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New Flagellates *Proteromonas kakatiyae* sp. n. of *Hemidactylus* and *Proteromonas warangalensis* sp. n. of *Mabuya carinata* from Warangal, Andhra Pradesh, India

Synopsis. Two new species of the genus *Proteromonas* Kunstler, 1883, *Proteromonas kakatiyae* sp. n. from *Hemidactylus* sp. and *Proteromonas warangalensis* sp. n. from *Mabuya carinata* are recovered from the rectal contents. Spatulated caudal end, dumble shaped nucleus, ribbon like paranuclear body, and parabasal body are recorded from *Proteromonas kakatiyae. Proteromonas warangalensis* is characterized by a laterally compressed paranuclear body, sub-blepharoplastic mass which is fused with blepharoplast and parabasal body. There are 10–15 darkly stained granules on either side of the parabasal body.

Number of parasites of the genus *Proteromonas* Kunstler, 1883 have been recorded from different hosts. Grassi (1879) for the first time recorded these parasites from lizards and placed them in the genus *Monocercomonas*. Later on Kunstler (1883) errected the genus *Proteromonas* for similar flagellate found by him in a Chelonian host. There was great confusion regarding generic identity and systematic position and it was cleared by Grasse (1926) who has revived the name *Proteromonas* erected by Kunstler.

So far eight species of this genus have been described, four of them from reptiles, one from amphibia and three from mammals.

Material and Methods

The animals were collected around Kakatiya University campus area (Warangal city). The incidence of infection of *Proteromonas* was heavy in *Hemidactylus* and associated with *Hypotrichomonas* and *Chilomastix*. Twelve lizards of the genus *Mabuya* were also examined, eight of them were harbouring heavily this infection.

The parasites were studied in living condition with the help of 0.85 per cent normal saline. For permanent preparations Methonal and Schaudinn's fluids were used as fixitives. Later on stained with Giemsa's and Iron haematoxylin stain. All the drawings were made with the aid of Camera lucida at a magnification of 2200×.

Results

Proteromonas kakatiyae sp. n. from *Hemidactylus* sp.

Morphology

In living condition the parasites were elongated or spindle shaped with fast jerky movements. On fixation the shape of the body was maintained in most of the parasites but in few it tends to become fusiform or oval.

In stained preparation the body is long and slender (Fig. 1 *3*), the length ranges from 10–14.09 μm and the breadth is uniform upto 2/3 of the body measuring 2.72–3.86 μm (Table 1). The posterior end slightly narrows in a typically spatulate form (Fig. 1 *1–3*). Sometimes the spatulate end tapers into a caudal spine (Fig. 1 *1–3*).

The nucleus is spherical or dumble or oval (Fig. 1 *1–3*) and sometimes sausage shaped situated at the anterior third of the body. There are 3 to 4 darkly stained chromatin granules observed in the haematoxylin stain (Fig. 1 *4–6*).

The para-nuclear body is found in the middle or slightly towards posterior side of the body but very rarely seen just below the nucleus (Fig. 1 *5,6*). It is ribbon like or rod like sometimes spherical.

A single blepharoplast is situated at the anterior end of the organism.

Table 1

The dimensions of *Proteromonas kakatiyae* sp. n., from *Hemidactylus* of 100 individuals (in μm)

	Minimum	Maximum	Average
Body length	10.0	14.09	12.04
Body breadth	2.72	3.86	3.29
Nucleus length	1.36	2.72	2.01
Nucleus breadth	1.36	2.72	2.06
Paranuclear body	0.9	3.86	2.38
Flagellum (thicker) length	15.0	22.72	18.86
Flagellum (thinner) length	12.95	19.09	16.02
Blepharoplast diameter	0.45	0.9	0.84

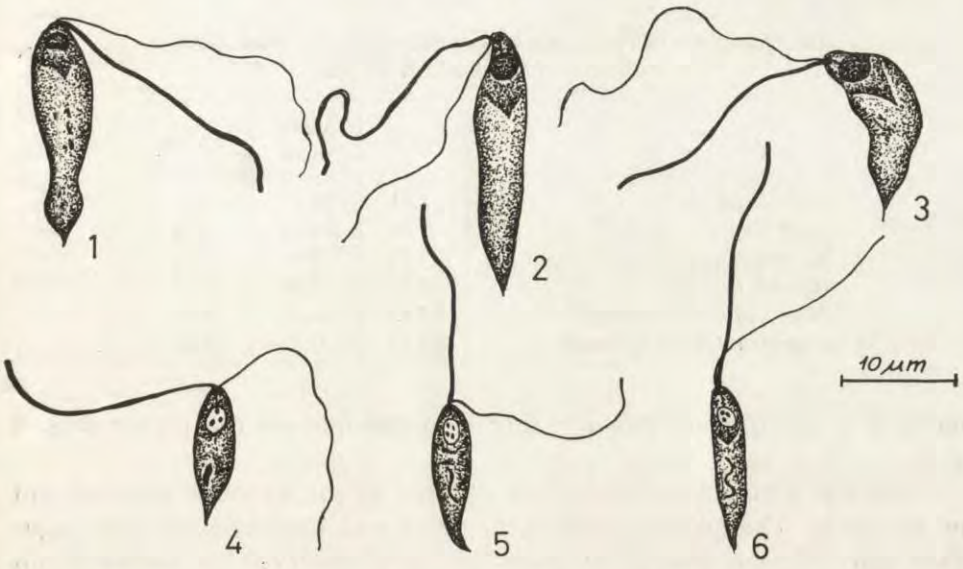

Fig. 1. *Proteromonas kakatiyae*, sp. n., *1–3* fixed in methyl alcohol and stained with Giemsa's stain, *4–6* fixed with schaudinn's fluid and stained with haematoxyline, *1–3* long slender form showing the spatulate end, *4–5* showing the spherical or dumble nucleus with *3–4* darkly stained chromatin granules. *5–6* showing paranuclear body

It measures 0.45–0.9 µm in diameter from which two dissimilar flagella arise (Fig. 1 *1–3*). One is thicker and larger than the other (Fig. 1 *1–3*). Both the flagella are longer than the body length. Sub-blepharoplastic mass is absent.

In few individuals thread like rhizoplast extends from blepharoplast to nuclear membrane. The rhizoplastic ring or parabasal body is absent.

Proteromonas warangalensis sp. n. from *Mabuya carinata*

Morphology

The organism in living condition was round or pear shaped, sometimes slightly long but tends to become round or oval on fixation.

In stained smears the parasite was round (Fig. 2 *1–3*), pear shaped or oval (Fig. 2 *5, 6*). It measures 6.81–11.1 × 3.86–7.72 µm (Table 2).

The nucleus is spherical (Fig. 2 *1, 2*) or ellipsoidal (Fig. 2 *3*) situated at the anterior half of the body or sometimes towards the middle. Endosome is compact situated in the centre of the nucleus. In few individuals 3 large granules are seen (Fig. 2 *6*). Nucleus measures 1.81–2.4 µm × 1.36–2.49 µm, para-nuclear body laterally compressed or oval and situat-

Table 2

The dimensions of *Proteromonas warangalensis* sp. n., from *Mabuya carinata* of 100 individuals (in μm)

	Minimum	Maximum	Average
Body length	6.81	11.1	8.5
Body breadth	3.86	7.72	5.94
Nucleus length	1.81	2.4	2.11
Nucleus breadth	1.36	2.49	1.77
Flagellum (thicker) length	14.13	22.71	18.32
Flagellum (thinner) length	13.12	23.8	18.46

ed just below the nucleus or adheres to the nuclear membrane (Fig. 2 *1, 3*).

There is a single blepharoplast situated at the extreme anterior end of the body. The sub-blepharoplastic mass and blepharoplast fuse together and forms a triangular mass has been observed in haematoxylin stain (Fig. 2 5–7).

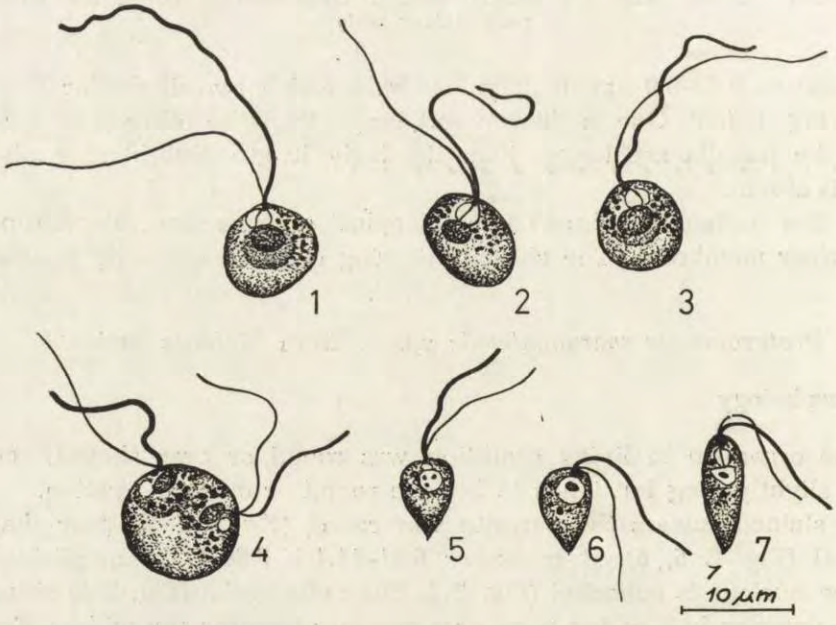

Fig. 2. *Proteromonas warangalensis*, sp. n., *1–4* fixed in methyl alcohol and stained with Giemsa's stain, *5–7* fixed with schaudinn's fluid and stained with haematoxyline, *1–3* round form with unequal, dissimilar flagella, rhizoplastic ring, *5–6* pear shaped or oval, *5* shows three chromatine granules, *6, 7* shows compact endosome, *4* — divisional form showing two nuclei, two pairs of mastigont elements

There is a thread like filamentous rhizoplast joining the blepharoplast and nuclear membrane (Fig. 2 *1–3*). The perirhizoplastic ring or parabasal body situated about midway between blepharoplast and nucleus and the rhizoplast passes through it. There are 10–15 darkly stained granules present on either side of the body (Fig. 2 *1–3*). Two unequal flagella arise from the blepharoplast, one is thicker and longer than the other but sometimes the tinner flagellum is longer than the thicker one. The length ranges from 14.13–22.71 μm (thicker), 15.12–23.8 μm (thinner).

Cytoplasm staines homogenously and devoid of bacteria. In few individuals one or two vacuoles are seen.

Few individuals (Fig. 2 *4*) show the divisional forms.

Discussion

A comparison of the structure of the present two species with those of the four already described species from reptiles shows marked differences in many respects (Table 3).

Table 3

Dimensions of different *Proteromonas* species (in μm)

	Length	Breadth	Average
P. lacerta viridis	11—13	2—5	—
P. uromastix	12.8—30.0	1.5—4.0	23.0×3.10
P. chameleoni	10.0—15.5	4.0—10.0	12.6×6.7
P. hemidactyli	12.9—25.2	2.1—5.7	17.4 ×3.6
P. kakatiyae sp.n.	10.0—14.09	2.72—3.86	12.04×3.29
P. warangalensis sp.n.	6.81—11.1	3.86—7.72	8.5×5.94

In its general appearance the parasite somewhat resembles *Proteromonas uromastix* Janaki Devi, 1962, which has a long tail like process; two very long equal length flagella of which one is thicker and coarser than the other; a sub-blepharoplastic body in the form of a horizontal bar; a crescentic or irregular paranuclear body and crescentic parabasal body; a nucleus with central endosome and peripheral chromatin granules. While the *P. kakatiyae* sp. n. has a spatula shaped tail end; unequal short flagella, filamentous loop like sub-blepharoplastic body. The nucleus has three blocks of chromatin granules. The second one *P. warangalensis* sp. n. is round and has unequal, dissimilar flagella but twice as long as body length. Nucleus with large chromatin blocks and thread like parabasal body.

In *P. lacerta viridis* the flagella are unequal and dissimilar, paranuclear body is dens ribbon like structure, while in *P. kakatiyae* the

flagella are unequal in length, parabasal body is filamentous loop like structure. However, in *warangalensis* flagella are unequal but very long, crescent shaped paranuclear body, and the nucleus possesses three large chromatine granules.

P. chameleoni Krishnamurthy, 1963 has unequal, dissimilar, very long flagella, with hemi-spherical paranuclear body. The nucleus with chromatine granules. While the two species show a marked differences in the size and the shape of the flagella, paranuclear body and nuclear structure.

P. hemidactyli Krishnamurthy, 1968 larger in size, subequal and similar flagella with 5 or 8 shaped ribbon like paranuclear body. *P. kakatiyae* sp. n. is smaller in size, unequal, dissimilar flagella; paranuclear body thick, rod like and nucleus with three blocks of chromatin granules. While *P. warangalensis* sp. n. smaller in size with unequal dissimilar flagella; paranuclear body crescent shaped around the nucleus, and nucleus contains three large chromatine granules.

These differences justify the recognition of the parasites as new species and it is, therefore, proposed to designate them as *Proteromonas kakatiyae* sp. n. after the Kakatiya University and *Proteromonas warangalensis* sp. n. after the place Warangal, Andhra Pradesh. India.

The type slides are deposited in Protozoology section, Zoology Department, Kakatiya University, Warangal, A. P. India.

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We are grateful to Prof. K. Venkata Ramiah, Vice-Chancellor of Kakatiya University for constant encouragement.

ZUSAMENFASSUNG

Zwei neue Arten aus der Gattung *Proteromonas* Kunstler, 1833, werden beschrieben. *Proteromonas kakatiyae* sp. n. aus *Hemidactylus* sp. ist durch die Verbreiterung des Körperendes, das kugelige oder hantelartige Kern, den bandartigen Paranuclearkörper und das Blepharoplastvorkommen charakterisiert. *Proteromonas varangalensis* sp. n. aus *Mabuya carinata* ist durch den laterlageplätten Paranuclearkörper und das Vorkommen der mit dem Blepharoplast verbundenen Sub-blepharoplastmasse charakterisiert.

REFERENCES

Grasse P. P. 1926: Contribution aletude des flagelles parasites. Arch. Zool. Exp. Gen., 65, 345–602.

Janaki Devi K. 1962: A new species of proteromonas from the spiny tailed lizard.; Arch. Protistenk., 105, 450–454.

Krishnamurthy R. 1963: On a new species of proteromonas from the *Chameleon*. J. Biol. Sci., 6, 14–18.

Krishnamurthy R. 1968: A new flagellate of the genus *Proteromonas* Kunstler, 1883 from an Indian lizard. Parasitology, 58, 531–534.

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Trypanosoma gangetica sp. n. from a Fresh Water Turtle *Trionyx gangeticus* Cuvier

Synopsis. The paper deals with a new species of monomorphic Trypanosome, *Trypanosoma gangetica* sp. n. (*Trypanosomatidae*) from a soft leathered turtle *Trionyx gangeticus* Cuvier collected from West Bengal, India. The morphology of the haemoflagellate has been described and it is compared with other known chelonian trypanosomes to consider it as new species.

Trypanosomes have been reported from the peripheral blood of different species of chelonians. Laveran and Mesnil (1902) described *Trypanosoma damoniae* from the tortoise *Damonia reevesii*. Dutton and Todd (1903) and Dutton et al. (1907) noted the presence of trypanosomes in different tortoises of Gambia. Bouet (1909) reported *Trypanosoma pontyi* from a tortoise *Sternotherus derbianus* of Africa. *Trypanosoma chelodina* was recorded from *Chelodina longicollis* by Johnson (1907) from Australia. Walliker (1965) reviewed the occurrence of reptilian trypanosomes and enlisted twelve chelonian trypanosome of which two were reported as *Trypanosoma* sp. In India, studies on chelonian trypanosomes have not so far been recorded.

The turtles *Trionyx gangeticus* Cuvier, were collected from local markets of Bongaon, West Bengal, India in March 1975 with a view to study haematozoa. Fifteen turtles were examined and five were positive for a haemoflagellate of the genus *Trypanosoma* in the circulating blood. No ecto-parasitic infestation was noticed. Thin blood films were drawn and stained with Giemsa and Leishman stain. Drawings and measurements were made with the aid of camera lucida, along a line drawn from posterior to anterior end of the flagellate at the uniform magnification of 1500 ×.

Results

Trypanosoma gangetica sp. n.

Type Host: *Trionyx gangeticus* Cuvier
Type Locality: Bongaon, 24 Parganas, West Bengal, India.

Trypanosoma gangetica sp. n. showed monomorphic form and were abundant in the blood film. All the forms were long and slender. This form (Fig. 1 *1–4*) of flagellate is elongated with sharply pointed ends, Cytoplasm is homogenous and stains light pink with Giemsa. It contains 7–10 vacuoles. Nucleus is round or oval in shape. It always lies at the middle of the body with a distinct nuclear membrane. Kinetoplast is a spherical dot like structure situated away from the posterior part of the nucleus and takes deep stain with Giemsa and Leishman. Flagellum is found to emerge from the kinetoplast and has 6 to 8 attachments with the undulating membrane proper and leaves it as a distinct free flagellum. A well stretched form exhibits the maximum width of the

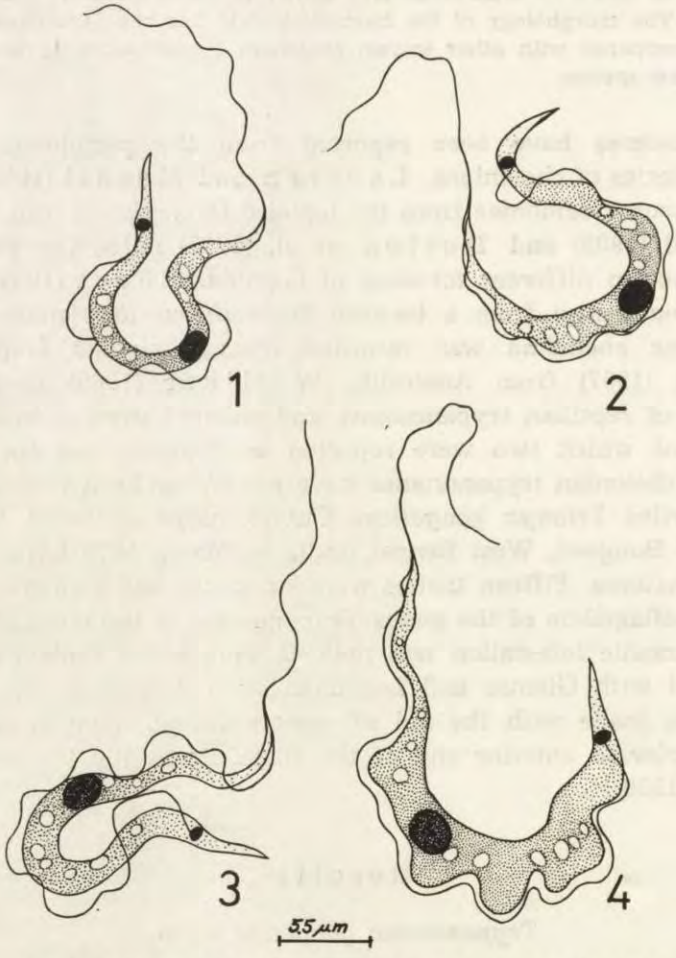

Fig. 1. *1–4. Trypanosoma gangetica* sp. n. Camera lucida drawings. *1–3* — Long slender form, note the distinct free flagellum and the vacuoles. *4* — Well stretched condition of the long slender form

Table 1

Measurements of *Trypanosoma gangetica* sp. n. (in μm)

Characters	Range	Mean
Total length of the parasite (including free flagellum)	53.4–58.0	55.7
Length of the cell body	33.7–36.7	34.7
Breadth of the cell body	1.8– 4.8	2.45
Length of the free flagellum	19.2–21.9	20.9
Length of the nucleus	2.2– 2.4	2.3
Breadth of the nucleus	1.3– 1.8	1.5
Length of the kinetoplast	0.6– 0.9	0.8
Breadth of the kinetoplast	0.7– 1.0	0.8
Post-kinetoplast distance	4.1– 4.6	4.38
Anterior edge of kinetoplast to posterior edge of nucleus	12.1–14.50	13.55
Anterior edge of nucleus to anterior end of cell body	12.8–14.80	13.7

cell body (Fig. 1 4). No divisional phase has been observed so far (Table 1).

Diagnosis of *Trypanosoma gangetica* sp. n.

The described haemoflagellate is monomorphic measuring 55.7 μm by 2.45 μm in total length including the free flagellum. Cytoplasm is homogenous having 7–10 vacuoles and without any volutin granules. Kinetoplast is always away from the posterior part of the nucleus. Nucleus is always centrally situated in the cell body. A prominent undulating membrane with 7–8 folds. The body cytoplasm does not enter inside the fold of it. A long free flagellum measuring 19.2 μm is also the characteristic feature of this parasite.

Discussion

Trypanosomes appeared to be non-pathogenic to their host. The peculiarity of the present species of trypanosome described in the paper is that it has a long free flagellum measuring 19.2–21.9 μm and mean length being 20.9 μm. Generally vertebrate trypanosomes reveal more than one morphological feature in the peripheral blood. Robertson (1909) discovered *Trypanosoma vittatae* in the soft tortoise *Emyda vittata* which was polymorphic. The parasite under report lacks this feature.

Trypanosoma vittatus was also recorded by Robertson (1908) from a tortoise *Lissemys punctata granosa* from Ceylon. The parasite

was 70 μm long and is different from the present form. The species under report draws a close affinity with *Trypanosoma chrysemydis* Roudabush and Coatney (1937) from *Chrysemys belli marginata* but it differs in many respect. *T. chrysemydis* measures 46.8–50.0 μm by 3.15–4.05 μm. Moreover, it has got a short free flagellum measuring 13.05 μm. However, this flagellate differs from all other known chelonian trypanosomes. Considering all these aspects the trypanosome from *Trionyx gangeticus* Cuvier, has been assigned a new species status and named *Trypanosoma gangetica* sp. n. This turtle is recorded for the first time as a host for trypanosome. The holotype and paratypes of *Trypanosoma gangetica* sp. n. will be deposited in the national zoological collection of Zoological Survey of India, Calcutta in due course.

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The author is indebted to Dr. B. Dasgupta, Principal, Darjeeling Government College for his valuable guidance and constant encouragement during the course of observations. Thanks are due to Shri Nilachal Chattaraj and Shri Sukdeb Sinha for their manifold help.

ZUSAMMENFASSUNG

Trypanosoma gangetica sp. n. wird von einer Süsswasser Schildkröte *Trionyx gangeticus* Cuvier beschrieben. Die Morphologie des Parasiten und seine Beziehungen zu anderen bekannten Arten Wird diskutiert.

REFERENCES

Bouet G. 1909: Sur quelques trypanosomes des vertébrès à sang froid de l'Afrique occidentale française. C. r. Seanc. Soc. Biol., 66, 609.

Dutton J. E. and Todd J. L. 1903: First report of the Trypanosomiasis Expedition to Senegambia (1902). Liverp. Sch. Trop. Med., Mem. 11, 1.

Dutton J. E., Todd J. L. and Tobey E. N. 1907: Concerning certain parasitic protozoa observed in Africa. Ann. Trop. Med. Parasitol., 1, 287.

Johnson E. A. 1907: A trypanosome found in the River Murray turtle (*Chelodina longicollis*). Australas. Med. Gaz., 26, 26.

Laveran A. and Mesnil F. 1902: Sur quelques Protozoaires parasites d'une tortue d'Asie (*Damonia reevesil*). C. r. Acad. Sci., 135, 609–614.

Robertson M. 1908: A preliminary note on Haematozoa from some Ceylon reptiles. Spolia Zeylan., 5, 178–185.

Robertson M. 1909: Studies on Ceylon Hematozoa. No. I. The life cycle of *Trypanosoma vittatae*. Q. J. microsc. Sci., 53, 665.

Roudabush R. L. and Coatney G. R. 1937: On some blood protozoa of reptiles and amphibians. Trans. Am. Microsc. Soc., 56, 291–297.

Walliker D. 1965: *Trypanosoma superciliosae* sp. nov. from the lizard *Uranoscodon superciliosa*. Parasitology, 55, 601–606.

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Maria WOLSKA

Tripalmaria dogieli Gass., 1928 (*Ciliata, Entodiniomorphida*). Structure and Ultrastructure. Part I. Light-microscope Investigations [1]

Synopsis. Results of silver-impregnation of *Tripalmaria dogieli* are presented and the infraciliature of the examined species was compared with those of other species of *Cycloposthiidae* which were silver-impregnated. A great similarity between the infraciliatures of *Tripalmaria dogieli* and *Cycloposthium* was shown.

Until quite lately *Entodiniomorphida* were considered to be relatives of *Heterotrichida* and assigned to the class *Spirotricha*; now the authors of recently proposed systems of *Ciliata* have excluded them from *Spirotricha*. Jankowski (1973) put *Entodiniomorphida* into one of the five *Ciliophora* subclasses, while Puytorac et al. (1974) and Corliss (1975) consider them as an order of the *Vestibulifora* subclass.

New techniques have been used not long ago for studies on *Entodiniomorpha*. In the electron microscope Noirot-Timothée (1960) examined the family *Ophryoscolecidae*, and Grain (1966) did *Cycloposthium bipalmatum* of the *Cycloposthiidae* family. In the light microscope Wolska (1965) investigated the infraciliature of some representatives of *Ophryoscolecidae* and *Cycloposthium*. The mentioned papers of Noirot-Timothée and Grain and perhaps also those of Wolska (1966 a, b) have subserved the change of *Entodiniomorphida's* taxonomic position. The belief on a close affinity between *Entodiniomorphida* and *Blepharocorythidae* has been reflected in the system proposed by Corliss (1975).

The opinion about the affinity between these groups and their relationships with *Buetschliidae* (*Gymnostomata*) was expressed by Wolska

[1] This investigation was supported by Committee of Cytobiology of Polish Academy of Sciences.

(1966 a, b) and developed in her further studies on *Blepharocorythidae* (Wolska 1967 a, 1969, 1971). Basing on descriptions of some new genera of *Blepharocorythidae* presented by Wolska (1967 b, 1968), Noirot-Timothée (1969) upheld this opinion. Thurston and Grain (1971) have also admitted the relations between *Blepharocorythidae, Gymnostomata* and *Entodiniomorphida* in their descriptions of some new species of *Holotricha* found in hippopotami.

