PL ISSN 0065 - 1583

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

VOLUME 23

Number 2

WARSZAWA 1984

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

International Journal of Protozoology

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ACTA PROTOZOOLOGICA appears quarterly. The indexes of previous volume will appear in No. 1 of the next volume.

Indexed in Current Contents and in Protozoological Abstracts.

ACTA PROTOZOOLOGICA
Vol. 23, No. 2, pp. 77–83 (1984)

Morphologie et infraciliature de *Kahlilembus fusiformis* (Kahl, 1926) gen. nov., scuticocilie dé sol

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Received on 10 October 1983

Synopsis. La morphologie et l'infraciliature de *Kahlilembus fusiformis* nov. gen. sont décrites. Le genre est caractérisé par les structures buccales comprenant une M1 allongée, triangulaire, une M2 plus massive et une M3 subdivisée en 2 parties. Le pore de la vacuole pulsatile est situé entre les cinéties 1 et n. La stomatogenèse est de type *Uronema* — *Parauronema*. La M1 ciliée et le scuticus non cilié permettent de classer le genre dans les *Philasteridae*.

Les Ciliés du sol sont actuellement l'objet de nombreux travaux systématiques et écologiques effectués, en particulier, par Buitkamp et Wilbert (1974), Bick et Buitkamp (1976), Buitkamp (1977–1979), Foissner (1980–1981–1982), Foissner et al. (1982), Coûteaux (1983). L'étude des Protozoaires d'un sol provenant de la Forêt de Fontainebleau nous a permis de trouver l'espèce *Cohnilembus fusiformis* décrite par Kahl (1926) récemment revue par Foissner (1981) et dont l'étude nous montre qu'elle ne peut appartenir à ce genre.

Matériel et méthodes

Les Ciliés proviennent du sol de la Station Biologique de Foljuif située dans la forêt de Fontainebleau, sur la commune de St-Pierre-les-Nemours, en bordure du bois de la Commanderie. Le sol y est un néopodzol à tendance moder, avec micropodzolisation locale. L'humus dans lequel les Infusoires ont été récoltés a une épaisseur de 3 à 4 cm et a un pH de 4,14.

La végétation dominante est:

— strate arborescente: *Quercus sessiliflora*

— strate arbustive: *Corpinus betulus*

— strate sous-arbustive: *Lonicera periclymenum, Rubus sp.*
— strate herbacée: *Deschampsia flexuosa, Poa nemoralis, Holcus mollis*
— strate muscinale: *Hypnum sp., Leucobryum glaucum, Polytrichum sp.*

Afin de favoriser le développement des Infusoires de l'humus étudié et d'obtenir un maximum de matériel, les échantillons ont été conditionnés en microcosmes, selon la technique de Coûteaux (1983), dans des seringues aménagées et incubées pendant 4 mois. L'eau interstitielle est extraite en comprimant l'humus à l'aide du piston de la seringue.

Dans cette eau interstitielle le Scuticocilié cohabitait avec *Spathidium muscicola, Drepanomonas revoluta, Cyrtolophosis muscicola, Platyophrya vorax, Cyclidium glaucoma, Blepharisma hyalinum* et *Uroleptus kahli.*

La morphologie générale et les stades de stomatogénèse ont été étudiés à l'aide d'imprégnations argentiques au protéinate d'argent, selon la technique de Bodian modifiée par Grolière (1980). Toutes les mensurations ont été effectuées sur des animaux fixés et après application de cette technique. Les différentes dimensions indiquées ont été obtenues sur 30 individus.

Observations

Morphologie générale

Aplatie latéralement, la cellule est de forme très allongée, ses deux pôles, effilés, arrondis à leur extrémité présentant chacun une légère courbure, vers la gauche pour la région antérieure et la droite pour la postérieure. Ces courbures paraissent plus ou moins accentuées sur le vivant, lors de la locomotion de l'Infusoire. Dans le milieu, cette dernière est fort lente et la cellule, presque incolore, est souvent difficile à déceler. Le cytoplasme renferme des vacuoles digestives contenant les bactéries dont le Cilié se nourrit.

La plus grande largeur du corps (6–10 µm); les dimensions moyennes, les plus couramment trouvées, sont 7,5 × 48 µm. Les cils somatiques, de taille importante, sont assez espacés sur les cinéties. Celles-là, au nombre de 8, débutent antérieurement par une paire cinétosomienne et délimitent, à ce pôle, une très petite calotte glabre. Les cinéties sont ensuite constituées sur le reste de leur trajet par des cinétosomes toujours isolés. Les cinéties 1 et 8 s'écartent ventralement au niveau de la plus grande largeur de la cellule (Fig. 1). Quatre ou cinq cils caudaux, plus longs que les autres cils somatiques occupent le pôle postérieur de la cellule.

Le cytoprocte s'ouvre ventralement, dans la partie moyenne du Cilié, en arrière des structures buccales, entre les cinéties 1 et 8, il est marqué sur les imprégnations argentiques par une ligne argentophile. La vacuole pulsatile, facilement observable sur le vivant, située au niveau du 2ème tiers de la cellule s'ouvre à l'extérieur par un pore excréteur unique situé entre les cinéties 1 et 8, en arrière du cytoprocte (Fig. 2).

— strate sous-arbustive: *Lonicera periclymenum, Rubus sp.*
— strate herbacée: *Deschampsia flexuosa, Poa nemoralis, Holcus mollis*
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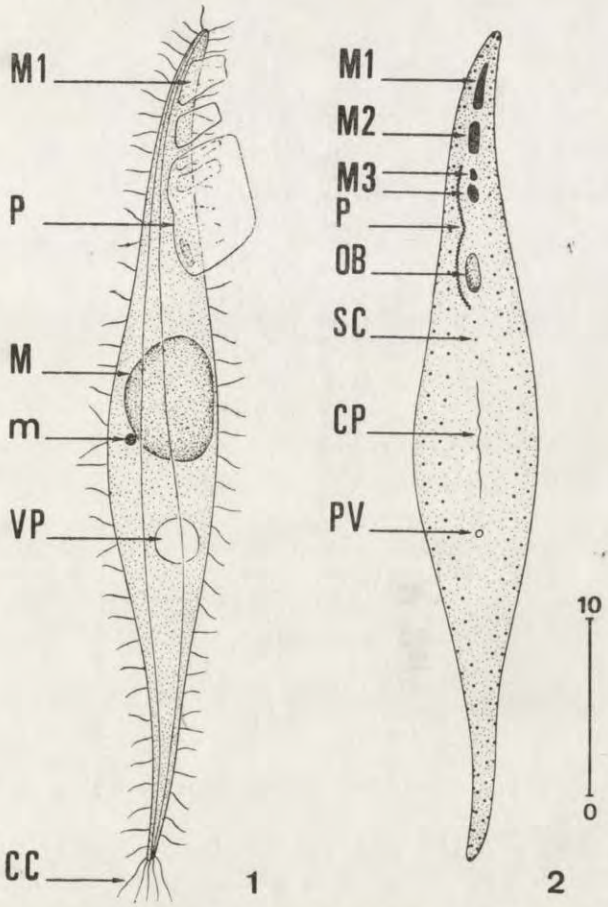

Fig. 1–2. *Kahlilembus fusiformis*. 1 — Aspect général sur le vivant avec indication du 1er organelle adoral (M1), de la parorale (P), du macronoyau (M), du micronoyau (m), de la vacuole contractile (VP), des cils caudaux (CC). 2—Aspect général après imprégnation au protéinate d'argent avec indication des 3 organelles adoraux (M1, M2, M3), de la parorale (P), de l'ouverture buccale (OB), du scuticus (SC), du cytoprocte (CP) et du pore de la vacuole contractile

L'appareil nucléaire comprend un macronucleus ovoïde, médian de 6 à 9 µm de diamètre et un petit micronoyau (0,5–0,7 µm) disposé à proximité du macronoyau.

Organisation buccale

La cavité buccale, antérieure, débute à 1,3–1,7 µm de l'apex de la cellule. Elle occupe un territoire étroit, allongé, de 14 µm (12,2–16,3 µm) soit approximativement le tiers de la longueur de l'Infusoire. Trois ensembles infraciliaires équidistants (M1, M2, M3), dont l'un, M3, est subdivisé en 2 parties, sont disposés en file méridienne. Une quatrième structure, la parorale est située à leur droite (Fig. 2–3).

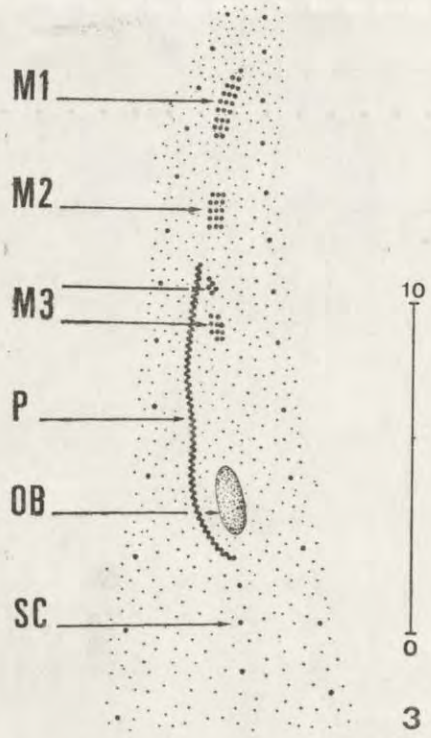

Fig. 3. Détail de l'infraciliature buccale

M1, de forme allongée, épouse vers la gauche, la courbure de la partie proximale de la cellule, elle mesure 3 µm de long (2,7–3,5 µm). Son infraciliature peut varier dans le détail. Les cinétosomes se répartissent selon 8 ou 9 niveaux; le premier est toujours constitué par un élément isolé; lui font suite une ou deux paires cinétosomiennes puis 5 ou 6 rangées de 3 cinétosomes. Cette base infraciliaire se termine toujours par une paire cinétosomienne. M1 apparaît toujours ciliée.

La base infraciliaire de M2 est plus massive que celle de M1, sa longueur maximum étant 2 µm. Un 1er niveau de 2 ou 3 cinétosomes puis 4 ou 5 niveaux de 3 éléments chacun la constituent (Fig. 3). M3 apparaît formée de 2 parties distinctes se succédant; d'abord, un groupe de 1 puis 2 fois 2 cinétosomes, disposé obliquement par rapport à l'axe longitudinal de la cellule donne un aspect triangulaire à cette structure; après un espacement de 0,6 µm se trouvent des cinétosomes disposés sur 4 niveaux; leur agencement et leur nombre varient; le plus souvent, le 1er comprend 2 éléments, les 2 suivants 3 et le dernier 2, les cinétosomes les plus à droite se disposant selon une ligne courbe. L'appartenance de la 1ère base infraciliaire à M3 plutôt qu'à M2 est indiquée par des stades de stomatogénèse.

La parorale (6 à 8 µm de longueur) a son extrémité antérieure immédiatement au dessus de la partie proximale de M3, c'est-à-dire en arrière de l'intervalle séparant M2 et M3; elle n'atteint jamais la 2ème membranelle adorale. Des cinétosomes disposés en zig-zag la constituent sur l'ensemble de son trajet. Sa courbure postérieure n'entoure pas complètement le cytostome qui apparaît comme une fente ovalaire. La parorale se continue postérieurement par généralement 3, plus rarement 4 éléments, isolés, assez espacés et alignés entre les cinéties 1 et 8, en avant du cytoprocte (Fig. 3). Ces cinétosomes constituent le scuticus.

Des stades de stomatogénèse nous ont montré que la division de ce Cilié est caractéristique des *Scuticociliatida* et plus particulièrement des *Philasterina*, les structures buccales tirant leur origine de champs cinétosomiens développés les uns (M1, M2) à partir de la parorale, les autres (M3) à partir du scuticus. Cette stomatogénèse correspond ainsi au 3ème type défini chez les *Philasterina* (Grolière 1974) pour *Uronema* et *Parauronema* et retrouvé chez *Philasterides* (Grolière 1980).

Discussion

Par la forme générale, les dimensions, la disposition de la vacuole pulsatile, la présence d'une touffe de cils caudaux et malgré une importance relative supérieure du péristome, il paraît possible d'assimiler le Cilié du sol que nous venons de décrire à *Lembus fusiformis*, décrit par Kahl en 1926, cet auteur ayant par la suite donné le nom de *Cohnilembus* au genre *Lembus*. Notre Infusoire paraît également proche, en particulier pour les dimensions du péristome, de *C. anguilla* trouvé par le même auteur, mais *C. anguilla* est marin alors que *C. fusiformis* a été signalé dans des mousses et vu dans des sols par différents auteurs, dont Stout (1960), Chardez (1967) et Foissner (1982).

Les structures buccales du genre *Cohnilembus* ont été décrites pour l'espèce *C. verminus* par Borror (1963), Evans et Thompson (1964), Thompson (1968) puis Didier et Detcheva (1974). Elles sont caractérisées par une M1 très allongée, constituée d'une rangée unique de cinétosomes alignés dans le sens longitudinal de la cellule, une M2 formée de 5 groupes individualisés de cinétosomes et une M3 représentée par "un ensemble argentophile quadrangulaire" selon Didier et Detcheva (1974). Le pore de la vacuole pulsatile s'ouvre ventralement entre les cinéties 1 et 2, dans la partie postérieure de la cellule. La stomatogénèse de *C. verminus* décrite par Small (1967) puis par Didier et Detcheva (1974) est également caractéristique, la parorale de la cellule-mère est à l'origine de la parorale et du scuticus de l'opisthe alors que M1, M2 et M3 proviennent de champs issus du

scuticus. Cette stomatogénèse appartient au 1er type défini par Groliere (1974) chez les *Philasterina*. Le Cilié que nous venons de décrire, par ses structures buccales, en particulier par sa M1 beaucoup plus massive, de forme triangulaire ne peut appartenir au même genre que *C. verminus*. En effet, chez le *Scuticociliatida* l'organisation et la disposition des 3 organelles adoraux, en particulier de M1 sont actuellement retenues pour différencier les genres. Le mode de stomatogénèse également différent dans les 2 cas peut être un argument de plus à l'idée que ces Ciliés ne peuvent être mis sous le même nom générique. Dans le sous-ordre des *Philasterina* auquel appartient notre Cilié tant par son mode de stomatogénèse que par ses structures buccales, il existe de nombreux genres ayant une M1 triangulaire à pointe antérieure tels *Parauronema, Miamensis, Potomacus, Anophryoides, Paralembus, Philasterides*, mais aucun n'a de M3 ou de M2 divisées transversalement en 2 parties. Pour cet ensemble de raisons, nous proposons de créer un genre nouveau *Kahlilembus* pour l'espèce *K. fusiformis*. La présence d'une M1 ciliée, d'un scuticus non cilié et le mode de stomatogénèse indiquent que ce genre peut être inclus dans la famille des *Philasteridae*.

Diagnose du genre *Kahlilembus* nov. gen.

Cilié de petite taille à corps fusiforme et cavité buccale antérieure comprenant une M1 allongée, triangulaire, une M2 plus massive et une M3 subdivisée en 2 parties et dont le pore de la vacuole pulsatile est situé entre les cinéties 1 et n. Ce genre fait partie de la famille des *Philasteridae*.

BIBLIOGRAPHIE

Bick H. et Buitkamp U. 1976: Ciliated *Protozoa* from canadien grassland soils. Trans. Am. Microsc. Soc., 95, 490–491.

Borror A. C. 1963: Morphology and ecology of some uncommon Ciliates from Alligator Harbor, Florida. Trans. Am, Microsc. Soc., 82, 125–131.

Buitkamp U. 1977: Die Ciliatenfauna der Savanne von Lamto (Elfenbeinküste). Acta Protozool., 16, 249–276.

Buitkamp U. 1977: Über die Ciliatenfauna szeier mitteleuropaischer Bodenstandorte (*Protozoa, Ciliata*). Decheniana (Bonn), 130, 114–126.

Buitkamp U. 1979: Vergleichende Untersuchungen zur Temperaturadaptation von Bodenciliaten aus klimatisch verschiedenen Regionen. Pedobiologia, 19, 221–236.

Buitkamp U. et Wilbert N. 1974: Morphologie und Taxonomie einiger Ciliaten eines kanadischen Prärieboden. Acta Protozool., 13, 201–210.

Chardez D. 1967: Infusoires Ciliés terricoles (*Protozoa, Infusoria, Ciliata*). Rev. Ecol. Biol. Sol., 4, 289–298.

Coûteaux M. M. 1983: Utilisation des microcosmes pour l'analyse des fonctions écologiques des Protozoaires de l'humus. Oecologia Generalis (sous presse).

Didier P. et Detcheva R. 1974: Observations sur le Cilié *Cohnilembus verminus* (O. F. Müller 1786). Morphogenèse de bipartition et ultrastructure. Protistologica, 10, 159–174.

Evans F. R. et Thompson J. C., Jr. 1964: *Pseudocohnilembidae* n. fam., a Hymenostome Ciliate family containing one genus, *Pseudocohnilembus* n. g., with three new species. J. Protozool., 11, 344–352.

Foissner W. 1980: Colpodide Ciliaten (*Protozoa*: *Ciliophora*) aus alpinen Böden. Zool. Jb. Syst., 107, 391–432.

Foissner W. 1981: Morphology and taxonomy of some new and little know kinetofragminophorous Ciliates (*Protozoa*: *Ciliophora*) from alpine soils. Zool. Jahrb. Abt. Syst. Okol. Geogr. Tiere, 108, 264–297.

Foissner W. 1981: Morphologie und Taxonomie einiger neuer und wenig bekannter kinetofragminophorer Ciliaten (*Protozoa*: *Ciliophora*) aus alpinen Böden. Zool. Jb. Syst., 108, 264–297.

Foissner W. 1982: Ökologie une Taxonomie der *Hypotrichida* (*Protozoa*: *Ciliophora*) einiger österreichischer Böden. Arch. Protistenk., 126, 19–143.

Foissner W., Adam H. et Foissner I. 1982: Morphologie, Infraciliatur und Silberlinienensystem einiger wenig bekannter *Scuticociliatida*. Zool. Jb. Syst., 109, 443–468.

Foissner W., Franz H. et Adam H. 1982: Terrestrische Protozoen als Bioindikatoren im Boden einer planierten Ski-Piste. Pedobiologie, 24, 45–56.

Grolière C. A. 1974: Etude comparée de la stomatogénèse chez quelques Ciliés Hyménostomes des genres *Paralembus* Kahl, 1933, *Philaster* Fabre-Domergue, 1885, *Parauronema* Thompson, 1967, *Tetrahymena* Furgasson, 1940. Protistologica, 10, 319–331.

Grolière C. A. 1980: Morphologie et stomatogénèse chez deux Ciliés *Scuticociliatida* des genres *Philasterides* Kahl, 1926 et *Cyclidium* O. F. Müller,, 1786. Acta Protozool., 19, 195–206.

Kahl A. 1926: Neue und wenig bekannte Formen der holotrichen und heterotrichen Ciliaten. Arch. Protistenk., 55, 197–438.

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

Stout J. D. 1960: Biological studies of some Tussock-grassland soils. XVIII. *Protozoa* of two cultived soils. N.Z.J. argric. Res., 3, 237–244.

Thompson J. C. Jr. 1968: A description of *Cohnilembus verminus* from Eniwetok Atoll. J. Protozool., 15, 396–399.

ACTA PROTOZOOLOGICA
Vol. 23, No. 2, pp. 85–92 (1984)

Photophobic Responses in *Euglenina*: 2. Sensitivity to Light of the Colorless Flagellate *Astasia longa* in Low and High Viscosity Medium

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Received on 29 November 1983

Synopsis. The photobehaviour of *Astasia longa* was examined in low and high viscosity medium. Stimulated by white light *Astasia longa* exhibits the step-up photophobic response when rinsed with a solution different from the culture medium.

In low viscosity medium the stimulated cells exhibit the turn response. In high viscosity medium, body contractions accompany the photophobic response (maximal reorientation) of the flagellum.

Astasia longa is considered to be an apochlorotic mutant of *Euglena gracilis* (Zumstein 1900, Pringsheim 1942, 1948, Pringsheim and Hovasse 1948). It lacks not only chloroplasts and stigma but also a paraflagellar body (PFB). Till the fifties of this century it was generally accepted that the lack of these organelles is the only feature making a difference between these two forms.

The late fifties to seventies brought further biochemical, cytochemical and morphological studies of *Astasia longa*. They re-vealed many other differences between *A. longa* and bleached *Euglena gracilis*. For example, there is a difference in the appearance of the pellicle if viewed with ultraviolet light. *Astasia pellicle* has an uneven pellicle outline, while colorless *Euglena* has a very smooth one (Roger and Kimzey 1972). According to Blum and coworkers (1965), under the same

conditions of growth, these two forms differ in: doubling time (in *Astasia* 11 h, *Euglena* 22 h), sensitivity to growth inhibitors (*Astasia* is more sensitive), ability to incorporate exogenous amino acids (*Astasia* does not have the ability to incorporate several amino acids, while *Euglena* does), synthesis of an induced acid phosphatase, and the level of constitutive acid phosphatase activity (the level in *Euglena* is more than twice the level of *Astasia*).

According to previous data, *Astasia longa* exhibits neither negative nor positive phototaxis (Mainx 1927, Pringsheim and Hovasse 1948). Gössel (1957), examining the behaviour of *A. longa*, did not observe any response to the light.

For many years, it has been known that *Amoebae* (Mast 1911, Chatterjee and Bhattacharjee (1975), and the colorless flagellate *Peranema trichophorum* (Mast and Hawk 1936, Shettles 1937) are sensitive to light. Moreover Iwatsuki and Naitoh (1982) demonstrated that *Paramecium caudatum* and *P. tetraurelia* also respond to the light. In the light of these data, it seems to be necessary to reexamine *Astasia longa* in respect to its photosensitivity. Results shown in this paper, approved the supposition of sensitivity of *A. longa* to an increase of light intensity. The step-up photophobic response (for terminology on this phobic response see Diehn et al. 1977) occurs in cells rinsed with a solution different from the culture medium. The phenomenon of increased sensitivity of cells to the light stimulation as a consequence of changes in the chemical composition of external medium, was also recently observed in *Euglena gracilis* (Mikołajczyk and Pado 1981, Mikołajczyk 1983).

The light stimulation of *Euglena gracilis* causes not only flagellar reorientation, but, in some circumstances, also evokes cell contraction. Generally speaking in *Euglena* the "minimal" reorientation of the flagellum (to a position perpendicular to the body) is sufficient for the cell to change the direction of swimming. Cell contraction occurs when the flagellum is "maximally" reoriented (Pl. I), in other words it has position in front of the cell (Mikołajczyk and Diehn 1976, Mikołajczyk and Kuźnicki 1981). Maximal photophobic flagellar reorientation followed by body contraction, commonly occurs in *Euglena gracilis* in high viscosity medium (Mikołajczyk and Diehn 1976). Whereas in the medium of low viscosity, the high intensity white light causes "minimal" flagellar reorientation and the turn response. In the presence of a mechanical factor (methylcellulose), stimulated by white light *Astasia longa* exhibits a step-up response which is accompanied by body contraction.

