciliaten ab, daß eine Einordnung in diese Gruppe kaum möglich erscheint. Dasselbe trifft auch für die teilweise mundlosen Vertreter der Familie *Actinobolinidae* zu (s. Kahl 1931). Corliss (1961, 1975) hat diese Arten daher unter die *Gymnostomata* eingereiht. Somit war bisher kein freilebender Infusor bekannt, das auf Grund seiner Mundlosigkeit in die Ordnung der *Astomatida* eingeordnet hätte werden können.

Bei meinen im Rahmen des österreichischen MAB-6 Programmes der UNESCO durchgeführten Untersuchungen über Hochgebirgs-Ciliaten fand ich einen sehr interessanten, freilebenden, mundlosen Ciliaten, der auch in der übrigen Körperorganisation so deutliche Beziehungen zu den *Astomatida* aufweist, daß ich ihn als neue Familie in diese Ordnung einreihen möchte — auch auf die Gefahr hin, daß dadurch ein wesentli-

ches Merkmal dieser Ordnung, nämlich die ausschließlich endosymbiotische oder parasitische Lebensweise (s. Corliss 1975) eine Bedeutungsminderung erfährt.

Untersuchungsmethoden

Die Morphologie und Cytologie von *Archiastomata adami* wurde wegen des begrenzten Tiermaterials vorwiegend mit einem REICHERT-Phasenkontrastmikroskop studiert. Die Lebendbeobachtung wurde allerdings durch die große Beweglichkeit und die Voluminösität dieses Ciliaten sehr erschwert. Zum Studium des Verlaufes der Cilienreihen und der argyrophilen Strukturen verwendete ich meine trockene Versilberungsmethode Foissner (1967, 1968) und die Opalblaumethode von Bresslau (1921). Die Färbung des Zellkernes erfolgte mit Orcein-Essigsäure.

Beschreibung von *Archiastomata adami* nov. spec. (Genotyp.)[1]

Archiastomata adami wird außer den unten angegebenen Familien — und Genuseigenschaften noch durch folgende Species-Merkmale charakterisiert: Das Infusor ist 220–250 µm lang, 140–170 µm breit und an einem Pol merkbar verjüngt. Da sich die Tiere mit diesem Pol voran bewegten, bezeichne ich ihn als den apikalen. Bei maximaler Füllung der Zentralvakuole (s. unten) ist der Querschnitt des Tieres kreisrund. Trotz dieser plump-eiförmigen Gestalt bewegte sich dieser Ciliat gewandt und schnell zwischen den Detrituspartikelchen und Pilzfäden umher, wobei er langsam um die Längsachse rotierte.

Die Bewimperung ist kurz, aber sehr dicht. Der Abstand der 120–150 Cilienreihen voneinander beträgt nur etwa 1,5 µm. Am apikalen Pol stoßen die Cilienreihen der "ventralen" und "dorsalen" Seite entlang einer Nahtlinie zusammen (Abb. 3d). Dadurch entsteht das für viele astomate Ciliaten typische „Système sécant" (s. z. B. Lom 1957, Puytorac 1954, 1959, 1960). Vom posterioren Pol erhielt ich leider keine günstigen Präparate, so daß ich über den Verlauf der Cilienreihen in dieser Region keine Angaben machen kann. Das Silberliniensystem ist ein sehr engmaschiges Gitter (Abb. 3b) mit etwa rechteckigen Maschen. In den Silberlinien, die teilweise aufgezweigt sind, liegen unregelmäßig verstreut kleine Kumulierungen argyrophiler Substanz, die wohl Relationskörper der Protrichocysten (s. unten) sind.

Das Ektoplasma ist vom Entoplasma deutlich abgegrenzt. Die Pellicula wird durch die Wimperreihen schwach gekerbt. An ihr sind viele, etwa 3 µm große, stäbchenförmige Protrichocysten befestigt (Abb. 3a), die bei Reizung (Deckglasdruck) ausgestoßen werden und das Tier als dünne

[1] Diese Art widme ich Herrn Univ. Prof. Dr. Hans Adam, durch dessen grosszügige Unterstützung diese Arbeit ausgeführt werden konnte.

Hülle umgeben. Das Cytoplasma ist dicht mit 0.5–1.5 µm großen, glänzenden Einschlüssen unbekannter Natur gefüllt. Typische Nahrungsvakuolen waren bei zehn daraufhin sorgfältig untersuchten Tieren nie zu erkennen. Auffällig waren jedoch viele, etwa 3–5 µm große, im Phasenkontrast dunkel erscheinende, unregelmäßig geformte lockere Zusammenballungen (Abb. 3a), die jedoch von keiner Membran begrenzt zu sein schienen. Diese Beobachtungen sprechen eindeutig dafür, daß dieser Ciliat tatsächlich mundlos ist, jedenfalls aber keinen funktionierenden Mund mehr besitzt.

Der Makronucleus ist lang, etwa 12 µm breit und an den Enden leicht keulenförmig erweitert (Abb. 1). Die Lage und Form sind nicht

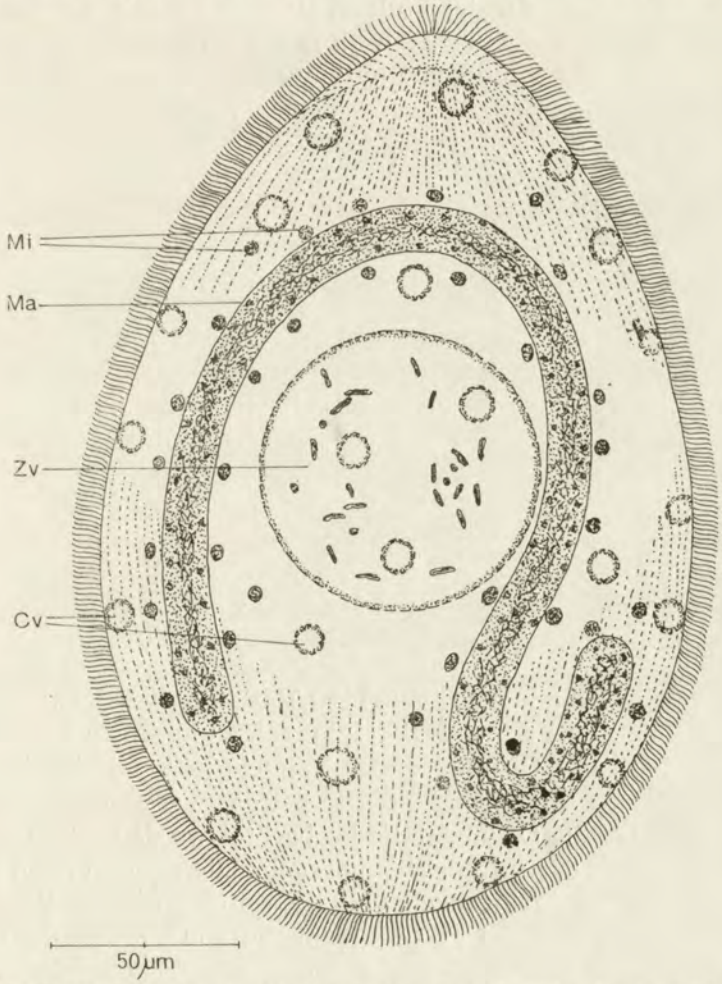

Abb. 1. *Archiastomata adami* (Genotyp.). Gezeichnet nach Lebendbeobachtungen im Phasenkontrastmikroskop. Cv — kontraktile Vakuolen, Ma — Makronucleus, Mi — Mikronuclei, Zv — Zentralvakuole

konstant, was vermutlich durch die große Zentralvakuole (s. unten) bedingt ist, die den Makronucleus je nach Füllungszustand wohl mehr oder weniger zur Seite drängt. Das Chromatin ist deutlich netzförmig angeordnet und im Zentrum des Kernes am dichtesten (Abb. 1). Es sind viele kleine Nucleolen erkennbar. Auffällig ist die große Anzahl von Mikronuclei, die rund bis leicht oval sind und einen Durchmesser von 4–5 µm haben. Bei zwei daraufhin untersuchten Tieren wurden 32 bzw. 35 Mikronuclei gezählt. Sie liegen meist dem Makronucleus an, finden sich aber manchmal auch ziemlich weit von diesem entfernt (Präparationsartefakt?).

Das wohl auffälligste Merkmal von *A. adami* ist eine riesige Zentralvakuole, die bei maximaler Füllung einen Großteil seines Innenraumes einnimmt (Abb. 1 Zv, 2 a, Taf. I 1, 2). Diese Zentralvakuole entleert sich in einem etwa $^1/_2$ — stündigen Rhythmus, wobei viele stäbchenförmige, stark lichtbrechende Kristalle ausgeschieden werden (Abb. 2 b, Taf. I 3).

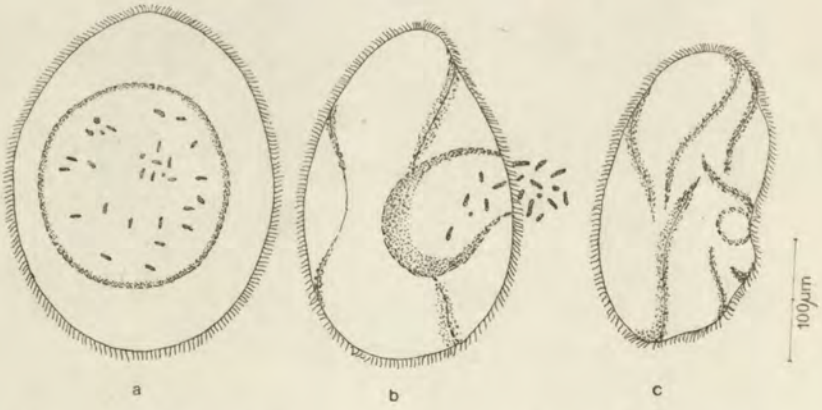

Abb. 2. Drei Stadien der Entleerung der Zentralvakuole. Nähere Erklärungen im Text

Zurück bleibt eine kleine Restvakuole (Abb. 3 c, Taf. I 4), aus der sich dann wieder die große Zentralvakuole bildet. Zuführende Kanäle wurden nicht festgestellt. Die Öffnung dieser Vakuole befindet sich etwa in der Mitte des Tieres. Leider konnte weder bei der Lebendbeobachtung noch in den Präparaten die Öffungsstelle beobachtet werden. Zugleich mit der Entleerung beginnt das Infusor sich unregelmäßig zu verformen (Abb. 2 b, 7, Taf. I 3) und erscheint nach völliger Entleerung der Vakuole ganz zerknittert (Abb. 2 c, Taf. I 4). In diesem Zustand ist auch die Bewegung deutlich behindert, was sich darin zeigt, daß sie unregelmäßig und torkelnd wird. Sobald die Vakuole wieder halb gefüllt ist (nach etwa 5–10 min), wird auch die Bewegung wieder normal. Diese Zentralvakuole

erfüllt offensichtlich hauptsächlich die Funktion einer Cytopyge, worauf besonders die ausgeschiedenen Kristalle hindeuten. Daß sie ähnlich wie ein hydrostatisches Organell wirksam ist, wie dies André (1915) bei *Acaryophrya helenae*, einer Form mit einer sehr großen kontraktilen Vakuole, vermutet, erscheint wenig wahrscheinlich, wenn man in Erwägung zieht, daß das Infusor bei der Entleerung in der Bewegung gehindert wird.

Neben dieser großen Zentralvakuole besitzt *A. adami* noch viele (über 30) unregelmäßig verteilte, etwa 15 µm große kontraktile Vakuolen,

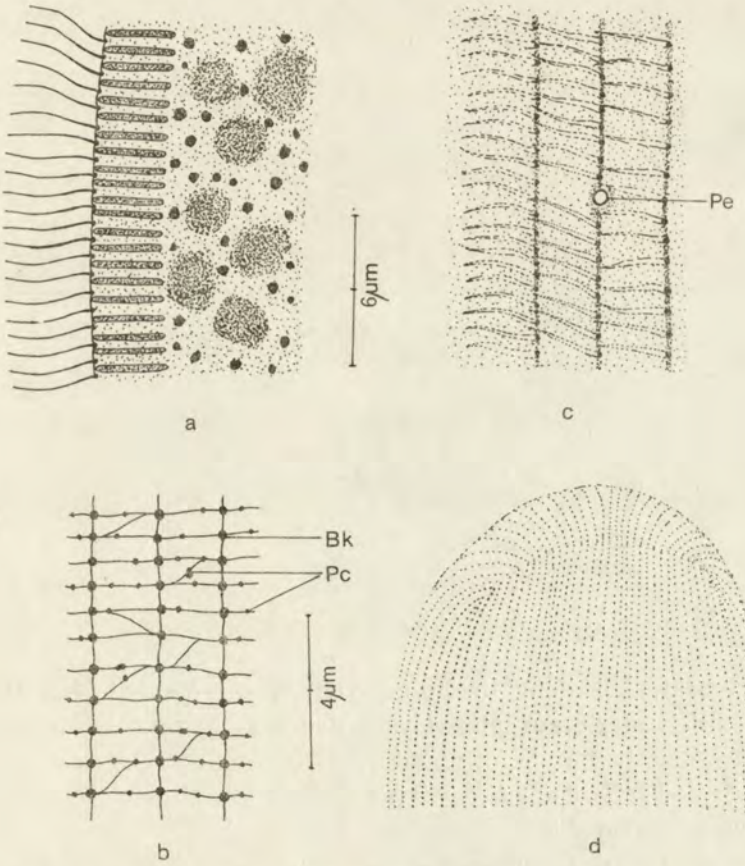

Abb. 3 *Archiastomata adami*. a — Peripherer Teil der Zelle bei starker Vergrößerung. An der Pellicula sind viele stäbchenförmige Protrichocysten befestigt. Das Entoplasma enthält kleine, stark lichtbrechende Einschlüsse und größere lockere Zusammenballungen b — Teil des Silberliniensystems. Die Basalkörper (Bk) und die Protrichocysten (Pc) werden durch Silberlinien untereinander verbunden c — Teil der Pellicula nach Präparation mit Opalblau. Der Exkretionsporus (Pe) der kontraktilen Vakuole liegt in der Cilienreihe d — Schematische Darstellung des Verlaufes der Cilienreihen am apikalen Pol. Die Cilienreihen stoßen entlang einer Nahtlinie zusammen, sodaß ein "Système sécant" entsteht

die sich pro Minute etwa einmal entleeren. Jede Vakuole besitzt einen Porus, der stets in der Cilienreihe liegt (Abb. 3c), so daß die Cilienreihe dort unterbrochen ist.

Excystierung: Schon bei der Lebendbeobachtung von *A. adami* fielen mir in den Präparaten sehr große, ovale 170 × 150 µm durchmessende Cysten auf, die die in Abb. 4 dargestellte Struktur hatten. In

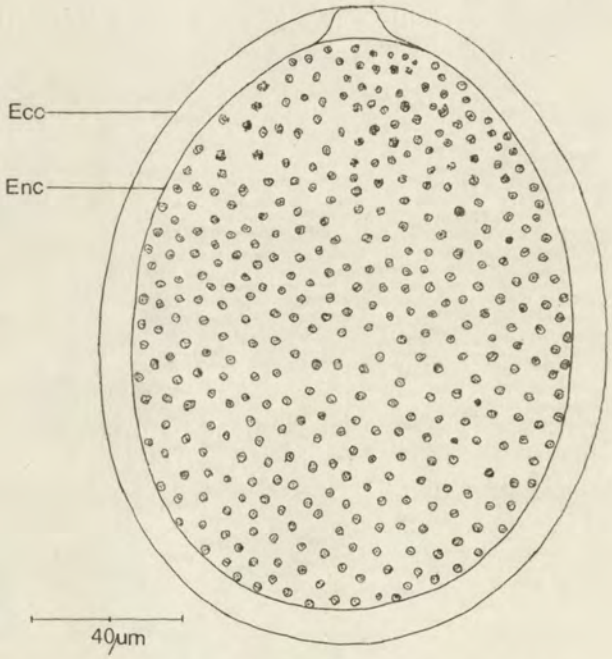

Abb. 4. Cyste von *Archiastomata adami*. Ecc — Ektocyste, Enc — Entocyste

einem Präparat, bei dem in Folge von Wassermangel das Deckglas bereits ziemlich stark auf eine Cyste drückte, konnte ich beobachten, daß sie an der halbkugelförmigen Vorwölbung aufplatzte. Daraufhin begann sich der Inhalt der Cyste zu bewegen und nach etwa 10 min (dem Präparat wurde wieder Standortwasser zugesetzt) schlüpfte ein Exemplar von *A. adami* aus, das noch keine Zentralvakuole erkennen ließ. Diese bildete sich erst etwa 10 min nach dem Schlüpfen. Die Encystierung wurde nicht beobachtet.

Die Cysten sind stets leicht oval und von einer ca. 10 µm dicken, strukturlosen Hülle umgeben. Die Entocyste läßt die Schlüpföffnung, die in Form einer mehr oder weniger halbkugelförmigen Erhebung ausgebildet ist, deutlich erkennen. Die Cysten waren dicht mit derselben kleinen

Granula wie die lebenden Tiere gefüllt. Die Zentralvakuole und die kontraktilen Vakuolen waren nicht feststellbar.

Locus typicus: *Archiastomata adami* wurde in insgesamt 30 Exemplaren in einem Aufguß von Algen und Pflanzenresten eines stark eutrophierten Viehweidetümpels unterhalb des Wallack-Hauses (Hohe Tauern, Großglockner-Hochalpenstraße, 2575 m ü. d. M.) gefunden. Der Aufguß hatte eine sehr dicke, aus Bakterien und Pilzfäden zusammengesetzte Kahmhaut, in der noch folgende andere Ciliaten festgestellt wurden: *Colpoda* sp., *Bursaria truncatella* und *Epistylis alpestris.*

Es sei hier auch angemerkt, daß die Möglichkeit in Erwägung gezogen wurde, daß *A. adami* vielleicht durch Zerquetschen eines Oligochäten, Turbellars oder einer Schnecke freigeworden sein könnte. Eine genaue Untersuchung des Aufgusses zeigte aber, daß dieser keine Oligochäten, Turbellarien und Schnecken enthielt.

Diagnose von *Archiastomata* nov. gen.

Auf Grund der im vorhergehenden geschilderten Merkmale von *A. adami* kann kein Zweifel darüber bestehen, daß für dieses Infusor ein neues Genus und eine neue Familie errichtet werden muß. Für das Genus schlage ich folgende Diagnose vor: Große, mehr oder weniger eiförmige, im Querschnitt runde Archiastomatidae, deren Zentralvakuole sich in einem etwa $^1/_2$ — stündigen Rhythmus entleert, wobei stäbchenförmige Kristalle ausgeschieden werden. Zahlreiche kleine kontraktile Vakuolen. Der Makronucleus ist lang und unregelmäßig verbogen. Bewimperung gleichmäßig und vollständig. Die Cilienreihen bilden zumindest am Bewegungspol ein "Système sécant".

Diagnose von *Archiastomatidae* nov. fam.

Freilebende, mundlose Ciliaten mit einer großen, sich in längeren Zeitabständen entleerenden Zentralvakuole.

Diskussion

a. Zur systematischen Einordnung von *Archiastomata adami*

Archiastomata adami hat mit Ausnahme der Zentralvakuole eine so große Ähnlichkeit mit vielen Arten der Familie *Anoplophryidae* (vgl. Cepede 1910, Puytorac 1954), daß man ihn ohne Bedenken in

diese Familie einordnen würde, wenn man ihn im Verdauungstrakt eines Oligochäten, wo die *Anoplophryidae* gewöhnlich leben, fände. Da *A. adami* aber ökologisch und morphologisch (Zentralvakuole) von allen bisher bekannten Familien der *Astomatida* getrennt ist, erscheint die Aufstellung einer neuen Familie gerechtfertigt. Für die Einreihung in die Ordnung der *Astomatida*, die bisher nur endosymbiotische oder parasitische Ciliaten enthielt, sprechen folgende Merkmale (vgl. Corliss 1975): (1) Die Mundlosigkeit und das Fehlen von typischen Nahrungsvakuolen. (2) Die gleichmäßige, holotriche Bewimperung und die große Körpergröße. (3) Die Ausbildung eines "Système sécant". (4) Die zahlereichen kontraktilen Vakuolen und der lange Makronucleus. (5) Das ähnliche Silberliniensystem.

Freilich kann die Einordnung von *A. adami* in die *Astomatida* nur eine provisorische sein, da dieser Ciliat auch deutliche Beziehungen zu den *Gymnostomata* aufweist (s. unten). Ich habe aber hier denselben Weg wie Puytorac (1959) eingeschlagen, der einen mundlosen Ciliaten, der deutliche Beziehungen zu den *Trichostomatida* aufweist, ebenfalls in die *Astomatida* eingeordnet hat. Kozloff (1954) hat dagegen bei *Curimostoma renalis*, ein mundloser Ciliat, der deutliche Beziehungen zu den *Tetrahymenidae* aufweist, den umgekehrten Weg eingeschlagen, indem er dieses Infusor in die *Tetrahymenidae* eingegliedert hat. Kozloff (1954) rechtfertigt diesen Schritt damit, daß die Einordnung in die *Astomatida* die Heterogenität dieser Gruppe nur vermehren würde. Dies ist sicherlich richtig, aber es muß auch bedacht werden, daß andererseits die Familie *Tetrahymenidae* ihre Homogenität durch diesen Schritt verliert. Dies hat Corliss (1961) wohl auch dazu bewogen, *C. renalis* unter die nicht einzuordnenden Genera zu stellen.

b. Bemerkungen zur Bedeutung von *Archiastomata adami* für die Phylogenie der astomaten Ciliaten

Die astomaten Ciliaten sind bisher einer einheitlichen Klassifikation entwichen. Sowohl von Puytorac (1954) als auch von Corliss (1956, 1975) wurde eine Auftrennung dieser Ordnung in Erwägung gezogen. Die heutige Auffassung geht dahin, daß sie eine polyphyletische Gruppe sind, deren Ahnen *Thigmotrichina, Trichostomatida* (Puytorac 1954, 1959), *Apostomatida* (Corliss 1956) und *Hymenostomata* (Kozloff 1954) gewesen sind. Die Mundlosigkeit wird in Anpassung an die endosymbiotische oder parasitische Lebensweise als sekundär erworben angesehen. Demgegenüber steht die Ansicht älterer Forscher (z. B. Calkins 1933), die die Mundlosigkeit als ursprüngliches Merkmal deuteten und daher die astomaten Ciliaten als die primitivsten ansahen.

Corliss (1959) hat darauf aufmerksam gemacht, daß sich ein Teil

der *Astomatida* auch von primitiven *Gymnostomata* und *Hymenostomata* ableiten ließe. Darauf hat gleichzeitig auch Puytorac (1959) hingewiesen, der das Genus *Lubetiella* von den *Trichostomatida* ableiten möchte. Daß derartige Möglichkeiten tatsächlich in Betracht gezogen werden müssen, zeigt das schon einleitend erwähnte Vorkommen freilebender, mundloser "gymnostomater" Ciliaten, bei denen vorstellbar ist, daß sie auch endosymbiotisch oder parasitisch leben könnten. Wie im folgenden zu zeigen versucht werden wird, bietet *A. adami die* Möglichkeit, die Entwicklung von astomaten Ciliaten aus prostomen *Gymnostomata* zu verstehen (Abb. 5).

Die wesentlichen Stützen dieses Ableitungsversuches sind die Zentralvakuole und das "Système sécant" von *A. adami.* Bei der Durchsicht der mir zur Verfügung stehenden Literatur fielen mir mehrere Ciliaten auf, die eine ähnlich große "Zentralvakuole" wie *A. adami* besitzen. Es sind dies die Arten der Gattung *Bursella* (s. Schmidt 1920, Kahl 1930–35) und *Coelosomides marina* (Anigstein 1912). Die Gattung *Bursella,* bei der der Mund nur eine spaltförmige Öffung ist, die sich in eine große Grube ausweitet, kann ohne Schwierigkeiten von so ursprünglich organisierten Prostomatina wie etwa der Gattung *Holophrya* abgeleitet werden (vgl. Kahl 1930–35). Als drittes Glied dieser Entwicklungsreihe könnte man den Ciliaten *Coelosomides marina* ansehen. Dieser eigenartige, von Fauré-Fremiet (1950) in eine besondere Familie, zu der Corliss (1961) unter anderen auch das Genus *Bursella* hinzugefügt hat, gestellte Ciliat, besitzt ebenfalls eine außerordentlich große Vakuole. Diese dient der Verdauung der Nahrung, die über einen engen, bewimperten Schlund in die Vakuole transportiert wird. Sowohl bei *Bursella* als auch Coelosomides ist diese Vakuole eine beständige Einrichtung, die auch dann vorhanden ist, wenn gerade keine Nahrung aufgenommen wird!

Um *Archiastomata adami* von Coelosomides abzuleiten, muß angenommen werden, daß der bewimperte Schlund im Verlaufe der Evolution wieder aufgegeben worden ist, die Empfangsvakuole aber erhalten geblieben ist und nun als Exkretionsorganell funktioniert. Die Mundanlage könnte sich als "Système sécant" erhalten haben. An welche Gruppe der Astomatida nun *A. adami* anzuschließen ist, kann beim Stand der heutigen Kenntnisse kaum entschieden werden. Die hier vorgeschlagenen Genera *Corlissiella* (Puytorac 1960) und *Jirovecella* (Lom 1957) der Familie *Anoplophryidae* bzw. *Hoplitophryidae* sind lediglich zwei Möglichkeiten, die durch das "Système sécant", die große Zahl der Cilienreihen und das ähnliche Silberliniensystem gestützt werden. Die große Zentralvakuole von *A. adami* könnte man in Anpassung an die endosymbiotische Lebensweise als verlorengegangen betrachten.

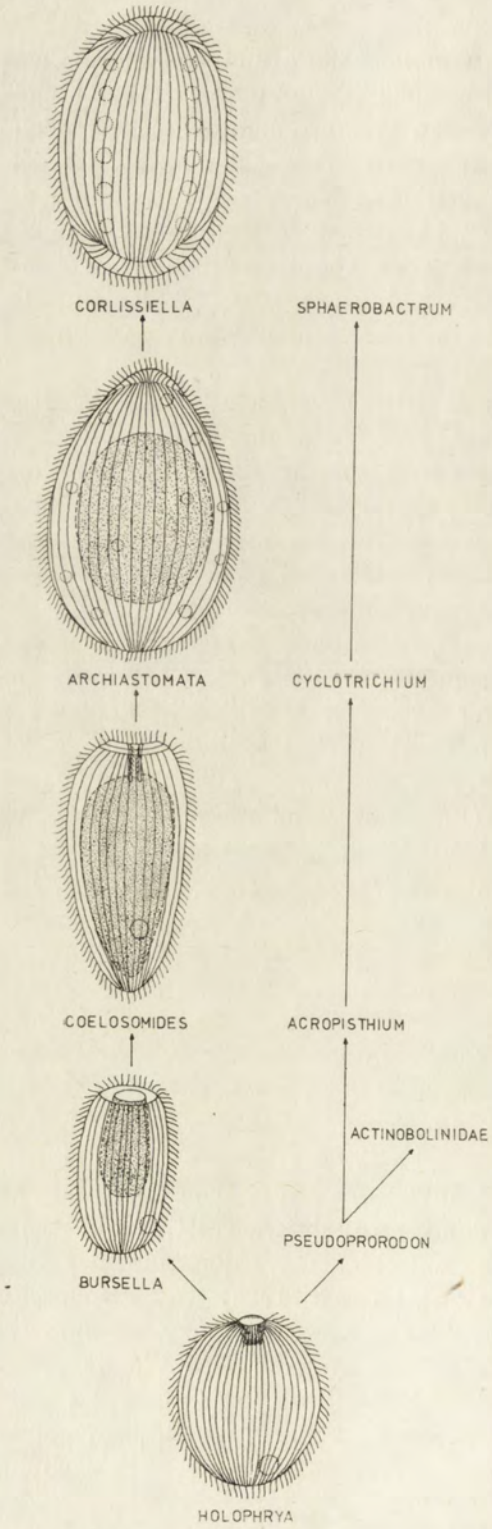

Abb. 5. Zwei mögliche Entwicklungswege von mundlosen Ciliaten aus prostomen *Gymnostomata*. Der noch freilebende astomate Ciliat *Archiastomata adami* wird als Übergangsform zu den *Astomatida* s. str. angesehen. Nähere Erklärungen im Text. *Holophrya nigricans* (nach Kahl 1930–35), *Bursella* sp. (nach Kahl 1930–35), *Coelosomides marina* (nach Anigstein 1912), *Corlissiella criodrili* (kombiniert nach Puytorac 1959)

Der vorliegende Ableitungsversuch ist natürlich mit vielen Unsicherheiten behaftet, vor allem auch deswegen, weil wir über die argyrophilen Strukturen von *Bursella* und *Coelosomides* keine Angaben besitzen. Es muß weiters auch in Erwägung gezogen werden, daß es sich bei *A. adami* um einen sekundär wieder freilebend gewordenen Astomaten handelt. Man muß sich jedoch auch darüber klar sein, daß der Ableitungsversuch der meisten *Astomatida* von *Thigmotrichina* (s. Puytorac 1954) ebenfalls keine besonders große Wahrscheinlichkeit besitzt, wenn man bedenkt, daß sich von dieser Gruppe auch die *Peritricha* herleiten sollen (s. Corliss 1956, Lom et al. 1968). Es ist kaum anzunehmen, daß sich gleich zwei so hoch evolutionierte Ordnungen wie die *Peritrichida* und *Astomatida* von den doch recht spezialisierten *Thigmotrichina* ableiten lassen (vgl. Raabe 1964, 1967). Daß gewisse *Thigmotrichina* recht enge Beziehungen zu den *Gymnostomata* aufweisen, konnte vor kurzem auch elektronenmikroskopisch demonstriert werden (Lom and Kozloff 1969). Daher scheint mir die von Corliss (1956) geäußerte Ansicht, daß sich ein Teil der *Astomatida* und die *Thigmotrichina* unabhängig aus einer gemeinsamen Wurzel entwickelt haben, glaubhafter.

Von den prostomen *Gymnostomata* führt möglicherweise eine zweite Entwicklungslinie, die hier aber nicht näher besprochen werden soll, zu mundlosen Ciliaten. Die bereits einleitend erwähnten zwei mundlosen Ciliaten *Sphaerobactrum warduae* und *Cyclotrichium gigas*, könnten über die Didiniidae von der Gattung *Prorodon* oder *Pseudoprorodon* abgeleitet werden (vgl. Fauré-Fremiet 1924 und Kahl 1930–35). Von der Gattung Prorodon sind nach Kahl (1931) auch die mundlosen Vertreter der Familie *Actinobolinidae* abzuleiten.

Danksagung: Mit dankenswerter Unterstützung des österreichischen MAB-6 Programmes der UNESCO, des Fonds zur Förderung der wissenschaftlichen Forschung (Projekt 1838 und No. 39), der Jubiläumsstiftung der Österreichischen Nationalbank und der Gesellschaft zur Förderung der Hochschule für Bodenkultur.

Summary

A free-living astomatous ciliate occurring in a heavy eutrophous cattle-range pool of the Austrian Alps is described. This ciliate has a very prominent central-vacuole that discharges its contents twice an hour. Accordingly, *Archiastomata adami* nov. spec. is a representative of a new family, *Archiastomatidae* nov. fam., which is referred provisionally to the order astomatida. This step seems to be justified by a number of morphological similarities (e.g., mouthless, "système sécant", many contractile vacuoles) with some families of the order astomatida. But *Archiastomata adami* give us a change to understand the evolution of astomatous ciliates from prostomatous gymnostomata. A phylogenetic tree for this course of development is discussed.

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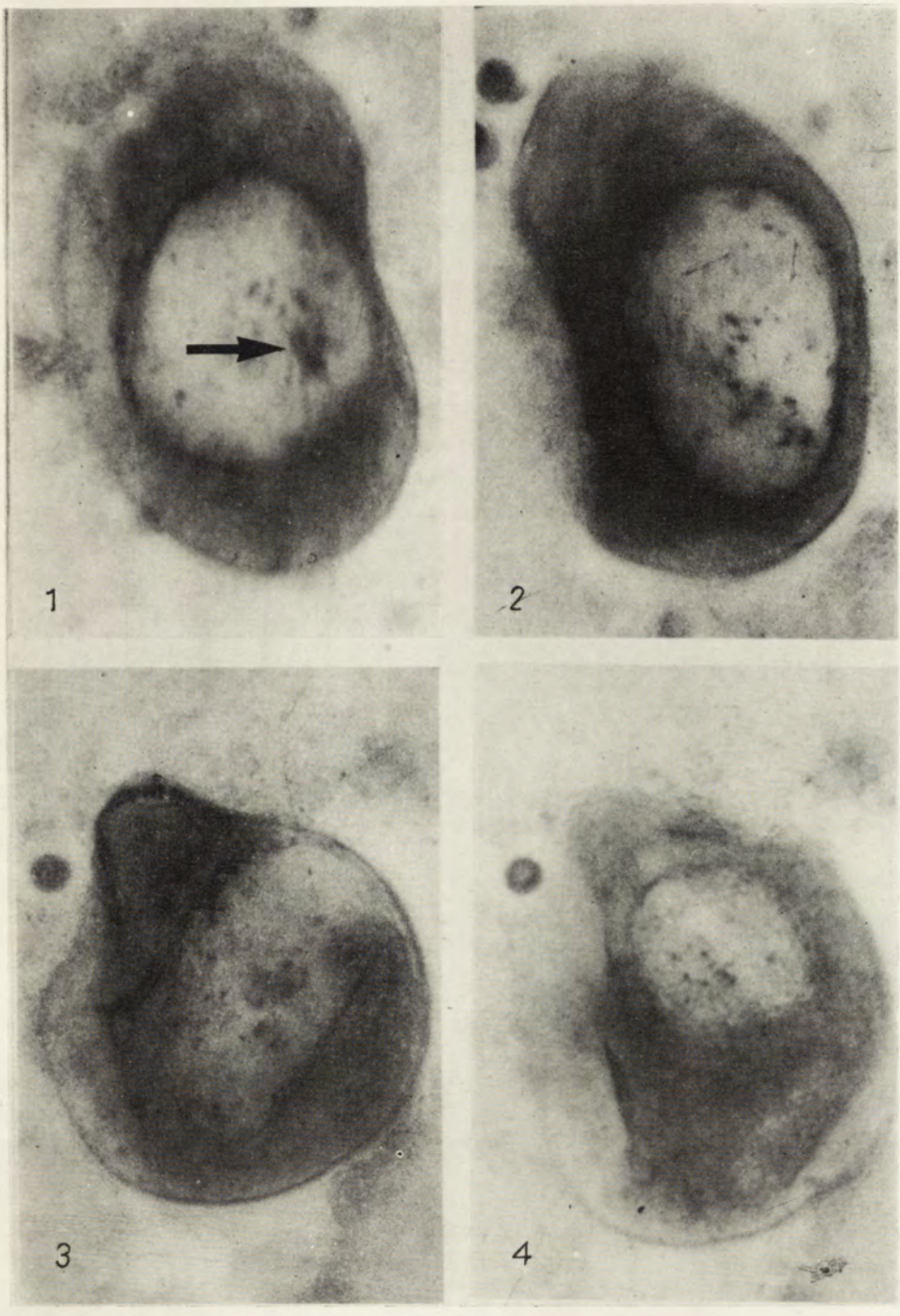
LEGENDE ZU DER TAFEL I

1: *Archiastomata adami* n. gen., n. sp. mit der typischen, grossen Zentralvakuole, in der viele stäbchenförmige Kristalle sind (Pfeil) 2–4: Drei Stadien der Entleerung der Zentralvakuole. Nähere Erklärungen in Text. Alle Aufnahmen wurden mit einem Dunkelfeldmikroskop angefertigt



W. Foissner auctor phot.

ACTA PROTOZOOLOGICA
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Zoological Survey of India, 8, Lindsay Street, Calcutta-16, India

A. K. MANDAL and K. N. NAIR

Ptychostomum drawidi sp. n. and *Plagiotoma pellogasteri* sp. n. — New Ciliates from Earthworms of Orissa, India

Synopsis. Two new species of ciliates collected from the terrestrial Oligochaetes of Koraput District, Orissa State, India are described. One is a thigmotrich ciliate belonging to the genus *Ptychostomum* Stein, 1860 of the *Hysterocinetidae* collected from the intestine *Drawida willsi* Michaelsen and the second is a heterotrich ciliate belonging to the genus *Plagiotoma* Dujardin, 1841 of the family *Plagiotomidae* collected from the coelom of *Pellogaster bengalensis Michaelsen.* This is the first record of the genus *Ptychostomum* in India.

A new thigmotrich ciliate of the genus *Ptychostomum* Stein, 1860 and a new heterotrich ciliate of the genus *Plagiotoma* Dujardin, 1841 are described in this paper. The former is the intestinal parasite of the terrestrial oligochaete *Drawida willsi* Michaelsen and the latter is from the coelom of *Pellogaster bengalensis* Michaelsen. Both the hosts are collected from Biswanathpur, Koraput Dist., Orissa State, India.

The type specimens are deposited in the National collection of Zoological Survey of India, Calcutta.

Material and Methods

Nine species of terrestrial oligochaetes collected during the faunistic Survey of three districts of Orissa namely Koraput, Kalahandi and Phulbani in July, 1974 were examined for the parasitic ciliates occurring in them. Twenty one specimens of *Drawida willsi* were examined and five of them were seen with an interesting species of thigmotrich ciliate. In each, the number of parasite varies from eight to ten. Out of the twelve specimens of *Pellogaster bengalensis*, eight were found to be infected with an interesting species of heterotrich ciliate. Number of parasites in each host varies from twelve to eighteen. The ciliates were isolated and observed in Physiological saline. For making permanent preparations the specimens were fixed in hot Schaudinn's fluid and followed by staining in Heidenhains' Iron-haematoxylin, Klein's dry silver method was employed in some specimens to observe the kineties. All the measurements were taken with the aid of a calibrated ocular micrometer.

Description of Species

Ptychostomum drawidi sp. n.

Body elongated, flat, anterior half narrow ending in a blunt cone, posterior half broad truncated (Fig. 1). Twenty one specimens are measured. Body length varies from 56–89 μm (average 69.7 μm), width from 26–39 μm at the broadest area (average 32.2 μm) and the thickness 6–8 μm. Body ciliation is dense with closely set kineties numbering 66–69. The kineties terminate around the sucker at more or less uniform

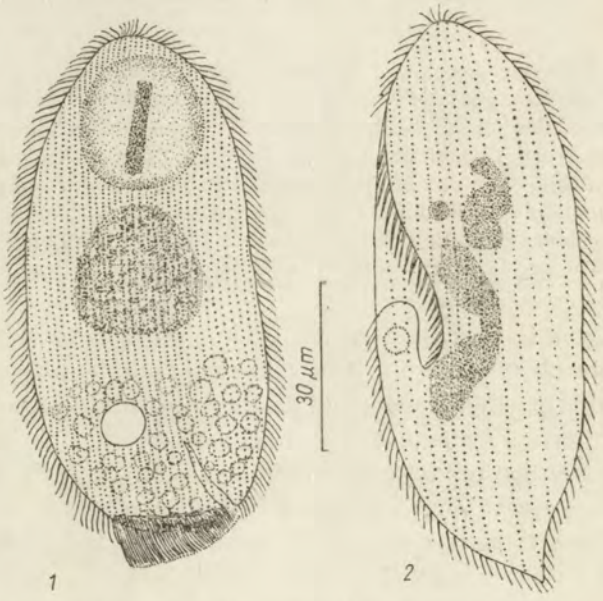

Fig. 1 — *Ptychostomum drawidi* sp. n., 2 — *Plagiotoma pellogasteri* sp. n.

intervals at the anterior portion. The sucker is prominent, 16–18 μm in diameter, having six to seven small kineties of uneven length placed close to each other. Fine strands of cytoskeletal fibres, transverse from the margin of sucker obliquely at different levels are also visible in side. Body cilia measure 7–9 μm long. The peristome runs transverse just above the truncated posterior end and provided with an undulating membrane. The cytopharynx is a small tapering tube 6–7 μm long and placed at a right angle to the peristome towards one side. The food vacuoles are concentrated in the posterior part of the body. The contractile vacuole is prominent and located at the posterior third (Fig. 1). The macronucleus is plum-shaped, prominent, size 13.2–23.1 × 11.5–19.5 μm and placed in the middle of the body. The micronucleus is not observed.

Discussion

The elongated shape of the body, the round sucker having small interrupted kineties enclosed within it, the structural details of the peristome and the restricted zone of food vacuoles in the posterior portion of the specimens encountered, render them to place in the genus *Ptychostomum* Stein. Raabe (1972) recognized 23 species of *Ptychostomum*. The range of size is attributable to the growth differences of the specimens encountered. Among the described species, *Ptychostomum campelomae* Kozloff is the one having some superficial resemblance to the form described here due to the shape of sucker. The size range of the present specimens (56–89 × 26–39 μm) is considerably lesser than that of *P. compelomae* (57–187 μm by 30–102 μm). Number of body kineties (66–69) of the specimens dealt with is also lesser than that of *P. campelomae* (95–115). The number of small kineties (7–8) within the sucker of the former is also less than that of the other (11–12). The plum-shaped macronucleus of the present species is unlike the varied shape described in *P. campelomae*. Hence, it is described as new and named *Ptychostomum drawidi* sp. n.

Species: *Ptychostomum drawidi* sp. n.
Host: *Drawida willsi* Michaelsen
Habitat: Intestine
Type-locality: Biswanathpur, Koraput District, Orissa, India
Holotype: Z.S.I. Reg. No. Pt. 1819
Paratype: Z.S.I. Reg. No. Pt. 1820, 1821, 1822, 1823, 1824.

Plagiatoma pellogasteri sp. n.

Description: Body elongated, flat, anterior end bluntly pointed, posterior end attenuated (Fig. 2). Measurements of twenty two specimens show body size range 92.4–105.6 μm in length average 98.5 μm and 33–46.2 μm in breadth average 37.5 μm, thickness varies from 8–12 μm. Body ciliation uniform, number of kineties 8–13, interval between two kineties (Fig. 2) is 2.5–3.3 μm. Taking the origin from the anterior, the peristome extends lateral to the length of 39.6–45 μm. The cytopharynx measures 6.5–9.9 μm in length. The peristome and cytostome are provided with membranellae. In the majority of specimens, eighteen, the macronucleus consists of two unequal fragments and in five into three fragments. Size of fragments varies from 4.5 × 3 μm and 8.25 × 3.3 μm. Micronucleus single, 2.25 μm diameter, located at the side of the anterior third. The cytoplasm of the anterior portion has more granules than the posterior.

Discussion

The gradually narrowing posterior portion with an attenuated end is the characteristic feature in the specimens examined. Macronuclear fragments numbering two and rarely three are different from the two known species *P. lumbrici* Dujardin (12–20) and *P. dichogasteri* Mandal and Nair (8–13). Micronucleus is single in the specimens encountered while in the earlier described species possess invariably two and occassionally three. Hence the species is described as new and named *Plagiotoma pellogasteri* sp. n.

Species: *Plagiotoma pellogasteri* sp. n.
Host: *Pellogaster bengalensis* Michaelsen
Habitat: Coelom
Type-locality: Biswanathpur, Koraput District, Orissa, India
Holotype: Z.S.I. Regd. No. Pt. 1825
Paratype: Z.S.I. Regd. No. Pt. 1826, 1827, 1828, 1829

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RESUME

Deux espèces nouvelles des ciliés sont décrites, provenant des oligochètes terrestres dans le district de Koraput, l'état Orissa, aux Indes. La prémiére est une thigmotriche du genre *Ptychostomum* Stein, 1860, de la famille *Hysterocinetidae*, trouvée dans l'intestin de *Drawida willsi* Michaelsen, et l'autre est une hétérotriche du genre *Plagiotoma* Dujardin, 1841, de la famille *Plagiotomidae*, prélévée du coelome de *Pellogaster bengalensis* Michaelsen. Ceci constitute le prémier rapport sur la présence du genre *Ptychostomum* aux Indes.

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Strombidium grande Levander

Synopsis. L'auteur décrit la morphologie d'un ciliè appartenant au genre *Strombidium*, qui a été trouvé sur la côte de la mer Noire. L'espèce trouvée est probablement identique avec *Strombidium grande* décrit par Lavander. Le trait caracteristique pour elle est la bande de trichocystes qui commence dans la partie equatoriale de la cellule court à droite, descend en spirale sur le côté dorsal et rejoint son commencement en suivant une ligne verticale. La structure reticulée de la surface, typique pour le genre *Strombidium* occupe ici la partie centrale du corps entre AZM et la bande de trichocystes et non la partie postérieure du corps comme chez les autres espèces.

Parmi les représentants de l'ordre *Oligotricha* il y a plusieurs espèces qui depuis la description originelle n'ont pas été retrouvées et décrites d'après les techniques modernes. A ces espèces, peu connues appartient *Strombidium grande* décrite par Levander en termes suivantes: „Gross, cylindrisch, beide Körperenden stumpf abgerundet. Vorderende mit hervorstehendem Apikalzapfen am Stirnfelde. Stachel am Ende fehlt. Ein langes gleichbreites Trichocystenband windet sich schraubenförmig um den Körper und endigt in der hinteren Körperspitze. Plasma grobkörnig. Bräunliche Nahrungskörper, Diatomeen etc. Fölisö, Uferpfütze".

Cette déscription peu exacte accentue pourtant le caractère le plus important pour cette espèce à savoir la ligne spirale suivie par la bande de trichocystes qui entoure le corps. D'après la monographie de Kahl chez deux espèces seulement la bande de trichocystes a la forme de spirale: chez *Strombidium grande* et *Strombidium obliquum*. Chez toutes autres les trichocystes forment une ceinture ou des lignes verticales. Ces deux espèces citées se distinguent bien l'une de l'autre par la forme du corps. Les auteurs contemporains qui s'occupent des cilliés marins psammiques comme Borror (1963, 1965, 1968, 1972), Dragesco (1965) Fauré-Fremiet (1948, 1951), Agamaliev (1972), Burkovsky

(1970) n'ont pas trouvé une forme pareille parmi les oligotriches qu'ils ont rencontrés.

J'ai trouvé un cilié correspondant à la déscription de Levander dans un prélèvement d'eau marine provenant de la mer Noire. Le prélèvement a été pris du sable humide sur une plage en Roumanie et gardé pendant quelques semaines au laboratoire dans un bocal. Les ciliés se nourrissaient de bactéries et de diatomés. Ils se sont multiplié si bien qu'il était possible de faire les préparations. Les préparations imprégnés à l'argent d'après la méthode de Chatton ont revelé la bande de trichocystes et la structure de la surface du corps, d'autres préparations faites avec le proteinate d'argent d'après la méthode de Tuffrau ont montré le noyau, les membranelles et la stomatogenèse.

La longueur du corps ovoïde est moyenne — environ 50 μm bien que le nom donné par Levander suggère une taille plus grande. Le puissante frange adorale entoure en spirale la protuberance sur le pôle antérieur et descend vers le cytostome sur le côté ventral. Elle compte environ 45 membranelles. Au-dessous d'elle, dans la partie equatoriale du corps commence la bande de trichocystes qui court à droite, descend en spirale sur le côté dorsal et rejoint son commencement en suivant une ligne verticale (Fig. 1, Pl. I). La surface du corps contenue entre la frange adorale et la bande de trichocystes montre une structure qui ressemble à un reseau de fibrils composé d'irregulières mailles polygonales. La partie postérieure du corps a la surface lisse. Le noyau est sphérique ou ovoïde.