To confirm this view, it is necessary to examine some more representatives of those groups using the newest techniques.

The study on the *Tripalmaria dogieli* of the *Cycloposthiidae* family was initiated, to examin infraciliature (adoral first of all) and ultrastructure of the cortex. Part I of this study concerns infraciliature and topography of particular parts of the adoral region. Morphology and inner structure of *T. dogieli* were described in extensive and excellent papers by Strelkov (1931, 1939). However, there cannot be found any detailed information on ciliature which can be obtained by using the silver impregnation technique. The general description of the protozoan will be neglected in the present paper. Instead, the ciliature and infraciliature of *T. dogieli* will be here described and compared with the infraciliature of those representatives of *Cycloposthiidae* which were subjected to the silver impregnation.

Material and Methods

The studied material included samples of contents of colons of some horses born near the town of Łódź, and one sample of horse's faeces where *T. dogieli* occurred abundantly and could be silver impregnated.

Silver impregnation was performed as follows: protozoans were fixed in 10% formalin solution, then washed with water and closed on slides in a thin layer of gelatin according to Chatton. Such preparations obtained in this way were treated with silver solution according to Bielschowsky, and then with 10% formalin solution. When dehydrated the preparations were closed in the Canada balsam.

There were also prepared the so-called semi-thin sections using the ultramicrotome. Semi-thin sections 1 μm thick were made of protozoans fixed with osmium tetroxide and embedded in the Epon similarly as if they were to be examined under the electron microscope. The sections were slightly stained with toluidine blue with addition of Azur II.

Results

The adoral infraciliature of *Tripalmaria dogieli* Gass., 1928 becomes very distinct after silver impregnation (Fig. 1); however, as a whole, it can hardly be presented by microphotography. During fixation the

ciliophore gets drawn into the infundibulum and squeezes. On the other hand, the ciliophore of *Cycloposthium* (Pl. I 1) is a rigid formation and can keep its unchanged shape even when having been invaginated and upturned (cf. illustrations in Fernandez-Galiano 1959).

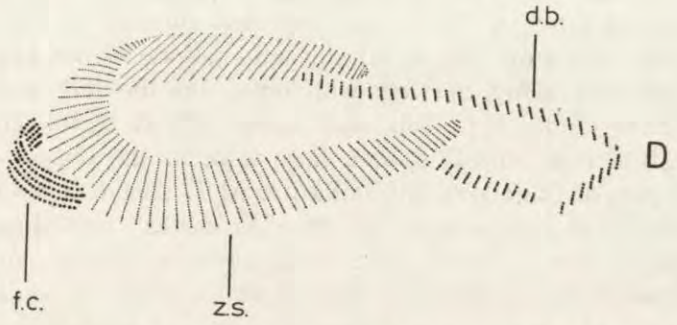

Fig. 1. *Tripalmaria dogieli,* adoral infraciliature (scheme). z.s. — the zone of syncilia, d.b. — dorsal bande of short rows, f.c. — rows of kinetosomes bearing free cilia, D — dorsal side, V — ventral side

The ciliophore of *Tripalmaria dogieli* ever becomes upturned and deformed, and fragments of its infraciliature overlap making the picture dimmed. The general outline of the drawn, relatively little altered ciliophore is presented in Pl. I 2. A number of photographs will show the whole of adoral infraciliature.

A main, largest part of the adoral ciliature of *T. dogieli* consists of syncilia (previously believed to be membranelles) growing on the lateral surface of the ciliophore. Syncilia can easily be visible in alive protozoans. When silvered the region as a whole looks like a semicircular zone with its ends approaching on the dorsal side (Pl. I 2, 4). The zone is composed of diagonal, dense, evenly spaced raws of kinetosomes (Pl. I 4, 5). On the left arm of the zone the raws of kinetosomes are ventrally-dorsally inclined, while on the right one — the inclination is dorsally-ventrally oriented. Such a pattern of raws of kinetosomes can be found in "la banda bucal" according to Fernandez-Galiano (1959) in *Cycloposthium edentatum.*

At the base of the described zone of syncilia, on the ventral wall of the infundibulum, there occur rather short raws of kinetosomes, in number from a few to a dozen or so, which are markedly larger than those occurring in the region of syncilia (Pl. I 4, 5, 6, 7). Similarly arranged kinetosomes in Cycloposthium edentatum were described by Fernandez-Galiano (1959) as "cinecias independientes" and by

Wolska (1971) as "free cilia". The kinetosomes sprout short cilia to be seen in sections of *T. dogieli* (Pl. II 11, 12).

The third, dorsal part of the adoral infraciliature is composed of short raws of kinetosomes situated on the wall of the hollow occurring just before the cytostome-cytopharyngeal complex (Pl. I 3). These short raws compose two uneven segments beginning at the ends of the semicircular zone of syncilia. The longer segment runs along the right wall of the hollow, bends on the dorsal side and passes to the left wall. The shorter band runs along the left wall only. On the left wall the both ends of the segments approach each other (Pl. II 8, 9). The raws of kinetosomes are less densely spaced here than in the region of syncilia. This dorsal part of the adoral infraciliature of *T. dogieli* looks like a torn, narrow band. It is homologous of "cinecias cortas" according to Fernandez-Galiano (1959). The short raws of kinetosomes of this band give rise to cilia which are seen in sections (Pl. II 13, 14, 15).

The system of parallel, evenly spaced raws of kinetosomes can also be observed at the base of three somatic tufts (caudalia) of *T. dogieli*. The raws are arranged more or less along the long axis of the protozoan's body. They cover slightly concave, rhombapproximating areas (Pl. II 10).

Discussion

The obtained results of silvering of *Tripalmaria dogieli* confirm the already known fact that *Cycloposthiidae* like all the *Entodiniomorphida* have membranelles neither in the adoral nor in the somatic ciliature. Within all ciliary areas there occur parallel, evenly spaced raws of kinetosomes.

The adoral infraciliature in *T. dogieli* is of the same kind as that in *Cycloposthium edentatum* described by Fernandez-Galiano (1959).

We deal here with the same topography of the three ciliary formations similar in both genera. Like in *Cycloposthium,* the zone of syncilia in *T. dogieli* ("la banda bucal" according to Fernandez-Galiano) is collar-shaped and covers the slopes of the ciliophore protuberance. The raws of kinetosomes on the "collar" run diagonally like in *Cycloposthium.* The ends of the "collar" arms, dorsally oriented, touch the ends of the short kineties band — proper buccal ciliature.

The system of short kineties of *T. dogieli* is interrupted on the left side (Pl. II 8). In *Cycloposthium edentatum* such a discontinuity in the similar system ("cinecias cortas" of Fernandez-Galiano) does not appear during interdivision. However, according to results obtained

in my earlier observations on the genus *Cycloposthium* (Wolska 1965), the system comes into being from two primordia during the division. Therefore, also these dorsal parts of adoral infraciliature of *Tripalmaria* and *Cycloposthium* are comparable.

The appearance and the arrangement of the small ventrally located part of the adoral ciliature of *T. dogieli* are, in general, the same as those of *Cycloposthium.* Few raws of large kinetosomes are slightly arched at the base of the ciliophore on the ventral side ("cinecias independientes" according to Fernandez-Galiano or "free cilia" according to Wolska). In *Cycloposthium* they lie just at the ciliofor base while in *Tripalmaria* they go up to the lateral wall of the infundibulum. Owing to such position, when the ciliophore is drawn, they are usually visible in front of the syncilia region (Pl. I 4, 6, 7). The infraciliature of the caudalia of *Tripalmaria* and *Cycloposthium* are also distinctly similar. In *Cycloposthium* there are two wide bands with rounded ends, arranged more or less across the longer axis of the body, and covered with diagonal raws of kinetosomes. Each band is twice as long as it is wide. In *Tripalmaria* there are three bands (as three caudalia occur) which are distinctly shortened and they are rhomb--shaped.

The comparison with *Triplumaria selenica* Latteur, Tuffrau et Wespes, 1970 is rather difficult but some differences can be revealed in this way. The silvering method according to Bodian was used for this species. In *Triplumaria selenica,* there can be distinguished three main parts of the adoral infraciliature and a small, rhomb-shaped region of kinetosomes. These are: (1) "the region of apical infraciliature" similar in shape to the syncilia regions in *Tripalmaria* and *Cycloposthium,* situatet at the top of the ciliophore; (2) "marginal polykineties" situated on the slopes of the ciliophore protuberance, which in the authors' opinion, seem to emerge on the dorsal side from the rhomb-shaped region of kinetosomes (the latter being situated on the dorsal side between the apical region and the cytostome); (3) "pericytosomal kineties" emerging from the same region as the "marginal kineties" do, and settling the cytostome edge.

As to their topography, it seems that the three main parts correspond to the three parts of the adoral ciliature of *Tripalmaria* and *Cycloposthium,* but — nevertheless — there is something doubtful. We may assume that the system of kinetosomes in the "apical region" is the same as that in the zone of syncilia of *Tripalmaria* and *Cycloposthium.* Also, the "marginal kineties", due to the ciliophore base may be recognized as homologues of "free cilia" in the two mentioned species. But, those in *T. selenica* are the long formations enveloping the whole ciliofor

also from the dorsal side, and reaching the rhomb-shaped region of kinetosomes. However, no such rhomb-shaped region occurs in *Tripalmaria* and *Cycloposthium*. Moreover, considering the dorsal side — it may well be that "pericytostomal kineties" of *Triplumaria selenica* are similarly arranged as bands of "short kineties" in *Tripalmaria* and *Cycloposthium*, but no certain conclusions may be drawn on the basis of picture of *T. selenica*, the more so as the "pericytostomal kineties" are connected with the rhomb-shaped region which is not the case for *Cycloposthium* and *Tripalmaria*.

There are also differences between the compared genera in the areas of kinetosomes bearing caudalia. In *Cycloposthium* and *Tripalmaria* there are shorter or longer bands, while in *Tripalmaria selenica* cilia of each tuft originate, according to authors' opinion from the narrow, ring-shaped area of the infraciliature.

As results from the above-discussed comparison, the genera *Cycloposthium* and *Tripalmaria* reveal close resemblance in their both adoral and somatic infraciliatures, while their relative *Triplumaria*, unexpectedly, such a great similarity did not display.

It seems necessary to examine infraciliatures of other representatives of *Cycloposthiidae*, especially those with three caudalia.

RÉSUMÉ

L'infraciliature de *Tripalmaria dogieli* est révélée et comparée avec celle des autres *Cycloposthiidae*. On a constaté que l'infraciliature de *T. dogieli* et de *Cycloposthium* sp. présentent des ressemblances étroites.

REFERENCES

Corliss J. O. 1975: Taxonomic characterization of the suprafamilial groups in a revision of recently proposed schemes of classification for the phylum ciliophora. Trans. Am. microsc. Soc., 94, 224–267.

Fernandez-Galiano D. 1959: La infraciliacion en *Cycloposthium endentatum*. Boln. R. Soc. esp. Hist. Nat. 57, 139–150.

Grain J. 1966: Etude cytologique de quelques ciliés holotriches des ruminants et des équidés. Protistologica, 2, 59–141.

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

Latteur B., Tuffrau M., Wespes G. 1970: *Triplumaria selenica* n. sp, cilié *Spirotriche* du colon de l'elephant d'Afrique. Protistologica, 6, 3, 319–327.

Noirot-Timothée C. 1960: Etude d'une famille de ciliés: les *Ophryoscolecidae*. Structures et ultrastructures. Ann. Sci. Nat. Zool., 2, 527–718.

Noirot-Timothée C. 1969: Les affinités des *Entodiniomorphida* (*Ciliata*). Progress in Protozoology Abstr. Third Int. Congr. Protozool., Leningrad 1969, Nauka, 371.

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

Strelkov A. 1931: Morphologische Studien ueber Oligotriche Infusorien aus dem Darme des Pferdes. III. Körperbau von *Tripalmaria dogieli* Gassowsky. Arch. Protistenk., 75, 221–254.

Strelkov A. 1939: Parasitičeskie infuzorii iz kišečnika neparnokopytnych semejstwa *Equidae*. Učen. Zap. Leningr. Pedag. Inst., 17, 1–262.

Thurston J. P. and Grain J. 1971: Holotrich ciliates from the stomach of *Hippopotamus amphibius*, with description of two new genera and four new species. J. Protozool., 18, 133–141.

Wolska M. 1965: Remarks on the adoral ciliature in the order *Etnodiniomorphida*. Acta Protozool., 3, 322–325.

Wolska M. 1966 a: Division morphogenesis in the genus *Didesmis* Fior. of the family *Buetschliidae* (*Ciliata, Gymnostomata*). Acta Protozool., 4, 15–18.

Wolska M. 1966 b: Study on the family *Blepharocorythidae* Hsiung. I. Preliminary remarks. Acta Protozool. 4, 98–104.

Wolska M. 1967 a: Study on the family *Blepharocorythidae* Hsiung. II. *Chronina ventriculi* (Jameson). Acta Protozool., 4, 279–284.

Wolska M. 1967 b: Study on the family *Blepharocorythidae* Hsiung. III. *Raabena bella* gen. n., sp. n. from the intestine of the Indian elephant. Acta Protozool. 4, 285–290.

Wolska M. 1968: Study on the family *Blepharocorythidae* Hsiung. IV. *Pararaabena dentata* gen., sp. n. from the intestine of Indian elephant Acta Protozool., 5, 219–224.

Wolska M. 1969: *Triadinium minimum* Gassovsky. Its phylogenetic significance. Rev. Soc. Mexic. Hist. Nat. 30, 65–73.

Wolska M. 1971: Studies on the family *Blepharocorythidae* Hsiung. VI. Phylogenesis of the family and the description of the new genus *Circodinium* gen. n. with the species *C. minimum* (Gassovsky, 1918). Acta Protozool., 9, 171–194.

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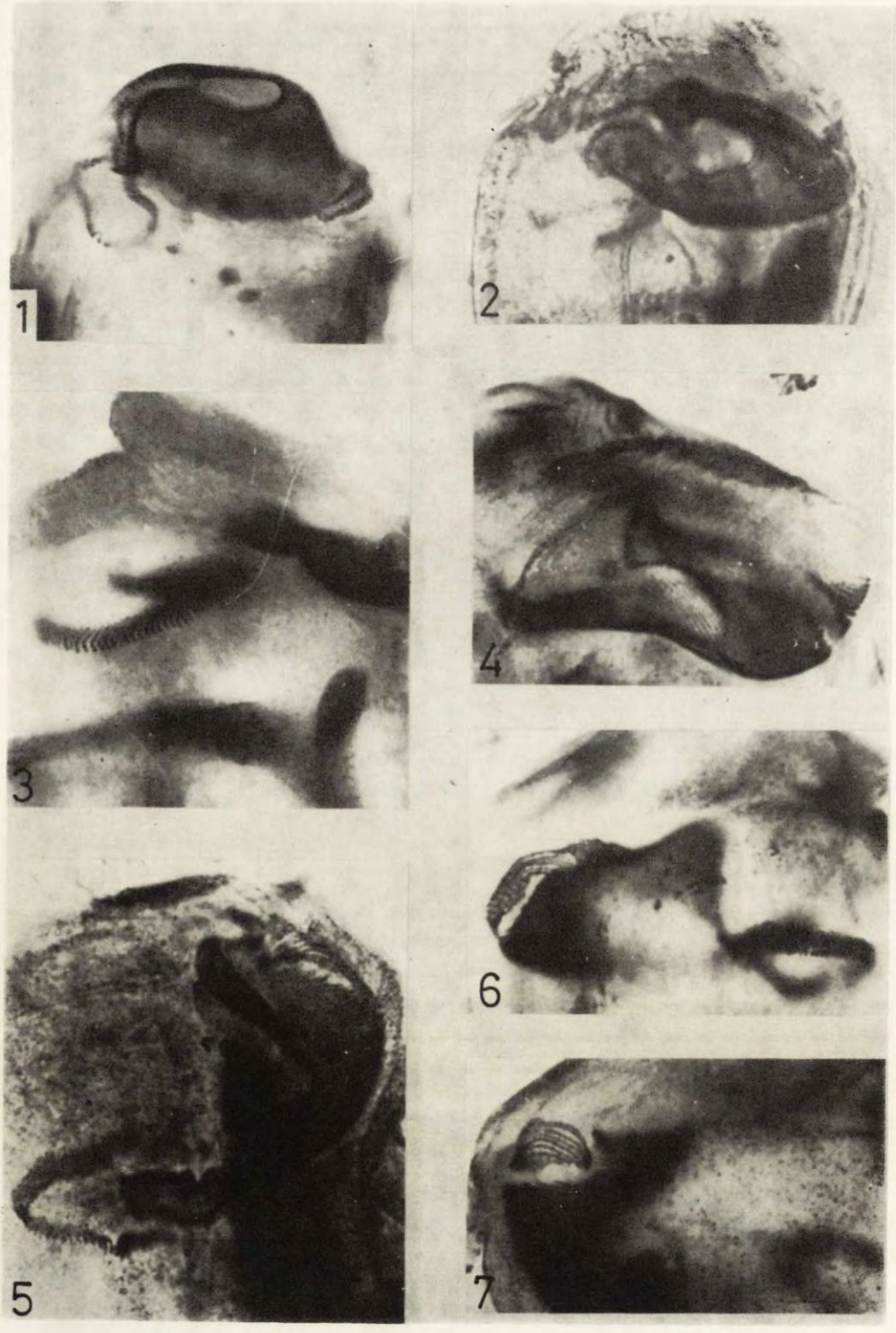
EXPLANATION OF PLATES I–II

1: *Cycloposthium.* The completely devaginated ciliophore. Visible is the dorsal side of the adoral zone i.e., "cinecias cortas" according to Fernandez-Galiano (on the left). On the ventral side, at the base of the ciliophore, "cinecias independientes" or "cilias independientes" according to Fernandez-Galiano can be seen (on the right)
2: *Tripalmaria dogieli.* The outline of the invaginated ciliophore and short kineties
3: *T. dogieli.* A part of the short kineties system
4. *T. dogieli.* The upturned ciliophore. Visible are diagonal raws of kinetosomes in the syncilia zone and large kinetosomes at the ventral edge, which form a base for "free cilia" according to Wolska, corresponding to "cilias independientes" according to Fernandez-Galiano
5: *T. dogieli.* The outline of the adoral ciliature. The view from anterior body pole
6, 7: *T. dogieli.* Raws of large kinetosomes ("free cilia") as seen at diverse positions of the ciliofor
8, 9: *T. dogieli.* Short kineties of the same specimen. 8 — left-side view, 9 — right-side view
10: *T. dogieli.* The base of the dorsal, anterior caudal tuft
11, 12: *T. dogieli.* Sagital section. Visible are long cilia of the ciliophore (the syncilia zone) and short cilia ("free cilia") on the wall of the infundibulum (on the right)
13–15: *T. dogieli.* Sections through the hollow preceding the cytostome-cytopharynx complex. Visible are cilia emerging from the short kineties
1–10 — Photomicrographs of silver-impregnated specimens × 1500,
11–15 — Photomicrographs of semi-thin sections. × 1500



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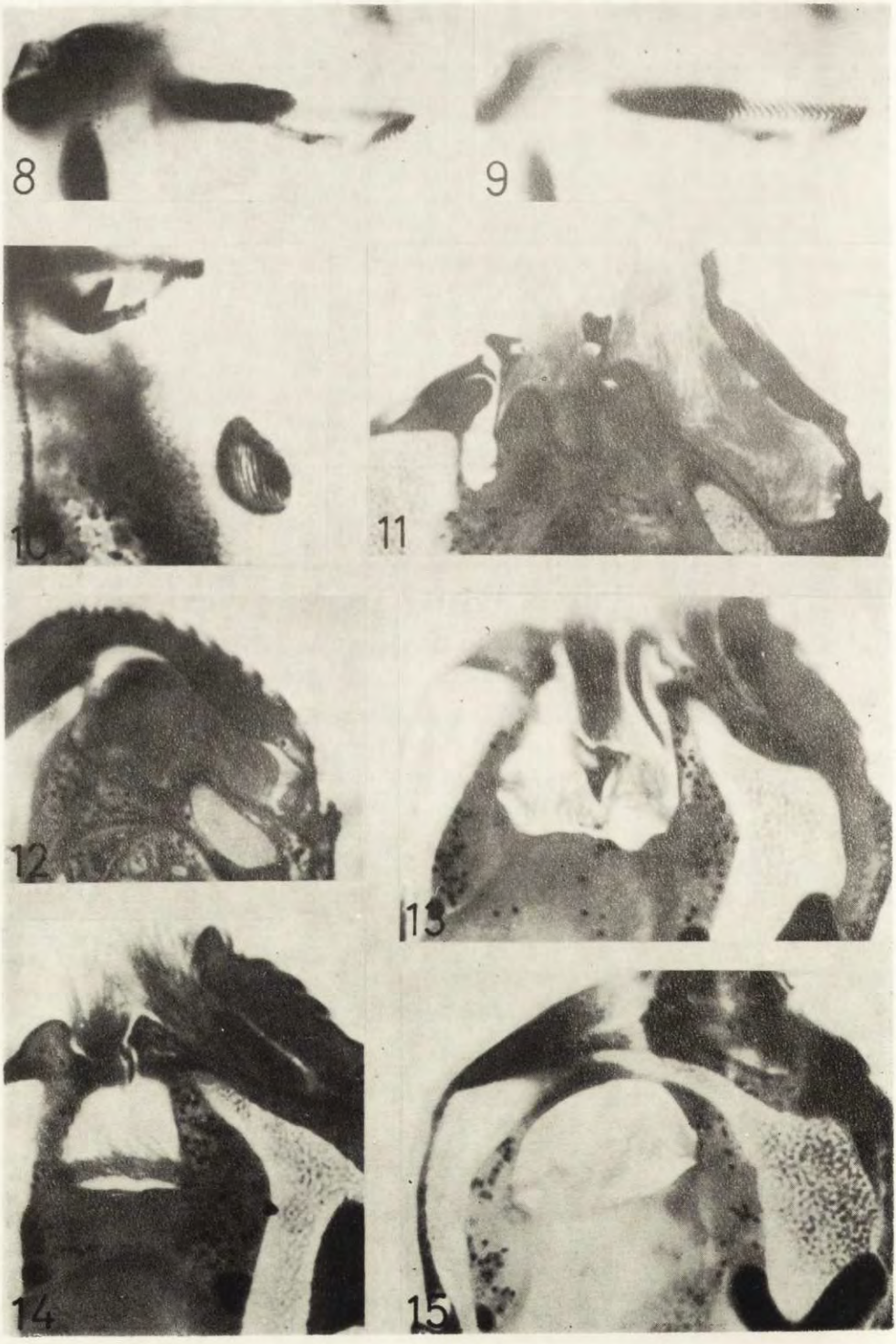

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Tripalmaria dogieli Gass., 1928 (*Ciliata, Entodiniomorphida*). Structure and Ultrastructure. Part II. Electron-microscope Investigations [1]

Synopsis. The structure of the ectoplast of *T. dogieli* is different from any till now-studied *Ophryoscolecidae* and *Cycloposthiidae*. The difference concerns a structure of the sheets underlying the microtubular layer. In *Tripalmaria*, there occurs a structureless, electron-dense substance which forms a kind of net, while in *Cochliatoxum* the corresponding formation is composed of lamellae of the dense substance. Under the net short barren kinetosomes are scattered, which are in touch with a middle sheet of microfibrils of the ectoplast. In ciliary regions the dense rods underline the raws of kinetosomes, like "baguettes infraciliaires" in *Ophryoscolecidae*. The adoral ciliature of *T. dogieli* is similar as that of *Cycloposthium*. The cytostome-cytopharyngeal complex is of similar nature as in other *Entodiniomorphida*.

The aim of the present study was to characterize the cell cortex (more exactly, the whole ectoplast) and the cytostome–cytopharyngeal complex.

Material and Methods

The source of material were samples of contents of horse colons. The samples were taken to thermos bottles immediately after killing the horses at the Konstantynów slaughterhouse near the town of Łódź. At the laboratory in Łódź the ciliata were prepared and fixed according to Grain (1966) manner. They were fixed in the mixture of 2% osmium tetraoxide and Hungate fluid as 1 : 1 for about 1 h end embeded in Epon 812. Sections were cut on III LKB ultramicrotome and picked up on formvar-coated grids. The sections were contrasted with uranyl acetate and lead citrate, according to Reynolds. The grids were examined with an electron microscope Tesla BS, 513A.

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Moreover, the so-called semi-thin sections were prepared according to the procedure described in Part I (Wolska 1977), and photographed with an optical microscope (Pl. II 10, 11, 12).

Results

It is worthy of emphasis that in *Tripalmaria dogieli* ectoplast is markedly separated from endoplast by fibrous ecto-endoplast boundary. The body surface is covered with a single membrane-unit membrane. The membrane looks typically; it extends over the cilia where it can be best visible (Pl. I 1, 2). In the parts of the body devoid of cilia the membrane is covered with amorphous substance of uneven density (Pl. I, 1), which resembles to mucoidal layer described by Senaud and Grain (1972) in *Cochliatoxum,* the *Entodiniomorphida* belonging to the family *Ditoxidae* according to Strelkov (1939), to the family *Spirodiniidae* according to Latteur et Dufey (1967). Under the unit membrane of *T. dogieli,* there is a thin, structureless, electron-dense layer of epiplasm. Still deeper run regularly spaced bundles of longitudinal microtubules (Pl. I 1, 7).

This groups of microtubules are underlined with an electron-dense substance in the form of thick trabeculae or bars. They sprout lateral branches (transverse strands) of diverse shapes at many levels. All this makes a three-dimensional reticulum or net specifically-looking (Pl. I 1, 7, 8, 9). Further on we will call it a dense net. In the major part of the body the longitudinal bars of the dense net are parallel to each other. In the other, especially in the posterior part, this arrangement is not regular any more (Pl. I 9).

Deeper on, nearly in the middle of the ectoplast thickness, there is a sheet of circular microfibrils (Pl. I 1), sometimes broken into bunchs (Pl. IV 20). This sheet is very prominent and compact near the ciliary regions where microtubules and the dense net disappear (Pl. I 4). Moreover, all the ectoplast above the microfibrillar sheet and deeper on up to the boundary with the endoplast is threaded with loosely scattered microfibrils. In some places the microfibrils compose huge agglomerations (Pl. I 6), for example, when joining the main skeletal plate with the small, mobile part of the skeleton (the finger like part according to Strelkov (1931, 1939)), or when joining the syncilia region of the ciliophore with the ventral surface of the body (Pl. III, 13, Pl. IX 44). In the ectoplast, especially between the middle sheet of microfibrills and the ectoplast/endoplast boundary, the ergastoplasm occurs abundantly (Pl. I 1).