Material and Methods

Astasia longa strain 1204/17D Pringsheim arised from *Euglena gracilis* was grown in Bloomington medium at pH 6.9 (Starr 1964) at 24–25°C in darkness. Prior to experimentation, 7–10 day-old cultures were gently centrifuged, rinsed twice with the solution containing 0.125 mM $MgCl_2$, 0.5 mM $CaCl_2$ and 1 mM KCl (Doughty and Diehn 1979, 1982) and buffered, to pH 8.0, with 2.5 mM Hepes-Pipes and NaOH (the pH of 7–10 day-old culture medium is 8.2) and resuspended in the rinsing medium. Control cells were centrifuged and rinsed with culture medium supernatant.

The photophobic response was examined microscopically under a NU microscope (Carl Zeiss Jena) one minute after cells were placed on a microscope slide and covered with a cover slip. Such constant delay was necessary because rinsed cells stimulated directly after preparation frequently showed a sensitivity to light different from those stimulated 30 s to 1 min later. After 1 min cells were usually more sensitive than those examined just after preparation. The pattern of swimming generally changed to a screw-like path with a large diameter and the direction of movement changed rather frequently.

The observations of cell photobehaviour were carried out on the maximum of five specimens at a time. At least 150 individuals were observed at each light intensity. The cells were stimulated only once with an ordinary white light and then discarded. The turn response in 100% of the examined cells was taken as "standard" response. The minimum light intensity necessary to elicit the standard photophobic response was used to measure cell sensitivity and to establish the threshold of this response.

The viscosity of the medium was increased by methylcellulose dissolved in the same buffer as for washing. The cells were mixed 1 : 1 with a methylcellulose solution directly after washing. The final viscosity of the cell medium was about 3000 cP. Such viscosity stops completely the forward swimming of immersed cells. Observations were carried out 1, 3 and 24 h after mixing.

Observations of behaviour of the cells in a high viscosity medium were carried out on only one cell at a time, and the cell was stimulated with white light only once. Ten individuals were tested in each experiment for a given light intensity (leight experiments were carried out). As a response to light stimulus we assumed body contraction which appeared within 15 s from the moment of light stimulation (measured with the stop watch).

Light intensity was measured with BPW 20 photodiode (Siemens). An HBO 101 W halogen lamp mounted to the microscope was used as the light source to induce the photophobic response. Light stimuli were applied through a condensor controlled by a photographic shutter. A red (690 nm) light at very low intensity was applied through the objective as a background illumination. For further details of the optical system see Mikołajczyk (1984).

The whole cells fixed with mercuric chloride and impregnated with protargol (according to procedure modified by Golińska 1984) were examined with the bright light (Leitz) microscope.

For electron microscopy, cells were fixed in a mixture of glutaraldehyde (2.5%), acrolein (0.12%) and osmium tetroxide (1%) buffered with 0.2 M cacodylate buffer at pH 7.4 for 1 h on an ice bath, then washed in cacodylate buffer, dehydrated in an ethanol series, and embedded in Epon 812 using propylene oxide to facilitate infiltration. The microsections were stained with uranyl acetate and lead citrate. A JEM 100 B electron microscope operating at accelerating voltage of 80 kV was used to examine the thin sections.

Results

Whole cells of *Astasia longa* fixed with mercurie chloride (Pl. I 1–3), and thin sections show no stigma or PFB (Pl. I 4, 5)

Photobehaviour of Cells in Low Viscosity Medium

Suspended in the culture medium, or rinsed with culture supernatant *Astasia longa* does not respond to a sudden increase of white light intensity up to 1.34×10^4 W/m^2 (the maximum light intensity available). However, it was observed that *Astasia longa* becomes sensitive to white light stimulation when the chemical composition of the external medium is changed from culture medium to buffer with Ca-Mg-K ions. An increased sensitivity to light appears within one hour after rinsing with a buffer, but the level of the light intensity threshold is stabilized after 3 h. After that stimulation with white light between 1.54×10^2 and 1.34×10^4 W/m^2 causes a step-up response in 100 per cent of the observed individuals. Body contractions do not accompany this reaction. The turn response may last as long as the light stimulation (3 s) or may be limited to a single change of swimming direction by 90 or 360°.

Below 1.54×10^2 W/m^2 the percentage of cells showing the step-up response diminishes. At 1.11×10^2 W/m^2 50 per cent and at 0.30×10^2 W/m^2 only about 10 per cent of the cells respond to the light.

The increased sensitivity of *Astasia longa* to white light may persist over 24 h at the same level as noted 3 h after rinsing. Mostly, however, the light intensity threshold rises considerably so that after 24 h the standard step-up response does not appear below a light intensity of 4.00×10^3 W/m^2.

Photobehaviour of Cells in High Viscosity Medium

In high viscosity medium *Astasia longa* exhibits the step-up photophobic response expressed as a maximal flagellar reorientation which is followed by body contraction. The flagellar response and the body contractions appear within 1 h after cell washing and mixing with a methylcellulose solution. The level of sensitivity to the light stabilizes within the next two hours and does not undergo any significant changes during 24 h, while the level of sensitivity of free swimming cells is usually increased (see previous part).

The dependence of the percentage of cells exhibiting the step-up response on the intensity of illumination is well approximated by the curve shown in Fig. 1. It suggests that the step-up response depends

on the logarithm of light intensity. The stimulation of cells by light intensity of 1.30×10^3 W/m^2 causes body contraction in 95 per cent of the examined cells. The slight increase of the light intensity to 1.46×10^3 W/m^2 causes contractions in all stimulated cells (80 individuals). Of course, higher light intensities (from 1.46×10^3 to 1.34×10^4 W/m^2) also cause body contractions in 100 per cent stimulated cells (within 15 s.).

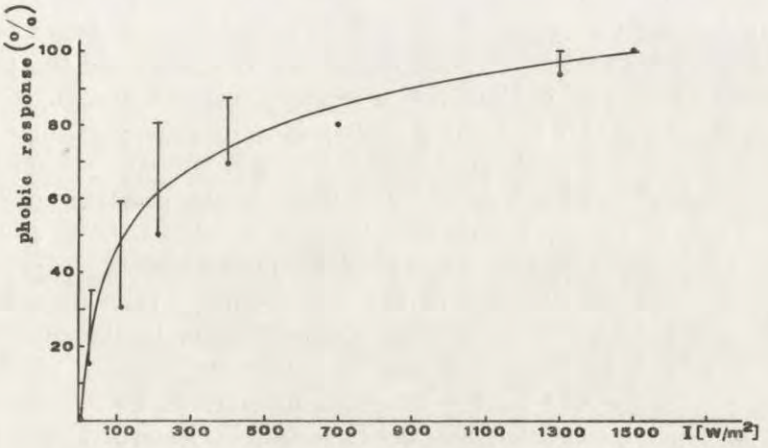


Fig. 1. Diagram of a step-up photophobic response, expressed as a body contraction, caused by a white light stimulation. *Abscissa*: percentage of responding cells, *ordinate*: light intensity in W/m^2

A decrease of light intensities results in a decrease number of cells contracting within 15 s. At stimulation with light intensity of 4.00×10^2 W/m^2 body contractions are observed in 70 per cent cells, of 2.14×10^2 W/m^2 in 50 per cent, of 1.11×10^2 W/m^2 in 30 per cent and of 0.30×10^2 W/m^2 in 15 per cent. So, the light intensity threshold evoking the body contractions in 100 per cent of the individuals is much higher than for the standard photophobic response of free swimming cells — 1.46×10^3 W/m^2 for body contractions, while 1.54×10^2 W/m^2 for free swimming cells.

It was observed that with a decrease of light intensity, the transduction time (time from stimulus application to beginning of the contraction) increases. A decrease of light intensity from 1.34×10^4 W/m^2 to 1.30×10^3 W/m^2, in the range in which the cell contractions are observed in 95–100 per cent stimulated cells, the transduction time is increased by about a factor of 2. At light intensity 1.34×10^4 W/m^2 contractions appear after 2.73 ± 0.5 s, while at 1.30×10^3 W/m^2 after 6.21 ± 3.3 s.

Discussion

According to light and electron microscope studies, *Astasia longa* does not possess a stigma and a PFB. Although it was reported earlier that *Astasia longa* lacks these organelles, but since it is sensitive to light and since some free living *Astasia*, for example *A. linealis* (Pringsheim 1937) and those parasitising in the *Copepoda Parastasia macrogranulata* (Wita 1984) have a well developed stigma, the absence of these organelles in *Astasia longa* had to be ascertained.

What are the consequences of the fact that *Astasia longa* is sensitive to a sudden increase of light intensity? This suggests the presence of a pigment sensitive to light and a sensory transduction chain. In the previous paper (Mikołajczyk 1984) it was suggested that appart of flavins located in PFB, an accessory pigment exists, which is probably responsible, at least partly, for the step-up reaction in *Euglena gracilis*. Thus, if we assume that *A. longa* is a relative of *E. gracilis*, it may be supposed that the same kind of photopigment is present in both organisms. The character of the photopigment in *A. longa* and of the accessory pigment in *E. gracilis*, as well as its localization in both organisms, remain to be established. The supposed location of this pigment in *Euglena* would be the flagellar membrane (Mikołajczyk 1984). The same localization can be expected in *Astasia longa*, but its presence in other parts of the cell body cannot be ruled out. Experiments with photobehaviour of the colorless flagellate *Peranema trichophorum* (Mast and Hawk 1936, Shettles 1937) showed that the entire body of this organism is sensitive to light, but the strongest sensitivity was found at the anterior end of the cell. Moreover, the separated flagella of this organism also respond to light stimulation (Shettles 1937). Therefore a definite answer to the question of localization of the photopigment in *Astasia* might be obtained by stimulation of various parts of the cell by light.

The influence of the change of the chemical composition of the cell external medium on photobehaviour (expression of the step-down photophobic response) of *Euglena gracilis*, was first observed and then studied extensively by Doughty and Diehn (1979, 1983). The experiments with the behaviour of *Astasia* to light stimulation show that changing of the chemical composition of the medium from culture to buffer containing K-Ca-Mg ions causes the increase of the cell sensitivity to light. The increased sensitivity to light stimulation was also observed in rinsed light- and dark-grown *Euglena gracilis* (Mikołajczyk and Pado 1981, Mikołajczyk 1984). Thus, the phenomenon of the decrease of the light intensity threshold for the step-up response, as a consequence of the change in the chemical composition of the external medium, appeared to be common for both *Euglena gra-*

cilis and *Astasia longa*. However, the ionic mechanism of this phenomenon is still unknown.

In cell evolution the photopigment in *Astasia longa* and an accessory photopigment in *Euglena gracilis* would be the primary one acting to protect cells against dangerous light irradiation (Mikołajczyk and Kuźnicki 1983). The appearance of PFB and stigma enable the cells to respond to changes of light intensities. *Euglena gracilis*, possessing these organelles, has the ability to respond to an increase as well as to a decrease of the light intensity thus providing these cells an opportunity to find the optimal life conditions.

The behaviour of *Astasia longa* in high viscosity medium is similar to that of *Euglena gracilis*. The stimulation with white light causes the maximal flagellar reorientation and body contraction. The light intensity necessary to evoke the body contractions in the high viscosity medium has to be much higher compared to that evoking the turning response in the low viscosity medium. Probably the reason for this is that the lower light intensities are sufficient to evoke the photophobic response in the low viscosity medium (by causing only a minimal flagellar reorientation) while they are not sufficient to force the flagellum against the high viscosity medium to get the position in front of the cell, necessary for body contraction.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Profs. Wilhelm Nultsch, Patricia Walne and Leszek Kuźnicki for critical reading of the manuscript and valuable suggestions. Thanks are also due to dr. George J. Morris for the generous gift of the *Astasia longa* cultures.

REFERENCES

Blum J. J., Sommer J. R. and Kahn V. 1965: Some biochemical, cytological and morphogenetic comparisons between *Astasia longa* and bleached *Euglena gracilis*. J. Protozool., 12, 202–209.

Chatterjee S. and Bhattacharjee S. K. 1975: Affect of near ultraviolet and visible light on amoeba. J. Cell Sci., 19, 117–126.

Diehn B., Feinleib M. E., Haupt W., Hildebrand E., Lenci F. and Nultsch W. 1977: Terminology of behavioral responses of motile microorganisms. Photochem. Photobiol., 26, 559–560.

Doughty M. J. and Diehn B. 1979: Photosensory transduction in the flagellated alga, *Euglena gracilis*. I. Action of divalent cations, Ca^{2+} antagonists and Ca^{2+} ionophore on motility and photobehavior. Biochim. Biophys. Acta, 588, 148–168.

Doughty M. J. and Diehn B. 1982: Photosensory transduction in the flagellated alga, *Euglena gracilis*. III. Induction of Ca^{2+}-dependent responses by monovalent cation ionophores. Biochim. Biophys. Acta, 682, 32–43.

Doughty M. J. and Diehn B. 1983: Photosensory transduction in the flagellated alga, *Euglena gracilis*. IV. Long term effects of ions and pH on the expression of step-down photobehaviour. Arch. Microbiol., 134, 204–207.

Golińska K. 1984: Diminution of microtubular organelles after experimental reduction of cell size in a ciliate *Dileptus*. J. Cell Sci., (in press).
Gössel I. 1957: Über das Aktionsspektrum der Phototaxis chlorophyllfreier Euglenen und über die Absorption des Augenflecks. Arch. Mikrobiol., 27, 288–305.
Iwatsuki K. and Naitoh Y. 1982: Photoresponses in colorless *Paramecium*. Experientia, 38, 1453–1454.
Mainx F. 1927: Beiträge zur Morphologie und Physiologie der Eugleninen. II. Untersuchungen über die Ernährungs und Reizphysiologie. Arch. Protistenkd., 60, 355–414.
Mast S. O. 1911: Light and the Behavior of Organisms. John Willey and Sons, London: Chapman and Hall, Limited, New York, pp. 410.
Mast S. O. and Hawk B. 1936: Response to light in *Peranema trichophorum*. I. Relation between dark-adaptation and sensitivity to light. Biol. Bull., 70, 408–412.
Mikołajczyk E. 1984: Photophobic responses in *Euglenina*: 1. Effects of excitation wavelength and external medium on the step-up response of light- and dark-grown *Euglena gracilis*. Acta Protozool., 23, 1–10.
Mikołajczyk E. and Diehn B. 1976: Light-induced body movement of *Euglena gracilis* coupled to flagellar photophobic responses by mechanical stimulation. J. Protozool., 23, 144–147.
Mikołajczyk E. and Kuźnicki L. 1981: Body contraction and ultrastructure of *Euglena*. Acta Protozool., 20, 1–24.
Mikołajczyk E. and Kuźnicki L. 1983: Speculation on the origin of two photoreception systems in *Euglena*. Postępy Biol. Kom. (in press).
Mikołajczyk E. and Pado R. 1981: The kinetics of photobehavior modification upon change of the suspention medium of *Euglena gracilis*. Progress in Protozoology Abstr. Sixth Int. Congr. Protozool., Warszawa, Poland, July 5–11, 1981, 252.
Pringsheim E. G. 1937: Über das Stigma bei farblosen Flagellaten. Cytologia, vol. 8, 234—255.
Pringsheim E. G. 1942: Contribution to our knowledge of saprophytic algae and flagellata. III. *Astasia, Distigma, Menoidium* and *Rhabdomonas*. New Phytol., 41, 171–205.
Pringsheim E. G. 1948: Taxonomic problem in the *Euglenineae*. Biol. Rev., 23, 46–61.
Pringsheim E. G. and Hovasse R. 1948: The loss of chromatophores in *Euglena gracilis*. New Phytol., 47, 52–87.
Roger T. D. and Kimzey S. L. 1972: Rapid scanning microspectrophotometry of colorless *Euglena gracilis* and *Astasia longa*. A basis for differentiation. J. Protozool., 19, 150–155.
Shettles L. B. 1937: Response to light in *Peranema trichophorum* with special reference to dark-adaptation and light-adaptation J. Exp. Zool., 77, 215–249.
Starr R. C. 1964: The culture collection of algae at Indiana University. Am. J. Bot., 51, 1013–1044.
Wita I. 1984: A new flagellate species *Parastasia macrogranulata* sp. n. (*Euglenida*) parasitizing *Copepoda* in the environs of Leningrad. Acta Parasitol., 29, (in press).
Zumstein H. 1900: Zur Morphologie und Physiologie der *Euglena gracilis* Klebs. Jb. wiss. Bot., 34, 149–198.

EXPLANATION OF PLATE I

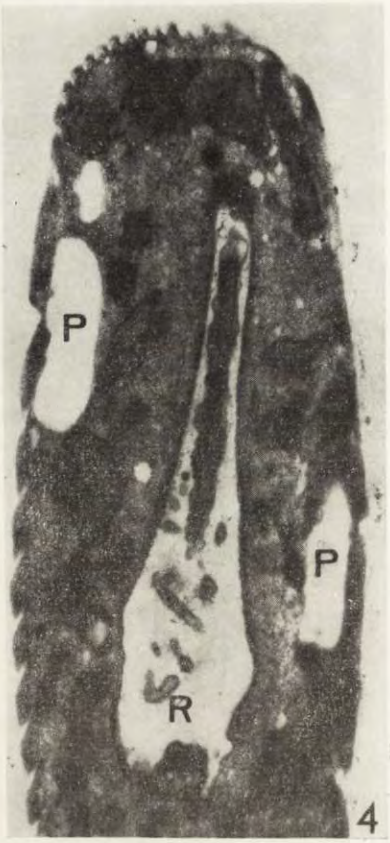
1–3: *Astasia longa* fixed with mercuric chloride and impregnated with protargol. Bright-field optics. LM. × 1000

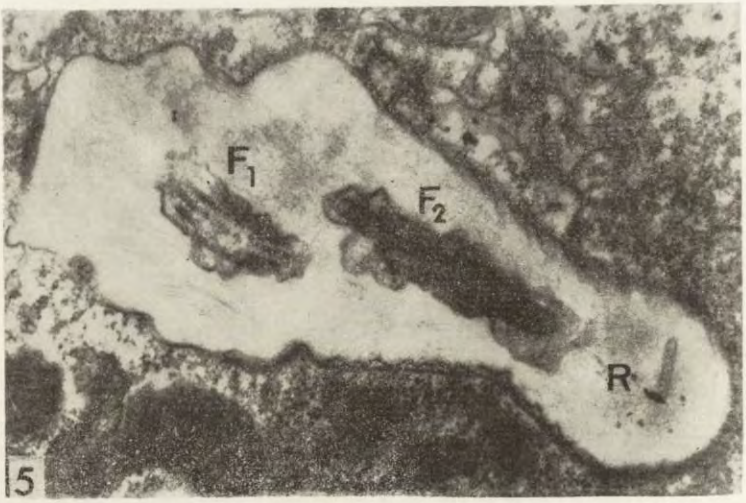
1: Emergent flagellum (F_1) is in trailing position
2: Two flagella (F_1 and F_2) well visible
3: Emergent flagellum is maximally reoriented

4,5: Electronmicrographs of the reservoir and flagella

4: magnification 12 000 ×
5: magnification 19 000 ×

Abbreviations: F_1, F_2 — flagella; P — paramylon; R — reservoir





E. Mikołajczyk auctores phot.

ACTA PROTOZOOLOGICA
Vol. 23, No. 2, pp. 93–105 (1984)

Possible Role of Cilia in the Control of Cytoplasmic Streaming in *Paramecium tetraurelia*

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Received on 12 October 1983

Synopsis. Paramecium tetraurelia cells immobilized by 0.25 mM nickel chloride lost differentially cilia from their body surface. Similarly 5% ethanol caused partial or complete surface deciliation. Deciliation proceeded from the anterior end of the body surface towards the posterior. Cytostomal cilia are more resistant and remain unaffected. Shortly after deciliation the plasma membrane heals over the remaining kinetosomes. We found that with the decrease in area of the ciliated surface the velocity of cytoplasmic streaming decreased. However, cytoplasmic streaming is maintained even in paramecia with no cilia on their body surface provided that the cytostomal cilia are beating. When the cytostomal cilia stop beating cytoplasmic streaming ceases as well and there are present only some discordant movements of the small crystals which are used as markers in the microscopic observation of the movement of the cytoplasm. It is suggested that food particles when driven into by the beating of the cytostomal cilia stimulate the mechano-receptors located on the ventral and cytostomal surface of *Paramecium* cells. Consequently the changes in the velocity of cytoplasmic streaming are the responses of the cytoplasm to the mechanical stimulation.

The use of nickel ions to immobilize paramecia by stopping their ciliary activity was described originally by Gelei (1935) and introduced to the investigation of cytoplasmic streaming by Sikora and Wasik (1978). Sikora (1981) has reviewed further studies on the use of nickel ions to immobilize paramecia and to study their cytoplasmic streaming. Sikora and Wasik (1978) showed that samples of paramecia treated with increasing concentrations of nickel chloride exhibited decreasing proportions of animals with perceptible cytoplasmic streaming. Simultaneously with increasing concentrations of nickel ions

the numbers of immobilized animals increased. However, in these experiments there was also an increase in the numbers of animals showing traumatic effects which led to irreversible changes and death. For this reason it was thought that the decrease in the velocity of cytoplasmic streaming caused by the nickel chloride treatment was due to a general toxic injury to the cells without taking into account the possibility that deciliation as such may have a more direct influence on cytoplasmic streaming.

The present communication reports new experimental observations indicating that there is a good correlation between the degree of deciliation in *Paramecium* cells induced by means of 0.25 mM nickel chloride or 5% ethanol and the velocity of cytoplasmic streaming.

Material and Methods

The investigations were carried out using *Paramecium tetraurelia* cells, stock 51 d4-90, cultured at 27°C in an aqueous infusion of Scottish grass inoculated with *Klebsiella* (*Aerobacter*) *aerogenes* (Sonneborn 1970). For experiments which were carried out at room temperature (20 ± 2°C) paramecia were filtered throught absorbent cotton wool, concentrated by centrifugation at 350 × g for up to 1 min and rinsed with the maintenance solution (M.S.) containing 5 mM Tris-HCl buffer at pH 7.3 and 1 mM of $CaCl_2$, 1 mM $MgCl_2$ and 1 mM KCl. This maintenance solution was also used as a solvent for nickel chloride (B.D.H.), ethanol (James Burrought), dibucaine hydrochloride (Sigma) and chloral hydrate (B.D.H.). Before use in experiments the paramecia were preincubated in M.S. for 2–24 h at room temperature.

In the first part of these investigations, while looking for the most suitable methods for partial or complete deciliation, the following were used: (a) treatment with 5% ethanol according to the modified method of Ogura (1977) based on shaking the suspension of animals for 3–4 min after introducing a concentrated sample of cells directly to excess 5% ethanol in M.S. (b) the same treatment as (a) but leaving the animals for 3–4 min without shaking. (c) treatment with 5% ethanol dissolved in M.S. according to the modified method of Sonneborn (1970) where an equal volume of 10% ethanol dissolved in M.S. is injected into the suspension of paramecia in M.S. In this way the final concentration of ethanol is 5%. Prepared samples were kept 1–16 min without shaking. (d) treatment with 0.25 mM of nickel chloride dissolved in M.S. according to Sikora and Wasik (1978) for 1–16 min. (e) the same treatment as (d), but shaken for 3–4 or 7 min. (f) treatment with dibucaine hydrochloride according to the method of Thompson et al. (1974). In this case as a maintenance solution the phosphate buffer (pH 7.2) according to Dryl modified by Grębecki and Kuźnicki (1963) was used. (g) treatment with chloral hydrate according to method of Kuźnicki (1963a). (h) osmotic shock according to the method of Rosenbaum and Carlson (1969).