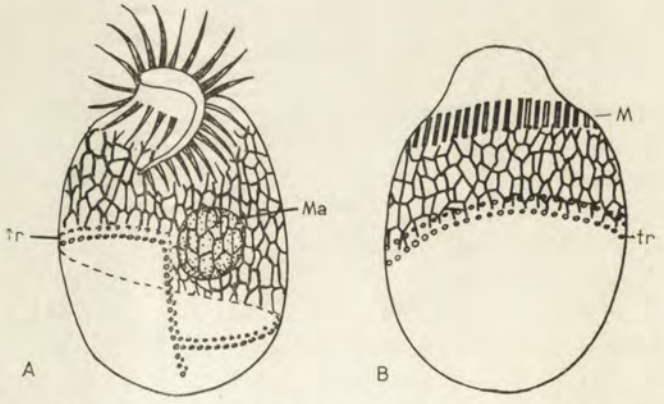

Fig. 1. La morphologie de *Strombidium grande* Levander. A — l'animal vu du côté ventral, B — le côté dorsal, tr — la bande de trichocystes, Ma — macronucleus, M — les bases des membranelles

Le trait le plus curieux dans la morphologie de ce cilié est la situation du réseau qui occupe la partie centrale du corps tandis que chez les autres espèces de ce genre la partie basse du corps. Quant à la nature de cette structure les opinions sont divisées. Fauré-Fremiet (1948)

qui l'a examiné chez *Strombidium oculatum* constate que ce sont les plaquettes d'hydrates de carbone Borror (1965) qui a décrit la même structure chez *Strombidium sulcatum* prétend qu'il s'agit d'une couche d'alvéoles aplaties.

SUMMARY

The morphology of the ciliate of the genus *Strombidium*, found at the Black Sea shore, is described. The species is probably identical with that described by Levander under the name *Strombidium grande*. The characteristic feature of this ciliate is the shape of trichocyst zone, which begins in the equatorial part of the cell, goes to the left side and then, spirally passes down to the dorsal side. Its posterior end joins the anterior part through a perpendicular line. The reticular structure of the body surface, typical of the genus *Strombidium*, occupies the median part of the body, between the zone of membranelles and the zone of trichocysts, not the posterior body part as in other species.

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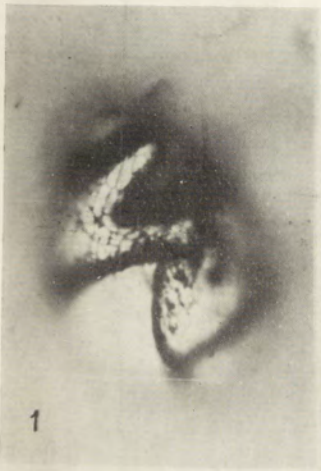
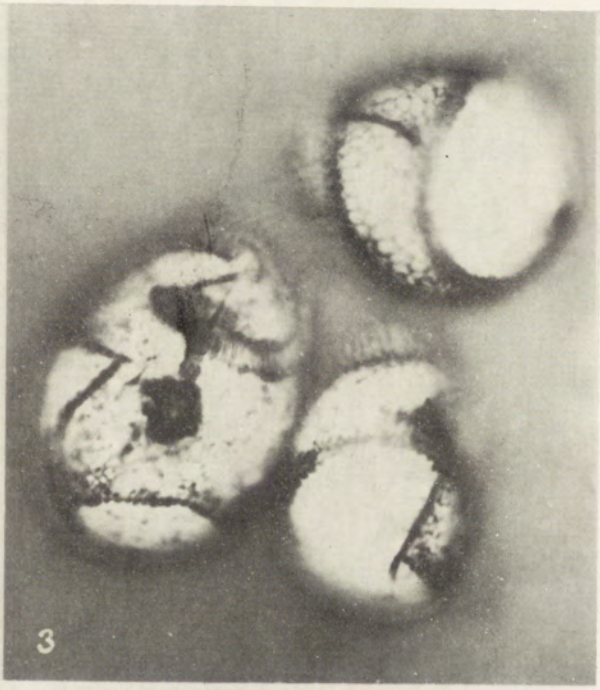
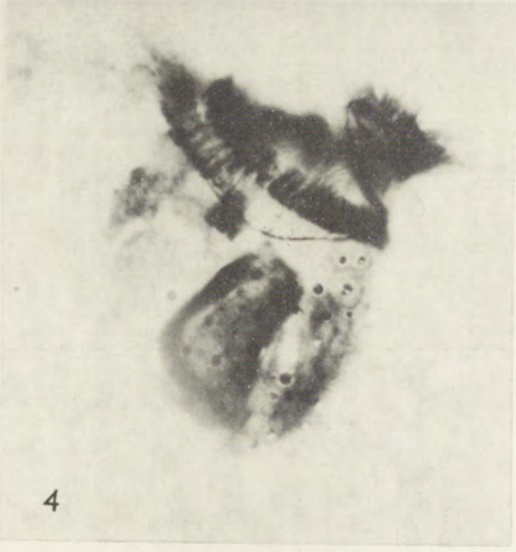
EXPLICATION DE PLANCHE I

Strombidium grande Levander

1: Animal vu du coté ventral
2: Le côté dorsal
3: Trois individus dans differentes positions montrant la bande de trichocystes. Chez l'individu à gauche AZM de l'opisthe est en train de se former
4: La puissante frange adorale est visible. Au-dessus du noyau AZM de l'opisthe







A. Czapik auctor phot.

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ANNA CZAPIK et ANNA JORDAN

Les observations sur les ciliés d'une mare

Synopsis. On observait pendant une année une petite mare qui apparaissait après chaque pluie sur une prairie. On a constaté la présence d'une microfaune riche et variée. On a trouvé 25 espèces de ciliés dont une nouvelle. La nourriture de base pour les ciliés consistait en bactéries, flagellés verts et Cyanophycés.

Nous avons étudié pendant une année les ciliés vivant dans une petite mare qui apparaissait après chaque pluie sur une grande prairie située tout près de l'Institut Zoologique. Cette mare a attiré notre attention par son fond bleu-vert qui laissait supposer l'existence des différents organismes. Le premier prélèvement a confirmé cette supposition.

Le sol de la prairie est bien gras grâce à un troupeau de vaches qui parfois demeurent ici et aux nombreux chiens qui s'y promènent. Notre mare avait environ 0.5–2 m² et son profondeur ne dépassait jamais 4 cm (le plus souvent 1–2.5 cm). En automne la mare persistait 2–3 jours après la pluie; en été elle disparaissait plus vite. Le monde vivant apparaissait déjà en quelques heures après la pluie.

La temperature de l'eau dans la mare était changeante, selon la temperature de l'air et la force du soleil. Elle était le facteur le plus important qui déterminait la vie dans la mare. Au début d'avril quand la température de jour oscillait autour de 10°C la microfaune était pauvre; on ne rencontrait que des individus isolés. L'augmentation de la température dans la deuxième moitié de ce mois était suivie d'un brusque développement des algues ainsi que des animaux. Vers la fin du novembre avec les premiers gels la vie dans la mare commençait à disparaître.

pH oscillait entre 6.4–7.8. L'eau de la mare riche en substances organiques présentait un milieu favorable au développment des flagellés verts et des cyanophycés; *Euglena viridis, Chlamydomonas* sp. et *Pandorina* sp. flottaient abondamment dans l'eau; sur la vase s'étalaient les filaments de *Phormidium subfuscum.* Toutes ces formes assuraient la nourriture de base pour les ciliés algivores. Les métazoaires étaient représentés par quelques espèces des rotifères qui, surtout en été, apparaissaient en grand nombre. (*Epiphanes senta, Philodina citrina, Brachionus urceolaris).*

On prenait les prelèvements à l'aide d'une cuillère à soupe en râclant la couche superficelle de la boue avec de l'eau. Au laboratoire on mettait les échantillons dans de petits aquariums Après une heure on commençait les observations. Les ciliés étaient d'abord étudié in vivo; ensuite, si leur nombre le permettait, on faisait les préparations en utilisant la méthode de Chatton modifiée par Corliss et celle de Bodian modifiée par Tuffrau.

La liste des espèces trouvées

Pseudoprorodon niveus Ehrenberg, 1838

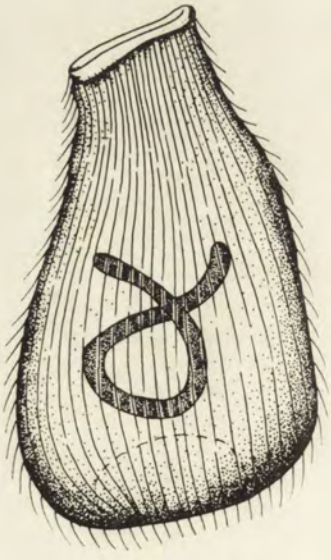
Le cilié identifié par nous comme Pseudoprorodon niveus ressemblait bien à la déscription de Kahl (1930). Nous l'avons trouvé seulement une fois en octobre en trois exemplaires.

Didinium nasutum O. F. Müller, 1786

Askenasia volvox Claparède et Lachmann, 1858

Ces deux ciliés correspondaient bien aux déscriptions des auteurs précedents (Kahl 1930, Dingfelder 1962)

Spathidium puteolagris Baumeister, 1930



Ce cilié était trouvé seulement deux fois: par Baumeister in Kahl (1930) et par Dingfelder (1962) dans l'eau polluée par le purin. Nous avons rencontré *Spathidium puteolagris* seulement une fois en deux exemplaires (Fig. 1) En général ils correspondaient bien aux déscriptions précedentes mais nous ajoutons quelques observations plus précises. De 160–180 μm de long et 50 μm de large dans sa partie postérieure le cilié porte 48–50 cinéties. La brosse est peu distincte sur l'animal vivant, les cils assez longs et fins. Les trichites de la bouche sont puissant.

Fig. 1. *Spathidium puteolagris* Baumeister

Nassulopsis elegans (Ehrenberg 1838)

Cette espèce a été décrite par des auteurs anciens (Ehrenberg 1838, Kahl 1930, Gelei 1950) sous le nom de *Nassula elegans*. Elle se distingue par le corps allongé, parfois vermiforme, très légèrement courbé à gauche, qui contient un macronucleus fusiforme. Les vacuoles contractiles au nombre de 5 forment une rangée longitudinale. La frange ciliaire fait un tour complet autour du corps (Pl. I 1). Chez tous les autres représentants du genre *Nassula* le macronucleus est ovoïde et la frange ciliaire fait un demi-tour. En s'appuyant sur ces deux caractères Fauré-Fremiet (1959) a crée pour cette espèce le genre nouveau — *Nassulopsis*. Cette espèce était fréquente dans la mare et pendant la saison chaude parfois très nombreuse. Elle se nourrissait de la cyanophycée *Phormidium subfuscum*. Les vacuoles digestives d'abord bleu-vert devenaient rosâtres au fur et à mesure que la digestion avançait.

Nassula tumida Maskell, 1887

La population de cette espèce trouvée dans la mare correspondait bien à la déscription de Kahl (1930), seulement les individus étaient plus petits; la longueur du corps oscillait entre 80–100 μm Le cytoplasme jaunatre de ces ciliés était rempli de vacuoles vertes contenant les cyanophycés (*Phormidium subfuscum*). La frange ciliaire de cette espèce commence au-dessous du cytostome et court vers le côté gauche où elle se termine (Pl. I 2,3). Cette espèce était fréquente dans la mare pendant la saison chaude.

Nassula citrea Kahl, 1930

Ce cilié dont les caractères ont été très bien précisés par Kahl (1930) (le corps elipsoide, le cytoplasme jaune, une tâche orange au-dessous du pôle antérieur) montre une variabilité de forme assez remarquable. Kahl a remarqué qu'à côté des individus moyens dont le corps mesure 70–90 μm de long, une petite forme de 30–40 μm aparaît. En cultivant une population de *Nassula citrea* dans les boîtes de Petrie nous avons constaté qu'on y peut distinguer trois formes selon le dégré d'alimentation. Les individus qui viennent d'absorber une grande quantité de la nourriture (la cyanophycé *Phormidium subfuscum*) augmentent leur dimensions (le corps atteint 120 μm de long) et le cytoplasme se remplit de vacuoles digestives vertes qui masquent tous les détails morphologiques. Si l'on isole de tels individus en les privant de nourriture, ils s'enkystent, et après avoir digéré les algues ils quittent les kystes

sous la forme habituelle, moyenne. S'ils ne trouvent pas de nourriture ils s'enkystent de nouveau et ensuite abandonnent les kystes sous la petite forme d'environ 40 μm. La frange ciliaire de cette espèce commence juste au-dessous du cytostome et court à gauche suivant la ligne perpendiculaire à l'axe longue du corps; après avoir atteint le côté gauche elle descend obliquement sur le côté dorsal où elle se termine (Pl. I 4). Cette espèce était fréquente et parfois nombreuse pendant la saison chaude. En comparant la déscription de *Nassula rotunda* Gelei, 1950 avec les caractères de *Nassula citrea* Kahl, 1930 on a l'impression qu'il s'agit de la même espèce.

Nassula pratensis **n. sp.**

En juin une autre espèce de *Nassula* a apparu dans la mare; elle se distinguait des autres espèces décrites jusqu'à présent. Le corps qui mesure 300–400 μm de long est largment ovoïde; les individus fort alimentés sont presque spheriques. Le cytoplasme est jaunâtre, fort vacuolisé. La pellicule contient des trichocystes en forme de fuseau. La nasse est longue (120–160 μm), composée d'environ 16 trichites, la ciliature très dense. Une simple vacuole contractile est située dans la partie equatoriale du corps à gauche. Le frange ciliaire qui commence à coté gauche du cytostome court vers le flanc du corps et descend sur la surface dorsale où elle se termine après avoir fait un demit-tour. Chaque pinceau de cils qui forment la frange consiste en 5 cinétosomes. Les bases de ces cils sur la surface ventrale se superposent comme les tuiles du toit (Pl. II 5–7), ce n'est qu'après avoir passé sur le coté dorsal qu'elles forment une ligne continue comme chez les autres espèces de *Nassula* (Fig. 2) Les individus trouvés dans notre mare se nourrissaient de *Phormidium subfuscum*. Leur vacuoles digestives d'abord vertes devenaient jaune-brune au

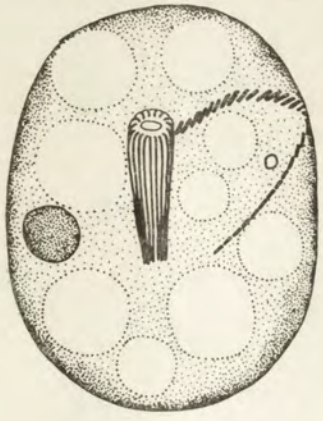


Fig. 2. *Nassula pratensis* n. sp.

fur et à mesure que le procès digestif avançait. Nous avons trouvé la même espèce dans une autre mare dans la vallée de la rivière Prądnik. Là-bas les animaux se nourrissaient de *Phormidium uncinatum* qui donnait à leurs vacuoles la couleur jaune-orange. Les kystes qu'elles formaient aussitôt que la nourriture manquait, étaient de la même couleur. Elles mesuraient environ 200 μm en diamètre; leurs parois étaient épaisses.

En lisant l'étude de Dingfelder (1962) nous avons eu l'impression que l'animal qu'il décrivait sous le nom de *Nassula ornata* est identique avec notre espèce nouvelle. Malheureusement l'auteur allemand ne faisait pas de préparations imprégnées à l'argent qui auraient pu confirmer cette supposition.

Cyclogramma trichocystis (Stokes, 1894)

Cette espèce était assez nombreuse en juin. Elle se nourrissait de *Phormidium subfuscum*. Les individus trouvés étaient typiques et s'accordaient avec la déscription de Fauré-Fremiet (1967 a, b).

Colpoda penardi Kahl, 1930

Rencontré une seul fois le cilié correspond bien à la déscription de Kahl (1930).

Tillina magna Gruber, 1880

Tillina magna ainsi que l'espèce suivante font partie d'un grupe des ciliés vivant dans la mare pendant toute la saison chaude c'est-à-dire du printemps jusqu'à l'automne. C'est un cilié omnivore; les individus observés avaient les vacuoles digestives remplies d'habitude par les petits ciliés. Une excellente déscription de cette espèce a été donné par Dingfelder (1962).

Woodrufia rostrata Kahl, 1930

Nous rencontrions ce cilié (Pl. II 8) aussi souvent que *Tillina magna*. Il se distinguait par une assez grande variabilité de taille et de forme. La population vivante dans la mare ingérait les flagellés du genre *Pandorina*. Kahl a observé la population qui se nourrissait exclusivement de *Oscillatoria*.

Frontomia acuminata Ehrenberg, 1833

Paramecium caudatum Ehrenberg, 1838

Disematostoma bütschli Lauterborn, 1894
Disematostoma invallatum Gelei, 1954
Lembadion lucens (Haskell, 1887)

Ces espèces bien connues et communes ont été décrites par plusieurs auteur (Gelei 1950, Roque 1961, Dingfelder 1962). Nous n'avons rien à ajouter à ces déscriptions sauf les microphotographies faites des préparations imprégnées à l'argent de *Disematostoma bütschli* et *D. invallatum* (Pl. II 9–11 et 12–13).

Sathrophilus muscorum (Kahl, 1930)

Nous avons rencontré quelques exemplaires de ce cilié seulement une fois en octobre (Fig 3). Ils correspondaient exactement aux déscriptions de Kahl (1930) et de Stout (1956).

Blepharisma lateritium (Ehrenberg, 1831)

Les exemplaires trouvés par nous correspondaient bien aux déscriptions précedentes (Kahl 1930, Hirshfield et al. 1973). Nous avons observé les individus qui avaient le cytoplasme transparent, incolore (Fig. 4).

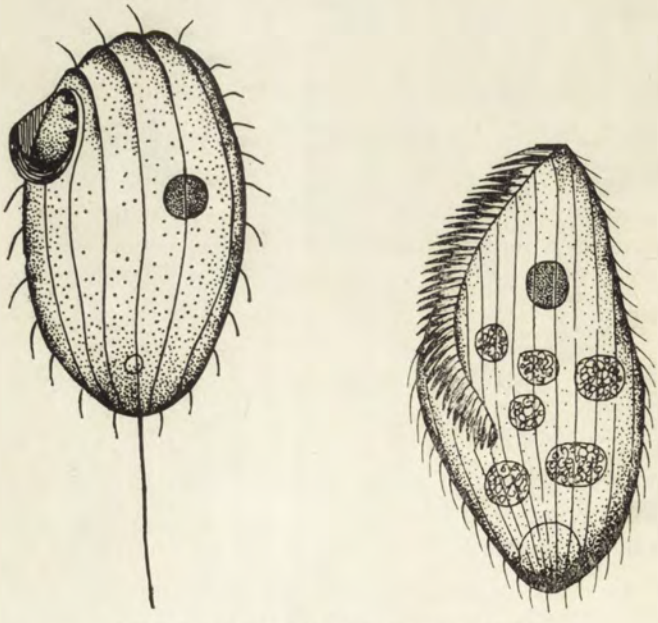


Fig. 3. *Sathrophilus muscorum* (Kahl)
Fig. 4. *Blepharisma lateritium* (Ehrenberg)

Bursaria truncatella O. F. Müller, 1786

Ce grand cilié dont une excellente redéscription a été donné par T u f f r a u (1967) apparaissait assez souvent dans la mare pendant la saison chaude. Cette espèce omnivore a été notée dans différents types de réservoirs d'eau et semble être un ubiquiste.

Halteria grandinella (O. F. Müller, 1786)

On a trouvé cette espèce commune plusieurs fois. Les individus étaient typiques; ils se nourrissaient de bactéries.

Stichotricha secunda Perty, 1852

Nous avons rencontré ce cilié quelques fois toujours en grand nombre (Fig. 5). K a h l (1930) en a donné une déscription très détaillée à laquelle nous n'avons rien à ajouter. Notre population se nourris sait de flagellés *Chlamydomonas* et *Euglena*.

Fig. 5. *Stichotricha secunda* Perty

Fig. 6. *Paruroleptus strenuus* Dingfelder

Paruroleptus strenuus Dingfelder, 1962

Ce curieux hypotriche a apparu en grand nombre seulement en septembre quand l'eau de mare avait la temperature 8–12°C. Son identification était très difficile; il ressemble fort *Paruroleptus strenuus* décrit par Dingfelder (1962) qui l'a trouvé dans le même biotope. Borror (1972) n'a pas pris en considération le travail de Dingfelder. Avant qu'on entreprenne les études sur la morphogenèse lesquelles nous puissent mieux renseigner sur sa position systematique il nous semble mieux de redécrire ce cilié sous le vieux nom *Paruroleptus strenuus.*

Les individus trouvés par nous ont montré quelques différences concernantes surtous l'infraciliature en comparaisson avec la déscription originale (Fig. 6). Il est possible que ces différences resultent des observations faites par Dingfelder uniquement sur le materiel vivant. La taille de l'animal est relativement grande (250–500 μm); nos individus étaient plus grands que ceux decrits par Dingfelder (103–326 μm). La région apicale est élargie, la region caudale pointue et un peu courbée à droite. L'observateur est surtout frappé par l'énorme péristome (sa longueur totale peut atteindre 180–200 μm) flanqué par 60–70 membranelles adorales. Dingfelder a observé les exemplaires plus petits qui en portaient 50–60. La membrane ondulante (UM) a 120–180 μm de long. L'infraciliature est constituée par trois rangées des cirres marginaux, trois rangées de cires ventraux, un groupe de cirres frontaux et 2–6 cirres tansversaux. Les cirres marginaux droites en nombre de 40–44 débutent au voisinage des premières membranelles adorales et descendent jusqu'au pôle posterieur pour joindre 22–23 cirres marginaux gauches. Les cirres ventraux sont groupés en trois rangées (Pl. IV 14–15). La première à gauche debutante au centre de l'animal consiste en 7 cirres et mene jusqu'au deuxième cirre transversal. La deuxième rangées commence sur le bord droit du corps près du pole antérieur du cilié; il consiste en 14 cirres dont le dernier est aussi puissant que les cirres transversaux. La troisième rangée, la plus courte consiste en 6 cirres. Le groupe de cirres frontaux consiste en trois cirres allongeant le pôle antérieur et deux rangées longitudinales, chacune à quatre cirres. Chez quelques exemplaires nous avons observé encore un cirre de plus dans le rang le plus proche du péristome. Au bout du corps il y a deux cirres qui ressemblent les cirres caudales. L'appareil nucléaire se compose de quatre macronuclei. C'est vrai que d'après Dingfelder cette espèce possède 8 macronuclei mais probablement l'auteur a observé les individus qui entraient dans le stade division. Nous avons remarqué que longtemps avant que la division de la cellule commence le nombre de macronuclei double. *Paruroleptus strenuus* se nourrit de petits ciliés aussi bien que

de flagellés et de diatomés (le même a été observé par Dingfelder). En avenir il sera probablement nécessaire de revenir à l'étude de cette espèce pour établir sa position dans la systématique des ciliés hypotriches.

Telotrochidium hennegui (Fauré-Fremiet, 1906)

Ce cilié qui est typique pour les eaux riches en bactéries a été trouvé aussi dans de petites mares par Gelei (1950) et Dingfelder (1962). Il montre une certaine variabilité de forme. La population que nous avons rencontrée se composait des formes larges et courtes; la forme longue était rare (Pl. IV 16–17).

Hastatella radians Erlanger, 1890

Ce peritriche interéssant a été redécrit par Fauré-Fremiet (1924) et Dingfelder (1962). Il est typique pour les eaux contenant beaucoup de bactéries dont il se nourrit. Nous l'avons trouvé en juin en nombre assez grand (Pl. IV 18 a, b).

Discussion

Les premiers chercheurs qui on pris l'intéret des petits réservoires d'eau ephémères pouvaient constater que ceux-ci sont habités par une microfaune non seulement riche mais abondante en formes inconnues, absentes dans d'autres milieux. Gelei (1950) a découvert dans les mares aux différents endroits de la Hongrie plusieurs espèces nouvelles, entre autres les bizarres hypotriches planktoniques (*Spiretta, Hypotrichidium).* Dingfelder 1962) a enfermé les résultats de ses recherches sur les ciliés des mares dans une vaste étude écologique en précisant les conditions physico-chimiques ainsi que nutritives de ce milieu. Récemment Buitkamp et Wilbert (1974) ont étudié quelques espèces des ciliés trouvées dans le sol d'une prairie canadienne.

Dans l'assemblée des ciliés de notre mare deminaient les formes algivores: toutes les espèces de *Nassula* et *Disematostomma, Cyclogramma trichocystis, Woodrufia rostrata, Stichotricha secunda, Frontonia acuminata*; elle étaient nombreuses et parfois apparaissaient en masse. Les ciliés microphages étaient moins nombreux; on trouvait sporadiquement les individus isolés de *Paramecium caudatum, Sathrophilus muscorum, Colpoda penardi, Blepharisma lateritium*; seulement *Halteria grandinella* et *Telotrochidium hennegui* étaient plus fréquents. Les ciliés omnivores

étaient représentés par *Bursaria truncatella* et *Tillina magna*. Les deux espèces étaient fréquentes prédateurs étaient rares; on a rencontré deux fois quelques individus de *Didinium nasutum* et une fois *Pseudoprorodon niveus*.

Les espèces apparaissaient et disparaissaient d'une facon imprevue. Après presque chaque pluie la mare renaissante présentait une image différente. *Disematostoma bütschli* s'est montré comme l'espèce la plus résistante, qui vivait depuis le printemps jusqu'aux derniers jours avant le gel.

Il faut encore accentuer qu'il y a des espèces qui semblent préferer ce milieu peu durable, aux autres, constantes. On y peut classer par exemple *Woodrufia rostrata* qui d'après nos expériences est plutôt rare; nous n'avons rencontré que quelques individus dans une rivière, tandis que dans la mare elle apparaissait périodiquement en grand nombre. L'autre exemple est *Nassula pratensis* et *Paruroleptus strenuus*, qu'on a trouvé exclusivement dans la mare.

SUMMARY

Rich and diverse microfauna has been observed during one year in a small rainy pool situated in the meadow. In this pool 25 species of ciliates have been found, one of them appeared to be new. Bacteria, green flagellates and cyanophyces constituted main food for them.

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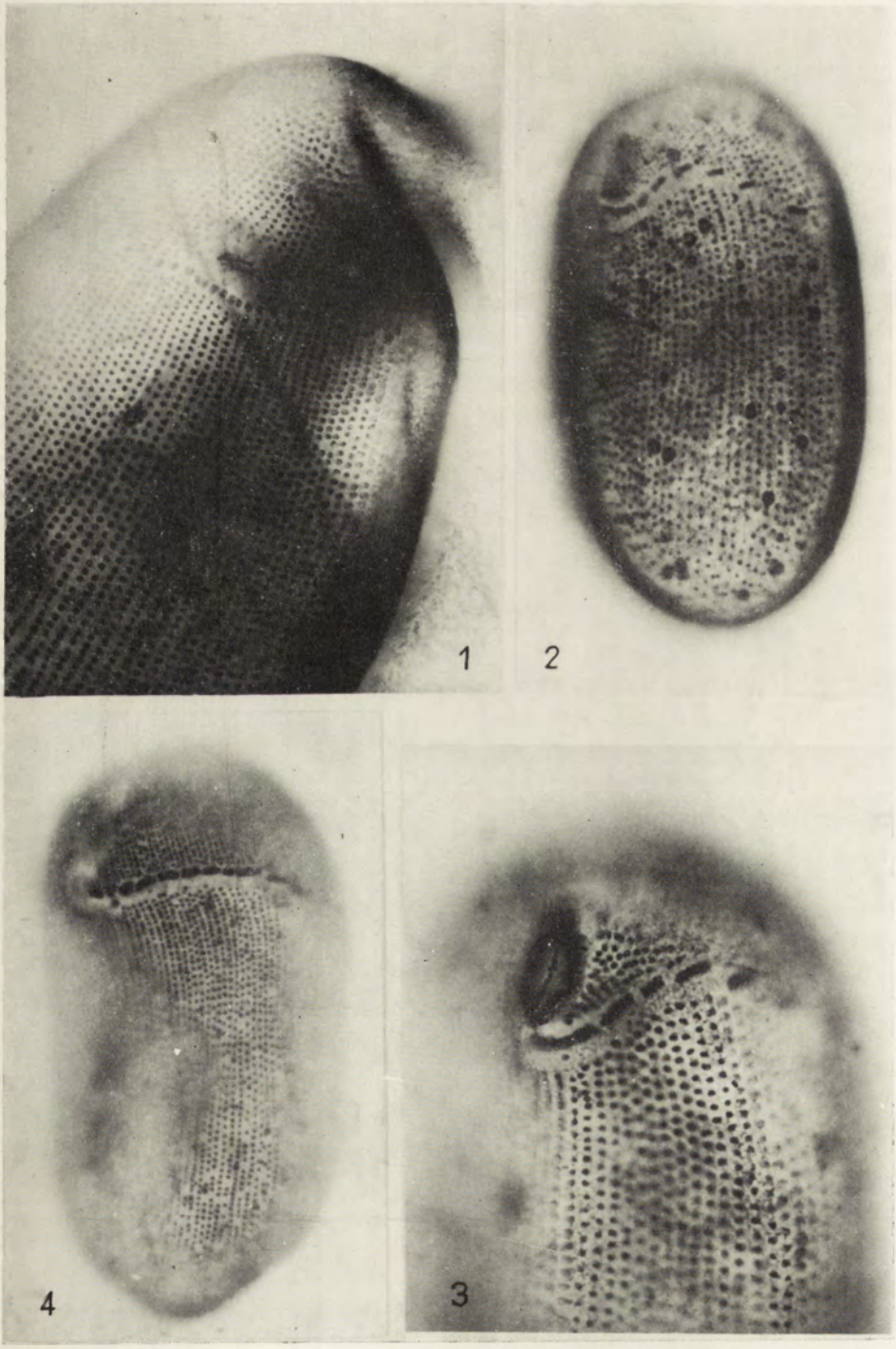
EXPLICATION DES PLANCHES I–IV

1: *Nassulopsis elegans* (Ehrbg.)
2–3: *Nassula tumida* Maskell, 2 — vue générale de l'animal, 3 — frange ciliaire
4: *Nassula citrea* Kahl
5–7: *Nassula pratensis* n. sp., 5 — vue générale de l'animal, 6—7 la frange ciliaire sur le coté ventrale
8: *Woodrufia rostrata* Kahl — le pole anterieur
9–11: *Disematostoma butschli* Lanterborn, 9 — vue générale, 10 — bouche avec les cinéties vestibulaires, 11 — penicules
12–13 *Disematostoma invallatum* Gelei, 12 — bouche avec les cinéties vestibulaires, 13 — penicules
14–15: *Parauroleptus strenuus* Dingfelder, 14 — vue générale de l'animal, 15 — péristome
16–17: *Telotrochidium hennegui* (Fauré-Fremiet)
18 a, b: *Hastellata radians* Erlanger



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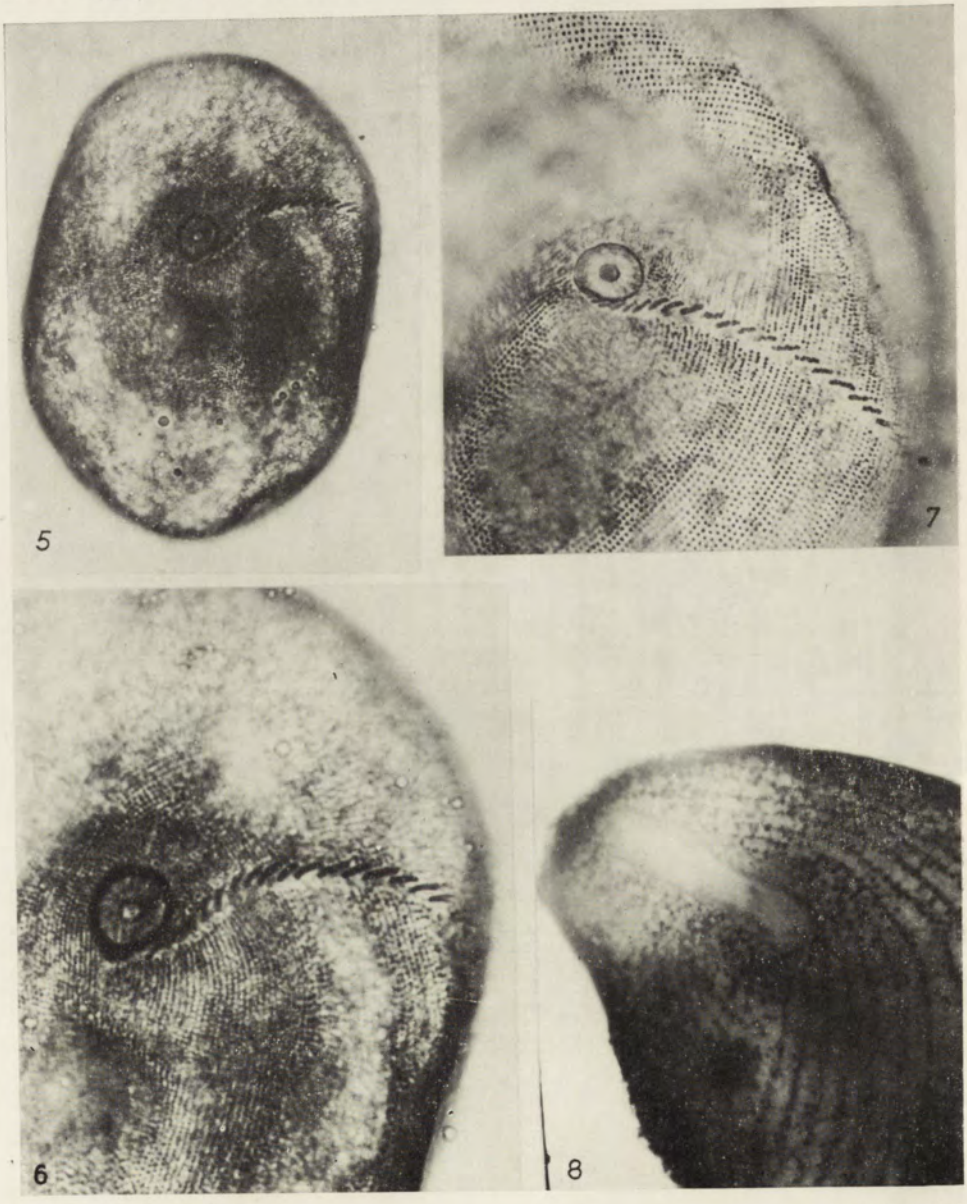

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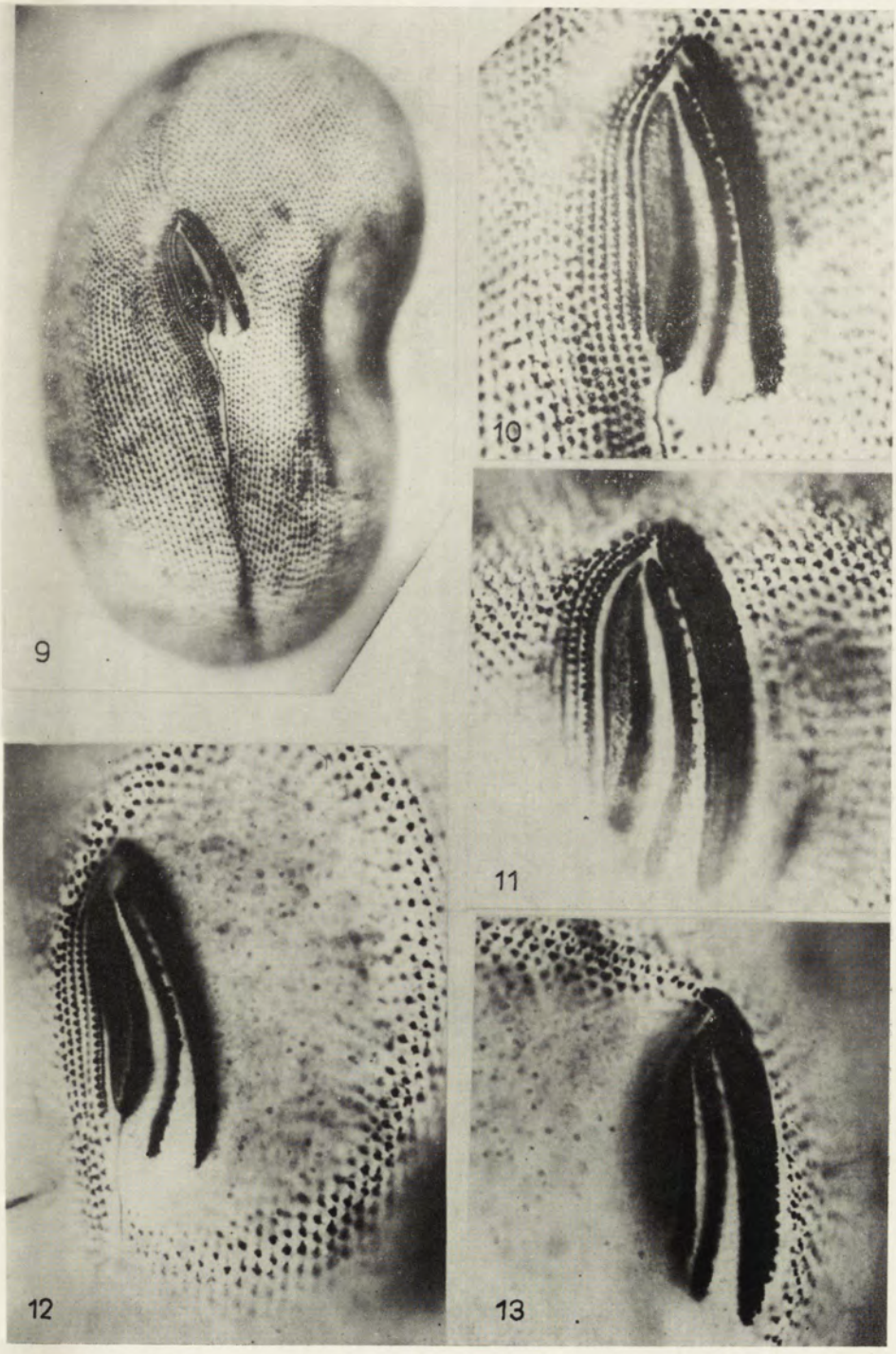

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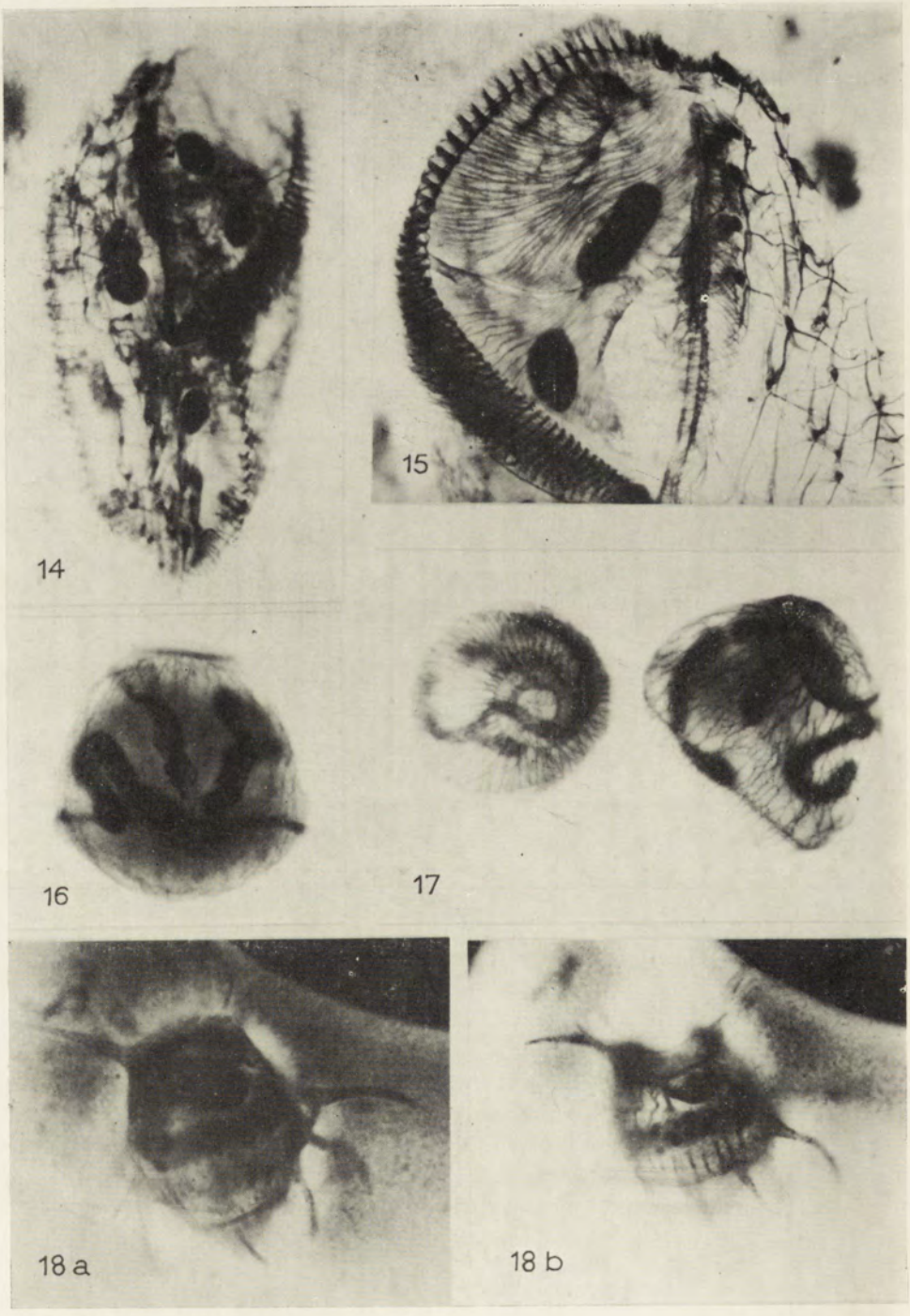

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Chilomastix hemidactyli n. sp. from a Lizard *Hemidactylus* of Warangal, A. P. India

Synopsis. Chilomastix hemidactyli n. sp. was found in the rectal contents of a lizard *Hemidactylus*. It is round or oval in shape with a length of (7.0–15.6 μm) 22.3 μm and a breadth or (4.3–8.6 μm) 13.85 μm. The nucleus is round with distinct endosome. Blepharoplasts are 2 or 3 in number, cystostome is small 1/4 or 1/2 of the body length with a distinct cytostomal fibrils.

Parasites of genus *Chilomastix* Alexeieff, 1912 (Order *Retortamonadidae* Grassi, 1912, Family *Retortamonadinae* Wenrich, 1932) are common in the rectum of various vertebrates. A number of species have been described from mammals but very few from reptiles. Moskowitz (1951) described *Chilomastix bursa* from reptiles and in 1961 Janaki Devi has reported another parasite *Chilomastix wenyoni* from *Calotes nemorcola*. Wenyon (1920) reported a *Chilomastix* from an Egyptian lizard and did not give any name. In course of study of Protozoan fauna of lizards, an interesting species of *Chilomastix* was encountered from a species of *Hemidactylus* lizard. The present communication gives a full account of the morphology and a detailed discussion justifying its recognition as a distinct species.

Material and Methods

For the study of the intestinal flagellates of reptiles, the lizards of the genus *Hemidactylus* were examined. The animals were brought alive to the laboratory for examination and they were examined immediately. This parasite was abundant in the rectum of only one lizard out or 20 lizards examined and this was found in association with *Proteromonas* and the infection was heavy. It could easily be distinguished by its characteristic movement. The movement was comparatively slow and the body propelled forwards by twisting itself in a clockwise direction. The flagella were clearly seen beating the body. Permanent slides were made by

fixing the smears in methonal and staining with Giemsa's stain or fixing in Schaudinn's fixative and stained with Haidenhains iron haematoxylin. One hundred individuals were taken into account for measurements. All the drawings were made with the help of camera lucida at a magnification of 2200 ×.

Morphology

The shape of the body is spherical (Figs. 1–3, 7) or slightly oval (Figs. 4–6) with abruptly pointed posterior end which is drawn out into a short spike (Figs. 1–7). In few forms posterior end is rounded. The cytoplasm is vacuolated and contains bacteria and other granules. The pellicle is very thin and is not distinguishable. The nucleus is situated very close to the anterior extremity and is spherical in shape. It possesses

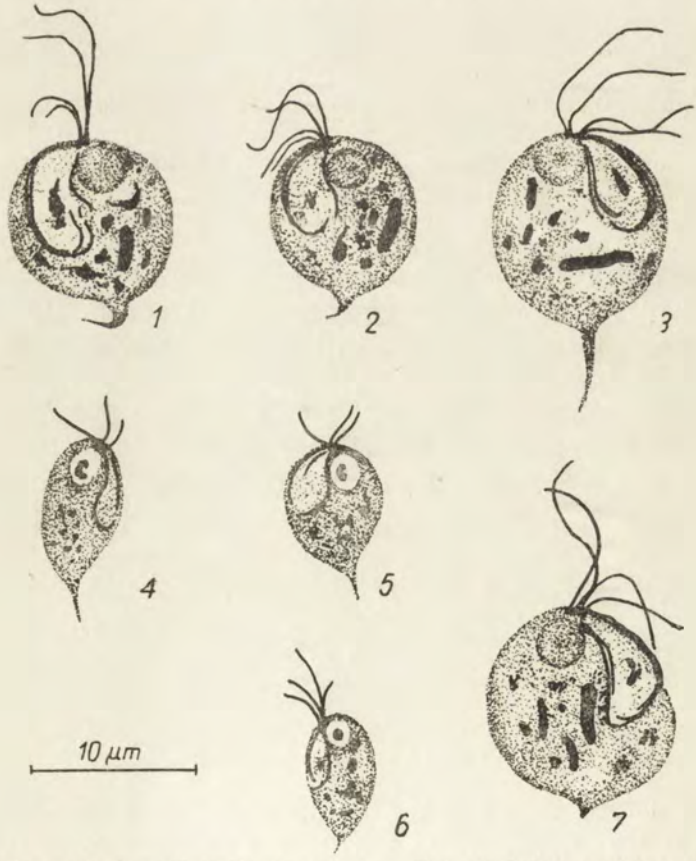

Fig. 1–7. *Chilomastix hemidactyli* sp. n. 1–3 — Spherical form with a short spike showing sac-like cytostome with distinct cytostomal flagellum, two blepharoplasts, two short and two long flagella, 4, 5 — Oval forms showing irregular endosome in the nucleus, 6 — Oval form showing spherical endosome, 7 — Showing sac-like cytostome and three blepharoplasts.

(Figs. 1–3, 7, fixed in methonal and stained with Giemsa's stain. Figs. 4–6, fixed in Schaudinn's fixative and stained with Heidenhain's iron haematoxylin)

a distinct nuclear membrane with irregular endosomal mass in the centre and a clear space around it (Figs. 4–6).

The blepharoplasts are two to three in number and are quite away from each other. One blepharoplast is always situated just at the origin of the left fibril of the cytostome. Two flagella originate from one blepharoplast and the cytostomal flagellum originates from the blepharoplast which is close to the cytostome. The flagella are unequal in length two being long and two short of which one extends into the cytostome.