At the level of microtubules there are quite numerous vesicles (Pl. IV 19, Pl. VII 38). In some places pores occur (Pl. I 5), which may suggest that vesicles excrete a substance to cover the surface of the protozoan's body.

Between the dense net and the middle microfibrillar sheet of the ectoplast, the barren kinetosomes are scattered all over the non ciliated body region (Pl. I 3, 7, 8, 9, Pl. IV 19, 20). They are short and always connected with microfibrills of the ectoplast (Pl. IV 18, 19, 20).

Another sheet of variously oriented microfibrills with deeper lying and adhering to it longitudinal microtubules produces the boundary between the ectoplast and the endoplast — EE. (Pl. I 1, Pl. IV 20).

On the left side of the body the boundary runs parallelly to the surface of the cell. Here the ectoplast is a thin layer. On the dorsal and ventral sides the ectoplast/endoplast boundary begins to move away the surface, the ectoplast becoming a broad layer enveloping the nucleus, caudal tufts, the skeleton on the right side and the anterior part of the body including the adoral ciliature. A difference in the ectoplast thickness between the left and the right side may be seen in the cross-section through the posterior part of the body (Pl. II 10 — semi-thin section). Running away from the surface, the ectoplast-endoplast microfibrillar layer sprouts branches to the ectoplast (Pl. XII 35).

In the middle part of the body, on the left side, the microtubules of the boundary form small assemblages, while the microfibrillar layer becomes wavy (Pl. VII 39).

On the right side of the body, along the skeleton, the boundary layer creases forming prominent folds or septa protruding into the endoplast (Pl. II 10, Pl. IV 18, Pl. VII 39). In this way, the endoplasmic sac (the endoplast), resembling in outline a short-neck bottle, with left anteriorly-oriented recess, is bordered on the right with longitudinal folds, which usually may be seen under the light microscope in form of streaks or septa (Pl. II 11, 12). The septa cling to the skeleton in the posterior and middle parts of the body (Pl. II 10, Pl. IV 18), but in the anterior part they come off the skeleton plate, where the endoplast narrows to become a neck with its opening entering through the cytostome into the infundibulum (for more details see below). The interior of each septum is filled with microfibrills of the ectoplast/endoplast boundary, and outside there run two layers of tubular fibrils. On the septular surface (towards the endoplast) the cytoplasm is condensed, sometimes granular (Pl. III 15, 16). As described by Strelkov (1931, 1939), the skeleton of *T. dogieli* consists of tightly coherent grains. And such it looks in electron micrographs. The whole skeletal plate is threaded with twisting,

branched channels filled with cytoplasm. Single grains occur but along the edges (Pl. I 3, Pl. IV 18).

In enlarged parts of the ectoplast, near the ciliary regions, around the nucleus and contractile vacuoles the structures, which must be considered as mitochondria, are crowded (Pl. VII 35, 36, Pl. V 27). In the ciliates from the alimentary tract of ruminants end horses, the mitochondria are non-typically structured. They are single membrane limited so they have neither cristae nor tubules (Grain 1966, Gaumont and Grain 1967), and they are distributed similarly as the mentioned formations occurring in *T. dogieli*.

The surface of the nucleus lying in the ectoplast is coated by a dense, thin layer of microfibrills (Pl. VII 36, 37). The cytoplasm around the contractile vacuoles have an aspect of spongioplasm (Pl. VII 36, 37).

Kinetosomes of *T. dogieli* are typically structured. Usually, beneath the septum there is one or more axial grains (Pl. VIII 43, Pl. VI 34). Never more than four axial grains in a kinetosome happened to be found. Both in the somatic and adoral regions, proximal ends of kinetosomes are closed and surrounded by a thick layer of electron-dense material (Pl. VIII 43). Framings of the dense substance surrounding individual kinetosomes of each raw adhere to each other by their basal parts and form a bedding for the same raw (Pl. V 23, 25–28). This dense rods of any neighbouring raws are interconnected by means of specifically looking transversal strands. They are threads of the dense substance, markedly thickened in their middle parts (Pl. V 25, 28). These junctions lie below the proximal ends of kinetosomes, as appears from cross-sections on diverse levels of the very same somatic ciliary zone (Pl. V 23, 25, 26). The transversal junctions occur also in the adoral infraciliature: in the syncilia region (Pl. VI 31) and in the dorsal region, the so-called short-raws band (Pl. VI 33). Sometimes the junctions give the impression of fibrous formations with condensation nodes (cf. Pl. VI 32), and resemble, to some extent, reticular fibrils. Most probably, however, this is a structureless, specifically shaped substance.

Basing on the obtained electron micrographs, it is difficult to say whether similar junctions appear also in infraciliature of the so-called "free cilia" (cf. Wolska 1977). Their kinetosomes lie between high folds of the ventral wall of the infundibulum. At their bases the dense substance is less abundant than in other ciliary regions (Pl. IX 45). In foldings of the wall of the infundibulum, transvers sections of tubular fibrils are visible, similarly as in the case of *Cycloposthium* (Grain 1966).

From among the typical fibrils associated with kinetosomes in *T. dogieli* there occur, transver fibrils (Pl. V 24) and, perhaps, kinetodesmal

ones (Pl. VI 29, Pl. III 14). From the dense rods underlying the raws of kinetosomes arise variously oriented nemadesmata. The initial plate of the nemadesma may be variously oriented with regard to the dense rod (Pl. V 27, Pl. VI 34), in which a distinct overgrowth happens to be observed in the place where a nemadesma begins (Pl. VI 34). Most often, nemadesmata are strongly developed and numerous (Pl. VI 30, 33). Bundles of microfibrils are usually associated with them (Pl. VI 29, Pl. V 23).

One of the obtained cross-sections through the cilia of the syncilia zone reveals connections of cilia. A hooked appendix of one cilium catches another hook of the nearest cilium to be caught itself by the next one (Pl. IV 22), etc. All these hookings take place along a certain line, but how it is oriented towards kineties — we cannot judge of, for there is too small visible area of the section. It may well be that the appendices of cilia are nothing but artifacts appearing in result of unsatisfactory fixation, but this seems impossible when considering their regular arrangement.

We have already mentioned the longitudinal folds or septa at the ectoplast/endoplast boundary on the right side of the body. A little above the anterior, dorsal caudalium and the transversal part of the nucleus, there appear less pronounced folds also on the left body side (Pl. III 7), almost entirely enveloping the narrowed part of the endoplast. On the left side where they meet, there appears a plug caving into the endoplast, composed of irregular assemblages of nemadesmata and membraneous system (Pl. VIII 42). Loosely spaced such membraneous formations have already been found in the endoplast. They appear also in the ectoplast, at the plug level. Near the septa the microtubules occur in small groups (Pl. IV 21) or individually. Further on, towards the anterior end of the cell, instead of the isolated or assembled microtubules, we can see more and more nemadesmata (Pl. VIII 41), and the septa gradually disappear there. The plug disappears, too; the microfibrils become very frequent; nemadesmata and single differently running tubular fibrils occur also in the ectoplast. Finally, the broad layer of closely packed nemadesmata and single tubular fibrils composes a cover of the short cytopharynx and cytostome provided with characteristically arranged microtubules — "Rideaux de tubules" (Pl. IX 46).

Discussion

Senaud and Grain (1972) compared the cortex of *Cochliatoxum periachtum* with that of *Cycloposthium* (described in Grain 1966) and

of *Ophryoscolecidae* (described in Noirot-Timothée 1960). The present paper extends such a comparison to *Tripalmaria dogieli.*

In *Cochliatoxum,* as was stated by authors, lamellae of dense substance (lames denses) lie between the microtubular and the microfibrillar layers thus being similarly situated as plates of the polysaccharide skeleton in *Cycloposthium.* In *Tripalmaria dogieli* the same position is held by the dense net made of structureless, electron-dense substance; thus the net corresponds with lamellae of *Cochliatoxum,* although it is differently shaped. Besides, below the dense net, on the right side of *T. dogieli,* a thick plate of polysaccharide skeleton occurs, similarly as polysaccharide plates lying in some places in *Ophryoscolecidae.*

The cortex of *T. dogieli* is similar to those of *Cycloposthiidae* and *Ophryoscolecidae* also in the presence of the osmiophylic layer underlying the cell membrane. Such a layer does not occur in *Cochliatoxum.*

In *T. dogieli,* outside the cell membrane there occurs a substance which seems to be the mucus excreted by vesicles lying near the tubular fibres. This resembles *Cochliatoxum periachtum* with its numerous mucocystes excreting the substance to cover the cell membrane. Up to now such a system has been observed neither in *Ophryoscolecidae* nor in *Cycloposthium.*

In *T. dogieli,* short kinetosomes without cilia, rather regulary spaced, underlie the dense net, like in *Cochliatoxum periachtum* where they underlie the lamellar layer.

Do those kinetosomes play any role in formation of ciliary primordia during the cell division? The answer to this question, asked already by Senaud and Grain (1972), cannot be done.

In the ectoplast of the anterior part of *T. dogieli,* there occur membraneous formations, looking in the sections as curved tubes, sometimes strongly twisted or ring-shaped. For their extremely tangled shapes I call them lianas. The wall of a liana looks identically as the cell membrane, as appears at great magnifications (Pl. VIII 40). The so-called lianas occur also in the endoplast. They may, perhaps, be fragments of the cell membrane pinched from the surface at the cytostome, and removed to the ectoplast at the plug where they appear to be especially abundant and where ectoplast/endoplast boundary is disorganized. It is hard to say what is their origin and significance; nevertheless, they are characteristic of *T. dogieli,* being not, up to now, observed in any other *Entodiniomorphida.*

Kinetosomes of *T. dogieli* are closed, similarly as those of *Cycloposthium* and *Ophryoscolecidae,* but differently from those of *Cochliatoxum periachtum* which are but partly closed. Kinetosomes of ciliary zones of *T. dogieli* are arranged in raws which seem to be kineties.

The raws are parallel each other and regularly spaced. Such an arrangement may be observed in caudalia and in the adoral region, in its both dorsal and ventral parts, around the cytostome where the raws are spaced at greater distances than those in the ventral part. Again there is confirmed that neither membranells nor cirri occur in *Entodiniomorphida*. In *T. dogieli*, transverse microtubular fibrils are connected with kinetosomes, and it seems that there are poorly developed kinetodesmal fibres. These two kinds of fibrils associated with kinetosomes occur in *Cochliatoxum* (Senaud and Grain 1972).

The dense substance, observed also in other *Entodiniomorphida*, which sustain the ciliary raws in *T. dogieli* is much similar to "baguettes infraciliaires" of *Ophryoscolecidae* (Noirot-Timothée 1960), but the shape of transversal connections is specific in *T. dogieli*.

The electron micrographs of the ventral wall of the infundibulum of *T. dogieli* assure that there exist a group of cilia lying in the pellicular furrows whose kinetosomes blacken at the silver impregnation procedure according to Bielschowsky (Wolska 1977). A similar group of kinetosomes in *Cycloposthium edentatum* was described by Fernandez-Galiano (1959) and called by him as "cinecias independientes". Fernandez-Galiano indicated also that the dorsal part of the adoral infraciliature in *Cycloposthium* is composed of short raws of kinetosomes. The raws are more rarely spaced than those in the ventral part. The similarly structured is the dorsal part of *T. dogieli*. Thus, the adoral ciliature of *T. dogieli* is similar as that of *Cycloposthium* and different from that of *Ophryoscolecidae*.

In *T. dogieli*, similarly as in *Ophryoscolecidae* and *Cycloposthium*, the ectoplast is demarcated from the endoplast with a fibrous boundary. However, that of *T. dogieli* is more compact and rich in microtubules.

The mouth parts of *T. dogieli* are similar as that of other *Entodiniomorphida*. "Rideaux de tubules" and the surrounding nemadesmata begin, surely, at kinetosomes of the dorsal part of the adoral infraciliature surrounding the cytostome, although electron micrographs to show this are lacking. The outer cover of the cytofarynx constitute foldings of the ectoplast/endoplast boundary.

To summarize, it should be stated that some features of *T. dogieli* are the same as those of *Cycloposthidae* (first of all, the structure of the adoral ciliature, and some other — as those of *Ophryoscolecidae*. Similar conclusions on characteristics of this species were offered by Strelkov (1931, 1939).

The occurrence of the so–called dense net in *T. dogieli* is interesting; as to its situation and consistency the net resembles lamellae of *Cochliatoxum*, and, on the other hand, it reminds "travèes opaques transver-

sales" and "baguettes squelettiques" of *Didesmis quadrata* (Grain 1966) of the family *Buetschliidae*. The association with *Buetschliidae* especially with *Didesmis*, is also justified by the presence in *T. dogieli* of kinetosomes in the body parts devoid of cilia, and makes still more probable the supposition (Wolska 1966) that *Entodiniomorphida* are the *Gymnostomata's* descendants. It appears clear that further studies on the ciliates from alimentary tract of herbivorous mammals are needed.

RÉSUMÉ

Tripalmaria dogieli se distingue des *Cycloposthiidae* et des *Ophryoscolecidae*, étudiés jusqu' à présent, par la structure d'ectoplaste. Sous une couche microfibrillaire d'ectoplaste on trouve chez *T. dogieli* une substance dense arrangér en réseau, comparable aux lanus denses de *Cochliatoxum*. Sous les réseau des courts cinétosomes sont dispersés. Dans les zones ciliées une substance dense soutienne les rangés de cinétosomes, comme les "baguettes infraciliaires" chez les *Ophryoscolecidae*. La ciliature adorale est formée comme chez *Cycloposthium*. Le complexe cytostome — cytopharynx ne différe pas d'autres *Entodiniomorphida*.

REFERENCES

Fernandez-Galiano D. 1959: La infraciliacion en *Cycloposthium edentatum* Strelkov. Boln. R. Soc. esp. Hist. nat., 57, 139–150.

Gaumont R. et Grain J. 1967: L'anaerobiose et les mitochondries chez les protozoaires du tube digestif. Ann. Univ. A.R.E.R.S. Reims, 5, 174–176.

Grain J. 1966: Etude cytologique de quelques Ciliés Holotriches endocommensaux des Ruminants et des Equides. Thèse Clermont–Ferrand, editions du C.N.R.S., 1–141.

Latteur B. de Dufey M. M. 1967: Réforme systématique de la famille des *Cycloposthiidae* Poche, 1913. Acta Zool. Pathol. Antverpiensia, 44, 125–139.

Noirot-Timothée C. 1960: Etude d'une famille de Ciliés: les *Ophryoscolecidae*. Structures et ultrastructures. Ann. Sci. Nat. Zool., 2, 517–718.

Senaud J. and Grain J. 1972: Etude ultrastructurale préliminaire de *Cochliatoxum periachtum* Gassovsky, 1919, cilié Entodiniomorphe endocommensal du cheval. Protistologica, 8, 65–82.

Strelkov A. 1931: Morphologische Studien über oligotriche Infusorien aus den Darme des Pferdes. III. Korperbau von *Tripalmaria dogieli* Gassowsky. Arch. Protistenk., 75, 221–254.

Strelkov A. 1939: Parasitičeskie infuzorii iz kišečnika neparnokopytnych sem. *Equidae*. Učen. Zap. Leningrad. Pedagog. Inst. Gercena 17, 1–262.

Wolska M. 1966: Division morphogenesis in the genus *Didesmis* Fior. of the family *Buetschliidae* (*Ciliata, Gymnostomata*). Acta Protozool., 4, 15–18.

Wolska M. 1977: *Tripalmaria dogieli* Gass., 1928 (*Ciliata, Entodiniomorphida*) structure and ultrastructure. Part I. Light-microscope investigations. Acta Protozool., 17, 15–21.

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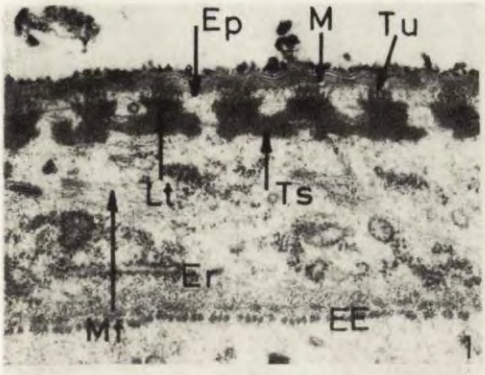
EXPLANATION OF PLATES I–IX

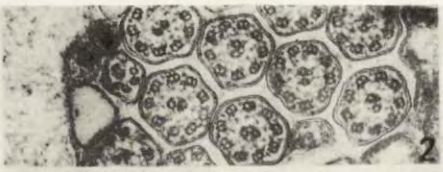
Tripalmaria dogieli Gass., 1928

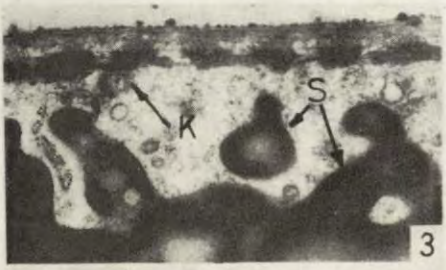
1: The transverse-section of the ectoplast, ventral side. Cell membrane (M), epiplasm (Ep). Longitudinal microtubules (Tu), Longitudinal trabeculae of the dense net (Lt), Transverse strand of the dense net (Ts), Microfibrils (Mf), Ergastoplasm (Er), The ectoplasm/endoplasm boundary (EE) × 26,700
2: Cilia of the adoral zone in the transverse-section × 44,200
3: The outer layer of the ectoplast on the right body side. Transverse-section of the kinetosome (K), Skeleton (S), × 23,200
4: The cross-section through the cuff surrounding the caudalium. Microfibrils (Mf) × 11,200
5: A fragment of the body surface with an empty mucocyste(?) — arrow. × 25,500
6: The assemblage of microfibrills connected with the skeleton plate × 12,300
7: The cross-section almost parallel to the body surface. On the left — the level of tubular fibres, in the middle — the level of the dense net, on the right — the level of kinetosomes. × 11,200
8: The cross-section through the left body side, similarly taken as that in phot. 7. × 11,200
9: The cross-section tangenial to the surface near the cytopyge. Irregularly running longitudinal trabeculae of the dense net. × 9500
10: The transverse-section at the level of the posterior, ventral caudalium. Ectoplast (Ek), Endoplast (En), The ectoplast/endoplasm boundary (EE), Skeleton (S), Macronucleus (Mn), × 1500
11: Oblique cross-section. Partly visible are longitudinally cut folds (F), Skeleton (S). × 1500
12: The cross-section similar as in phot. 11, Taken nearer the left body side. × 1500
13: Microfibrils uniting the two parts of the skeleton. × 12,300
14: A fragment of syncilia zone. Kinetodesma(?) — arrow. × 25,700
15: Transverse section of folds. Microfibrils (Mf), Microtubules (Tu). × 44,200
16: Longitudinally sectioned folds. × 12,300
17: Folds in the left, anterior body side. × 44,200
18: The cross-section through the ectoplast of the middle part of the body, the right side. Kinetosome (K), Skeleton (S). × 12,300
19: Oblique cross-section, near the surface. Longitudinal microtubules (Tu), Dense net (Dl). Microfribrils in the middle part of the ectoplast (Mf), Vesicles (V). × 26,900
20: The ectoplast of the left body side cut along the kinetosome (K). Microfibrils (Mf), Microtubules (Tu). × 44,200
21: Folds of the EE in the anterior part. Assemblages of microtubules — arrow. × 25,500
22: Cilia of the syncilia zone. Hooks of cilia (arrow) × 44,200
23: The cross-section through kinetosomes of the anterior, caudal tuft. Nemadesmata arising from the dense substance under lying kinetosomes. Numerous microfibrils. × 12,300
24: A fragment of phot. 23. Transverse fibrils — arrow. × 30,200
25, 26: Fragments of the zone presented in phot. 23 at deeper levels Transverse strands of the dense substance, more numerous in phot. 26. × 12,300
27: Fragments of the syncilia zone. Mitochondria — arrow. × 12,800
28: Another fragment of the syncilia zone. × 12,300
29: The transverse-section through kinetosomes of the posterior caudal tuft. Kinetodesmal fibres(?) — arrow. × 8000
30: An assemblage of nemadesmata near the ciliary zone. × 27,000
31: A fragment of the adoral zone. Transverse connections of the dense substance — arrow. × 12,300
32: A fragment of the adoral zone. Transverse connections of the dense substance — arrow. × 44,200
33: Short rows band. Transverse connections of the dense substance — arrow. × 23,500

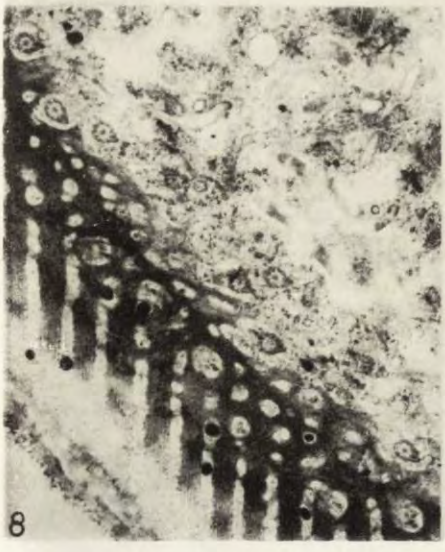
34: The connection of nemadesmata with the dense substance. × 23,500
35: The cross-section of the ectoplast of the ventral side. The EE departing the surface. Microfibrils arising from the boundary — arrow. Mitochondria — double-arrow × 26,000
36: The cross-section through the contractile vacuole and the nucleus. Mitochondria (arrow) around the vacuole. × 5800
37: A fragment of phot. 36. Spongioplasm — arrow. Microfibrils (Mf). × 12,300
38: The oblique section through the cortex. Vesicles between the tubular fibrils — arrow. × 11,200
39: The cross-section of the middle body part. The EE on the left body side — arrow. Folds on the right side (F). Ectoplast (Ek), Endoplast (En). × 26,700
40: Membranous formations, the so-called lianas (arrow). × 44,200
41: The boundary of the endoplast in the anterior part, the left side. Numerous nemadesmata, disappearing folds. × 12,300
42: The plug on the left side of the endoplast. Nemadesmata (N). Lianas (L). × 12,300
43: The longitudinal section of kinetosome. × 23,200
44: Microfibrils connecting the ciliophore with the ventral body side. × 6900
45: The cross-section through the ventral wall of the infundibulum. Kinetosomes in deep furrows. Cross-sections through microtubules (Tu) in folds of the infundibulum. × 12,300
46: The cross-section through the cytostome region "Rideaux de tubules" (arrow). × 12,900

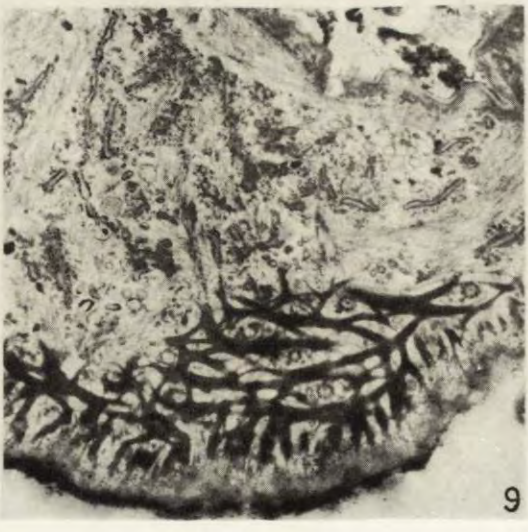
10–12: Photomicrograph of semi-thin sections.