The last three methods were discontinued as they were unsatisfactory (see Results).

For control purposes paramecia were kept in M.S. and shaken for the same time as the experimental samples.

In the preliminary investigations to examine whether deciliation was partial or complete, the paramecia after treatment were gently centrifuged (approximately at 100 × g for 30 s) and then fixed with 3% glutaraldehyde in 0.05 M cacodylate buffer pH 7.2 and examined by phase contrast microscopy. Later, for *in vivo* observation of the degree of deciliation and simultaneous observations of cytoplasmic streaming, only methods a, c and d were used.

The durations of treatment using the last two methods were adjusted so as to obtain a mixture of approximately equal proportions of partially and completely deciliated cells. In all cases after treatment paramecia were rinsed with M.S.

During the first 30 min after rinsing, the animals were examined under the phase contrast microscope and the velocity of cytoplasmic streaming was estimated by measuring the time for the movement of small crystals (1–3 μm in diameter) suspended in the cytoplasm (Kuźnicki and Sikora 1973) to travel between two points marked by scale divisions of an eye-piece graticule. Similarly the velocity of movement of cytoplasmic streaming in control paramecia thigmotactically settled was measured.

To evaluate the degree of deciliation and to make observations on the cytostomal cilia, samples of paramecia treated with 5% ethanol, 0.25 mM nickel chloride and untreated control cells were fixed with 1% osmium tetroxide in 0.05 M cacodylate buffer (pH 7.2), dehydrated and embedded in Araldite (TAAB).

Ultrathin sections stained with 1% potassium permanganate and 2.5% uranyl acetate were examined using an AEI EM6B electron microscope. The same material was used for preparing 0.5 μm thick sections stained with toluidine blue, dried, covered with coverslips using Canada balsam and examined under a phase contrast microscope.

Results

Out of all methods of deciliation tested only two methods using 5% ethanol (Ogura 1977, Sonneborn 1970) and one with nickel chloride (Sikora and Wasik 1978) appeared to be suitable for obtaining partially or completely deciliated paramecia (Table 1). Dibucaine hydrochloride, chloral hydrate and osmotic shock did cause deciliation of paramecia but this was immediately followed by death of cells which rendered impossible the *in vivo* observations of the motility of the cytoplasm.

The progress of deciliation in *Paramecium tetraurelia* induced by the factors tested followed the scheme described by Kuźnicki (1963 b) for *Paramecium caudatum* when deciliated by the use of chloral hydrate. In the first phase paramecia lost the cilia from the anterior part of their body, then from the middle part and eventually from the posterior part of the cell. The most resistant cilia were those of the vestibulum and cytostome.

To facilitate evaluation of the degree of deciliation, an arbitrary

Table 1

Relationship between the time of deciliation and its degree. Before examination paramecia were fixed with 3% glutaraldehyde

Method of treatment	Time without shaking min (f)	Time of shaking min (f)	Degree of deciliation						Dead cells	
			0		1-2		3			
			n	%	*n*	%	*n*	%	*n*	%
Control (g)	—	—	170	100	0	—	0	—	0	—
Control (h)	—	—	97	100	0	—	0	—	0	—
Control	—	3–4	107	100	0	—	0	—	0	—
5% ethanol (a)	—	3–4	0	—	1	1.1	176	98.9	0	—
% ethanol (b)	3–4	—	0	—	50	53.8	43	46.2	0	—
% ethanol (c)	1	—	3	2.2	35	25.9	97	71.9	0	—
5% ethanol (c)	2	—	2	1.3	33	21.0	122	77.7	0	—
5% ethanol (c)	4	—	0	—	42	23.1	140	76.9	0	—
5% ethanol (c)	8	—	0	—	48	33.8	94	66.2	0	—
5% ethanol (c)	16	—	1	0.5	44	23.8	140	75.7	0	—
0.25mM $NiCl_2$ (d)	1	—	173	97.2	5	2.8	0	—	0	—
0.25mM $NiCl_2$ (d)	2	—	183	89.3	22	10.7	0	—	0	—
0.25mM $NiCl_2$ (d)	4	—	139	73.5	50	26.5	0	—	0	—
0.25mM $NiCl_2$ (d)	8	—	164	68.3	76	31.7	0	—	0	—
0.25mM $NiCl_2$ (d)	16	—	82	41.3	95	47.7	22	11.0	30	13.1
0.25mM $NiCl_2$ (e)	—	3–4	125	87.4	18	12.6	0	—	3	2.0
0.25mM $NiCl_2$ (e)	—	7	54	29.3	130	70.7	0	—	12	6.1

(a-e) — See Material and Methods, (f) — in deciliation solution, (g) — additional time of incubation in M.S. for 16 min and (h) — for 120 min

scale of stages was adopted (Fig. 1). Stage 0 — paramecia with the ciliature unaffected. Stage 1 — paramecia without cilia on the anterior one third of the body. Stage 2 — paramecia without approximately 2/3 of the body cilia. Stage 3a – paramecia without any cilia on their body but with the cytostomal cilia beating. Stage 3b — paramecia without any external cilia and with cytostomal cilia motionless.

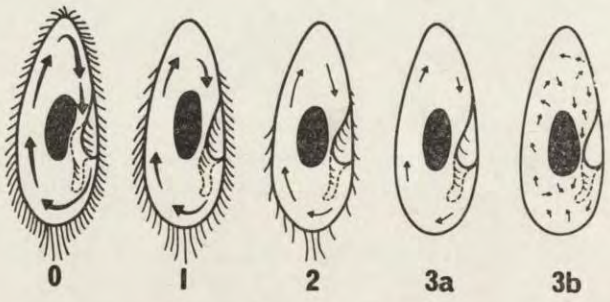


Fig. 1. Diagrammatic presentation of the arbitrary scale of deciliation. Arrows indicate the direction of cytoplasmic streaming

As shown in Table 1, in control experiments M.S. by itself or shaking in this medium did not cause deciliation. Incubation in 5% ethanol according to the modified method of Sonneborn (1970) caused an immediate deciliation but extension of the time of treatment did not change the proportion of partially-deciliated animals (stages 1 and 2) to completely deciliated ones (stage 3). However, the modified method of Ogura (1977) followed by shaking increased decisively the number of completely deciliated animals.

Partial and complete deciliation can be achieved also by incubating paramecia in 0.25 mM nickel chloride. Longer treatment caused an increase in numbers of partially-deciliated animals and eventually after 16 min there appeared wholly deciliated animals. Shaking paramecia in the nickel chloride solution did not increase the number of completely deciliated animals unlike shaking in 5% ethanol prepared according to the modified method of Ogura (1977) where complete deciliation took place in almost all animals. Examination by light microscopy of 0.5 μm Araldite sections of control paramecia and those treated with 5% ethanol or nickel chloride showed that the cytostome did not become deciliated (Pl. I 2).

Electron microscope examination of ultrathin sections of treated paramecia showed that cilia became broken off as a rule at a level of

Table 2

Numbers (n) and percent (%) of *Paramecium tetraurelia* cells with and without cytoplasmic streaming during the first 30 min after deciliation

Method of treatment	Parameters	Degree of deciliation							Total number of cells examined
		Cytoplasmic streaming present			Cytoplasmic streaming ceased				
		0	1–2	3a	0	1–2	3a	3b	
Untereated (thigmotactic) cells in M.S.	n	50	0	0	0	0	0	0	50
	%	100	0	0	0	0	0	0	
5% ethanol (Ogura 1977) modified, shaken 2–4 min	n	5	40	500	0	0	0	0	545
	%	0.9	7.3	91.7	0	0	0	0	
5% ethanol (Sonneborn 1970) modified incubation 7–20 min	n	24	56	302	0	10	18	0	410
	%	5.8	13.6	73.6	0	2.4	4.4	0	
0.25 mM $NiCl_2$ Sikora and Wasik (1978) incubation 7–11 min	n	482	312	256	0	36	127	212	1425
	%	33.8	21.9	18.0	0	2.5	8.9	14.9	

the ciliary-basal body junction distally to the terminal plate just above the axial granule level. After examination of a number of electron micrographs it appears that when cilia break off the plasma membrane heals almost immediately over the exposed kinetosome.

Subsequently the cytoplasm penetrates between the plasma membrane and outer alveolar membrane separating them widely and forms accumulations containing normal constituents of the cytoplasm and numerous vesicles up to 0.2 μm in diameter. These accumulation are limited on the outside by the plasma membrane and on the inside by the outer alveolar membrane. In the same time the affected alveoli become drastically reduced in size. Later these accumulations of the cytoplasm become reabsorbed and the regeneration of cilia begins. In

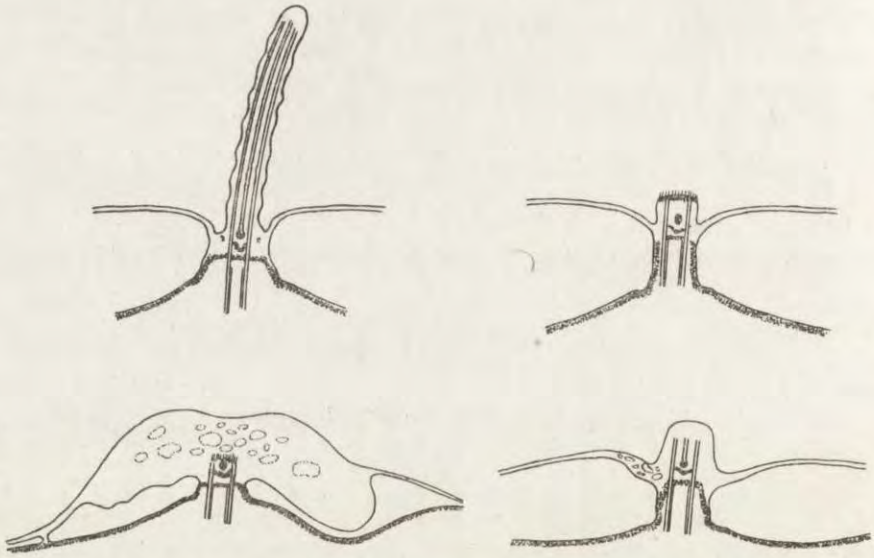


Fig. 2. Diagrammatic presentation of the ultrastructural features of the deciliation and the beginning of regeneration of cilia in *Paramecium tetraurelia* after treatment with 5% ethanol or with 0.25 mM nickel chloride. Transverse sections through membranes are represented by single lines. The diagram is based on Pl. II 3–9. Magnification approximately × 30 000

longitudinal sections passing through the central plane of the remaining kinetosome it was found that the original plasma membrane covering the surface area adjacent to kinetosomes was continuous over the area where cilia were broken off. This indicates that deciliated paramecia regenerate the outer membrane of the pellicle, which does not differ in any way from the normal membrane, in a matter of a few minutes after deciliation. The successive stages of repair and healing are shown in electron micrographs Pl. II 3–9, and diagrammatically in Fig. 2.

In vivo observations on the motility of the cytoplasm and its relation

to the degree of deciliation and to the activity of the cytostomal cilia are shown in the Table 2. Various times of treatment in experiments using 5% ethanol (two methods) and 0.25 mM nickel chloride were chosen so as to obtain in the same samples non-deciliated animals (stage 0), animals deciliated over 1/3 to 2/3 of the external surface (stages 1 and 2) and completely deciliated animals (stage 3).

Further detailed observation of animals without any cilia on their body surface allowed two subgroups to be distinguished, namely stage 3a in which the cytostomal cilia continued to beat and the stage 3b in which the cytostomal cilia were present but immobilized.

Cytoplasmic streaming was considered to be taking place if it was possible to estimate the velocity of the movement of granules or crystals carried by the cytoplasm along a distance of 8 μm and if the streaming was proceeding according to a definite pattern (Sikora 1981).

In Table 3 there is shown the relationship between the degree of deciliation of the *Paramecium* cells (see Fig. 1) and the velocity of their cytoplasmic streaming.

Table 3

The effect of deciliation of *Paramecium tetraurelia* on cytoplasmic streaming velocity expressed in μm s^{-1} ± standard deviation

Method of treatment	Degree of deciliation			Total number of cells examined
	0	1–2	3a	
Untreated (thigmotactic) cells in M.S.	4.65±1.30	—	—	50
5% ethanol (Ogura 1977) modified, shaken 2–4 min	(a)	3.34±1.22	3.20±1.10	100
5% ethanol (Sonneborn 1970) modified incubation 7–20 min	(a)	3.55±1.23	3.12±1.12	100
0.25 mM $NiCl_2$ (Sikora and Wąsik 1978) incubation 7–11 min	2.02±0.59	1.43±0.37	0.63±0.12	150

(a) — Cytoplasmic streaming present but estimation of its velocity was impossible because paramecia were in constant motion.

Measurement of the velocity of cytoplasmic streaming in fully ciliated paramecia (stage 0) was possible only in animals which adhere thigmotactically to the substrate and also in animals immobilized by using nickel ions. Animals treated witm 5% ethanol but which retained their full ciliature (stage 0) did not exhibit any thigmotaxis and therefore the velocity of cytoplasmic streaming could not be measured in this group. Only in animals treated with 5% ethanol and which were deciliated partly or completely (stages 1, 2 and 3) could the velocity of

cytoplasmic streaming be measured and compared with that in untreated animals.

A test of significance (at the level $P=0.01$) based on unit standard deviation (Bailey 1981) has been applied to estimate of the mean differences in velocity of cytoplasmic streaming in cells with different degrees of deciliation. Thus the differences are statistically significant between the velocities in control paramecia ($v=4.64$ μm s^{-1}) and in partially or completely deciliated animals using 5% ethanol according to the method of Ogura (1977) (3.34 and 3.20 μm s^{-1}) as well as according to the method of Sonneborn (1970) (3.55 and 3.12 μm s^{-1}). However, the differences between the velocities in partially and completely deciliated paramecia after ethanol used according to any of the two methods are not significant.

In paramecia treated with 0.25 mM nickel chloride solution, which not only immobilized the animals but also caused deciliation of some cells, there were found statistically significant differences between the velocities of cytoplasmic streaming in fully ciliated animals (stage 0), partially deciliated animals (stage 1 and 2) and in those completely without cilia on their body (stage 3a).

Immobilization of the cytostomal cilia (stage 3b) was observed only in some paramecia without any cilia on their body and this condition was always well correlated with a cessation of cytoplasmic streaming. There were present, however, in the cytoplasm some irregular movements of crystals or other solid inclusions (Sikora et al. 1979). Some partly or completely deciliated animals did not show any motility of the cytoplasm (see Table 2). This occurred mainly after the longer treatments with deciliation agents and may have been due to side effects.

Stoppage of cytoplasmic streaming was observed to occur simultaneously with the cessation of activity of the cytostomal cilia and was preceded by a slowing down of the velocity of cytoplasmic streaming which accompanied the loss of cilia from the body of the animals. These correlations suggest there may be a causal relationship between the activity of cytoplasmic streaming and the activity of cilia, particularly of those in the cytostome.

Discussion

Paramecia of each species in this genus show characteristic average velocities of cytoplasmic streaming (Yamada 1969). The velocity in a given species depends on various factors within the surrounding environment as well as on the particular phase of the life cycle (for review see Sikora 1981).

It was shown that during an intensive food intake by paramecia there is always observed an increased rate of cytoplasmic streaming (Wasik and Sikora 1981). Wasik (1983) has shown that in *Paramecium bursaria* there is a distinct relationship between the concentration of particles in the culture medium and the velocity of cytoplasmic streaming. This suggests that there is a positive interaction between cytoplasmic streaming and the activity of both cilia on the body surface and those which propel food bacteria down the buccal cavity and the cytostome where food vacuoles are formed. This view is at variance with the opinion of the inverse relation between the ciliary activity and the velocity of the cytoplasmic streaming, put forward by Kuźnicki and Sikora (1972) for ciliates immobilized by means of homologous antiserum. The possible existence of a positive interaction was the subject of the present investigations.

When cells were treated with 5% ethanol most of them showed partial or complete deciliation on the external surface of the cell and there were only a few which retained their cilia. On the other hand after using 0.25 mM nickel chloride approximately equal proportions of cells were found to retain their external cilia or undergo partial or complete deciliation. The additional advantage of the nickel chloride method lay in the possibility of measuring the velocity of cytoplasmic streaming in animals with the full complement of cilia, because after incubation for at least 7 min the animals were completely immobilized.

Satir et al. (1980) concluded that the process of deciliation as well as ciliary reversal, ciliary stoppage, cell contraction and trichocyst discharge are all the results of an influx of calcium ions from the surrounding medium to the interior of the cells. The ethanol used in this work was most probably the factor which induced an influx of calcium ions from the surrounding medium (Matt et al. 1978) and therefore caused deciliation of the *Paramecium tetraurelia* cells. This assumption is supported by the observation that paramacia become deciliated by ethanol only in the presence of calcium in the medium (Sikora, unpublished observations).

The mechanism of deciliation by nickel chloride is difficult to understand. One can only assume that nickel ions have perhaps stronger affinity for the calcium binding sites which are localized, according to Fisher et al. (1976) in the region of the curved septum just below the axial granule of the cilia and in the region of the terminal plate of the basal granules. The nickel ions might therefore displace calcium by ionic substitution and give an increased concentration of free calcium sufficient to cause deciliation. Alternatively it is possible that nickel chloride so changes the permeability of the plasma membrane that an influx of calcium causes deciliation.

The observed differences between the velocity of cytoplasmic streaming in animals deciliated to various degrees by means of 5% ethanol or 0.25 mM nickel chloride seem to indicate the existence of a dependence between the extent of the ciliated surface area which the cell has retained and the velocity of cytoplasmic streaming. It was observed that there is a cessation of the cytoplasmic streaming when the beating of the cytostomal cilia is suspended. This probably indicates that not only the presence of the cytostomal cilia but also their activity are essential for the maintenance of cytoplasmic streaming.

Sattler and Staehelin (1974) have shown in *Tetrahymena pyriformis* that some cytostomal cilia have bristles. These authors have suggested that these bristles are receptors which control the selection of food particles by modifying the activity of the cytostomal cilia. Apart from that according to investigations of Wasik (1983) velocity of cytoplasmic streaming in *Paramecium bursaria* depends on the concentration of the suspended solid particles in the medium.

There are then sufficient grounds to assume that stimulation of the cell by food particles driven by cilia is sufficient to stimulate the cytoplasm and to accelerate its movement. In other words, immobilization of the cytostomal cilia as well as deciliation of the cell body seem to be responsible in some way to reduce the velocity of cytoplasmic streaming probably because the stimulation of mechanoreceptors is decreased (Wasik et al. 1983). To sustain cytoplasmic streaming the activity of the cytostomal cilia is sufficient, but then its velocity is many times slower than in fully ciliated and actively feeding paramecia.

In untreated paramecia, most of the endoplasm flows round the cell along a specified pathway always in the same direction (Kuźnicki and Sikora 1971). However, there are small numbers of granules and crystals present in the cytoplasm which move in directions other than of the majority of granules. It is possible that this phenomenon is due to the saltatory character of cytoplasmic streaming in *Paramecium* cells (Sikora 1981). This type of movement was described for *Paramecium tetraurelia* in the cortical layer of the cytoplasm, (Aufderheide 1977) and in the streaming of the endoplasm itself (Sikora et al. 1979).

The present observations on the movement of cytoplasmic granules in deciliated cells have shown that with the substantial decrease in velocity of the streaming movement of the majority of granules along the main track the number of granules displaying irregular movement increases. Rough estimates indicate that in cases where the velocity of streaming falls below approximately 0.5 $\mu m\ s^{-1}$ the number of granules showing irregular movement increaes to such an extent that eventually it becomes impossible to estimate the velocity of the main movement.

As it was shown by Ogura (1981) and according to our observations during deciliation of *Paramecium* cells cilia break off just above the level of the axial granule located distally to the curved septum. The basal granules with the terminal plates remain intact. This region of the ciliary-basal granule junction is recognized as a universal breaking point in cilia and flagella (Blum 1981, Kennedy and Brittingham 1968).

Calcium-mediated regulation of cellular processes, particularly of those associated with *Paramecium* motility is well documented (Eckert et al. 1976). Since calcium deposits have been shown at the base of cilia (Fisher and Kaneshiro 1975, Fisher et al. 1976) it seems probable that during deciliation induced by ethanol or nickel ions, disturbance of the balance of divalent cations, particularly of calcium takes place to such an extent, that cilia at the base become fragile and break off readily when *Paramecium* encounters obstacles.

Regeneration of the normal, membrane covering the site left after the break off of cilium takes place in a matter of a few minutes. This is not surprising as it is known that the entire penicular cilia, during the normal development of the cytostome, grow from basal granules in as little as 10 min (Ehret and de Haller 1963).

In general the ultrastructural features of deciliation and subsequent healing of the plasma membrane are essentially the same after treatment with ethanol (Ogura 1981) or nickel chloride as those after treatment with chloral hydrate described for *Paramecium caudatum* (Kennedy and Brittingham 1968).

Cilia in *Paramecium tetraurelia* and in ciliates in general have two functions, one for propulsion during swimming and the other to drive the food particles into the buccal cavity.

In this report we suggest a third function to ensure that food particles driven in by the cilia collide with the mechanoreceptors located on the cytostomal cilia and thus stimulate cytoplasm to accelerate and to assure efficient intracellular transportation of food vacuoles.

REFERENCES

Aufderheide K. J. 1977: Saltatory motility of uninserted trichocysts and mitochondria in *Paramecium tetraurelia*. Science, 198, 299–300.

Bailey N. T. 1981: Statistical Methods in Biology. 2nd ed. Hodder and Stoughton, London, Sydney, Auckland and Toronto, 216 pp.

Blum J. J. 1971: Existance of a breaking point in cilia and flagella. J. Theor. Biol., 33, 257–263.

Eckert R., Naitoh Y. and Machemer H. 1976: Calcium in the bioelectric and motor functions of *Paramecium*. In: Calcium in Biological Systems, Symp. Soc. Exp. Biol., Cambridge University Press, pp. 233–255.

Ehret C. F. and de Haller G. 1963: Origin, development and maturation of organelle systems of the cell surface in *Paramecium*. J. Ultrastruct. Res., 6, (Suppl.), 3–42.