The cytostome is short and extends either $^1/_4$ or $^1/_2$ of the body length but never more than that. The cytostome is sac-like with anterior end narrow and posterior end broad. It is supported by two distinct fibrils,

Table 1

Chilomastix hemidactyli sp. n. The measurements of various organelles (in μm)

	Length range	Average	Breadth range	Average
Body	7.0–15.6	22.3	4.3–8.6	13.85
Nucleus	1.36–2.9	4.5	1.36–2.9	4.5
Cystostome	2.5–5.4	4.3	1.36–2.7	2.3
Flagellum I	2.5–5.9	3.7		
Flagellum II	2.2–6.3	3.9		
Flagellum III	2.5–6.3	4.1		
Flagellum IV	2.7–6.6	4.4		

the left one is thick and originates from the blepharoplast from which the cytostomal flagellum originates and takes a deep stain (Figs. 1–8). The right is thin its origin is not clear. Measurements are given in Table 1.

Discussion

A detailed study of the parasite shows that it differs considerably from the species of this genus described from reptiles so far. *Chilomastix bursa* Moskowitz, 1951 differs from the present species in number of characters. It ranges in length from 0.8–13.0 μm while the species under discussion is 7.3–14.5 μm .In the known form blepharoplast is only one where as in the new form two or three are noticed. Cytostome in *C. bursa* is about more than half of the body length while in new species it is less than half measuring about 4.9 μm in length.

Chilomastix wenyoni Janaki Devi, 1961 is elongated oval, posterior end is drawn out into a long spike-like tail. It measures 6.5–16.0 μm × × 2.5–10.5 μm wit han verage of 11.2 × 6.0 μm. Whereas the present form is round in shape with small posterior spike. It differs in measurements. Nucleus is at the anterior end with chromatin plaques in *C. wenyoni* while in new species a distinct endosome is situated in the center. Cytostome in known form extends $^1/_2$ or $^2/_3$ of the body length but in the new form it is less than $^1/_2$ of the body length. Besides these characters the known form has four or three blepharoplasts while in the new species only two or three.

By the foregoing comparison it appears that the present species is different from all other species so far reported and therefore it is proposed to designate this flagellate from the lizard *Hemidactylus* as *Chilomastix hemidactyli* species nova, after the generic name of the host.

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RÉSUMÉ

Chilomastix hemidactyli n. sp. a été trouvé dans l'intestin droit du lézard *Hemidactylus*. Son corps est rond ou oval, long de (7.0 μm–15.6 μm) 22.3 μm et large de (4.3 μm–8.6 μm) 13.85 μm. Le noyau est rond avec l'endosome distinct. Il y a 2 ou 3 blépharoplastes le cytostome est petit mésurant $^1/_2$–$^1/_4$ de le longueur du corps, avec des fibrilles cytostomeles bien visibles.

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The Morphology and Life History of a New Microsporidian Parasite, *Thelohania orchestii* n. sp. from the Muscles of an Amphipod *Orchestia platensis* Kr. and Experimental Infection

Synopsis. The morphology and life-history of a new species of microsporidian *Thelohania orchestii* n. sp. from the body muscles of *Orchestia platensis* Kr. is described. The spores are found in the body muscles in 8 days after experimental infection and the hosts died in 17 days. The parasite is host specific and is lethal to the host.

Numerous species of *Thelohania* have been reported from a variety of invertebrate and vertebrate hosts. Five of the species described from invertebrates are from amphipods of which one is from a talitrid amphipod (Mercier 1906). The present form from *Orchestia platensis* Kr. is an account of the morphology and life-history of a new form which for reasons discussed below is considered a new species for which the name *Thelohania orchestii* n. sp. is proposed.

Material and Methods

The hosts were collected from damp soil underneath banana plantations and maintained in glass jars (60 × 30 cm) in the laboratory. Care was taken to see that the soil was always kept moist as otherwise the animals die. They were fed on raw potato chips and powdered *Ipomea leaves.* Infected amphipods could be distingushed by their sluggish movement and the presence of a whitish pach at the junction of the cephalothorax and abdomen on the dorsal side.

Smears prepared from the infected hosts were fixed in Schaudinn's fluid or Carnoy's fluid and stained with Heidenhain's iron haematoxylin or by Feulgen's technique. Smears were also fixed in methyl-alcohol and stained with Giemsa after an initial hydrolysis in 1 N HCl. Infected amphipods fixed in alcoholic Bouin's fluid were sectioned at 8 μm thickness and were stained with iron haematoxylin.

Observations

Host species: *Orchestia platensis* Kr.
Host tissue involved: Body muscles.
Type locality: Viskhapatnam, 530002, India.

We did not observe nuclear division during merogony but meronts with variable number of nuclei have been seen in the body muscles. They measure 10.0–12.6 × 5.6–7 μm and contained 20–25 nuclei (Fig. 2).

Sporogony: Sporogonial plasmodia showing 2–8 nuclei can be seen clearly in smears stained with Giemsa. They measured 10.0–12.0 × × 8.0–10.0 μm and gave rise to eight sporoblasts. The nucleus of the sporogonial mother cell undergoes division forming eight binucleate sporoblasts, each of which develops into a spore (Fig. 3).

Description of the spore: Fresh smears from the infected muscle revealed a large number of pansporoblasts each containing eight spores (Fig. 4). They are oval in shape measuring 12.0–14.0 × 9.0–10.0 μm with a thin limiting membrane. As the spores mature they tend to separate.

Mature spores are oval or cylindrical in shape with rounded ends and measured 5.0–5.4 × 2.0–2.5 μm. Fresh spores showed a small vacuole at the posterior end (Fig. 5). No internal structure could be made out. The spore wall is refractive and thick. Spores stained with haematoxylin showed the sporoplasm in the form of a band extending across the spore lying between the polaroplast and posterior vacuole (Fig. 6). The nuclei and the polar filament are clearly seen in preparations stained with chrome haematoxylin, after an initial hydrolysis in 1 HCl for 10 min. Nuclei were usually paired but sometimes only one nucleus was seen in the sporoplasm (Fig. 7). The nuclei appeared as tiny pink dots when stained according to Feulgen's technique. A crescent shaped PAS positive polar cap is present at the anterior end just above the polaroplast (Fig. 8). The polar filament was released when the fresh spores were treated with hydrogen peroxide. The polar filament was seen to make vigorous uncoiling movements when observed under dark ground illumination and was finally ejected out with considerable force. The spores in the pansporoblast get separated during filament extrusion. A fully extruded polar filament is uniformly thin and measured 35–40 μm in length (Fig. 9).

Discussion

Lipa (1967) listed eight species of microsporidians from gammarids (*Amphipoda*) to which a ninth was added by Blunheim and Vavra (1968) from *Gammarus duebani*. Of these four species belong to the genus *Thelohania* Henneguy.

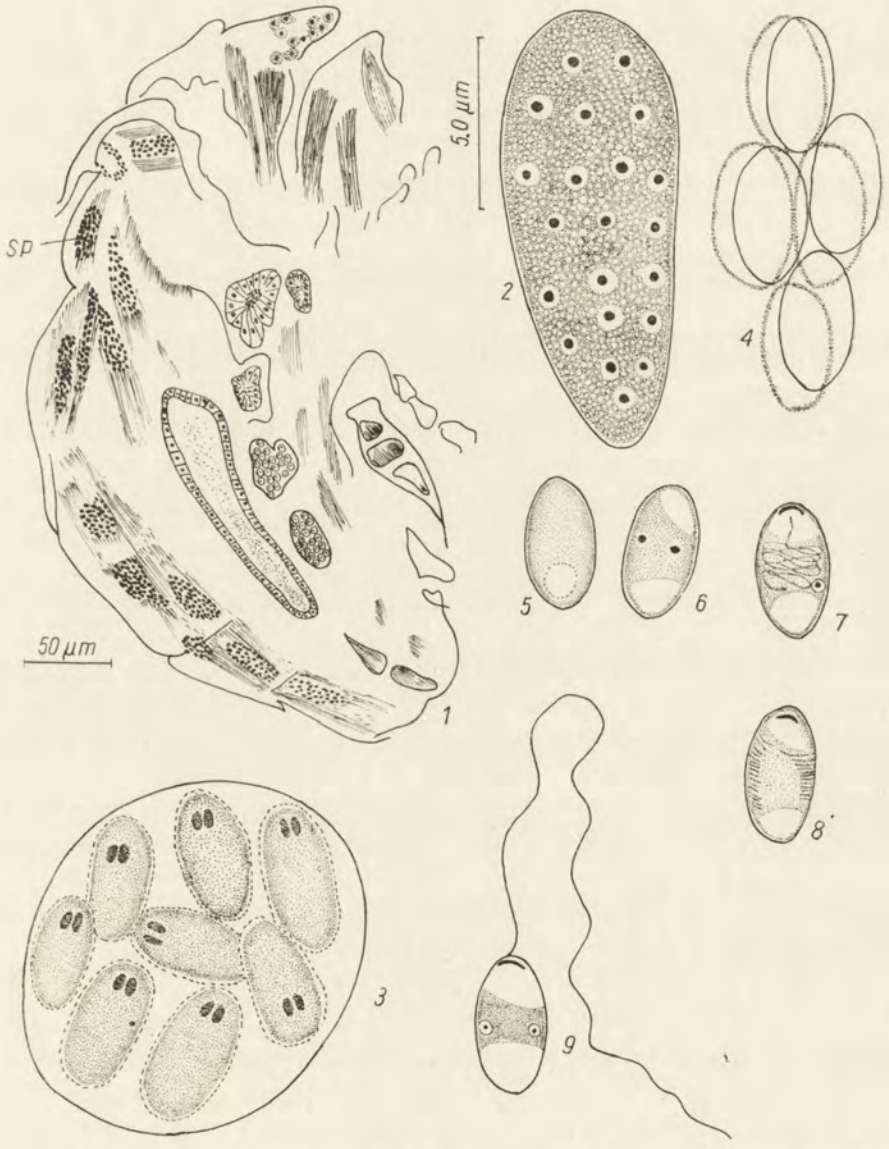

Fig. 1–9, *Thelohania orchestii*. *1* — Section of an amphipod showing the extent of infection of muscles, *2* — A multinucleate meront, *3* — A pansporoblast with developing spores, *4* — Pansporoblast showing the arrangement of spores in the fresh condition, *5* — A fresh spore showing the posterior vacuole, *6* — Spores stained with Heidenhain's iron haematoxylin, 7 — Spore stained with chrome haematoxylin, *8* — Spore stained according to the PAS technique, *9* — Spore with extended polar filament

The only previous report of a species belonging to the genus *Thelohania* from a talitrid is that of Mercier (1906) from the muscles of the heart and body of *Talitrus* sp. (?) obtained from Roscoff, France. He has not given any characters of taxonomic value apart from stating that the spores occur in groups of eight which made him include the form in the genus *Thelohania*. He was unable to demonstrate the presence of a polar filament in the spore (quoted from Kudo 1924).

The present microsporidian from *Orchestia platensis* Kr. belongs to the genus *Thelohania* because eight spores are formed from each pansporoblast. Mercier's account did not give the details of the spore and as such it is not possible to make a comparison of this form with the present species. As this is the first report of a species belonging to the genus *Thelohania* from *Orchestia platensis* Kr. we consider it new to science for which the name *Thelohania orchestii* n. sp. is proposed.

Experimental infection

The amphipod hosts collected from different localities were maintained in the laboratory for a period of one month and periodically examined for microsporidian infection. The hosts were separated into four size groups (1–3 mm, 3–5 mm, 5–7 mm and 7–9 mm). Two or three specimens were kept in each petri-dish on a moist filter paper and fed with raw potato chips and powdered *Ipomea* leaves. The filter paper was always kept moist and changed frequently. Young ones isolated from the brood pouch of the uninfected females were also similarly maintained in the laboratory for one month and used for experimental infection.

The hosts were starved for at least two days before the experimental infection. Spores collected from the infected hosts were tested for their viability by treating them with a suitable method for the extrusion of the polar filament before they were used for infecting clean hosts. Potato chips contaminated with the spores and the carcasses of the infected individuals were fed to clean hosts. They fed on these types of food without any difficulty. The animals fed with the infected material were sacrificed and fixed in alcoholic Bouin's fluid at hourly intervals for the first six hours and thereafter at 24 h intervals for the next ten days. Sections of entire animals were cut at 8 μm thickness and stained with iron haematoxylin. Smears prepared from the infected hosts were air-dried and stained with Giemsa solution. Each experiment was repeated at least three times before any conclusions were drawn.

Results

Large number of spores were found in the stomach at the end of one hour and they get dispersed into the intestine, digestive caeca, excretory tubules and rectum in the course of about 6 h. The spores in the rectum appeared empty as only the spore wall could be made out in stained preparations.

At the end of 24 h meronts appeared in the epithelial cells of the midgut region. A few uninucleate bodies were seen sparsely distributed in the muscle cells by the end of the third day and by the fourth day such bodies were seen in the muscles. Sections of the amphipods taken five days after the infection showed large number of sporogonial plasmodia and a few earlier stages in the body muscles. Smears prepared on the sixth day showed 1–4 nucleate sporogonial plasmodia which completed their development into eight nucleated stages by the eighth day. It is not clear how the sporoplasm which entered the epithelial cells reached the body muscles. Most of the hosts lost their ability to hop by about the fifth day of infection and appeared sluggish. External indications of infection such as white patches on the body surface at the junction between the cephalothorax and abdomen were clearly seen by the seventh day. In about 15 days after the infective feed 70% of the amphipods died, apparently due to the damage caused by the parasite to the body muscles and intestinal epithelium. When such specimens were examined by preparing smears and sections they were found to harbour large number of spores in the body muscles (Fig. 1).

The juvenile forms (1–3 mm size group) picked up the infection quicker than the mature forms and they did not survive for more than 7–10 days. There was no growth during the period of experimental infection. Most of the specimens which were 3–5 mm, 5–7 mm and 7–9 mm in length died on the 15th day after being given infective feeds and the remaining few died on the 17th day. Controls maintained under identical conditions showed an increase in length by about 1–2 mm in 15 days while the experimentally infected forms did not show any growth during the same period.

Discussion

Many workers have studied the influence of microsporidian parasites on the longevity and physiology of the hosts in view of the fact that some of them could be used in microbial control. Experimental infection of the hosts showed that the longevity of the hosts was reduced consi-

derably due to microsporidian infection. Oshima (1935, 1937) infected the silk-worm moth experimentally and found that the body anterior to the fifth segment showed heavy infection on the seventeeth day and all of them died soon. Kudo and Decoursey (1940) experimentally infected *Hyphantria cunea* with *Nosema bombycis* and observed that the spores were formed in 12 days and the larvae died subsequently. Borchert (1940) found that the bees infected with *Nosema apis* died sooner than the uninfected ones. Hassanein (1951) infected bees with *Nosema apis* and spores were produced in 8 days. Thomson (1955) observed that "young" larvae at room temperature die within 7 or 8 days after infection but 5th and 6th instar larvae are sometimes able to survive extremely heavy infections. Hassanein and Ibrahim (1963) observed that the cotton-leaf worm *Prodemia litura* infected with *Nosema bombycis* showed that the 1st and 2nd instar larvae died without moulting and only 56 pupated amongst 4th and 5th instar larvae. Kramer (1965) has shown that there is no significant reduction in the locomotor activity upto 11–13 days in *Phormia regina* infected with *Octosporea muscae-domesticae* Flu but in flies which did not succumb to the infection in 14–19 days there is a definite reduction in locomotor activity. In *Phormia regina* the intestinal epithelium is destroyed and the life expectancy is reduced when infected with *Octosporea muscae-domesticae* (Kramer 1966). The longevity of the infected larvae to *Heliothis* infected with *Nosema heliothidis* was highly reduced by the parasite and on an average death occurred between 12 and 14 days after infection but it varied between 18–22 days depending upon the number of spores ingested (Lipa 1968). In the gypsy moth infected with *Plistophora schubergi* Zwolfer the infected larvae increased to four times their weight by sixth or seventh day (Issi 1968). Kellen and Lindengren (1968, 1969) infected the indian meal-worm *Plodia interpunctella* with *Nosema plodiae* and observed that the larvae with acute infection showed signs and symptoms in 6 days after feeding. Such larvae were stunted and usually succumbed before pupation.

In the present instance the external indications of infection were seen on the seventh day after infection and the spores were formed on the eighth day. 70% of the hosts died by the 15th day and all of them died on the 17th day. It has also been observed that the infected forms were stunted and did not increase in size during the period of experimental infection. The control group, however, showed an increase in length of about 1.0–2.0 mm.

Malek and Steinhaus (1948) followed the invasion route of *Nosema* sp. in potato tuber worm (*Gnorimoschoma operculella*) by ligaturing experiments and found that the route was by way of alimentary

canal and through the wall of the fore gut and mid gut. They further observed that though the parasite was capable of passing through the fore gut and hind gut the invasion through the wall of the mid gut and Malpighian tubules takes place regularly. The results obtained in the present investigation suggest that the microsporidian infecting *Orchestia platensis* Kr. probably follows the same route. The presence of the spores in the mid-gut and excretory tubules suggest that the invasion of the muscles is probably through the mid-gut region and through the excretory tubules.

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RÉSUMÉ

La morphologie et le cycle décrits d'une espèce nouvelle de microsporidie *Thelohania orchestii* n. sp. en provenance des muscles de l'*Orchestia platensis* Kr. Dans 8 jours lors d'une infection expérimentelle on trouve des spores dans les muscles et dans 17 jours l'hôte succombe. Le parasite est spécifique par rapport à l'hôte et sa présence est létale pour ce dernier.

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A. V. USPENSKAJA

The Nuclear Cycle of *Myxosporidia* According to Cytophotometry

Synopsis. The cytophotometric investigation of DNA content in the nuclei of a Myxosporidian *Sphaeromyxa elegini* Dogiel, 1948 was made. It was found that not only the nuclei of sporoplasm but also capsulogenic and valvogenic nuclei are haploid. The doublets of nuclei which some authors regard as the nuclei of macro- and microgametes are diploid. The sporoblast nuclei are diploid. The one-step meiosis takes place after the third mitotic division of sporoblast nuclei. The vegetative nuclei are polyploid (tetraploid in average). The new scheme of nuclear cycle for *Sphaeromyxa* is proposed.

Up to now there is no uniformity in opinions on the nuclear cycle of *Myxosporidia* in spite of numerous morphological investigations (Naville 1931, Noble 1944, Tuzet et Ormieres 1957, Schulman 1966, 1972, Lom 1973, Shulman and Semenowitch 1973).

Schulman (1966, 1972) gave a detailed review of the literature on the nuclear cycle of *Myxosporidia* and classified all the papers into several groups. There are four different points of view.

(1) According to Averinzev (1908), Mercier (1909) the formation of gametes and their fusion takes place before sporogenesis. Marco- and microgametes, formed by means of reduction division, fuse inside the cytoplasm of trophozoit and synkaryon gives rise to the pansporoblast. It is a single site of a sexual process. There is no sexual process inside the spore. So all the nuclei of sporoblasts as well as the nuclei of sporoplasm are diploid.

The Schröder's (1907) finding of the fusion of the nuclei inside the sporoplasm made some authors to deny this point of view.

(2) According to Parisi (1913), Southwell and Prashad (1918), Naville (1931) the formation of the gametes and the fusion of their nuclei occurs twice during the life cycle of *Myxosporidia*. It takes

place before pansporoblast formation and in the sporoplasm inside the spore. At the beginning of sporogenesis sporoblast nuclei are diploid and then meiosis takes place (during the second division in monoblastic species and the third division in diblastic species). The nuclei of sporoblasts of the following stages of sporogony, capsulogenic, valvogenic and sporoplasm nuclei are haploid. Then the nuclei of sporoplasm fuse and a zygote is formed.

(3) According to Auerbach (1912), Erdman (1924), Naville (1931) and Schröder (1910) the haploid nuclei are formed long before the spores formation, but their fusion is retarded and takes place only inside the sporoplasm. Thus all the nuclei of sporoblasts, valvogenic, capsulogenic and sporoplasm nuclei are haploid.

(4) According to Noble (1944), Schulman (1966, 1972) a single sexual process takes place in sporoplasm. The meiosis occurs only before the formation of sporoplasm nuclei which are haploid while other nuclei in the life cycle are diploid. This hypothesis is widely spread at present.

The variety of the interpretation of the nuclear cycle of *Myxosporidia* leads to the different views on the toxonomic position and the phylogeny of these animals (Tuzet et Ormieres 1957, Grassé 1960, Schulman 1966, Schulman and Semenovitch 1973, Lom 1973).

All these differences are due to the difficulties of morphological investigation of a very small nuclei of *Myxosporidia* as well to the asynchronous character of sporogenesis inside a trophozoit.

Electron microscopy does not help to the interpretation of the nuclear cycle. A necessity arises to use some other methods such as cytophotometry and autoradiography.

In the present paper I make an attempt to solve the problem with the help of Feulgen cytophotometry and to measure the amount of DNA in the nuclei of different stages of the life cycle of *Myxosporidia*.

Material and Methods

The *Sphaeromyxa elegini* Dogiel, 1948 from the gall-bladder of *Eleginus gracilis* (Tilesius) from the Pacific Ocean is the most suitable species for the purposes of cytophotometry among all the species available for us.

As it has been observed (Uspenskaja 1975) the trophozoite of *S. elegini* forms a large, flat and round plasmodium up to 1.5 cm in diameter. The large size of the plasmodium makes it possible to measure the great amount of the nuclei in the same preparation, minimizing a possible error of the photometry which is due to the inaccuracy of the hydrolysis and staining time. Besides, the capsulogenic nuclei of this species degenerate rather late and enable us to measure the

amount of DNA contained in it. We also succeeded in finding the initial stage of sporogoenesis when valvogenic nuclei had not yet been degenerated.

Sometimes we found doublets of the nuclei in our material, one of the nuclei being smaller then the other. They were similar to the stage described by many authors as the stage preceding the pansporoblast formation (Schröder 1907, Auerbach 1912, Naville 1931, Lom 1969, and others).

Thus the amount of DNA was measured in the vegetative nuclei of the trophozoite, in the above mentioned doublets of nuclei and in the sporoblast nuclei at different stages of division (two, four, eight nuclei in one pansporoblast). Because of the enormous amount of the nuclei in plasmodia it is difficult to identify the stage. Therefore the 50 nuclei of each mentioned stage of the sporoblast were measured as well about 250 nuclei of sporoblasts without the exact identification of the stage of their division.

The amount of DNA was measured in sporoplasm nuclei, capsulogenic and valvogenic nuclei at the different stages of spore formation:

(1) At the beginning of the process when all the nuclei forming spore cells are round in shape without signs of degeneration.

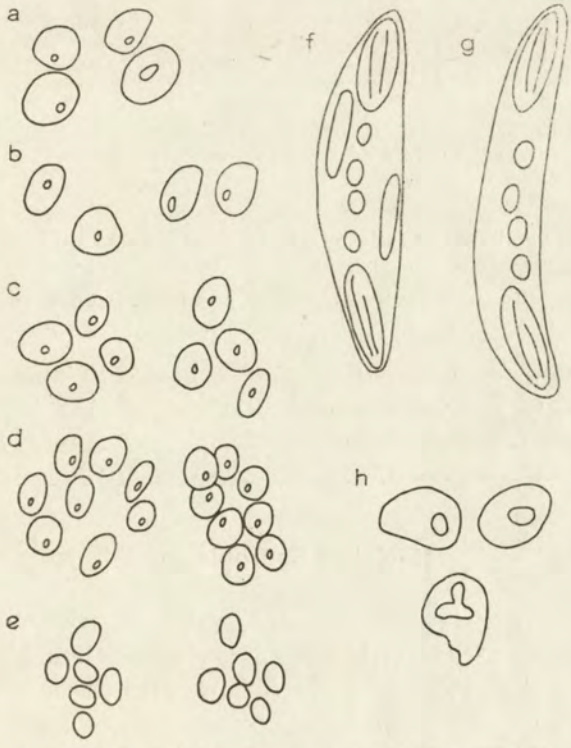


Fig. 1. The groups of nuclei of *Sphaeromyxa elegini* investigated cytophotometricaly (× 290). a — doublets of nuclei, b — two sporoblast nuclei inside the pansporoblast, c — four sporoblast nuclei inside the pansporoblast, d — eight sporoblast nuclei inside pansporoblast, e — the spore of the 1st stage, f — the spore of the 2nd stage, g — the spore of the 3rd stage, h — vegetative nuclei

(2) In mid course of the process when the valvogenic cells have elongated the viscosity of their cytoplasm has increased and the valvogenic nuclei have assumed a sousage-like shape.

(3) In the mature spore, when valvogenic nuclei have disappeared and capsulogenic are still present.

To make it shorter we shall further denominate these stages as the spores of the 1st, the 2nd, the 3rd stage in Tables and in the Figures. It must be pointed out that at the two latter stages the nuclei were measured through the spore valves which in spite of their transparency can be the cause of a systematic error leading to the overestimate of the amount of DNA in these nuclei. In the Fig. 1 the nuclei of all the stages of *Sphaeromyxa elegini* investigated are shown. The sketches were made from the negative image projected by means of a photomagnifier on paper.

The smears fixed in the Carnoy mixture were hydrolyzed in 1 N HCl at 60°C for 8 min and were placed into Schiff's reagent prepared according to "cold Schiff" method (Lilie 1969). Nuclei of *Myxosporidia* stained more intensive by this method.

Nuclear DNA was measured by photometry. Nuclei were photographed by microscope MUF-6 in monochromatic green light (546 nm). The densities of the negatives were measured with a scanning microphotometer MF-4. The nuclear area was measured by planimetry on drawings enlarged by photomagnifier (× 540). The results were expressed in arbitrary units.

Results and Discussion

The mean DNA content for all investigated groups of the nuclei is shown in the Table 1. According to the average amount of DNA in the gametic haploid nuclei of sporoplasm it seems possible to conclude that capsulogenic and valvogenic nuclei are haploid, while the sporoblast nuclei are diploid. The amount of DNA in sporoplasm nuclei. Small and large nuclei of the doublets are diploid. The vegetative nuclei are tetraploid. However, the examination of histogram (Fig. 2) reveals some facts which must be discussed.

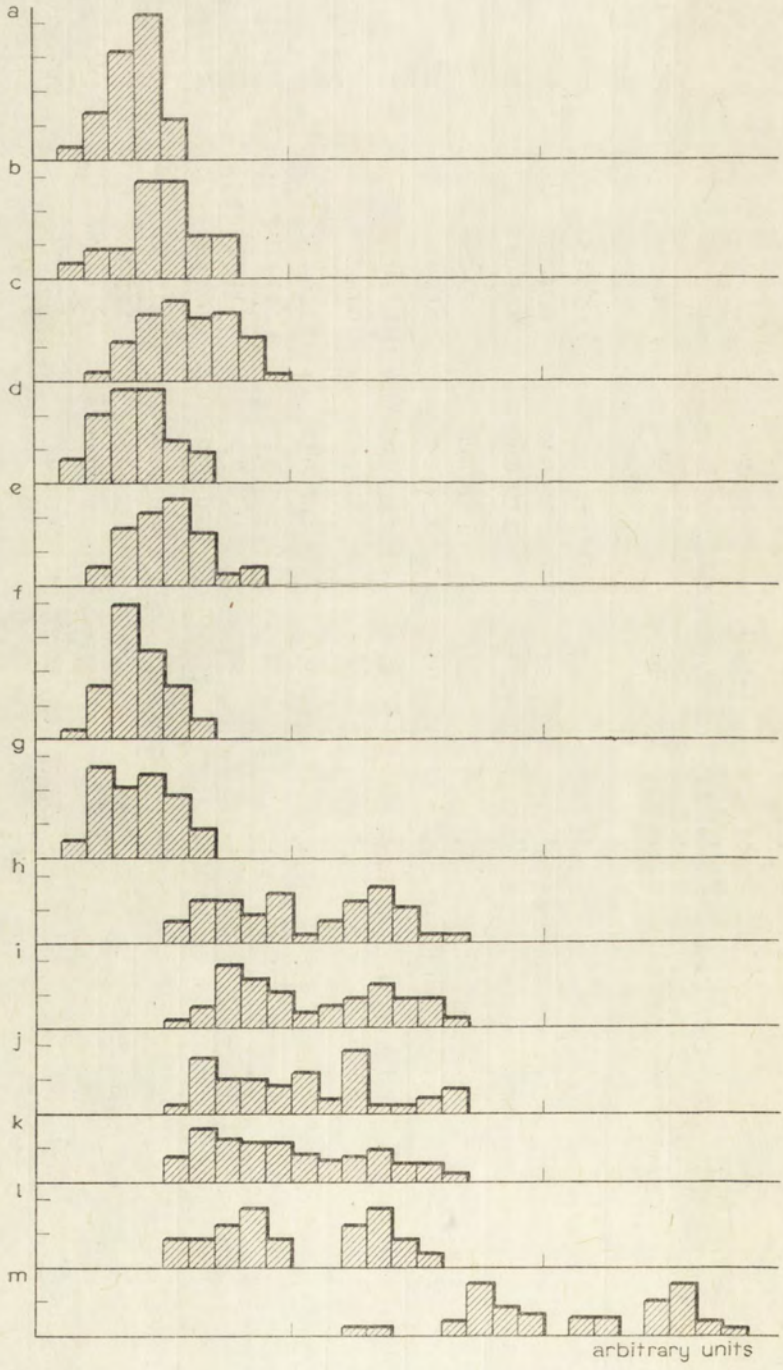
Nuclei of Sporoplasm

The frequency distribution of DNA value in sporoplasm nuclei is normal according to χ^2 test but rather a great variation of this character is surprising (Fig. 2 a, b, c). The coefficients of variation are 23%, 28% and 26% for three investigated groups respectively (Table 1 a, b, c). We can not expect the synthesis of DNA in sporoplasm nuclei because they are gametic ones. This is confirmed by their further fusion inside the sporoplasm. The attempt was made to verify the possible error of DNA measurements in sporoplasm nuclei. For this purpose the nuclei of sporoplasm from the same spore were measured twenty times. A rat-

Table 1

Mean DNA Content in Nuclei of Different Stages of *Sphaeromyxa elegini* (in arbitrary units)

The nuclei investigated		Number of measurements	Mean DNA value	Ranges	Coefficient of variation
(1) From sporoplasm	(a) spores of the 1st stage	62	1.78±0.05	0.90–2.54	23%
	(b) spores of the 2nd stage	25	2.24±0.12	0.97–3.36	28%
	(c) spores of the 3rd stage	175	2.32±0.05	1.06–3.65	26%
(2) Capsulogenic	(a) spores of the 1st stage	58	1.80±0.06	0.79–2.96	25%
	(b) spores of the 3rd stage	50	2.26±0.09	1.08–3.68	28%
(3) Valvogenic	(a) spores of the 1st stage	61	1.81±0.05	0.85–2.88	23%
	(b) spores of the 2nd stage	50	1.76±0.08	0.76–2.93	30%
(4) From pansporoblasts	(a) stage of 2 sporoblast nuclei	50	4.07±0.17	2.16–6.42	30%
	(b) stage of 4 sporoblast nuclei	50	4.01±0.17	2.13–6.91	34%
	(c) stage of 8 sporoblast nuclei	50	4.07±0.20	2.14–6.96	35%
	(d) different stage of division	248	4.00±0.08	2.12–6.96	32%
(5) Doublets of nuclei	(a) together	24	4.16±0.37	2.12–7.08	43%
	(b) small	12	4.12±0.44	2.12–7.08	37%
	(c) large	12	4.20±0.50	2.14–5.70	42%
(6) Vegetative		48	9.19±0.44	6.62–17.43	30%



her great range of variation of DNA amount for each of these two nuclei was shown. The coefficient of variation for each nuclei was 15% (Table 2).

Table 2

The Measurement Error of DNA Content in each of the two Sporoplasm Nuclei of *Sphaeromyxa elegini*

Nucleus	Mean DNA value	Limits	Coefficient of variation
the 1st	1.73±0.06	1.38–2.13	15%
the 2nd	1.64±0.06	1.15–2.29	15%

The amount of DNA was measured 20 times in each of the two nuclei.

The recent data show that the nuclei lacking the synthesis of DNA, where DNA content must be stable (nuclei of blood cells, nuclei of spermatozoids), may give different Feulgen values because of the changes in the DNA — protein relation in the chromatin (Atkin and Richards 1956, Hale and Willson 1961, Muller 1967, Ringerts et al. 1970, Marschak et al. 1970, Noeske 1971). It is pointed out that there are two types of chromatin which differ in DNA — protein linkage stability. The mean value of DNA changes depending on the quantitative correlation between the two types of chromatin (Sibatini 1953, Bonner 1965, Renandes and Keyl 1976, Noeske 1971).

The changes in physico-chemical state of the chromatin may explain the wide variability of DNA content in sporoplasm nuclei.

As we can see from the Table 1 the mean amount of DNA in sporoplasm nuclei from mature spores (Table 1, 1 c) is higher then that in the sporoplasm nuclei from the spores without refracting valves (Table 1, 1a). This fact is connected with the systematic error discussed in the part "Material and Methods".

The mean value of DNA corresponds to the haploid amount of DNA in *Sphaeromyxa elegini* is 1.78 ± 0.05 arbitrary units.

Fig. 2. Frequency distribution of DNA value of nuclei of the different stages of *Sphaeromyxa elegini*. a — sporoplasm nuclei from the spores of the 1st stage, b — sporoplasm nuclei from the spores of the 2nd stage, c — sporoplasm nuclei from the spores of the 3rd stage, d — capsulogenic nuclei from the spores of the 1st stage, e — capsulogenic nuclei from the spores of the 3rd stage, f — valvogenic nuclei from the spores of the 1st stage, g — valvogenic nuclei from the spores of the 2nd stage, h–k — sporoblast nuclei (h — two, i — four, j — eight nuclei inside the pansporoblast, k — nuclei at the different stages of division, l — doublets of nuclei, m — vegetative nuclei

Capsulogenic Nuclei

The frequency distribution of DNA value in capsulogenic nuclei in both groups is normal according to χ^2 test (see histogram, Fig. 2 d e). The ranges of variation are correlated with those in sporoplasm nuclei of corresponding groups. The average amount of DNA of capsulogenic and sporoplasm nuclei are almost equal (the difference is insignificant according to Student's test). There is no doubt that capsulogenic nuclei are haploid.

Valvogenic Nuclei

As stated above (see "Material and Methods") the amount of DNA in valvogenic nuclei was measured at the beginning of sporogenesis and then after the formation of valves when valvogenic nuclei became sousage-like in shape. In the mature spores valvogenic nuclei are absent.

The comparison of the mean DNA content in valvogenic nuclei with that in sporoplasm nuclei by means of Student's test show that the difference between them is insignificant.

Valvogenic nuclei are also haploid (Table 1, 3 a, b); histogram Fig. 2 f, g): There is no significant decrease of DNA content in sausage-like valvogenic nuclei but the appearance of a peak in the left part of the histogram (Fig. 2 g) shows that the amount of nuclei with smaller DNA value increases for this group.

The Sporoblast Nuclei

The frequency distribution of DNA value in sporoblast nuclei is abnormal according to χ^2 test. The frequency curve has two peaks (see histogram Fig. 2 h–k).

For example the histogram of sporoblast nuclei which belong to the group without the exact identification of the stage of division (Fig. 2 k) has an excess equal to –0.8.

The double peak in the frequency curve shows unhomogeneity of the group. There may be two reasons of the unhomogeneity: (1) The presence of haploid and diploid nuclei or (2), the presence of pre- and postsynthetic nuclei. The following facts seem to contradict to the first supposition (the presence of haploid and diploid nuclei):

(1) The mean DNA value calculated for the two subgroups of this group (Fig. 2 k) of sporoblast nuclei are 3.24 ± 0.06 for the left peak and 5.70 ± 0.70 for the right peak. The mean value for the left subgroup significantly differ (according to Student's test) from the mean amount of DNA in sporoplasm nuclei and their ratio is 1.94.

(2) Frequency distribution of DNA value in all the stages of sporoblasts division (two, four, eight nuclei inside the pansporoblast, Fig. 2 h–j) have the same ranges. This would be impossible if the nuclei of one of these stages were haploid and the other diploid.

(3) The lowest DNA value for all the groups of sporoblast nuclei is twice as large as the lowest DNA value in sporoplasm nuclei.
The number of measurements in these groups is enough not to consider the fact to be an accidental one.

(4) Finally the mean value of DNA in all the groups of sporoblasts nuclei (Table 1) is twice as large as the mean value of DNA in haploid sporoplasm nuclei.

The enumerated facts speak in favour of the diploidy of sporoblast nuclei at all the stages of division. All of them are diploid but at the different stage of premitotic synthesis.

The frequency curve of sporoblast nuclei is similar to that characteristic for nuclei with high mitotic activity (Sandritter 1969). And indeed the nuclei of sporoblasts divide many times mitoticaly during the sporogenesis.

Doublets of Nuclei

Doublets or pairs of the nuclei, one of which is smaller than the other, have been found in our material rather rarely. They correspond morphologicaly to the stages described as the binuclear pansporoblast (Schröder 1907, Erdmann 1924) but as it has been found in the present investigation the small and the large nuclei in doublets are diploid. It means that these nuclei are not gametic one and the difference in their size does not influence their ploidy. It seems that "a" and "b" groups of the nuclei in Fig. 1 is one and the same stage of division and the difference in the dimension has no significance.

There is no doubt that these nuclei are not macro- and microgametes. The frequency distribution ranges of this group coincide with those of sporoblast nuclei (Fig. 21).

If, according to Georgevitsch (1937) and Noble (1944), we consider the binuclear pansporoblast to be a result of nuclear division of a generative cell the diploidy of this stage will be understandable.

Vegetative Nuclei

Vegetative nuclei of the trophozoite morphologicaly differ from sporoblast nuclei. They are larger than the latters, irregular in shape and have large nucleoli.

The vegetative nuclei are polyploid, tetraploid in average (Table 1 6, Fig. 2 m). It may be either the degenerative polyploidy due to the destruction of the division mechanism, or the polyploidy connected with the high metabolic activity of the nuclei in such a large trophozoite where the great amount of metabolic materials is needed for spores formation.

Scheme of the Nuclear Cycle of *Sphaeromyxa*

The attempts to investigate the nuclear cycle of the members of the genus *Sphaeromyxa* by morphological methods have been made many times. The majority of the authors working on *Sphaeromyxa* share the third point of view on the nuclear cycle of *Myxosporidia* (see Introduction).

According to Schröder (1907) and Naville (1931) the sexual process in *Sphaeromyxa* consists of two steps: (1) plasmogamy, before the spores formation and (2) karyogamy at the end of sporogenesis inside the sporoplasm. Binuclear pansporoblasts arise as a result of fusion of cytoplasm of two haploid gametic elements, micro- and macrogametes. As karyogamy is retarded all the nuclei of sporoblasts are diploid.

Debaisieux (1924) also shares this point of view but more carefuly. As far as *Sphaeromyxa* is concerned he thinks that there is another possible way of pansporoblast formation.

Georgevitsch (1937) assumed that in *Sphaeromyxa*, as well as in *Myxosporidia* as a whole, the binuclear pansporoblast was not a result of the fusion of two cells but a result of the division of the generative cell nucleus. All the nuclei of sporoblasts are diploid. Meiosis takes place only before the formation of the two nuclei of sporoplasm. It is a single haploid stage.

According to the scheme of Schröder (1907, 1910) and Debaisieux (1924) the residual nuclei of pansporoblast arise at the last stage of nuclear division. In this case the pansporoblast must contain two, four, eight and 14 nuclei subsequently. According to the scheme of Naville (1931) and Georgevitsch (1937) the residual nuclei of pansporoblast cease to divide after the first division of the nuclei of binuclear pansporoblast. Valvogenic nuclei do not take part in the last division of pansporoblast nuclei. Therefore inside the pansporoblast there are two, four, six and ten nuclei subsequently.

As we can see from our material the groups of two, four, eight and 14 nuclei are found inside the pansporoblasts. So we can suppose that the residual nuclei of pansporoblast arise at the stage of eight nuclei inside the pansporoblast and do not take part in the last division.

The data on the ploidy of the nuclei in different stages of the life cycle of *Sphaeromyxa* obtained by us in the present investigation do not fit no one of the schemes proposed before. We can not agree with the schemes of Schröder (1907), Mercier (1909), Debaisieux (1924), Naville (1931), Tuzet et Ormieres (1957) because according to them all the nuclei of sporoblasts are haploid. Our data show that the stages with two, four, and eight nuclei inside the pansporoblast are diploid. We must leave apart the schemes of Georgevitsch (1937), Noble (1944) and others because the capsulogenic and valvogenic nuclei as well as the nuclei of sporoplasm are haploid in *Sphaeromyxa elegini*.

The only scheme of the nuclear cycle of *Sphaeromyxa* we can propose on the basis of Feulgen cytophotometry is that shown in Fig. 3.

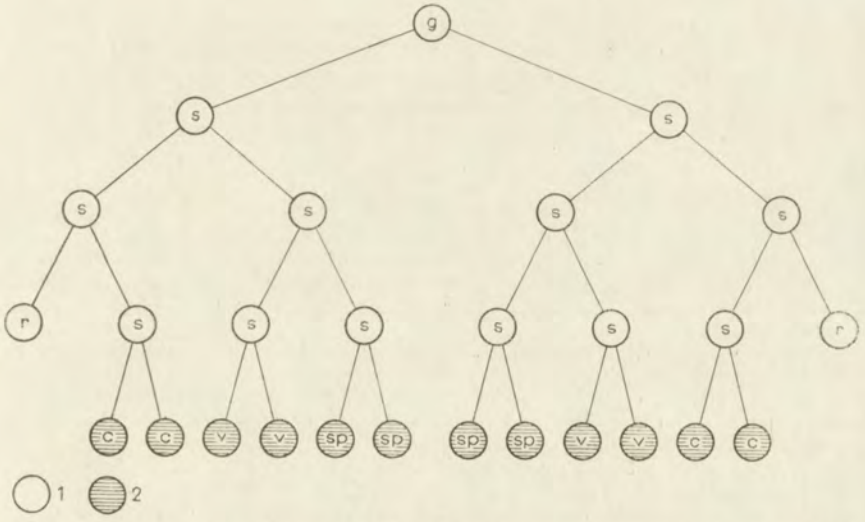


Fig. 3. The scheme of nuclear cycle of *Sphaeromyxa*. g — generative nuclei, s — sporoblasts nuclei, r — residual nuclei of pansporoblast, c — capsulogenic nuclei, v — valvogenic nuclei, sp — nuclei of sporoplasm, 1 — diploid nuclei, 2 — haploid nuclei

According to this scheme *Sphaeromyxa* can have one-step meiosis, which occurs after the third mitotic division of diploid sporoblast nuclei. Only six nuclei inside the pansporoblast take part in the mitotic division. Two of the eight nuclei of pansporoblast do not divide and become the two residual nuclei of pansporoblast. The haploid capsulogenic, valvogenic and sporoplasm nuclei arise from mitotic division of six sporoblast nuclei. They are surrounded by separate cytoplasm and two valvogenic, two capsulogenic and one binuclear sporoplasm cells are formed. The sexual

process takes place inside the sporoplasm. The generative nuclei of trophozoite are diploid up to the stage of eight nuclei (included) inside the pansporoblast. Pansporoblast arises most likely as a result of the division of the generative cell nuclei. The vegetative nuclei are polyploid (tetraploid in average). As it was mentioned above it may be either the degenerative polyploidy or the polyploidy connected with high metabolic activity of those nuclei.

РЕЗЮМЕ

Проведено цитофотометрическое определение количества ДНК в ядрах различных стадий жизненного цикла миксоспоридии *Sphaeromyxa elegini* Dogiel, 1948. Установлено, что не только ядра амебоидного зародища, но тоже капсулогенные и вальвогенные ядра гаплоидны. Парные ядра, которые рассматривались некоторыми авторами как макро- и микрогаметы диплоидны. Ядра споробластов тоже диплоидны. Одноступенчатый мейоз имеет место после третьего деления ядер споробласта. Вегетативные ядра являются полиплоидны (обычно тетраплоидны). Предлагается новая схема ядерного цикла *Sphaeromyxa*.

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Ультраструктура сидячей инфузории *Cavichona elegans* (*Chonotricha*). I. Непочкующиеся особи

Ultrastructure of the Sessile Ciliate *Cavichona elegans* (*Chonotricha*). I. Non-dividing Animals

Синопсис. Инфузория *Cavichona elegans* Swarczewsky (*Chonotricha*) покрыта клеточной мембраной, под которой находится эпиплазматической слой, пронизанный многочисленными пелликулярными порами. Эпиплазма подостлана продольно ориентированными триадами микротрубочек. Предротовой ресничный аппарат представлен несколькими кинетами с одиночными кинетосомами, под основаниями которых проходят субкинетальные ленты микротрубочек. Стенка глотки образована 28–32 плоскими лентами микротрубочек и снабжена системой "сложных трубочек". Экскреторный аппарат состоит из системы канальцев гладкой эндоплазматической сети ("нефридиальная плазма"), одной сократительной вакуоли, ее приводящих каналов и выводного канала, образованного инвагинацией пелликулы и укрепленного микротрубочками. Железистая органелла, выделяющая прикрепительный диск, состоит из пальцевидных секреторных ампул, открывающихся у основания тела. Основание предротовой воронки, глотка и железистая органелла укреплены арматурой из микрофибриллярных тяжей, заякоренных на внутренней стороне эпиплазмы. Ядерный аппарат представлен одним гетеромерным макронуклеусом последовательного типа и 1–3 микронуклеусами. Ортомер макронуклеуса содержит сеть хроматиновых тяжей, парамер образован фибриллярным матриксом, эндосома образуется лишь в конце интерфазы, ядрышки присутствуют в обоих сегментах ядра и имеют фиброгранулярное строение. В матриксе обоих сегментов макронуклеуса отмечены также интерхроматиновые и перихроматиновые гранулы. В начале интерфазы в макронуклеусе имеется "эпимер"-хромофобный сегмент, лежащий на стороне, противоположной парамеру и имеющий ту же ультраструктуру, что и парамер.

Представители аберантной группы сидячих инфузорий—подкласса *Chonotricha* — слабо изучены электронномикроскопически, хотя их организация уникальна во многих отношениях. В отличие от подавляющего большинства остальных инфузорий, *Chonotricha* обладают сложным "гетеромерным" макронуклеусом, состоящим из хромофильной и хромофобной частей и содержащим хроматиновую "эндосому", которая появляется лишь на определен-

ных стадиях цикла деления макронуклеуса. Ход деления гетеромерного макронуклеуса весьма сложен (Tuffrau 1953, Guénin et Fahrni 1971). *Chonotricha* характеризуются также весьма своеобразным прикрепительным аппаратом, а также сложно устроенным предротовым ресничным аппаратом (см. Янковский 1973).

Объект нашего исследования — *Cavichona elegans* (Swarczewsky) — первоначально описан как вид рода *Spirochona*. Это эктокомменсал, обитающий на жаберных листках байкальского бокоплава *Eulimnogammarus verrucosus* (см.: Янковский 1973).

Материал и методика

Материал был собран на Байкальской биологической станции Иркутского университета (пос. Большие Коты) летом 1968 и 1973 гг. Инфузории были зафиксированы непосредственно на отпрепарированных жаберных листках 1% OsO_4, на фосфатном буфере (pH 7,2) в течение 1-1,5 часов при комнатной температуре и залиты в аралдит. Ультратонкие срезы приготовлялись на ультратоме LKB, окрашивались 4–6 часов насыщенным водным раствором уранилацетата и затем 10–15 минут цитратом свинца. Срезы изучались в электронном микроскопе JEM-7.