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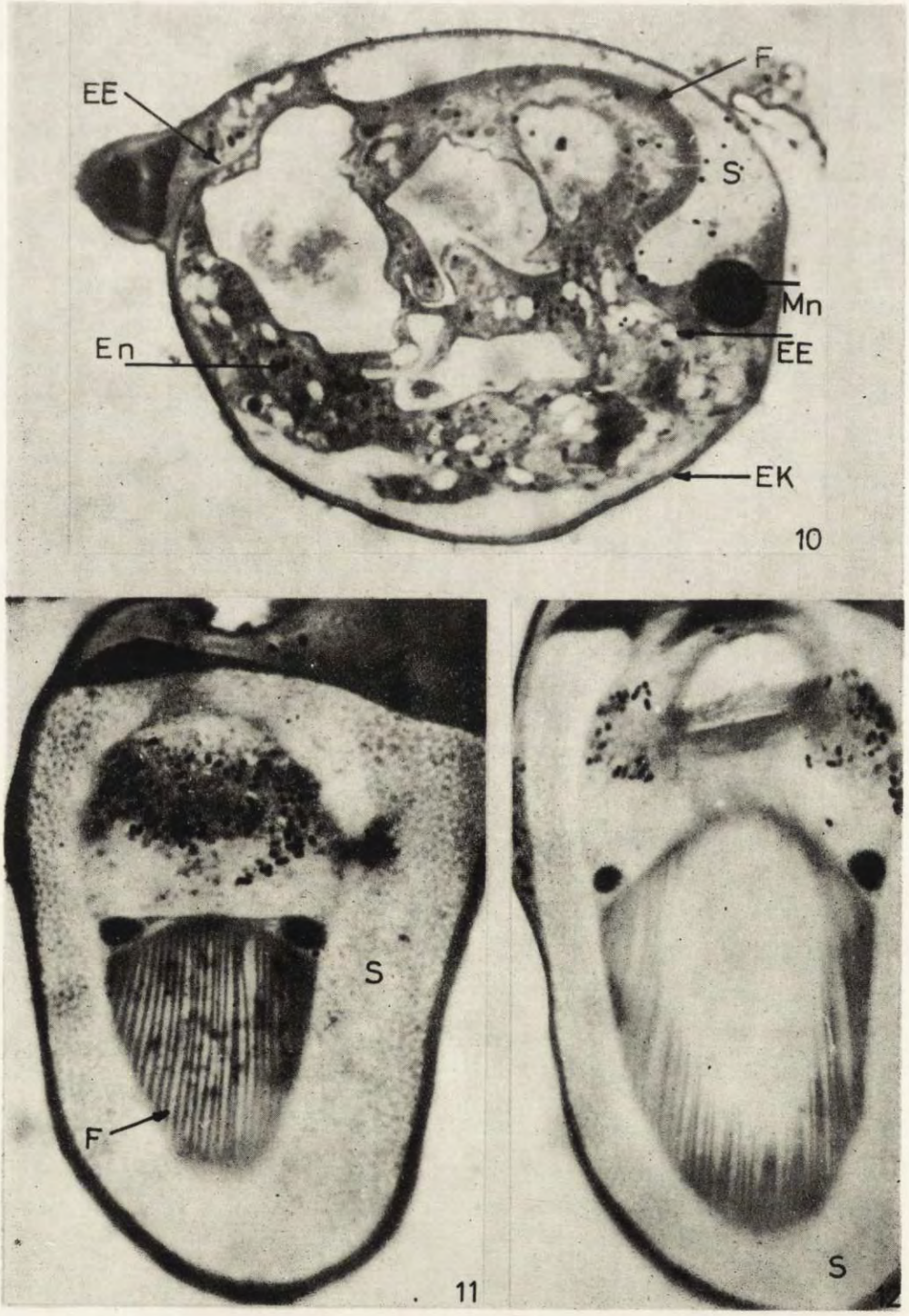

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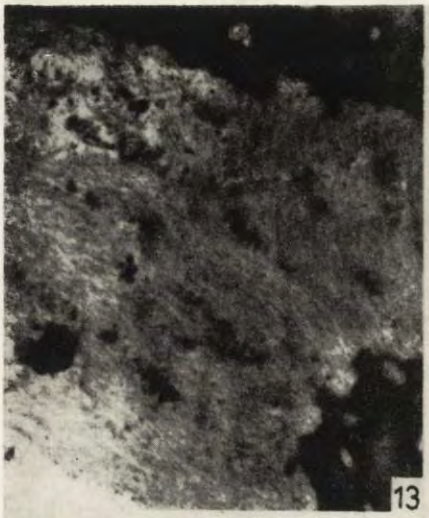

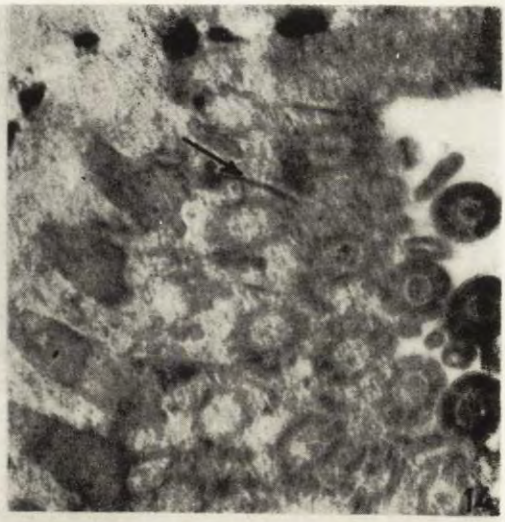

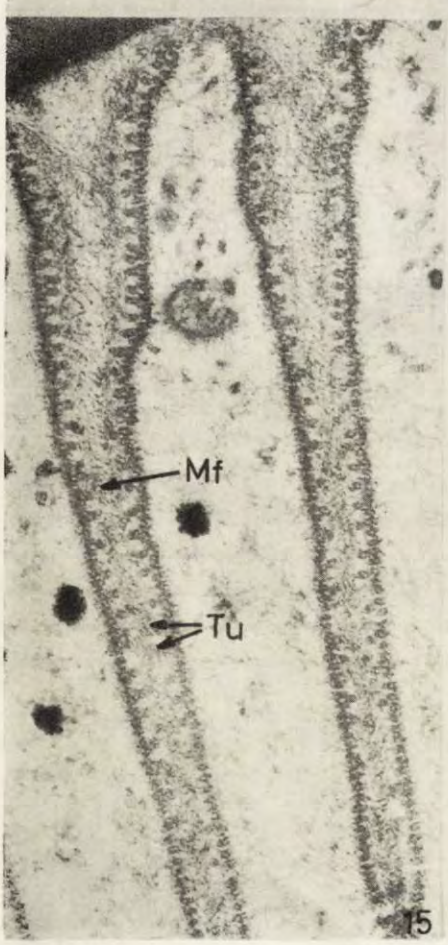

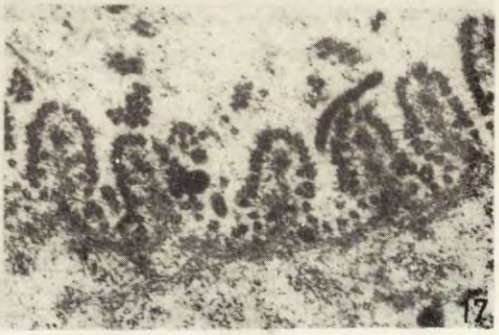

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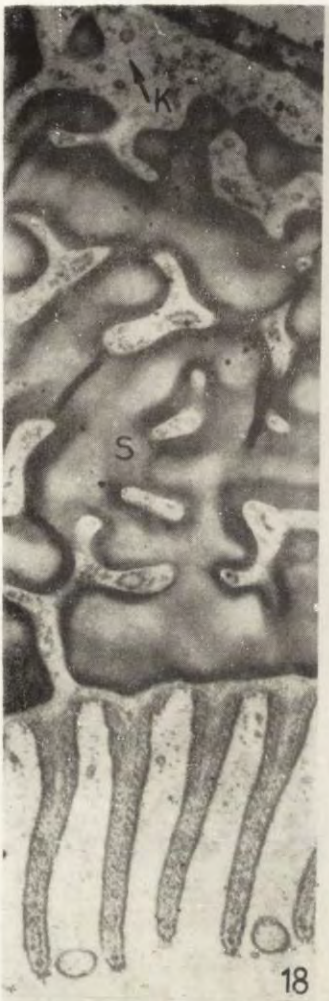

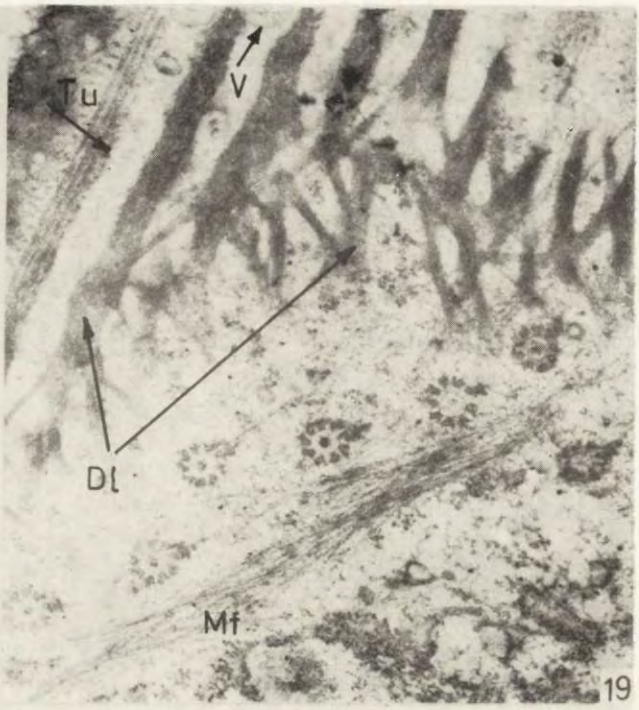

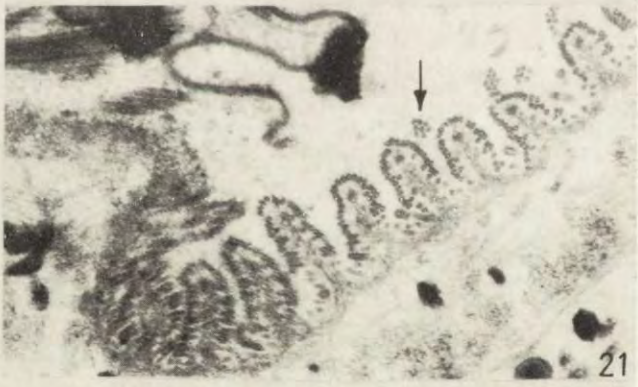

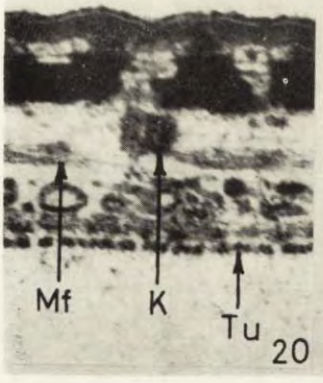

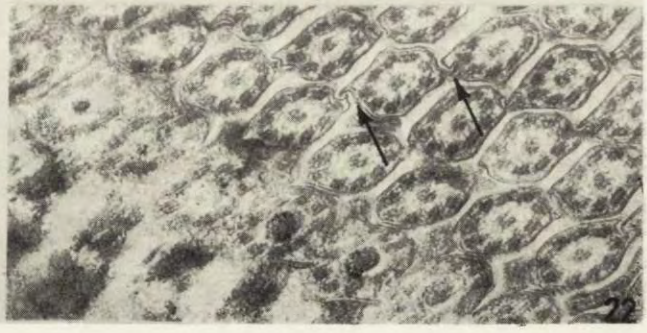

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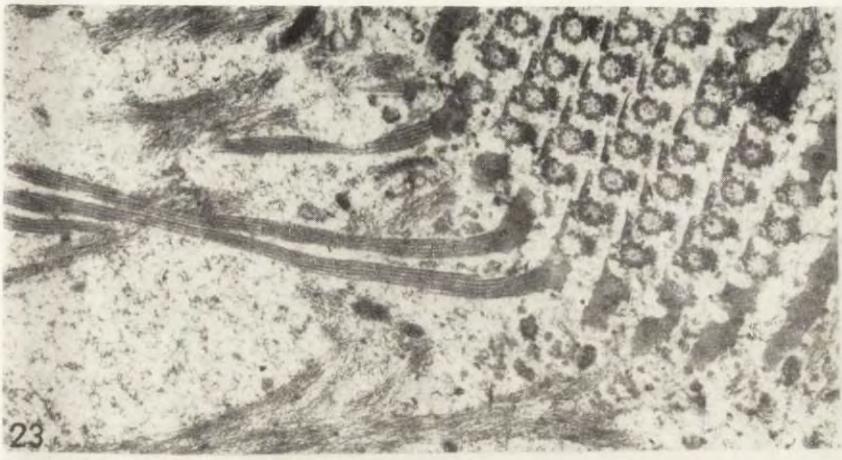

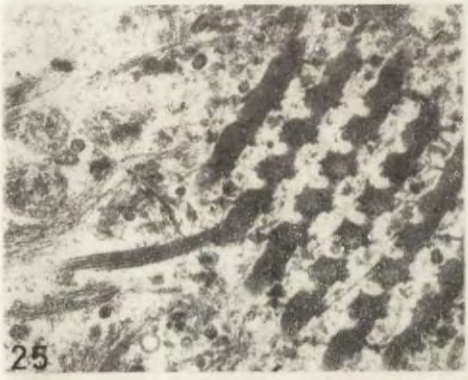

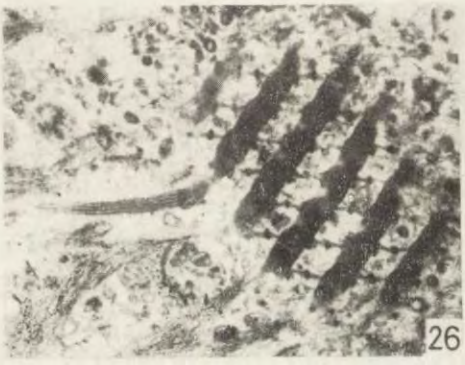

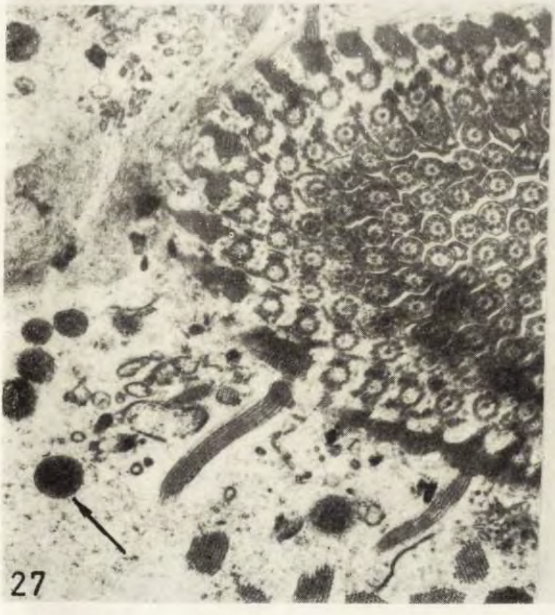

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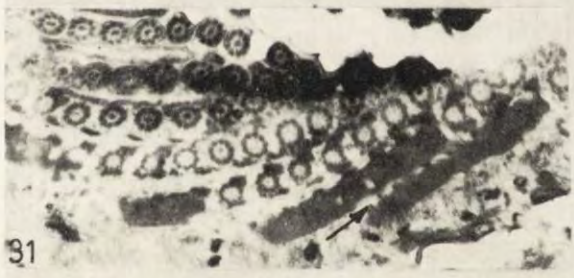
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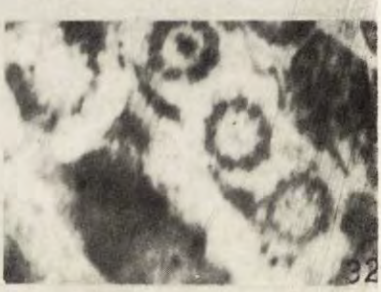
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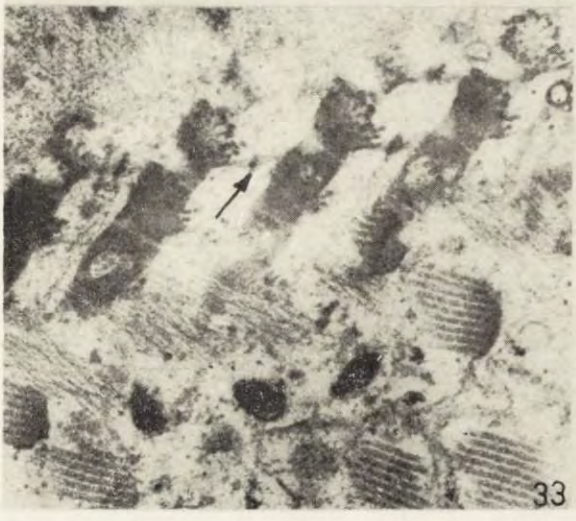
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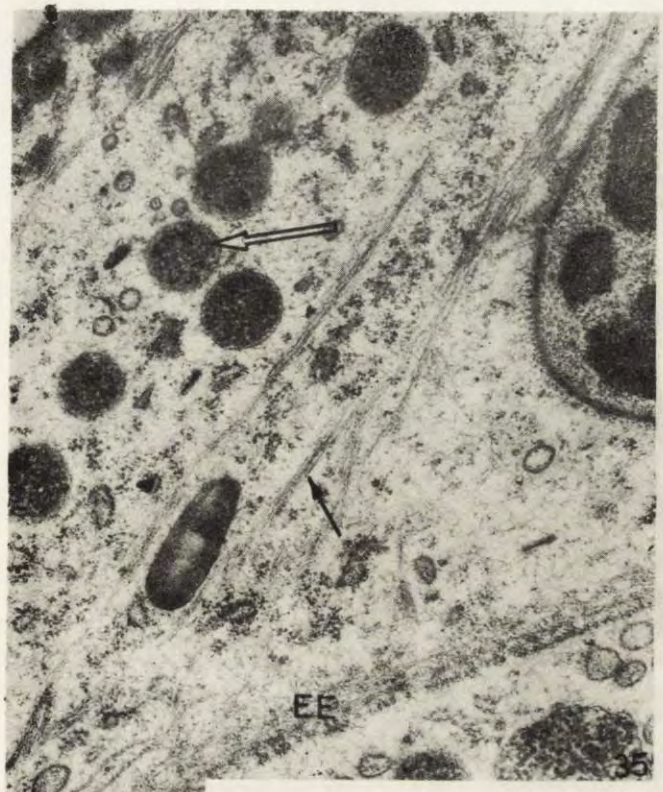

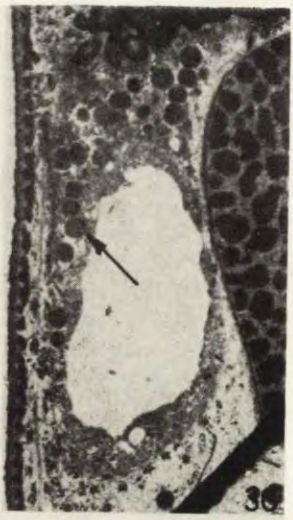

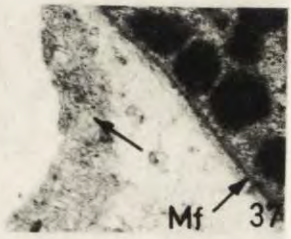

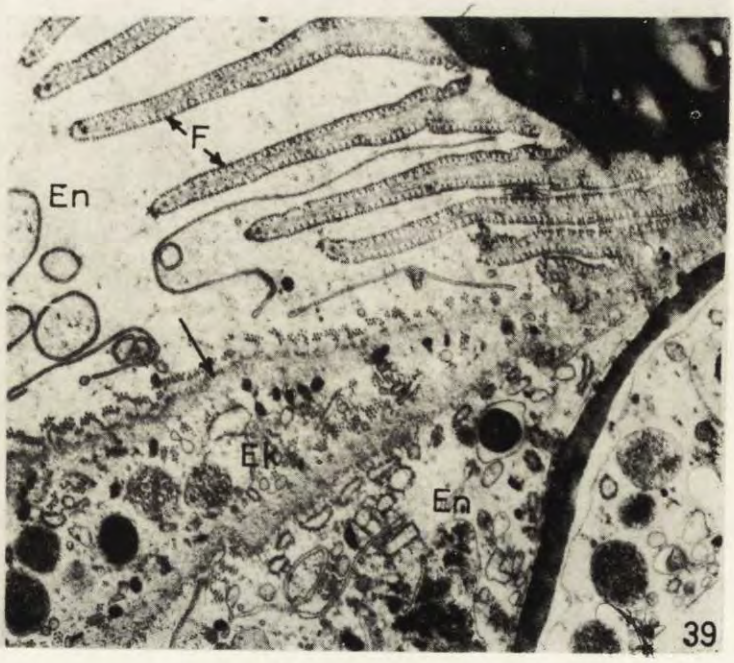

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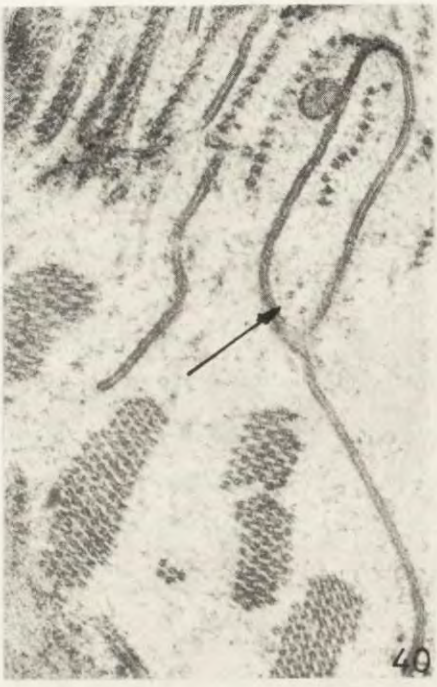

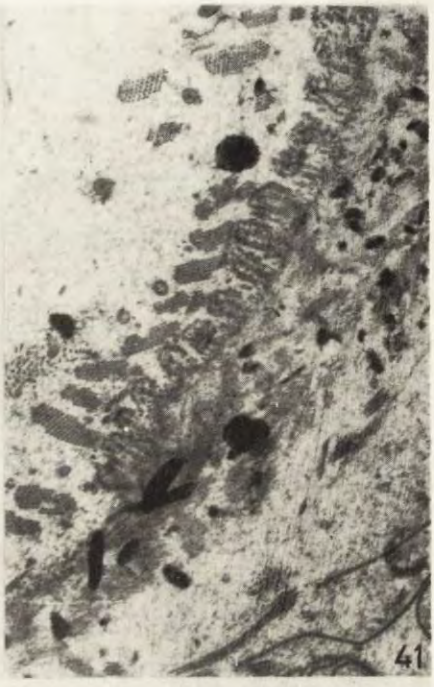

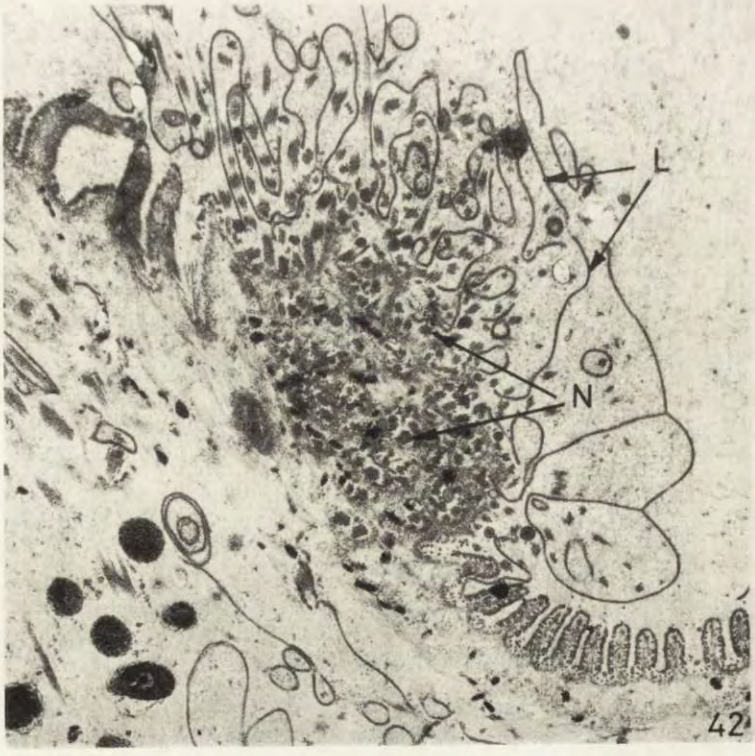

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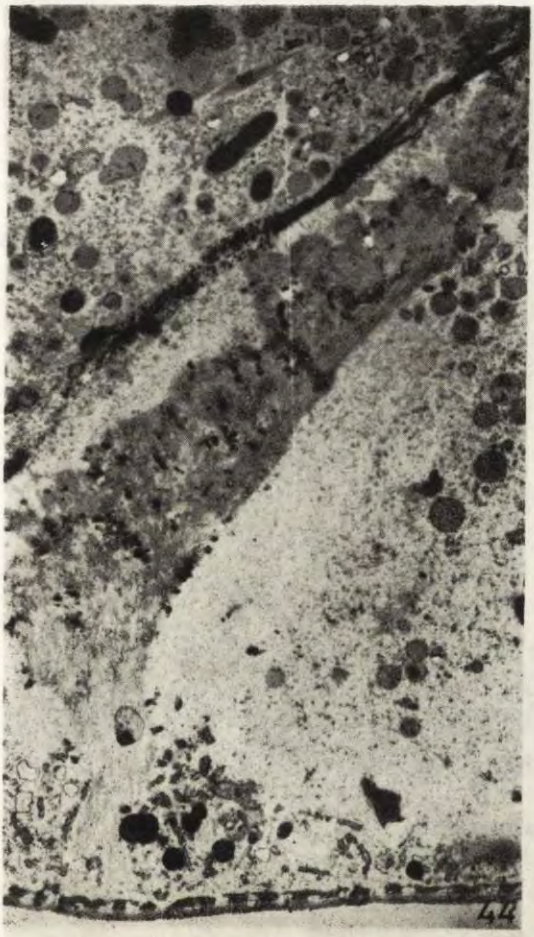

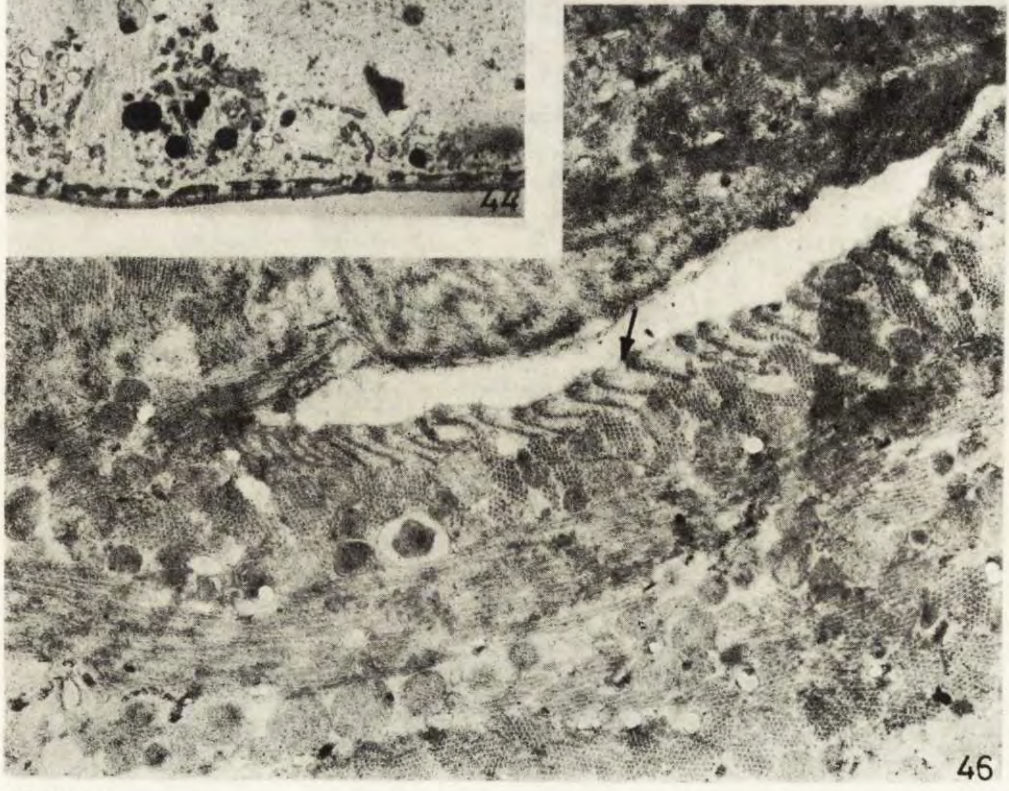

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Dedifferentiation of Fibrillar Structures during Encystment of *Dileptus visscheri* (*Gymnostomatida*)

Synopsis. The dedifferentiation of tubular and microfibrillar structures during the encystment of *Dileptus visscheri* has been studied and the successive stages of dedifferentiation of the ciliated oral and somatic kinetosomes are described. It has been found that the ciliated kinetosomes are displaced into cytoplasm together with their cilia and resorbed there without participation of autophagic vacuoles. The process of desintegration of paracytostomal oral kineties, representing a complex pattern of ciliature, is described, as well as the structural changes occurring in microfibrillar material during successive stages of encystment.