Fisher G. W. and Kaneshiro E. S. 1975: Ultrastructural localization of calcium deposits in cilia of *Paramecium aurelia*. J. Cell Biol., 67, 115 a.
Fisher G. W., Kaneshiro E. S. and Peters P. D. 1976: Divalent cation affinity sites in *Paramecium aurelia*. J. Cell Biol., 69, 429–442.
Gelei J. 1935: Ni-Infusorien im Dienste der Forschung und des Unterrichtes. Biol. Zentralbl., 55, 57–74.
Grębecki A. and Kuźnicki L. 1963: The influence of external pH on the toxicity of inorganic ions for *Paramecium caudatum*. Acta Protozool., 1, 157–164.
Kennedy J. R. Jr. and Brittingham E. 1968: Fine structure changes during chloral hydrate deciliation of *Paramecium caudatum*. J. Ultrastruct. Res., 22, 530–545.
Kuźnicki L. 1963 a: Recovery in *Paramecium caudatum* immobilized by chloral-hydrate treatment. Acta Protozool., 1, 177–185.
Kuźnicki L. 1963 b: Reversible immobilization of *Paramecium caudatum* evoked by nickel ions. Acta Protozool., 1, 301–312.
Kuźnicki L. and Sikora J. 1971: Cytoplasmic streaming within *Paramecium aurelia*. I. Movements of crystals after immobilization by antiserum. Acta Protozool., 8, 439–446.
Kuźnicki L. and Sikora J. 1972: The hypothesis of inverse relation between ciliary acivity and cyclosis in *Paramecium*. Acta Protozool., 11, 243–250.
Kuźnicki L. and Sikora J. 1973: Cytoplasmic streaming within *Paramecium aurelia*. III. The effect of temperature on flow velocity. Acta Protozool., 12, 59–66.
Matt H., Bilinski M. and Plattner H. 1978: Adenosinetriphosphate, calcium and temperature requirements for the final steps of exocytosis in *Paramecium* cells. J. Cell Sci., 32, 67–86.
Ogura A. 1977: Non-lethal deciliation of *Paramecium* with ethanol. M. S. thesis, University of Tokyo (cited after Machemer and Ogura 1979).
Ogura A. 1981: Deciliation and reciliation in *Paramecium* after treatment with ethanol. Cell Struct. Funct., 6, 43–50.
Rosenbaum J. L. and Carlson K. 1969: Cilia regeneration in *Tetrahymena* and its inhibition by colchicine. J. Cell Biol., 40, 415–425.
Satir B. H., Garafalo R. S., Giligan D. M. and Maihle N. J. 1980: Possible functions of calmodulin in Protozoa. Ann. N.Y. Acad. Sci., 356, 83–91.
Sattler C. A. and Staehelin L. A. 1974: Ciliary membrane differentiations in *Tetrahymena pyriformis*. *Tetrahymena* has four types of cilia. J. Cell Biol., 62, 473–490.
Sikora J. 1981: Cytoplasmic streaming in *Paramecium*. Protoplasma, 109, 57–77.
Sikora J. and Wasik A. 1978: Cytoplasmic streaming within Ni^{2+} immobilized *Paramecium aurelia*. Acta Protozool., 17, 389–397.
Sikora J., Wasik A. and Allen R. D. 1979: The role of single particle saltations in *Paramecium* cytoplasmic streaming. Acta Protozool., 18, 201.
Sonneborn T. M. 1970: Methods in *Paramecium* research. In: Methods in Cell Physiology, 4, (Prescott D.M. ed.), Academic Press, New York, pp. 241–339.
Thompson G. A. Jr., Baugh L. C. and Walker L. F. 1974: Nonlethal deciliation by a local anesthetic and its utility as a tool for studying cilia regeneration. J. Cell Biol., 61, 253–257.
Wasik A. 1983: Effect of external agents on cytoplasmic streaming in *Paramecium*. I. Influence of carmine suspension. Acta Protozool., 22, 183–189.
Wasik A. and Sikora J. 1981: Acceleration of cytoplasmic streaming velocity by external stimuli in *Paramecium bursaria*. In: Progress in Protozoology, Abstracts of papers, Sixth Int. Congr. Protozool., Warszawa, Poland, July 5–11, 1981, p. 387.
Wasik A., Sikora J. and Kuźnicki L. 1983: Effect of different suspensions on phagocytosis and cytoplasmic streaming in *Paramecium bursaria*. Post. Biol. Kom., (in press).
Yamada K. 1969: A comparative study on the cyclosis in *Paramecium*. J. Sci. Hirosh. Univ., Ser. B., Div. 1, 22, 1–153.

EXPLANATIONS OF PLATES I–II

2: Light micrographs of 0.5 μm Araldite sections stained with toluidine blue. a — control, b — a cell partially deciliated by means of 0.25 mM nickel chloride (anterior end uppermost), c — a cell after 5% ethanol treatment completely without cilia on its outer surface but with the cytostomal cilia retained. × 1100

3: Transmission electron micrograph of a control cilium in *Paramecium tetraurelia*. Bar 0.5 μm. × 40 000

4: Two remaining basal bodies after deciliation with 5% ethanol. Note that the stump on the right-hand side shows some reconstruction of the membrane across the exposed interior of the basal body. Bar 0.5 μm. × 60 000

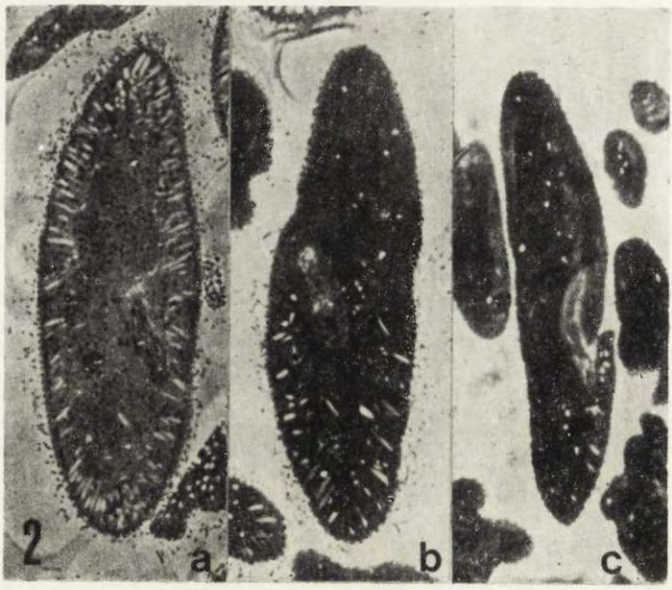
5: Remaining basal body after ciliary breakage due to treatment with 0.25 mM nickel chloride. The reconstruction of the membrane across the basal body is more progressed than that in Phot 4. Bar 0.5 μm. × 60 000

6: The ultrastructural features of the penetration of the cytoplasm into the space between the plasma membrane and the outer alveolar membrane with simultaneous collapse of alveoli on both sides after treatment with 5% ethanol. The accumulation of the cytoplasm is covered from outside by a single plasma membrane and the stump is devoid of membrane. Bar 0.5 μm. × 60 000

7: Similar section through a ciliary unit showing accumulation of the cytoplasm between the plasma membrane and outer alveolar membrane after treatment with 0.25% mM nickel chloride. Note numerous vesicles about 60 nm in the accumulation of cytoplasm. Bar 0.5 μm. × 60 000

8: Commencement of regeneration of cilium after deciliation with 5% ethanol. Note the stump smoothly covered by the ciliary plasma membrane. Curved septum and terminal plate are well formed. The accumulations of cytoplasm are still present and the alveoli are collapsed. Bar 0.5 μm. × 60 000

9: Similar section to that in Phot. 8, but after deciliation with 0.25 mM nickel chloride. Regeneration has progressed further than in Phot. 8. Accumulations of cytoplasm are absent and alveoli are normal again. Bar 0.5 μm. × 60 000



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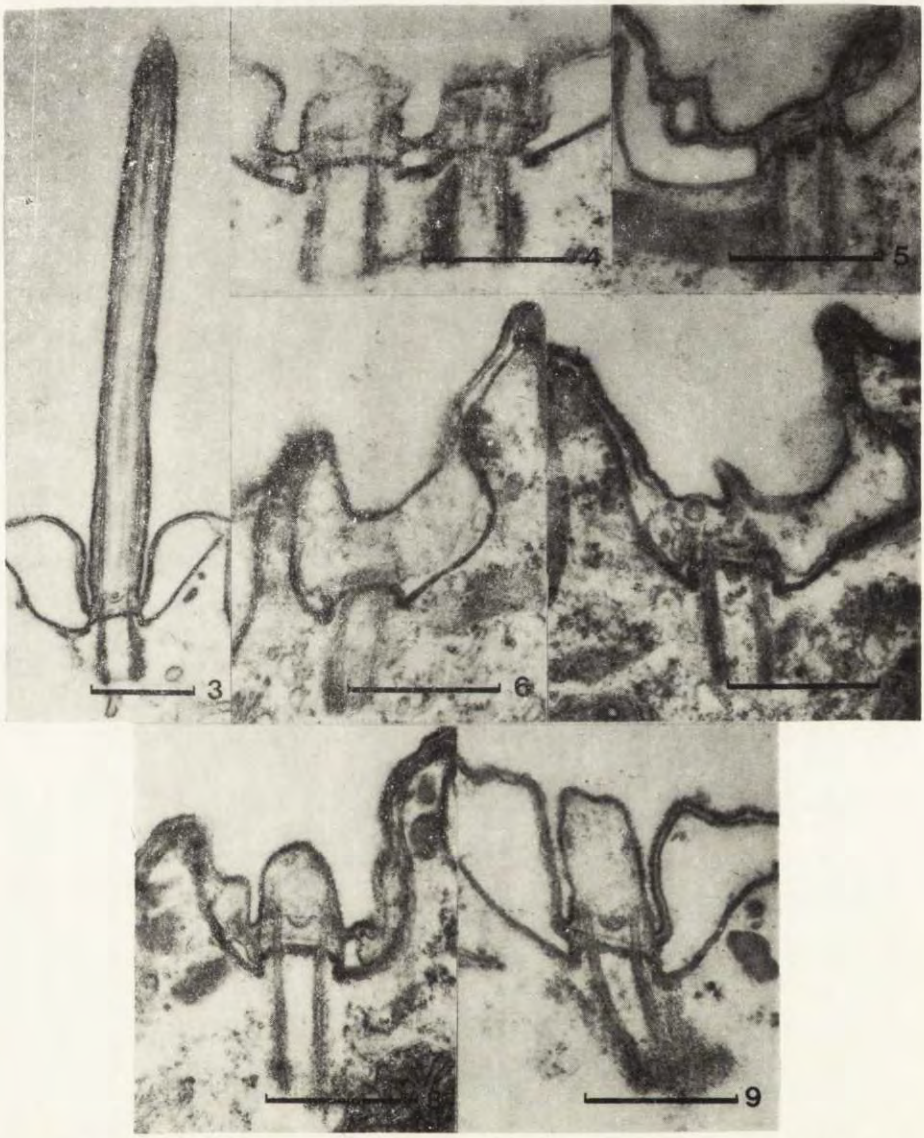

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ACTA PROTOZOOLOGICA
Vol. 23, No. 2, pp. 107–113 (1984)

Effect of External Agents on Cytoplasmic Streaming in *Paramecium*. II. Influence of Media Free of Suspension

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Received on 28 November 1983

Synopsis. Solutions of orange G, neutral red and bovine albumin deprived of solid impurities do not evoke food vacuole formation and at the same time do not change the velocity of cytoplasmic streaming in *Paramecium bursaria*. In contrast, incubation of the cells in the above mentioned solutions in the presence of carmine particles (10^8 particles ml^{-1}) induces an increase of the rate of endocytosis and cytoplasmic streaming flow. The intensisty of both phenomena is at the level estimated for carmine particles acting alone. The presented evidence strongly supports the view that particulate material present in the surrounding medium plays an important role in enhancement of endocytosis and cytoplasmic streaming velocity in *Paramecium*.

The effect of suspensions of particles in the medium on food vacuole formation rate in *Paramecium* has been previously investigated (Bozler 1924, Ricketts 1971, Nilsson 1972, Pavlovskaya 1973, Railkin 1981). Recently it was shown (Wasik 1983, Wasik et al. 1984) that inedible particles like carmine and barium chromate or eatable ones like the bacteria *Enterobacter aerogenes* enhance in *Paramecium bursaria* cells not only the rate of food vacuole formation, but also accelerate the cytoplasmic streaming velocity. The intensity of both phenomena is related to the concentration of particles in the medium. This leads to the supposition that the activity of both processes is under control of external factors. The solid particles in medium might collide with cilia or/and with the body surface and stimulate hypothetical mechanoreceptors (Sikora and Jurand 1984). This stimulation of surface receptors might be the first link of the chain leading to the process responsible for propulsion of cytoplasmic streaming and endocytosis.

Grębecki and Kuźnicki (1956) showed that in *Paramecium caudatum* some dyes induce food vacuole formation. It was found that especially basic dyes, like for example neutral red and toluidine blue, were condensed within food vacuoles, while acidic dyes like orange G and Congo red were not. However, all of those dyes were taken up through food vacuoles. Grębecki (1963) postulated the mechanism of condensation of basic dyes within food vacuoles which depends on: the charge of the coloured ion, ionic potential of the dye and electrokinetic potential of the *Paramecium* surface. The nature of this phenomenon believed to be generally related to electroadsorption. It was suggested thereby that solutions of some basic dyes are responsible for stimulation of endocytosis, especially in the case of vital dyes.

The aim of the present contribution is to determine whether non-particulate agents would induce food vacuole formation and changes in the velocity of cytoplasmic streaming in *Paramecium bursaria*.

Material and Methods

The ciliate used in this investigation was *Paramecium bursaria*, bred in darkness (Sikora et al. 1979). The cells were grown at room temperature 19° ± 2°C, in Scottish grass medium with *Enterobacter aerogenes* according to Sonneborn (1970).

Two exemplary vital dyes belonging to: the acidic — orange G and basic group — neutral red, were used. Both dyes were dissolved in maintenance solution (M.S.) consisting of 5 mM Tris-HCl buffer of pH 7.25 with 1 mM KCl and 1 mM $CaCl_2$ added. The vital harmless concentrations of dyes used were: 5 mM of orange G and 0.0001 mM of neutral red. The fraction V of bovine albumin dissolved in M.S. was used in concentrations ranging from 0.06% to 2%. All solutions used were deprived of possible particulate impurities by means of centrifugation at 13 000 × g for 20 min or by filtration through a 0.6 μm Synpor No. 5 membrane filter.

To determine the food vacuole formation rate and cytoplasmic streaming velocity in *Paramecium bursaria* cells, the following procedure was applied. Before experiments, the centrifuge-concentrated (up to 300 × g for 1 min) paramecia were preincubated for 16–20 h in M.S. Then the paramecia were treated with dyes or bovine albumin solutions for 3 min. In some cases carmine suspension was added. The control cells were incubated in fresh M.S. Concentration of *Paramecium* cells used in experiments was about 1000 specimens ml^{-1}. After treatment paramecia were immobilized for 10 min by means of $NiCl_2$ according to Sikora and Wasik (1978). Washed in M.S. motionless specimens were observed under bright-light or phase-contrast microscope for 30 min and the velocity of cytoplasmic streaming was recorded (Sikora et al. 1979). The number of food vacuoles formed was counted in cells fixed with 3% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.3, after 3 min exposure to the given dye or albumin in the presence of carmine or without it, depending on the experiment.

All experimental procedure was performed at room temperature 19° ± 2°C.

Results

The purpose of our study was to estimate whether solutions of some dyes would change the velocity of cytoplasmic streaming as it is observed in the case of particulate material which enhances distinctly both the velocity of cytoplasm and food vacuole formation rate in *Paramecium bursaria* (Wasik 1983, Wasik et al. 1984, Sikora et al. 1984).

To avoid possible side effects of solid particles remaining in the solutions to be used in experiments, centrifugation and filtration of these solutions was performed. It was found that centrifugation at the 13 000 × g for 20 min or filtration through Synpor No. 5 membrane filter was sufficient to remove impurities which could influence the results.

Among acidic dyes, orange G was chosen because it was found to be quite harmless for *Paramecium bursaria* even in 5 mM concentration. The advantage of this dye is its intensive colour which should be easily seen in newly formed food vacuoles.

Table 1

Effect of incubation of *Paramecium bursaria* in 5mM orange G on food vacuole formation rate and cytoplasmic streaming velocity. Carmine suspension used in concentration 1×10^8 particles ml^{-1}. S.E. — Standard error

Treatment	Mean velocity of cytoplasmic streaming		Mean number ±S.E. of food vacuoles formed in 3 min incubation	Total number of specimens examined
	in $\mu m\ s^{-1}$ ± S.E.	percent of the control		
Control	2.13±0.06	100	0	90
Orange G	2.20±0.07	103.3	0	90
Carmine	3.29±0.05	154.5	9.6±0.4	90
Orange G + Carmine	3.23±0.06	151.6	9.8±0.7	90

As shown in Table 1 there is no significant difference in the velocity of cytoplasmic streaming in the control and orange G-treated paramecia and food vacuole formation was not observed. Paramecia treated with orange G in the presence of 10^8 particles ml^{-1} of carmine suspension show consistent acceleration of cytoplasmic streaming accompanied by formation of food vacuoles filled with carmine particles. The velocity of cytoplasm and the rate of food vacuoles formation is the same as observed in the case of carmine acting alone.

As observed in preliminary experiments 0.0001 mM of neutral red has been estimated to be the highest vital harmless concentration for

Table 2

Effect of incubation of *Paramecium bursaria* in 0.0007 mM neutral red on food vacuole formation rate and cytoplasmic streaming velocity. Carmine suspension in concentration 1×10^8 particles ml^{-1}. S.E. — Standard error

Treatment	Mean velocity of cytoplasmic streaming		Mean number ± S.E. of food vacuoles formed in 3 min incubation	Total number of specimens examined
	in $\mu m\ s^{-1}$ ± S.E.	percent of the control		
Control	2.74±0.07	100	0	90
Neutral red	2.70±0.07	98.5	0	90
Carmine	3.31±0.07	123.7	10.4±0.8	90
Neutral red + Carmine	3.49±0.08	127.4	11.3±0.7	90

Paramecium bursaria (at least for 1 h exposure). In paramecia exposed to neutral red (Table 2) no changes in the cytoplasmic streaming velocity were observed and no food vacuoles formed. A predominant effect of particulate material suspended in neutral red on regulation of cytoplasmic streaming velocity and food vacuole formation was noted.

Bovine albumin, as suggested by Brutkowska (1969) for *Tetrahymena pyriformis*, stimulates food vacuole formation process. Preliminary experiments with albumin not centrifuge-purified of particulate impurities (Wasik and Sikora 1981) indicated that this medium enhances both endocytosis and cytoplasmic streaming velocity of *Paramecium bursaria*. Table 3 shows the influence of particle-free bovine albumin on *Paramecium*. Regardless of the concentration used no distinct changes in the rate of cytoplasm flow velocity were observed. Treatment

Table 3

Effect of bovine albumin fraction V on cytoplasmic streaming velocity in *Paramecium bursaria*. S.E. — Standard error

Concentration of albumin in w/v percent	Mean velocity of cytoplasmic streaming		Total number of specimens examined
	in $\mu m\ s^{-1}$ ± S.E.	percent of the control	
0	1.97±0.05	100	90
0.06	1.94±0.05	98.5	90
0.25	1.81±0.07	91.9	90
0.5	1.92±0.05	97.5	90
1	1.96±0.06	99.5	90
2	2.02±0.07	102.5	90

Table 4

Influence of 1% bovine albumin fraction V and 10^8 particles ml^{-1} of carmine suspension of food vacuole formation rate and cytoplasmic streaming velocity in *Paramecium bursaria*. S.E. — Standard error

Treatment	Mean velocity of cytoplasmic streaming		Mean number±S.E. of food vacuoles formed in 3 min incubation	Total number of specimens examined
	in $\mu m\ s^{-1}$ ±S.E.	percent of the control		
Control	2.04±0.07	100	0	90
Albumin	2.08±0.07	102	0	90
Carmine	2.90±0.08	142.2	12.30±0.25	90
Albumin + Carmine	2.94±0.07	114.6	12.56±0.41	90

of paramecia with 1% bovine albumin in the presence of 10^8 particles ml^{-1} of carmine suspension evoked a marked increase of endocytosis and cytoplasmic streaming velocity (Table 4).

Discussion

It is likely that particulate material in the medium is a factor of far reaching importance for the food vacuole formation process in some ciliates as it has been suggested by Bozler (1924) and Mast (1947) in *Paramecium caudatum* and by Nilsson (1972) in *Tetrahymena pyriformis*. These suggestions have been confirmed in *Paramecium bursaria* (Wasik 1983, Wasik et al. 1984) either by means of inedible or eatable particles. Furthermore, it was found that different suspensions change the velocity of cytoplasm depending on the concentration used. An increase of suspension concentration enhances the food vacuole formation rate and cytoplasmic streaming velocity to a great extent.

The question arises whether the particles which fill the newly formed vacuoles or the food vacuole itself are responsible for cytoplasmic streaming velocity acceleration. This activity might be only associated with the process leading to food vacuole formation. To elucidate this problem non-particulate factors, considered as inducers of endocytosis (Chapman-Andresen 1967, Stockem and Wohlfarth-Bottermann 1969) were suggested as fulfilling this role. Among suitable dyes (Grębecki and Kuźnicki 1955, Grębecki 1963) orange G and neutral red, while among proteins fraction V of bovine albumin (Brutkowska 1969) were selected. These factors were used to test

their effectivness in stimulation of food vacuole formation and influence on cytoplasmic streaming velocity in *Paramecium bursaria*.

It was expected that if paramecia would form easily visible coloured food vacuoles even in solutions of dyes deprived of particulate material, this would indicate a close relation between the process of food vacuole formation and the mechanism responsible for propulsion of the cytoplasm. Otherwise more probable will be the relation between particles and cytoplasmic streaming intensity, based on the assumption that susceptible mechanoreceptors (Sikora and Jurand 1984) are involved in transmission of information to the interior of the *Paramecium* cell, on the presence of supposed eatable particulate food in the close neighbourhood.

From the results here presented it appeared that both dyes and bovine albumin, free of particles, do not induce food vacuole formation. Neither do these factors evoke changes in the velocity of cytoplasmic streaming, in contrast to the particulate material introduced into the surrounding medium. This supports firmly the view presented previously (Wasik 1983, Wasik et al. 1984) that the role of suspension in stimulation of endocytosis and cytoplasmic streaming is predominant.

In contrast to the previous studies on *Paramecium caudatum* (Grębecki and Kuźnicki 1955, Grębecki 1963), in *Paramecium bursaria* incubated in solutions free of particles, neither food vacuoles nor condensation of dyes in vacuoles were observed. The discrepancies in data are probably due to differences in the experimental approach and species used what should be kept in mind.

The presented evidence supports the suggestion that suspension of particles in the surrounding medium is an important factor in formation of food vacuoles. The question, whether acceleration of cytoplasmic streaming velocity is under control of the suspension in the medium or of the food vacuole formation process, will be presented elsewhere (Wasik and Sikora 1984).

REFERENCES

Bozler E. 1924: Über die Morphologie der Ernährungsorganelle und die Physiologie der Nahrungsaufnahme von *Paramecium caudatum* Ehrenberg. Arch. Protistenkd., 49, 163–215.

Brutkowska M. 1969: Fluids intake by *Tetrahymena pyriformis*. In: Progress in Protozoology Abstr. Third Int. Congr. Protozool., Leningrad 1969, "Nauka", 135–136.

Chapman-Andresen C. 1967: Studies on endocytosis in *Amoeba*. The distribution of pinocytotically ingested dyes in relation to food vacuoles in *Chaos chaos*. I. Light microscopic observations. C. R. Trav. Lab. Carlsberg, 36, 161–186.

Grębecki A. 1963: Electrobiologie d'ingestion des colorants par le cytostome de *Paramecium caudatum*. Protoplasma, 56, 89–98.

Grębecki A. and Kuźnicki L. 1956: Autoprotection in *Paramecium caudatum* by influencing the chemical properties of its medium. Acta Biol. Exp., 17, 71–107.

Mast S. O. 1947: The food vacuole in *Paramecium*. Biol. Bull., 92, 31–72.

Nilsson J. R. 1972: Further studies on vacuole formation in *Tetrahymena pyriformis* GL. C. R. Trav. Lab. Carlsberg, 39, 83–110.