Результаты и обсуждение

Cavichona elegans (Swarczewsky) имеет удлиненное, лишенное ресничного покрова тело веретенообразной формы, в котором можно различить четыре морфологически отличных отдела (Рис. 1, табл. I 1). (1) перистом, включающий в себя хорошо развитый завиток предротовой воронки, несущей ресничные полосы, а также ротовое отверстие, (2) суженную шейку, в которой располагается экскреторный аппарат, (3) среднюю, наиболее широкую часть тела, характеризующуюся плотной гомогенной цитоплазмой, в которой располагаются ядра, вакуоли и цитофарингеальная трубка и (4) стебелек (псевдостил, по Янковскому 1973) с прикрепительным диском на конце. На границе между вторым и третьим отделом в цитоплазме находится зона липидных гранул.

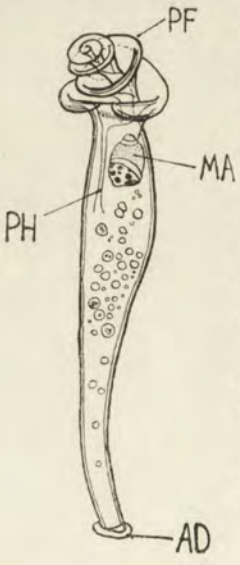

Рис. 1. *Cavichona elegans* (Swarczewsky). Общий вид (по Янковскому 1973). PF — предротовая воронка, PH — глотка, MA — макронуклеус, AD — прикрепительный диск

Fig. 1 *Cavichona elegans* (Swarczewsky), general aspect (after Jankowsky 1973). PF — preoral funnel, PH — cytopharynx, MA — macronucleus, AD — attachment disc

Поверхность тела

Наружная поверхность тела *C. elegans* покрыта пелликулой, состоящей из одной клеточной мембраны и электронноплотного подмембранного слоя (Табл. II 4, III 5), под которой находится сильно развитый эпиплазматический слой толщиной 350 nm (Табл. I 1, II 4, III 5). Эпиплазма не ограничена от цитоплазмы мембраной, но подостлана со стороны цитоплазмы продольно ориентированными микротрубочками диаметром 20 nm (табл. I 2, II 3). На поперечных срезах тела видно, что микротрубочки собраны в группы по три-две в наружном ряду, одна во внутреннем (Табл. II, 4 VIII 16). Сходная система продольно ориентированных субпелликулярных микротрубочек описана у многих инфузорий из числа *Hypotricha* (Grim 1967, Fauré-Fremiet et Andre 1968, Tuffrau et al. 1968), а также у филогенетически связанных с *Chonotricha* инфузорий *Chlamydodontidae* (Sołtyńska 1971) и *Dysteriidae* (Lom and Corliss 1971). По крайней мере у последних эти микротрубочки представляют собой постцилиарные отростки соматических кинетосом. Эпиплазму *C. elegans* пронизывают многочисленные поры, расположенные более или менее правильными продольными рядами. Они имеют строение глубоких впячиваний клеточной мембраны, достигающих внутренней стороны эпиплазмы. На поперечном срезе пора круглая, с диаметром просвета около 35 nm. Стенка поры образована двумя концентрическими кольцами; внутреннее кольцо — это клеточная мембрана, а внешнее — электронноплотный подмембранный слой (Табл. I 2, II 3, 4, III 5). На конце пор, под эпиплазмой, обычно образуются ампулоподобные пузырьки, ограниченные только клеточной мембраной (электронноплотный слой здесь исчезает — Табл. II 3, 4). Некоторые пузырьки содержат оформленные включения (Табл. III 5). По-видимому, пузырьки могут терять связь с эпиплазматической порой и уходить вглубь цитоплазмы. Таким образом, их можно трактовать как пиноцитозные пузырьки, формирующиеся на концах постоянно имеющихся эпиплазматических пор.

Сама эпиплазма имеет структуру в виде палочек с размытыми концами, ориентированных перпендикулярно поверхности тела (Табл. II 3, III 5).

Эпипалзма внутренней стороны предротовой воронки значительно тоньше (42 nm), чем эпиплазма остальных отделов тела (Табл. III 6, IV 9, V 10)

Ультраструктура кортекса *Cavichona elegans* мало отличается от кортикальных структур немногих изученных до сих пор *Chonotricha*. Хорошо развитый слой эпиплазмы, которая пронизана порами и которую подстилают ряды продольно ориентированных микротрубочек, описан у *Chilodochona quennerstedti* (Grain et Batisse 1973, 1974). Однако, в отличие от *Ch. quennerstedti*, на нашем материале мы не обнаружили альвеолярного слоя, находящегося непосредственно под клеточной мембраной (т.е., снаружи от эпиплазмы). Хорошо развитый альвеолярный слой описан также у предков *Chonotricha* — инфузорий семейства *Dysteriidae*. Так, он имеется у *Brooklynella hostilis* (Lom and

Corliss 1971) и у других *Dysteriidae*, для которых характерно также и заметное развитие эпиплазматического слоя, образующего панцирь, жесткость которого обусловлена содержанием мукополисахаридов (Fauré-Fremiet et al. 1956). Это несомненный гомолог эпиплазмы *Chonotricha*.

Предротовая воронка

Как и все спирохониды (Янковский 1973), *C. elegans* обладает хорошо развитой предротовой воронкой, (Табл. III 6) образующей спиральный завиток и несущей на своей внутренней стороне две ресничные зоны. Количество витков предротовой спирали — 4–4½. Эпиплазма предротовой воронки, как и эпиплазма остальной части тела, подостлана продольно ориентированными микротрубочками. Цитоплазма предротовой воронки гомогенная, содержит лишь отдельные липидные гранулы, митохондрии и рибосомы. В верхней части спирального завитка слой цитоплазмы становится очень тонким.

Основание предротовой воронки поддерживается мощными анастомозирующими пучками микрофиламентов, расположенными в цитоплазме завитка, под эпиплазмой. Пучки филаментов прикреплены своими нижними концами к внутренней стороне эпиплазмы в области шейки (Табл. VII 14).

Ресничный аппарат развит только на внутренней стороне завитка предротовой воронки и состоит из двух ресничных зон (Янковский 1973). Первая зона имеет вид длинной узкой полосы, проходит по всему завитку предротовой воронки и состоит из 5–9 продольных рядов ресничек, разделенных пелликулярными гребнями (Табл. I 1, III 6, IV 9). Вторая зона короткая и широкая, включает около 20 ресничных рядов и находится вблизи цитостома (Табл. I 1, IV 8). В ресничных рядах все кинетосомы одиночные и несут реснички (Табл. I 1, III 7, IV 8, 9, VII 14). Кинетосомы имеют типичную структуру: в их дистальной части располагается крупная аксосома, под которой находится вогнутая септа (Табл. III 7). Под основаниями кинетосом каждого ряда проходит лента субкинетальных микротрубочек, которая состоит из 4–6 рядов микротрубочек (Табл. III 7, IV 8). Субкинетальные микротрубочки особенно выражены в широкой ресничной зоне (Табл. IV 8). Кинетосомы каждого ресничного ряда несут три типичных фибриллярных отростка — постцилиарные, трансверсальные, кинетодесмальные. Постцилиарные фибриллы образованы микротрубочками, располагаются в гребнях пелликулы, разделяющих ресничные ряды (Табл. VII 14). Кинетодесмальные отростки кинетосом выражены очень слабо.

Строение ресничных рядов *C. elegans* сходно со строением соматических ресничных рядов вентральной поверхности тела дистериид — *Brooklynella hostilis* (Lom and Corliss 1971). У *B. hostilis* кинетосомы также одиночны, ресничные ряды разделены гребнями, а под основаниями кинетосом проходят мощные ленты субкинетальных микротрубочек.

Цитофаринкс и "сложные трубочки"

Ротовое отверстие, расположенное на дне предротовой воронки, ведет в длинную узкую глотку, или цитофаринкс (Табл. V 10). Глотка состоит из 28–32 плоских лент микротрубочек, проходящих в продольном направлении и спирально накладывающихся друг на друга, что видно на поперечном срезе глотки (Табл. VI 11). Выходя к поверхности тела, на уровне ротового отверстия, ленты микротрубочек становятся субпелликулярными и проходят под тонким эпиплазматическим слоем внутренней поверхности предротовой воронки, будучи ориентированы ребром к пелликуле (Табл. IV 9, V 10, стрелки). Возможно, что это — продолжение трансверсальных микротрубочек кинетосом предротовой воронки. На поперечном срезе, на некотором расстоянии от ротового отверстия, цитофарингеальная трубка имеет почти круглое сечение (Табл. VI 11). Полость цитофарингеальной трубки наполнена тонкогранулированной цитоплазмой, содержащей плотные пузырьки, митохондрии, элементы эндоплазматического ретикулума и практически очень мало отличается от окружающей цитоплазмы. Таким образом, у *Cavichona elegans* постоянно имеется только арматура глотки (ленты микротрубочек), но постоянно существующего глоточного канала нет. Это довольно типично для *Gymnostomatida*, от которых произошли *Chonotricha* — например, для *Didinium* (Wessenberg et Antipa 1968), *Lacrymaria* (Bohatier 1970), *Chilodonella* (Sołtyńska 1971), *Brooklynella* (Lom and Corliss 1971).

Глотка *C. elegans* очень длинная, заканчивается несколько ниже нижнего конца макронуклеуса (Табл. I 1). На этом уровне ленты микротрубочек теряют правильное спиральное расположение и стенка глотки распадается на отдельные группы микротрубочек. Здесь в цитоплазме много пищеварительных вакуолей (Табл. VIII 15, 16).

Похожая система микротрубочек, образующих цитофарингеальную трубку, недавно описана у *Chilodochona quennerstedti* (Grain et Batisse 1974). Цитофарингеальная трубка всех изученных до сих пор с электронным микроскопом представителей отрядов *Dysteriidae* и *Chlamydodontidae* — *B. hostilis* (Lom and Corliss 1971), *Chlamydodon pedarius* (Kaneda 1962), *Chilodonella cucullus* (Sołtyńska 1971), *Phascolodon vorticella* (Tucker 1972), также образована лентами микротрубочек, спирально накладывающихся друг на друга. Однако у дистериид и хламидодонтид (а также у многих других *Gymnostomatida*) глотка поддерживается также лежащей снаружи от нее арматурой из немадесм, состоящих из гексагонально упакованных микротрубочек.

Как и основание предротовой воронки в целом, глотка *C. elegans* поддерживается анастомозирующими пучками микрофиламентов (Табл. V 10, стрелка). Большое количество таких ветвящихся пучков имеется у верхнего конца глотки, в средней части количество их уменьшается. Трудно сказать, являются ли эти микрофиламенты только опорными элементами или обладают и сокра-

тимой функцией, растягивая ротовое отверстие и глотку при заглатывании пищи.

Вблизи ротового отверстия, между лентами микротрубочек и снаружи от них, мы наблюдали большое количество так называемых "сложных трубочек" с гладкой мембраной, которые на поперечном срезе выглядят круглыми или слегка гексогональными (Табл. IV 9, V 10, VI 11). Наружный диаметр этих трубочек 42 nm, внутренний 13 nm. На продольных срезах эти трубочки имеют вид извитых каналов (Табл. IV 9). Концы некоторых "сложных трубочек" вплотную подходят к клеточной мембране в области цитостома и могут даже впадать в полость ротовой ямки. Вероятно, связь между клеточной мембраной в области ротового отверстия и "сложными трубочками" лабильна. Никакой связи "сложных трубочек" с другими цитоплазматическими структурами обнаружено не было.

Аналогичные "сложные трубочки" были впервые детально описаны у *Chilodonella* (Pyne 1970, Pyneet Tuffrau 1970). Эти авторы полагают, что "сложные трубочки" могут играть роль в выработке некоторых ферментов, участвующих в переваривании пищи. Трубочки такого же строения вблизи ротового отверстия были отмечены у *Brooklynella hostilis* (Lom and Corliss 1971), *Phascolodon vorticella* (Tucker 1972), *Chilodochona quennerstedti* (Grain et Batisse 1974). Можно, однако, предположить и то, что „сложные трубочки" представляют собой „резерв" мембранного материала, наподобие дисковидных мешочков, встречающихся в околоротовой цитоплазме *Euplotes*, который потребляется при формировании пищеварительных вакуолей (Kloetzel 1974).

Сократительная вакуоль

Литературные данные о наличии сократительной вакуоли у *Chonotricha* очень скудны и противоречивы. Многие авторы (Mohr et al. 1963, Matsudo and Mohr 1966, Янковский 1973 и др.) считают, что у хонотрих нет сократительной вакуоли. Янковский (1973) объясняет отсутствие сократительной вакуоли у хонотрих наличием у них прочной эпиплазмы, ограничивающей водообмен, а также предположительно морским происхождением подкласса *Chonotricha*.

Однако, у изученного нами вида мы обнаружили обычный комплекс структур экскреторного аппарата, ничем принципиально не отличающийся от такового у других инфузорий. Этот комплекс состоит из одной сократительной вакуоли, ее приводящих каналов и длинного выводного канала, образованного инвагинацией клеточной мембраны и подмембранного слоя пелликулы и соединяющего полость сократительной вакуоли с внешней средой.

Сократительная вакуоль *C. elegans* располагается в районе шейки (между дном предротовой воронки и макронуклеусом) (Табл. I 1, V 10, VI 12). Она окружена особой канально-пузырьковой зоной цитоплазмы — "нефридиальной плазмой", которая состоит из густой сети ветвящихся трубочек гладкой эндоплазматической сети. В нефридиальной плазме различимы и более толстые

(приводящие) каналы (Табл. V 10, VII 14); наблюдалось впадение в них тонких трубочек (Табл. VI 13) во время диастолы приводящих каналов и систолы самой сократительной вакуоли. Стенки тонких каналов нефридиальной плазмы образованы одной элементарной мембраной и могут непосредственно переходить в мембрану ампулы, которая представляет собой концевое расширение приводящих каналов (Табл. VI 13). Стенки приводящих каналов, в отличие от тонких канальцев, снабжены электронноплотным примембранным слоем (Табл. VI 13, VII 14). На нашем материале, фиксированном OsO_4, мы не обнаружили фибриллярных структур или микротрубочек в стенках приводящих каналов.

Похожая система трубочек, образующих нефридиальную плазму, которая окружает сократительную вакуоль, описана у *Peritricha* (Faurè-Fremiet et al. 1959). Электронномикроскопически нефридиальная система подробно изучена у *Paramecium caudatum* и *P. aurelia* Schneider (1960). Наличие нефридиальной зоны цитоплазмы вокруг сократительной вакуоли Pitelka (1963) считает одной из общих черт организации простейших.

Стенка самой сократительной вакуоли *C. elegans* состоит из мембраны и прилегающего к ней снаружи толстого слоя аморфного вещества, толщиной 80 nm (Табл. I 1, VI 12). Никаких фибриллярных структур здесь, как и в стенках приводящих каналов, обнаружено не было. Во время диастолы сократительной вакуоли стенка ее гладкая, а во время систолы образует многочисленные складки. По-видимому, наполнение сократительной вакуоли может происходить до полного расправления ее стенок; дальнейшие поступления жидкости в сократительную вакуоль будут вызывать выбрасывание ее содержимого через выводной канал. Наблюдалось впадение приводящих каналов в сократительную вакуоль (Табл. VI 12). При наполнении вакуоли стенка ее, по-видимому, только расправляется, но не растягивается. Толщина аморфного слоя вокруг мембраны сократительной вакуоли в состоянии диастолы и систолы одинакова.

Выводной канал (Табл. V 10, VII 14) очень длинный, извитой; он является постоянной структурой экскреторного аппарата. В его стенке, снаружи от мембраны, обнаруживается слой продольных микротрубочек, расположенных в один ряд (Табл. VII 14). Пока неясно, являются ли эти микротрубочки опорными или сократимыми элементами.

Выводной канал сократительной вакуоли, как и глотка, поддерживается пучками связанных между собой микрофиламентов, расположенных под эпиплазмой (Табл. V 10, VII 14). В цитоплазме вокруг сократительной вакуоли и ее приводящих каналов наблюдается скопление митохондрий и свободных рибосом.

Эндоплазма и её органеллы

Эндоплазма *C. elegans* плотная, содержит митохондрии, элементы эндоплазматического ретикулума, аппарат Гольджи, пищеварительные вакуоли, рибосомы.

Митохондрии мелкие (около 200 nm), многочисленные; особенно много их в зоне нефридиального аппарата (Табл. V 10, VII 14) и в цитоплазме предротовой воронки (Табл. I 1, III 6). Митохондрии *C. elegans* по своему строению не отличается от митохондрий большинства свободноживущих простейших (Vivier 1966) — они округлой или эллипсоидной формы, с многочисленными трубчатыми кристами (Табл. VIII 15, IX 17).

Эндоплазматическая сеть в основном агранулярная; вне зоны нефридиальной плазмы она представлена главным образом короткими каналами (Табл. IX 17). Большое количество каналов эндоплазматической сети обнаруживается в районе стебелька; в этой же зоне встречаются скопления агранулярных уплощенных цистерн; возможно, это — гомологи диктиосом (Табл. IX 17). Вблизи них, как правило, много пузырьков с электроннoплотным содержимым, вероятно, отделяющихся от цистерн аппарата Гольджи (Табл. IX 17).

В матриксе цитоплазмы много свободных, не прикрепленных к мембранам рибосом (Табл. II 3, 4, VI 11, VII 14, VIII 15, 16, IX 17).

В цитоплазме *C. elegans*, в основном ниже макронуклеуса, обнаруживается большое количество пищеварительных вакуолей (Табл. VIII 15, 16). Их особенно много на уровне нижнего конца глотки. Стенка пищеварительной вакуоли обычно образована одной элементарной мембраной. В пищеварительных вакуолях обнаруживаются бактерии на разных стадиях переваривания, которые, по-видимому, и являются основной пищей *Cavichona elegans* (Табл. VIII 15). На поздних стадиях переваривания пищеварительные вакуоли содержат только скопления бактериальных мембран (Табл. VIII 16).

Прикрепительный диск и секреторные ампулы

Cavichona elegans прочно прикрепляется к жаберным листочкам хозяина с помощью секрета, выделяемого внутриклеточной железистой органеллой. Ультраструктура железистой органеллы сходна с таковой у *Chilodochona carcini* (Faurè-Fremiet et al. 1956) и несколько отличается от структуры прикрепительного диска дистериды *Hartmannula oliva* (Fauré-Fremiet et al. 1968). У *Hartmannula oliva* секреторный комплекс состоит из (1) многочисленных секреторных пузырьков ("гетеросом"), окружающих (2) большой общий резервуар и (3) собственно железы, соединенный тонким каналом с ампулой. Общий резервуар железистого аппарата у *Cavichona elegans* полностью отсутствует. Железа, вырабатывающая аморфный клей у *C. elegans*, состоит из розетки отдельных пальцевидных ампул, открывающихся в основание тела (Табл. IX 18). Ампулы представляют собой впячивания пелликулы и ограничены мембраной, к которой со стороны цитоплазмы прилегает слой аморфного вещества, толщиной 90 nm (Табл. IX 19). Внутренность ампулы занята аморфной или тонко-волокнистой массой, более плотной у её основания. Fauré-Fremiet et al. (1956) считает, что ампулы секретируют мукопротеиды, застывающиеся при контакте с морской водой. Секрет ампул образует у *C. elegans*

толстый диск („подошву"), с воротникообразным расширением посередине, который и соединяет тело инфузории с эпиплазмой жаберного листка (Табл. IX 18).

Цитоплазма, окружающая железистую органеллу, менее плотная, чем в остальной части тела. От стенок всех ампул вглубь цитоплазмы, в сторону верхнего конца тела, отходят пучки микрофиламентов, которые соединяются друг с другом и постепенно становятся субкутикулярными (Табл. IX 18, 19). Пелликула в районе „ножки", в отличие от пелликулы остальной части тела, обнаруживает продольные бороздки.

Ядерный аппарат

Ядерный аппарат *C. elegans* состоит из одного гетеромерного макронуклеуса и 1–3 микронуклеусов. Количество микронуклеусов варьирует, но преобладают особи с двумя микронуклеусами.

Гетеромерные макронуклеусы встречаются у представителей двух специализированных групп — у *Holotricha Hypostomata* (сем. *Chlamydodontidae*, *Dysteriidae*) и у *Chonotricha*, произошедших от дистериид (Fauré-Fremiet 1957, Dobrzańska-Kaczanowska 1963).

В группе *Hypostomata* встречаются гетеромерные макронуклеусы как с концентрическим расположением кариомеров, когда хромофильная зона окружает со всех сторон хромофобную, так и с последовательным расположением ортомера и парамера. Концентрический гетеромерный макронуклеус наиболее подробно изучен у *Chilodonella*, как светооптически (Seshachar 1950, 1953, Radzikowski 1965) так и электронномикроскопически (Radzikowski 1973). Гетеромерный макронуклеус последовательного типа цитохимически подробно изучен у *Dysteria monostyla* (Fauré-Fremiet 1957), а в световом и электронном микроскопе у *Chlamydodon pedarius* (Kaneda 1960, 1961 a, b).

В подклассе *Chonotricha* встречаются гетеромерные макронуклеусы только с последовательным расположением ортомера и парамера, весьма сходные по структуре с таковыми *Dysteridae* и части *Chlamydodontidae*.

Макронуклеус *C. elegans* овальной формы (длина 7.5, μm ширина 6.0 μm), обычно состоит из двух резко отграниченных друг от друга сегментов—хромофильного (ортомера) и хромофобного (парамера) (Табл. I 1, X 20, XI 22). Макронуклеус лежит в верхней трети тела и обращен ортомером вверх (Табл. I 1). Морфология сегментов макронуклеуса сильно зависит от стадии клеточного цикла. В данной статье мы описываем только интерфазный макронуклеус, но и на протяжении интерфазы его морфология меняется. Размеры ортомера и парамера обычно примерно одинаковы (Табл. X 20), или же ортомер несколько крупнее парамера (Табл. I 1, XI 22). Оба сегмента макронуклеуса окружены общей двухмембранной оболочкой (Табл. X 21), между ортомером и парамером нет никакой мембранной перегородки (Табл. X 20, XI 22). Особой светлой "септы", которая, по данным некоторых светомикроскопических работ,

разграничивает парамер и ортомер, здесь также нет (Табл. X 20); по-видимому, её появление — результат сжатия, вызванного фиксацией. Тем не менее граница ортомера и парамера весьма резкая, благодаря тому, что по ней, как правило, проходит непрерывный диск хроматина ортомера (Табл. I 1, XI 22), отмеченный также у *Spirochona gemmipara* (Guénin et Fahrni 1971). Однако у макронуклеусов, имеющих эндосому, такого слоя нет (Табл. X 20).

Хроматин ортомера представлен электроннoплотной хроматиновой сетью, состоящей из тяжей толщиной около 100 nm и отдельных хроматиновых глыбок (Табл. X 20, XI 22). Последние, возможно, являются на самом деле поперечными срезами тяжей. Тяжи состоят из тонких (10 nm) элементарных фибрилл высокой электронной плотности (Табл. XII 23). Промежутки между тяжами заполнены фибриллярным матриксом низкой электронной плотности.

Парамер состоит из рыхлого сплетения тонких фибрилл низкой электронной плотности, сходных с таковыми матрикса ортомера (Табл. X 20, XI 21, XII 22). В первой половине интерфазы парамер не содержит конденсированного хроматина (Табл. I 1, XI 22). На более поздних стадиях в парамере появляется видимая и в световом микроскопе резко Фёльген-положительная кариосома ("эндосома") (Табл. X 20). В электронном микроскопе материал кариосомы сходен с хроматином ортомера и состоит из плотного клубка тонких фибрилл. Кариосома четко отграничена от остальной части парамера, хотя никаких мембранных структур вокруг неё не обнаружено. Аналогичное строение имеет и кариосома парамера *Chilodonella* (Radzikowski 1973).

Как парамер, так и ортомер содержат большое количество ядрышек, которые, как правило, оттеснены к периферии макронуклеуса (Табл. X 20), но иногда встречаются как в толще парамера и ортомера, так и вблизи их границы (Табл. I 1, XI 22, XII 23). Ядрышко состоит из фибриллярного компонента, занимающего его центральную часть, и гранулярного, занимающего периферическую зону и отвечающего, по-видимому, гранулярной форме РНП.

В апикальной части ортомера (со стороны, противоположной парамеру) в начале интерфазы различается третий сегмент — „эпимер" (Янковский 1973). Эпимер напоминает по своей ультраструктуре парамер. Он также не отделен от ортомера никакими мембранами (Табл. XI 22).

В матриксе как парамера, так и ортомера (между хроматиновыми тяжами) мы наблюдали скопления интерхроматиновых гранул, со средним диаметром около 20 nm (Табл. XII 23). Часто эти гранулы расположены вблизи ядрышек. Кроме интерхроматиновых гранул, в обоих сегментах макронуклеуса обнаруживаются и более крупные электроннoплотные перихроматиновые гранулы, диаметром около 40 nm (Табл. XII 23). Как предполагают Monneron et Bernard (1969), оба типа гранул представляют собой рибонуклеопротеиды, содержашие мРНК, и возможно, играют роль в переносе информации из ядра в цитоплазму. Как правило, перихроматиновые гранулы располагают-

ся по периферии компактного хроматина ортомера, часто непосредственно под ядерной оболочкой. Их также можно наблюдать вблизи ядрышек.

Микронуклеусы *C. elegans* сферические: у особей с кариосомой они крупные и лежат в непосредственной близости от макронуклеуса, а их хроматин диспергирован. В начале интерфазы микронуклеусы мельче и их хроматин конденсирован (Табл. I 1, XII 24). Как обычно у *Ciliata*, микронуклеус *C. elegans* окружен двухмембранной оболочкой и в интерфазе равномерно заполнен фибриллярным материалом, по-видимому, представляющим собой нити ДНП.

Таким образом, ультраструктура кортекса, ресничных рядов, цитофарингеальной стенки, прикрепительного диска и гетеромерного макронуклеуса, характерные для *Cavichona elegans*, мало отличается от строения этих же структур у представителей двух семейств отряда *Gymnostomatida—Chlamydodontidae* и особенно *Dysteriidae*. Изучив аналогичные структуры у *Chilodochona* Grain et Batisse (1974) предлагают пересмотреть систему инфузорий и установить два новых подотряда (*Chonotricha* и *Cyrtophorina*) в отряде *Hypostomatida*. Признавая несомненное происхождение *Chonotricha* от *Dysteriidae* и вызванное этим сходство их ультраструктур, мы считаем тем не менее более правильной точку зрения Янковского (1973), который выделяет *Chonotricha* в отдельный подкласс класса *Ciliata*. Справедливо принимая, что доказанное происхождение одного таксона от другого еще не является основанием для включения первого во второй, Янковский приходит к такому выводу на основании большого морфологического своеобразия *Chonotricha* большого числа родов и семейств в этой группе и значительной дивергенции, имевшей место внутри данного таксона.

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SUMMARY

The ciliate *Cavichona elegans* (Swarczewsky) (*Chonotricha*) is covered by the plasma membrane, under which there is an epiplasmic layer pierced by numerous pelicular pores. The epiplasmic is underlain by longitudinally oriented triads of microtobuli. The pellicular pores are places of formation of pinocytotic vesicles.

The helical prebuccal funnel forms 4–4.5 turns. Its inner side carries the ciliary bands. These consist of several kineties, composed of single kinetosomes and giving rise to subkinetal ribbons of microtubuli which pass under kinetosomal bases. The cytostome is located at the base of the funnel and leads into the cytopharynx. The pharyngeal wall is formed by 28–32 longitudinal flat ribbons of microtubuli which are superposed in a spiral-like manner. A system of "complex tubuli" exists between the ribbons and outside of them. The pharyngeal armature includes no nemadesmata.

The excretory apparatus consists of a system of channels of the smooth endoplasmic reticulum ("nephridioplasm"), a contractile vacuole, its feeder canals and

its discharge canal. The latter is formed by an invagination of the plasma membrane and strengthened by microtubules.

The glandular organelle, secreting the adhesive disc, consists of finger-like secretory ampullae which open at the base of the body. The base of the preoral funnel, the pharynx, and the glandular organelle are attached to the cuticle by an armature consisting of anastomosing microfibrillar bundles.

The nuclear apparatus consists of one heteromerous macronucleus of juxtaposed type and of 1 to 3 micronuclei. The orthomere of the macronucleus contains a network of chromatin strands. The paramere has a fibrillar matrix and forms an endosome by the end of the interphase. Nucleoli exist in both segments of the macronucleus and have fibrogranular structure. Interchromatin and perichromatin granules occur in both nuclear segments. Early interphasic macronuclei display also an "epimere", a chromophobic segment located opposite to the paramere and having the same fine structure as the latter.

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ПОДПИСИ К ТАБЛИЦАМ I—XII

Ультраструктура *Cavichona elegans* (Swarczewsky)
1: Обзорный снимок продольного среза через переднюю часть тела. Видны обе ресничные зоны-узкая (слева) и широкая (справа). 8000 ×
2,3: Тангенциальные срезы поверхности в средней части тела. Видны поперечные срезы пор и микротрубочки под эпиплазмой. Ряды пор расположены между пучками микротрубочек. 98000 ×, 97 000 ×
4: Поперуечный срез тела. Виден продольный профиль пелликулярной поры и триады микротрубочек. 94000 ×
5: Пелликулярная пора с пузырьком, содержащим включения. 93 000 ×

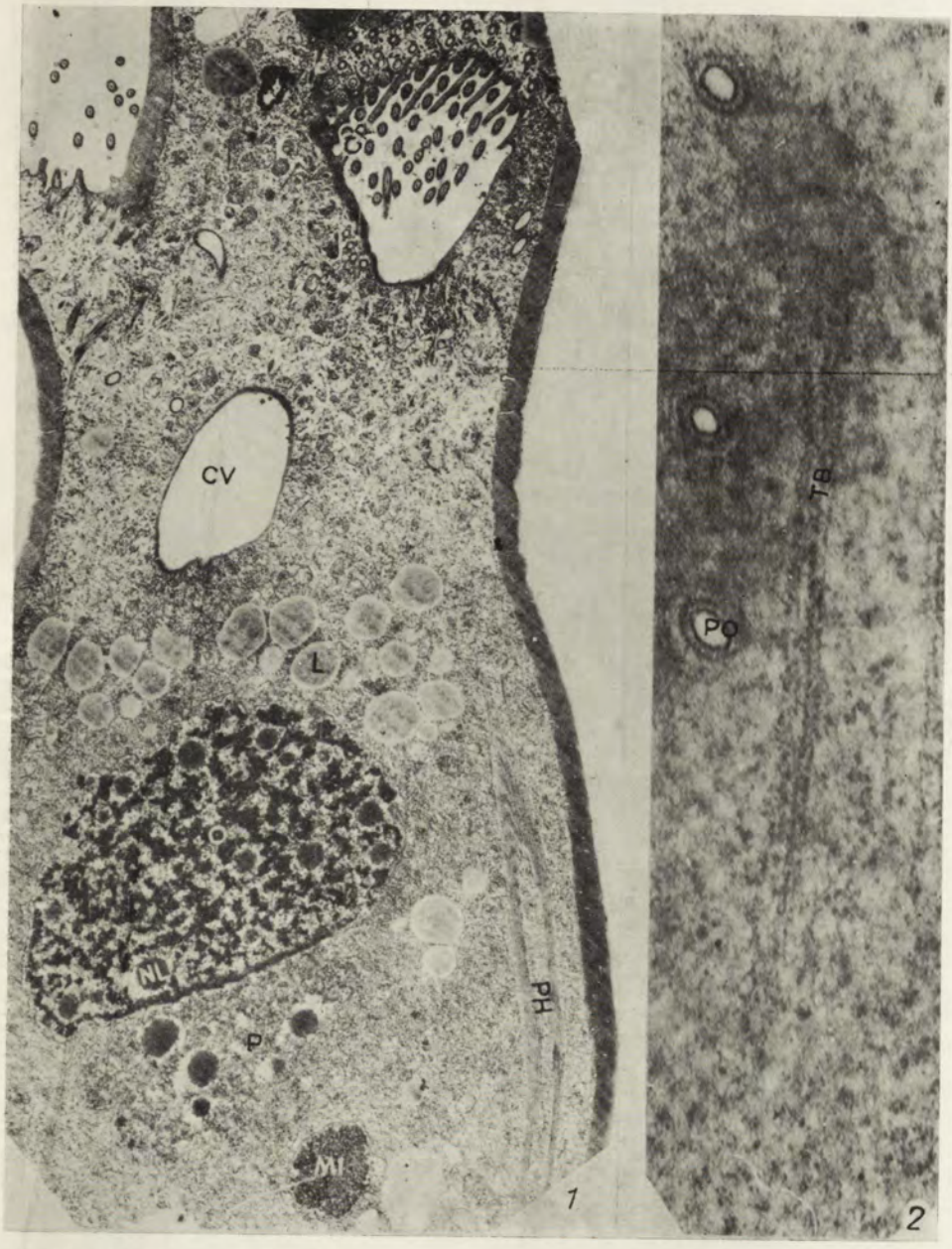
6: Часть предротовой воронки с перерезанной в нескольких местах узкой ресничной зоной. 6000 ×
7: Две кинетосомы одного ряда. Видны субкинетальные микротрубочки. 96 000 ×
8: Тангенциальный срез через широкую ресничную зону. Видны субкинетальные ленты микротрубочек. 62 000 ×
9: "Сложные трубочки" у поверхности перистома вблизи ротового отверстия. Видны ленты микротрубочек глотки, ставшие субпелликулярными (стрелки). Узкая ресничная зона (6 кинет) на поперечном срезе. 29 000 ×
10: Основание предротовой воронки: продольный срез через глотку и отверстие выводного канала сократительной вакуоли. 15 000 ×
11: Поперечный срез через глотку. 48 000 ×
12: Сократительная вакуоль в диастоле. 32 000 ×
13: Концевое расширение приводящего канала и впадение в него тонких каналов нефридиальной плазмы. 50 000×
14:Нефридиальная зона цитоплазмы с приводящими каналами и выводной канал сократительной вакуоли. Видны пучки микрофиламентов, поддерживающих предротовую воронку. 48 000 ×
15: Пищеварительные вакуоли с заглоченными бактериями. Видны эндоплазматическая сеть и митохондрии. 48 000 ×
16: Поздние стадии переваривания бактерий. Видны триады микротрубочек под эпиплазмой. 48 000 ×
17: Цитоплазма в районе стебелька. 64 000 ×
18: Общий вид "ножки" с железистым аппаратом. 6000 ×
19: Ампула железистого аппарата. 24 000 ×
20: Общий вид гетеромерного макронуклеуса с кариосомой в парамере; видны два микронуклеуса. 18 000 ×
21: Часть ортомера макронуклеуса: видна двухмембранная оболочка макронуклеуса. 72 000 ×
22: Молодой макронуклеус на продольном срезе, состоящий из трех частей — эпимера, ортомера и парамера (без кариосомы). Виден микронуклеус. 15 000 ×
23: Часть макронуклеуса, показывающая структуру компактного хроматина ортомера, часть парамера и ядрышко с гранулярной и фибриллярной частями. 40 000 ×. Видны интерхроматиновые и перихроматиновые гранулы.
24: Общий вид интерфазного микронуклеуса. 64 000 ×
Используемые обозначения: A — аксосома, AD — прикрепительный диск, AM — ампула, секретирующая прикрепительный диск, C — реснички, CC — конденсированный хроматин, CR — цитоплазматические гребни, CT — сложные трубочки, CV — сократительная вакуоль DC — выводной канал сократительной вакуоли, EP — эпимер, ER — эндоплазматическая сеть, FC — приводящие каналы сократительной вакуоли, FP — фибрилярная часть ядрышка, FV — пищеварительная вакуоль, GP — гранулярная часть ядрышка, IG — интерхроматиновые гранулы, K — кариосома, L — липидные гранулы, M — митохондрии, MA — макронуклеус, MI — микронуклеус, N — шейка, NE — ядерная оболочка, NL — ядрышко, NP — нефридиальная плазма, O — ортомер, P — парамер, PF — предротовая воронка, PH — глотка, PG — перихроматиновые гранулы, PO — кутиркулярные поры, R — рибосомы, S — септа в кинетосоме, SK — субкинетальные микротрубочки, TB — микротрубочки, подстилающие кутикулу.

EXPLANATION OF PLATES I–XII

Fine structure of *Cavichona elegans* (Swarczewsky)
1: Survey micrograph of a longitudinal section through body end showing both ciliated zones, the narrow (at left) and the broad (at right). 8000 ×
2, 3: Tangential section of the cell surface in the medium body part. Transverse cuts of pores and subcuticular microtubules are seen. Rows of pores alternate with microtubular bundles. 98 000 ×, 97 000 ×
4: Cross section of the body showing the longitudinal profile of a cuticular pore and transversely cut triads of microtubules. 94 000 ×
5: Cuticular pore with an inclusion-containing vesicle. 93 000×
6: Part of the preoral funnel with several sections through the narrow ciliary zone. 6000 ×
7: Two kinetosomes of the same kinety showing subkinetal microtubuli. 96 000 ×

8: Tangential section through the broad ciliary zone. Subkinetal ribbons of microtubuli are seen. 62 000 ×
9: "Complex tubules" approaching the peristomal surface near the cytostome. Ribbons of pharyngeal microtubules here become subpellicular (at arrows). The narrow ciliary zone (6 kineties) seen in transverse section. 29 000 ×
10: The base of the preoral funnel showing the cytopharynx in longitudinal section and the outer opening of the discharge canal of the contractile vacuole. 15 000 ×
11: Cross section through the cytopharynx. 48 000 ×
12: Contractile vacuole in diastole. 32 000 ×
13: Terminal dilatation of the feeder canal and thin channels of the nephridioplasm opening into it. 50 000 ×
14: Nephridial zone of the cytoplasm showing feeder canals and the discharge canal of the contractile vacuole. Bundles of microfilaments support the preoral funnel. 48 000 ×
15: Food vacuoles containing bacteria, endoplasmic reticulum and mitochondria are also seen. 48 000 ×
16: Late stages of digestion of bacteria. Triads of microtubules visible under the cuticle. 48 000 ×
17: Cytoplasm of the stalk region. 64 000 ×
18: General view of the "foot" with the glandular apparatus. 6000 ×
19: Secretory ampulla of the glandular apparatus. 24 000 ×
20: General aspect of the heteromerous macronucleus containing a karyosome in its paramere; two micronuclei are seen. 18 000 ×
21: Part of the ortomere showing the two-membraned envelope of the macronucleus. 72 000 ×
22: Longitudinal section of a post-division macronucleus showing three parts, the epimere, the orthomere and the paramere (without karyosome). Micronucleus seen. 15 000 ×
23: Part of macronucleus showing fine structure of the compact chromatin of the orthomere, a part of the paramere and a nucleolus with its granular and fibrillar zones. Interchromatin and perichromatin granules are seen. 40 000 ×
24: General aspect of an interphase micronucleus. 64 000 ×

Abbreviation used: A — axosome, AD — attachment disc, AM — secretory ampullae of the glandular apparatus, C — cilia, CC — condensed chromatin, CR — cytoplasmic ridges, CT — complex tubules, CV — contractile vacuole, DC — discharge canal of the contractile vacuole, EP — epimere, ER — endoplasmic reticulum, FC — feeder canals of the contractile vacuole, FP — fibrillar part of the nucleolus, FV — food vacuole, GP — granular part of the nucleolus, IG — interchromatin granules, K — karyosome, L — lipid granules, M — mitochondria, MA — macronucleus, MI — micronucleus, N — "neck", NE — nuclear envelope, NL — nucleolus, NP — nephridioplasm, O — orthomere, P — paramere, PF — preoral funnel, PH — pharynx, PG — perichromatin granules, PO — cuticular pores, R — ribosomes, S — septum (in the kinetosome), SK — subkinetal microtubules, TB — subcuticular microtubules.



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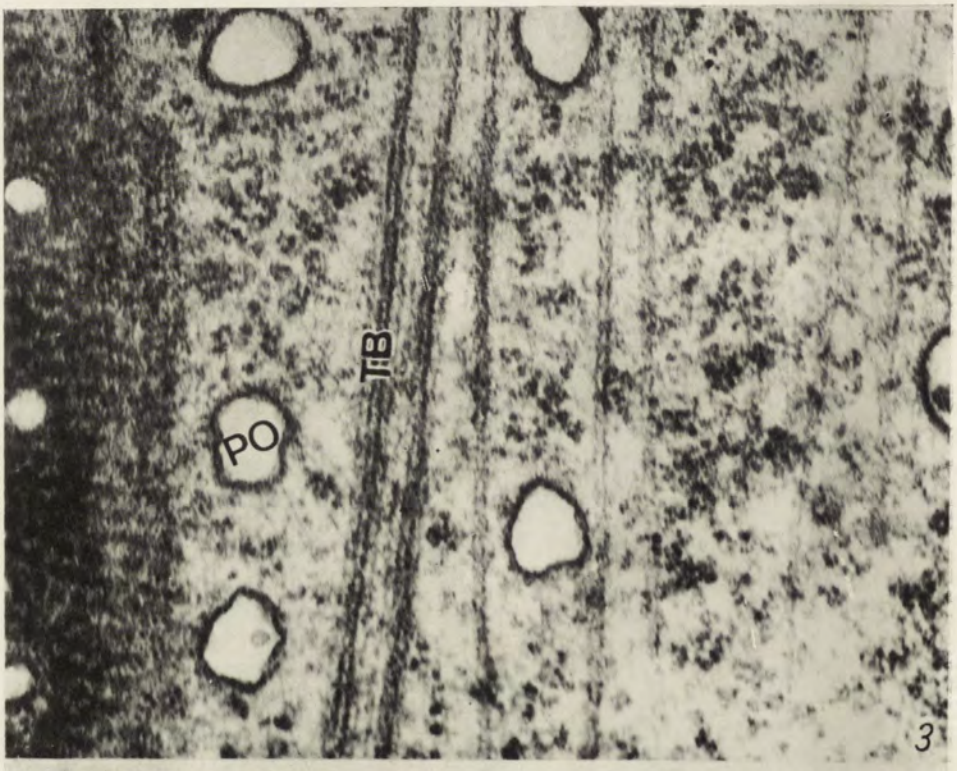

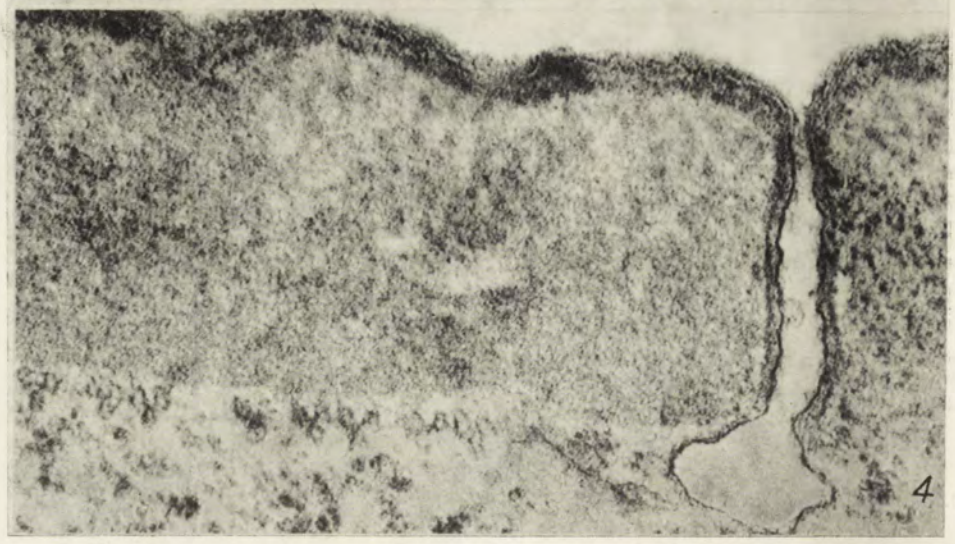

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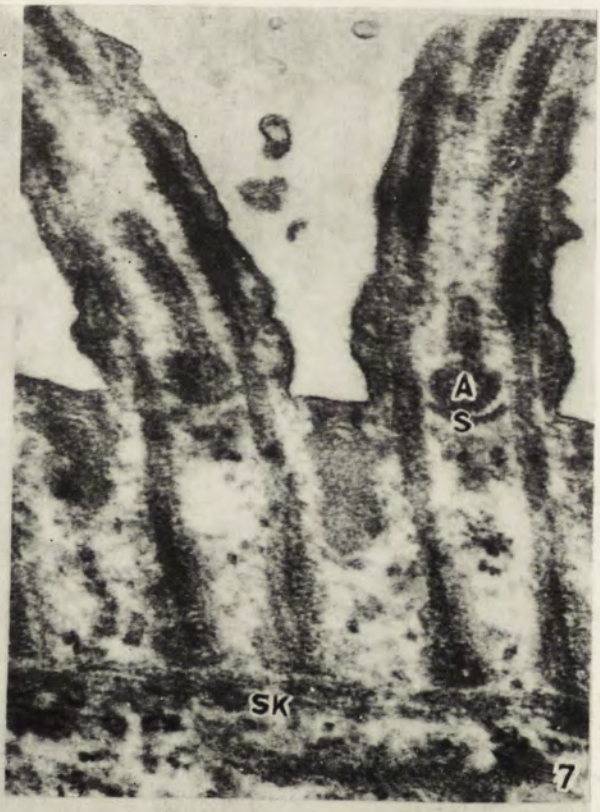

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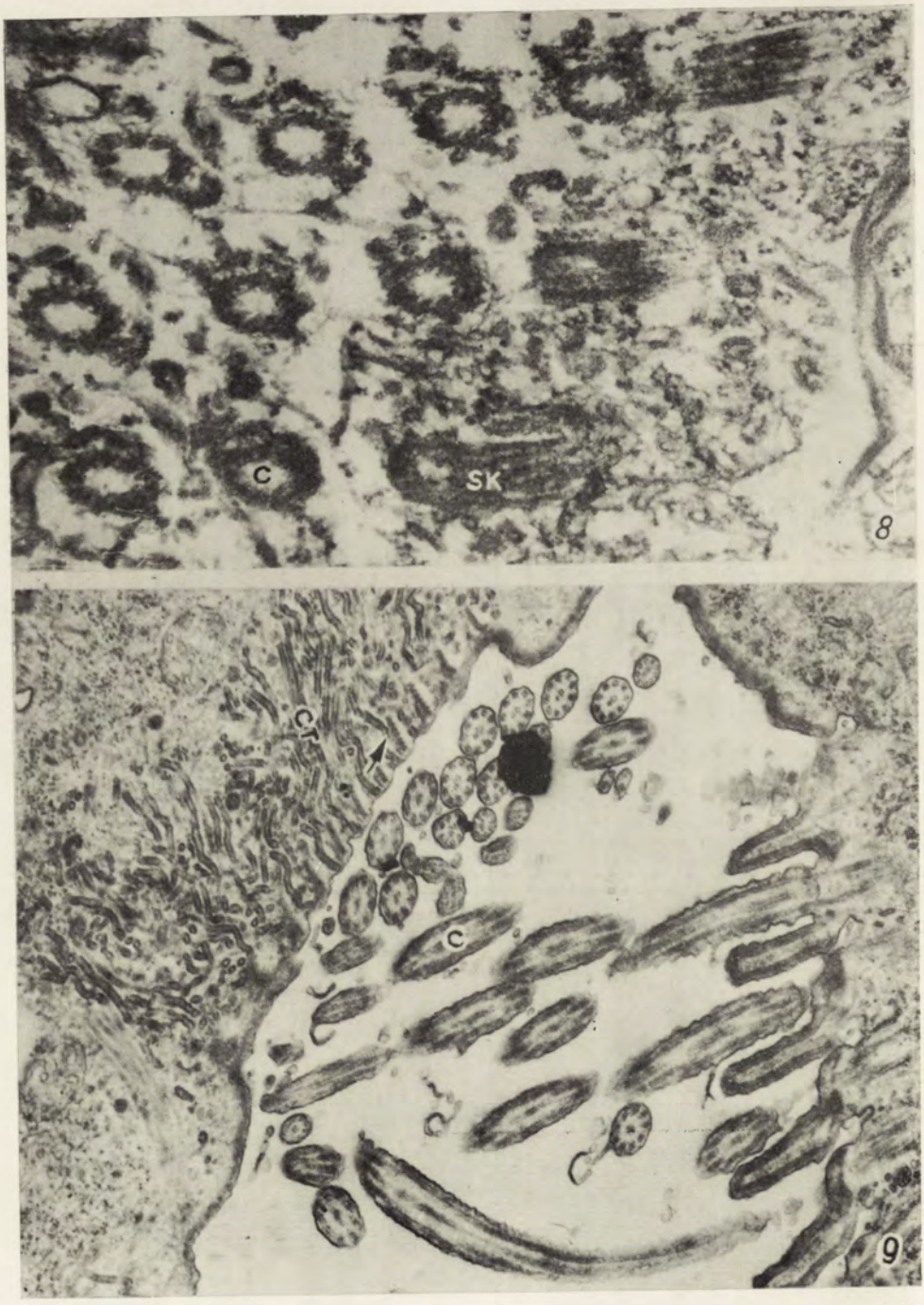