Differences in the mode of resorption of ciliated and nonciliated kinetosomes and the role of cilia in this process, as well as the possibility of occurrence of the resorption *in situ* during the encystment of *Dileptus* are discussed.

The anterior and the posterior fragments of the ciliate *Dileptus*, obtained as a result of traumatic impairment, quickly reconstitute the primary shape and proportions of the cell. Simultaneously with this process takes place regulation of the pattern of oral and somatic ciliature (Golińska and Kink 1976, 1977, Golińska 1977). Regulation of the ciliature comprises proliferation of new somatic and oral kinetosomes, as well as resorption of corresponding basal bodies; proliferation and resorption take place in defined places on cell surface.

It is known that in resting cysts of this ciliate only single kinetosomes without cilia are preserved (Kink 1973). Therefore an investigation of these changes, at ultrastructural level, gives an insight into the process of dedifferentiation of the somatic and oral ciliature, as well

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as the possibility of comparison of these processes with those, occurring during regeneration *Dileptus* cell (Golińska and Kink 1976, Golińska 1977).

Material and Methods

Observations were made on ciliates *Dileptus visscheri* fed on *Colpidium* sp. The cultures of *Dileptus* were maintained in beakers in Pringsheim solution. The culture medium was not renewed, only an amount of liquid was added to prevent evaporation. The ciliates, intensively fed during several days, began the mass encystment.

For light microscopy the encystants were silver impregnated according to protargol method by Tuffrau (1967). The encystants having already formed cyst wall, corresponding to the stage 4 of encystment in the present paper, after fixation were submitted to a procedure which specifically removed the cyst wall, which otherwise obscured the picture of cell surface. The encystants enclosed in cysts were washed in distilled water after fixation in Holand's fluid. Then, some drops of 1% Javel's water was added. As a result swelling of the ciliates inside the cysts occurred leading to rupture of the cyst wall and release of slightly swollen encystants. Similar procedure was used in the case of resting cysts of *Dileptus*. After washing in water the cells were impregnated with protargol.

For electron microscopy the cells in successive stages of encystment were fixed in a mixture of 2% OsO_4 and 6% glutaraldehyde dissolved in 0.5 M cacodylate buffer at pH 7.2 during 1 h. Encystants having already formed cyst wall were fixed together with their cyst walls. Agar blocks were embedded in Epon 812 and sectioned on LKB ultramicrotome. The sections were stained and contrasted in uranyl acetate and lead nitrate and examined with JEM 100 B.

Results

During encystment of *Dileptus* four stages may be distinguished when changing body shape is taken as a criterion. Stage 1 (Pl. I 2) is represented by the ciliates with slightly thickened body and shortened proboscis; their posterior body end is rounded up rather than sharply pointed (cf. trophont in Pl. I 1). In stage 2 the ciliates with distinctly rounded up body and strongly shortened proboscis were placed (Pl. I 3), while encysting cells with rounded up body lacking proboscis were placed in stage 3 (Pl. I 4) and those being in course of the formation of either cyst wall or having already formed the outer wall were assigned to stage 4.

(1) Changes During the Stage 1 of Encystment

In protargol stained preparations no loss have been observed in kinetosomal pattern, such as loosening of kinetosomes or discontinuity of kineties. The sections examined with the electron microscope showed the following changes: (a) dedifferentiation of somatic ciliature, (b) dedifferentiation of oral ciliature, and (c) structural changes in microfibrillar layer, separating endo- and ectoplasm.

(a) In the encysting cells of the stage 1, in various parts of the cell body, numerous cilia displaced into ectoplasm have been observed (Pl. II 8). They were laid parallely or slightly diagonally to the cell surface, being connected with kinetosomes perpendicular to the surface and bearing fibres (Pl. II 8).

Distribution and length of these cilia suggest the following way of their displacement inside the cell: the cilium lies down on the body surface, its membrane and the membrane covering the ectoplasm come into contact, then the lysis of the membrane fragments adhering to each other occurs. The cilia present in ectoplasm retain their typical tubular pattern (9 + 2) and no traces of desintegration of their structure have been observed in this stage of encystment. Thus, it seems that as the first step of dedifferentiation of the somatic ciliature may be regarded the displacement of the cilia into ectoplasm, with unchanged tubular structure and preserved contact with kinetosomes (Pl. IV 19).

(b) In the first stage of encystment some changes in the structure of paracytostomal kineties have been observed along the whole proboscis. The sections through distal (Pl. II 10) and basal (Pl. II 11) parts of proboscis show numerous cilia in the oral field. More frequently than in the case of somatic cilia one may observe the displacement of oral cilia (together with their kinetosomes), inward the cytoplasm perpendicular to the body surface. This is probably due to the lack of homogenous microfibrillar layer in the proboscis which could form a mechanical obstacle for the cilia displacing into cytoplasm.

The paracytostomal kineties in trophonts are composed of a pairs of kinetosomes, the ciliated and nonciliated one. In each pair the kinetosomes are joined together and the pairs situated in the right paracytostomal kinety are connected by an additional fibre (Goliń-ska and Kink 1976).

In encystants tangential sections at the level of kinetosomes of paracytostomal kineties show that in the oral ciliature the ciliated kinetosomes dedifferentiate first (Pl. III 12, 13), while those without cilia remain in their original positions, at as judged by the position of the transverse fibres. Certainly, this fact concerns recently desintegrating

segments of paracytostomal kineties, and it cannot be stated whether all ciliated kinetosomes from pairs are pulled into cytoplasm always at first, being followed by the nonciliated ones.

In the case of the left paracytostomal kinety separation of kinetosomes within pairs seems to be connected with desintegration of junctions between their bases, while release of ciliated kinetosomes of the right kinety would be additionally connected with deterioration of the structure of the additional fibre integrating the kinety (Pl. III 12).

In the oral cilia pulled into cytoplasm the disruption of the circle of outer tubules in one (Pl. III 12) or in several places (Pl. III 13) has been observed. It would indicate that the regression of oral cilia begins with the lysis of junctions between the pairs of tubules. In such cilia the central tubules are clearly seen. In neighborhood of the oral ciliature some sections have been found through the cilia with completely changed structure; there were pairs of tubules loosely distributed, therefore it was impossible to determine which of them were outer and which central ones (Pl. IV 18).

The oral cilia are desintegrated directly in the cytoplasm. Neither the pictures of autophagic vacuoles filled exclusively with cilia, also no cilia were found in vacuoles filled with other cell organelles as trichocysts or mucocysts.

Sumarizing the changes in the oral ciliature one can say that in the stage 1 of encystment the dedifferentiation of this ciliature begins in various parts of paracytostomal kineties; from each pair of oral kinetosomes the ciliated one is pulled at first into the cytoplasm together with its cilium; the nonciliated kinetosomes rest in their original places; shortly after pulling down the cilium or even in the course of its displacement to the cytoplasm begins the desintegration of its tubular pattern; desintegration of cilia takes place in the cytoplasm, apart of autophagic vacuoles.

In this stage of encystment no changes in the structure of circumcytostomal kinety, participating in the pharyngeal complex, have been observed.

(c) In the stage 1 of encystment some changes in the structure of microfibrillar ecto- endoplasmic layer are observed. In trophonts (Pl. V 22) this layer, littering the ectoplasm, is surrounded by numerous rough and few smooth vesicles of cytoplasmic reticulum. Proximal ends of somatic kinetosomes are fixed in this layer. In encysting ciliates discontinuity of this layer is clearly visible (Pl. II 8); it is disrupted in many places. Some kinetosomes seem to be shifted from this layer towards cell surface. In the places of disruption the cilia are frequently visible, probably those penetrating the endoplasm. One may suppose

that the disruption of this layer is a result of mechanical injury caused by the cilia passing to endoplasm, or, it may be regarded as a process independent of dedifferentiating ciliature.

No changes in the appearance or in the number of vesicles of rough reticulum have been observed.

(2) Changes During the 2nd Stage of Encystment

(a) Dedifferentiation of somatic ciliature. Protargol stained preparations of the ciliates in this stage showed discontinuity of somatic kineties. Some bare fields may be observed on the cell surface in which there are rows of kinetosomes without cilia. These rows are of various length (Pl. I 7). There are also some groups of kinetosomes (Pl. I 7). It is possible that the kinetosomes, pulled together with cilia into the ectoplasm, are displaced under cell surface, e.g., due to the movement of cytoplasm.

In EM preparations numerous somatic cilia have been found in the ectoplasm as well as single cilia or groups of somatic cilia in the endoplasm (Pl. II 9). This indicates that the process of dedifferentiation of the somatic ciliature, which has begun in the stage 1 by displacement of somatic cilia into ectoplasm, is continued. Simultaneously the cilia, probably those which have been pulled earlier into ectoplasm, penetrate endoplasm together with their kinetosomes. In the cilia found in endoplasm breaking of the circle of outer pairs of tubules has been observed similarly as in the case of oral cilia. The process of desintegration of both types of cilia seems to have the same course, the more so, that the somatic cilia desintegrate directly in cytoplasm, apart of autophagic vacuoles.

In endoplasm numerous longitudinal and transverse sections through the somatic kinetosomes were observed; these kinetosomes were provided with infraciliary fibres, transverse and postciliary ones as well as kinetodesmes (Pl. II 9). However it was not possible to examine the succeeding stages of kinetosome desintegration, so, it is not known whether they become separated from the cilia before the beginning of resorption, when and in what sequence the accompanying fibres are lysed, and in what manner proceeds the resorption of 9 triplets.

In the stage 2 of encystment the process of dedifferentiation of the somatic ciliature is continued; some cilia are displaced into ectoplasm, others, together with their kinetosomes and accompanying fibres, penetrate endoplasm and are resorbed there.

(b) Dedifferentiation of oral ciliature. In protargol stained specimens of the stage 2 clearly shortened paracytostomal kineties were observed, similar to oral kineties of the trophont or of the specimens in the

stage 1 of encystment (Pl. I 5, 6). The palisade of nemadesmata is visible at the base of proboscis, however the nemadesmata do not form a regular basket being somewhat in disorder. It is possible that this picture corresponds to the desintegration of nemadesmata at their ends, observed by Tucker (1970) in *Nassula*.

In sections examined with EM all the pictures of dedifferentiation of paracytostomal kineties were observed which had been found in the stage 1. In the desintegrating pairs of kinetosomes the ciliated kinetosome penetrates the cytoplasm while the nonciliated one rests in its place. There were found also desintegrating cilia with characteristics disruption of the circle of pairs of outer tubules. However, the longitudinal sections which would give the unquestionable picture of nonciliated oral kinetosomes has not been found, so it is not sure whether they are resorbed in situ or migrate to endoplasm.

In the stage 2 of encystment the desintegration of paracytostomal kineties makes progress but no changes have been found in the structure of circumcytostomal kinety (Pl. III 14). In its vicinity some resorbing cilia of somatic or oral origin (from paracytostomal kineties) have been observed, however the structure of this kinety, formed exclusively of nonciliated kinetosomes, seems to rest unchanged.

(c) Changes in the structure of microfibrillar layer, already observed in the stage 1, are visible also in the stage 2 of encystment. The layer is disrupted in many places. Typical of trophic forms, flattened canals of endoreticulum are present, however there is clearly less elements of rough reticulum accompanying this layer from the side of ectoplasm (Pl. V 23).

No changes have been found in the structure of microfibrillar bands in the proboscis as well as in microfibrills integrating the external and internal basket of nemadesmata.

(3) Changes in the Stage 3 of Encystment

Protargol stained preparations revealed some bare fields on the cell surface, similarly as in the stage 2. No remnants of paracytostomal kineties were observed in the cell completely devoid of the proboscis, while in those having a small protuberance in the place of proboscis very short paracytostomal kineties were found.

Moreover, clearly visible changes occur in the shape of palisade of external nemadesmata, which from original circular transforms into ellipsoidal one (Pl. I 4). Silvering with protargol did not reveal the internal basket of nemadesmata.

In this stage EM reveals very few somatic cilia in the ectoplasm.

Sections through the pharyngeal complex give pictures of single, big nemadesmata hanging on the non-ciliated kinetosomes (Pl. III 15). In this region some resorbing cilia have been observed which may be the remnants of dedifferentiated paracytostomal kineties.

In the encystants of the stage 3 aggregation of microfibrillar material which had not been observed earlier was found in the neighborhood of the cytopharynx (Pl. IV 20). It is in form of bands, somewhat similar to microfibrillar bands in the proboscis of trophic specimens or of the ciliates in the stage 1 of encystment (Pl. IV 21). The bands are accompanied by numerous vesicles of smooth reticulum. These microfibrillar structures are situated directly in the cytoplasm being not surrounded by the wall of autophagic vacuole. They were found only in this place, near the nemadesmata.

The microfibrills forming the layer, are still visible between ecto- and endoplasm, although the ectoplasm has changed its original organisation. In comparison with a trophont this layer contains distinctly less vesicles of rough reticulum; there are also few mucocysts and typical of endoplasm of *Dileptus* mitochondria otherwise. Flat alveoles adhering the surface membrane from the side of ectoplasm are still visible.

Thus, the deformation of the palisade of nemadesmata of the external basket and aggregation of great amount of microfibrillar material with smooth vesicles near the oral structures may be regarded as a characteristic feature of the stage 3 of encystment.

(4) Changes in the Stage 4 of Encystment

In protargol stained preparations of this stage bundles of fibres, loosely scattered in cytoplasm, were found as well as the bundles sticking perpendicularly to cell surface (Kink 1973). It is possible that these pictures reflect the process of desintegration of the basket of nemadesmata: detaching of groups of nemadesmata from kinetosomes in the case of external basket, or from the microfibrillar ring integrating the internal basket.

In EM the cilia in the endoplasm have been observed only sporadically indicating that the process of their dedifferentiation comes to the end.

In this stage of encystment numerous kinetosomes bearing fibres have been found sticking perpendicularly under cell surface (Pl. III 16, 17). Axosom and phragments of outer tubules, are observed at the apical plate of these kinetosomes. It is possible that the ciliary structures present at the kinetosomes are the remnants of cilia resorbed in situ, without penetration of the whole complex, cilium + kinetosome, into endoplasm. In general, the process of resorption in situ would concern

the cilia of these somatic kinetosomes which are preserved in resting cysts of *Dileptus* (Kink 1973).

In this stage of encystment the remnants of microfibrillar aggregations have not been found; they were not present in the cysts as well (Kink 1973). It shows that they are temporarily formed and desintegrate toward the end of encystment.

The microfibrillar layer and the ectoplasm show strongly changed organisation in comparison with a trophont (Pl. V 24), being analogical to those observed in the stage 3. Additionally, more smooth vesicles were observed in the vicinity of this layer than it had been found in previous stages.

Discussion

Taking into account the character of dedifferentiating fibrillar structures two phases may be distinguished during the encystment of *Dileptus visscheri*. In the first phase somatic ciliature undergoes desintegration (except the kinetosomes preserved in resting cysts, Kink 1973) as well as paracytostomal kineties of the oral ciliature. In the second phase the dedifferentiation of the circumcytostomal kinety and the pharyngeal complex occurs. Desintegration of microfibrillar material takes place in both these phases, however its aggregation near the buccal region is characteristic of the phase 2 of encystment.

Reduction of the number of elements of the somatic ciliature takes place during the encystment of *Dileptus* in clearly random way. The somatic cilia, together with their kinetosomes, are pulled out simultaneously from various places on cel surface. There are no defined places on the encysting surface in which the resorption of the somatic ciliature would take place earlier or later than in other ones.

It is known that in experimental injured cells of *Dileptus*, deprived of the proboscis or trunk, i.e., in the cells being in course of regulation of the body shape and proportions, the resorption of somatic ciliature takes place in all narrowed parts of examined phragments (Golińska and Kink 1976, 1977). In these phragments only some body parts are diminished and just to them the resorption is limited.

During the encystment the anterior (proboscis) and the posterior (tail) parts of somatic surface gradually diminish, up to complete disappearing; just in these parts occurs resorption. However the process of resorption spreads simultaneously over the median regions of the cell, which do not change so distinctly as the anterior and the posterior parts do, nevertheless their deformation is clearly visible when compared with

trophic cells. The resorption may appear in slight deformation of the cell, such as local narrowing of the proboscis, as well as in the change of body proportions, i.e., shortening of the proboscis, or in the complete deformation of the cell as in the case of encystment, when the shape of trunk changes from spindle-shaped to sphaerical.

Several stages of dedifferentiation of the ciliature may be distinguished in the encystment of *Dileptus*: (1) Dislocation of cilia into the ectoplasm being probably a result of fusion of ciliary membrane and the surface membrane of the body (plasmalemma). Such a way of losing the cilia and flagella was observed by Bloodgood (1974). In the case of encysting *Dileptus*, when hundreds of cilia disappear simultaneously from the cell surface, the fusion of membranes seems to be advantageous in attaining the minimum cyst surface and removing excessive membranes in relatively simple way. (2) Migration of the complex, kinetosome with infraciliary fibres + cilium, inside the cytoplasm. Similar phenomenon was described by Kormos (1971) in *Suctoria*. In the case of *Cyathodinium* the cilia, separating from kinetosomes and migrating to cytoplasm and resorbed there, were found (Paulin 1973). In *Tetrahymena* oral cilia digested in endoplasm were observed (Williams et al. 1972), however, it was not sure whether they had migrated alone or with kinetosomes. (3) Desintegration of cilia. This process takes place directly in cytoplasm and no autophagic vacuoles are formed around resorbed cilia. Resorption of cilia without vacuole formation occurs also in Suctoria (Kormos 1971), in *Cyathodinium* (Paulin 1973), and in *Tetrahymena* (Williams et al. 1972, Moore 1972), while in *Diplodinium* the resorption of cilia inside autophagic vacuoles has been described (Roth and Shigenaka 1964).

In *Dileptus* the desintegration of cilia begins with the disruption of junctions between pairs of outer tubules. The pictures given by Paulin (1973) indicate that the outer tubules disappear at first. Similarly Bloodgood (1974), summing up the data on ciliates and flagellates, shows on the outer tubules, although Roth and Shigenaka (1964) have stated that the central tubules first disappear in resorbed cilia. In the case of *Dileptus* desintegration of the cilium would begin with disappearance of links between outer pairs of tubules and nextly the radial links between outer and central pairs. Such liberated pairs of tubules would be resorbed later (detachment of subunits).

(4) Desintegration of basal bodies. It is known that the ciliated somatic kinetosomes, similarly as oral ones, migrate to endoplasm together with cilia. However, their further fate has not been investigated. It is possible that they separate from cilia before resorption.

Such process is known in *Chlamydomonas* (Johnson and Porter 1968) and in *Stigeoclonium* (Manton 1964). Paulin (1973) observed detaching of the cilium from kinetosome in subpellicular zone of *Cyathodinium,* so the kinetosomes remained in their places while the cilia migrated to cytoplasm. It is possible that in *Dileptus* the cilium and its kinetosome retain their structural contact when they desintegrate.

The problem of resorption of infraciliary fibres which persist with ciliated kinetosomes being already pulled down into cytoplasm remains unresolved. It has not been ascertained whether these fibres detach from kinetosomes before the beginning of resorption of the basal bodies.

In the case of oral ciliature these parts which dedifferentiate earlier, such as paracytostomal kineties may be distinguished and circumcytostomal kinety desintegrating just after paracytostomal ones. Probably it is a result of the fact that the paracytostomal kineties are situated in proboscis which, due to numerous observations on trophonts of *Dileptus,* is known to be a highly instabile part of the ciliate cell, quickly reacting to changing culture conditions by change of its length. It has been postulated that the regulation of the proboscis length would be performed by the proliferation center situated at the base of the proboscis (Kink 1976, Golińska and Kink 1976) and by the resorption center localized in the apical part of this processus (Golińska and Kink 1977). It is possible that during the encystment the proliferation center in oral region is disengaged. Thus, the only active one would be the resorption center acting along the whole length of paracytostomal kineties because desintegration of these kineties has been observed in various places, not only at the apex.

The paracytostomal kineties represent a complex type of organization of ciliature according to Jerka-Dziadosz and Golińska (1977). It has been found, due to the study on regulation of body proportions in anterior phragments of *Dileptus* (Golińska and Kink 1977) that during transformation of the basal parts of these kineties the desintegration of pairs of kinetosomes occurs, resulting in the resorption of the ciliated kinetosome and preservation of the nonciliated one. Transition from a complex (pairs of kinetosomes) to a simple (single kinetosomes) type of organization of the ciliature in the case of examined phragments was limited to rebuilding segments of paracytostomal kineties.

Similar, but more expanded process, proceeding along the whole length of these kineties, occurs during encystment. Simultaneous dedifferentiation of the ciliated and nonciliated kinetosomes from each pair was not observed. At first the ciliated kinetosome separates and passes into

endoplasm together with its cilium, so the complex pattern becomes simplified by selectively acting dedifferentiation mechanism.

In the encystment of *Dileptus* the problem of resorption of the nonciliated oral kinetosomes also attracts attention. The fact that these kinetosomes rest longer in their original positions than the ciliated ones, and the fact that they have not been found deeply in the cytoplasm allow to conclude that they are resorbed in situ. The study by Golińska and Kink (1977) gave similar conclusion about the resorption of nonciliated kinetosomes, being not found in the endoplasm. It may be supposed that in *Dileptus* only the ciliated kinetosomes, somatic as well as oral ones, are displaced to cell inside and resorbed in cytoplasm. Possibly, the cilium plays a certain role in their displacement, pressing on them and pushing them to cell inside. In *Cyathodinium* (Paulin 1973) the cilia detach from kinetosomes and then penetrate cytoplasm, while kinetosomes rest in their original positions. Kormos (1971) observed also the desintegration of kinetosomes devoided of cilia near their original positions.

There are suggestions that the kinetosomes preserved in the resting cysts of *Dileptus* lose their cilia by resorption in situ. In the late stage of encystment (stage 4) some kinetosomes bearing fibres, axosom and fragments of ciliary tubules, situated just under cell surface have been observed. Also in resting cysts of *Dileptus,* being in various age, some single somatic kinetosomes situated beneath cell surface were found (Kink 1973). Resorption of cilia *in situ* in *Dileptus* may proceede in the way as that known in *Suctoria* (Kormos 1971). Also the occurrence of two types of resorption of ciliated kinetosomes in *Dileptus* seems to be possible, as Kormos (1971) has found in *Suctoria* in the same simultaneous resorption of kinetosomes *in situ* and by retraction cell.

The differences in the mode of resorption of the ciliated and nonciliated kinetosomes in *Dileptus* seem to be due to the presence or lack of cilia. Both ciliated oral and somatic kinetosomes are resorbed inside the cytoplasm although they differ by accompanying infracillary fibres.

Dedifferentiation of pharyngeal complex in encystants takes place after desintegration of proboscis. As first the changes in the distal end of the basket of nemadesmata appear; its structure becomes loosen. Then, the distal end of this basket, originally circular, becomes ellipsoidal, however the nemadesmata are still hanging on kinetosomes. Similar changes were observed by Tucker (1970) in *Nassula,* but in the basket already detached from the surface and pulled

down into cytoplasm. Then, the gradual desintegration of the basket occurred. It seems that also in *Dileptus* the nemadesmata detach gradually, because at the end of encystment some of them may be observed hanging of kinetosomes. Moreover, the desintegration of nemadesmata is connected with the resorption of transverse junctions between tubules, i.e. with the disappearance of hexagonal structure, typical of nemadesmata. In the cysts of *Dileptus* the bundles of long, tubular fibres were observed having no interconnections between particular fibres (Kink 1973). These bundles are regarded as the remnants of the largest oral structure — the nemadesmata.

In division of *Nassula* (Tucker 1970) the old basket pulled down into cytoplasm becomes invisible as early as in 1 h after beginning of this process. Thus, the desintegration of this very complicated structure proceeds quickly. In the encysting *Dileptus* this process is also relatively quick, because the resorption of the basket begins at the end of encystment in the stage 3 and in young cysts (cysts with clearly visible two envelopes, 1 day after encystment) the basket is not present.

Desintegration of the inner basket, embedded only in microfibrillar material, seems to be connected with disappearance of microfibrillar structures.

Dedifferentiation of microfibrillar structures. In the encystment of *Dileptus* the following steps may be distinguished: (a) Gradual desintegration, up to complete disappearance of microfibrillar ecto- endoplasmic layer. The process begins in the stage 1 and proceeds up to the end of encystment. (b) Accumulation of ribbon- like microfibrillar structures near the pharyngeal complex in the stage 3. This phenomenon is limited only to this stage of encystment.

As main signs of the process of desintegration of this layer numerous breakings, diminution of the number of rough reticular vesicles from the side of ectoplasm, as well as the occurrence of smooth vesicles at both sides and in the places of breaking down may be regarded. Golińska (1974) has suggested that the vesicles of rough reticulum, situated in the vicinity of microfibrillar layer in *Dileptus*, may take part in the formation of the cell shape. In comparison with trophonts fewer rough vesicles accompanying this layer occur in encystants, however the shape of encysting cell completely changes. These observations supports the above suggestion about the role played by these vesicles in the maintenance of cell shape.

In the encysting ciliates more smooth vesicles are present than in the trophonts. Smooth reticular vesicles appear in all these places of the *Dileptus* cell where narrowing, shortening, or elongation occurs, i.e., in the furrow, at wound healing, in the most elastic part of the body —

in the proboscis; they always accompany microfibrilles (Golińska 1972, Grain and Golińska 1969). May be, they play certain role in disruption of the layer, cooperating with it during contraction. However, the enzymatic destruction of this layer is also possible.

Appearance of microfribrillar aggregations (step b) with big, smooth vesicles may be regarded as a result of contraction of the proboscis. The microfibrillar bands occurring in the proboscis would be passively pushed down and accumulated at its base when the proboscis becomes shorter.