Pavlovskaya T. V. 1973: Vlijanie uslovij pitanija na skorost' potreblenija pišči i vremja generacji infusorij. Zool. Zh., 52, 1451–1457.

Railkih A. I. 1981: Količestviennyj analiz fagocitoza *Paramecium caudatum* Ehrbg. i *Spirostomum ambiguum* Ehrbg. Acta Protozool., 20, 255–280.

Ricketts T. R. 1971: Periodicity of endocytosis in *Tetrahymena pyriformis*. Protoplasma, 73, 387–396.

Sikora J. and Jurand A. 1984: Possible role of cilia in the control of cytoplasmic streaming in *Paramecium tetraurelia*. Acta Protozool., 23, 93–105.

Sikora J. and Wasik A. 1978: Cytoplasmic streaming within Ni^{2+} immobilized *Paramecium aurelia*. Acta Protozool., 17, 389–397.

Sikora J., Wasik A. and Baranowski Z. 1979: The estimation of velocity distribution profile of *Paramecium* cytoplasmic streaming. Eur. J. Cell Biol., 19, 184–188.

Sikora J., Kubalski A., Wasik A. and Kuźnicki L. 1984: The role of mechanical stimuli in the control of cytoplasmic streaming in *Paramecium bursaria*. Postępy Biol. Kom. (in press).

Sonneborn T. M. 1970: Methods in *Paramecium* research. *In*: Methods of Cell Physiology 4, Academic Press, New York, 242–335.

Stockem W. and Wohlfarth-Bottermann K. E. 1969: Pinocytosis (endocytosis). In: Handbook of Molecular Cytology (ed. A. Lima-deFaria), North-Holland Publ. Comp., Amsterdam, London, 1373–1400.

Wasik A. 1983: Effect of external agents on cytoplasmic streaming in *Paramecium*. I. Influence of carmine suspension. Acta Protozool., 22, 183–189.

Wasik A. and Sikora J. 1981: Acceleration of cytoplasmic streaming velocity by external stimuli in *Paramecium bursaria*. In: Progress in Protozoology, Abstr. Sixth Int. Congr. Protozool., Warszawa, Poland, July 5–11 1981, 387.

Wasik A. and Sikora J. 1984: Effect of external agents on cytoplasmic streaming in *Paramecium*. III. Influence of endocytosis cessation. Acta Protozool. 23, 115–120.

Wasik A., Sikora J. and Kuźnicki L. 1984: Effect of different suspensions on phagocytosis and cytoplasmic streaming in *Paramecium bursaria*. Postępy Biol. Kom. (in press).

ACTA PROTOZOOLOGICA
Vol. 23, No. 2, pp. 115–121 (1984)

Effect of External Agents on Cytoplasmic Streaming in *Paramecium*. III. Influence of Endocytosis Cessation

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Received on 28 November 1983

Synopsis. Inhibition of food vacuole formation without decrease of ciliary activity of *Paramecium bursaria* does not prevent the acceleration of cytoplasmic streaming velocity, induced by particular material in the surrounding medium. This effect has been demonstrated by means of Alcian blue. Although this basis dye lowers slightly the velocity of cytoplasmic streaming it greatly reduces the food vacuole formation rate. Since an increase in concentration of carmine particles in the medium causes a substantial enhancement of cytoplasmic streaming velocity, even in the absence of newly formed vacuoles, there is a consistent and probably direct particle-dependent response of the propulsive mechanism of *Paramecium cytoplasm*. The relation between particulate material in the medium and cytoplasm motility is discussed in terms of the possible role of cilia, newly formed food vacuoles and mechanoreceptors.

Suspensions of solid particles of different nature evoke a distinct enhancement of endocytosis and cytoplasmic streaming in *Paramecium bursaria* cells (Wasik 1983, Wasik et al. 1984). It was found that (Wasik and Sikora 1984) solutions deprived of solid impurities do not induce food vacuole formation nor do they accelerate cytoplasmic streaming. This supports the idea of the important role of particulate material as stimulating factor of both physiological processes (Wasik 1983).

It seems quite probable that surface receptors susceptible to mechanical stimulation are responsible for transmission of information on the presence of suspension in the medium to the cell interior. The question remains unsolved, whether these hypothetical mechanoreceptors are involved in transmission of information directly to the motile cytoplasm, or the mechanism responsible for cytoplasmic motility is regulated

by the newly formed food vacuoles. Therefore, it was worth ascertaining whether a suspension of particles in the medium would accelerate cytoplasmic streaming velocity if endocytosis is inhibited.

Material and Methods

Paramecium bursaria grown in darkness on Scottish grass medium (Sonneborn 1970) with *Enterobacter aerogenes* at 19° ± 2°C was used. Paramecia before use were preincubated for 16–18 h in maintenance solution (M.S.) (Wasik 1983). For cessation or retardation of food vacuole formation paramecia were incubated in 0.0007% Alcian blue 8GX for 7 min. A carmine suspension in final concentration of 10^8 particles ml^{-1} was added to the Alcian blue solution for the last 3 min. The cells treated with Alcian blue and carmine or Alcian blue only, were afterwards immobilized by means of the nickel method for 10 min (Sikora and Wasik 1978).

In other experiments the effect of particles on cytoplasmic streaming velocity was tested in the already immobilized specimens. The carmine suspension in final concentration 10^8 particles ml^{-1} was added to the nickel-immobilized paramecia. Owing to the nickel method, the cessation of food vacuole formation is instantaneous (Brutkowska 1967).

Immobilized paramecia were rinsed thoroughly in M.S. prior to observation under a bright-light microscope lasting for 30 min. The velocity of cytoplasmic streaming and food vacuole formation rate were measured according to Sikora et al. (1979) and Wasik (1983). All experimental procedure was done at room temperature 19° ± 2°C, and all solutions used were deprived of particulate impurities (Wasik and Sikora 1984).

Results

The endocytosis was arrested to elucidate the question whether particulate material which induces food vacuole formation is directly involved in acceleration of cytoplasmic streaming or acts through newly formed food vacuoles. To stop food vacuole formation in *Paramecium bursaria* cells two of the selected inhibitors were used. The first one, Alcian blue, inhibits endocytosis at 0.0007%, as found in preliminary experiments. It was the highest concentration which did not evoke any visible harmful effects for at least 1 h after exposure of paramecia for 7 min to the dye. No distinct changes in ciliary activity have been noted. The number of food vacuoles formed during 3 min exposure to carmine particles in the presence of Alcian blue for 7 min is consistently reduced (Table 1). A solution of dye deprived of particulate material decreases slightly the velocity of cytoplasmic streaming. In cells treated with Alcian blue in the presence of carmine suspension a distinct acceleration of cytoplasmic streaming velocity occurs though it is not as high as in the

Table 1

Effects of 0.0007% Alcian blue and 1×10^8 particles ml^{-1} of carmine suspension on food vacuole formation rate and cytoplasmic streaming velocity in *Paramecium bursaria*. S.E. — Standard error

Treatment	Mean velocity of cytoplasmic streaming			Mean number ± S.E. of food vacuoles formed in 3 min incubation	Total number of specimens examined
	in $\mu m\ s^{-1}$ ±S.E.	percent of the control velocity	percent of Alcian blue velocity		
Control	2.63±0.06	100	—	0	180
Carmine	3.49±0.06	132.7	—	10.4±0.5	180
Alcian blue	2.40±0.08	91.3	100	0	180
Alcian blue + Carmine	3.17±0.07	120.5	132.1	2.3±0.4	180

case when carmine suspension acts alone. However, if one compares the values (Table 1) of cytoplasmic streaming velocities expressed in percentage it becomes evident that the acceleration of cytoplasmic streaming as consequence of action of carmine and Alcian blue is almost identical with the effect caused by the carmine suspension acting alone, if allowance for the restraining effect of Alcian blue on the cytoplasm will be made.

Since ciliary activity was not affected by the Alcian blue, it was worth to substitute a dye with a factor which would stop both food vacuole formation and motility of somatic cilia. In *Paramecium caudatum* nickelous ions evoke instantaneous and complete cessation of endocytosis (Brutkowska 1967) and ciliary activity disappears in a few minutes (Kuźnicki 1963). It was found that this factor stops food vacuole formation in *Paramecium bursaria* in 0.5 mM concentration.

To determine whether cytoplasmic streaming will be accelerated by the particulate material if endocytosis and ciliary activity have been

Table 2

Influence of carmine particles on cytoplasmic streaming velocity within the already Ni-imm obilize *Paramecium bursaria*. S.E. — Standard error

Concentration of carmine particles ml^{-1}	Mean velocity of cytoplasmic streaming		Total number of specimens examined
	in $\mu m\ s^{-1}$ ± S.E.	percent of the control	
0	2.44±0.05	100	90
1×10^8	2.46±0.05	100.4	90
9×10^8	2.49±0.05	101.6	90

inhibited, the Ni^{2+}-immobilized paramecia were exposed to a suspension of carmine. As shown in Table 2 these cells do not exhibit any visible changes in cytoplasmic streaming velocity in comparison with untreated cells. The concentration of $NiCl_2$ used and duration of exposure do not affect the motility of cytostomal cilia, the activity of which was insufficient to introduce particles into the cytostome.

Discussion

As shown elsewhere (Wasik 1983, Wasik et al. 1984), endocytosis and cytoplasmic streaming in *Paramecium bursaria* are affected by the particulate material in the surrounding medium. These two processes depend on the concentration of particles while they are likely to be independent of their nature (Wasik et al. 1984). In solutions deprived of particles no food vacuoles nor changes in cytoplasmic streaming velocity were observed (Wasik and Sikora 1984).

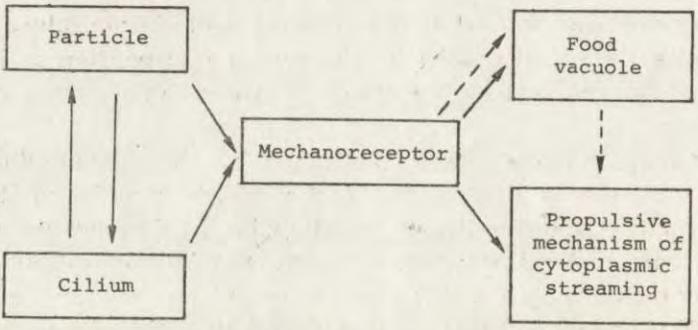

Fig. 1. Suggested pathways of transmission of the information on particles in the medium to the interior of the *Paramecium* cell. The oral groove cilia propelling medium containing particles evoke their collisions with mechanoreceptors. Due to excitation of mechanoreceptor a signal is processed and transmitted to induce a food vacuole formation, which after being intaked evokes acceleration of cytoplasmic streaming (dotted line). It is more probably that a signal from mechanoreceptor is transmitted directly to the mechanism responsible for propulsion of cytoplasm and vacuole formation as well (solid line). Cilia may passively conduct mechanical stimuli, too

As indicated on the scheme (Fig. 1) there are two putative pathways of transmission of information on the presence of particles in the medium within the *Paramecium* cell. This concept is based on the assumption that on the surface of the *Paramecium* body hypothetical mechanoreceptors are localized (Sikora and Jurand 1984). They must be capable of recognizing the input signal caused by collisions with solid particles. The signal coming from the mechanoreceptor after being processed reaches directly or indirectly the responding propulsive mechanism of the

cytoplasm and parallelly the food vacuole formation mechanism. Another pathway might be based on induction of endocytosis, and in consequence newly formed food vacuoles might influence the propulsive mechanism of the cytoplasm to enhance its flow.

To verify which of these two pathways is more probable, inhibition of endocytosis by means of Alcian blue was applied. This dye retards considerably endocytosis in *Paramecium bursaria,* while cytoplasmic streaming velocity is affected slightly. The almost identical enhancement of cytoplasmic streaming velocity in carmine-treated cells, irrespective whether food vacuoles were formed or not, leads to the conclusion that endocytosis does not play a crucial role in regulation of the cytoplasmic streaming velocity.

The overall mechanism of action of Alcian blue on living *Paramecium* cell is still unclear, although the presented evidence of its influence on the food vacuole formation seems to be substantial. The presented results are in contradiction with previous findings (Kaczanowska 1979) suggesting that, although Alcian blue combines with the *Paramecium tetraurelia* surface coat, it does not interfere significantly with the food vacuole formation process. The possibility of differences due to experimental conditions or species should not be disregarded.

It should be pointed out that conducting structures as well as mechanoreceptors, have not been discovered yet. Some sort of mechanoreceptors sensitive to mechanical stimuli have been suggested to be located on the somatic membrane of *Paramecium* cells (Machemer and Ogura 1979, Ogura and Machemer 1980). Also certain cilia in *Paramecium caudatum* have been shown to be passive structures conducting mechanical stimuli (Machemer and Machemer 1983). It is not clear yet, in what extent these mechanoreceptors in *Paramecium caudatum* which mediate changes in permeability to Ca^{2+} and K^{+} (Eckert 1972, Naitoh 1974) are related to the mechanoreceptors involved in the control of cytoplasmic streaming velocity (Sikora and Jurand 1984).

The role of somatic cilia in supplying the cytostome of *Paramecium* with particulate food has been emphasized in previous studies (Jennings 1897, Grębecki 1965). An alimentary current is caused by somatic cilia mainly at the oral groove. It would seem that particles of the suspension will collide either with the cilia or the cell body membrane and as result stimulate the sensitive area or points named here mechanoreceptors. This supposition corresponds well with recent contributions (Sikora and Jurand 1984, Sikora et al. 1984) emphasizing the crucial role of cilia and particulate material (Wasik 1983, Wasik and Sikora 1984) in the medium in the control of cytoplasmic streaming velocity. The lack of any response of cytoplasm to

externally applied carmine particles in the already immobilized paramecia confirms a substantial role of somatic cilia in providing food particles to the cytostome and their significant role in stimulation of mechanoreceptors.

One may argue that in the case of $NiCl_2$-immobilized paramecia, the food vacuole formation ability is excluded, therefore owing the lack of newly formed food vacuoles, the velocity of cytoplasmic streaming is modified. The role of food vacuoles seems to be only apparent since distinct acceleration of cytoplasm precedes food vacuole formation (Wasik 1983, Wasik et al. 1984). Furthermore inhibition of food vacuole formation by Alcian blue does not prevent an acceleration of cytoplasmic streaming if particulate material is present in the medium. Thus, the direct pathway of transmission of information on possibly edible particles in surrounding medium to the mechanism responsible for cytoplasm motility in *Paramecium* seems highly probable.

In conclusion it is suggested that the particles of suspension in medium are the chief factor responsible for stimulation of the hypothetical mechanoreceptors. It is likely that these receptors after being stimulated, transmit a signal about the presence of particles in medium to the cell interior. It is quite probable that this signal might directly reach the motile cytoplasm and trigger the propulsive mechanism, as well as the food vacuole formation mechanism.

REFERENCES

Brutkowska M. 1967: Immobilization effect of $NiCl_2$ and food vacuole formation in *Paramecium caudatum*. Bull. Acad. Pol. Sci., 15, 119–122.

Eckert R. 1972: Bioelectric control of ciliary activity. Science, 176, 473–481.

Grębecki A. 1965: Gradient stomato-caudal d'excitabilité des ciliés. Acta Protozool., 3, 79–100.

Jennings H. S. 1897: Studies on reactions to stimuli in unicellular organisms. I. Reactions to chemical, osmotical and mechanical stimuli in the ciliate infusoria. J. Physiol., 21, 258–322.

Kaczanowska J. 1979: Physiological dissection of various effects of ruthenium red dye on *Paramecium* cells. Experientia, 35, 1062–1064.

Kuźnicki L. 1963: Reversible immobilization of *Paramecium caudatum* evoked by nickel ions. Acta Protozool., 1, 301–312.

Machemer H. and Machemer-Röhnisch S. 1983: Tail cilia of *Paramecium* passively transmit mechanical stimuli to the cell soma. J. Submicrosc. Cytol., 15, 281–284.

Machemer H. and Ogura A. 1979: Ionic conductance of membranes in ciliated and deciliated *Paramecium*. J. Physiol., 296, 49–60.

Naitoh Y. 1974: Bioelectric basis of behavior in Protozoa. Am. Zool., 14, 883–893.

Ogura A. and Machemer H. 1980: Distribution of mechanoreceptor channels in the *Paramecium* surface membrane. J. Comp. Physiol., 135, 233–242.

Sikora J. and Jurand A. 1984: Possible role of cilia in the control of cytoplasmic streaming in *Paramecium tetraurelia*. Acta Protozool., 23, 93–105.

Sikora J. and Wasik A. 1978: Cytoplasmic streaming within Ni^{2+} immobilized *Paramecium aurelia*. Acta Protozool., 17, 389–397.

Sikora J., Wasik A. and Baranowski Z. 1979: The estimation of velocity distribution profile of *Paramecium* cytoplasmic streaming. Eur. J. Cell Biol., 19, 184–188.

Sikora J., Kubalski A., Wasik A. and Kuźnicki L. 1984: The role of mechanical stimuli in the control of cytoplasmic streaming in *Paramecium bursaria*. Postępy Biol. Kom., (in press).

Sonneborn T. M. 1970: Methods in *Paramecium* research. *In*: Methods of Cell Physiology 4, Academic Press, New York, 242–335.

Wasik A. 1983: Effect of external agents on cytoplasmic streaming in *Paramecium*. I. Influence of carmine suspension. Acta Protozool., 22, 183–189.

Wasik A. and Sikora J. 1984: Effect of external agents on cytoplasmic streaming in *Paramecium*. II. Influence of media free of suspension. Acta Protozool., 23, 107–113.

Wasik A., Sikora J. and Kuźnicki L. 1984: Effect of different suspensions on phagocytosis and cytoplasmic streaming in *Paramecium bursaria*. Postępy Biol. Kom., (in press).

ACTA PROTOZOOLOGICA
Vol. 23, No. 2, pp. 123–134 (1984)

Motive Force Generation Site in Plasmodium of *Physarum polycephalum*, a Dissection Study

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Received on 5 December 1983

Synopsis. Different cuts across the frontal zone and the network of veins demonstrate that the progression of frontal edge is faster when it is connected with a relatively larger system of veins. That speaks against considering the front as main source of the motive power. Frontal fragments separated from the network continue to move only if they contain some veins. Otherwise they stop until new veins differentiate inside them. New advancing edges are very easily formed along the lines of cutting which run across the frontal region. The undifferentiated protoplasm layer in the front seems to fulfil merely formative functions. It is concluded that the motive force of plasmodial locomotion is generated in every place where the protoplasm is differentiated into endoplasm and ectoplasm, i.e., along the whole system of ectoplasmic tubes as well in the network as inside the frontal region.

The well known dispute between the tail contraction (Goldacre and Lorch 1950) and frontal contraction (Allen 1961) theories of amoeboid movement had long no serious influence on the explanations given to the motile phenomena in slime moulds plasmodia. The classical contraction-hydrodynamic theory of Kamiya (1959) was in its main lines followed by all later authors (e.g. Jahn 1964, Stewart 1964, Nakajima and Allen 1965, Komnick et al. 1973, Wohlfarth-Bottermann 1975). According to that view the movement of the protoplasm along plasmodial veins is always directed toward the areas of lower pressure. The pressure differences inside the organism are due to the alternating contractions and relaxations of the walls of ectoplasmic tubes. Not much attention was paid to a possibility to localize the site of motive force generation only at one of the two opposite locomotion poles, either in the network of veins or in the frontal zone.

But the situation changed when Yoshimoto and Kamiya (1978) concluded that "...the active site of the rhythmic contraction and relaxation in the advancing plasmodium is restricted to its anterior zone...", whereas in the network of veins "...the radial activity of the strand(s) in situ is a passive phenomenon caused by the shuttle streaming..". The present authors, simultaneously and independently, came to the diametrically opposed concept that "...the entire system of major veins forming the network contracts synchronously..." and therefore "...general contraction of the network squeezes the protoplasm out toward the peripheral (= frontal) zone of the plasmodium..." (Grębecki and Cieślawska 1978). The present study was undertaken as an attempt to resolve that controversy by testing the effects of different dissections of plasmodia on their locomotion.

Special attention will be paid to the fact, often underestimated in the past, that the distinction between the network of veins and the frontal zone is not sharp. In fact the anterior region contains, besides the specific frontal material (the advancing ridge and the continuous protoplasm sheet), the very important system of channels and young veins.

Material and Methods

Small pieces of plasmodial mass from stock cultures were put on non-nutrient agar in Petri dishes. The experiments started 48 h later, when they turned out into plasmodia several cm long. The frontal and rear zones of plasmodia were dissected in various configurations. Three methods of dissecting were used: (1) cutting with a blunt needle which produced a shallow furrow on the surface of agar (as in Pl. I 3), (2) dissecting plasmodium together with the whole agar layer and pulling the excised fragment slightly apart (e.g., Pl. I 2); (3) effacing a part of plasmodium from the surface of agar (as in Pl. VI 12) or effacing a large stripe between two parts to be separated from one another (as in Pl. III 7).

The reactions of plasmodia were recorded, depending on the kind of experiment, either by time lapse filming with 16 mm camera, for 10 min before the operation and 10 min after it, or by taking a sequence of 6–8 photomacrographic still pictures at the intervals of 20 min between them (> 100 of the total observation time). Both these types of documentation were used to control the velocity of frontal progression of the dissected parts of plasmodia and to follow the changes in their configuration and orientation of movement. Moreover, the time lapse film records served to produce by photometric technique (Grębecki and Cieślawska 1978) the pulsation curves at several points along the veins, before and after cutting.

Results

Forward Movement of the Dissected fronts

The preliminary experiments, in which plasmodia were transversally cut across the network of veins behind the frontal zone, gave no con-

sistent results. The frontal velocities were measured during 10 min before and 10 min after the operations, on the film records. Sometimes a decrease and sometimes an increase of the frontal progression was obtained. But such activity fluctuations between two consecutive recording periods may be stated in the intact plasmodia as well. The experiment is thus not conclusive, because of the lack of a reliable control.

Such a control was ascertained in the next experiments by dissecting the frontal zone in such a way as to leave a part of it in connection with the network of veins. Then, the behaviour of the amputated fragments might be compared to that of the intact parts. Either just 1/2 of a front was unilateraly cut off (Pl. I 1, 2), or nearly 1/4 of the frontal mass was excised symmetrically on each side (Pl. I 3). The cuts were so deep, that the isolated fragments always contained the well developed young veins. The effects were recorded as well by filming (10 min), as by taking serial photomacrographs ($>$ 100 min).

Two opposite effects of such dissections might be expected, depending on which hypothetical localization of the motive force our previsions were based. If the protoplasm is pumped into the front by active contractions of the network of veins, the isolated fragment should slow down, while the remaining parts of frontal zone, still connected with the whole network, should receive more protoplasm than before and advance faster. And conversely, if the whole plasmodial mass is in some way dragged behind the active front, the amputated fragment should accelerate being relieved of the ballast, whereas the remaining parts of the frontal edge should be more hampered in their progression, than before cutting.

The most typical results are shown in the Plate I. It is clearly seen that the amputated half of a branched front (Pl. I 1) moves slower than before the operation and its remaining part, which is still connected with the network of veins, distinctly accelerates. In a fan-like frontal zone (Pl. I 2) the excision of its evidently more active half (lower part in a) leads very quickly to the massive protoplasm influx to the latent half (b) followed later on by its vigorous forward movement (c–d). The fan-like fronts also accelerated after symmetrical cutting out their side lobes, whereas both lateral amputated fragments were moving slower than before (Pl. I 3).