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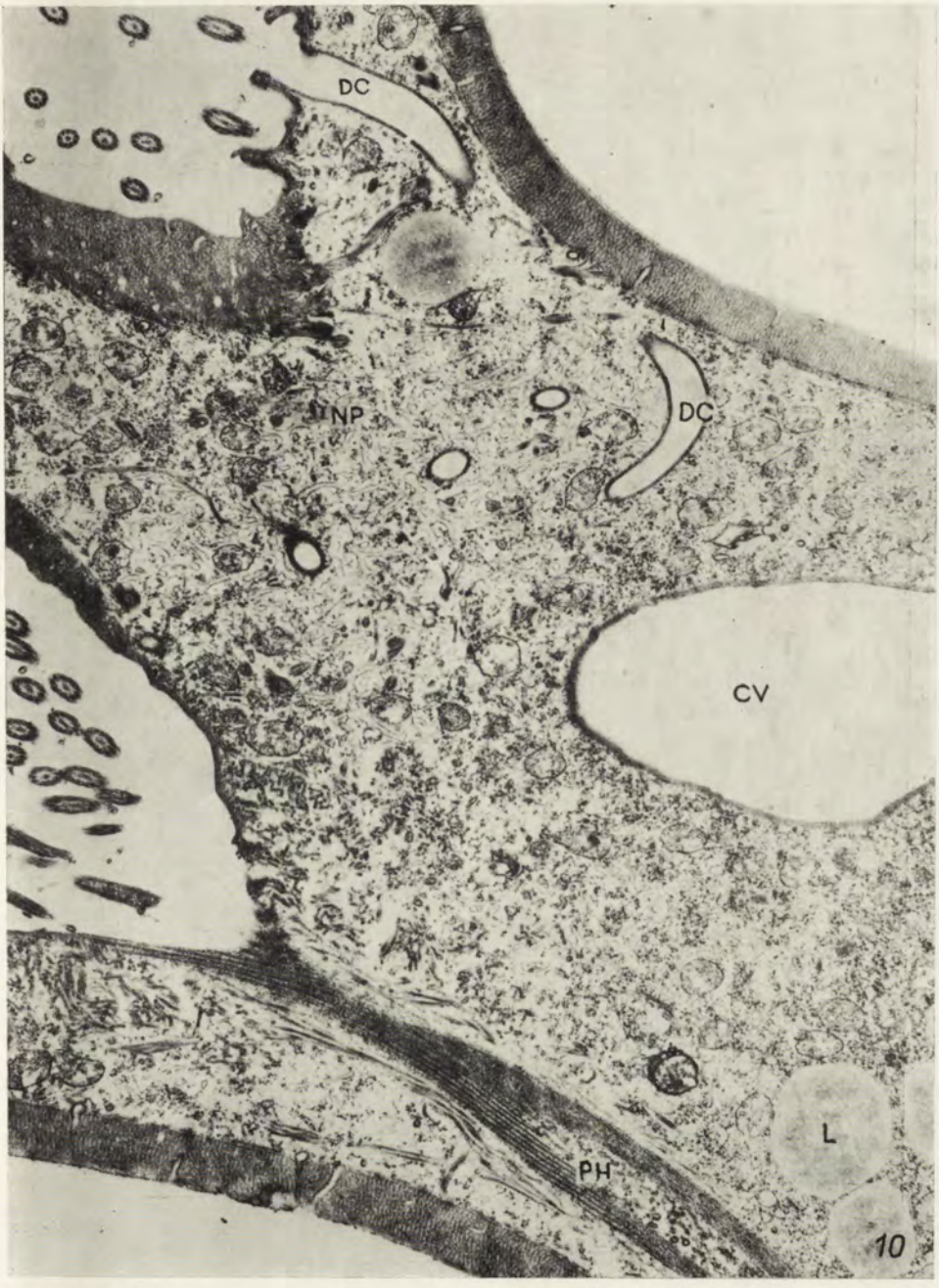

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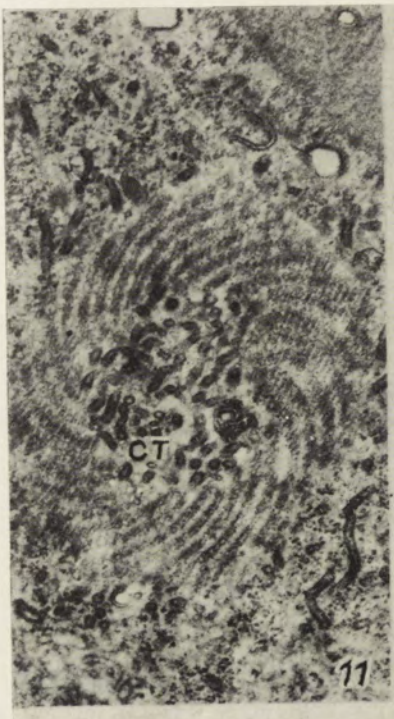

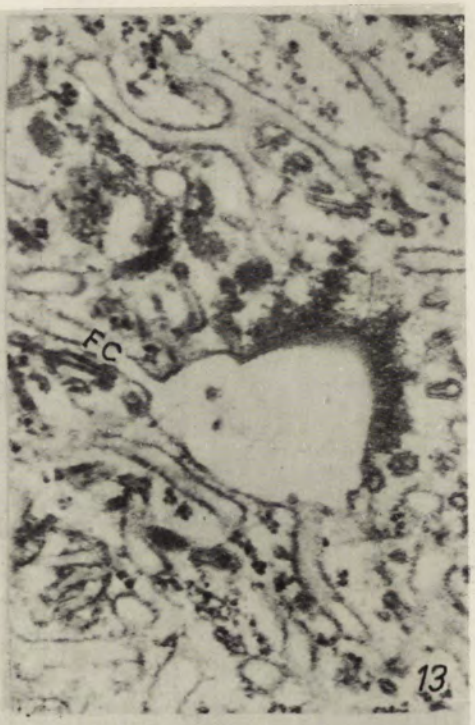

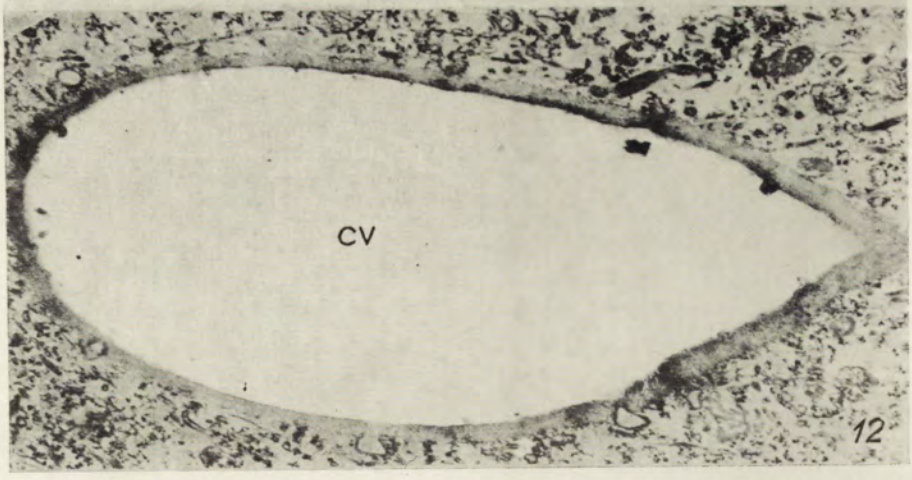

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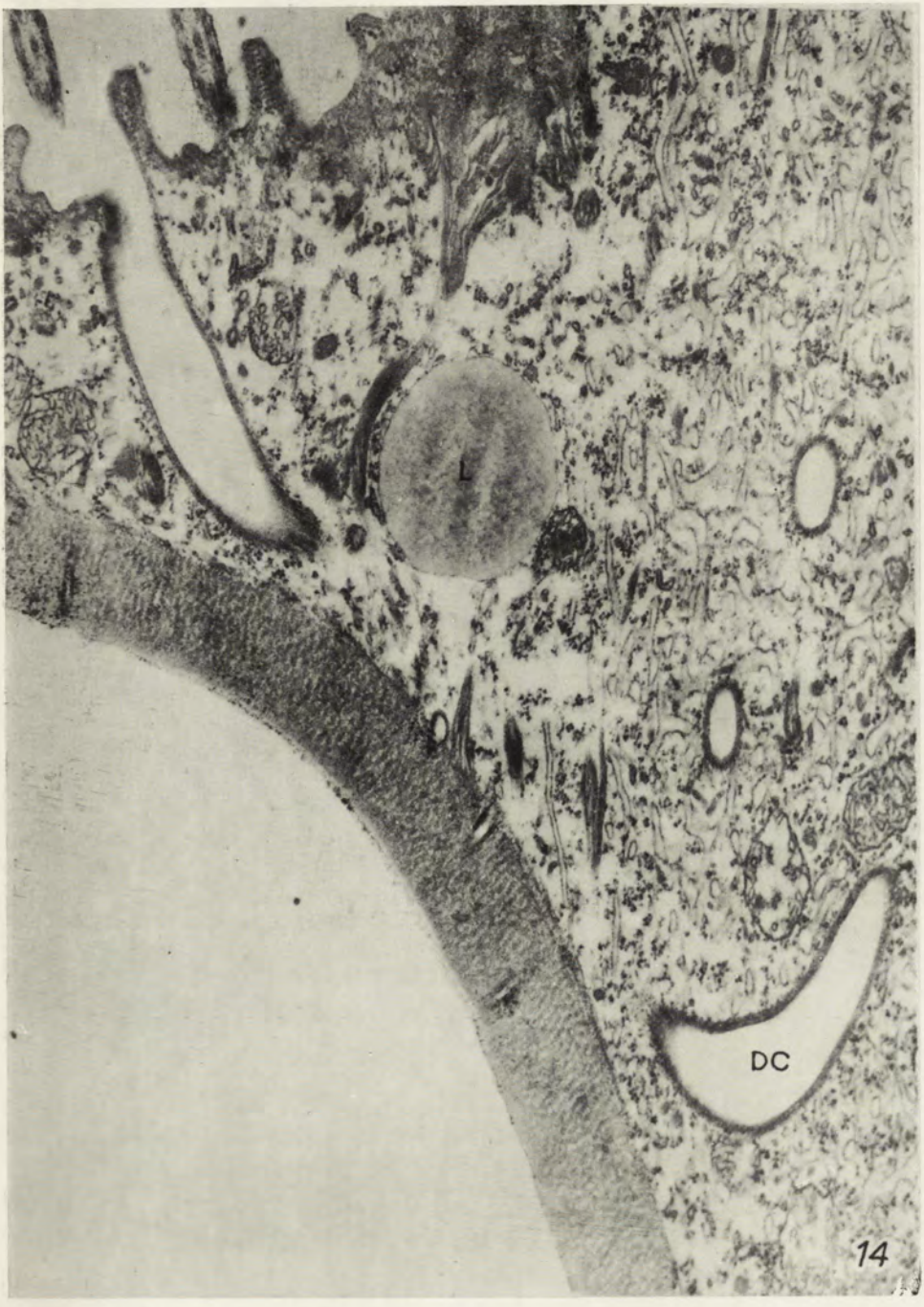

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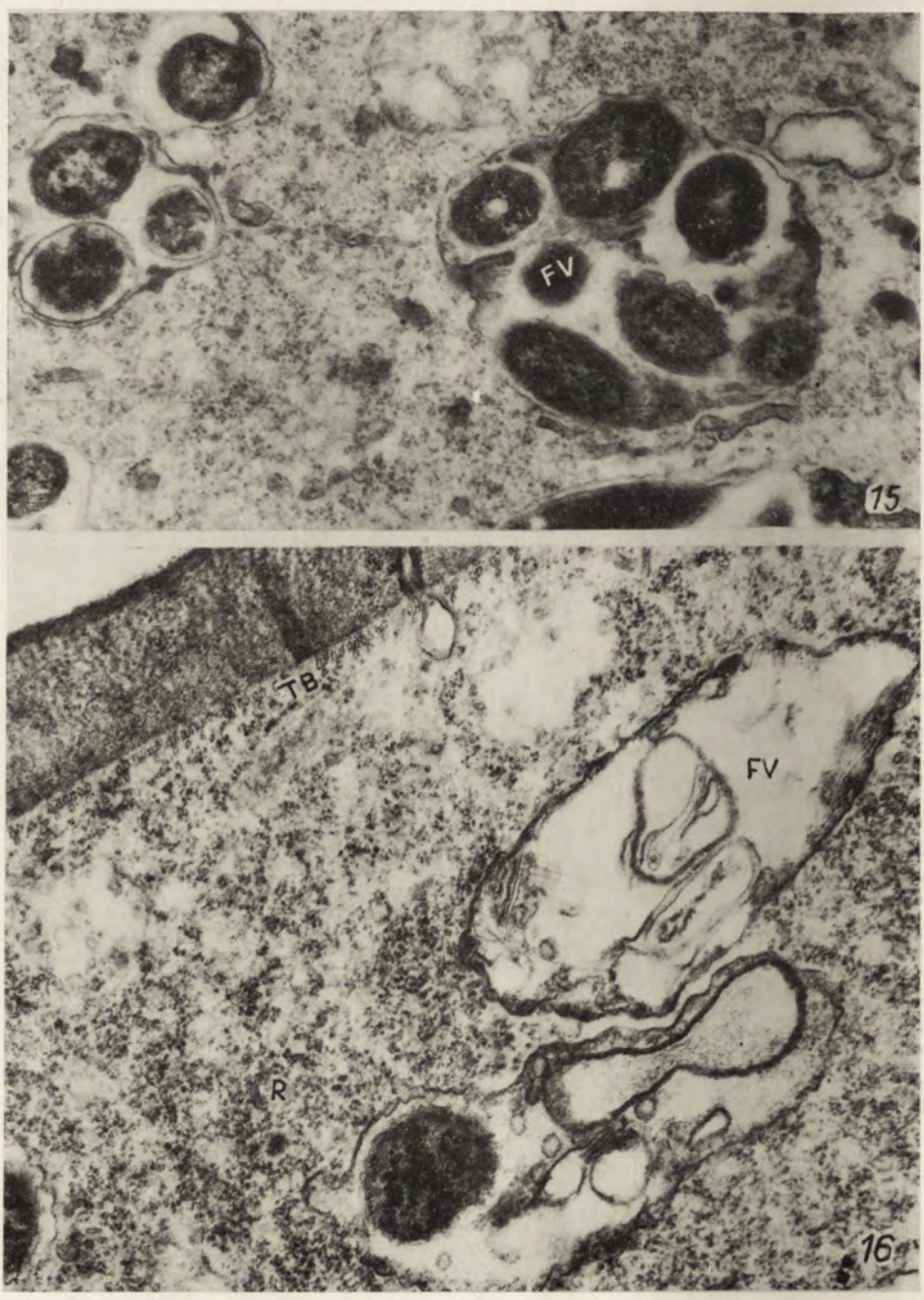

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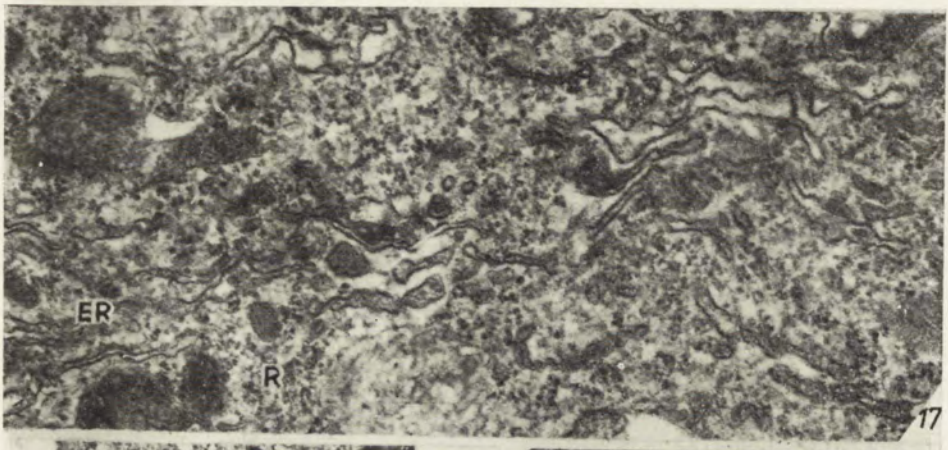

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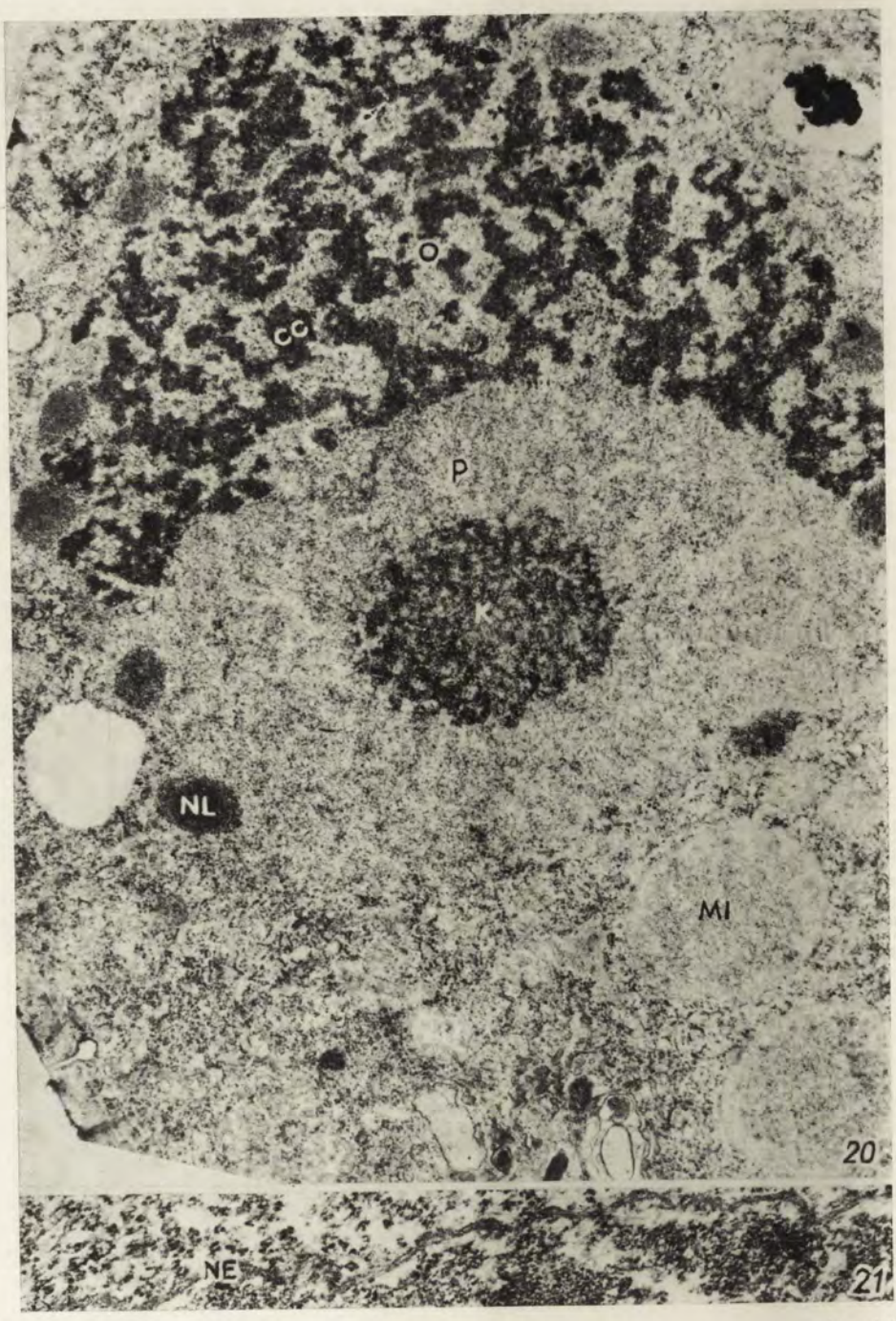

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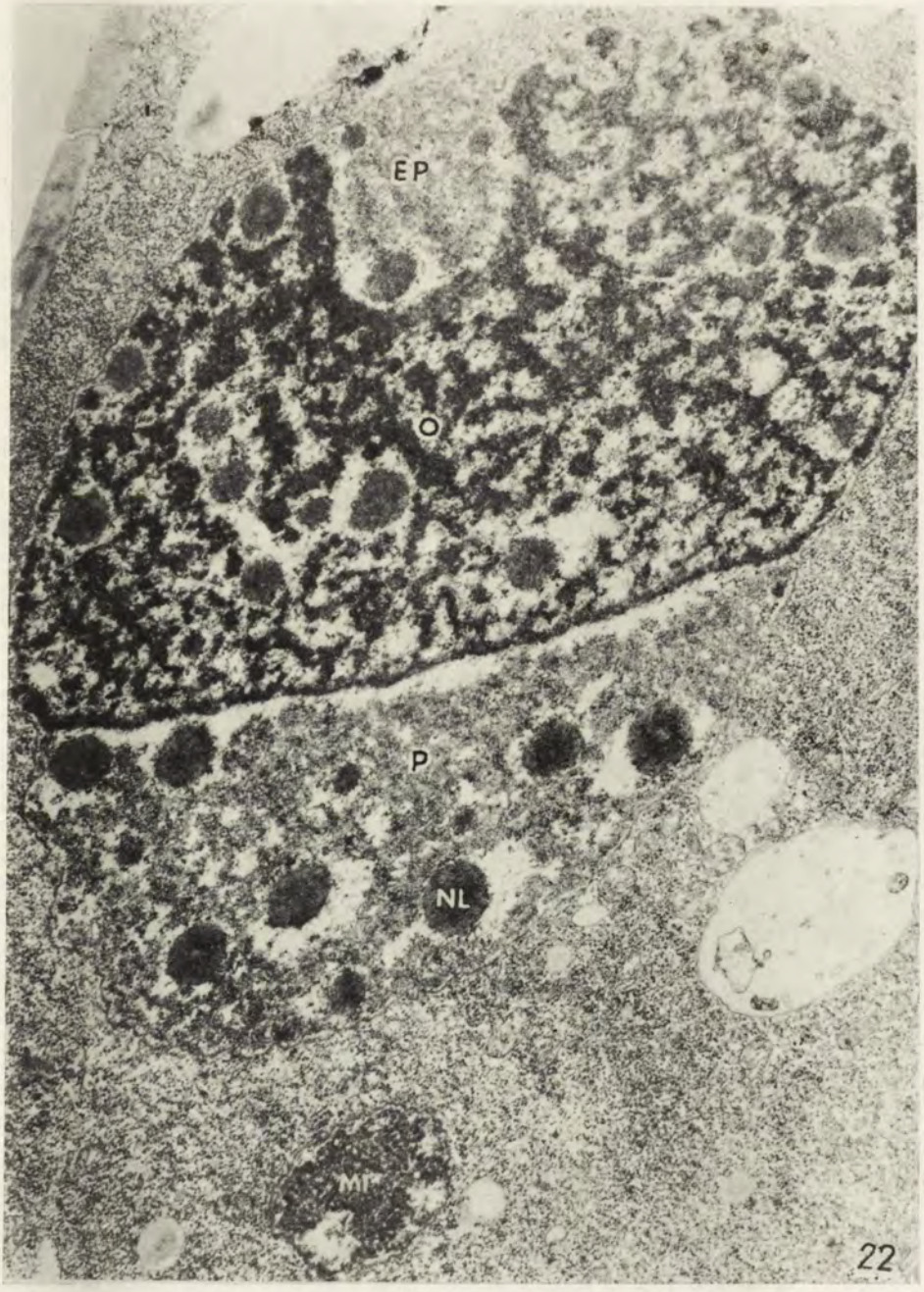

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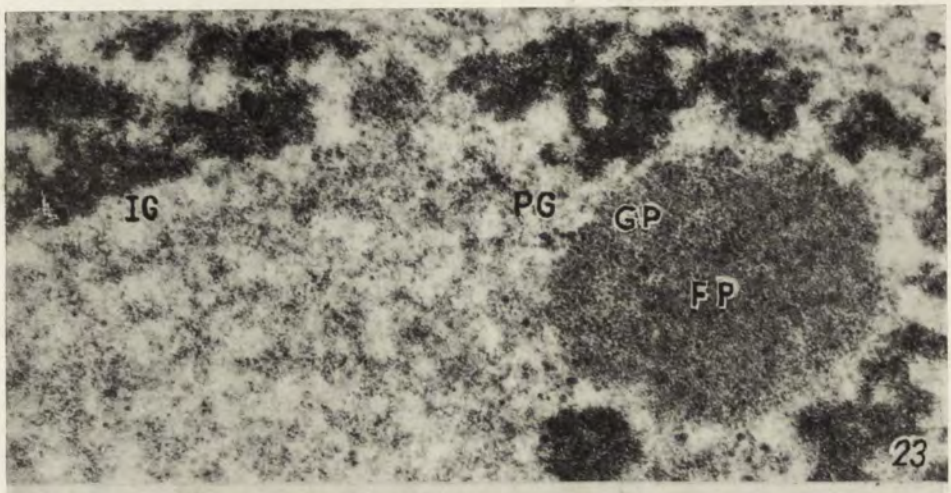

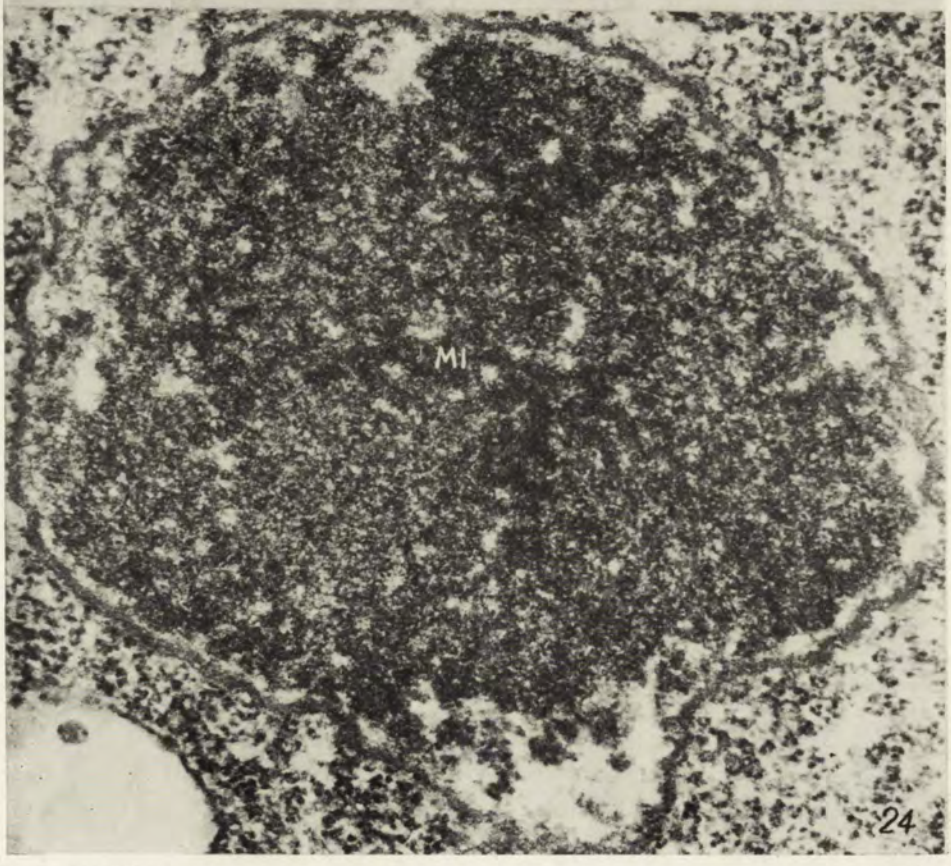

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Temperature Induced Abnormal Forms in *Stylonychia notophora* (Stokes)

Synopsis. Stylonychia notophora were subjected to a suboptimum temperature of 10± 1°C for a period of seven days. Cold treatment caused high mortality. However, the survivals during the course of treatment exhibited erratic binary fission resulting in daughter cells with abnormal body shape and nuclear apparatus. Some of them remained as doublets. These doublets and abnormal forms after a series of abnormal divisions under optimum conditions assumed normal body shape and nuclear make up.

It is well documented that temperature influences the metabolic processes of protozoans (Andrus et al. 1963) and thereby alters their fission rate (Kasturi Bai et al. 1969). Protozoans exposed to high temperatures exhibit reduction in the rate of cell division; when division occurs it results in abnormal forms lacking certain oral organelles or structural systems (Frankel 1962). Though resistance to low temperatures has been observed in many protozoans (Hall 1967) relatively very little is known about the biothermal relationship in protozoans exposed to very low temperatures. The present paper deals with such studies on the effects of a sub-optimal temperature on the induction of abnormal forms and the macronuclear changes during binary fission in a ciliate, *Stylonychia notophora* (Stokes).

Material and Methods

Stock cultures of *S. notophora* were grown in sterilized hay infusion inoculated with the bacteria, *Aerobacter aerogens* at 20 ± 1°C. Jars containing actively dividing animals in the log phase of growth were exposed continuously to a suboptimal temperature of 10 ± 1°C in a cold chamber and at the end of every 24 h

they were examined under microscope and abnormal forms were scored for 7 days. The percentage mortality during the exposure period was also recorded by subjecting a known number of animals (100) to cold treatment at one time. Cytokinetic forms were isolated during the exposure period, allowed to divide and the time taken by the daughter cells to divide under experimental conditions was noted. For recovery experiments, from animals subjected to low temperature for seven days, the abnormal forms were isolated and allowed to grow under optimum temperature (21 ± 1°C). All the experiments were repeated at least three times. The changes in nuclear morphology during the course of divisions of these abnormal forms were studied after fixing and staining them by Feulgen light green.

Results

Animals subjected to cold treatment became progressively sluggish and majority of them settled at the bottom by 24 h. On the first day, the treatment caused about 30% mortality and on the 7th day there were only 37% survivals (Table 1). Cytokinetic forms were detected in the

Table 1

Percentage Mortality in *Stylonychia notophora* Subjected to Cold Treatment

Period of cold exposure (days)	No. of animals surviving		Cumulative per cent mortality
	Before exposure	After exposure	
1	100	70	30
2	70	59	41
3	59	51	49
4	51	47	53
5	47	43	57
6	43	41	59
7	41	37	63

treated colony indicating that the survivals did multiply during treatment. However, the mean generation time was 33 h as compared to 9.5 h in the controls.

The treated animals on division produced abnormal forms which were smaller than the normal ones and were characterized by changes in their body shape and variations in the morphology of their nuclear apparatus. Such forms were either flattened in the region above the adoral zone of membranelles; or the entire body assumed a triangular or spherical shape (Pl. I 1); or had a distinct notch on one of the lateral

sides (Pl. I 2). A steady increase in the number of abnormal forms was observed when the treatment lasted for seven days (Table 2).

Table 2

Induction of Abnormal Forms in *Stylonychia notophora* Following Cold Exposure

Period of cold exposure (days)	Per cent abnormal forms	Various Abnormal Forms[1]		
		Having one macronucleus	Having two macronucleus	With deformed shape
1	18	4	2	12
2	30	8	8	14
3	36	9	11	16
4	40	12	10	18
5	37	13	9	15
6	44	19	12	13
7	48	23	11	14

[1] Each observation is the mean of two sets of experiments each consisting of 100 animals randomly picked up from a log phase culture which had been subjected to cold treatment.

Stylonychia normally posseses two cylindrical macronuclei oriented more or less in the centre of the animal (Pl I 3). However, the abnormal forms displayed variation with regard to the number, shape and location of the macronuclei. The spherical and triangular forms usually possessed a single spherical or elongated macronucleus located in the centre or more towards the anterior end (Pl. I 1). A few abnormal forms showed a single rod shaped, elongated macronucleus with variations in its morphology. Some had club shaped macronucleus with a notch giving a nodal appearance. Binucleate abnormal forms closely resembled the normal animals but the macronuclei were either unequal in size (Pl. I 4–5) or differed in their orientation as given in (Pl. I 6).

In recovery experiments the spherical and the triangular abnormal forms failed to multiply and cytolysed in 24 h. The other abnormal forms under optimum conditions divided rapidly but the macronuclear changes during fission differed from that of the normal animals. The results are summarized in Fig. 1. In most of the single macronucleated daughter cells the macronucleus divided alone and they became binucleated forms within 6 h of the previous division. After a long period of 10 h the animals showed normal division of the macronuclei (primary and secondary divisions) resulting in daughter cells with two macronuclei each. At times, after the primary division of the elongated macronucleus, a secondary division occurred in one of the macronuclei and the daughter cells accordingly possessed one or two macronuclei. This type of division was common in those abnormal forms which had a trinodal nucleus. These

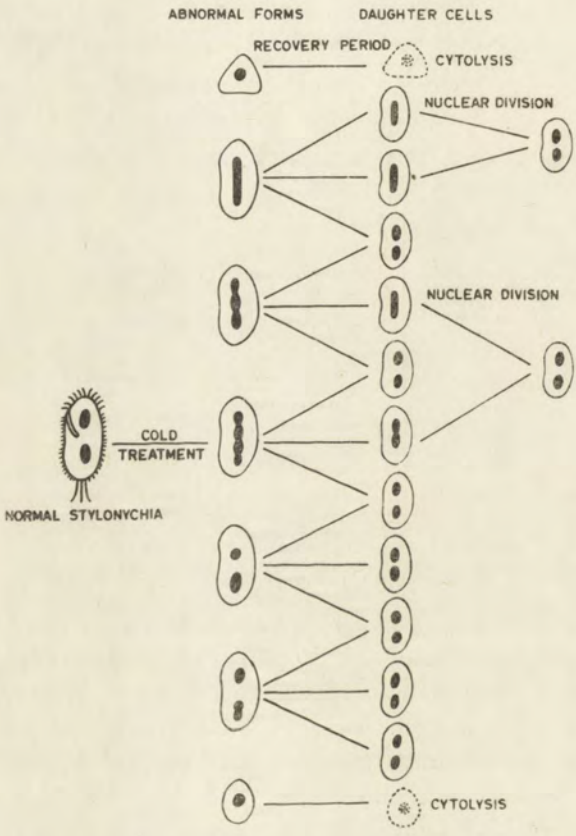

Fig. 1. Diagram showing the formation of abnormal forms in *Stylonychia notophora* on cold treatment and their division on recovery

abnormal forms became binucleated after two division cycles and acquired the shape, size and disposition of the normal animals by subsequent five division cycles. In the normal animals there is a fusion of the two macronucleus preceding primary and secondary divisions. Such a stage was not observed in the abnormal forms. However, binary fission in binucleated forms was normal.

While the majority of the abnormal forms divided but about 5% of the daughter cells remained united by a cytoplasmic bridge forming doublets (Pl. I 7) due to abortive division. The Figure 2 summarizes the formation and division of doublets. In these dublets, the proter remained attached to the opisthe and both were oriented at an angle and appeared L-shaped. They remained so only for a period of about 15 h after which they separated before the macronucleus showed secondary division and the resulting singlets possessed only a single elongated macronucleus.

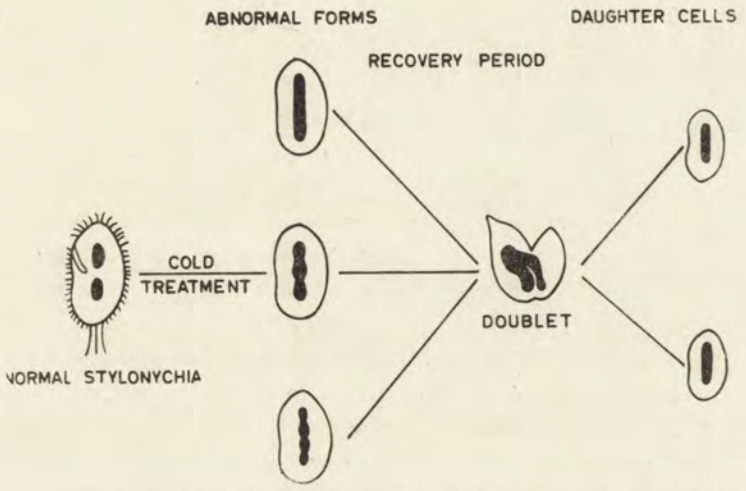

Fig. 2. Diagram showing the formation of doublets in *Stylonychia notophora*

Discussion

The present data clearly indicate that *S. notophora* subjected to cold treatment showed high mortality and delayed cell division in the survivals resulting in a number of abnormal forms. Factors such as temperature (Kasturi Bai et al. 1969), radiations (Jagger et al. 1969), operations (Gauvrin and Hirshfield 1967) have been reported to induce disturbances in the normal course of division of the ciliate. Thermic shocks imparted to *Stylonychia* do not impair the nuclear division (Totwen-Nowakowska 1965) though it distorts the normal body shape. However, in the present study it was observed that *Stylonychia* subjected to cold treatment exhibited erratic unequal divisions of the macronucleus. During division, the line of macronuclear division coincides with the cytokinetic furrow. This indicates that the site of cleavage in the cell appears to be not determined by the position of the macronucleus but rather by spatial relationship within the cortex. A similar observations was recorded earlier in *Tetrahymena* (Gavin and Frankel 1966). In *Stylonychia* doublets have been shown to arise spontaneously or due to high thermic shocks (Totwen-Nowakowska 1964, 1965) or under the influence of chemical factors or operation (Tchang Tso-sun et al. 1964). The present study shows that cold treatment also can result in doublet formation. But these doublets divide erratically unlike the heat-induced ones, which divide true to their type by fission (Totwen-Nowakowska 1965), and do not give rise to the next generation of doublets and hence exibit a very low degree of

stability through asexual reproduction. Inhibition of cytokinesis is responsible for the non-separation of daughter cells and in the formation of doublets. The delayed cytokinesis may be due to the inhibition of certain substances which are synthesized at this late stage and thus associated with cytokinesis (Tartar 1967 and Berkavitz et al. 1968).

The S phase in *Stylonychia* as in other hypotrichous ciliates is characterized by the appearance and movement of the replication band across the length of the two macronuclei and its disappearance (Raikov 1969). The replication band in normal *Stylonychia* appears after 2 h of growth and disappears after another 6 h thus demarcating the S phase from the preceding G_1 phase and the succeeding G_2 phase and division. However, in the present study, the abnormal forms which had a single macronucleus, did not show any replication band even under optimal conditions though they divided. In the normal animals after the S phase the two macronuclei fuse to form a compact body and this fusion stage which lasts for 30 min marks the end of G_2 period. This fusion stage was not observed during fission of the abnormal forms. It is likely that the single macronucleus in the abnormal forms may be the fusion nucleus of the preceding cell cycle which had undergone primary division only, during the D phase of the cell cycle thus resulting in mono-macronucleated abnormal forms.

The present study shows that a certain level of resistance to cold treatment persists among *Stylonychia*. But since all these forms had originated from the same clone they are bound to have the same genotype and should therefore express an identical degree of resistance. But the data clearly indicate a significant variation in their tolerance level to cold treatment. Similar variability in response to temperature changes in *Paramecium* with identical genomes was observed and this phenomenon has been attributed to epigenetic variability (Ossipov 1966 and Poljansky and Irlina 1973).

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RÉSUMÉ

Stylonychia notophora était éxposée, durant 7 jours, à la température suboptimale do 10 ± 1°C. Ce traitement provoquait une mortalité élevée. Cependant, les individus survivants ce traitement subissaient la division binaire défective qui

aboutissait à des cellules déscendantes anormales en ce qui concerne leur forme et leur appareil nucléaire. Quelques uns restaient comme formes doubles. Les individus doubles et ceux de forme anormale regagnaient la forme habituelle du corps et la constitution normale de l'appareil nucléaire, dans les conditions optimales et après une série de divisions anormales.

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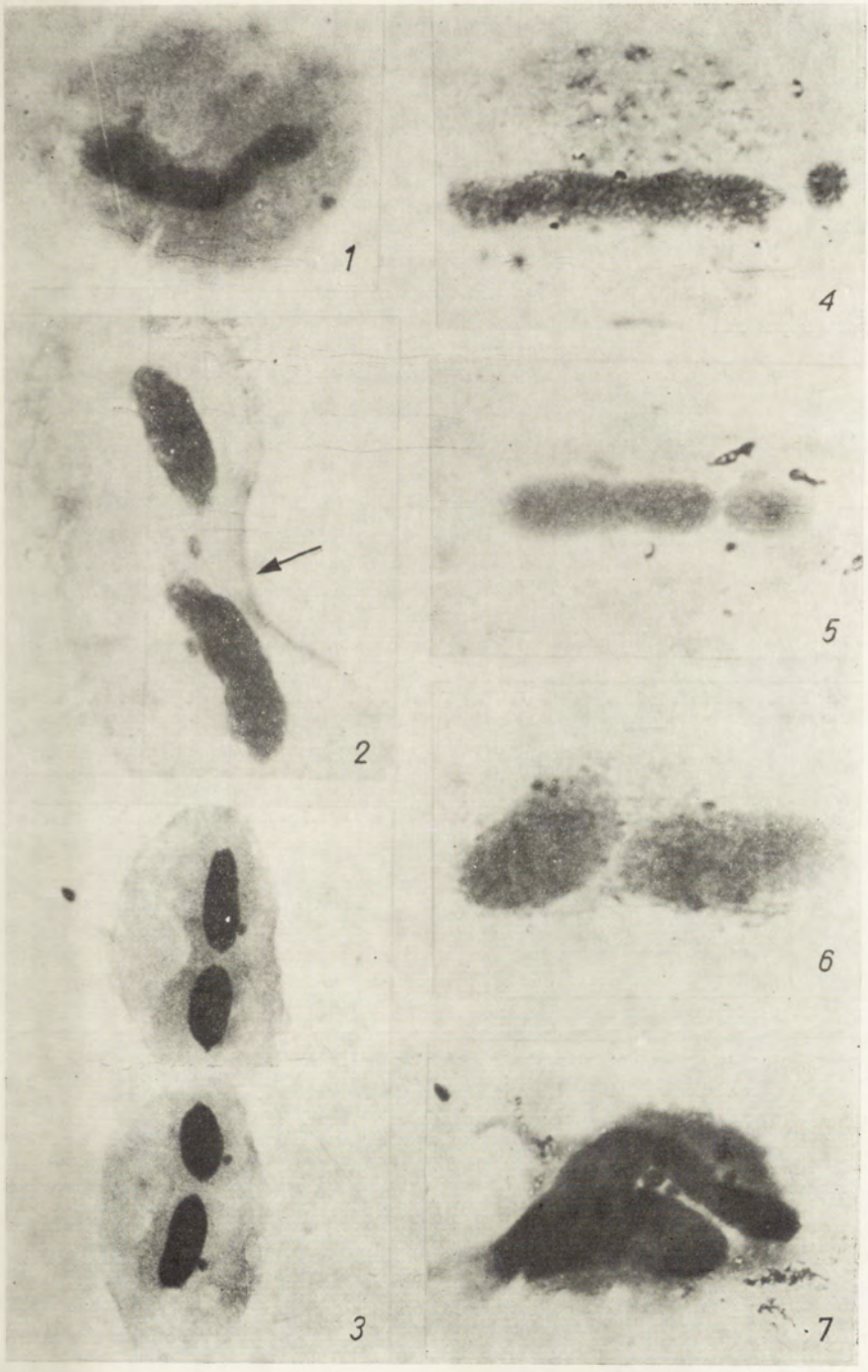
EXPLANATION OF PLATE I

1: A spherical, abnormal form of *S. notophora* with an elongated macronucleus. 400 ×
2: An abnormal form of *S. notophora* with a prominent lateral notch (arrow). 500 ×
3: A normal *S. notophora* just before division showing cylindrical macronuclei arranged in one line. 400 ×
4–5: Abnormal forms of *S. notophora* with macronuclei of unequal size. 400 ×
6: An abnormal *S. notophora* with two macronuclei oriented at an angle to each other. 400 ×
7: A doublet form of *S. notophora*. 400 ×



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Minimal Requirement of External Calcium of Motility of *Amoeba proteus*

Synopsis. The minimal calcium concentration required for amoeboid movement was detected. The range between 10^{-5} and 10^{-4} M Ca^{2+} was found as an optimal concentration for normal locomotion of amoebae. The $^{45}Ca^{2+}$ was applied for investigation of the amounts of calcium on the surface of amoeba. The amount of calcium adsorbed on the surface is proportional to the surrounding calcium concentration.

It has been recently proved that EGTA treatment results in sequences of changes in behaviour of amoebae, i.e., first they loose contact with substratum, what is followed by shortening of the body and finally by cessation of movement. These reactions were reversible only when 10^{-3} M calcium chloride was added (Hrebenda 1972). Now, the minimal concentration of calcium necessary to sustain all motile activities of *Amoeba proteus* has been determined.

Material and Methods

Amoeba proteus type C was cultured in Pringsheim solution at room temperature. Amoebae were fed with *Colpidium* sp. and washed with fresh medium two times a week. Prior to each experiment they were washed three times in 5 mM Tris-HCl buffer at pH 7. 1. Experiments were carried out in polyethylene or glass vessels. Glass was washed in 1 mM EDTA solution and deionised water.

Low concentrations of calcium ions were obtained through the use of calcium buffers. These were mixtures of EGTA and its calcium complex which also contains stabilized concentration of free calcium ions. The concentration of calcium ions was calculated according to Portzehl et al. (1964) with supplementary calculations referring to lower ionic strength (Ettori and Scoogan 1958).

In experiments with ^{45}Ca the radioactivity of supernatant was examined in "Nuclear Chicago D-17" gas scintillator. In such experiments a known number of

specimens was employed. Each sample contained from two to seven thousands of cells. The volume of cells was calculated as follows — amoebae were put into capillary of known diameter and their length measured. The volume was calculated from the formula for the cylinder. The volume of amoebae was necessary in this case to calculate the theoretical dilution of radioactive calcium added to cell suspension. The volume of one amoeba was equal to 2.9×10^6 cm^3.

Results

Amoebae washed three times and kept overnight in 5 mM Tris-HCl buffer at pH 7. 1 were transferred to vessels with calcium-EGTA buffers with concentration of free calcium ions equal to 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-7} M. Behaviour of specimens was observed in light microscope: (1) directly, after transfer, (2) for one hour after transfer, (3) after 24 h.