On the other hand the cooperation of the microfibrillar bands in the process of shortening of the proboscis is also possible. The microfibrilles with smooth vesicles may play the role of contracting elements. Such suggestion was pronounced by Allen (1971) for *Paramecium* and by Golińska (1974) for *Dileptus*.

In the resting cysts of *Dileptus* no remnants of these structures have been found (Kink 1973). Also the pictures of resting cysts of *Didinium* given by Holt and Champan (1971) showed no traces of microfibrillar ecto- endoplasmic layer, typical of trophonts of this ciliate. In *Acanthamoeba* microvilli containing great amount of fibrillar material completely disappear during the encystment and simultaneously actine is reduced (Rubin and Maher 1976). In all these cases the shape of encysting cells changes. Thus, the above data show that the fibrillar material plays great role in formation of the cell shape.

RÉSUMÉ

On a suivi la dedifférentiation des structures tubulaires et microfibrillaires chez *Dileptus visscheri* pendant l'encystation. La dedifférentiation des cinétosomes ciliés somatiques et orales a été divisé en plusieures étapes. Les cinétosomes munis de cils sont transloqués avec leurs cils et resorbés dans l'endoplasme sans participation des vacuoles autophagiques. On a décrit le mode de la desintégration des cinéties orales paracytostomales qui représentent le pattern complèxe de la ciliature. On a suivi les changements de structure du matériel microfibrillaire à travers les étapes de l'encystation.

La discussion concerne les différences de la résorption des cinétosomes ciliés et de ceux sans cils, le rôle du cil dans ce phénomène. La possibilité de leurs résorption *in situ* pendent l'encystation de *Dileptus*. La resorption de la ciliature en fonction des changements de la forme et des proportions de la cellule, et le rôle des structures microfibrillaires dans le développement de la forme de la cellule.

REFERENCES

Allen A. D. 1971: Fine structure of membraneous and microfibrillar systems in the cortex of *Paramecium*. J. Cell. Biol., 49, 1–20.

Bloodgood R. A. 1974: Resorption of organelles containg microtubules. Cytobios, 9, 143–161.

Golińska K. 1972: Studies on stomatogenesis in *Dileptus* (*Ciliata, Holotricha*) in the course of division processes. Acta Protozool., 9, 284–297.

Golińska K. 1974: Effect of puromycin on regeneration processes in *Dileptus anatinus* Golińska 1971. Acta Protozool., 12, 289–306.

Golińska K. and Kink J. 1976: The regrowth of oral structures in *Dileptus cygnus* after partial excision. Acta Protozool., 15, 143–163.

Golińska K. and Kink J. 1977: Proportional regulation of body form and cortical organelle pattern in the ciliate *Dileptus*. J. Cell. Sci (in press).

Grain J. and Golińska K. 1969: Structure et ultrastructure de *Dileptus cygnus* Claparede et Lachman, 1859, *Cilie Holotriche, Gymnostome*. Protistologica, 5, 269–291.

Holt P. A. and Chapman G. B. 1971: The fine structure of the cyst wall of the ciliated protozoan *Didinium nasutum*. J. Protozool., 18, 604–614.

Jerka-Dziadosz M. and Golińska K. 1977: Regulation of pattern in the ciliates. J. Protozool. (in press).

Johnson K. G. and Porter K. R. 1968: Fine structure of cell division in *Chlamydomonas reinhardii*. J. Cell. Biol., 38, 403–425.

Kink J. 1973: The organization of fibrillar structures in the trophic and encysted *Dileptus visscheri* (*Ciliata, Rhabdophorina*). Acta Protozool., 12, 174–194.

Kink J. 1976: A localized region of basal body proliferation in growing cells of *Dileptus visscheri* (*Ciliata, Gymnostomata*). J. Cell. Sci., 20, 115–133.

Kormos J. 1971: Two types of ciliary resorption. Acta Biol. Acad. Sci. Hung., 22, 245–260.

Manton J. 1964: Observations on the fine structure of the zoospore and young germling of *Stigeoclonium*. J. Exp. Bot., 15, 399–411.

Moore K. C. 1972: Pressure-induced regression of oral apparatus microtubules in synchronized *Tetrahymena*. J. Ultrast. Res., 41, 499–518.

Paulin J. J. 1973: The resorption of cilia in *Cyathodinium piriforme*. J. Protozool., 20, 281–285.

Roth L. E. and Shigenaka Y. 1964: The resorption of cilia. Zellforch., 64, 19–24.

Rubin R. W. and Maher M. 1976: Actin turnover during encystation in *Acanthamoeba*. Exp. Cell. Res., 103, 159–168.

Tucker J. 1970: Morphogenesis of a large microtubular organelle and its association with basal bodies in the ciliate *Nassula*. J. Cell. Sci., 6, 385–429.

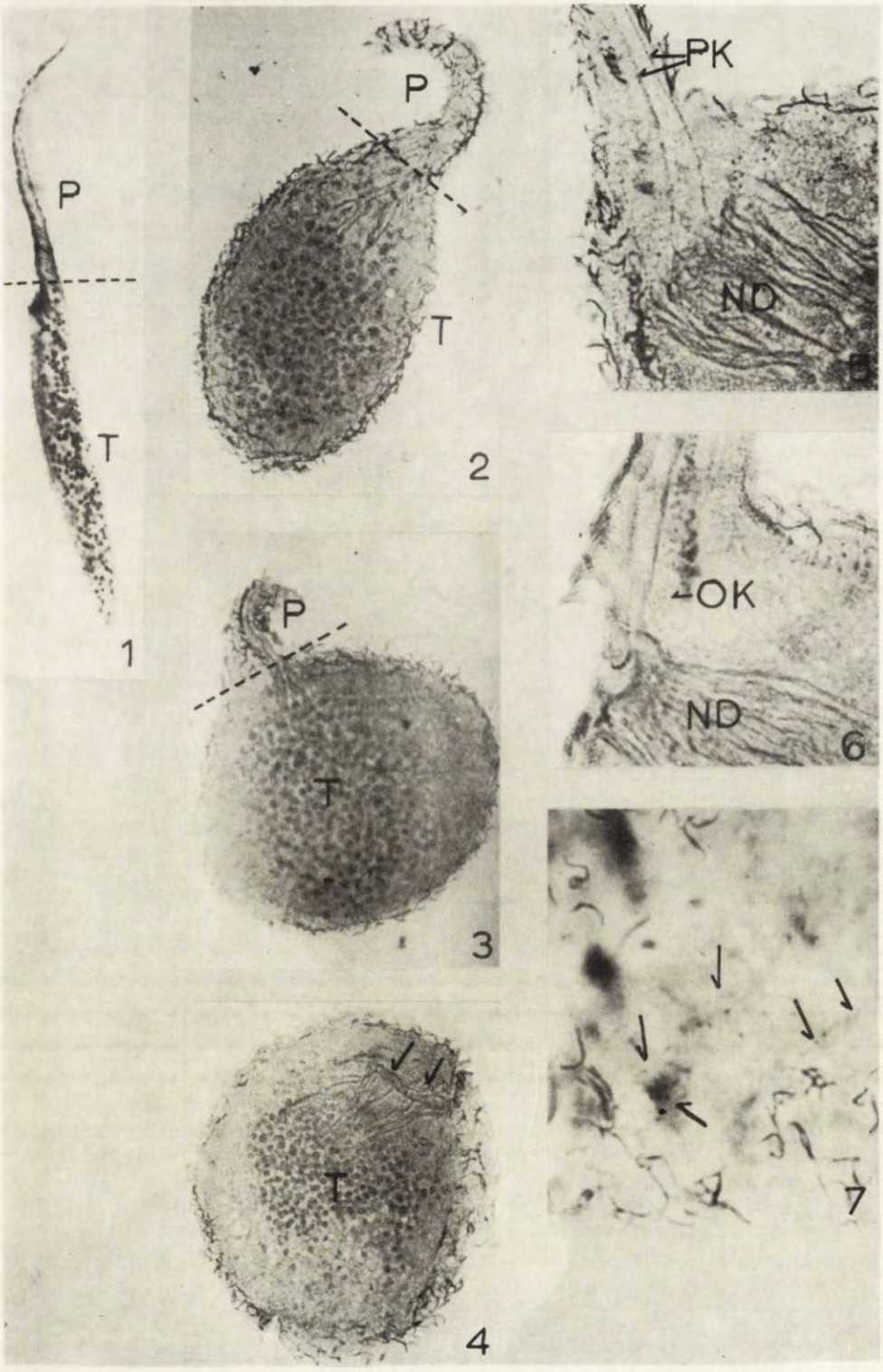
Tuffrau M. 1967: Perfectionnement et practique d'impregnation au protargol des *Infusoires Cilies*. Protistologica, 3, 91–98.

Williams N. E., Frankel J. and Jaeckel R. F. 1972: Electron microscopy of oral replacement in *Tetrahymena pyriformis*. J. Protozool., 19 (suppl.) 20–21.

Received on 19 March 1977

EXPLANATION PLATES I–V

1: Trophont ciliate *Dileptus visscheri*. Protargol stained. Obj. 10 ×. P — proboscis, T — trunk
2: Ciliate in stage 1 of encystment. Protargol stained. Obj. 20 ×. P — proboscis, T — trunk
3: Ciliate in stage 2 of encystment. Protargol stained. Obj. 20 ×. P — proboscis, T — trunk
4: Ciliate in stage 3 of encystment. Protargol stained. Obj. 20 ×. T — trunk. Arrows show the basket of nemadesmata.
5: Stage 2 of encystment. Protargol stained. Obj. 100 ×. PK — paracytostomal kineties, ND — nemadesmata
6: Stage 2 of encystment. Protargol stained. Obj. 100 ×. OK — oblique kineties, ND — nemadesmata
7: Stage 2 of encystment. Protargol stained. Obj. 100 ×. Arrows show disordered aggregations and rows of kinetosomes
8: Stage 1 of encystment. Section through ectoplasm, perpendicular to cell surface. Magn. 13 000 × c.ks. — ciliated kinetosome connected with cilium, mf. l. — microfibrillar layer. Arrows show somatic in endoplasm
9: Stage 2 of encystment. Section through ectoplasm (EKT) and endoplasm (END). Magn. 13 000 ×. Thick arrows show somatic kinetosomes displaced to cell inside together with fibres. Thin arrows show cilia in cytoplasm. mf. l. — microfibrillar layer
10: Stage 1 of encystment. Section through distal part of ventral band. Magn. 11 500 ×. Arrows show cilia in cytoplasm
11: Stage 1 of encystment. Section through basal part of ventral band. Magn. 9000 ×. s.ks. — somatic kinetosome. Numerous cilia (arrows) visible in cytoplasm
12: Stage 1 of encystment. Tangential section through the right paracytostomal kinety. Magn. 30 000 ×. s.ks. — somatic kinetosome, c.ks. ciliated oral kinetosome, n.ks. — nonciliated oral kinetosome.
Arrows show resorbing oral cilia
13: Stage 1 of encystment. Transverse section through nonciliated kinetosomes of the left paracytostomal kinety. Magn. 23 000 ×. n.ks. — nonciliated oral kinetosome, c — others resorbing cilia. Arrows show resorbing oral cilia
14: Stage 2 of encystment. Transverse section through circumcytostomal kinety fibrillar layer. Arrows show somatic cilia in endoplasm
(thick arrows). Thin arrows show resorbing cilia. Magn. 14 000 ×
15: Stage 3 of encystment. Longitudinal section through nonciliated kinetosome (thick arrow). Resorbing cilium (thin arrow). ND — nemadesmata. Magn. 15 000 ×
16: Stage 4 of encystment. Longitudinal section through somatic kinetosome provided with infraciliary fibres. Arrows show outer ciliary microtubules. Magn. 19 000 ×
17: Stage 4 of encystment. Longitudinal section through other somatic kinetosome. Arrows show outer ciliary microtubules. Magn. 22 500 ×
18: Section through desintegrating cilium (thick arrow). Other cilia in cytoplasm (thin arrows). Magn. 15 500 ×
19: Longitudinal section through kinetosome with cilium. Thick arrows show axoneme covered by cell membrane and proximal phragment of axoneme deprived of membrane. Magn. 15 500 ×
20: Stage 3 of encystment. Section through aggregation of microfibrillar material (mf) surrounded by numerous smoot vesicles Magn. 7500 ×
21: Stage 1 of encystment. Section through oral region. cph. — cytopharynx. Arrows show microfibrillar aggregation at the base of proboscis. Magn. 6000 ×
22: Microfibrillar layer (mf. l.) in trophic ciliate. EKT — ectoplasm. Arrows show rough vesicles of endoplasmic reticulum, m — mitochondrium. Mgn. 22000 × rough vesicles of endoplasmic reticulum, m — mitochondrium. Magn. 22000 ×
23: Stage 2 of encystment. Structure of ectoplasm (EKT) and of microfibrillar layer (mf. l.). m — mitochondrium. Arrows show flat vesicles of rough reticulum. Magn. 17000×
24: Stage 4 of encystment. Structure of ectoplasm (EKT). m — mitochondrium. Arrows show smooth vesicles of reticulum near microfibrillar layer. Magn. 11000 ×



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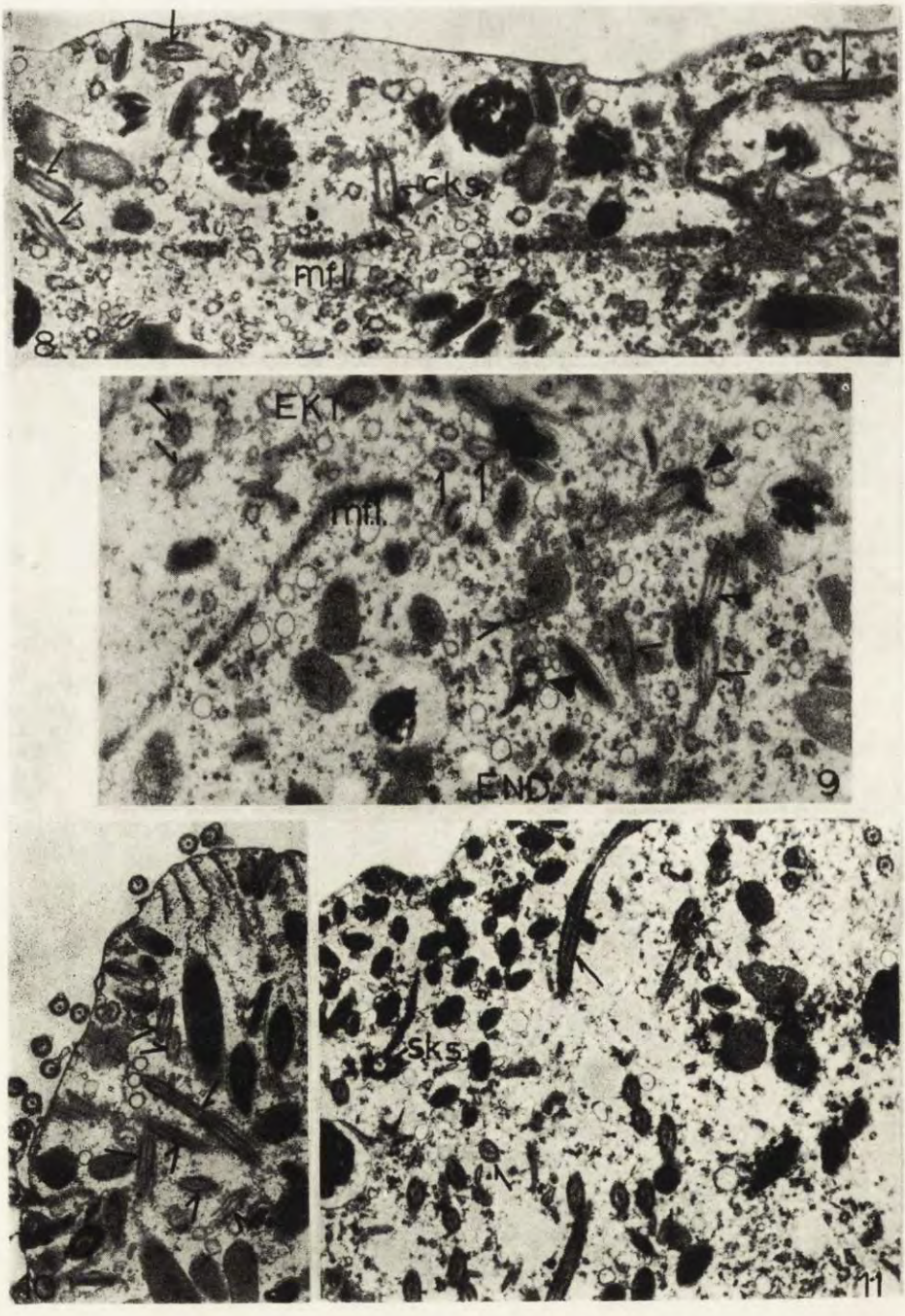

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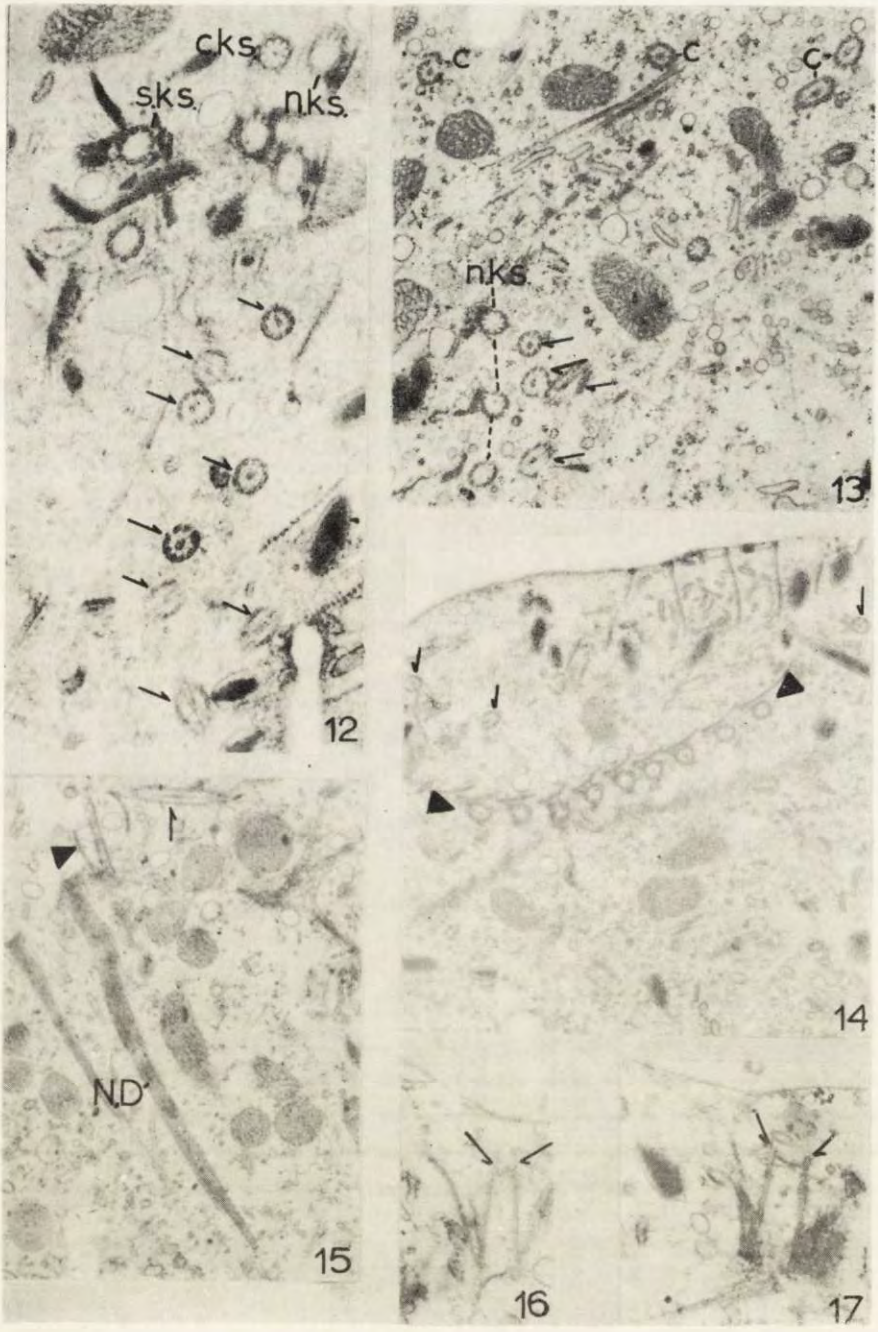

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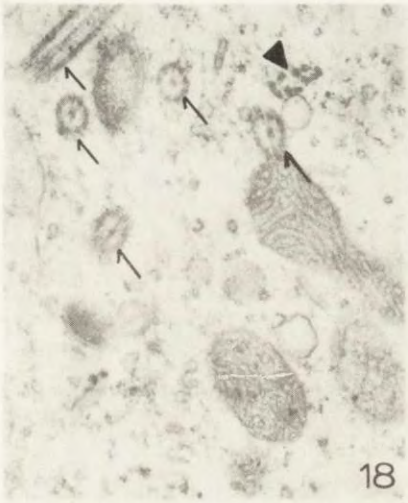

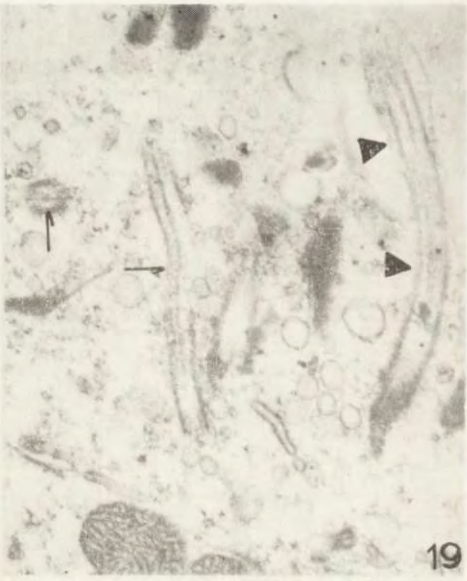

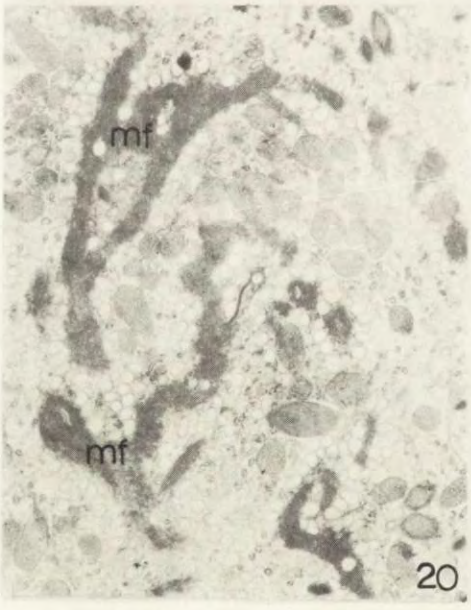

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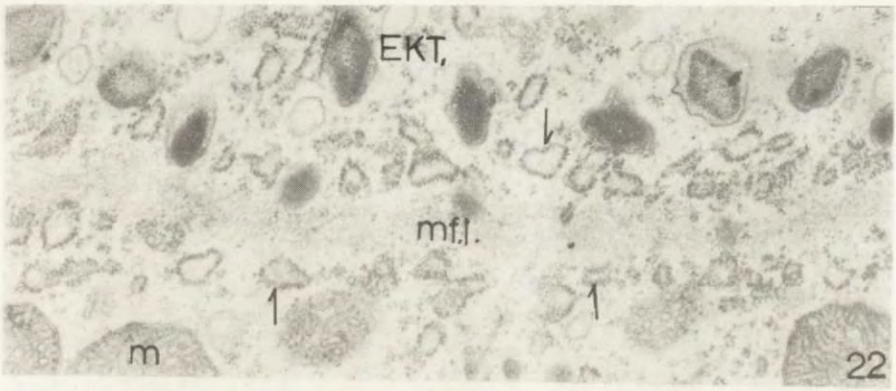

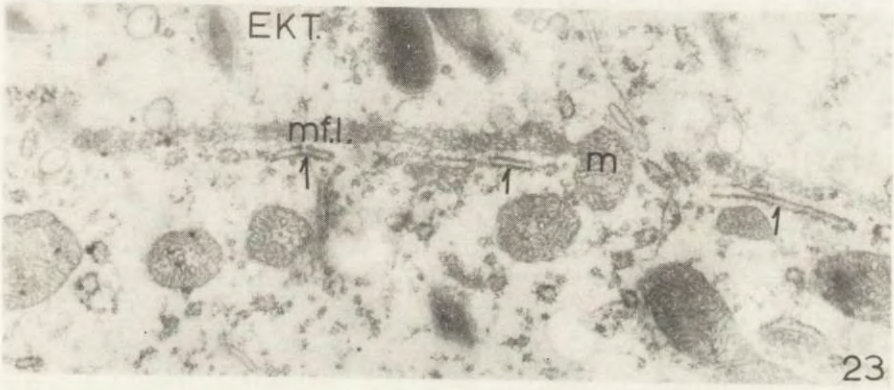

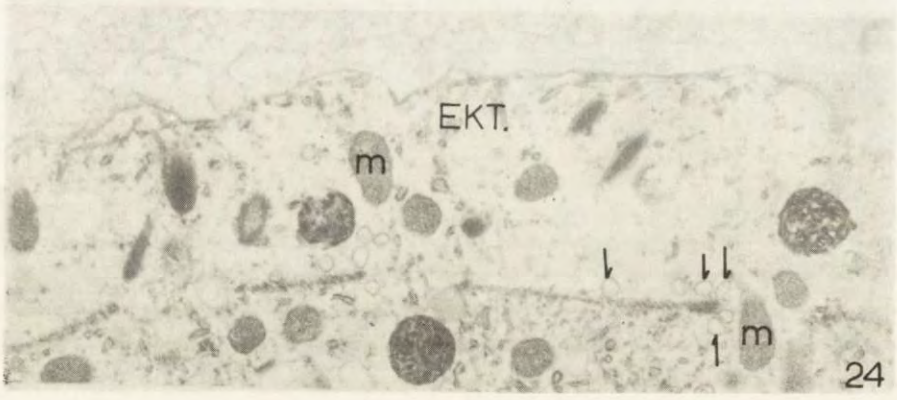

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The Course of *in situ* Remodelling of Injured Mouthparts in *Dileptus* (*Ciliata, Gymnostomata*)

Synopsis. The course of regeneration of oral structures with its proximal portion excised was studied in *Dileptus anser*, by means of light and electron microscopy. Partial dedifferentiation of old structures and differentiation of missing parts was observed. The process proceeds in the distal-proximal direction, new parts arising as a prolongation of the old ones. Some general aspects of the process, namely its relation to the last stages of normal stomatogenesis are discussed, as well as the way of formation of inner nematodesmal basket and phagoplasmic vesicles.