After all these types of operations the slowing down of amputated fragments was practically always stated, and the simultaneous acceleration of the intact parts occurred in a large majority of cases. Some of the exceptions were due to the polarity reversal of whole plasmodia, i.e., to the formation of new fronts in their rear regions (see Pl. II 5 and IV 9).

Role of Young Veins Inside the Frontal Zone

In next experiments the trapezoid fragments were cut out in the frontal zones, in three variants: (1) deep cuts producing fragments composed of frontal edge and continuous protoplasm layer with the young newly differentiated veins (Pl. II 4), (2) intermediate cuts without differentiated veins (Pl. II 5), (3) shallow cuts limited to the frontal ridge only (Pl. III 6). The objective was to test the role of channels and veins which are usually present in any larger fragment separated from the frontal zone.

The example given in Pl. II 4 demonstrates that the fragment which contains some veins (a) does not interrupt its forward motion after cutting it off (b) and, although some "hesitations" are possible (as in c), it eventually produces a completely differentiated plasmodium in miniature which advances slower than the mother organism (d–f). Its development was easily accomplished within the time limits of present observations.

When the cut contains only the frontal edge and a portion of the continuous protoplasm sheet without differentiated veins (as in Pl. II 5), it usually retracts after being excised (a–b) and does not move (c–d), until veins develop inside it. The differentiation of new channels and veins, and the start of locomotion, sometimes began at the end of the standard observation period but usually needed a longer lapse of time.

The most shallow cuts, limited to the frontal ridge alone (Pl. III 6), were never observed to move.

The necessity of veins and/or channels to support locomotion of the fragments cut out from the frontal zone is also confirmed by the reorganization taking place in the frontal zones which were separated as a whole from the plasmodial network (Pl. III 7). The wide continuous protoplasm sheet in a fan-like front (as seen in a) after cutting undergoes fragmentation and drastic reduction in size, in favour of the veins which share in the total volume is conspicuously increasing (b–d).

Reorganization and Reorientation after Dissections

There are two typical reactions of the network veins in response to the complete ablation of the frontal zone (Pl. IV 8). First of them is the leakage of protoplasm from the veins which were wounded (empty circles), the second one — formation of new fronts inside the network (arrowheads). Most usually (but not always) the leakage is slower than the development of new fronts in other places. Therefore, the networks

deprived of their former leading fronts very often change the direction of locomotion, or at least temporarily move in two divergent directions.

Formation of new fronts inside the network of veins was sometimes also seen after operations which only reduced the volume of the original frontal zone, without removing it completely. For instance, after amputation of both lateral lobes of the fan-like front (Pl. IV 9) a new front developed in the slightly bulged posterior segment of the main vein of the network (arrowheads in b–c). Excision of the central part of frontal zone, shown in Pl. II 5, brought similar effects. At the beginning the massive protoplasm inflow toward the wound was observed (b), but later the new fronts developed directing the plasmodium along divergent paths (c–d).

Simultaneously, the protoplasm is seen to leak out also from the cut border of the amputated frontal zone, provided that it contained the differentiated veins (Pl. IV 8, empty circles). It often results in temporary hampering the progression of the excised frontal fragment, or even in formation of a competitive new front which reorients the locomotion back, toward the mother plasmodium (Pl. IV 10).

The most spectacular changes of the direction of movement are shown in Pl. V 11. The rear part of a plasmodium was completely removed, but the whole front was left on the surface of agar together with a large major vein which formerly supplied it with the protoplasm (a–b). At the beginning the ouflow of protoplasm toward the place of injury did not suppress the advancing of untouched front in the original direction (c–d). Then, the "competition phase" came at which two mutually opposed fronts were simultaneously active (e–f). The process was accomplished when the plasmodium definitely adopted and followed the new reversed direction of locomotion (g–h).

It seems, however, that in general the cuts made across the frontal region are particularly capable to reorient locomotion by creating more rapidly a new advancing edge at the place of injury. The cuts across network veins lead to the endoplasm leakage which is slower and less efficient than the differentiation of new fronts. That conclusion, drawn from the experiments described above, is supported by the results of operation shown in Pl. VI 12. A part of small compact plasmodium was effaced from the surface of agar in such a manner that the plane of injury (dashed line) run across the advancing frontal zone and continued along the veins network (a–b). The protoplasm leakage from the posterior cut veins (empty circles in c–d) had no impact on locomotion, whereas the frontal region formed a new dominant advancing edge along its wounded side (arrows in c–g).

Synchronism of Veins Pulsation after Dissection of Fronts

The pulsation curves were photometrically plotted from the cinematographic records, in 2–3 veins for 10 min preceding the operation and 10 min following it. This procedure was applied only to the experiments leading either to the complete ablation of the whole frontal zone, or to the removal of about a half of frontal mass (unilaterally or symmetrically from both sides).

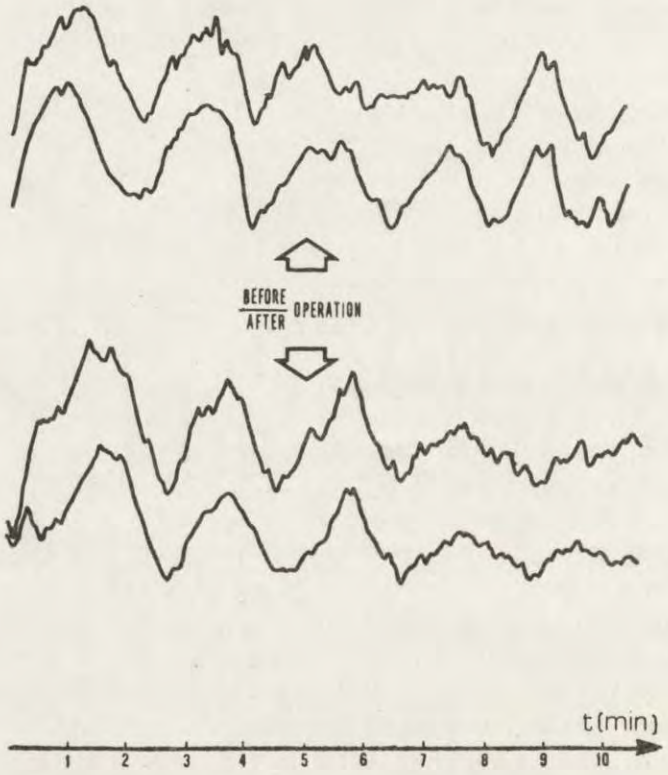


Fig. 1. Pulsation curves photometrically recorded at two control sites in the network of veins, before and after the operation which led to unilateral removal of a half of the frontal zone. Note that the synchronization of the contraction--relaxation cycles between both sites does not disappear after the operation

The complete or partial removal of the frontal zone was never observed to result in any appreciable deterioration of the synchronism of veins pulsation in the remaining part of plasmodium. Figure 1 brings an example of pulsation cycles recorded at two points in the network, prior to the operation and after it, in a plasmodium from which about a half of the frontal zone was unilaterally excised.

Discussion

The direct discussion of the conclusions of Yoshimoto and Kamiya (1978) is extremely difficult, because they were not precisely formulated in the terms of force generation site and manner of force transmission to other areas, which were supposed to be passive. Therefore, they may be understood in two different ways. (1) The motive force is generated only inside the frontal zone and transmitted to the network veins as negative hydrostatic pressure (suction) or mechanical tension (pulling); if so, the protoplasm inside the network is a passive ballast dragged behind the active front; this interpretation is analogous to the frontal contraction theory of amoeboid movement (Allen 1961). (2) It may be also understood that the network veins do generate the motive force, but it produces only a steady tension in them, whereas the front contains the modulating system, being therefore responsible for the control of movements and for their oscillatory character; if so, the network in fact is not passive, but works under the frontal control; this second interpretation would be more close to the generalized cortical contraction theory of amoeboid movement (Grębecki 1982).

The present experiments were planned to check the first interpretation, according to which the front is the only region producing the motive force in a complete locomoting plasmodium. The results speak against the validity of that concept. But the second interpretation, which attributes to the front the control functions and makes it responsible for the oscillatory manifestation of veins contraction in the intact plasmodium in situ, cannot be at present neither definitely rejected nor proved true. It needs further experimental testing.

The most general conclusion to be drawn from the present experiments is that the distinction between the network of veins and the frontal zone is so rough and unprecise that it is more misleading than helpful in resolving the question of localization of the motive force in a locomoting plasmodium. It should not be forgotten that the frontal zone, besides the advancing edge and the continuous protoplasm sheet, contains also the channels and veins, at very different stages of their differentiation, development and recoil. The present results strongly indicate that the motive force is generated everywhere along the veins system, as well in the posterior and central regions of plasmodium, where they form the network, as inside the frontal zone.

It means in other words that the motive force is generated in any place, where the protoplasm is differentiated into the gelated ectoplasm and solated endoplasm, and where the endoplasm is surrounded by contractile ectoplasmic tube walls. And on the contrary, any place where that differentiation is not yet accomplished (i.e., the frontal ridge and

the adjacent continuous protoplasm sheet) probably plays rather the formative than motor functions.

It seems that such a concept is well applicable to the interpretation of phenomena which were encountered in the present research:

(1) The protoplasm inside the network of veins cannot be considered neither as a passive material just sucked or pulled by the frontal activity (as it might be inferred from the statements of Yoshimoto and Kamiya 1978), nor as the only source of the motor power (as it can be concluded from our own earlier simplified scheme: Grębecki and Cieślawska 1978). According to the first concept the frontal zone should be enhanced, and according to the second one it should be hampered in its progressive movement, after being separated as a whole from the plasmodial network. But the results of such experiments were not conclusive, because both opposite working hypotheses localizing the motive power only at one of the two locomotion poles, were in the same degree inexact and oversimplified.

(2) When in the same plasmodium only one half of the frontal zone is separated from the network, whereas the other half is kept in connection with the rest of plasmodium, the movement of the isolated part is relatively suppressed in respect to the motility of the intact one, which is usually increased. It is easily explained by the fact that the share of the organized veins, which produce the motive force and actively supply the front with the endoplasmic material, becomes unequally redistributed between the two approximately equal parts of the dissected advancing edge. So, the motor functions of the ectoplasmic walls of all the veins in the whole plasmodium account for more clear effects of that type of dissections.

(3) The motor role of all the veins and channels accords well with the evidence that there is an internal overpressure along the whole veins system. It is demonstrated by the immediate protoplasm outflow after cutting them in any place, and by the fact that the protoplasm is then evacuated in both directions from both endings of each intersected vein. The same is proved by producing endoplasmic drops from the veins subject to puncture (Isenberg and Wohlfarth-Bottermann 1976, Baranowski 1980). But such drops are not obtained when the undifferentiated frontal protoplasm layers are punctured (Cieślawska, unpublished).

(4) The motor autonomy of the network veins is demonstrated by the fact (already earlier stated e.g., by Winer and Moore 1941, Wohlfarth-Bottermann 1975, Hülsmann and Wohlfarth-Bottermann 1978, Cieślawska 1980, Kołodziejczyk and Grębecki 1982) that they do not interrupt pulsation after being separated from the front, provided they are left after operation in situ,

on the substrate. Moreover, as indicated by the present experiments, they keep the synchronous pulsation pattern which indicates that also the oscillation regulating system may be omnipresent (being for instance metabolic, as recently proposed by Korohoda et al. 1983).

(5) The internal pressure in the network of veins surgically deprived of any front should then be raising, because of the lack of any way open to the endoplasm outflow. The leakage through the cut veins' endings is stopped very quickly, probably by rapid formation of new membrane. The endoplasm leaking from cut veins seems to be analogous to the naked endoplasmic drops which form the membrane within 6–30 s, but need 20–40 min to reconstruct the internal architecture enabling the rhythmic contractions (Isenberg and Wohlfarth-Bottermann 1976, Baranowski 1980). Therefore, the overpressure inside the "decapitated" veins region favours the development of new fronts scattered inside the network, as described in the present study. It is possible that they arise at the sites which were predisposed to front formation by their structure and/or internal ionic conditions. Formation of new fronts inside the network could be also induced by suppressing the activity of the original front by the blue light (Cieślawska, unpublished).

(6) The motor role of young channels and veins inside the frontal area is demonstrated by several observations: (a) all the fragments excised from that zone do not interrupt their progression if they contain the young veins, (b) their post-operational reorganization consists in raising the share of the veins in the total mass of the fragment, (c) fragments which contain the frontal ridge alone or with the adjacent continuous protoplasm sheet (but without organized veins or channels) are incapable to move, (d) their motility may be restored much later, after their constituent material differentiates into ectoplasm and endoplasm and forms ectoplasmic tubes. It should be noted that the active frontal fragments described by Yoshimoto and Kamiya (1978) corresponded in their composition and behaviour to the situation described under a.

(7) The cuts made across the frontal zone which contains the differentiated channels as well as the undifferentiated continuous protoplasm layer rapidly and intensely develop into the new advancing edges (instead of the simple leakage of endoplasm demanding a long reorganization time). It may be explained by the abundance, at the place of injury, of the protoplasmic material which already before cutting was well advanced in the redifferentiation process, necessary to build up a new front. Perhaps, the internal ionic conditions may also favour the front formation. It seems that the frontal continuous protoplasm sheet plays not the motor but the formative functions.

(8) When a new front is formed owing to the process discussed under 7, it starts competition with the old one. Usually, at the competition phase the system of veins linking two opposite fronts is not slackening nor torn in two pieces, but on the contrary, visibly reinforced. It speaks against the possibility of pulling the material in two directions by the two advancing poles, but in favour of the coherent force generation by the whole system of veins between the two frontal edges.

The main conclusion from the present study points to the basic parallel of most fundamental structural feature of motor machinery in slime moulds plasmodia and in amoebae. According to the generalized cortical contraction theory of amoeboid movement (Grębecki 1982) the motive force is generated along the whole ectoplasmic cylinder, it creates the internal overpressure, and then the local pressure drops in the fronts of advancing pseudopodia may induce, control and steer the endoplasmic streaming and cell locomotion. It seems that in *Physarum polycephalum* as well, the motive force is generated everywhere along the ectoplasmic tubes, in each body region, as well by the network veins as by the frontal channels. The formative functions of the second component of the frontal zone: the undifferentiated protoplasm sheet, obviously mean that by adding new segments to the ectoplasmic tubes system the front controls the direction and rate of locomotion. The first indications are also available (Kołodziejczyk and Grębecki 1983) that in *Physarum polycephalum*, as in *Amoeba proteus* (Grębecki 1981), the frontal zone is the area most sensitive to photic stimulation, being hampered by the contracting stimuli and enhanced by the relaxing ones. The basic concept that the ectoplasmic walls play the motor role everywhere they are present, while the steering functions are performed by the fronts, seems to be applicable equally well to both these motile systems.

Nevertheless, the differences between them should not be neglected. Besides the well known list containing the differences in size, geometrical complication, extension of the substrate attachment, oscillatory character of contraction and streaming phenomena, one difference more should be particularly stressed in the light of the present results. The endoplasm-ectoplasm conversion in amoeba is very quick and restraint to a narrow zone, whereas in plasmodium the reorganization seems to take much longer time and needs a large territory of undifferentiated protoplasm sheet. It complicates the morphology of the frontal part of plasmodium and makes more difficult to distinguish the formative and locomotory aspects of its functions.

REFERENCES

Allen R. D. 1961: A new theory of amoeboid movement and endoplasmic streaming. Exp. Cell Res., (Suppl.), 8, 17–31.

Baranowski Z. 1980: Kinetics of the regeneration of rhythmic contraction activity in *Physarum polycephalum* drops. Acta Protozool., 19, 67–76.

Cieślawska M. 1980: Dynamics of the ending veins in plasmodia of *Physarum polycephalum*. Acta Protozool., 19, 143–152.

Goldacre R. J. and Lorch I. J. 1950: Folding and unfolding of protein molecules in relation to cytoplasmic streaming, amoeboid movement and osmotic work. Nature, 166, 487–499.

Grębecki A. 1981: Effects of localized photic stimulation on amoeboid movement and their theoretical implications. Eur. J. Cell Biol., 24, 163–175.

Grębecki A. 1982: Supramolecular aspects of amoeboid movement. In: Progress in Protozoology, Proc. VI Int. Congr. Protozool., part I, 117–130.

Grębecki A. and Cieślawska M. 1978: Plasmodium of *Physarum polycephalum* as a synchronous contractile system. Cytobiologie, 17, 335–342.

Hülsmann N. and Wohlfarth-Bottermann K. E. 1978: Spatio-temporal relationships between protoplasmic streaming and contraction activities in plasmodial veins of *Physarum polycephalum*. Cytobiologie, 17, 317–334.

Isenberg G. and Wohlfarth-Bottermann K. E. 1976: Transformation of cytoplasmic actin. Importance for the organization of the contractile gel reticulum and the contraction-relaxation cycle of cytoplasmic actomyosin. Cell Tiss. Res., 173, 495–528.

Jahn T. L. 1964: Protoplasmic flow in the mycetozoan, *Physarum*. II. The mechanism of flow; a re-evaluation of the contraction-hydraulic theory and of the diffusion drag hypothesis. Biorheology, 2, 133–152.

Kamiya N. 1959: Protoplasmic streaming. In: Protoplasmatologia VIII 3a, Springer-Verlag, Wien, 1–199.

Kołodziejczyk J. and Grębecki A. 1982: Further studies on the relation between contraction and streaming oscillations in the plasmodial veins of *Physarum polycephalum*. Acta Protozool., 21, 37–53.

Kołodziejczyk J. and Grębecki A. 1983: Effects of white-red illumination changes on the coordination of some motor functions in plasmodia of *Physarum polycephalum*. Acta Protozool., 22, 19–31.

Komnick H., Stockem W. and Wohlfarth-Bottermann K. E. 1973: Cell motility: mechanisms in protoplasmic streaming and ameboid movement. Int. Rev. Cytol., 34, 169–249.

Korohoda W., Shraideh Z., Baranowski Z. and Wohlfarth-Botterman K. E. 1983: Energy metabolic regulation of oscillatory contraction activity in *Physarum polycephalum*. Cell Tiss. Res., 231, 675–691.

Nakajima H. and Allen R. D. 1965: The changing pattern of birefringence in plasmodia of the slime mold, *Physarum polycephalum*. J. Cell Biol., 25, 361–374.

Stewart P. A. 1964: The organization of movement in slime mold plasmodia. In: Primitive Motile Systems in Cell Biology, Academic Press, New York, 69–78.

Winer B. J. and Moore A. R. 1941: Reactions of the plasmodium *Physarum polycephalum* to physico-chemical changes in the environment. Biodynamica, 3, 323–345.

Wohlfarth-Bottermann K. E. 1975: Tensiometric demonstration of endogenous, oscillating contractions in plasmodia of *Physarum polycephalum*. Z. Pflanzenphysiol., 76, 14–27.

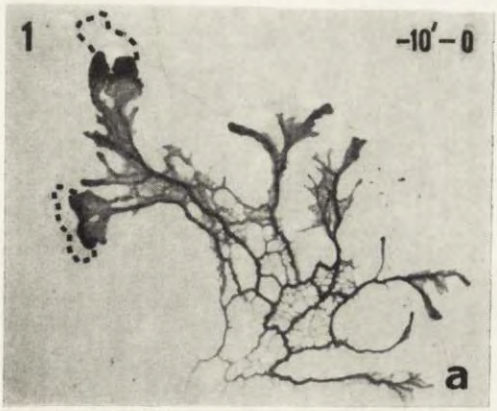
Yoshimoto Y. and Kamiya N. 1978: Studies on contraction rhythm of the plasmodial strand. IV. Site of active oscillation in an advancing plasmodium. Protoplasma, 95, 123–133.

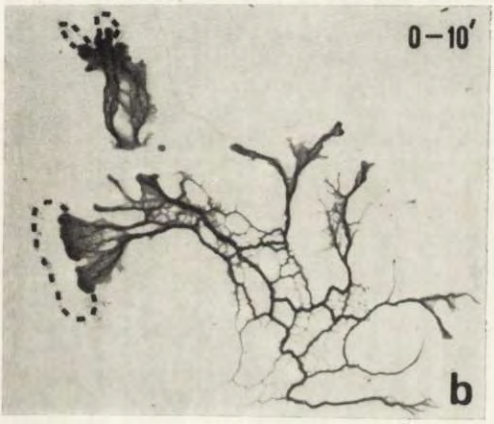
EXPLANATION OF PLATES I–VI

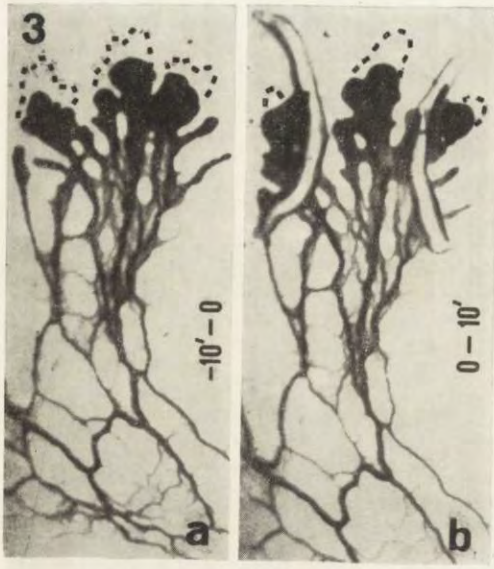
1: Separation of one half of the branched front by effacing its junction with the rest of plasmodium. Note the frontal velocity decreasing after operation in the isolated fragment and increasing in the part kept in connection with the network
2: Removal of a half of the fan-like front by deep cutting and pulling the excised fragment apart. It leads to the massive protoplasm influx to the intact part of the frontal edge and increase of its progression rate
3: Removal of a half of the frontal volume by two symmetrical cuts with a blunt needle on both sides of the front region. Note the velocity decrease in both isolated lateral fragments and increase in the central area which is kept in connection with the network veins
4: Excision of a frontal fragment containing the young organized veins. Note its locomotor capability and rapid transformation in a complete small plasmodium
5: Excision of a frontal fragment containing only the frontal edge with the undifferentiated protoplasm layer. Note its slight retraction and resting condition during the remaining part of observation time
6: Excision of a fragment of the frontal ridge alone. Note the lack of any activity of the excised piece
7: Separation of the whole frontal zone by effacing a stripe between it and the network of veins. Note the reduction and fragmentation of the continuous protoplasm sheet and the increase of veins during reorganization of the isolated front
8: The network region of the same plasmodium at the stage shown in 7 d. Note the locomotor inefficiency of the endoplasm leaking from the intersected veins and the development of new fronts inside the intact areas of the network of veins
9: Development of a new front from bulged segment of the network vein after symmetrical ablation of a half of the original frontal region
10: Reversal of motor polarity in a frontal fragment containing young veins. It leads to reorientation of its locomotion back toward the mother plasmodium
11: Effects of effacing the whole posterior network part of a plasmodium with thick compact front. Note the development of new front by a large intersected vein, its competition with the original one, and the eventual change of the direction of locomotion
12: Effacing a part of plasmodium along the cutting line running across the frontal zone and across the network. Note the vigorous new advancing margin developed from the cutting line on the frontal territory and the motor inefficiency of endoplasm leaking from the intersected network veins

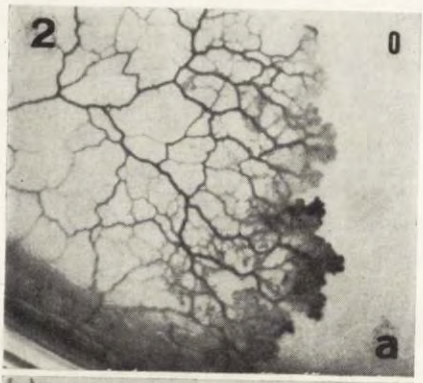
Symbols used in Plates

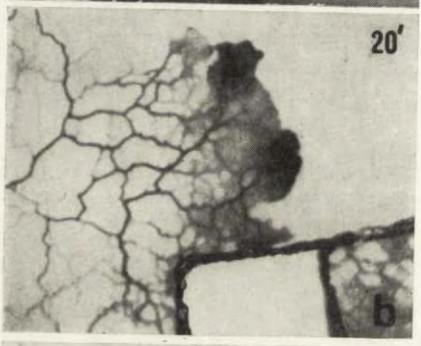
Timing in min is shown in all pictures, taking for 0 the stage just before the operation or just after it; any longer time periods before the operation are shown in negative values. Dashed lines in Pl. I mark the position of frontal margin 10 min after the moment of taking the picture, in Pl. V and VI they show the lines of cutting. Arrows indicate the direction of movement of frontal edges and asterisks the standstill. Arrowheads point to the new fronts formation sites, and the empty circles mark the sites of endoplasm leakage from wounded veins.