The first reaction observed was the elongation of pseudopodia — normal reaction of orientation. Then, in solution with low calcium concentration (10^{-7} M and 10^{-6} M) strong cytoplasmic streaming towards the inside took place. Finally, a rosette or spheric shape of the body of amoeba was observed. This took about 30 min, and resembled the behaviour of amoebae in solution of EGTA only. All cells kept in these solutions of EGTA-calcium buffers were destroyed after 24 h. A similar behaviour of amoebae could be seen in calcium concentration equal to 10^{-5} M, however, the cells were not lysed within 24 h.

Amoebae treated with calcium-EGTA buffer in concentration of free calcium ions 10^{-4} M maintained their normal polypodial shape and activities up to 24 h. Thus the range of concentrations between 10^{-4} M and 10^{-5} M was estimated as crucial for amoeboid movement.

Figure 1 presents the changes in amoebae population treated with two calcium-EGTA buffers, i.e., 10^{-4} M and 10^{-5} M. In these experiments motionless cells were counted at 5 min intervals for one hour. There no differences between these two groups of cells during the first 15 min, then the amount of immobilized cells grew rapidly in the solution containing lower concentration of calcium ions (10^{-5} M). During the next 45 min 96 per cent of cells were motionless, whereas others were ovoidal in shape with distinctly shortened body and weak motility inside. At the same time in the solution of higher calcium concentration (10^{-4} M), normal locomotion of amoebae was observed.

Confirmation of the results given above was sought for in the next experiment. Amoebae washed as usually were transferred for hour to calcium-EGTA buffer containing 10^{-5} M Ca^{2+}. This period of time was efficient to immobilize all the specimens. Their shape changed into spheres or rosettes. After this time the cells were divided into three groups:

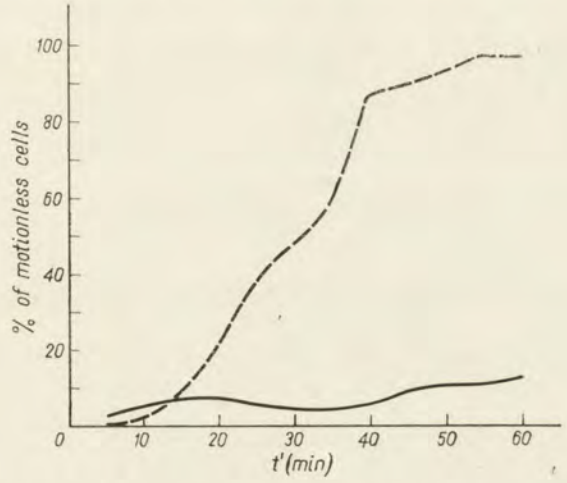

Fig. 1. Behaviour of amoebae incubated in Ca-EGTA buffers, mean value of three experiments cell conc. 100 cells/ml - - - - 10^{-5} M Ca^{2+}, ——— 10^{-4} M Ca^{2+}

(1) remaining in the same calcium buffer, (2) transferred to calcium Tris-HCl buffer and (3) transferred to calcium buffer with 10^{-4} M Ca^{2+}. After transfer the amoebae of the first and second groups were still motionless but those in the Tris-HCl buffer demonstrated stronger cytoplasmic streaming inside the cell. In spite of this in the group incubated in higher concentration of free calcium ions (10^{-4} M), 90 per cent of amoebae recovered locomotion. Their shape was polypodial or rarely monopodial — never spherical or rosette.

Preliminary attempts to calculate the amounts of calcium adsorbed on surface of amoebae were carried out. The investigations were performed with the aid of $^{45}Ca^{2+}$. The cells were maintained in calcium chloride solution at given concentration to obtain equilibrium between calcium ions in the medium and on the surface. Then $^{45}Ca^{2+}$ was added in the same concentration. The difference between the impulses counted 5 min after addition, and from theoretical dilution, was taken as the real number of impulses coming from the surface of amoeba. This number corresponding to calcium concentration permits to determine the approximate amount of calcium adsorbed on surface of amoeba.

Figure 2 gives as example three curves obtained from one of typical experiments. The radioactivity of the supernatant decreased rapidly in the first five minutes of the experiment. Probably in this period the main exchange between surface and medium took place. Then the curves stabilized. The amounts of calcium adsorbed for concentrations 10^{-5} M, 10^{-4} M and 10^{-5} M $CaCl_2$ were 0.000003 μg Ca, 0.00004 μg Ca and 0.0001 μg Ca per one amoeba, respectively.

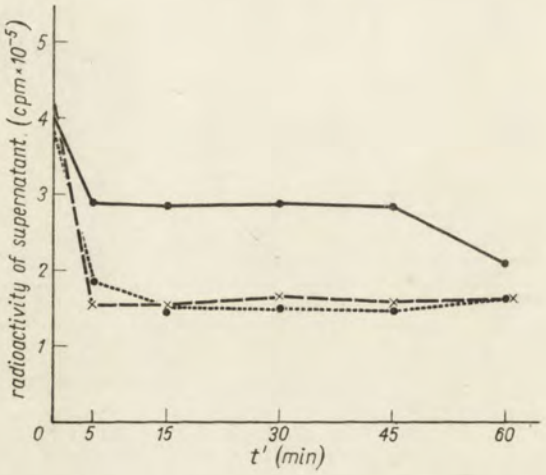

Fig. 2. Relationship between time of incubation and adsorption of calcium on cell surface ——— 10^{-5} M Ca^{2+}, -.-.-.-. 10^{-4} M Ca^{2+}, - - - - 10^{-3} M Ca^{2+}

Others kinds of experiments were carried out with cells previously decalcified by EGTA treatment. The radioactivity of supernatant was detected: (1) after addition of 10^{-3} M $^{45}CaCl_2$ solution, (2) with the first sign of renormalization, i.e., the contact with substratum, return of pseudopodial activity and (3) when all cells become normal with full motion activity. This process was connected with decreasing of radioactivity in supernatant and calcium adsorbed amounted 0.0016 μg per one amoeba.

Discussion

It has been stated many times that calcium ions in the medium are necessary to maintain all life activities in *Amoeba proteus*. The effect of Ca^{2+} deficiency is connected with decrease of the elasticity of amoeba surface structure (Kanno 1964), changes in permeability for ions (Bruce and Marshall 1965, Brandt and Freeman 1967 a, b, Prush and Dunham 1972), detachment from substratum and immobilization (Hrebenda 1972). Only the presence of calcium ions in the medium maintains the locomotion activity of amoeba *C. chaos* kept in distilled water (Bruce and Marshall 1965) or pseudopodial activity of *A. proteus* put into hanging droplet (Korohoda 1970). However, most observations on the role of calcium were carried out in the context of investigations on other ions such as Na^{1+} and K^{1+} (Bruce and Marshall 1965, Prush and Dunham 1972), H^{1+} (Braatz-

Schade and Stockem 1972), La^{3+} (Hawkes and Holberton 1973).

In this paper the minimal requirement of calcium necessary for ameboid movement was detected. By the use of Ca-EGTA buffers the influence of Ca traces originating from glass was omitted, this precaution being of prime importance with low concentrations of calcium investigated. The results obtained show that the calcium level in medium has to be relatively high, up to 10^{-4} M. The lowering of this level to 10^{-5} M results in reduced locomotion but didn't destroy the cells. This is in agreement with the observations of Kanno (1964) who found that the surface rigidity remained normal if the concentration of calcium ions in the medium was between 2.2×10^{-5} and 8.9×10^{-5} M.

The outer surface of amoeba is a mucopolisaccharide coat which increases the surface of amoeba many times. With respect to chemical composition it is chondroitine sulphate (Marshall and Nachmias 1965). The anionic sites on the surface are $-COO^-$ and $-SO^-_3$ groups which selectively bind cations from the environment. It has been stated that calcium ions have the highest affinity to these sites on amoeba surface (Hendil 1971, Brandt and Hendil 1972). In the investigations presented above the concentration of calcium ions adsorbed on amoebae is proportional to calcium concentration in the medium. The amount of calcium adsorbed increased when cells were treated with EGTA. Probably all anionic sites for calcium were exposed. The process was very rapid and main amounts were adsorbed in the first 5 min. In this reaction the slightly increase in radioactivity of supernatant after 30 min is unclear. It may be a result of lysis of cells during centrifugation with subsequent release of intracellular proteins which may affect the equilibrium of medium and cell surface.

RÉSUMÉ

On a déterminé la concentration minimale du calcium nécessaire pour soutenir le mouvement amoeboïde. La concentration optimale pour la locomotion normale de l'amibe se situe dans les limites de 10^{-5} à 10^{-4} MCa^{2+}. Le $^{45}Ca^{2+}$ a été utilisé pour une étude quantitative du calcium sur la surface de l'amibe. La quantité du calcium adsorbée par la surface est proportionelle à sa concentration dans le milieu ambiant.

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Experimental Study on Locomotion of *Amoeba proteus*. I. Movements in the Nucleated and Anucleated Fragments of the *Amoebae* after Removal of the Part of their Cyoplasm

Synopsis. The studies were carried out on the motility of the fragments of *Amoeba proteus* from which the major part of cytoplasm had been removed by the high speed centrifugation. It has been shown that in the anucleated amoeba fragments changes in the shape occur due to the activities of the surface cortex and not of the internal cytoplasm. The nucleated amoeba fragments locomoted for hours though their loose cytoplasm did not differentiate into the ectoplasmic cylinder and the endoplasmic stream. This locomotion occurred due to the contractile processes taking place in the cortex which moved forward as the entity. These results prove that in large amoebae the activities of the peripherical cytoplasm play a decisive role in the shape changes and in the locomotion. The need is discussed for the reinterpretation of the significance in the locomotion of normal *Amoeba proteus* of the processes assuring the ectoplasmic cylinder formation as well as the endoplasmic stream generation, and the endoplasm-ectoplasm mutual conversions.

In the recent works of Korohoda and Stockem (1973, 1975) it has been demonstrated that a thin hyaline layer separating the cell membrane from the granular cytoplasm in *Amoeba proteus* is built up with the dense, filamentous groundplasm. The E. M. morphology of an amoeba periphery, i.e., the cell cortex consisting of plasmalemma and the cortical cytoplasm containing contractile proteins, closely corresponds to the similar structures which are present in the tissue culture cells capable of amoeboid locomotion. It has been commonly accepted that in those cells the contractile processes occurring within their cortex are responsible for changes of cell shape and locomotion (Ambrose 1972, Goldman et al. 1973, Spooner et al. 1973, Wessels et al. 1973). Korohoda and Stockem (1973, 1975) have therefore postulated that also in large amoeba the cortex plays the similar functions, but the cortex activity is screened by other movements occurring within the amo-

eba cytoplasm. When this suggestion had been put forward at the International Congress of Protozoology in Clermont-Ferrand (1973) professor K. W. Jeon suggested to one of the authors (W. K.) that the amoebae, from which a major part of a cytoplasm is removed by high speed centrifugation, can be used for the study of the amoeba cortex activity. Consequently, such investigations have been performed and this communication presents the first results of studies on motility of the nucleated and anucleated fragments of *Amoeba proteus*, from which the cytoplasm was removed to a great extent.

Material and Methods

Amoeba proteus of the Princeton strain was cultured in Chalkley's medium as previously described (Korohoda and Kalisz 1970). To remove the particulate cytoplasm from amoebae the modified method of Jeon et al. (1970) was used. Before the experiments a few hundreds of specimens were chosen, washed in pure mineral Ca-free Chalkley's medium, and preincubated for 30 min at 4°C. Then the amoebae were centrifuged for 30 min at 3700 g, resuspended, and again centrifuged for 15 min at 11 300 g. The centrifugations were carried out with the high speed centrifuge (Type 310, Unipan, Poland) at 4° C. The centrifuged amoebae were again resuspended by a gentle shaking and transferred onto a watch glass filled with full Chalkley's mineral medium. The forms of amoeba fragments desired for observation were chosen with a Pasteur pipette, placed on three-chambers slides and observed under the PZO stereo contrast phase microscope (MB-30S, PZO, Poland) or under the polarization-interference microscope (MPI-3, PZO, Poland), The motile activities of amoebae and their fragments were recorded by still photography (with MF-matic, Carl Zeiss, Jena) and by time-lapse cinematography. The film was analysed with a single frame technique. The cine-film records were taken out on black-white 16 mm ORWO-UP 15 film at 90 frames per minute. For some observations only amoebae showing originally the fountain-like streamings of cytoplasm were chosen prior to centrifugation.

Results

The described method of amoeba centrifugation yielded in the variety of cell fragments. Besides the cell ghosts and the fragments filled with a dense, particulate cytoplasm the numerous hyaline anucleated and nucleated fragments of the amoebae were obtained. These fragments contained very loose cytoplasm which easily vacuolized. This cytoplasm did not differentiate into the plasmagel cylinder and streaming endoplasm. In spite of this the fragments showed intense motile activity and, therefore, were teken for the more detailed study. Among them one could distinguish the following main types:

(1) Anucleated fragments

(a) containing exclusively the hyaline cytoplasm,

(b) containing some minute particles (probably mitochondria within the loose cytoplasm,

(c) similar as 'b' but with great injury vacuoles,

(d) containing some remnants of the optically dense cytoplasm, great vacuoles and cytoplasmic fibrils; these fragments originated from the specimens showing originally the fountain-like cytoplasmic streamings.

(2) Nucleated fragments

(a) small fragments almost entirely free of particulate cytoplasm,

(b) containing very strongly vacuolized cytoplasm.

From all these fragments only those which contained a nucleus were capable of locomotion which persisted for hours. Nevertheless, all anucleated fragments also showed more or less organized movements and continuous changes in shape. Since the fragments' cytoplasm included a low number of minute particles the translocations of individual particles could be followed with contrast phase and difference contrast microscope. Simultaneously the observations could be performed at higher magnifications due to the relatively small dimensions of the fragments.

I. Movements in Anucleated, Hyaline Fragments of *A. proteus*

Among the various motile activities shown by the anucleated fragments one could find a whole gradation, from the simplest surface pulsations to the production of pseudopodia. The simplest form of motile activity was found in the spherical, small fragments. It was represented by localized surface pulsations which were not associated with any cytoplasmic currents nor with the translocations of vacuoles present in the fragment interior. The amplitude of these pulsations could be often limited to several micrometers (cf. Pl. I a–c, and Fig. 1). More frequently, hawever, the local surface protuberances were formed which shifted around the fragment. This could lead to changes in the fragment shape but again, the internal vacuoles and particles did not change position. Only some particles near the surface showed local translocations, perpendicular to the direction of the surface protuberance shifting.

A majority of the anucleated fragments had cylindrical rather than a spherical shape. Even completely hyaline fragments containing only injury vacuoles changed their shape continuously (cf. Pl. I f–i). In such fragments there were no particles which could made possible observations of their interior behaviour. However, if the particles were present then their translocations were rather chaotic and not correlated with the shape

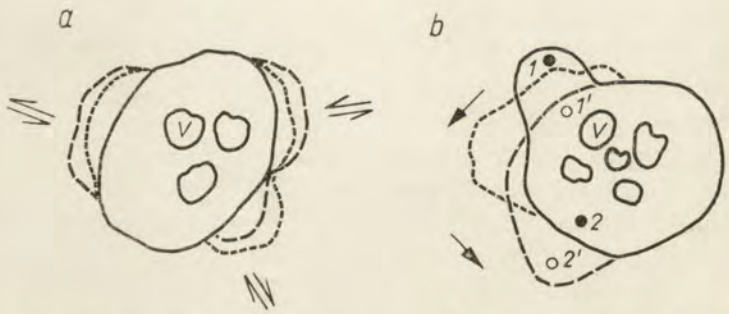

Fig. 1. The simplest forms of motility observed in the anucleated, spherical fragments of *Amoeba proteus*: a — the localized pulsations of a surface, b — the formation of a surface protuberance and its shifting around the fragment

changes. Numerous anucleated fragments contained great injury vacuoles (of. Pl. I f–i, II d–f), which occupied their interior. The contractile vacuole, if present, was readily distinguishable by the surrounding envelope of the dense hyaloplasm (Pl. I d–e). This vacuole showed normal activity. The injury vacuoles were also very active continuously changing their shapes, fusing together and dividing (Pl. II d–f).

The anucleated fragments which contained the relatively greater portion of an optically dense ctyoplasm could even produce and maintain pseudopodia. These pseudopodia were either quite hyaline or were almost entirely filled up with a great vacuole (Pl. II a–c). This did not prevent changes in their length and shape.

In some fragments containing more numerous cytoplasmic minute particles and small vacuoles, the formation of the structures was found, which correspond to the plasmagel sheets present sporadically in extending pseudopodia of the normal *Amoeba proteus* (Pl. III a–d). These sheets were formed at one pole of a fragment and were always anchored to the small cavities in the surface. When the plasmagel sheets moved posteriorly the cytoplasmic particles and even vacuoles were squeezed through it into the more hyaline region of an amoeba fragment. This was followed by the formation of a new plasmagel sheet and the described phenomena repeated. The travelling of the plasmagel sheet along a fragment of amoeba was accompanied by the parallel shifting of the surface cavity, which itself resembled a constriction ring. The inflow of particles and vacuoles into the more hyaline region of cytoplasm never resembled the fountain pattern.

The anucleated fragments which originated from the amoebae showing originally a fountain-like cytoplasmic streaming usually contained more of the optically dense cytoplasm. This cytoplasm formed clusters situated at the pole of the fragment opposite to the pole occupied by

great vacuole. Among the vacuoles and the clusters the fibrillar structures run. These fibrils, because of their high optical density were easily distinguishable (Pl. IV). They attached strongly to the injury or contractile vacuoles. Along the fibrils the movements of minute particles continued. Shortening of the fibrils caused visible deformations and stretchings of the vacuoles to which the fibrils were anchored. This suggests that the fibrils remained under some tension. The motility associated with the fibrils did not show any correlation with changes in the contour of amoeba fragments.

II. Movements and Locomotion in the Nucleated Fragments of Centrifuged *Amoeba proteus*

The nucleated amoeba fragments were usually greater than the anucleated. Their length ranged from 10 to 80% of the original length of normal *A. proteus* (cf. Pl. V–VIII). All of these fragments actively locomoted. The average speed of their movement was in the same range as in normal *A. proteus*, i.e., 1 to 4 $\mu m\ s^{-1}$.

The pattern of the nucleated fragments' locomotion depended on their size and on the amount of cytoplasm left in their interior. The small fragments (Pl. V–VII) showed movements distinctly different from the normal locomotion of *A. proteus*. As a rule they moved monopodially for hours. Any change in direction of movement was accompanied by the withdrawal of an old pseudopodium and the formation of new one (Pl. V c–e). No sign of any surface folding-unfolding processes, so characteristic for normal *A. proteus*, could be detected. The surface of moving fragments was smooth not only in their anterior but also in their posterior regions. In greater fragments, nevertheless, the locomotion much more resembled the normal movements of *A. proteus* as far as the cell shape changes are concerned (cf. Pl. VIII d–e). These last fragments produced more than one pseudopodium, and the anterior-posterior polarization of their morphology was much more pronounced (cf. Pl. VIII d–e and VI a–b or V d–f).

The nucleated amoeba fragments from which a major part of the cytoplasm had been removed were filled up with very loose, almost entirely hyaline cytoplasm. This cytoplasm did not differentiate into the normal ectoplasmic (plamagel) cylinder and streaming endoplasm. Very striking was the absence of an axial endoplasmic stream which in normal amoebae exceeds five to ten times the speed of pseudopodial extension (Allen and Roslansky 1959). Characteristic conversions of endoplasm into the plasmagel cylinder did not occur. There was therefore no circulation of the cytoplasm. The minute particles remained in

the same region of the amoeba fragment for the time period sufficient for the fragment to pass several times its own length. In Figure 2 there is shown the behaviour of minute particles during an extension of the

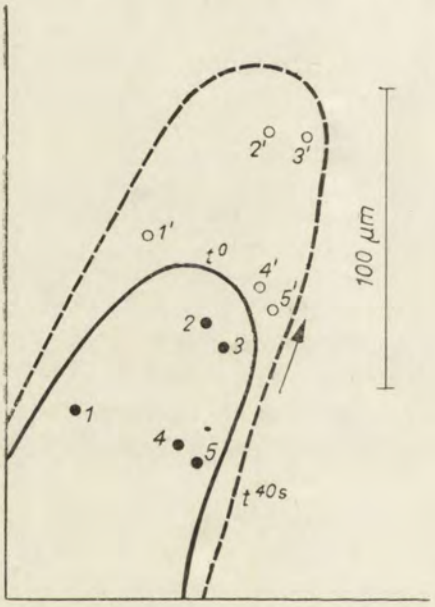

Fig. 2. The translocations of minute cytoplasmic particles within the extending pseudopodium of the amoeba shown in Pl. VII a–c

pseudopodium. This drawing has been made from the original photographs of the amoeba fragment shown in Pl. VII a–c. In the same fragment during its locomotion the nucleus occupied a middle part of the body and separated the posterior region of cytoplasm containing more numerous cytoplasmic particles.

Lack of the plasmagel cylinder formation had been visible in the fragments in which great vacuoles and nuclei moved within the loose cytoplasm without any obstacles. They did not disturb the locomotion though their diameter was only slightly smaller than the diameter of the amoeba fragments (cf. Pl. V c–e, VI c–d).

The absence of the cytoplasm circulation is shown in Fig. 3 illustrating the translocations of cytoplasmic particles during the locomotion of the amoeba fragment. Like in some species of small amoebae (S h a f f e r 1965 a, b) and in tissue culture cells (A m b r o s e 1972, S p o o n e r et al. 1973) the cytoplasm seemed to be transported in volume by the locomoting and contractile cell cortex.

In the hyaline, nucleated amoeba fragments the behaviour of their cortex could be easily followed. The film analysis showed that it moved forward as an entity, at the speed of the same order of magnitude as

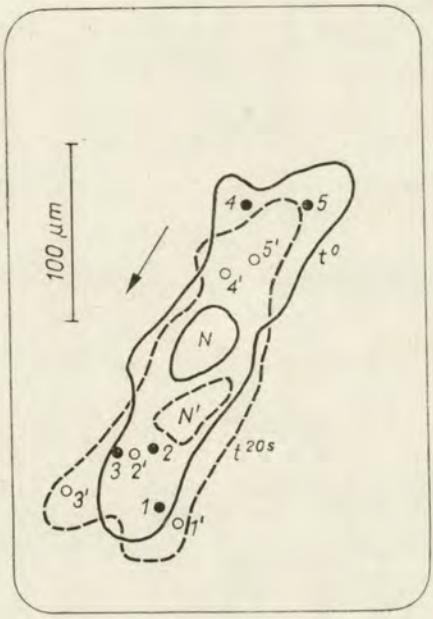

Fig. 3. Changes in the position of cytoplasmic particles occurring during 20 s in the locomoting fragment of *Amoeba proteus*. Drawing made from the original cine film record

the fragment locomotion. The minute particles situated within the posterior region of the cortex were not stationary in relation to the substratum but moved forward. The relative positions of the particles attached from outside on the surface of a fragment and the cortical particles remaining in their close vicinity did not change. But simultaneously, the forward translocation of the cortex was associated with a significant decrease in distances among the cortical particles laying in the posterior regions of the amoeba fragment, what shows the local contraction of the cortex (Pl. VI a–b, Fig. 4). The opposite phenomena were recorded at cell sides if the new lateral pseudopodium was formed.

In some nucleated amoeba fragments an extensive vacuolization of the entire, loose cytoplasm took place (Pl. VII d–f, VIII). This did not impair the fragments locomotion and it was possible to follow the behaviour of individual vacuoles in the moving fragments. It was found that at the anterior region of pseudopodia, approx. 20 µm behind their tips, great vacuoles were situated. These vacuoles occupied the regions in which in normal *A. proteus* the endoplasm converts into the plasmagel cylinder. The vacuoles flowing within the cytoplasm at the rate slightly higher than the speed of pseudopodium extension when approached this large vacuole fused with it. At the same time small vacuoles separated laterally from this large front-end vacuole. These new vacuoles remained attached to the hyaline cortex, shifted with it forward in relation to substratum but more slowly than the central cytoplasm, and when reached

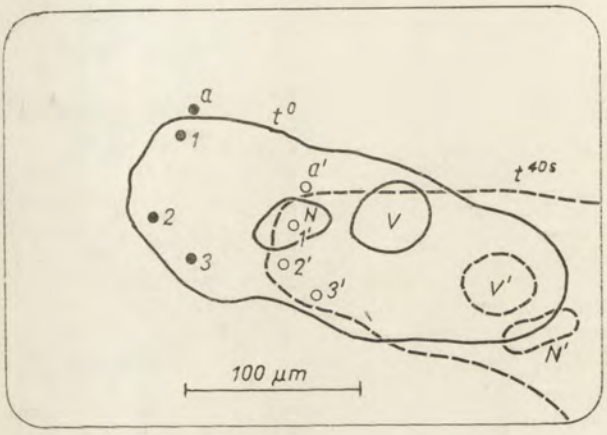

Fig. 4. Change in distances among minute cytoplasmic particles situated within the posterior region of the cortical cytoplasm and in their position in relation to a substratum, occurring during the locomotion of the nucleated fragment of *Amoeba proteus*. Drawing made from the original cine film record

the uroid joined the main cytoplasmic flow (Pl. VII d–f, Fig. 5). These processes occurred usually only in the greater fragments of amoebae and showed some similarity with the circulation of cytoplasm in normal

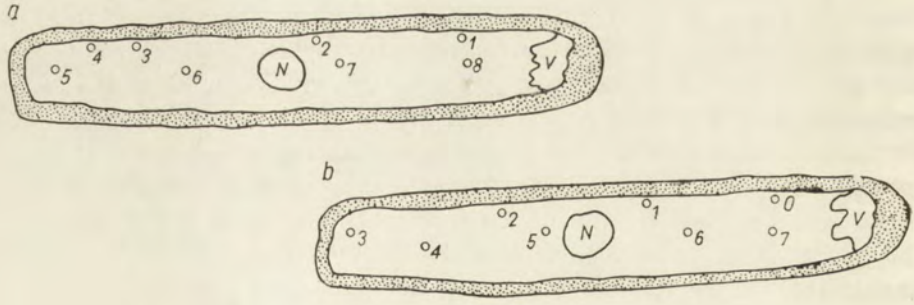

Fig. 5. Schematic representation of the vacuole circulation during the locomotion of the nucleated, strongly vacuolized and relatively great fragment of *Amoeba proteus*. N — nucleus, V — front-end vacuole, 5, 6, 7 — vacuoles flowing faster than the fragment progresses, 1, 2, 3 — vacuoles translocated slower than the fragment moves. Small vacuole 8 fussed with the front-end vacuole, and a new vacuole O was formed when the fragment moved from position a to b

A. proteus. There was, however, no evidence that the front-end vacuole can result from local syneresis. On the contrary, the formation and growth of this vacuole by coalescence with the vacuoles brought by the flowing cytoplasm was clearly visible.

Discussion

It has been recently proved that in large amoebae directly beneath the plasmalemma there is a thin layer of hyaline cytoplasm which discloses its very dense structure when studied in the electron microscopy (Korohoda and Stockem 1973, 1975, Rinaldi and Hrebenda 1975). In normal amoebae the study of functions of this structure are difficult. One difficulty stems from the presence of numerous crystals and food-residues which are distributed throughout the ectoplasmic cylinder and streaming endoplasm. This obstacle can be avoided, as it was shown by Allen and Francis (1965) by the centrifugation of amoebae at low accelerations (1000 g) for 2–6 min. In such amoebae, nevertheless, the fast flow of endoplasm and its conversions into ectoplasmic cylinder still continue and mask the behaviour of relatively thinner cell cortex. To discriminate between the functions of the cortex and of the ectoplasmic cylinder it appeared necessary to simplify the system studied. This has been achieved by the removal of the major part of cellular cytoplasm during the prolonged, high speed centrifugation of amoebae at 4°C. The amoeba fragments obtained in this way contained a very loose cytoplasm which did not differentiate into the ectoplasmic cylinder and streaming endoplasm. The cytoplasm remaining in the amoeba fragments contained only very small particles (probably mitochondria) which enabled us to follow its movements. If there were some remnants of a dense cytoplasm corresponding to the ectoplasmic cylinder or normal endoplasm they were readily detectable. These remnants formed sometimes fibrils which had much greater optical density than the surrounding cytoplasm (cf. Pl. IV). The loose structure of the cytoplasm of amoeba fragments caused its easy vacuolization.

The cytoplasm was removed in particular fragments to various extent what made possible to chose the whole gradation of the fragments, from such which were showing the most simple motile behaviour to those which actively locomoted for hours. The capacity of the fragments to locomote has been found, in agreement with the data of other investigators (Jeon et al. 1970, Willis 1916), to be inseparably connected with the presence of a nucleus. The anucleated fragments changed their shape but did not locomote because of the impairment of their attachment to glass.

The simplest patterns of motile activity found in the anucleated fragments of amoebae, i.e., the pulsations of their surface and the production of the surface protuberances, closely resembled similar phenomena occurring in the isolated drops of plant cell cytoplasm (Kamiya and Kuroda 1957) and in tissue culture cells, in which they were descri-

bed as blebbings (Weiss 1959). This activity involved only the cortex and it was not correlated with any organized currents within the cytoplasm. One can hence safely conclude that these changes in the shape of the amoeba fragments unquestionably originated from contractile processes taking part in the cortex i.e., in the outermost, hyaline layer of ectoplasm and the plasmalemma.

In the fragments in which more cytoplasm remained, the phenomena were recorded which occasionally appear in the extending pseudopodia of *Amoeba proteus*. Especially striking was the observation of plasmagel sheet formation followed by its translocation along the amoeba fragment and accompanied by the filtration through it vacuoles and minute particles (cf. Pl. III). This observation confirms strongly the conclusions of Mast (1931), Goldacre (1964) concerning the plasmagel sheet nature and behaviour.

The contractile vacuole if present showed normal activity. Since it was localized in hyaline cytoplasm one could easily discriminate it among other vacuoles. The contractile vacuole was always surrounded by the thin ring of an opticaly dense hyaloplasm (cf. Pl. I d, e, V f, g, VI a, b. VIII a, c).

In some amoeba fragments the fibrils of an optically dense cytoplasm were visible (cf. Pl. IV a–f). The fibrils attached as a rule to vacuoles and the remnants of dense cytoplasm. They often run perpendicularly to the surface protuberances formed by the fragments and were under visible tension, since the vacuoles to which they attached were strongly deformed. Anastomoses among the fibrils shifted along their length and minute particles in vicinity translocated. These observations suggest that the amoeba cytoplasm under natural condition can have also the considerable structure (cf. Taylor et al. 1973, Allen 1974).

The nucleated fragments which had a relatively simple morphology locomoted for hours though their pattern of movement significantly differed from the normal locomotion. The small fragments as a rule moved monopodially. The change in the direction of their locomotion was associated with the withdrawal of an old pseudopodium and the formation of a new one. Their surface was always smooth and the surface folding-unfolding processes which accompany the movement of normal *Amoeba proteus* (cf. Czarska and Grębecki 1966, Komnick et al. 1973) were absent. In their loose cytoplasm there was no sign of differentiation into the plasmagel or ectoplasmic cylinder and the endoplasmic stream. The minute particles flowed forward over the whole diameter of the fragments except of their hyaline cortex. This flow took place almost at the same speed as the speed of the nucleated fragments locomotion. Hence, the typical for normal *Amoeba proteus* conversions

of the endoplasm into the more stationary plasmagel cylinder did not occur (cf. Mast 1931, Jahn 1964, Komnick et al. 1973). The nucleus or a great vacuole could have the diameter only slightly smaller than the diameter of the amoeba fragment but it not disturbed the fragment locomotion (cf. Pl. V c, d, e, VI c, d, VII a, b, c). In some other fragments the entire interior had been strongly vacuolized and it again did not inhibit the locomotion (cf. Pl. VII d–f, VIII). The minute particles situated within the cortex showed that it moved forward as one entity at the speed only slightly slower than the fragment progression. Simultaneously, at its posterior regions some contraction was visualized by the decrease in distances among the neighbour particles (cf. Pl. VII a, b Fig. 3, 4). The particles attached to or localized within the cortex did not show any Brownian motion, in the contrary to the behaviour of those in the fragment interior. All these features point to the conclusion that the locomotion of the nucleated fragments of centrifuged *Amoeba proteus* occurred exclusively due to the contractile processes taking part within their cortex. This conclusion corresponds to contemporary view on the mechanism of the locomotion of tissue culture cells in which a distinct cytoplasmic cortex built of contractile proteins is also present (cf. Ambrose et al. 1970, Ambrose 1972, Goldman et al. 1973, Wessells et al. 1973).

If one accepts this conclusion than a need appears for the reinterpretation of the significance of various motile phenomena occurring in large amoebae of proteus-chaos group. There is a low probability that the phenomena studied in small, hyaline fragments of the centrifuged amoebae completely vanish in normal specimens. We would rather conclude that they are screened by other motile activities of amoeba cytoplasm and because of it they escaped so far the caution of investigators. According to our view the characteristic features of large amoeba movements, i.e. the fast axial stream of endoplasm into extending pseudopodia which occurs at the rate five to ten times higher than the rate of amoeba locomotion, the formation of thick plasmagel cylinder built of granuloplasm, the polarized and highly organized mutual conversions of endoplasm into the plasmagel cylinder as well as the characteristic foldings of the surface of uroid and retracting pseudopodia, all represent particular specializations of these cells and are associated with their size. The great dimensions of these amoebae can require the efficient supply of cytoplasm into extending pseudopodia, formation of ectoplasmic cylinder to assure the rigidity of stout pseudopodia, and the organized circulation of cytoplasm constituents to assure the fast distribution of metabolic products within the cell. These striking the eye phenomena can screen other motile activities which can be equally important for the locomotion of

these amoebae. This view can be supported by the fact, that numerous species of small amoebae and tissue culture cells locomote in an "amoeboid" manner but without so organized movements in their cytoplasm and without the formation of thick plasmagel cylinder (Abe 1962, Bovee 1964, Ambrose 1772, Spooner et al. 1973, Shaffer 1964, 1765 a, b).

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RÉSUMÉ

On a éxaminé la motilité des fragments de l'*Amoeba proteus* qui manguent de la partie majeure de leur cytoplasme évacué par la suite de la centrifugation à haute vitesse. Il a été démontré que chez les fragments depourvus de noyau les changements de forme dépendent de l'activité des structures superficielles corticales et non des couches intérieures du cytoplasme. Les fragments qui gardent leur noyau, continuent la locomotion pendant des heures, en dépit de ce fait que le peu du cytoplasme qui en reste ne s'organise pas en cylindre ectoplasmique ni en courant endoplasmique. Dans ce cas la locomotion est également due à des contractions ayant lieu dans le cortex qui se déplace progressivement en son entier. Ces phénoménes prouvent que c'est l'active du cytoplasme péripherique qui décide du changement de forme et de la locomotion des grandes amibes. On souligne la nécéssité d'une intérprétation nouvelle du rôle de la formation du cylindre ectoplasmique et du courant endoplasmique, ainsi que de l'interconversion réciproque de l'endoplasme et de l'ectoplasme, étant donné que l'importance de ces phénomènes pour la locomotion doit être remise en question même pour les cellules normales de l'*Amoeba proteus*.

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EXPLANATION OF PLATES I–VIII

I: Changes in the shape of *Amoeba proteus* fragments, from which a major part of the cytoplasm had been removed. The photographs a, b, c and f, g, h, i correspond to cine film frames taken at 10 s time intervals; the photographs d, e, at 30 s

II: a, b — relatively great fragment of *Amoeba proteus* which contains a greater portion of an optically dense cytoplasm. It maintains hyaline and vacuolized pseudopodia changing their shape and length. The photographs a, b were taken at the 10 s time interval, c — the vacuolized pseudopodium shown at higher magnification, d, e, f — changes in the shape of the strongly vacuolized fragment of *Amoeba proteus*. Photographs d–f taken at 15 s time intervals

III: An amoeba fragment containing some more dense cytoplasm left in its interior. Photographs taken at 10 s time intervals show the formation and translocation of structure corresponding to the plasmagel sheets

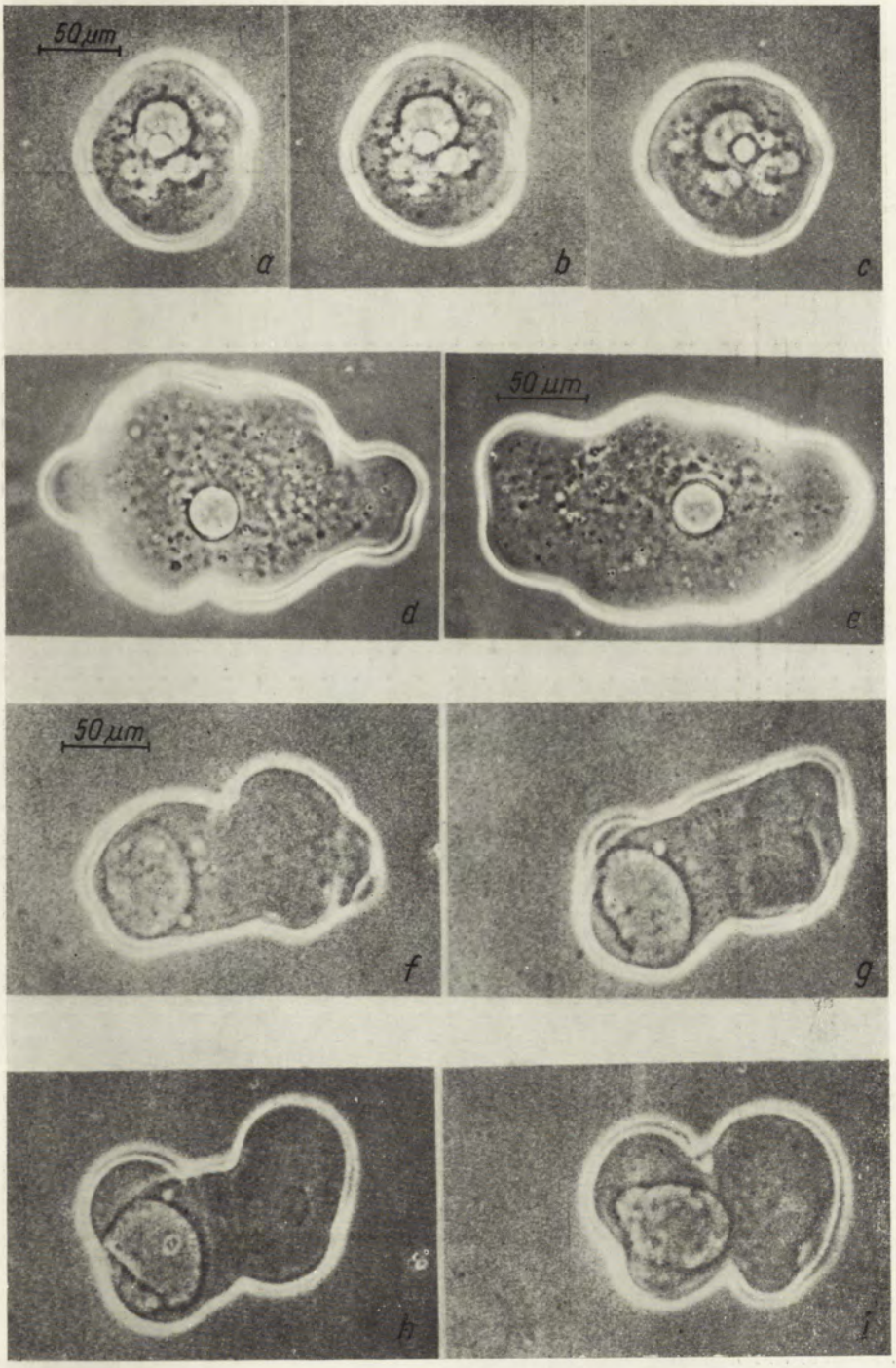
IV: The amoeba fragments which originated from the *Amoeba proteus* specimens showing originally the fountain-like cytoplasmic streamings. These fragments as a rule contain clusters of an optically dense cytoplasm, great vacuoles, and attached to them cytoplasmic fibrils. The photographs correspond to 10 s time intervals among the cine film frames. For more detailed explanation cf. the text.

V: a — an anterior, and b — a posterior region of a normal *Amoeba proteus* specimen; c, d, e — contrast phase photographs of the small, nucleated fragment of *Amoeba proteus* from which a major part of the cytoplasm was removed, photographed at 30 s time intervals; e, f — interference contrast photographs of the monopodial, nucleated fragment of *Amoeba proteus*

VI: a, b — frames of the cine film record of the locomotion of the nucleated fragment of *Amoeba proteus*; c, d — an amoeba fragment containing the great vacuole which does not disturb its locomotion

VII: a, b, c — contrast phase photographs of the nucleated fragment shown at three different magnifications. The photograph c exposed for 10 s to show the translocations within more particulate cytoplasm in the posterior region of the fragment; d, e, f — cine film record of the relatively great, highly vacuolized, nucleated fragment of *Amoeba proteus*; frames correspond to 20 s time intervals

VIII: Contrast phase photographs of the great fragments of *Amoeba proteus*, resembling in morphology the normal specimens but containing in their interior a strongly vacuolized cytoplasm. Photographs a, b, c — taken at 30 s time intervals



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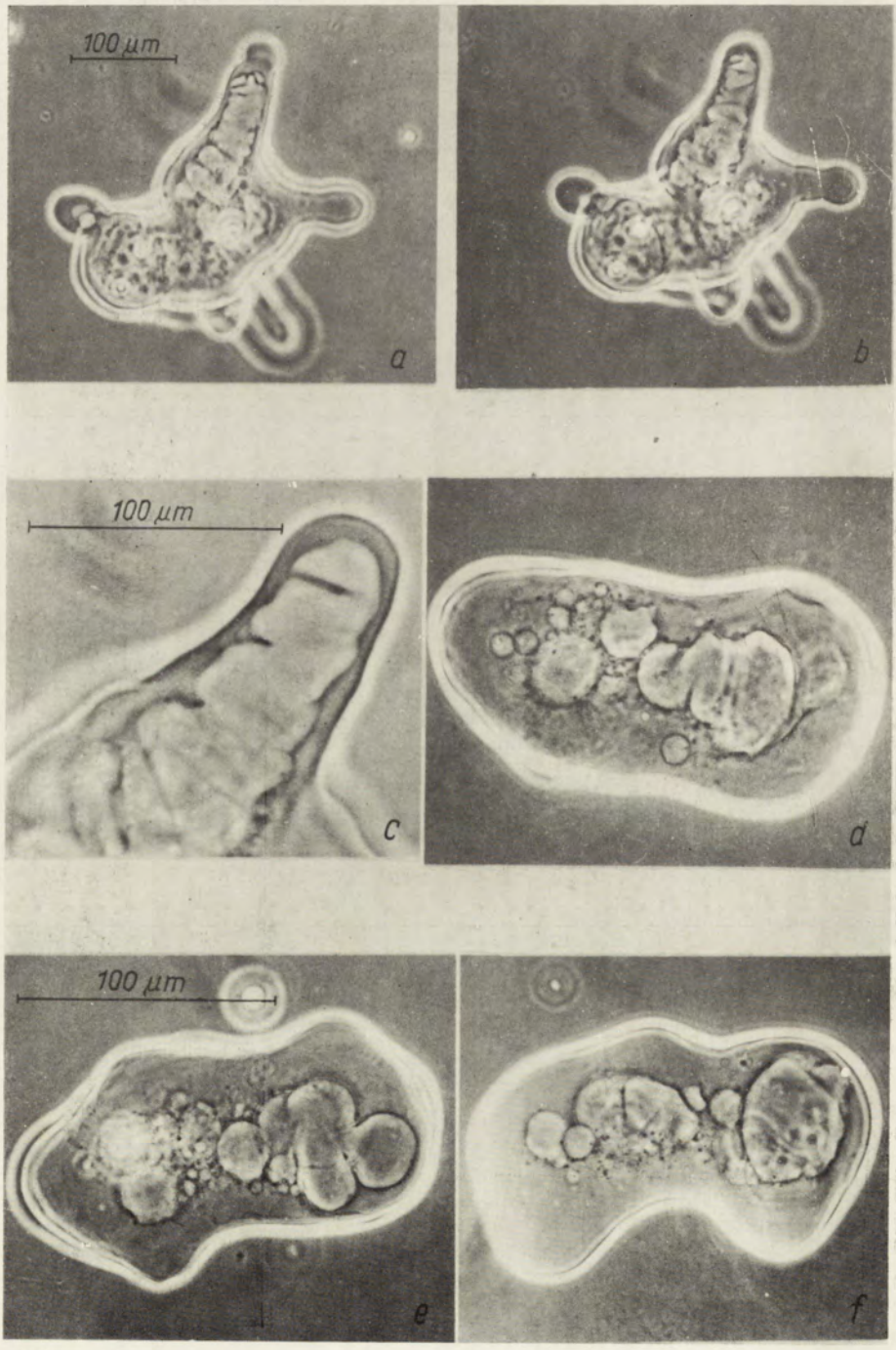

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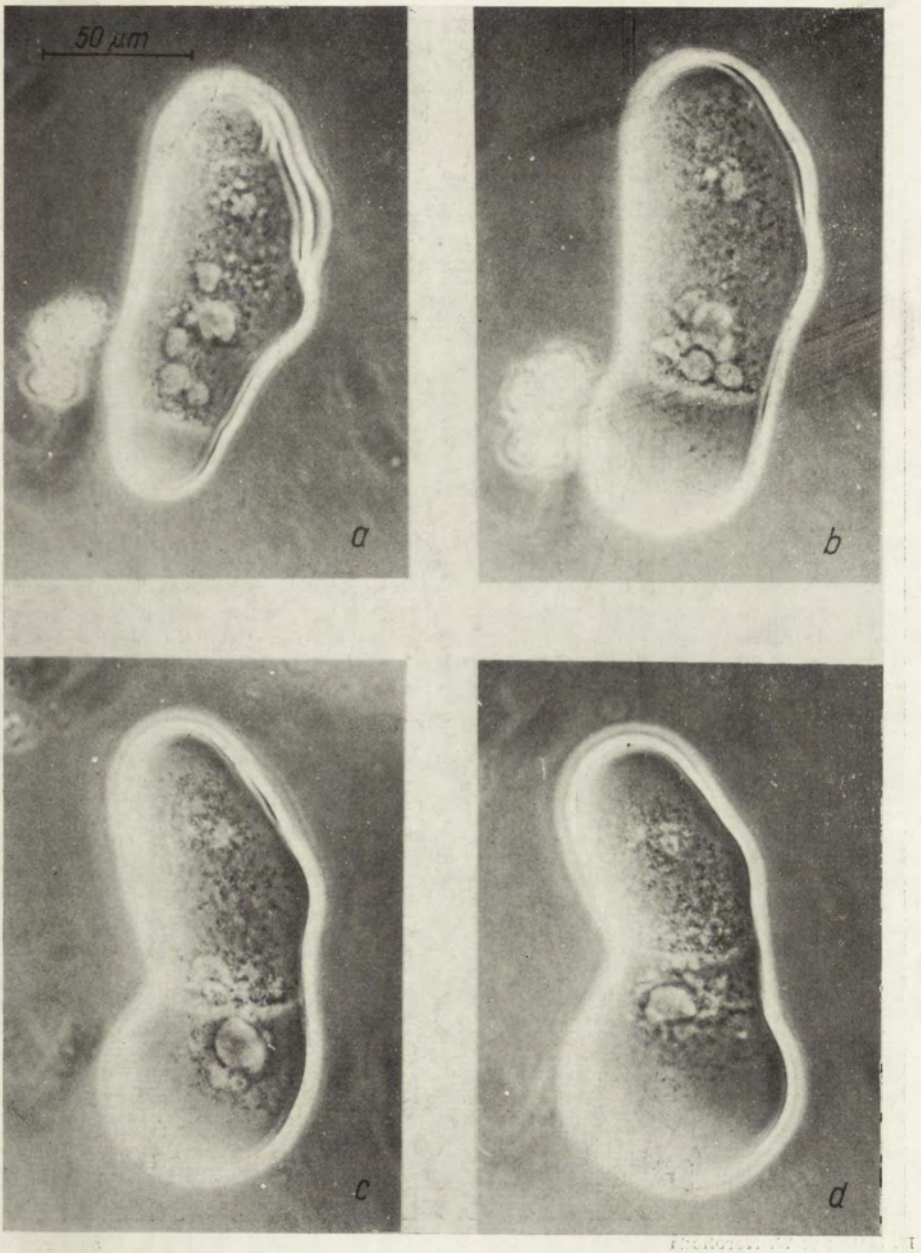