Exceptional abilities of *Dileptus* cells to repair *in situ* the damaged oral structures have been reported previously. Oral structures of *Dileptus* contain two regions: the distal region — so-called ventral band and proximally situated cytostomal field. Mouthparts of *Dileptus* after their distal part is excised are able to form the new structural elements within proximal portion (Golińska and Kink 1973). Distal structures then regrow, the new kinetosomal units being incorporated at the base of growing structure. Fragments with only distal oral structures left are also able to reorganize into complete and proportionate organism (Golińska and Kink 1977, Jerka-Dziadosz and Golińska 1977). Excision of the proximal oral structures is followed by an *in situ* formation of proximal parts, some distal structures being utilized in the process.

Other than *Dileptus* ciliates in response to excision of some portion of mouthparts, form the oral primordia and new complete mouth develops. This reaction being typical for ciliates served as a basis for the concept of inhibitory role of intact mouthparts in primordium

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formation (reviewed by Hanson 1967, Tartar 1967). Morphogenetic abilities found for the oral parts of *Dileptus* can be compared to those known for early oral primordia of *Stentor* (Tartar 1957). When proximal or distal portion of the early (before stage 3 of development) primordium of *Stentor* is removed, the remnant is able to form a complete and proportionate mouth through compensatory growth of primordium. It seems possible that the compensatory proliferation of kinetosomes takes place, like in *Dileptus*, within proximal portion of the structure. Primordia of *Stentor* after stage 4 of development are no more able to start the compensatory proliferation, and their morphogenetic abilities become restricted when compared to these of *Dileptus*.

The course of formation of complete mouth from its distal parts is the object of this study. There is no oral primordium formation during the process. Instead, there is partial dedifferentiation of the rear portion of distal mouthparts, and new oral structures arise as a continuity of the old ones. The process resemble the late stages of normal stomatogenesis. Some new details of stomatogenesis were found, namely the formation of the inner nematodesmal basket. Very unusual site for microtubule assembly was observed during the basket formation — new nematodesmata in this region appear close to subpellicular alveoli.

Materials and Methods

Dileptus anser O. F. M. was used for observations. Culture medium was Pringsheim solution. Cultures were kept in beakers and fed every other day with *Colpidium colpoda*.

Operations performed were transections at the base of proboscis made by hand, using a microscalpel. Anterior fragments, i.e., proboscises, were transferred into depression slides. Several dozen cells were operated upon during 30 or 60 min interval. Groups of several dozen proboscises were fixed in definite time after the end of operations.

For light microscopy the preparations were either impregnated with protargol after Dragesco (1962), or stained with acid fuchsin. The last procedure reveals the distribution of toxicysts. Saturated solution of mercuric chloride was used as a fixative. After fixation material was carefully washed in H_2O non buffered, then stained with acid fuchsin prepared accordingly to slightly modified recipe after Gurr (1962). First solvent was prepared: 9 parts of 30° ethyl alcohol to one part of acetic acid. Then slight amount of acid fuchsin was washed once, dehydrated in alcohols as quickly as possible, and mounted in Canada balsam.

Preparations for electron microscopy were made according to conventional methods, except for fixation. Cells were fixed with a mixture of two parts of 2% osmium tetroxide and 1 part of 6% glutaraldehyde in cacodylate buffer pH 7.2, freshly prepared and maintained in temperature 0°C during 1 h fixation. The sections were contrasted with uranyl acetate and lead citrate, examined with a JEM 100 B electron microscope.

Results

Structure of the intact oral parts of *Dileptus*

Detailed description of the oral parts of *Dileptus* was published elsewhere (Grain and Golińska 1969, Kink 1973, Golińska and Kink 1976). I believe it necessary, however, to remind this in general lines, with addition of some new details.

The mouthparts consist of the cytostome located on nonciliated oral field. Proximal portion of oral field is situated at the top of cylindrical trunk (Fig. 1 A), distal portion is situated along ventral side of body protrusion called proboscis. The field is enclosed by single row of oral infraciliature, and all kinetosomal elements of this row give rise to transverse fibers directed towards the cytostome. The infraciliature which encircles oral field has been previously termed oral kinety (Kink 1973,

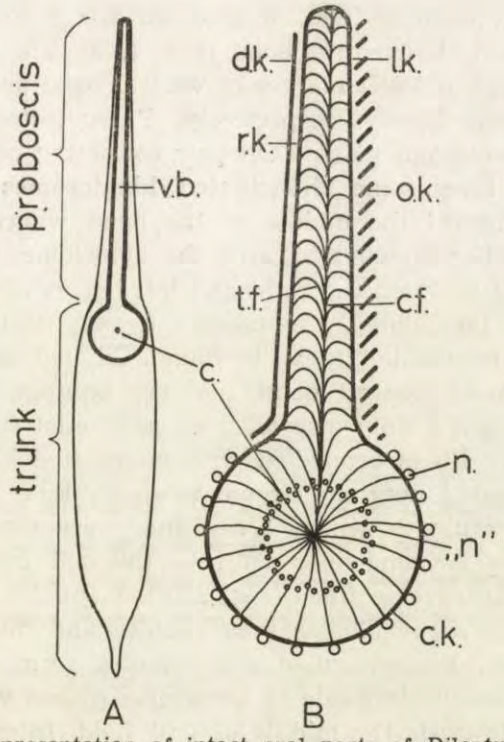

Fig. 1. Schematic representation of intact oral parts of *Dileptus*. A — Localization of mouthparts on the cell. B — Structure of oral field. (c.) — cytostome — center of proximal mouthparts, (v.b.) — central band — distal mouthparts, heavy lines are kineties: (d.k.) — dense kinety, (r.k.) — right paracytostomal kinety, (c.k.) — cirmumcytostomal kinety, (l.k.) — left paracytostomal kinety, (o.k.) — oblique kinety; (t.f.) — transverse fiber, (c.f.) — central fiber, (n.) — nematodesma of outer basket, ("n") — nematodesma of inner basket

Golińska and Kink 1976). Term kinety is used here in its morphological meaning, without implications concerning the origin of its elements. It is still possible that some segments of oral kinety may originate at the tops of several somatic kineties (Grain and Golińska 1969).

Proximal portion of oral field is circular, with centrally located cytostome. The segment of oral infraciliature which encircles this part of oral field (Fig. 1 B) is called circumcytostomal kinety. It is a row of single, nonciliated kinetosomes each bearing a transverse fiber and nematodesma. Nematodesmata connected with the circumcytostomal kinety form a palisade of external basket which extends deeply into endoplasm. The endoplasm inside the basket is filled with smooth elongated vesicles and is called phagoplasma (Fauré-Fremiet 1961). Within the external basket there is a smaller internal basket composed of numerous small nematodesmata hold together by a microfibrillar ring situated under the surface of cytostomal field.

Distal portion of oral field, located all along the ventral side of proboscis, is called the ventral band (Fig. 1, 2). The segments of oral kinety on the right and left margin of ventral band are called right and left paracytostomal kinety, respectively. Paracytostomal kineties are composed of kinetosomal pairs, each pair consisting of one ciliated and one nonciliated kinetosome. Nonciliated kinetosomes bear transverse fibers running toward the middle of the band where all these fibers form a central fiber directed towards the cytostome. The central fiber forms a boundary between the right and left halves of the ventral band. At the base of nonciliated kinetosomes a very thin nematodesmata, composed of 6–8 microtubules, can be found. Ciliated kinetosomes occupy the outer margin of ventral band, and are equipped with postciliary fiber, if in the right kinety also with an additional fiber.

The transverse fibers connected with nonciliated kinetosomes of the right paracytostomal kinety and circumcytostomal kinety are accompanied by peculiar microtubules which I call the perpendicular microtubules because they are perpendicular both to the cell surface and to the microtubules of transverse fiber (Fig. 2, Pl. I 10). No connectives were observed between perpendicular microtubules and microtubules of the transverse ribbon. Perpendicular microtubules form a row of single tubules situated on the left side of transverse ribbon when viewed from the kinetosome towards the middle of oral field. Intervals between the tubules within one row are only roughly regular. Below the surface of the cell perpendicular tubules join the subpellicular alveoli (Pl. I 11, IV 18). These small, flat alveoli are present under cell membrane all over the *Dileptus* body (Pl. I 9), but only in the described region there

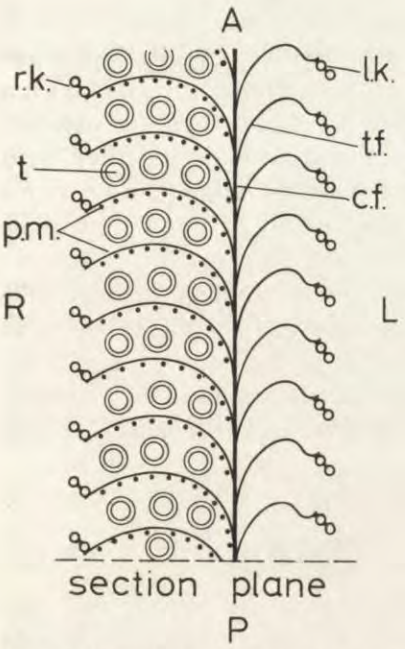

Fig. 2. Structure of ventral band. Anterior (A), posterior (P), left (L) and right (R) sides are those of the animal. (r.k.) — kinetosomal pair of the right paracytostomal kinety ,(l.k.) — kinetosomal pair of the left paracytostomal kinety, (t.) — toxicyst, (t.f.) — transverse fiber, (c.f.) — central fiber, (p.m.) — perpendicular microtubules

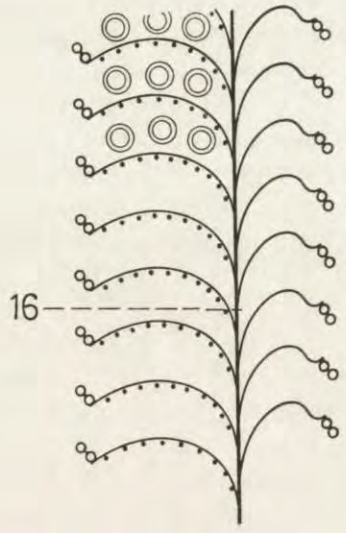

Fig. 3. Posterior portion of ventral band, stage 0–1 h after the operation. Toxicysts are withdrawn from the proximal portion of ventral band, and so are some posteriormost kinetosomal elements (compare to Fig. 1). Numbers marked on this and following drawings are the numbers of micrographs which illustrate places shown by broken line

are perpendicular microtubules attached to them. The other ends of perpendicular microtubules do not seem to join any particular structure.

Unlike circular portion of the oral field and the right part of ventral band, the left part of ventral band is deprived of the perpendicular microtubules. It is worthwhile to mention that kinetosomal pairs of the left paracytostomal kinety have reversed position when compared to kinetosomal pairs of the right paracytostomal kinety and to orientation of somatic ciliature of the rest of the body (Grain and Golińska 1969).

Somatic kineties situated on proboscis along the right and left side of paracytostomal kineties are called dense and oblique kineties (Golińska and Kink 1976). Dense kinety is composed of densely packed but otherwise normally oriented kinetosomes, and runs all along the right paracytostomal kinety (Fig. 1 B). Along the left paracytostomal kinety there are numerous oblique kineties (Grain and Golińska

1969), which have the same orientation as the left paracytostomal kinety, i.e., reversed in comparison to the rest of the ciliature. In *Dileptus anser* each oblique kinety contains usually four kinetosomes (Golińska 1971), and for every three or four kinetosomal pairs of left paracytostomal kinety there is one oblique kinety. These somatic kineties together with paracytostomal kineties were formerly called "feeding cilia" (Dragesco 1963), and it is probably the ciliary movement of all of them that gives metachronal waves on proboscis.

Toxicysts are fitted in between the transverse ribbons within right half of the ventral band (Fig. 2, Pl. I 10). Below the ribbons there is a microfibrillar meshwork running between deep portions of toxicysts. The meswork is continuous with microfibrillar layer situated between ecto- and endoplasm in the somatic region of proboscis (Pl. I 9) and rest of the body.

Formation of proximal mouthparts on isolated proboscises

The fragments studied were proboscises taken from the well fed individuals, cut off at the base of proboscis (identical to D fragments described by Golińska and Kink 1977). Fragments behave in different ways after the operation. Some of the proboscises regulate their form and reorganize into complete tiny dileptuses. Others degenerate soon after the operation — they become spheres with their ventral band spiralling on the surface (Pl. II 12). Some stay almost unchanged several hours, only slight shortening of the fragment (and its ventral band) occurring, with almost all of their toxicyst withdrawn from the ventral band; then die. I have tried to establish what is the percentage of fragments of each kind within the sample. There seems, however, to be no constant percentage of regenerating and not regenerating fragments in different samples. In present study only fragments apparently well regenerating were taken into consideration.

Fragments 0–1 h after the operation

During the first hour following operation two processes can be observed: resorption of kinetosomes at the hind end of ventral band, and withdrawal of toxicysts from proximal portion of ventral band (Fig. 3).

All observations concerning resorption of ciliature were made using electron microscope. There is no other method to visualize where cilia are withdrawn under the surface of the cell. The resorption affects both oral and somatic kinetosomes, and this was observed at the apex and at the rear end of fragment. The resorption located at the apex is probably related to the overall shortening of the ventral band (Golińska and

Kink 1977), while this at the rear end is probably the resorption which accompanies the healing of the wound (Golińska nad Grain 1969).

Withdrawal of toxicysts occurs simultaneously with the formation of narrow anterior and thick posterior portion of fragments; namely simultaneously with the first signs of shape regulation (Golińska and Kink 1977). Toxicysts disappear from the segment of ventral band which is situated on the thickening portion of fragment, i.e., on the future trunk (Pl. III 17). This was observed on preparations stained with acid fuchsin and confirmed by EM preparations (Pl. III 15, 16). The toxicysts are probably withdrawn into endoplasm of the forming trunk, because in there a dense accumulation of toxicysts can be seen (Pl. III 17). Toxicysts are withdrawn before microfilamentous meshwork disappears — this meshwork may be observed in segments of ventral band deprived of toxicysts (Pl. III 16). Later on the meshwork disappears too. The ciliature on both margins of the segment of ventral band without toxicysts does not, in this stage, differ from the ciliature bordering the normal ventral band.

The margin between the proximal and distal part of ventral band, marked in this early stage by presence and absence of toxicysts (Pl. III 17), is probably the future margin between cytostomal field and ventral band. This is confirmed by the fact that all subsequent changes leading to formation of cytostomal field occur along the ventral band without toxicysts. During subsequent stages there is probably no additional withdrawal of toxicysts from the proximal portion of ventral band: the segment deprived of toxicysts is the longest in about 1 h after the operation — later on it shortens.

Fragments 1–2 h after the operation

From 1 to 2 h after the operation there is resorption of accessory somatic ciliature on both sides of transforming segment of ventral band, formation of nematodesmata and resorption of some ciliated kinetosomes within proximal portions of paracytostomal kineties (Fig. 4). In this stage also smooth elongated vesicles appear within transforming segment of ventral band.

Resorption of accessory somatic ciliature was observed on protargol stained preparations (Pl. II 13, 14). The resorption within short oblique kineties occurs simultaneously all along the transforming segment of ventral band, but not all the ciliary units are resorbed at once. For some time ciliature on the left side of transforming band looks like disarranged (Pl. II 14 B), because when the number of kinetosomes diminishes, short oblique kineties cannot be easily traced. On the right side of proboscis kinetosomes of the dense somatic kinety become more spaced, especially

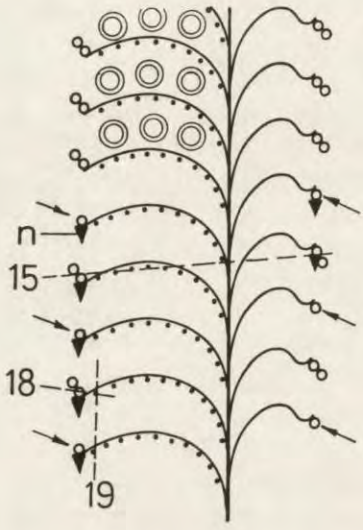

Fig. 4. Stage 1–2 h after the operation. Nematodesmata (n.) are formed, and some single kinetosomes can be found instead of kinetosomal pairs (arrows)

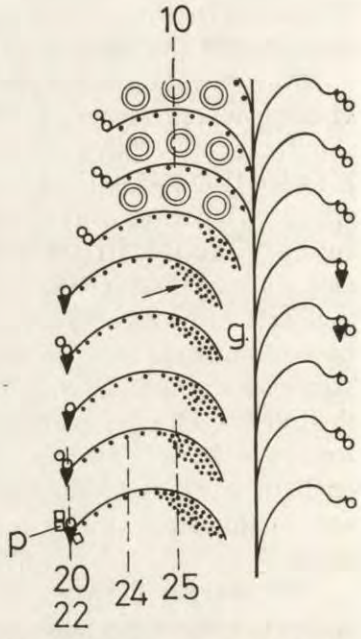

Fig. 5. Stage 2–3 h after the operation. There is a gap (g.) in between the transverse fibers of the right and left part of ventral band. Formation of groups of perpendicular microtubules (arrow) and proliferation of kinetosomes (p.) occurs

in the posterior region (Pl. II 13). The resorption does not, however, affect the whole segment of dense kinety which lies along transforming ventral band.

In the normal paracytostomal kineties at the base of nonciliated kinetosomes may be found very thin nematodesmata — built up of 6–8 microtubules. During the second hour following the operation at both margins of transforming ventral band nonciliated kinetosomes bear nematodesmata composed of 10 to 100 microtubules (Pl. IV 18, 19). Probably there is simply enlargement of the tiny nematodesmata going on in the proximal region of ventral band.

On the margins of transforming ventral band, within right and left paracytostomal kinety EM pictures showed resorption of ciliated kinetosomes out of kinetosomal pairs. On the sections parallel to the surface at the kinetosomal level (Pl. IV 21 s) some nonciliated kinetosomes (bearing transverse fiber) are single, not linked together with ciliated kinetosomes (bearing postciliary fiber). The single kinetosomes are always intermingled with kinetosomal pairs. In the neighbourhood the ciliary

shafts can often be seen withdrawn under cell surface. Sections just above the kinetosomes show some cilia from the groove of paracytostomal kinety embedded in the cytoplasm (Pl. IV 20), and show that transverse ribbons which enter the ventral band are more numerous than ciliary shafts in the groove (the supernumerary ribbons supposedly linked to single kinetosomes).

The segments of paracytostomal kineties on the margins of transforming ventral band resemble the so-called transitional segments of oral kinety (Golińska and Kink 1976) situated in the mature mouth between circumcytostomal and paracytostomal kineties. The transforming segments and transitional segments both contain single kinetosomes as well as kinetosomal pairs, nematodesmata, and transverse fibers which enter the oral field deprived of toxicysts. Also, the kinetosomal proliferation was not observed along the segments, only at their posterior ends. I suppose that the changed segments of paracytostomal kineties later on form the transitional segments of oral kinety.

In the transforming segment of ventral band, between the ribbons of transverse fibers, a smooth elongated vesicles of so-called phagoplasma appear (Pl. IV 18, V 24, 25). In this stage scarce, later on the vesicles fill in the nematodesmal basket. I was unable to detect what is the origin of these vesicles. Some EM micrographs (Pl. V 24) obtained from this and later stages show invaginations of cell membrane suggesting formation of some vesicles; this could be, however, formation of new subpellicular alveoli, not necessarily the phagoplasmic vesicles.

Fragments 2–3 h after the operation

All the processes observed during the period 1–2 h after the operation, last also during this stage. Moreover, the transforming segment of ventral band widens, and in this stage the proliferation of oral kinetosomes and formation of inner nematodesmal basket begins (Fig. 5).

The transforming segment of ventral band was observed to widen on protargol impregnated preparations. This was confirmed by EM pictures showing the segment wide and flattened (Pl. III 15); on some micrographs the gap between the free ends of the left and right transverse fibers can be observed — the fibers no longer meet in the middle of the band (Pl. V 25).

The proliferation of oral kinetosomes was observed to occur at the posterior end of the right paracytostomal kinety, sometimes also at the posterior end of the left paracytostomal kinety (Pl. IV 21) in both cases next to the single kinetosome bearing transverse fiber. On other micrographs a row of single nonciliated kinetosomes can be observed in

extension of the right paracytostomal kinety (Pl. V 22), with numerous new kinetosomes forming on its outer margin; what I think is a formation of circumcytostomal kinety. All kinetosomes within the row are equipped with transverse fibers, and on some micrographs perpendicular microtubules can be found in between the transverse fibers.

The formation of inner nematodesmal basket was first observed near to free ends of transverse fibers of the right transforming kinety (Pl. VI 25). On sections parallel to the surface beside the normally spaced perpendicular microtubules appear groups of microtubules in the middle region of transforming ventral band. Sections at the right angle to the surface show that several perpendicular microtubules join one subpellicular alveolus (Pl. III 15). Possibly each group is linked to one subpellicular vesicle in this stage. Groups of perpendicular vesicles later on form the nematodesmata of the inner basket.

Fragments 3–4 h after the operation

During this stage proliferation of oral kinetosomes and formation of inner nematodesmal basket goes on. Moreover, there is formation of cytostomal field, i.e., closing of oral kinety around proximal mouthparts (Fig. 6).

On protargol impregnated preparations oral field is entoured by

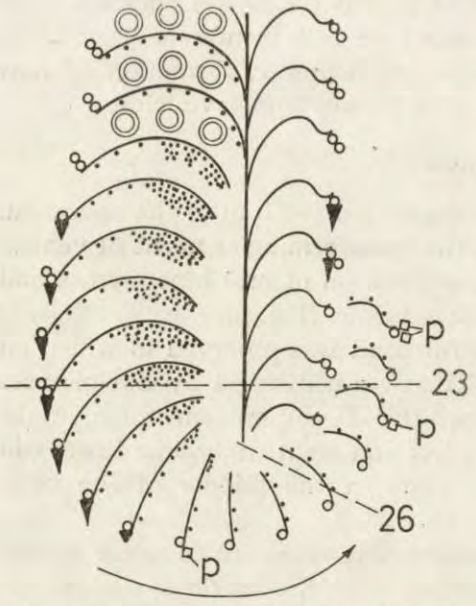

Fig. 6. Stage 3–4 h after the operation. The circumcytostomal kinety elongates making an U-turn (arrow). (p.) — proliferation of oral kinetosomes

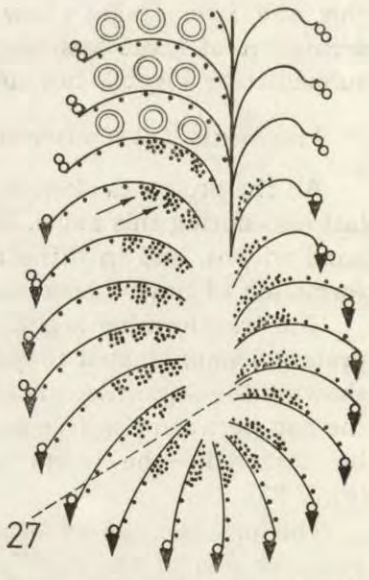

Fig. 7. Stage 4–5 h after the operation. Formation of "nematodesmata" of the inner basket (arrows)

a continuous line. Proximal portion is wide, somewhat irregular in shape. EM pictures show that growing circumcytostomal kinety makes an U-turn on ventral side of the fragment, and its anteriorly directed part encircles central fiber (Pl. VI 26) together with posterior portion of the left paracytostomal kinety. The latter observation is based on sections like the presented on Pl. V 23. In the left side of the micrograph (right side of the cell) there is the right paracytostomal kinety with nematodesmata of external basket. Toward the left of the cell there is the right portion of cytostomal field with transverse ribbons provided by the right paracytostomal kinety, and on some sections the groups of perpendicular microtubules are visible. Further to the left of the cell there is the central fiber and the left portion of ventral band with its transverse fibers, but no kinetosomes of the left paracytostomal kinety were found in their due place. Further to the left of the cell there is a cytoplasmic fold having under its surface the transverse ribbons provided by a row of nonciliated kinetosomes embedded in the groove at the base of the fold. The transverse ribbons on the fold are equipped with perpendicular microtubules. Such pictures are interpreted as encircling of the left part of transforming band by circumcytostomal kinety.

The circumcytostomal kinety joins somewhere the left paracytostomal kinety. Unfortunately I was unable to find the exact place of this junction. The joining is probably preceded by resorption of some supernumerary elements of the left paracytostomal kinety. This is indicated by the lack of kinetosomes in the segment of the left paracytostomal kinety encircled by circumcytostomal kinety, and by resorption within the left paracytostomal kinety not encircled by circumcytostomal, found in several fragments from this stage. On the other hand, proliferation of kinetosomes was found at the posterior end of some left transforming kineties in the earlier stage (Pl. IV 21). I believe that the resorption within posterior portion of the left paracytostomal kinety is not obligatory in the process, and joining of oral kineties may occur without encircling of some part of left transforming kinety by growing circumcytostomal kinety.

In this stage groups of perpendicular microtubules were found in proximity to transverse fibers of the left transitional segment, on the correct side of the transverse ribbon. I cannot say, however, whether the formation occurs before or after the circumcytostomal kinety joins the left transitional segment.

Fragments 4–5 h after the operation

In this stage proliferation of oral kinetosomes continues and there

is a beginning of the formation of nematodesmata out of groups of perpendicular microtubules (Fig. 7).

Some fragments on protargol stained preparations have in this stage cytostomal field circular in shape, although in most cases it is still irregular. EM micrographs show that oral kinetosomes still proliferate. This probably accounts for growth and change of shape of cytostomal field.