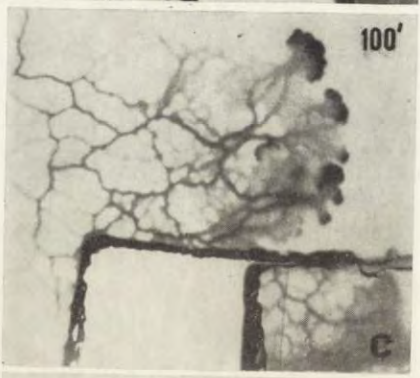


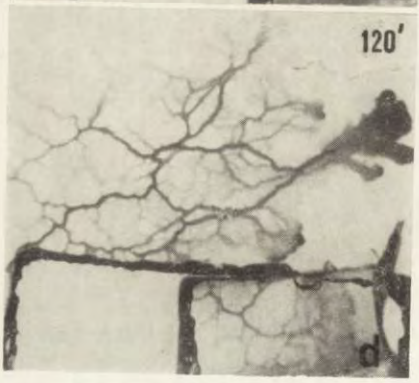












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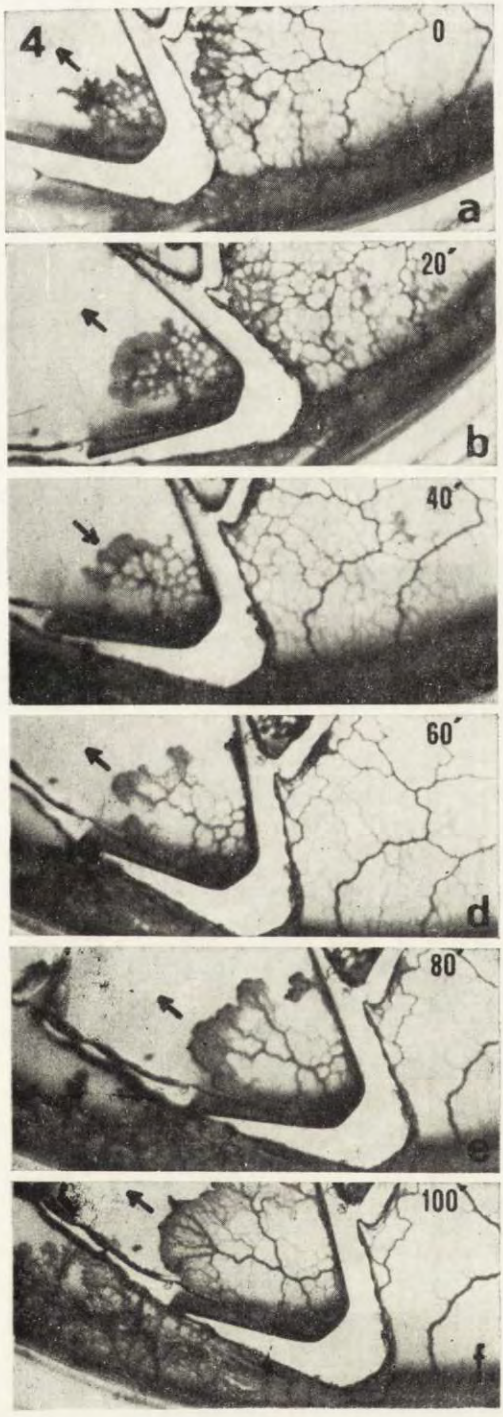

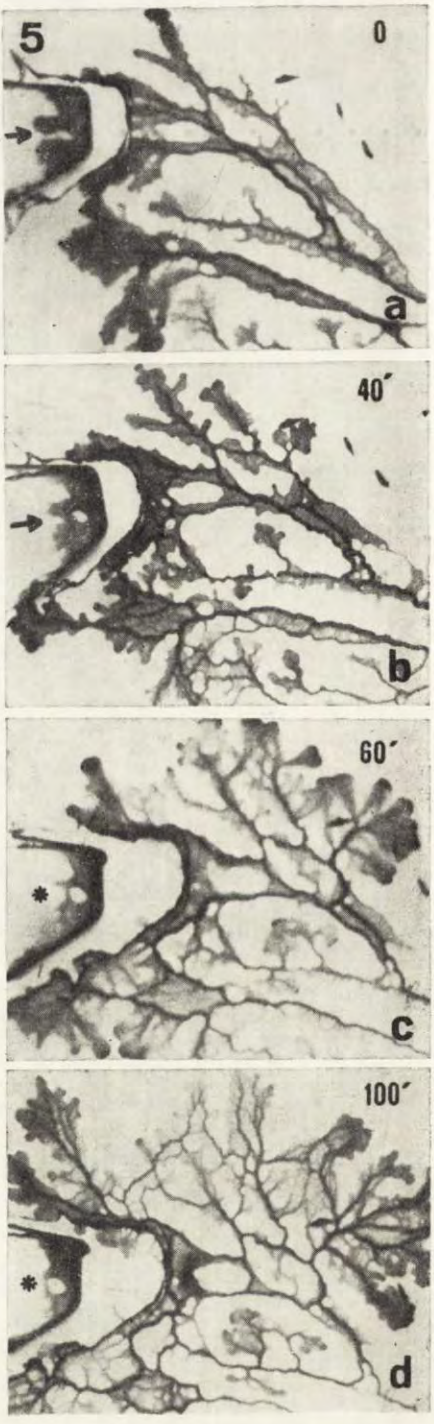

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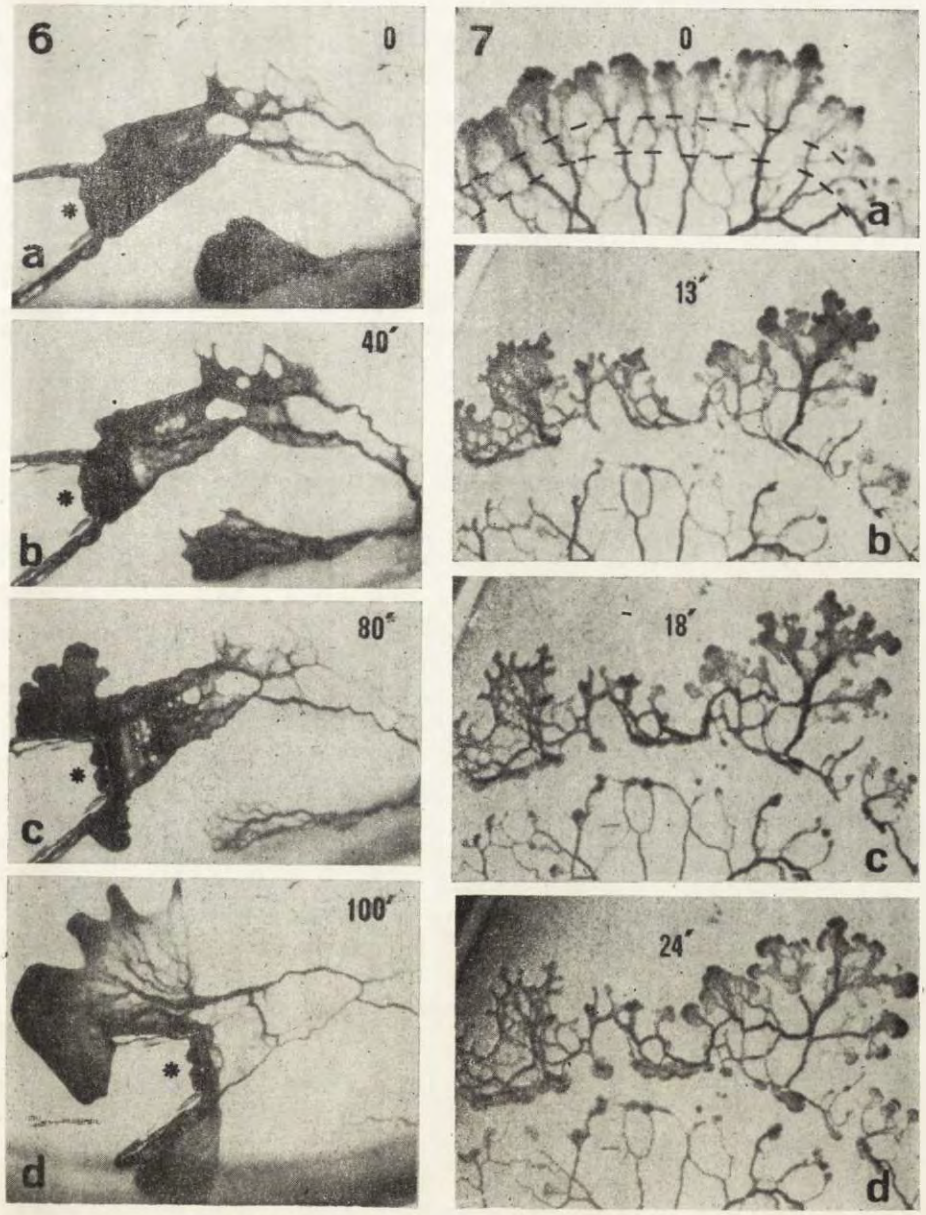

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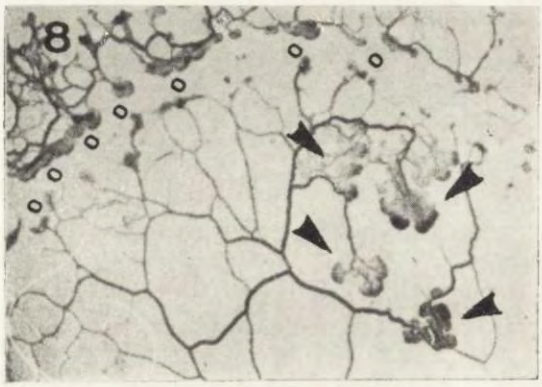

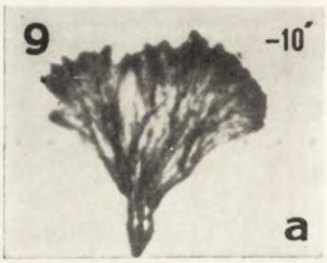

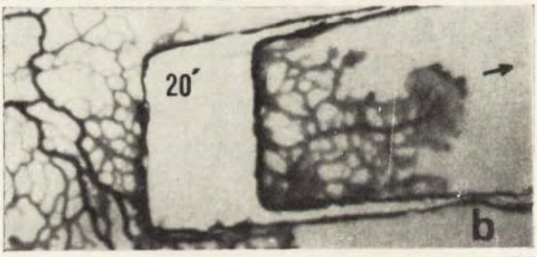

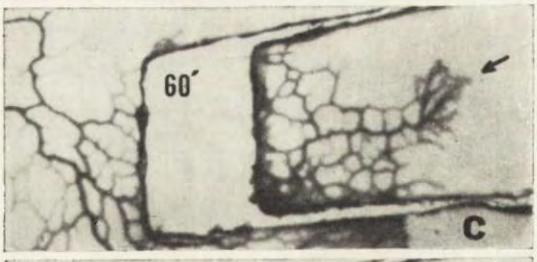

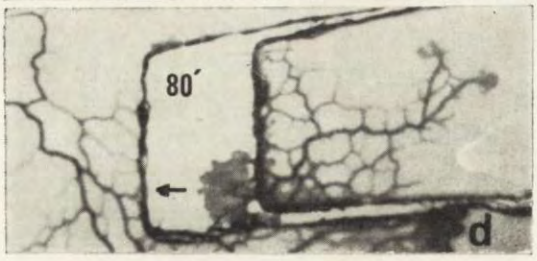

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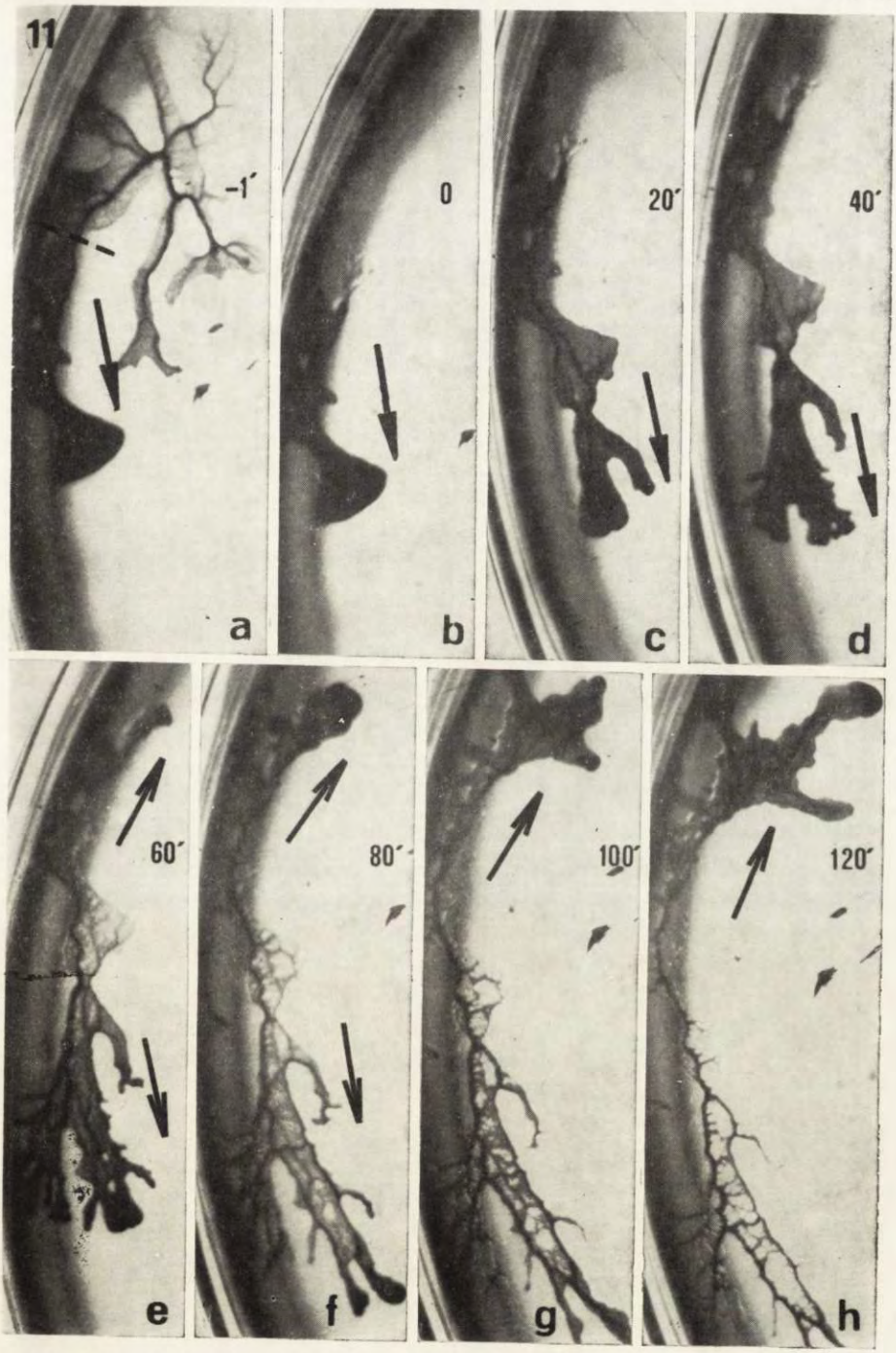

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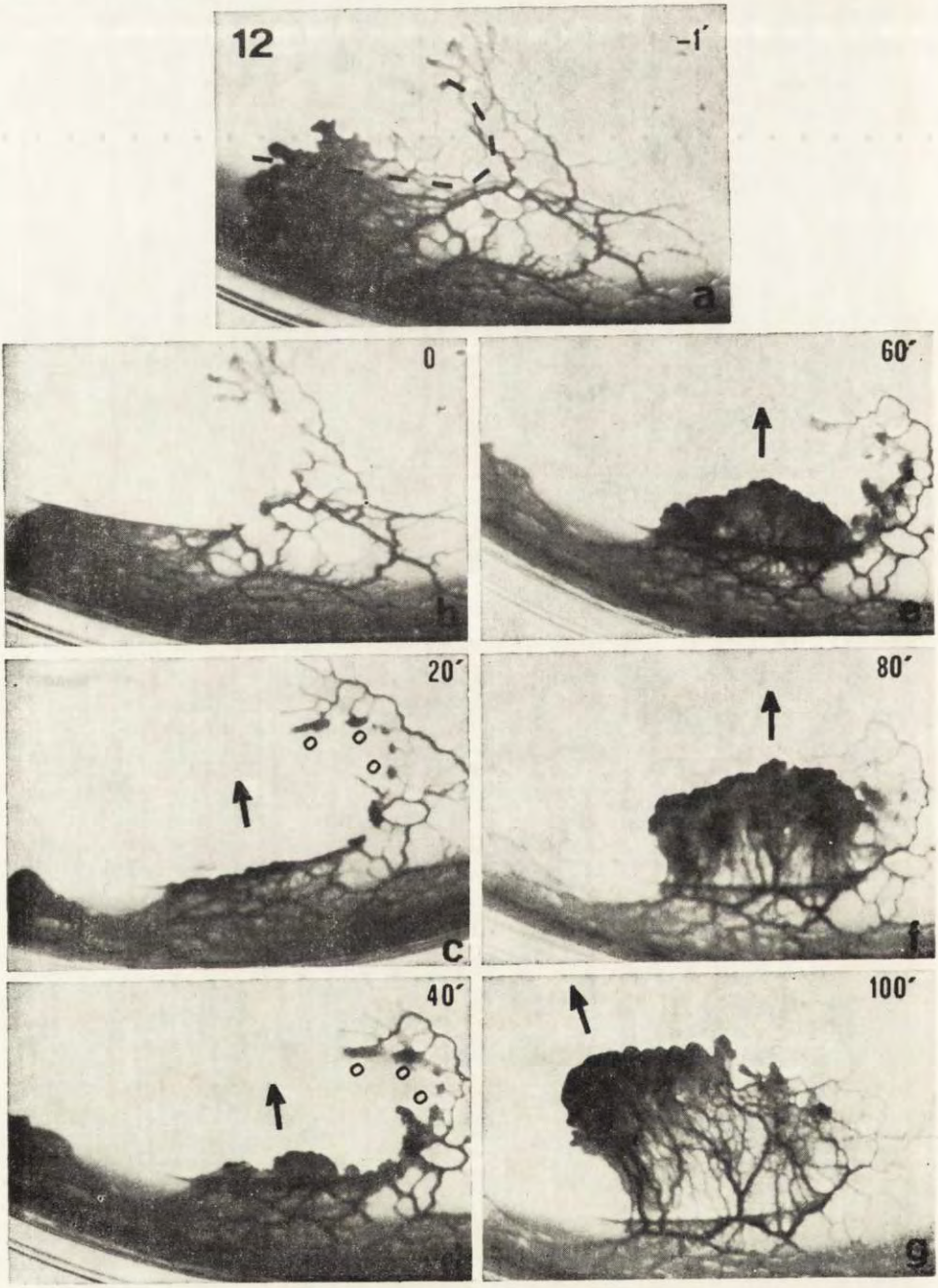

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ACTA PROTOZOOLOGICA
Vol. 23, No. 2, pp. 135–144 (1984)

Two New Species of Myxosporidians, *Chloromyxum tripathii* sp. n. and *Chloromyxum mitchelli* sp. n. from Fish *Therapon jarbua* (Forsskal)

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Received on 19 September 1983

Synopsis. Two new species of myxosporidians: *Chloromyxum tripathii* sp. n. and *C. mitchelli* sp. n. parasitic in the gall bladder and kidney of fish *Therapon jarbua* are described respectively. The seasonal distribution, prevalence and histopathology of *C. tripathii* are studied.

During the course of a survey of the local fresh water fishes for myxosporidian parasites, a total number of 296 *Therapon jarbua* (fam: *Theraponidae*) ranging in size from 7.5–12.0 cm, purchased from the local fish market were examined and 81 of them were infected with a myxosporidian parasite belonging to the genus *Chloromyxum* in the gall bladder and eight others harboured a different species of the parasite in the kidney. Other genera and species of the fish occurring in the same locality did not harbour either of the parasites. The two species are morphologically different and showed both host and organ specificity. The morphology and life-history of both species are reported in the present communication. The prevalence, seasonal distribution and histopathology of the parasite occurring in the gall bladder are also studied.

Material and Methods

The fish *Therapon jarbua* (Forsskal) was obtained from the local fish market which receives its supply from catches from different localities in and around Visakhapatnam (Andhra Pradesh, India). Fishes as soon as they were brought to the laboratory were dissected and all the internal organs were examined for myxosporidian parasites. Air dried smears were fixed in acetone-free methyl

alcohol and stained with Giemsa. Some of the smears were wet-fixed in hot Schaudinn's fluid (70°C) and stained with Heidenhain's iron haematoxylin. Bits of infected tissue were fixed in alcoholic Bouin's fluid or Carnoy's fluid or 10% formalin. They were sectioned at 8 μm thickness and stained with Heidenhain's iron heamatoxylin or Azocarmine or Mallory's triple stain or according to Feulgen's technique to study the developmental stages and histochemical and pathological changes. Aqueous saturated urea was used to release the polar filaments. All measurements were made from material suspended in normal saline and the figures are drawn with the aid of a camera lucida.

Observations

Chloromyxum tripathii sp. n.

Host: *Therapon jarbua* (Forsskal)
Site of infection: Gall bladder
Locality: Visakhapatnam (A. P., India)
Type slides: Author's collections and Dept. of Zoology, Andhra Univ.

Diagnosis

Trophozoites attain a maximum size of 48.0×42.0 μm with 16 generative nuclei and a number of somatic nuclei. Trophozoites generally formed of 2–4 disporous sporonts which were free in the bile. Spores spherical having a diameter of 10.8–12.0 μm. Sutural ridge straight. Spore wall smooth having 3–4 parietal folds at the capsular end. Four polar capsules of equal size measuring 4.2–5.0 μm. Sporoplasm hat-shaped located underneath the polar capsules. Polar filaments show 5–6 coils and coiled in opposite directions in adjacent capsules. They are uniformly thin and measure 28–34 μm in length.