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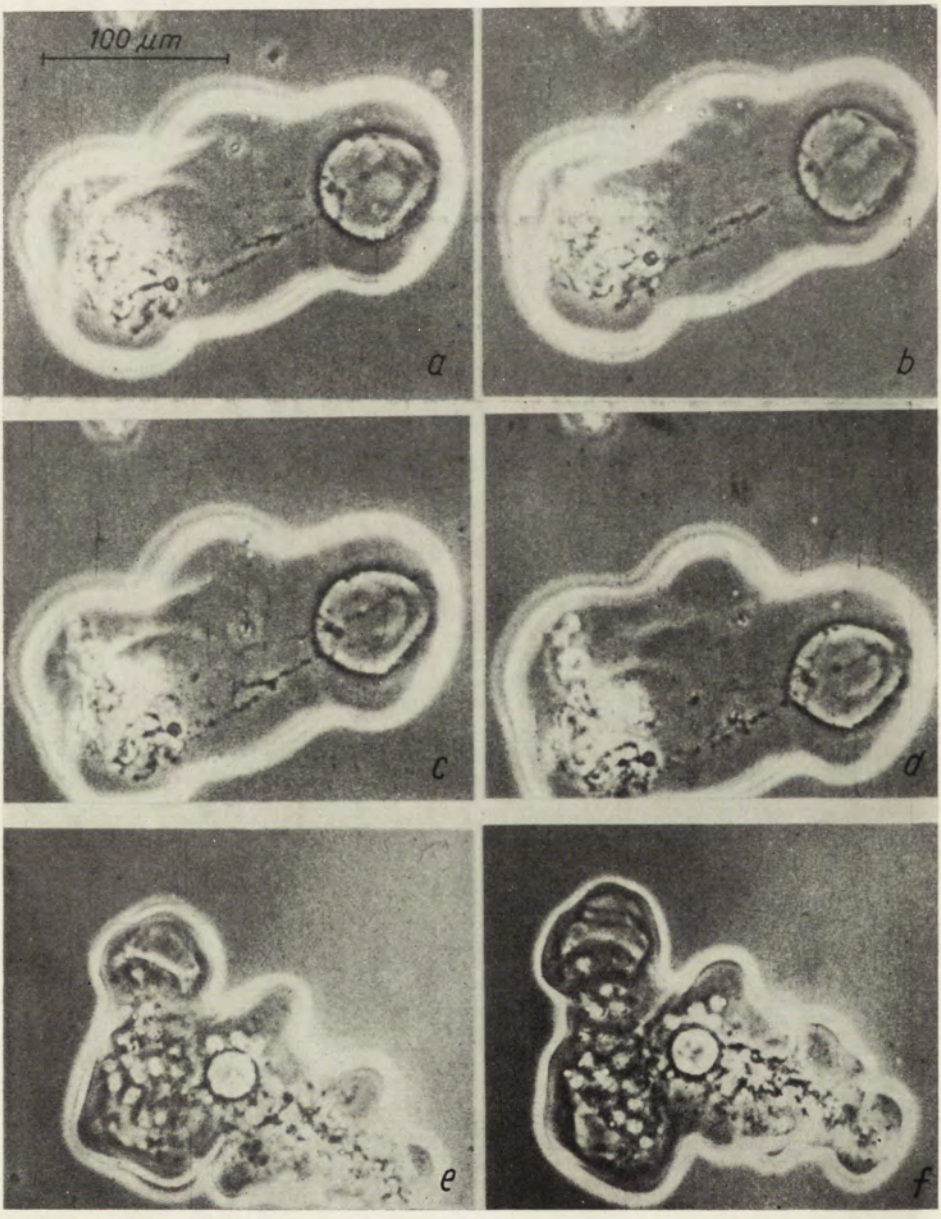

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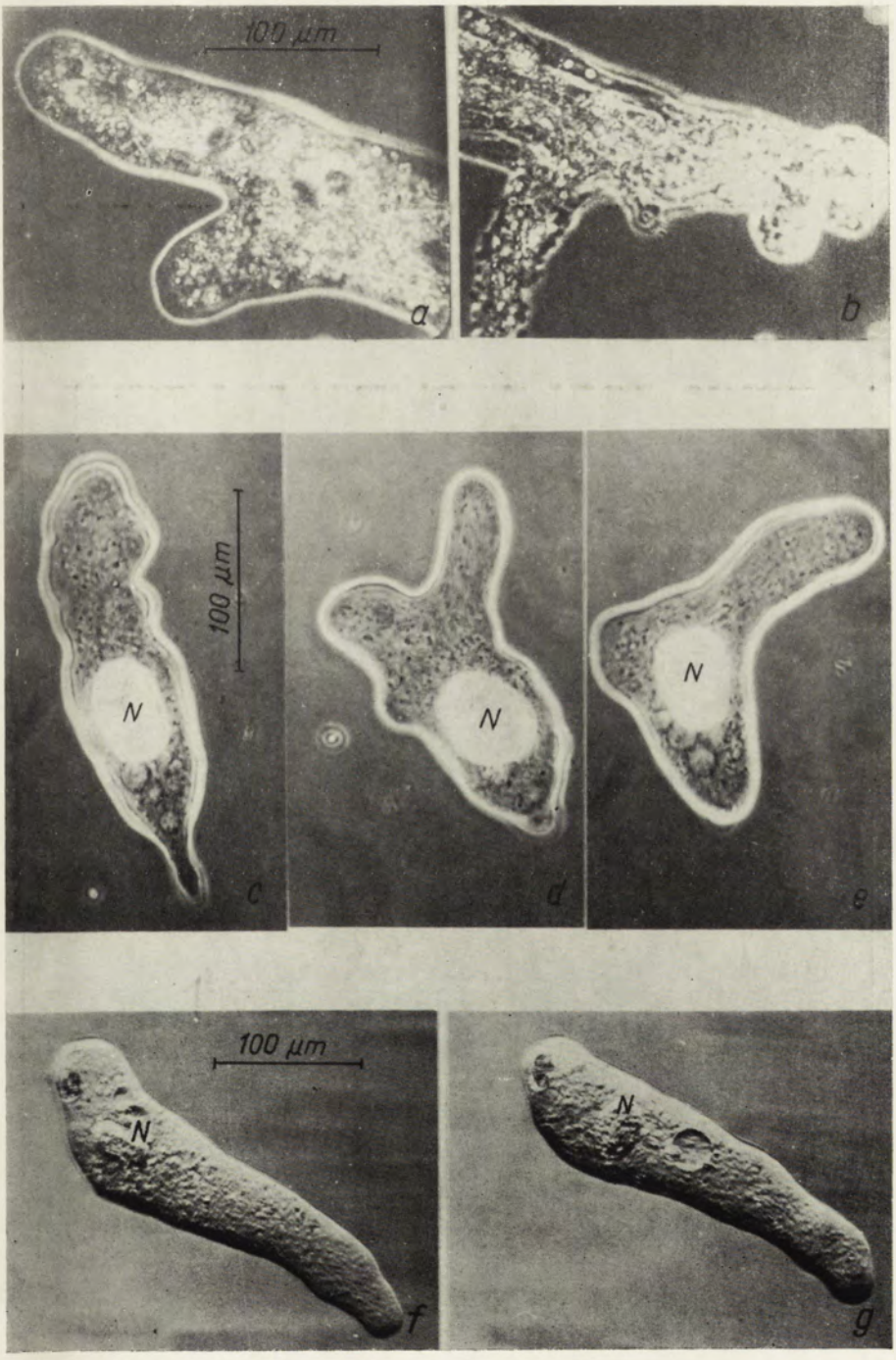

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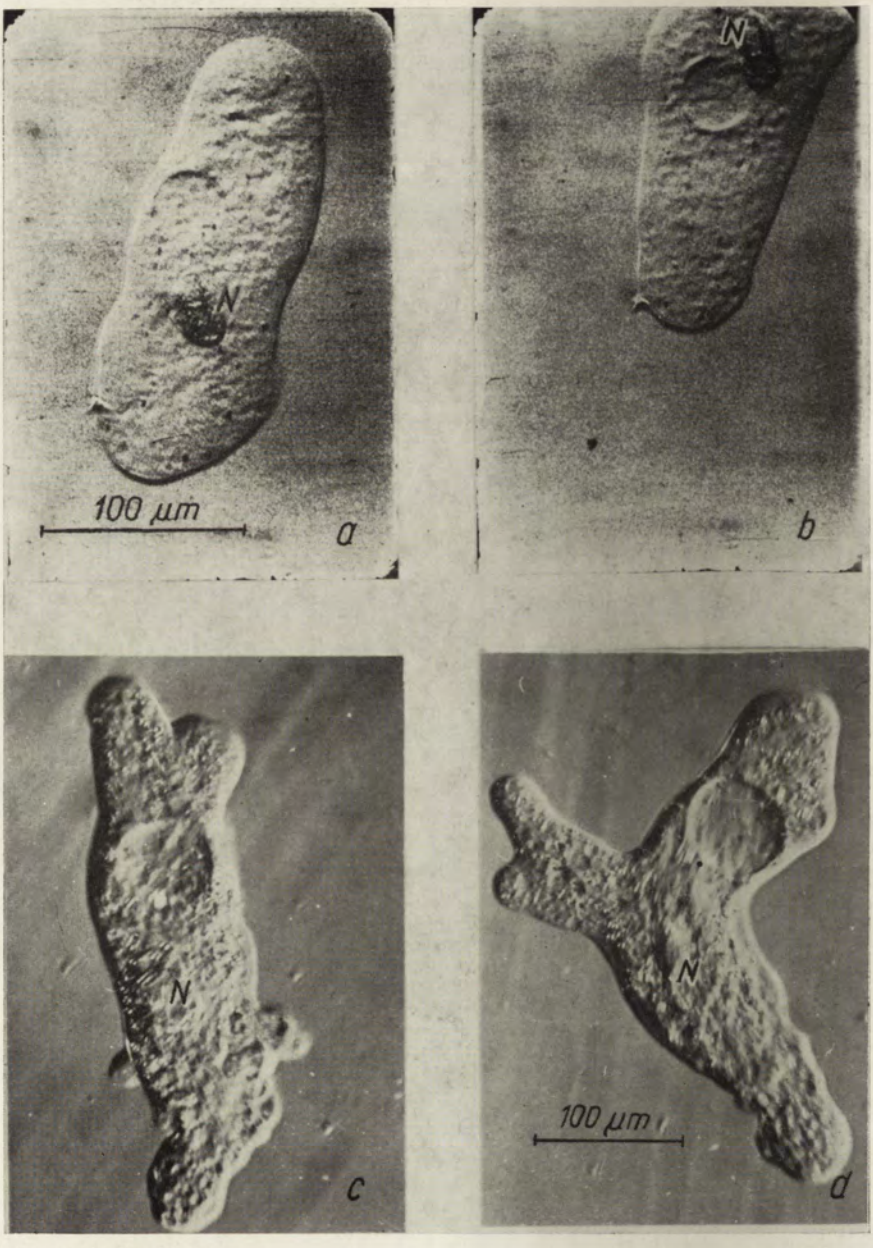

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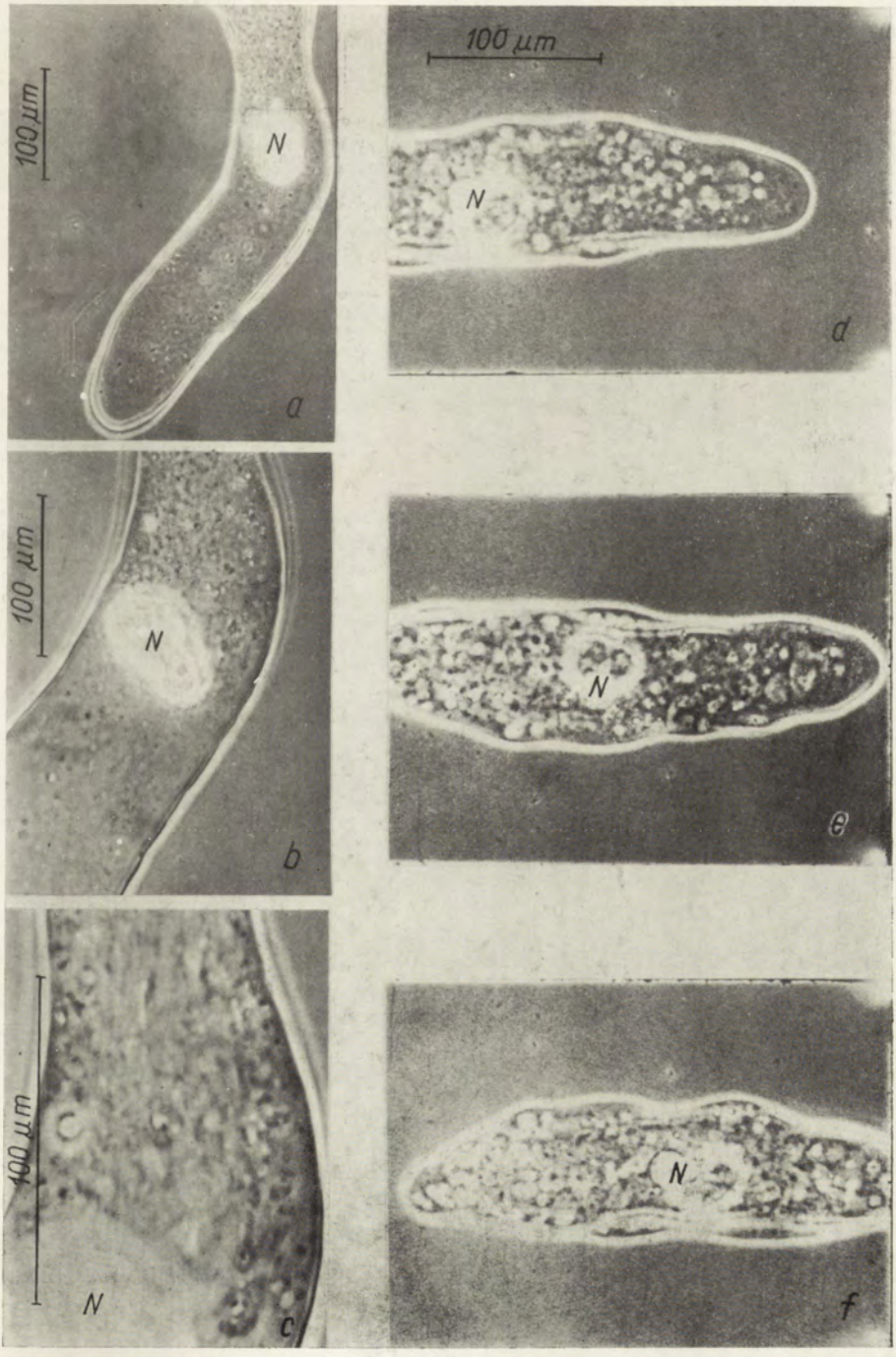

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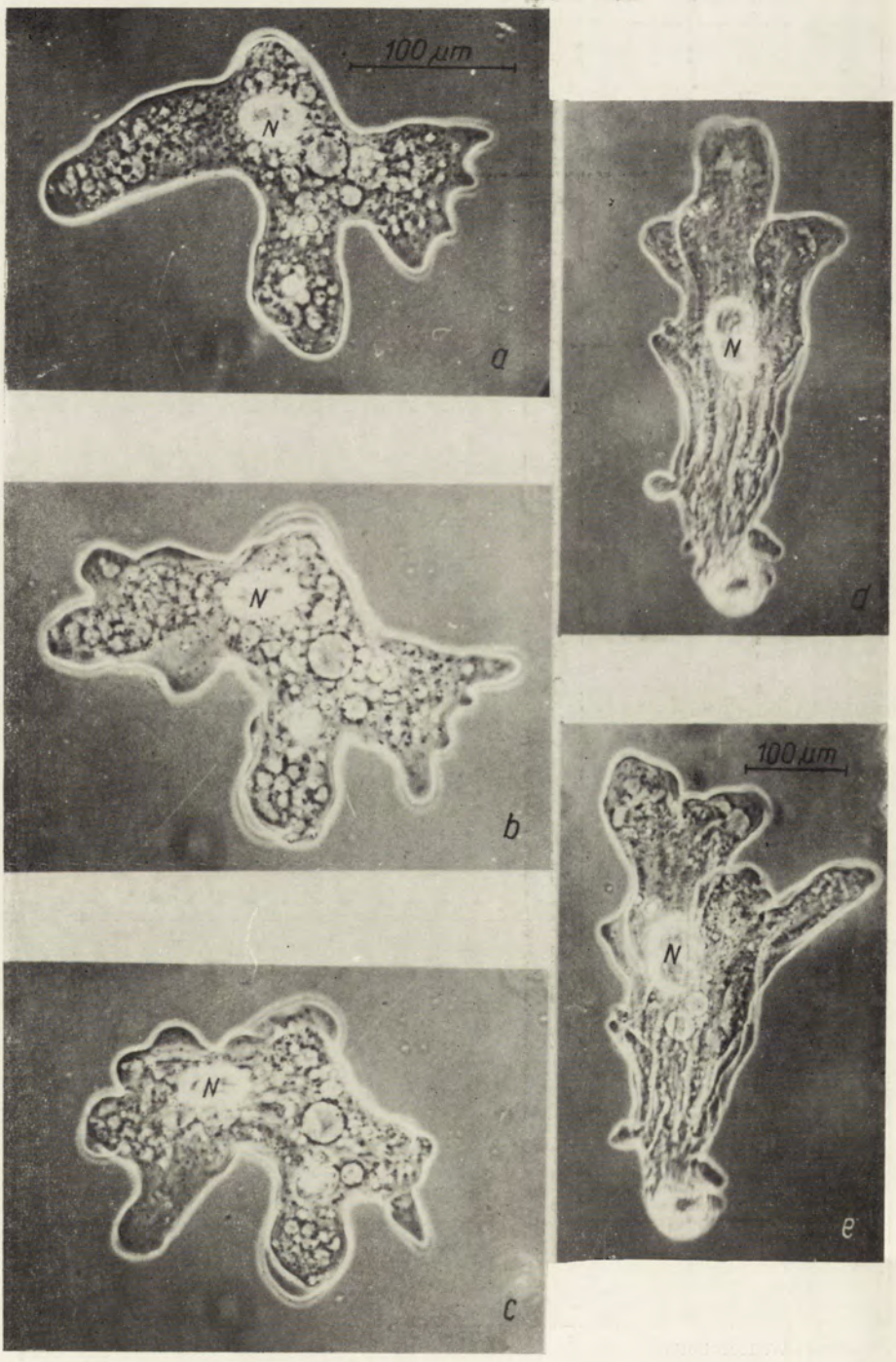

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Endocytosis in *Paramecium*. II. Effect of Lysozome and Neuraminidase

Synopsis. The treatment of *Paramecium caudatum* with lysozyme causes the interruption of food vacuole formation although no visible changes in surface coat covering the cytostome can be seen in cells examined in electron microscope. The effect of enzyme is reversible only when very low concentrations are used. Neuraminidase in the concentrations 25 or 50 units per ml does not affect neither the physiology of food vacuole formation, nor the ultrastructure of the surface coat.

It has been proved that in amoebae cell the surface coat covering the body plays an important role in endocytosis. The mucous layer is responsible for both: the stimulation of the engulfment and the accumulation of the ingested material (Chapman-Andresen and Holter 1964, Chapman-Andresen 1965, Christiansen and Marshall 1965, Holter 1965, Korn and Weisman 1967, Stockem and Wolfarth-Bottermann 1969, Hendil 1971, Braatz-Schade and Stockem 1973). Among ciliata the existence of the cell coat has been shown in *Tetrahymena* (Nilsson and Behnke 1971) and in *Paramecium* (Wyroba and Przełęcka 1973). The accumulation of dyes and particles has been observed in *Paramecium* (Mast 1947, Grębecki and Kuźnicki 1956, Grębecki 1963). The necessity of the concentration of nutrient material in *Tetryhamena* was postulated by Nilsson (1971). The influence of surface acting agents on phagocytis activity of *Tetrahymena* and *Paramecium* has been described by Brutkowska and Mehr (1976).

It has been stated recently that the destruction of the surface coat by proteolytic enzymes (trypsine and pronase) causes the disturbance in food vacuole formation in *Paramecium* (Tołłoczko 1975). In order to state which part of the mucous is important during the ingestion and

to exclude the possibility of destroying the plasma membrane — two other enzymes (lysozyme and neuraminidase) acting on the surface coat were tested.

Material and Methods

The ciliate *Paramecium caudatum* was used in this study. The cells were grown and prepared to the experiments as it had been described previously (Tołłoczko 1975) but citrate-phosphate buffer pH 6.3 (according to Grębicki and Kuźnicki 1963), instead of Tris HCl was used. The enzymes were diluted in the same buffer. Neuraminidase was prepared from the stock solution which contains 500 units per ml in 0.05 M acetate buffer pH 5.5. The experiments with lysozome were carried out in the room temperature (about 20° C) and with neuraminidase in 27° C. 2 ml samples containing about 400 cells each were treated with lysozyme in concentration 0.1–5 mg/ml or neuraminidase (25 or 50 units per ml). The citrate-phosphate buffer alone or with the appropriate amount of acetate buffer were used as control media. The suspension of latex particles (diameter 0.81 μm) was added after 5, 30, 60, 90, 120 min or 24 h of incubation with the enzyme for 15 min. The number of latex containing vacuoles was counted in the cells fixed with 10% neutralized formaline. The mean number of food vacuoles formed during 15 min and standard deviations were calculated from data obtained from at least three experiments. In experiments with lysozyme the enzyme was eliminated by washing cells with buffer after 30 or 60 min of incubation. The ability to form food vacuoles was tested 30 or 60 min later. Observations of living cells swimming in small droplets were also carried out.

The preparation for electron microscopy was the same as previously (Tołłoczko 1975). Fixation was carried out in 3.6% glutaraldehyde buffered with 0.15 M cacodylate buffer at pH 7.4 for 1 h. Then cells were postfixed in 1% OsO_4 buffered as above for 1 h. In the case of use of ruthenium red the procedure described by Luft (1971) was applied The cells were dehydrated through increasing ethanol series and two changes of propylene oxide and then embedded in Epon. The material was sectioned on LKB ultramicrotome. Sections were examined in the JEM 100 B electron microscope. Sections were examined both unstained and double stained with a saturated aqueous solution of uranyl acetate followed by lead citrate for 30 and 15 min, respectively.

Results

Only paramecia in lysozyme which concentration is lower than 0.5 mg/ml can live in enzyme till the next day. The food vacuole formation is diminished after 5 min treatment in all used concentrations of enzyme except 0.1 mg/ml (Table 1). The majority of the cells do not form vacuoles at all. Only few ciliates are able to ingest latex particles, but the vacuoles they form are smaller then in control cells. The number of paramecia without the latex containing vacuoles increases during the

Table 1

Mean Number of Food Vacuoles Formed during 15 min Exposition to Latex Particles after Different Time of Lysozyme Treatment

Time of treatment (min)	Concentration of lysozyme (mg/ml)						Control
	5	2.5	1	0.5	0.25	0.1	
5	1.19±0.92	2.36±1.89	2.86±1.01	2.78±0.79	2.64±1.05	4.76±0.94	5.04±1.84
30	0,70±0.76	0.78±0.72	1.05±0.92	1.29±0.84	1.56±0.87	3.24±1.51	4.58±1.37
60	0.38±0.67	0.54±0.42	0.78±0.60	1.25±0.67	1.45±0.65	2.21±0.97	4.41±0.96
90	0.12±0.64	0.40±0.71	0.11±0.42	0.52±0.93	0.64±1.00	1.80±2.32	2.96±1.12
120	0.13±0.55	0.27±0.99	0.41±1.46	0.58±1.80	0.19±0.64	1.65±1.27	3.57±1.29

Table 2

Mean Number of Food Vacuoles Formed during 15 min Exposition to Latex Particles after Removing of Lysozyme

Enzyme removed after 30 min of treatment

Time after washing (min)	Concentration of lysozyme (mg/ml)						Control
	5	2.5	1	0.5	0.25	0.1	
30	0.42±0.43	0.83±0.67	1.00±1.41	2.00±1.34	6.00±0.10	6.75±1.99	6.32±1.00
60	0.86±1.14	1.25±0.87	1.00±1.63	0.50±0.50	3.17±1.50	3.75±2.30	6 00±1.31

Enzyme removed after 60 min treatment

Time after washing (min)	Concentration of lysozyme (mg/ml)						Control
	5	2.5	1	0.5	0.25	0.1	
30	1.50±1.30	1.33±1.36	1.60±1.08	2.00±1.54	2.02±0.01	6.85±2.21	11.0±0.10
60	1.33±1.36	0.15±0.01	1.00±1.00	1.25±2.87	3.00±1.00	8.00±2.30	9.01±1.20

time of treatment — so the mean number of vacuoles decreases. It can be assumed that after 90 min of treatment no vacuoles are formed in all concentrations of enzyme. Only in 0.1 mg/ml lysozyme some cells are able to ingest latex but the mean number of vacuoles per cell is much lower than in control group (Table 1).

The observations of living cells show that in enzyme treated paramecia neither the aggregation of particles nor formation of the vacuoles without the suspension occurs. No enlargement of the lowest part of cytostome is visible, either.

The examination of ruthenium red stained paramecia does not reveal any visible differences in mucous layer between treated and untreated cell (Pl. I 1,2).

The influence of enzyme is reversible only in paramecia which were treated with 0.1 mg/ml and 0.25 mg/ml lysozyme 30 min and in paramecia treated with 0.1 mg/ml lysozyme 60 min (Table 2). Some of cells washed after 60 min of 0.25 mg/ml enzyme treatment are also able to form vacuoles with the latex suspension.

The treatment with neuraminidase in concentrations used in this study does not influence the vacuole formation to any considerable extent (Table 3). No changes in the surface coat in any part of the gullet can be seen in cells examined in electron microscope (Pl. I 3–6).

Table 3

Mean Number of Food Vacuoles Formed during 15 min Exposition for Latex Particles after Different Time of Neuraminidase Treatment

Time of treatment	Concentration of neuraminidase (mg/ml)		Control phosphate-citrate buffer with		
	50	25	5 mM acetate buffer	2.5 acetate buffer	without acetate buffer
30	2.90±0.52	5.85±0.64	6.53±0.62	5.42±0.54	5.57±0.47
60	3.44±0.72	5.00±0.69	3.00±1.25	1.25±0.47	3.33±0.73
120	5.20±0.40	2.81±0.49	5.93±0.76	5.29±0.54	6.13±0.39
24h	3.49±0.40	3.62±0.48	4.76±0.37	3.90±0.46	5.10±0.27
42h	2.73±0.57	3.31±0.44	4.08±0.38	4.73±0.48	2.67±0.44

Discussion

It has been proved, that proteolitic enzymes arrest the food vacuole formation in *Paramecium*. They remove the external part of surface coat (Tołłoczko 1975). In this study lysozome was used. It is mucopolisa-

ccharydase which acts on B 1.4 linkage between N-acetyl-muramic acid (or 2-acetamino-2-deoxy-D-glucose) and 2-acetamino-2-deoxy-D-glucose in mucopolisaccharides, mucopolipeptydes or chityne. Braatz-Schade and Stockem (1973) have shown that it changes the ultrastructure of the filamentous part of the mucous layer in amoeba promoting the process of pinocytosis. It seems possible, that in their experiments lysozome acts as a protein rather, than as an enzyme. In this study no changes in the ultrastructure of mucous coat of *P. caudatum* were observed. However, it might be assumed that (in spite of the fact that the changes are to small to be visible in electron microscope) lysozyme (acting as an enzyme) removes some part of mucopolisaccharides, reducing the number of anionic sites in the external part of the cell surface. It causes that the latex particles cannot accumulate and the stimulus for an engulfment cannot be produced. The effect of enzyme is reversible only when low concentrations are used in comparatively short period of time. Then the vacuole formation is not completely arrested. It is possible that in this case the enzymatic activity of lysozyme is very low and it acts only as a cationic polypeptyde blocking only in some cells anionic groups and protecting the surface from accumulation of particles or producing the stimulus. Since in the cells which are able to ingest latex in lysozyme the number of vacuoles is not higher than in control paramecia we can assume that the smaller diameter of vacuoles in treated cells is due to the lower ability of the accumulation of particles in this cells.

It has been stated that neuraminidase treatment changes the ultrastructure of surface coat in *Paramecium aurelia* (Wyroba and Przełęcka 1973). On the other hand it is known, that it influence the phagocytic activity of monocytes (Weiss et al. 1966). In this study different conditions of enzymatic treatment had to be used. Optimum temperature could not be applied because in most cases it is letal for *Paramecium caudatum*, or at least their physiology is disturbed to very high extent. Secondly it was impossible to use higher concentrations of enzyme because of high amount of toxic acetate buffer in stock solution.

There are three possible explanations of the lack of neuraminidase effect on food vacuole formation in *Paramecium caudatum*: (1) In conditions applied in this study neuraminidase does not act at all or the effect is to small to produce the differences in physiology and ultrastructure. (2) *P. caudatum* does not possess sialic acid which is removed by neuraminidase treatment. It is not very likely because it acts on *P. aurelia* membrane. (3) Anionic sites in sialic acid do not play any important role in process of endocytosis. However, a lot of other data show that the anionic sites must be responsible for ingestion in significant extend. As a consequence of this most probable is the first explanation.

ZUSAMMENFASSUNG

Die Behandlung von *Paramecium caudatum* mit Lyzozym ergab eine Unterbrechung der Nährungsvakuolenausbildung, obwohl keine Änderungen von Mucoidsdicht in Cytostome, sogar mit Hilfe des Elektronmikroskopes gesehen wurden. Der Einfluss der Enzyme kann nur dann beim Süplen entfernt worden, wenn die benutzte Konzentrazion niedrig ist. Neuraminidase in Konzentrazionen von 25 bis 50 Einheiten per ml hat keinen Einfluss weder auf die Fisiologie von Nährungsvakuolenausbildung, noch auf die Ultrastruktur von Mucoidschicht.

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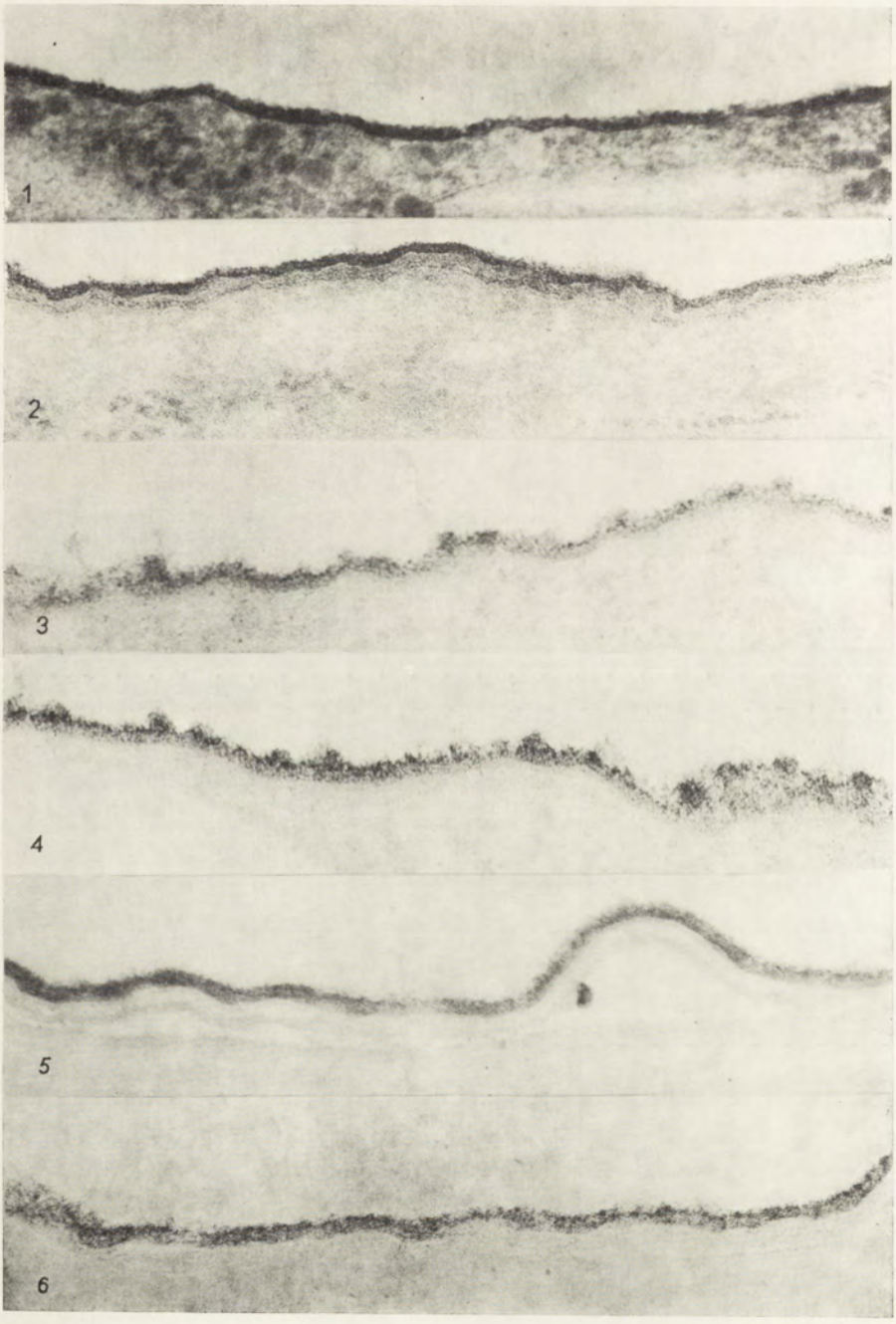
EXPLANATION OF PLATE I

Fragments of membrane of *Paramecium caudatum* in developing vacuole (3, 4) and in cytostome (7, 8). Ruthenium red staining

1: No enzyme treatment. Counterstained with uranyl acetate and lead citrate × 90 000
2: After lysozyme treatment (2.5 mg/ml) — 30 min. Counterstained with uranyl acetate and lead citrate × 90 000
3: No enzyme treatment, no counterstain × 120 000
4: After neuraminidase treatment (50 units per ml, 60 min). No counterstain × 120 000
5: No enzyme treatment, no counterstain × 120 000
6: After neuraminidase treatment (50 units per ml, 60 min). No counterstain × 120 000



B. Tołłoczko auctor phot.

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Comparative Effects of Methylcellulose, Gum Arabic and Carbopol-961 on Locomotion of *Polytomella agilis*

Synopsis. The thickening agents — methylcellulose, gum arabic and Carbopol-961 all depress the rate of locomotion of the flagellate *Polytomella agilis*. This effect may in part be due to increased viscosity but the greater reduction in speed in gum arabic and Carbopol-961 indicates complex viscosity changes at the cell surface and/or chemical reactions. For example, both gum arabic and Carbopol-961 reduced speed to less than 25% of normal at a relative viscosity of 2 while methylcellulose at a relative viscosity of 2.4 lowered speed to only 90% of normal.

A number of high molecular weight polymers are used as thickening, suspending, and emulsifying agents in formulations of pharmaceuticals and cosmetics (Goodrich et al. 1973, Rose and Rose 1966, Stecher 1968). Except for methylcellulose there is little information available on possible cellular effects of these chemicals. Methylcellulose has been used for many years to slow microorganisms (Brown 1944, Marsland 1943). The rate of movement of bacteria in methylcellulose initially increases as viscosity increases followed by a levelling or decline (Pijper 1947, Schneider and Doetsch 1974, Shoesmith 1960). Pigoń and Szarski (1955) found that speed of forward movement of *Paramecium caudatum* in solutions of gum arabic and polyvinyl alcohol was directly proportional to viscosity. The effect of methylcellulose on ciliary activity is also shown by its influence on the direction of spiralling in ciliates (Grębecki et al. 1967, Seravin 1970). The flagella of *Polytoma uvella* beat with a lower amplitude, wavelength and frequency in media to which methylocellulose has been added (Brokaw 1963). Aggregating behavior of the flagellate *Polytomella agilis* is modified in solutions of methylcellulose (Gittleson and Rogers 1972).

Higher than normal viscosity produced by methylcellulose in solutions

is generally considered the primary factor affecting motility. However, the same increases in viscosity brought about by gum arabic and Carbopol-961 result in a different effect on locomotion of *Polytomella agilis*.

Materials and Methods

Culturing Techniques. The flagellate *Polytomella agilis* was cultured in a medium consisting of 0.2 g yeast extract (Difco), 0.1 g bacto tryptone (Difco), and 0.1 g sodium acetate. $3H_2O$ per 100 ml of glass distilled water. This medium was used in all experiments. Axenic conditions were maintained by autoclaving the medium in 500 ml batches at 121°C and by using aseptic technique in transferring medium and cells. Temperature throughout was maintained between 24.5 and 25.5° C.

Preparation of Higher Viscosity Media. Carbopol-961 (B. F. Goodrich), gum arabic (Matheson, Coleman and Bell), and methylcellulose (Turtox) were added to the medium to raise the viscosity. Figure 1 shows the relationship between concentration of these polymers in g/100 ml of medium and relative viscosity (R. V.). R. V. were determined at 25°C with a Ubbelohde viscometer (size No. 1). Efflux times were measured with 15 ml samples. R. V. was calculated as the ratio of efflux time of an experimental solution to efflux time of glass distilled water.

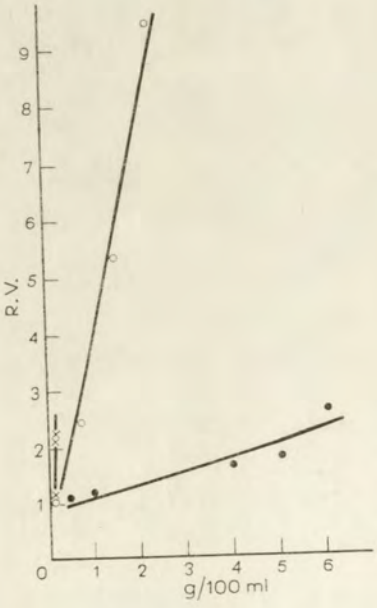

Fig. 1. Relationship between relative viscosity (R. V.) and concentration (g/100 ml) of Carbopol-961 (×), 10% methylcellulose (•), and gum arabic (○)

Carbopol-961 was added to the medium to make 0.1 and 0.2% (w/v) concentrations and then diluted with medium to obtain solutions of lower viscosity. Gum arabic solutions were prepared by adding 1, 2, 3, 4. 5, 6, and 15 g quantities

to the medium. Methylcellulose solutions were made by addition of 1, 5, 10, 15, and 20 g amounts of a 10% Turtox preparation to 100 ml of medium. Methylcellulose solutions were autoclaved as in the case of the other high viscosity polymer solutions but in addition were refrigerated at 4°C for 4–5 days to bring about clearing before use.

Measurement of Cell Locomotion. The speed of forward locomotion of *P. agilis* was measured in normal culture medium and in the same medium when mixed with solutions of Carbopol-961, gum arabic, and methylcellulose (prepared as described above). Drops of these media containing *P. agilis* were placed on a slide and covered with a coverslip edged with Dow Corning silicone stopcock grease. The mount was made at least 0.5 mm deep to allow freedom of movement of the approximately 10 μm diameter cells.

Timed-exposure photmicrographs were taken over a period of 15 min after allowing 15 min for equilibration. The moving organisms appeared as dark tracks on the negative. The lengths of these tracks were measured with a map measurer viewed with a microfilm reader. A photomicrograph of a slide micrometer taken under the same optical circumstances was used to correct for total magnification. Speed is expressed as the number of μm travelled/sec. Further discussion of this method may be obtained from Sears and Elveback (1961).

Summary of Properties of High Viscosity Agents. Carbopol-961 is a thickening, suspending, dispersing and emulsifying agent described by its manufacturer, B. F. Goodrich, as a carboxypolymethylene polymer. Carbopol-961 is a white powder slightly acidic in solution. Chemical properties are attributed to active carboxyl groups (Goodrich).

Gum arabic is the commercial term for acacia gum, the dried gummy exudate from the stems of *Acacia senegal*, used as a thickening agent and colloidal stabilizer. The central core is D-galactose and D-glucuronic acid to which are attached sugars such as L-arabinose and L-rhamnose. Gum arabic is a white powder which is acid in solution (Osol and Pratt 1973, Stecher 1968).

Methylcellulose is a thickening agent, stabilizer and emulsifier derived from cellulose. Methoxyl content is about 29%. The grayish white powder is neutral in aqueous suspensions (Rose and Rose 1966).

Results

The locomotion of *P. agilis* is reduced by increasing concentrations of methylcellulose, gum arabic and Carbopol-961 (Fig. 2.) However, the rate of decrease is significantly different for each polymer in reference to viscosity. At a R. V. of about 1. 1 speed is less than 70% of normal in Carbopol-961, 82% of normal in gum arabic but nearly normal in methylcellulose. With an increase in R. V. to 1.2 locomotion is still only slightly affected by methylcellulose (<5% change). Yet speed decreases to less than 80% of normal in gum arabic and to about 50% of normal in Carbopol-961. Speed of locomotion declines over 50% in Carbopol-961 from a R. V. of 1.1 to 1.2. The decrease in speed is less precipitous in gum arabic. Both gum arabic and Carbopol-961 reduced speed to less

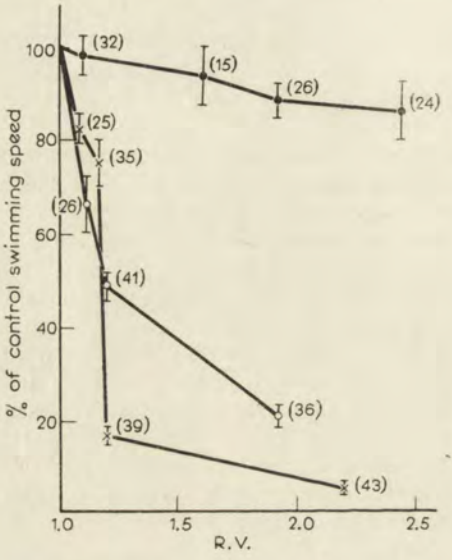

Fig. 2. Influence of methylcellulose (●●), *gum arabic* (○○), and Carbopol-961 (× - ×) on swimming speed of *Polytomella agilis*. Vertical bars deno te ± sample standard error of the mean. Number of measurements is shown in parentheses. There were 74 control measurements

than 25% of normal at a R.V. of about 2 while methylcellulose at a R.V. of 2.4 lowers speed to only about 90% of normal.

Measurement of osmotic pressure and pH in each experimental solution showed no changes over the range of concentrations used. The pH of the methylcellulose and gum arabic solutions was 6.3 and that of the Carbopol-961 solutions was 6.7.

Discussion

Cell motility measured as rate of locomotion provides a sensitive way of evaluating the influence of environmental conditions on an organism (Fenn 1969). Specifically, the experiments in this report were undertaken to evaluate how thickening agents compared in their effect on cell locomotion. The rate of locomotion of *P. agilis*, in general, was found to be inversely proportional to polymer concentration. Pigoń and Szarski (1955) obtained similar results for *Paramecium caudatum* swimming in gum arabic and polyvinyl alcohol solutions. In their study polyvinyl alcohol slowed movement to a greater extent than gum arabic which suggested to them that viscosity was not the only factor involved. We also have found differences in locomotion dependent upon the type of thickening agent. The following increasing order of effectiveness in reducing locomotion was measured up to a R. V. of about 1.2: methylcellulose < Carbopol-961 < gum arabic. At higher R. V. Carbopol-961 had a stronger effect than gum arabic.

Since viscous resistance in the viscometer and shearing forces on the organisms differ at various concentrations of methylcellulose then viscous effects *per se* are difficult to evaluate (Brokaw 1966, Shoesmith 1960). Differences in biological activity between these agents may also be related to specific chemical moieties such as oxidase enzymes and active carboxyl groups produced in solution by gum arabic and Carbopol-961, respectively. The inhibition in growth of *P. agilis* populations which we have measured in these same polymer solutions indicates that there is a chemical effect (Gittleson, unpublished measurements). Achemical effect is also indicated by reports that intravenous use of gum arabic and Carbopol-961 has resulted in renal and hepatic damage and allergic reactions (Osol and Pratt 1973).

In regard to studies of ciliary behavior in methylcellulose solutions we agree with Dryl (1974) that the results must be cautiously interpreted due to possible abnormal activity induced by thickening agent.

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RÉSUMÉ

Les agents augmentant la viscosité: la methylocellulose, la gomme arabique et le Carbopol-961 abaissent, tous, l'allure de la locomotion du flagéllé *Polytomella agilis*. Cependant, la gomme arabique et le Carbopol-961 réduisent la vitesse à moins de 25% de la normale, à une viscosité relative de 2, tandis que la methylocallulose, à une viscosité relative de 2.4, ne diminue la vitesse qu'à 90% de la normale.

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Studies on the Effects of Continuous Exposure to Light and Darkness on *Blepharisma intermedium*

Synopsis. Blepharisma intermedium were exposed to continuous light and darkness for a period of 14 days. The ciliates exposed to light lost their pink colour gradually where as the colour appeared brighter in those exposed to darkness. The volume increased gradually and later decreased in both and the maximum volume observed in the dark exposed form was greater than that of the light exposed form. Conjugants were seen in both light and dark exposed forms. The regeneration of the anterior and posterior fragments of *Blepharisma* that were cut into approximately two equal halves was accelerated in dark as compared to light. Initially, the division rate was enhanced under illumination but later it declined sharply. In darkness, the peak in division was reached later after which there was a gradual decline. Nuclear abnormalities were observed during the first three days of exposure in both light and dark exposed forms.

Blepharisma is a spirotrichous ciliate containing a red pigment which has been extracted and analysed and found to be photosensitive (Giese 1973). Most of the studies on the effects of visible light on *Blepharisma* have therefore been with reference to the reaction of this pigment rather than the reaction of the organism as a whole. Since visible light has the capacity of repairing to a certain extent the damage caused by ultraviolet irradiation, effects of visible light on *Blepharisma* with reference to this photoreversal phenomenon has also been studied (Giese and Lusignan 1961). In this investigation, the effects of continuous illumination and darkness separately on the division rate, morphology and regeneration rate of *Blepharisma intermedium* have been studied.

Material and Methods

Stock cultures of *Blepharisma intermedium* were grown at 28° C ± 1° C in hay infusion fortified with horlicks. While being exposed to light, cultures were kept at a constant distance of 18 cm from a 40 watt non-fluorescent bulb emitting

white ligh t(68 foot candles). Filters were not used to cut off any wavelengths. Since the temperature was observed to be 28° C ± 1° C with these conditions, the animals that were exposed to dark were kept in incubators at the same temperature.

For general observations, ciliates from the stock cultures were inoculated into petri dishes containing 10 ml of hay infusion medium fortified with horlicks (10 mg/100ml) so that a proportion of 100 ciliates/ml was maintained. The petri dishes were kept under light or in darkness separately. The ciliates were fed once in three days. Observations were made after every 24 h on the general morphology and behaviour for a period of 14 days. For a period of one week, daily the ciliates were fixed in carnoy's fluid, stained in Shiff's reagent and counterstained in light green. The number of ciliates showing normal and abnormal nuclei in the stained preparations were recorded and expressed as percentages.

To study volume changes, the length and width of the stained ciliates were measured on camera lucida drawings in milimeters, converted to microns using a stage micrometer (8 × eye piece and 10 × objective) and the cell volumes in μ^3 were computed from the formula for a prolate spheroid:

$$V = 4/3\ \Pi\ (A/2)\ (B/2)^2$$

in which A is the major axis and B is the minor axis as described by Scherbaum (1965), Thormar (1962) and Kasturi Bai and Tara (1974).

When conjugants were observed, the number of conjugants and non-conjugants were recorded. A few conjugants were isolated soon after pairing commenced and exposed to the same conditions in which they were formed (light or dark). The time taken for their separation was noted.

For studies on regeneration rate, 20 ciliates from the stock culture were cut into approximately two equal halves on a watch glass. Immediately, the anterior and posterior fragments were separately isolated into cavity dishes containing 5 ml of hay infusion. The dishes were placed under light or in darkness and observed at half hourly intervals. The time taken for regeneration was recorded. Experiments were run in triplicate and repeated ten times.

For division rate studies, four ciliates from the stock culture were inoculated into petri dishes containing 10 ml of hay infusion and exposed to light or dark. The individuals in each petri dish were counted once in 24 h and four were re-inoculated into the same medium. This process was repeated for a period of 14 days. Culture conditions were maintained as described already. Experiments were run in triplicate and repeated twice. The division rate was calculated as described elsewhere (Kasturi Bai and Manjula 1974).

Results

The ciliates exposed to light lost their pink colour gradually where as the colour appeared brighter in those exposed to darkness.

In both light and dark exposed forms, the volume increased gradually

and later decreased. The volume of the dark exposed forms was greater than the light exposed forms (Fig. 1).

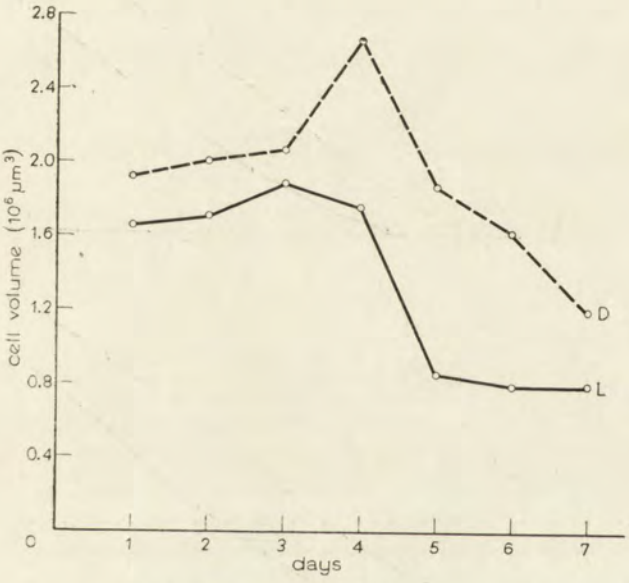

Fig. 1. Volume changes in *Blepharisma intermedium* exposed to continuous light (L) and darkness (D)

Regeneration was accelerated in dark than under light in both anterior and posterior fragments of cut *Blepharisma* (Fig. 2).

In the culture exposed to light, conjugation occurred in 1–2% of the organisms between the 3rd and 4th day while it occurred from 6–10% between 4th and 8th day in the dark exposed ciliates. The conjugating pairs separated by 24 h and divided normally in both.

Initially, division rate was enhanced under continuous illumination, the peak was reached on the second day after which there was a sharp decline. In darkness, the peak was reached on the 3rd day after which there was a gradual decline. From the 5th day onwards under light and from the 8th day onwards under darkness, the division rate was one per day (Fig. 3).

Observations on the nuclear apparatus of the stained preparations revealed a large number of abnormalities. Double (Pl. I 1), broken (Pl. I 2 and 4) and forked (Pl. I 3 and 5) macronuclei were seen in both light and dark exposed forms, the forked type being more numerous. Micronuclei appeared larger than their original size. The abnormalities were found in large numbers during the first three days of exposure (Fig. 4).

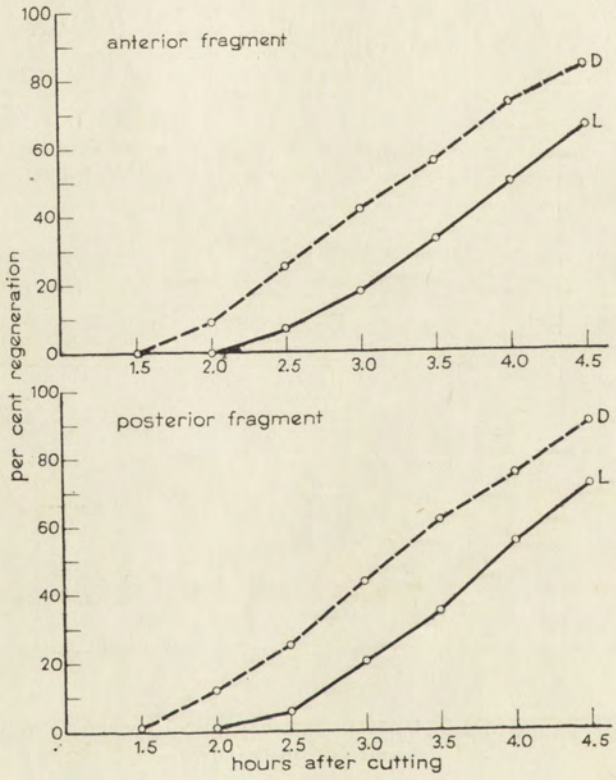

Fig. 2. Regeneration rate in the anterior and posterior fragments of *Blepharisma intermedium* exposed to continuous light (L) and darkness (D)

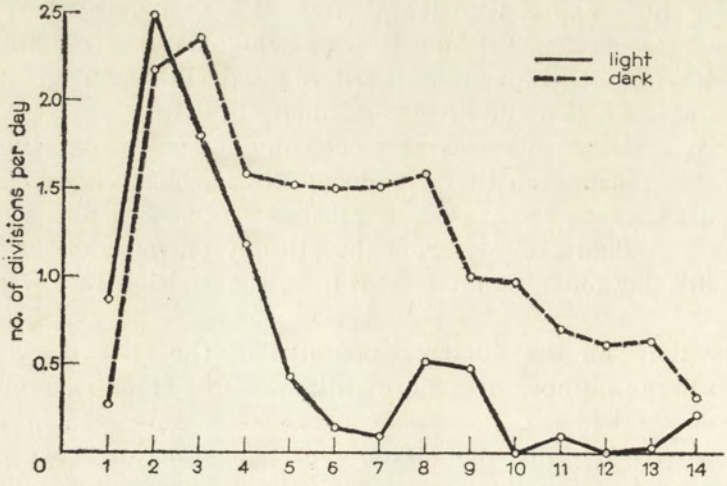

Fig. 3. Division rate in *Blepharisma intermedium* exposed to continuous light (L) and darkness (D)

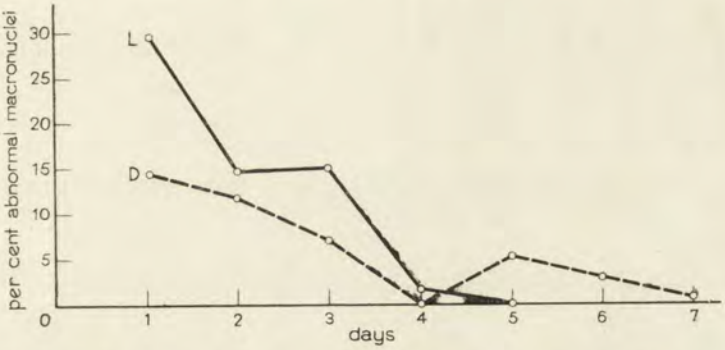

Fig. 4. Percentage of abnormal macronuclei occurring in *Blepharisma intermedium* exposed to continuous light (L) and darkness (D)

Discussion

The bleaching effect of light on *Blepharisma* is a well established fact. Giese (1973) records that a light of relatively low intensity (150 foot candles) evokes a slow extrusion of pigment in *Blepharisma* where as intense light causing a violent pigment extrusion always kills the cell. Under dim visible light or near UV light for 96–150 h, blue *Blepharsima* were completely bleached. According to him, the time required for the colour change depends on the physiological state and initial pigmentation of *Blepharisma*. In this investigation, the ciliates were isolated from a logarithmic culture before exposure and were coloured brightly and well fed. Gradually the bright pink colour faded and the ciliates turned pale.

It was observed that continuous exposure to light or darkness reduces the volume of the ciliate.

Low intensity visible light (74 foot candle) was not injurious to regenerating cells of *Blepharisma undulans* but considerably delayed the process where as an intensity of 100–250 foot candles cytolysed the cells at some time between 2–12 h of exposure (Giese and Lusignan 1961). It was also observed that division rate was not affected after 12 h exposure to light. In this investigation it was observed that division rate was affected when *Blepharisma* was continuously exposed to visible light and also that regeneration was delayed under light as compared to darkness.

Light is an important factor affecting conjugation especially in the coloured ciliates. In *Paramecium bursaria*, a green coloured ciliate the time of the day when the clones are mixed ei an important factor in regard to the occurrence of the mating reaction (Wichterman 1953).

In contrast, light or darkness have little effect on the mating process in an axenic culture of *Tetrahymena* which is a colourless ciliate (Elliot and Hayes 1953). In the present investigation it was observed that continuous light or darkness induces conjugation in *Blepharisma intermedium.*

Giese and Lusignan (1961) note that weak visible light of 74 foot candles applied for 12 h did not alter division rate in *Blepharisma undulans japonicus.* They used day light fluorescent lamp which was filtered through water to remove heat and through a filter to cut off wavelength between 4160 and 4360 Å. Giese (1967) also reports that yellow light from a sodium lamp had no effect upon the division or regeneration of *Blepharisma,* Edmunds (1974) exposed axenic cultures of *Tetrahymena pyriformis* to alternate light and dark periods of different lengths and found that division virtually ceased during at least a portion of the light periods and rhythmic division bursts persisted for at least six days with a circadian period if the culture was placed in constant darkness. In this study it was noticed that initially light increased division rate, but retarded division after prolonged exposure.

Under continuous exposure to light or darkness, nuclear abnormalities were observed namely forked, broken and double macronuclei. Buffaloe (1959) mentions that when *Chlamydomonas eugametos* and *C. moewussi* were cultured under light of 800 foot candle intensity rather than 100 foot candle, cells multiplied more slowly, enlarged and became polyploid. However, he has not observed any change in the morphology of the nucleus. It is interesting that nuclear abnormalities are induced during light and dark exposures. Since the normal functioning of the macronucleus is essential for binary fission (Raikov 1969), it would be of interest to study how the mode of binary fission appears to be normal inspite of these abnormalities.

ZUSAMMENFASSUNG

Blepharisma intermedium wurden 14 Tage dauerndem Licht bzw. Dunkel ausgesetzt. Die dem Licht ausgesetzten Ziliaten verloren allmählich ihre rötliche Farbe, während die Farbe der dem Dunkel ausgesetzten Ziliaten heller wurde. Bei beiden Versuchsarten nahm das Volumen zunächst allmählich zu und später ab; das beobachtete Volummaximum war jedoch bei den dem Dunkel ausgesetzten Ziliaten grösser als bei den dem Licht ausgesetzten. Konjuganden wurden bei beiden Versuchsarten beobachtet. Die Regenerierung der vorderen und hinteren Fragmente von *Blepharisma,* die in 2 nahezu gleiche Teile seziert wurden, ging im Dunkel beschleunigter vor als im Licht. Die Aufspaltungsrate vergrösserte sich zunächst bei Belichtung, liess aber dann stark nach. Das Maximum der Aufspaltung wurde

im Dunkel später erreicht; danach zeigte sich eine allmähliche Abnahme. Nukleare Unregelmässigkeiten wurden während der ersten 3 Tage der Aussetzung sowohl im Licht als auch im Dunkel beobachtet.

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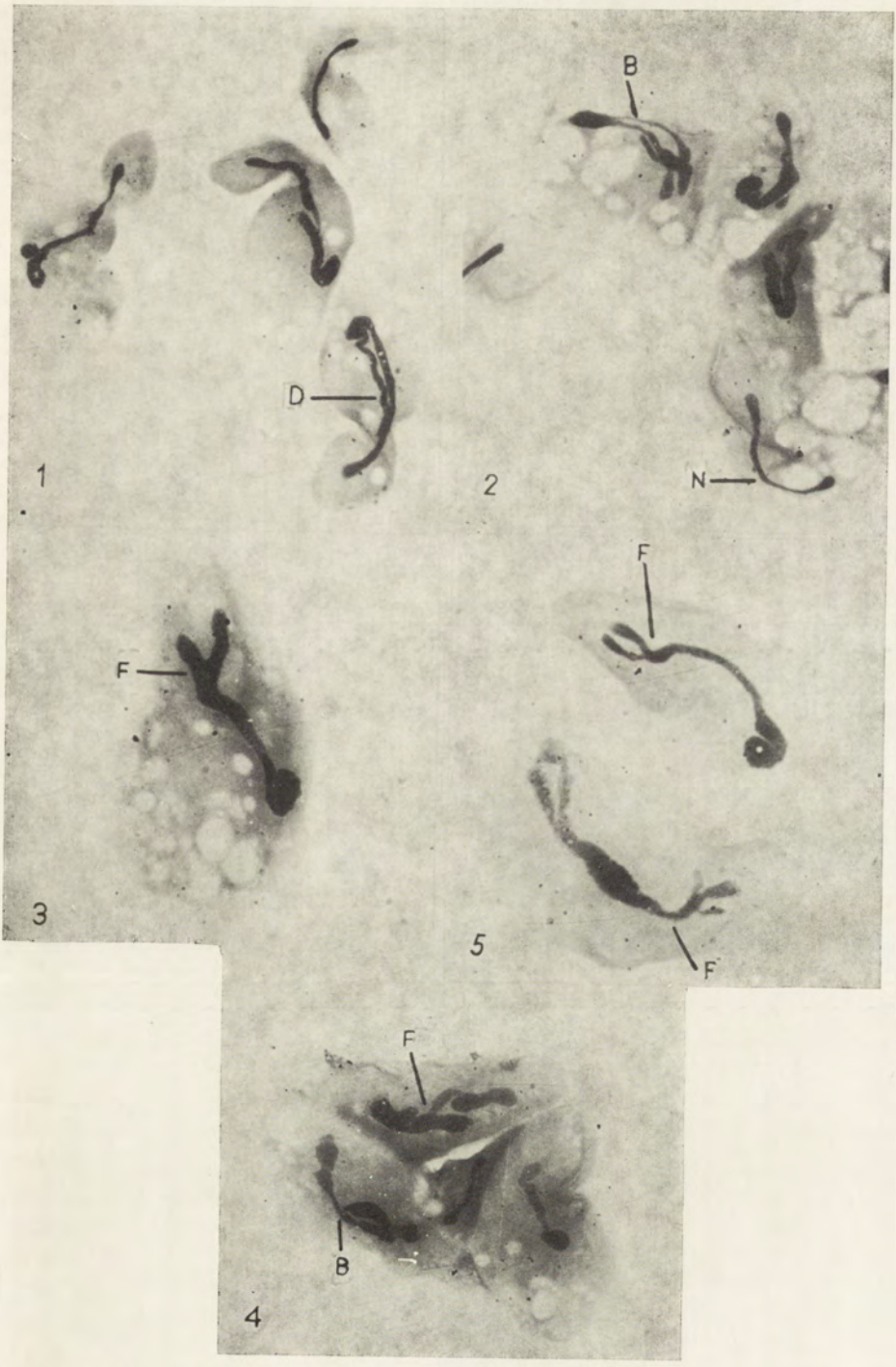
EXPLANATION OF PLATE I

Macronuclear changes observed in *Blepharisma intermedium* exposed to continuous light and darkness and stained with Feulgen and light green
N — Normal Macronucleus, F — Forked Macronucleus, B — Broken Macronucleus and D — Double Macronucleus
1: — Exposure to light for 24 h 2: Exposure to light for 48 h 3 and 4: Exposure to light for 72 h. 5: Exposure to darkness for 24 h



A. R. Kasturi Bai et M. K. Manjula auctores phot.

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Experimental Transmission of *Trypanosoma evansi* to Chicken

Synopsis. The present work was carried on the transmission of *Trypanosoma evansi* to chicken. The positive results of inoculation were found in some of the birds. The parasites were observed in the peripheral blood films only. Organ smears of the inoculated chickens did not show any positive result. Divisions by binary fission were seen. Mensurally *T. evansi* from chicken was smaller than the parasites from their original host, cow.

The observation clearly testify to the plasticity of the parasite which manifests itself by a wide range of host specificity.

Introduction

Trypanosoma evansi Steel, 1885, is world wide in its distribution. It was the first pathogenic trypanosome discovered causing the dreadful mammalian disease "Surra" which was known in India since time immemorial. Morphologically *T. evansi* is typically represented by their trypomastigote stage comprising of slender and intermediate forms corresponding to those of *T. brucei* (Hoare 1956). Hoare's standard measurement of *T. evansi* is 15–34 μm in length with a mean of 24 μm.

So far no report has been made on the experimental transmission of *T. evansi* to a bird. The present work deals with the experimental transmission to chicken of *T. evansi*. Surprisingly, the results were positive. These throw new light on the point of interclass host range of *T. evansi* hitherto unnoticed. The observations also provided us with the understanding of the plasticity and biology of the haemoflagellate in an unnatural host. Earlier, Misra and Choudhury (1975 b) have reported the results of brief.

The effect of experimental transmission of *T. evansi* to most laboratory mammals is positive (Choudhury and Misra 1972, Misra and Choudhury 1975 a). Brumpt (1949) reported that, some birds can be infected by blood inoculation of *T. rhodesiense* and sometimes

by tse-tse fly and they may also infect new glossinas, Weinman (1953) reported a positive experimental transmissin of *T. gambiense* and *T. rhodesiense* to embryonated chicken egg.

Materials and Methods

The present strain of *T. (T.) evansi* was isolated from a cow and was named BB 24. This strain is now being maintained in albino rats. To perform the transmission experiment with *T. (T.) evansi* in chicken, three day old fresh birds were selected as hosts. The chickens were kept in moderately large cages and fed with chicken mash. Animal cages were cleaned thrice a week and disinfected with lyzol. Approximately 10 000 trypanosomes were inoculated subcutaneously to six chickens. Similar inoculam was administered to six albino rats as a control. Six chickens also received 0.5 ml citrated saline. Appearance of trypanosome in circulation and intensity of parasitaemia during and in between peaks were studied by means of a wet preparation of blood mixed with citrated saline. The blood films were prepared from infected hosts and were stained with Leishman's stain. Measurements of the trypanosomes were followed after Davis (1952) with a slight modification in the procedure after K. K. Misra (unpublished).

Results

Only two chickens from the infected group were found positive. The trypanosomes were first encountered on the peripheral blood after the ninth day of inoculation. Maximum number of trypanosomes were found on the 11th day. On the 12th day after infection the number of trypanosomes depleted and on the 13th day the vanished altogether. So the 11th day is considered as the peak. After a few days all the ifected chickens died without showing any pathologie changes. The heart, lung and bone marrow of these dead chickens were examined but no trace of trypanosomes was found.

The experiment was repeated and the trypanosomes were found only in a small number of chickens and almost in all cases the peak of the parasitaemia was observed on the 11th day after infection.

T. evansi found in the blood of chicken is essentially similar to those found in the blood film of cow or albino rat. The parasite is slender with narrowly pointed anterior end (Pl. I 1–3). The posterior end of the parasite is sometimes blunt on pointed. Kinetoplast is situated at the posterior end. Occasionally it leaves a small space from the posterior end. It is round to ovoid in shape and stains bright in Leishman/Giemsa. A flagellum arises from the kinetoplast, wraps along the body as free

flagellum. The cytoplasm of the parasite stains blue. Vacuoles are found to be present in the cytoplasm. No granules or pigments are observed. Nucleus is oval in shape and situated at the middle of the cell body proper. It is granulated and stains red in Giemsa/Leishman. The cell body is sometimes narrow. The free flagellum is quite long. The comparative mensural account of the parasite from three different hosts is shown in Table 1.

Table 1

(Measurements in μm)

		Cow	Chicken	Albino rat
Length	Mean	30.02	24.45	26.7
	Range	23.0-36.5	17.5-34.0	24.5-31.0
	S.D,	3.154	4.603	2.2
	S. E.	± 6.575	± 0.758	± 0.73
Breadth	Mean	2.33	2.66	1.55
	Range	2.0–3.0	2.0–4.0	1.0–2.0
	S. D.	0.33	0.408	0.35
	S. E.	± 0.06	± 0.067	± 0.46
Nucleus	Mean	2.73	2.57	2.65
	Range	2.0–5.0	1.0–4.5	2.0–3.0
	S. D.	0.78	0.706	0.31
	S. E.	± 0.141	± 0.116	± 0.1
Free flagellum	Mean	7.09	6.22	8.3
	Range	4.0–10.0	3.0–11.0	5.5–10.0
	S. D.	1.451	2.305	1.1
	S. E.	± 0.265	± 0.379	± 0.36
Kinetoplast to middle of the nucleus	Mean	9.53	8.79	7.47
	Range	6.0–15.5	6.0–14.0	6.0–10.5
	S. D.	2.24	1.621	1.24
	S. E.	± 0.407	± 0.266	± 0.41
Middle of nucleus to anterior end	Mean	11.24	10.07	8.82
	Range	7.2–15.5	4.0–15.0	7.0–11.5
	S. D.	1.76	2.575	1.4
	S. E.	± 0.32	± 0.423	± 0.46
Post kinetoplast	Mean	2.06	0.78	1.6
	Range	5.0–6.0	0.5–3.0	1.0–2.0
	S. D.	1.035	0.793	0.13
	S. E.	± 0.188	± 0.13	± 0.043
Cell body	Mean	27.96	19.49	18.4
	Range	19.0–26.5	14.0–33.5	19.0–21.0
	S. D.	5.638	4.006	2.28
	S. E.	± 1.025	± 0.658	± 0.31

Some divisional stages are found in the blood film. The division follows the same pattern as observed in case of other mammalian trypanosomes. Kinetoplast divides into two (Pl. I 4) prior to the division of the nucleus. The resultant two trypanosomes are quite similar. No dyskinetoplastic form was observed. No multiple fission was found. All the divisional stages were found in the peripheral circulation. Amastigote and sphaeromastigote stages were not found in the smears of heart, lung and choroid plexus.

Discussion

Successful transmission of *Trypanosoma evansi* among interclass vertebrates clearly signifies that members of the Subgenus *Trypanozoon* are highly plastic in relation to their host specificity. They not only survive in the blood of avian host but also multiply very actively. It is clear from the present experiment that the parasite of "Surra" disease also maintains its general morphology and biology in the chicken, an unnatural host. Mensurally, *T. evansi* in chicken stands behind the

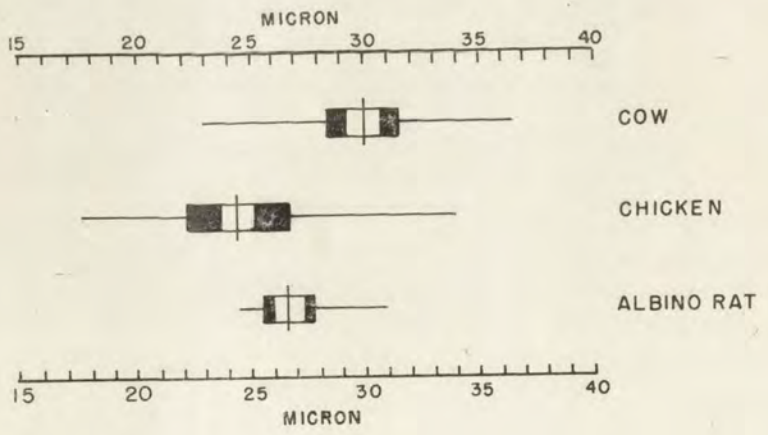

Fig. 1. Graph showing the mensural variation (total length) of *Trypanosoma evansi* when infected to heterologous hosts, The straight line (——) represents the mensural range; vertical line (|) indicates the mean length; black rectangle elucidates the standard deviation (DV) of the population and white rectangle represents twice the standard error (SE) of the population

original host, cow and the laboratory host, albino rat (Fig. 1 and 2). *T. evansi* from chicken significantly differs from *T. evansi* in original host in the post-kinetoplast distance, which is very short in the former. However, *T. evansi* from chicken is shorter than the *T. evansi* from cow and albino rat. Cytologically, parasites from cow and chicken differ in

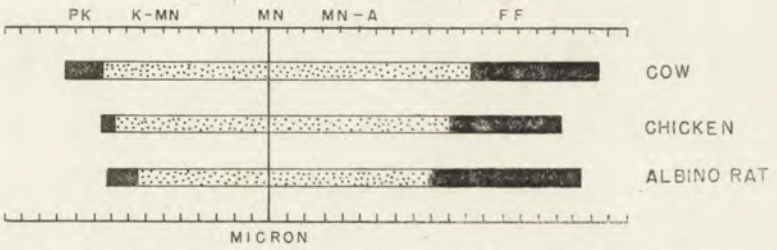

Fig. 2. Graph elucidating the mensural detail of *T. evansi* populations maintained in heterologous hosts. PK — distance between posterior end and kinetoplast, K-MN — distance between kinetoplast and middle of the nucleus, MN — middle of the nucleus, MN-A — distance between middle of the nucleus and anterior end FF — free flagellum

some points. Giemsa positive granules are present in the parasites from cow, but absent in those from chicken. Moreover, the cytoplasm of the parasites from the above mentioned hosts also differ to some extent in their tinctorial properties.

Only Brumpt (1949) have reported that chickens, guineahens and francolins can be infected by blood inoculation of *brucei* trypanosomes. These birds may be infected sometimes by tsetse fly and they may also infect new glossinas. However, no detail biology of the parasite in these birds has been studied. Experiments have been designed with *brucei* trypanosomes and embryonated chicken egg by Weinman (1953). He reported that both the culture and blood form of *brucei* can develop in the embryonated chicken egg. Moreover, chicken egg supports the growth of both blood and culture trypanosomes, but it proved impossible to convert one type to the other (Weinman 1953). He showed that blood forms multiply in the blood of the embryo.

However, the present observation records for the first time the growth and the development of *T. evansi* in the chicken. Detail morphology and biology of the haemoflagellate shows that the parasite can maintain its originality in a heterologous vertebrate host belonging to a different class. Further, it proves a wide range of host specificity of *T. evansi*. This observation may throw some new light on the relationship and pathogenicity of the trypanosomes of warm blooded vertebrates.

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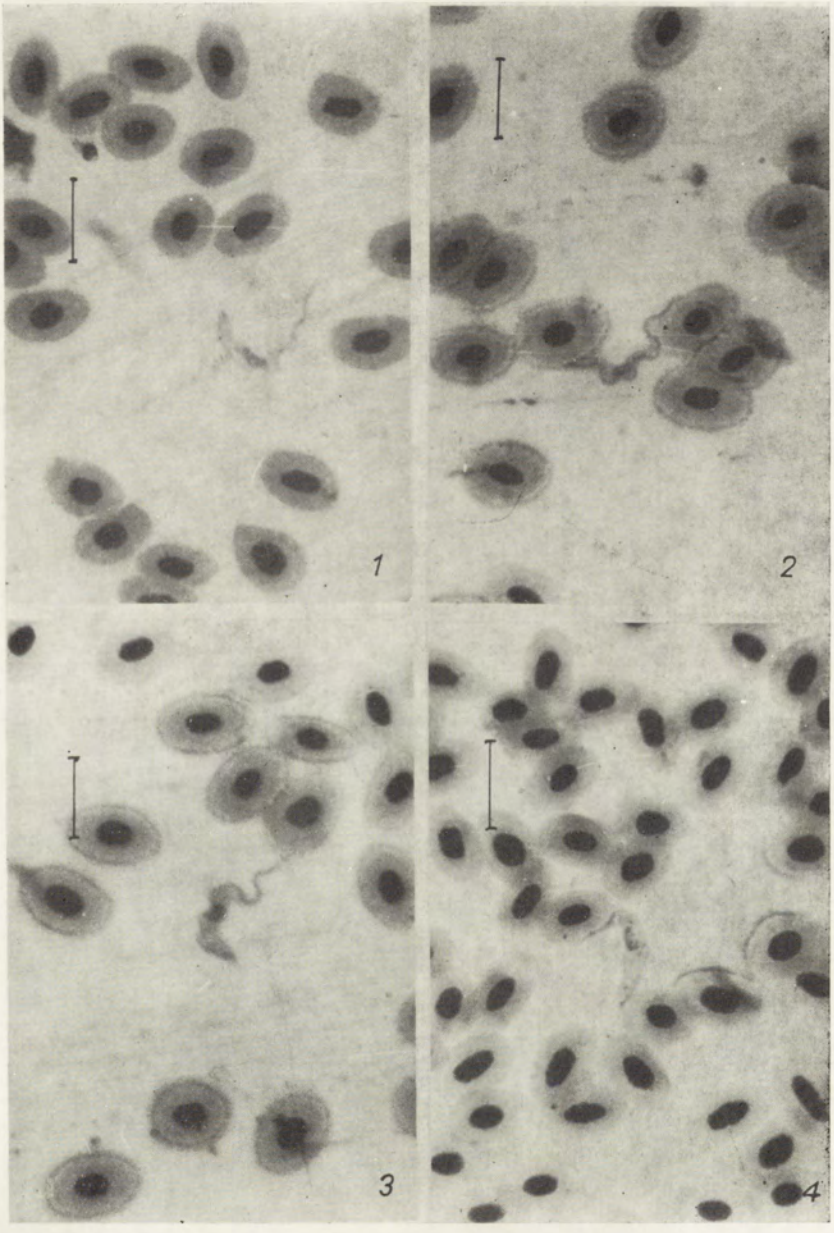
RÉSUMÉ

Le travail présent concerne la transmission de la *Trypanosoma evansi* aux poulets. L'inoculation s'est avérée efficace chez quelques individus. Les parasites ont été rétrouvés seulement dans le sang périphérique. Les préparations des organes n'ont montré aucun résultat positif. La reproduction du parasite par division binaire était observée. Par ses dimensions *T. evansi* du poulet est moins importante par rapport aux éxemplaires provenant de son hôte originaire la vache. Ces observations démontrent clairement qu'il s'agit d'un organisme parasitaire plastique dont la spécificité par raport à l'hôte est très étendue.

EXPLANATION OF PLATE I

1–4: Photomicrographs of *Trypanosoma evansi* from artificially infected chicken. Dividing kinetoplast may be seen in Fig. 4. Scale represents 10 μm



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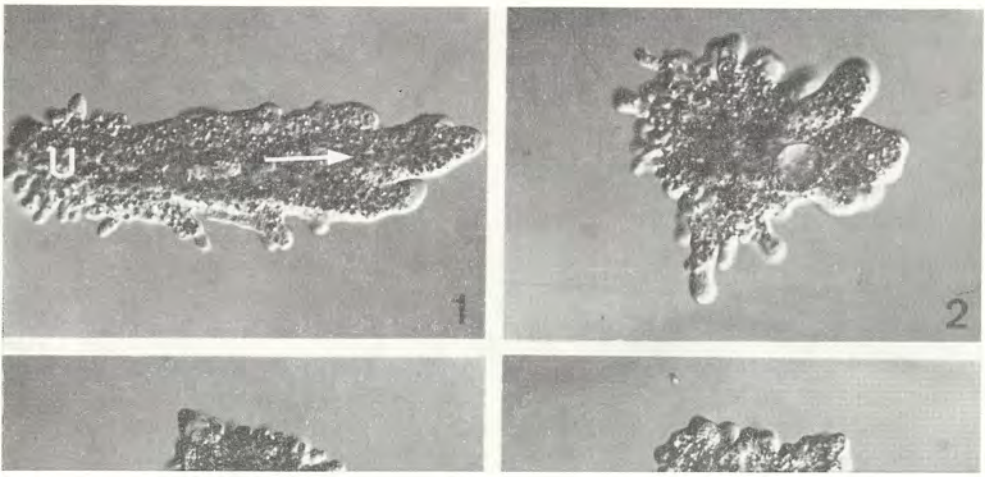

Omitted Plates I-IV to the paper of

N. Hülsmann, W. Korohoda and W. Stockem,
"Reversible Disorganization of Motile Activities
in *Amoeba proteus* Induced by General Anaesthetics"

Acta Protozoologica 15(2), 187-194

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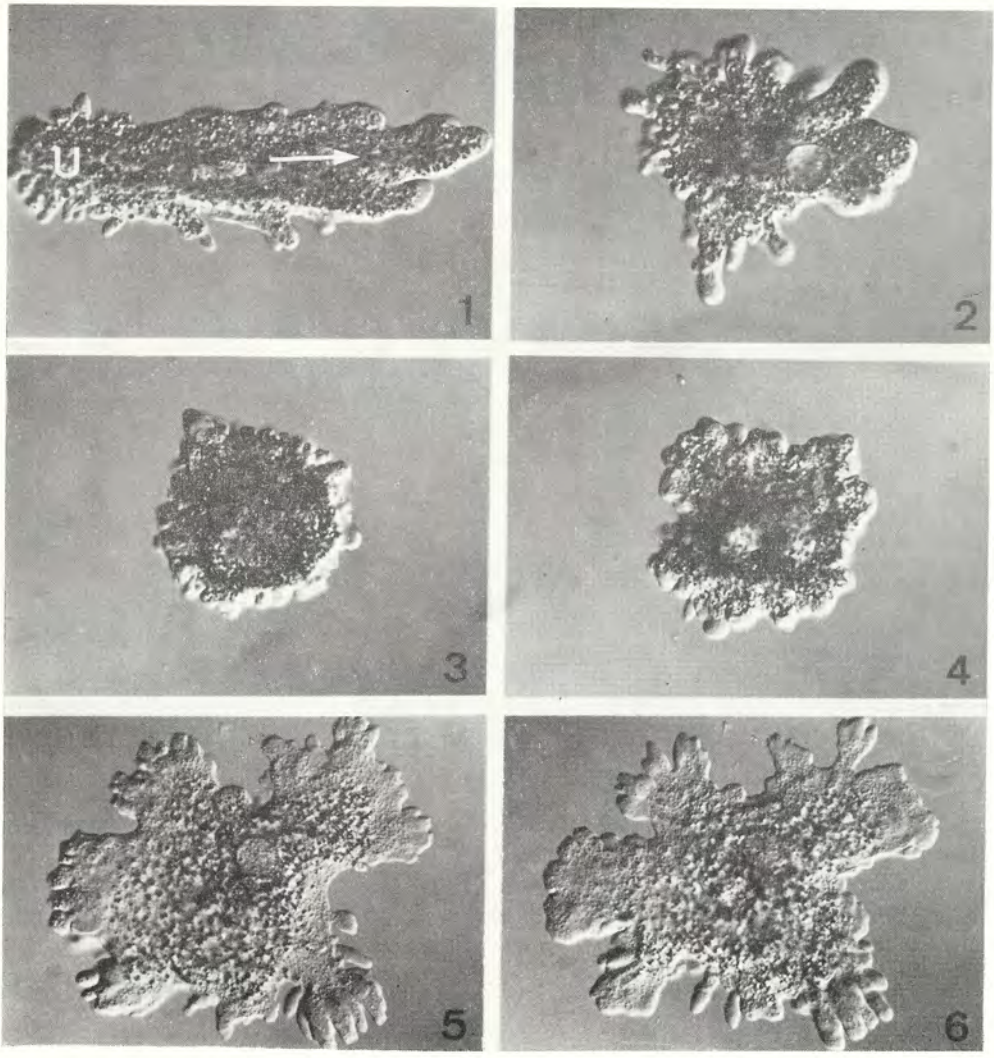

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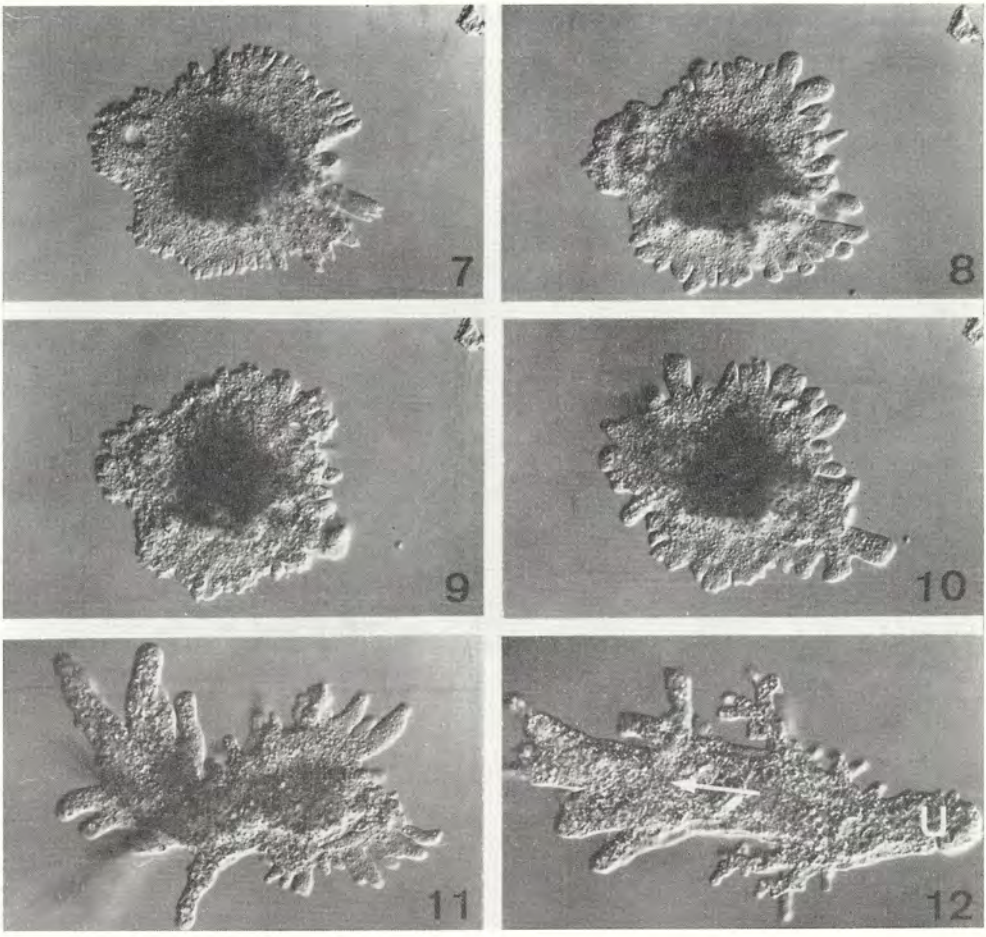

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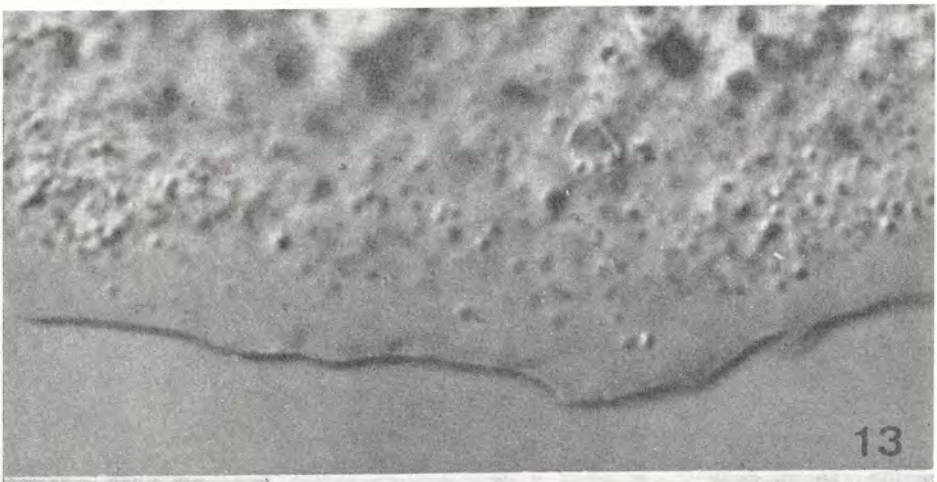

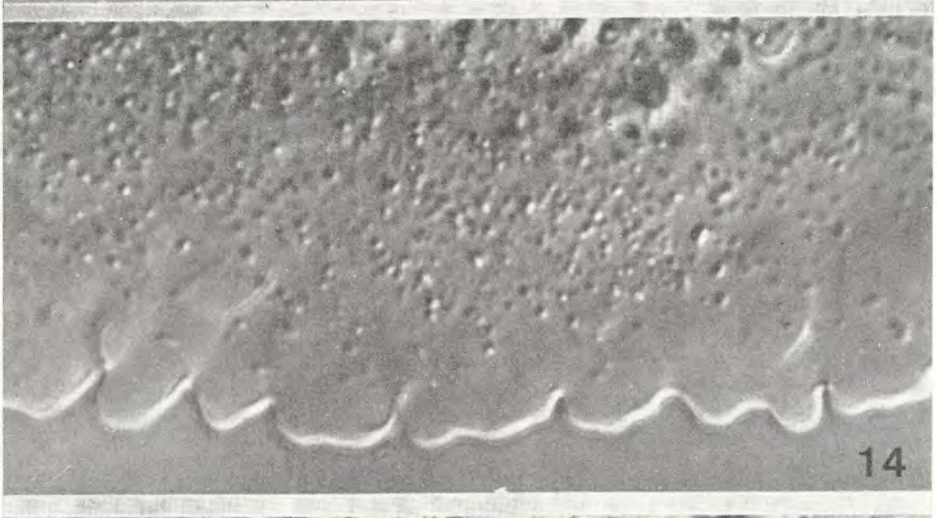

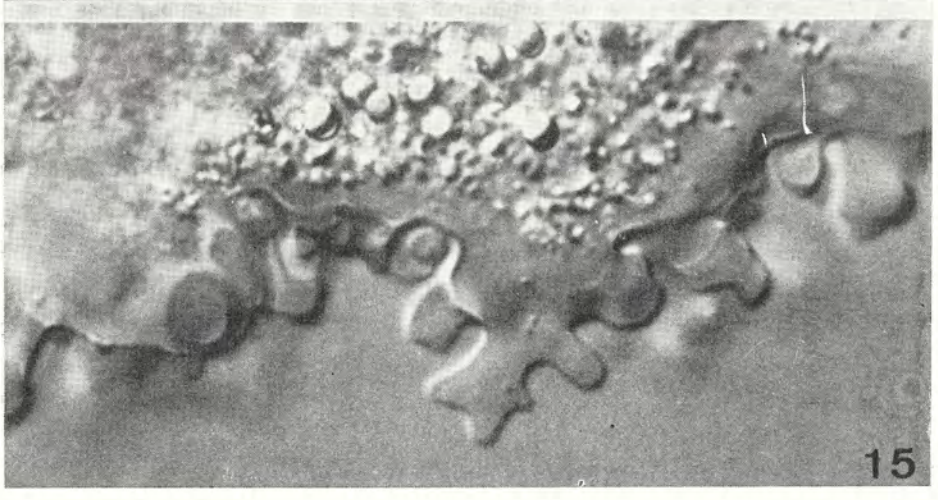

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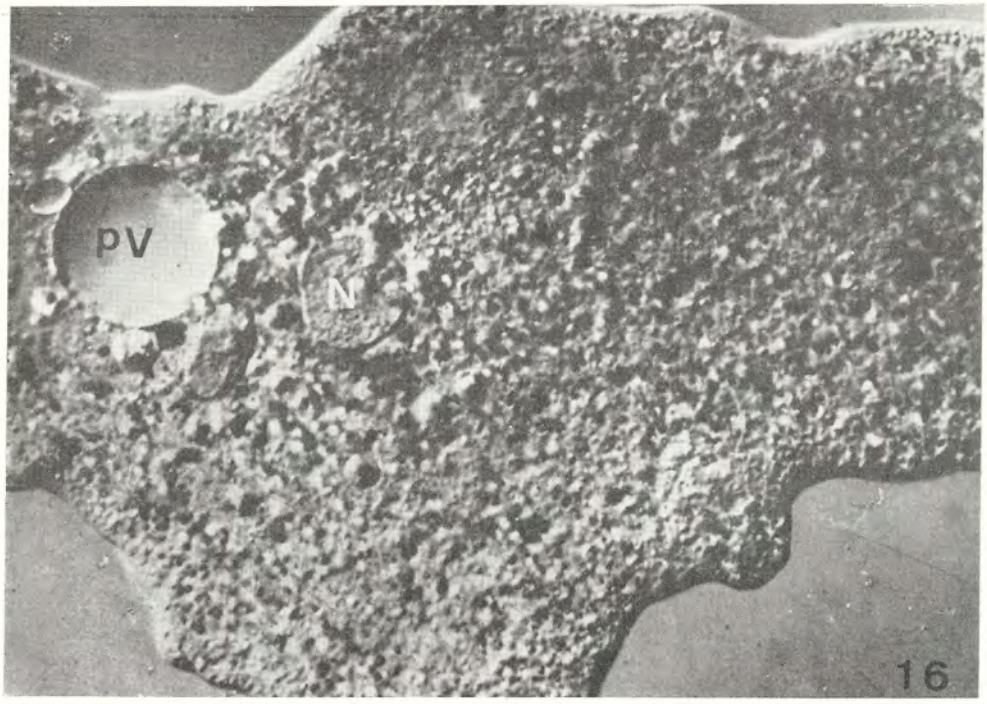

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In preparation:

W. Foissner: *Wallackia schiffmanni* nov. gen., nov. spec., (*Ciliophora, Hypotrichida*) ein alpiner hypotricher Ciliat — C. K. Sinha and A. K. Mandal: *Trypanosoma enhydris* sp. n. from a Fresh Water Snake *Enhydris enhydris* (Schneider) — V. Golemansky: Three New Coccidian Species (*Coccidia : Eimeridae*) Found in Wild Birds from Bulgaria — R. Mandal: *Caryospora bengalensis* sp. n. and *Eimeria fibrilosa* sp. n., New Coccidia (*Protozoa : Eucoccida*) from a Fresh-water Snake, *Enhydris enhydris* (Schneider) — C. Kalavati: Four New Species of Microsporidians from Termites — A. Czapik et A. Jordan: Les ciliés psammophiles de la mer Baltique aux environs de Gdańsk — Г. А. Штейн: Паразитические инфузории (*Peritricha, Urceolariidae*) рыб Белого моря — B. L. Bergquist and E. C. Bovee: Cadmium: Quantitative Methodology and Study of its Effect upon the Locomotor Rate of *Tetrahymena pyriformis* — M. Opas: Course of Glycerination of *Amoeba proteus* and Contraction of Glycerinated Models — S. Dryl and K. Mehr: Physiological and Toxic Effects of Detergents on *Paramecium caudatum* — В. Д. Каллиникова: Иммунологическая дискинетопластия у *Crithidia oncopelti* — J. Lipa and K. Borusiewicz: 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

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