Life Cycle

The earliest stage of the development of the parasite observed in smears prepared from the contents of the gall bladder was a plasmodium measuring 10.0–12.6 μm containing 6–12 vesicular nuclei each having 1–2 deeply stained chromatin granules (Fig. 1 *1*). The trophozoites attained a maximum size of 48.0×42.0 with as many as 16 generative nuclei and a number of somatic nuclei. The somatic nuclei were smaller in size and contained a single deeply stained centrally placed dot-like chromatin granule. The cytoplasm was filled with a number of refringent granules (Fig. 1 *2*). Trophozoites were generally formed of 2–4 disporous sporonts which are free in the bile. Occasionally isolated disporous sporonts were also seen in the smears. They were irregular in shape and contained 16 somatic and 4 generative nuclei (Fig. 1 *3*).

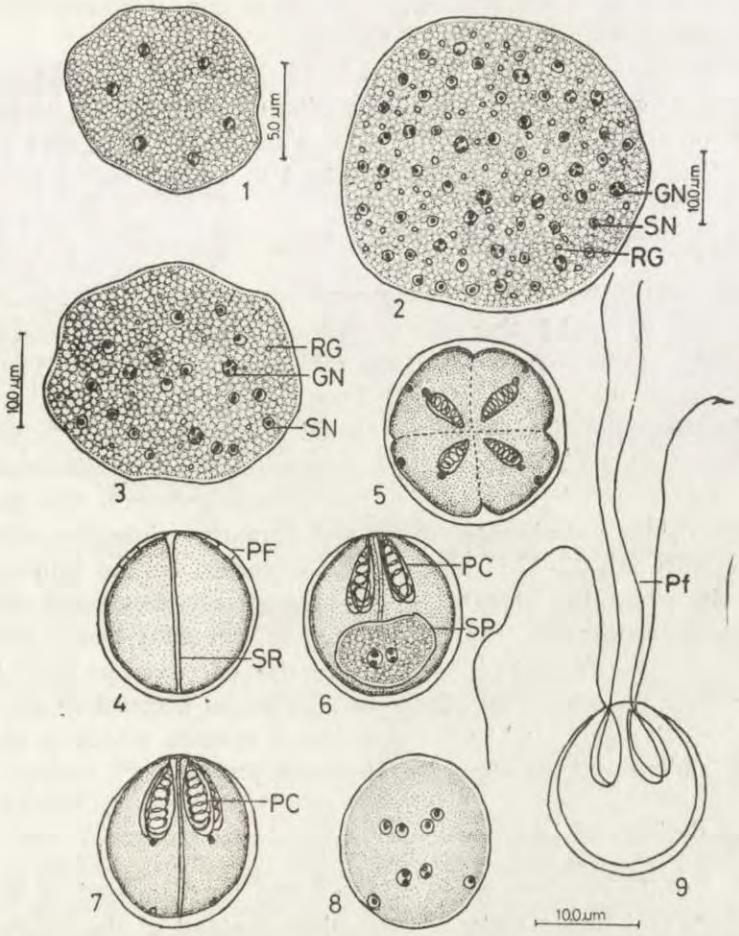

Fig. 1. *Chloromyxum tripathii* sp. n. *1* — An early plasmodial stage, *2* — A mature trophozoite, *3* — A disporous sporont, *4* — Fresh spores, *5* — Spore stained with Giemsa (polar view), *6* — Spores stained with Giemsa (side view), *7* — Spore stained with iron haematoxylin, *8* — A sporoblast stained according to Feulgen's technique, *9* — Spore with extruded polar filaments

Spores were spherical having a diameter of 10.8–12.0 µm (average 11.2, n=50). The sutural ridge was straight. The spore wall was smooth having 3–4 parietal folds at the capsular end which were more apparent in a side view (Fig. 1 *4*). The four polar capsules were similar in size measuring 4.2–5.0 µm (average 4.8, n=50)×2.1–2.6 (average 2.4, n=50) (Fig. 1 *5*). In a side view two of the polar capsules appear to be superimposed over the other two (Fig. 1 *6*). The hat-shaped sporoplasm measuring 6.5×3.0 µm was located underneath the polar capsules and sometimes extends into the intercapsular region. The polar filaments show 5–6 coils and are coiled in opposite directions in adjacent capsules (Fig. 1 7). Developing sporoblasts stained with Giemsa clearly show two

centrally placed sporoplasmic nuclei and four capsulogenous nuclei and four valvulogenous nuclei (Fig. 1 *8*).

The polar filaments were released in 60–70% of the spores by adding a drop of saturated urea. The polar filaments range in length from 23–34 μm (average 32, n=50). They were uniformly thin and were extruded at the same point in the spore (Fig. 1 *9*).

Seasonal Distribution and Prevalence

Eighty one of the 296 fishes examined showed infection. Sexually mature forms showed higher prevalence of infection when compared to the sexually immature forms. Both males and females were infected (48/146 females and 26/78 males). The prevalence increased with the age of the fish. Smaller fishes examined during the months May–July did not show any infection. Young trophozoites could be observed in the months August and September. The percentage infection was maximum during the colder months December and January when the water temperatures were ca. 23–27°C and slowly decreased in the hotter months March–July when the stream was comparatively dry and the water temperatures reached a maximum of 34°C in May–June period. As suggested by Mitchell et al. (1980) the decrease in prevalence of infection during summer months may also be on account of the regular emptying of the gall bladder in the winter months which is the active period of feeding and breeding for *Therapon jarbua*.

Histopathology of Infection

Infected gall bladders were enlarged and hard instead of being soft and resilient and had a dirty brown colour instead of the normal moss-green colour. Microscopic examination of the contents of the gall bladder showed bits of the epithelial tissue and the growing trophozoites freely floating in the bile. Sometimes a few refringent granules were seen in the trophozoites. Groups of trophozoites adherent to the epithelial cells were also observed (Fig. 2 *10*). The cells of the mucosal layer of the gall bladder showed frayed out margins and the cytoplasm was highly vacuolated. The nucleus was pushed to a side and showed hypertrophy. Occasionally in heavily infected fishes, the mucosal layer showed groups of growing plasmodia with a lining of stratified epithelial cells, probably due to metaplasia. The submucosal layer showed local thickening in the infected area with accumulation of leucocytes (Fig. 2 *11*). Sometimes the parasites were found in the bile duct also, where the tissue reaction was more pronounced. However, blocking of the bile duct was not observed.

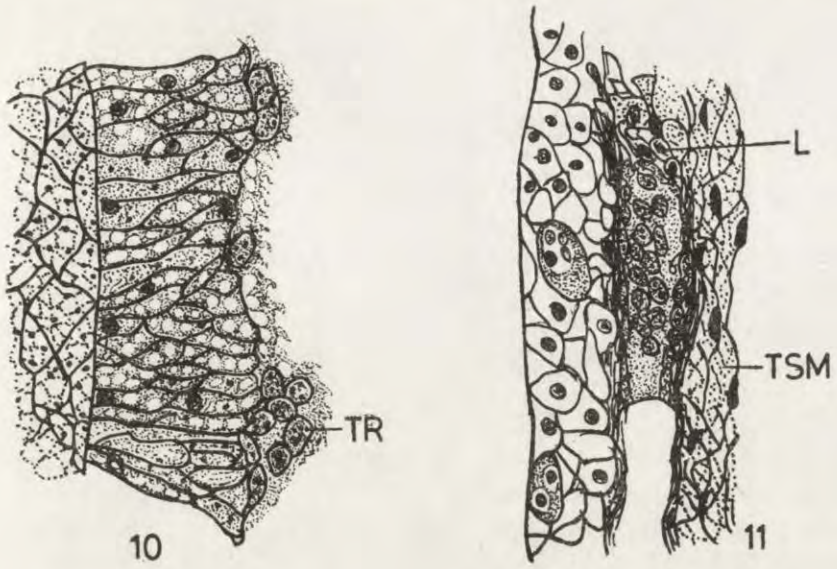

Fig. 2. *10* — Section of the gall bladder showing trophozoites, *11* — Section of the gall bladder showing submucosal thickening

Taxonomic Position

The genus *Chloromyxum* was established by Mingazzini for a myxosporidian parasite, *C. leydigi* parasitic in the gall bladder of the marine fish, *Rhina squatina* and other skates and rays. Since then several myxosporidians have been reported. Kudo (1919) in his "synopsis of genera and species of myxosporidia" listed 22 species of which 18 were in the body cavity and 4 were in the tissue. The same author (Kudo 1934) listed 41 species of *Chloromyxum.* Meglitsch (1947 a) while describing *C. renalis* added 14 species some of which were described after 1934 and others missed by Kudo (1934). Meglitsch (1947 b) established the new genus *Kudoa* and transferred to it eight species of *Chloromyxum* (*C.* sp. Tyzzer, 1900, *C. quadratum* Thelohan, 1895, *C. funduli* Hahn, 1915, *C. clupeidae* Hahn, 1917, *C.* sp. Davis, 1944, *C. thrysites* Gilchrist, 1924, *C. histolytica* Perard, 1928 and *C. rosenbuchi* Gelormini, 1944). He designated *C. clupeidae* Hahn, 1917 as the type species of the new genus established by him. He stated that a marked homogeneity of this grouping is that it removed all the histozoic forms previously referred to the genus *Chloromyxum* and included no coelozoic members of that genus. At the same time it removes from the genus *Chloromyxum* all forms which are not spherical in shape or somewhat longer than broad. More species were added in subsequent

years and to date there are as many as 58 species of *Chloromyxum* excluding the eight species of *Chloromyxum* transferred by Meglitsch to the genus *Kudoa*. Among them only five species, *C. amphipnoui* Ray, 1933, *C. mrigale* and *C.* sp. Tripathi, 1952, *C. hoarei* Lalitakumari, 1969 and *C. meglitschi* Sarkar, 1982 are from Indian fishes and all of them are from fresh water.

A perusal of the literature shows that the present form resembles to a certain extent *C. protei* Joseph, 1905, *C. cristatum* Leger, 1906, *C. dubium* Auerbach, 1908 and *C. fugitai* Kudo, 1916 in the spore measurements and some features of the spore morphology. However, *C. protei* is from the renal tubules of an amphibian, *Proteus sanguineus* and hence is not comparable to the present form. *C. cristatum* is from the gall bladder of a marine fish, *Tinca vulgaris* and has four unequal polar capsules one pair being smaller than the other and has ten marginal ridges running antero posteriorly on the valves and hence differs from the present form. *C. dubium* is from the gall bladder of *Lota vulgaris* and the spore valves have variable number of longitudinal ridges on the valves (six in figure) which are absent in the present form. *C. fugitai* is from the gall bladder of the fresh water fish, *Misgurnus anguillicaudatus* but the former has a very thick shell and 20–22 ridges on the valves which are absent in the present form and hence differs from the present form.

The present form does not also resemble any one of the species of *Chloromyxum* described from fishes of India although all of them are from fresh water fishes and from the gall bladder because the spores in the present form are bigger than all those forms. In view of what is stated the present form is considered new to science and the name *Chloromyxum tripathii* sp. n. is proposed for the same.

Chloromyxum mitchelli sp. n.

Host: *Therapon jarbua* (Forsskal)
Site of infection: Kidney
Types of slides: Authors' collections and Dept. of Zoology, Andhra University

Diagnosis

Spores pyriform with broadly anterior and bluntly pointed posterior ends measuring 18.0–22.0×16.5–21.0 μm. Four polar capsules at the anterior end. Two of larger capsules measured 6.5–8.5×2.0–2.5 and the polar filament inside the capsule showed eight coils and when fully extruded measured 70–80 μm. The two smaller capsules measured

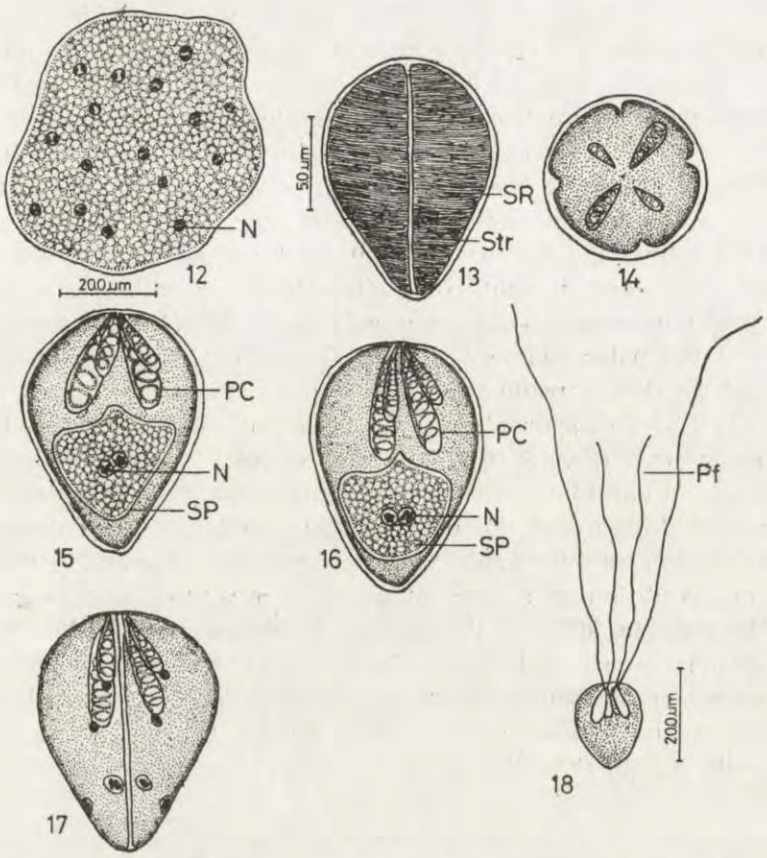

Fig. 3. *Chloromyxum mitchelli* sp. n. *12* — A trophozoite, *13* — A fresh spore (surface view), *14* — A spore stained with Giemsa (polar view), *15* — A spore stained with Giemsa (side view), *16* — A spore stained with iron haematoxylin, *17* — A sporoblast stained according to the Feulgen's technique, *18* — A spore with extruded polar filaments

Key to lettering: GN — Generative nucleus, SN — Somatic nucleus, PF — Parietal folds, Pf — Polar filament, PC — Polar capsule, RG — Refringent granules, N — Nucleus, L — Leucocytes, TSM — Thickened sub mucosa, Tr — Trophozoite, Sp — Sporoplasm, Str — Striations, SR — Sutural ridge

4.5–5.5×1.2–2.0 µm. The polar filament showed five coils and when fully extruded measured 45–55 µm.

Eight of the 296 *Therapon jarbua* collected during 1981–1982 were infected with a new species of *Chloromyxum* in the kidney. Infected kidneys showed pale patches. Smears prepared from such areas showed the spores of the myxosporidian. The intensity of infection was rather low and only a few spores were seen in each smear. Plasmodial stages having an irregular shape and hyaline cytoplasm containing variable number of nuclei were found free in the renal tubules. They measured 40–55×60–75 µm. The nuclei were vesicular and contained irregular

masses of chromatin (Fig. 3 *12*). Fresh spores were pyriform with broadly oval anterior and bluntly pointed posterior ends and measured 18.0–22.0 (average 21.0, n=50)×16.5–21.0 (average 20.5, n=50). The spore walls were symmetrical and united along a thick straight sutural ridge. Numerous transverse parallel striations were seen on the spore valves (Fig. 3 *13*). Four oval polar capsules were found at the anterior end. They were dissimilar in size, two of them were larger and measured 6.5–8.5 (average 8.0, n=50)×2.0–2.5 (average 2.2, n=50) and the polar filament showed eight coils. The other two polar capsules were smaller and measured 4.5–5.5 (average 5.0, n=50)×1.2–2.0 (average 1.5, n=50) and the polar filament showed five coils. In a surface view the larger and smaller capsules were located in diagonally opposite sides (Fig. 3 *14, 15*). The sporoplasm was binucleate and extended into the intercapsular area (Fig. 3 *16*). The two vesicular nuclei contained irregular masses of chromatin. In immature spores four capsulogenous nuclei were seen at the base of the polar capsules and the two valvulogenous nuclei were seen on either side of the sporoplasm adherent to the valves (Fig. 3 *17*). Addition of a drop of aqueous saturated urea released the polar filaments in 50% of the spores. Fully extruded polar filaments wer dissimilar in size and those released from the larger polar capsules were longer and measured 75–80 μm (average 78.0, n=50) while those released from the smaller capsules were shorter and measured 45–55 μm (average 50, n=50) (Fig. 3 *18*).

Taxonomic Position

The spores of the present form resemble to some extent those of *Chloromyxum caudatum* but the later are from the gall bladder of an amphibian, *Molge cristatus* and hence does not warrant a comparison. The present form also resembles *C. kabatae* from the gall bladder of *Squatina californica* and also *C. ferrugineum* from the same host. The present form differs from both these species in having unequal polar capsules. By virtue of this feature the present form resembles *C. amphipnoui, C. renalis* and *C. majori* but the spores in the present form are bigger than all of them and as such the present form is considered new to science for which the name *Chloromyxum mitchelli* sp. n. is proposed.

SUMMARY

Two new species: *Chloromyxum tripathii* sp. n. and *C. mitchelli* sp. n. parasitic in the gall bladder and kidney respectively of *Therapon jarbua* are described. The seasonal distribution, prevalence and histopathology of *C. tripathii* are studied.

In *C. tripathii* parasitic in the gall bladder of *Therapon jarbua* the trophozoites attain a maimum size of 48.0 × 42.0 μm and were generally formed of 2–4 disporous sporonts. Spores were spherical having a diameter of 10.8–12.0 μm. Sutural ridge straight. Spores measuring 4.2–5.0 μm. Sporoplasm hat-shaped and was present underneath the polar capsules. Polar filament showed five coils and was coiled in opposite directions in adjacent capsules. Polar filament uniformly thin and measured 28–34 μm when fully extruded.

Sexually mature fishes showed higher prevalence of infection. The prevalence increased with the age of fish. Both males and females were infected. The percentage infection was maximum in colder months and slowly decreased in hotter months.

Infected gall bladders were enlarged and hard instead of being soft and resilient. They had dirty brown colour instead of a moss-green colour. Cells of the mucosal layer of the gall bladder showed frayed out margins and the cytoplasm was highly vacuolated. The nucleus was pushed to a side and was hypertrophied. The submucosal layer showed local thickening in the bile duct and the tissue reaction was more prominant. Blocking of the bile duct was not observed.

In *C. mitchelli* sp. n. the spores were pyriform with broady anterior and bluntly pointed posterior ends and measured 18.0–22.0 × 16.5–21.0 μm. Four polar capsules present at the anterior end, two of the larger ones measured 4.5–5.5 μm × × 1.2–2.0 μm. The polar filament showed five coils and when fully extruded measured 45–55 μm in length. The two smaller capsules measured 4.5–5.5 × 1.2–2.0 μm. The polar filament showed five coils and when fully extruded measured 45–55 μm in length.

REFERENCES

Auerbach M. 1908: Bemerkungen über Myxosporidien heimischer susswasserfische. Zool. Anz., 32, 456–465.

Gelormini N.* 1944: Un nuevo parasito de la merbura. Univ. Buenos Aires, Rev. Fic. Agron. Y. Vet. 10, 458–463.

Gilchrist J. D. F. 1924: A protozoan parasite (*Chloromyxum thrysites* sp. n.) of the cape sea fish the snoek (*Thrysites atun* Euphr.). Trans. R. Soc. S. Afr., II, 263–273.

Hahn C.* 1915: Sporozoan parasites of certain fishes in the vicinity of Woods Hole. Mass. Bull. Bur. Fish., 33, 193–214.

Hahn C. W. 1917: The sporozoan parasites of the fishes and vicinity. 3. On *Chloromyxum clupeidae* of *Clupea harengus* (Young) *Pomolobus pseudoharengus* (Young) and *P. aestivalis* (Young). J. Parasitol., 4, 13–20.

Jameson A. P. 1931: Notes on Californian *Myxosporidia*. J. Parasitol., 18, 59–68.

Joseph H. 1905: *Chloromyxum protei* sp. n. Zool. Anz., 29, 450–451.

Kudo R. R. 1916: Contributions to the study of parasitic protozoa. 3. Notes on *Myxosporidia* found in some fresh water fishes of Japan with description of 3 new species. J. Parasitol., 4, 141–147.

Kudo R. R. 1919: Studies on *Myxosporidia*: A synopsis of genera and species of *Myxosporidia*. III. Bio. Mono., 5, 1–265.

Kudo R. R. 1934: Studies on parasitic protozoa (*Myxosporidia*) of Illinois. Ill. Bio. Mono., 13, 7–44.

Lalitakumari P. S. 1969: Studies on parasitic protozoa (*Myxosporidia*) of fresh water fishes of Andhra Pradesh, India. Rev. Parasitol., 30, 153–225.

Leger L.* 1906: Myxosporidies nouvelles, parasites des Poissons. I. Sur une nouvelle maladie myxosporienne de la truite indigene. Ann. Univ. Grenoble, 18, 267–270.

Meglitsch P. A. 1947a: Studies on *Myxosporidia* of the Beaufort region. II. Observations on *Kudoa clupeidae* (Hahn) gen. nov. J. Parasitol., 33, 271–277.

Meglitsch P. A. 1947b: Studies on *Myxosporidia* of the Beaufort region. I. Observations on *Chloromyxum renalis* n. sp. and *C. granulosum* Davis. J. Parasitol., 33, 271–277.

Mitchell L. G., Listevarger J. K. and Bailey W. C. 1980: Epizootiology and histopathology of *Chloromyxum trijugum* (*Myxospora, Myxosporidia*) in centrarchid fishes from Iowa. J. Wildl. Dis., 16, 233–236.

Moser M. and Noble E. R. 1977: Myxosporidian genera *Auerbachia, Sphaerospora, Davisia* and *Chloromyxum* in Macruroid fishes and sable fish, *Anaplasma fimbria*. Z. Parasitenkd., 51, 159–163.

Perard C. 1928: Sur une maladie du maquereau (*Scomber scomber* L.) due à une myxosporidie *Chloromyxum histolyticum* n. sp. C. R. Acad. Sci. Paris, 186, 108–110.

Ray H. N. 1933: Preliminary observations on *Myxosporidia* from India. Curr. Sci. 1, 349–350.

Sarkar N. K. 1982. On the three myxosporidian parasites (*Myxozoa*) of the ophicephalid fishes of West Bengal, India. Acta Protozool., 21, 239–244.

Thelohan P.* 1895: Recherches sur les myxosporidies. Bull. Sci. France et Belg., 26, 100–394.

Tripathi Y. R. 1952: Studies on parasites of Indian fishes: I. *Protozoa Myxosporidia* together with a check list of parasitic *Protozoa* described from Indian fishes. Rec. Ind. Mus., 50, 63–88.

Tyzzer E. E.* 1900: Tumours and sporozoa in fishes. J. Boston Soc. Med. Sci., 5, 62–68.

Yasutake W. T. and Wood E. M. 1957: Some *Myxosporidia* found in Pacific Northwest salmonoids. J. Parasitol., 43, 633–637.

* Not referred to in original.

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