Within older (right) part of cytostomal field large groups of perpendicular microtubules are in this stage broken into several small groups (Pl. VII 27). This is the formation of nematodesmata of the inner basket. The formation is probably mediated by microfilamentous elements, which appear at the tips of some microtubular groups embedded deeply in the cytoplasm and no longer ending at the subpellicular alveoli. A true microfibrillar ring characteristic for the mature inner basket is not yet formed. Smaller groups of perpendicular microtubules may probably remain connected with the alveoli also in mature structure; some pictures of these were found. Another possibility is that formation of nematodesmata of the inner basket is not restricted to stomatogenesis, and these elements (like elements of the outer basket) may continuously be formed if the need exists.

The nematodesmata of the inner basket during their formation and when mature are deprived of apical, electron-dense cap. This together with the fact that the arrangement of microtubules in bundles is more loose in the inner than in the outer basket, makes incorrect calling "nematodesmata" the elements of the inner basket.

Fragments 5–6 h after the operation

In this stage a cytostome is formed (Fig. 8). In some fragments EM micrographs show that all transverse fibers of circumcytostomal kinety end at elongated depression deprived of any microtubular elements (Pl. VII 27). In other fragments a cytostome can be observed — the depression shaped as a keyhole (Pl. VII 28) where all the transverse fibers meet. Some new cytostomes were found to be encircled by transverse fibers with grouping perpendicular microtubules, some encircled by transverse fibers with "nematodesmata" of the inner basket. There is apparently no constant sequence in formation of cytostome and "nematodesmata".

In this stage all structures of the proximal portion of oral field of *Dileptus* are formed, but growth of oral field proceeds much longer. The process of formation of proximal oral parts lasts about 6 h only in quickly regenerating animals. This process was however often observed to be retarded (unlike the normal stomatogenesis), sometimes it was retarded

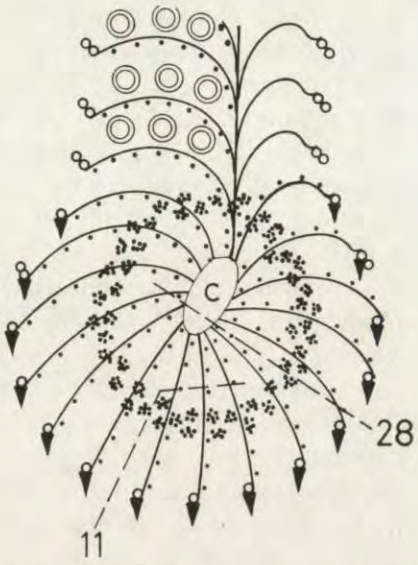

Fig. 8. Stage 5–6 h after the operation. Formation of cytostome (c.) and closing of the inner "nematodesmal" basket, makes the mouthparts complete

several hours. Fragments retarded were still able to form the complete and proportionate cell. I believe that the delay of regeneration might be a result of a very small size of the fragments studied — size limiting the regenerative capacities of cells.

Discussion

The regrowth of proximal oral structures from the distal ones in *Dileptus* has several interesting features. Mainly, it resembles the last stages of stomatogenesis. During this "partial stomatogenesis" the onset of oral kinetosomes proliferation is much delayed in comparison to a normal stomatogenesis, leaving a time for dedifferentiation processes and probably for the localization of proliferation area. The way of formation of internal "nematodesmal" basket implies that membranous structures play some role in microtubule assembly during this process.

The process studied resembles the last stages of stomatogenesis in the way and sequence of mouthparts forming. To recall the normal stomatogenesis of *Dileptus* it should be mentioned that oral kinetosomes develop not in the anarchic field but their formation is followed by immediate differentiation of ciliary units (Golińska and Grain 1969, Golińska 1972). The process of formation starts on the dorsal side of animal and proceeds toward its ventral side. First the units for the future ventral band, i.e., the distal mouthparts differentiate. The site of units formation moves toward the ventral side, where proximal units (for the circumcytostomal kinety) differentiate. On the isolated

proboscises, stomatogenesis proceeds not from the beginning, but from the moment of switch from forming the kinetosomal pairs for paracytostomal kineties to forming single kinetosomes for circumcytostomal kinety. Aside of dedifferentiation, the way and sequence of oral structures formation is the same as in late stomatogenesis, since the situation when ventral band is present and the rest of the oral field is missing, is very similar to the situation which occurs in normal stomatogenesis.

In normal stomatogenesis when proliferation of two-kinetosomal units for ventral band ends and proliferation of one-kinetosomal units for cytostomal field begins, formation of the middle portion of oral field bordered by transitional segments of oral kinety commence. In the partial stomatogenesis, formation of this area is not based on proliferation of new units but on the dedifferentiation of the posterior parts of paracytostomal kineties. Dedifferentiation processes consist of the withdrawal of toxicysts and resorptions of kinetosomes and their derivatives out of posterior segments of paracytostomal kineties until the transitional segments are formed. This dedifferentiation is accompanied by simultanous formation of the structures characteristic for the oral field along the transforming segment of kinety, namely the formation of internal basket of "nematodesmata", nematodesmata of external basket, phagoplasmic vesicles. Formation of elements of the oral field proceeds like in normal stomatogenesis.

There is evidence that *Dileptus* is not the exception among ciliates, and some other organisms are able to undergo a partial stomatogenesis. In many ciliates, during binary fission, oral structures of the proter undergo incomplete dedifferentiation, followed by the dedifferentiation of the missing parts only (Eberhardt 1962, Lom 1964, Williams 1975, Sawyer and Jenkins 1977). The course of the process is not known, however, not all the oral structures are uniformly dedifferentiated, and the most affected are the proximal pharyngeal parts. Some kind of partial stomatogenesis occurs in *Stentor* during reorganization process, or in its fragments deprived of proximal mouthparts (Schwartz 1935, Tartar 1961). A new primordium is formed in usual way and site, at some distance to the old mouthparts. During the development, however, a large portion of old membranellar band remains unchanged while primordium joins it and forms the proximal mouthparts only. The fact that differentiation of the newly formed units is not into complete mouth but into missing structures, makes the process similar to the partial stomatogenesis of *Dileptus*. *Dileptus*, however, does not form separate primordia for its missing structures, the partial stomatogenesis being the *in situ* regrowth of proximal mouthparts.

It seems probable that ability to undergo partial stomatogenesis is the attribute of these ciliates which differentiate their mouthparts in the distal-proximal sequence. Incorporation of old distal structures into new mouth means that differentiation of new oral structures starts not from the very beginning, but repeats some late stages of differentiation. It implies interesting questions concerning the control mechanisms of the partial stomatogenesis, such as the influence of the old structures upon the differentiation of new ones. This question is related to the problem of timing of oral primordia formation in fragments containing some portion of the old mouth. Regeneration has been found to be retarded in such fragments of *Stentor* (Weisz 1948), *Uronychia* (Taylor 1928), or *Condylostoma* (Suhama 1957). Moreover, the more of the membranellar band was removed, the sooner regeneration of *Stentor* fragments followed (Weisz 1948, Tartar 1959). The inhibitory role played by the old structures in regeneration may be true only in cases when some distal portions of the old mouth are destined as a distal parts for the new mouth, i.e., when structures which are first to differentiate in development, are already present on the fragment. The interesting observation made by Bohatier et al. (1976) that large proximal parts of the mouth left on *Condylostoma* fragment do not delay stomatogenesis, may be further indication of the special role of distal structures in partial stomatogenesis. This supposition is however highly speculative.

During the partial stomatogenesis of *Dileptus* the onset of oral kinetosomes proliferation is much delayed in comparison to normal stomatogenesis. In this study proliferation was first observed in fragments 2 h after the operation. During regrowth of proboscis (Golińska and Kink 1976) and during normal stomatogenesis in posterior fragments (Golińska and Grain 1969) the proliferation begins immediately after wound healing — 15 to 30 min after the operation. Kinetosomal units for the circumcytostomal kinety during normal stomatogenesis were first observed in fragment 1 h after the operation. This delay of the onset of proliferation during partial stomatogenesis may be discussed in two aspects. One aspect is related to the sequence found in this study: firstly there is dedifferentation of proximal parts of paracytostomal kineties, resulting in formation of some one-kinetosomal units out of two-kinetosomal. This is followed by proliferation of oral kinetosomes in vicinity of one-kinetosomal units. This confirm the previously found regularity that kinetosomal proliferation may occur next to one-kinetosomal units, but does not occur next to multikinetosomal units with intact inter-kinetosomal connective tracts (Jerka-Dziadosz and Golińska 1977). Formation of one-kinetosomal units might be a pre-

requisite for oral kinetosomes proliferation. Another aspect of the delay of proliferation onset in partial stomatogenesis is localization of the proliferation site. It was found here that proliferation starts not in the vicinity of every one-kinetosomal unit within transformed paracytostomal kinety, but only in the vicinity to the posterior-most unit. This suggests that precise localization of the proliferation site may also be a prerequisite for oral kinetosome proliferation to start.

One of the events which in partial stomatogenesis begins earlier than kinetosomal proliferation is formation of internal basket. In previous publication (Golińska and Grain 1969) this basket was supposed to be formed, during normal stomatogenesis, in connection with kinetosomes of the circumcytostomal kinety, and moved toward the center of oral field, while the outer basket would be formed in connection with the same kinetosomes during next stage. Grain considered (personal communication) that the way of the inner basket formation is different in partial than in normal stomatogenesis. I am inclined to an alternative possibility that the nematodesmata of the outer basket form and stay linked to the kinetosomes of the circumcytostomal kinety, while the inner basket — in every instances of stomatogenesis — is formed out of a very special microtubules described here as the perpendicular microtubules.

Dileptus is not the only ciliate equipped with perpendicular microtubules. The microtubules can be traced in some *Ophryoscolecidae* (Noirot-Timothée 1960, Pl. III Fig. 1, Pl. X Fig. 3), in *Litonotus* (Bohatier and Njine 1973, Fig. 6, 8, 17), perpendicular microtubules forming "nematodesmal" basket were found in *Didinium* (Yagiu and Shigenaka 1965, Wessenberg and Antipa 1968) and *Monodinium* (Rodrigues de Santa Rosa and Didier 1975). Moreover, some nematodesmata of uncertain origin, found in *Balantidium* (Grain 1966) or *Tillina* (Lynn 1976) might be analogous to the "nematodesmata" of the inner basket of *Dileptus*.

Assembly of perpendicular microtubules of the inner basket of *Dileptus* starts not in the proximal vicinity of oral kinetosomes, but close to the membrane of the subpellicular alveoli. Membranous structures are rarely found to be microtubular sites of termination. Usually, some amorphous dense matter was found to terminate the cytoplasmic microtubules, and this matter was considered as a morphopoietic factor (Fauré-Fremiet 1970), serving as a template for microtubular patterning (Pearson and Tucker 1977, Tucker 1977). In some heliozoans, however, nuclear membrane is the site of termination of microtubular structure (Tilney and Porter 1965, Bardele 1972, Bardele and Roth 1974, Cachon et al. 1977), and the membrane was consi-

dered as a nucleating site for the microtubules (Bardele 1972). The nucleating role of the membrane itself is not proved, since there exists some dense matter in between microtubular ends and the nuclear membrane (Tilney and Porter 1965, Cachon et al. 1977) — this may be true for perpendicular microtubules of *Dileptus* too.

During regrowth of proximal oral structures of *Dileptus* there is formation of smooth elongated vesicles of so-called phagoplasma. I believe that the vesicles are formed as invaginations of the cell membrane. This is based on observation that the vesicles first appear very close to the surface — they fill the deeper parts of pharyngeal structures during more advanced stages of development. Similar vesicles in other ciliates originate from the membranes of food vacuoles (McKanna 1973 b, Allen 1974) and are transported along the microtubules toward pharyngeal structures (McKanna 1973 a, Nilsson 1976) where join the surface of the cytostome or the surface of food vacuoles (Bradbury 1973, Schneider 1964). The same may be true for *Dileptus* during usual membrane flow in digestive system, but the origin of vesicles may change when food vacuoles are no more available. The study presented in this paper concern only the regulation of oral cortical pattern in isolated proboscises. Further studies dealing with the regulation of body shape and somatic ciliature will be published elsewhere.

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RÉSUMÉ

Le processus de regénération chez *Dileptus anser* qui suit l'amputation de la partie proximale de son appareil oral était étudiée en microscopie optique et électronique. On a constaté une dedifférentiation partielle des structures anciennes et le différentiation des parties absentes. La différentiation progresse de la zone distale wers la portion proximale, les structures nouvelles se reconstituant en prolongation des structures anciennes. La discussion concerne des certains aspects généraux de ce phénoméne, sa rélation aves les derniers étapes de la stomatogenèse normale et le mode de la formation du panier intérieur des nemadesmes et des vésicules phagoplasmiques.

REFERENCES

Allen R. D. 1974: Food vacuole mebrane growth with microtubule-associated membrane transport in *Paramecium*. J. Cell Biol., 63, 904–922.

Bardele C. F. 1972: Cell cycle, morphogenesis, and ultrastructure in the pseudoheliozoan *Clathurina elegans*. Z. Zellforsch. Mikrosc. Anat., 130, 219–242.

Bardele C. F. and Roth L. E. 1974: Microtubule patterning in centrohelidian *Heliozoa*. J. Protozool., 21, 415 (Abstr. 8).

Bohatier J. and Njine T. 1973: Observations ultrastructurales sur le Cilié *Holotriche Gymnostome Litonotus quadrinucleatus* Dragesco et Njine, 1971. Protistologica, 9, 359–372.

Bohatier J., Tuffrau M. and Tuffrau H. 1976: Morphogenèse de régénération dans le genre *Condylostoma* (Ciliés Hétérotriches). Protistologica, 12, 295–306.

Bradbury P. C. 1973: The fine structure of the cytostome of the apostomatous ciliate *Hyalophysa chattoni*. J. Protozool., 20, 405–414.

Cachon J., Cachon M., Tilney L. G. and Tilney M. S. 1977: Movement generated by interactions between the dense material at the ends of microtubules and non-actin-containing microfilaments in *Sticholoncha ranclea*. J. Cell Biol., 72, 314–338.

Dragesco J. 1962: L'orientation actuelle de la systematique des Ciliés et la technique d'impregnation au protéinate d'argent. Bull. Microsc. Appl., 11, 49–58.

Dragesco J. 1963: Révision du genre *Dileptus* Dujardin, 1871 (*Ciliata, Holotricha*) (Systématique, Cytologie, Biologie). Bull. Biol. Fr. Belg. 97, 103–145.

Eberhardt R. 1962: Unterschungen zur Morphogenese von *Blepharisma* und *Spirostomum*. Arch. Protistenk., 106, 141, 241–341.

Fauré-Fremiet E. 1961: Le cytoplasme stomopharyngien des Ciliés Cyrtophores. C. r. Acad. Sci. Paris 253, 357–362.

Fauré-Fremiet E. 1970: Microtubules et mécanismes morphopoietiques. Ann. Biol., 9, 1–61.

Golińska K. 1971: Comparative studies on the morphology of *Dileptus anatinus* sp., n. (*Holotricha, Gymnostomata*). Acta Protozool., 8, 367–377.

Golińska K. 1972: Studies on stomatogenesis in *Dileptus* (*Ciliata, Holotricha*) in the course of division processes. Acta Protozool., 9, 283–197.

Golińska K. and Grain J. 1969: Observations sur les modifications ultrastructurales lors de la régénération chez *Dileptus cygnus* Clap. et Lachm., 1859, Cilié Holotriche Gymnostome. Protistologica, 5, 447–464.

Golińska K. and Kink J. 1976: The regrowth of oral structures in Dileptus cygnus after partial exision. Acta Protozool., 15, 143–163.

Golińska K. and Kink J. 1977: Proportional regulation of body form and cortical organelle pattern in the ciliate *Dileptus*. J. Cell. Sci., 24, 11–29.

Grain J. 1966: Confirmation, par la microscopie électronique, de la place des *Balantidium* dans l'ordre des *Trichostomatida*. C. r. Acad. Sci. Paris, 263, 1864–1867.

Grain J. 1977: (personal communication).

Grain J. and Golińska K. 1969: Structure et ultrastructure de *Dileptus cygnus* Claparède et Lachmann, 1859, Cilié Holotriche Gymnostome. Protistologica 5, 269–291.

Gurr E. 1962: Staining animal tissues. Practical and theoretical. London, Leonard Hill (Books) Ltd.

Hanson E. D. 1967: Protozoan Development. In: Chemical Zoology, I, (ed. Florkin M. and Scheer B. T), Academic Press, 359–539.

Jerka-Dziadosz M. and Golińska K. 1977: Regulation of ciliary pattern in ciliates. J. Protozool., 24, 19–26.

Kink J. 1973: The organization of fibrillar structures in the trophic and encysted *Dileptus visscheri* (*Ciliata, Rhabdophorina*) Acta Protozool., 12, 173–194.

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

Lynn D. H. 1976: Comparative ultrastructure and systematics of the *Colpodida*. Fine structural specializations associated with large body size in *Tillina magna* Gruber, 1800. Protistologica, 12, 629–648.

McKanna J. A. 1973 a: Cyclic membrane flow in the ingestive-digestive system of peritrich protozoans. I. Vesicular fusion at the cytopharynx. J. Cell Sci., 13, 663–675.

McKanna J. A. 1973 b: Cyclic membrane flow in the ingestive-digestive system of peritrich protozoans. II. Cup-shaped coated vesicles. J. Cell Sci., 13, 677–686.

Nilsson J. R. 1976: Physiological and structural studies on *Tetrahymena pyriformis* GL. C. R. Trav. Lab. Carlsberg, 40, 215–355.

Noirot-Timothée C. 1960: Étude d'une famille de Ciliés: les "Ophryoscolecidae". Structures et ultrastructures. Ann. Sci. nat. Zool. 12-e série, 2, 527–718.

Pearson P. J. and Tucker J. B. 1977: Control of shape and pattern during the assembly of a large microtubule bundle. Evidence for a microtubule-nucleating-template. Cell Tissue Res., 180, 241–252.

Rodrigues de Santa Rosa M. and Didier P. 1975: Remarques sur l'ultrastructure de Cilié Gymnostome *Monodinium balbiani* (Fabre Domergue, 1888). Protistologica, 11, 469–479.

Sawyer H. R. and Jenkins R. A. 1977: Stomatogenic events accompanying binary fission in *Blepharisma*. J. Protozool., 24, 140–149.

Schneider L. 1964: Elektronenmikroskopische Untersuchungen an den Ernährungsorganellen von *Paramecium*. I. Der Cytopharynx. Z. Zellforsch. Mikrosc. Anat., 62, 198–224.

Schwartz V. 1935: Versuche über Regeneration und Kerndimorphismus bei *Stentor coeruleus* Ehrbg. Arch. Protistenk., 85, 100–139.

Suhama M. 1957: The change of surface structure in *Condylostoma spatiosum*, during the binary fission, regeneration and physiological reorganization J. Sci. Hiroshima Univ., 17, 137–143.

Tartar V. 1957: Delation experiments on the oral primordium of *Stentor coeruleus*. J. exp. Zool., 136, 53–74.

Tartar V. 1959: New findings on oral regeneration in *Stentor coeruleus*. J. Protozool., Suppl. 6 (abstr.) 32.

Tartar V. 1961: The Biology of *Stentor*. Pergamon Press.

Tartar V. 1967: Morphogenesis in *Protozoa*. In: Research in Protozoology, 2, (Ed. Tze-Tuan Chen), Pergamon Press, 1–116.

Taylor C. V. 1928: Protoplasmic reorganization in *Uronychia uncinata* sp. n. during binary fission and regeneration. Physiol. Zool., 1, 1–25.

Tilney L. G. and Porter K. R. 1965: Studies on microtubules in heliozoa. I. The fine structure of *Actinosphaerium nucleofilum* (Barret), with particular reference to the axial rod structure. Protoplasma, 60, 317–344.

Tucker J. B. 1977: Shape and pattern specification during microtubule bundle assembly. Nature, 266, 22–26.

Weisz P. B. 1948: Time, polarity, size and nuclear content in the regeneration of *Stentor* fragments. J. Exp. Zool., 107, 269–288.

Wessenberg H. and Antipa G. 1968: Studies on *Didinium nasutum*. I. Structure and ultrastructure. Protistologica, 4, 427–448.

Williams N. E. 1975: Regulation of microtubules in *Tetrahymena*. Int. Rev. Cytol., 41, 59–86.

Yagiu R. and Shigenaka Y. 1965: Electron microscopy of the ectoplasm and the proboscis in *Didinium*. J. Protozool., 12, 363–381.

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EXPLANATION OF PLATES I–VII

Dileptus anser O. F. M. Figs. 11–13 and 16 are taken from light microscope preparations, all the others are EM micrographs

9: Section through the somatic area of proboscis 2–2.5 h after the operation. Microfibrillar layer (m.) separates ecto and endoplasm. Under the cell membrane there are subpellicular alveoli (arrows), postciliary fibers (p.f.) run below the alveoli. × 34,000

10: Tangential section through the right side of distal portion of ventral band 2–3 h after the operation. Toxicysts (t) are packed between the transverse ribbons (t.f.), (m.c.) — mucocysts, (p.m.) — perpendicular microtubules. × 36,000

11: Section through the cytostomal field 6–6.5 h after the operation. Subpellicular alveoli (arrows) are in contact with perpendicular microtubules (p.m.), (t.f.) — ribbons of transverse fibers. × 34,000

12: Degenerating fragment 0.5–1 h after the operation, with its ventral band spiralling on the surface. Protargol impregnation. × 1200

13: Right side of the fragment 1.5–2 h after the operation. Dense kinety (d.k.) has spaced kinetosomes in its posterior portion (arrows). (r.k.) — right paracytostomal kinety. Dark spheres are nuclei. Protargol impregnation. × 1200

14: Left side of fragment 1.5–2 h after the operation. (a) — Focus on the oblique kineties (o.k.). (l.k.) — left paracytostomal kinety. (b) — Focus on the region where resorption of kinetosomes of oblique kineties occurs (r.) Protargol impregnation × 1200

15: Section through the proximal portion of ventral band 1–1.5 h after the operation. In the upper part of the micrograph there is right paracytostomal kinety (r.k.), followed by flattened right portion of ventral band where one subpellicular alveolus (arrow) contact several perpendicular microtubules (p.m.). Below the central fiber (c.f.) there is the left part of ventral band ending at left paracytostomal kinety (l.k.). Nematodesmata (n.) are present at the base of both paracytostomal kineties. × 12,000

16: Tangential section of the proximal part of the ventral band 0.5–1 h after the operation. In the upper part of the micrograph there is the dense somatic kinety (d.k.). Arrow shows the direction toward the posterior end the fragment. (r.k.) — right paracytostomal kinety. Right portion of ventral band, down to the central fiber (c.f.) is deprived of toxicysts but microfibrillar meshwork (m.) is still present. × 18,000

17: Fragment 0.5–1 h after the operation. Dark rods are toxicysts, stained with acid fuchsin. In the posterior part of the fragment the large segment of ventral band is deprived of toxicysts (t.s.). Dark mass inside the posterior part is composed of toxicysts. × 1000

18: Section through the right paracytostomal kinety 1–1.5 h after the operation. There is nematodesma (n.) under the nonciliated kinetosome out of kinetosomal pair. (t.f. — transverse fiber of the right paracytostomal kinety, some perpendicular microtubules can be seen (p.m.) ending at subpellicular alveoli (arrow). (p.v.) — elongated vesicles of phagoplasma. × 27,000

19: Tangential section of the right paracytostomal kinety 1–1.5 h after the operation. Kinetosomal pairs are visible each equipped with transverse fiber (t.f.). Large nematodesmata (n) are situated in prolongation of the kinety. × 32,500

20: Tangential section just above the right paracytostomal kinety 1.5–2 h after the operation. (d.k.) — dense somatic kinety. Number of cilia in the groove of the right paracytostomal kinety is smaller than number of transverse fibers (t.f.). Arrow points to the cilium which is embedding in the cytoplasm. × 23,000

21: Tangential section through the posterior portion of the left paracytostomal kinety 2–2.5 h after the operation. There are some single (s.) kinetosomes between kinetosomal pairs proliferation (p.) occurs at the end of kinety. (t.f.) — transverse fiber, arrow points at the resorbed cilium. Identical pictures were obtained for proximal end of the right paracytostomal kinety in this stage. × 27,000

22: Tangential section of growing circumcytostomal kinety (c.k.) 2–2.5 h after the operation. There is proliferation of kinetosomes (p.) (t.f.) — transverse fiber, (g.) — groove above the posterior end of circumcytostomal kinety — no ciliary shafts can be seen in the groove. × 23,500

23: Section through the oral field 3.5–4 h after the operation. r.k.) — right paracytostomal kinety, (c.f.) — central fiber, arrow shows the place where left paracytostomal kinety should be, (f.) — cytoplasmic fold with transverse fibers of circumcytostomal kinety. (c.k.) — circumcytostomal kinety, (g.) — groove above the circumcystosomal kinety. × 11,000

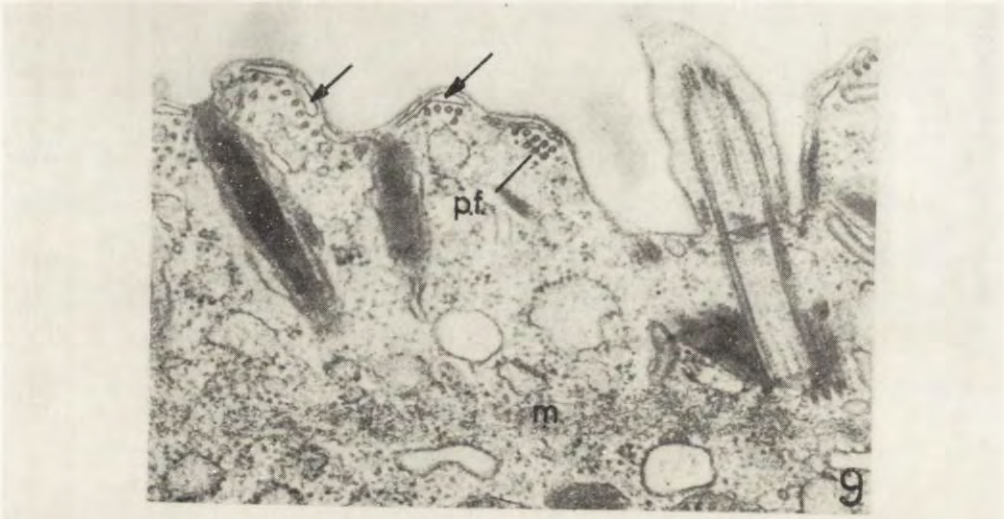
24: Tangential section of the right part of ventral band 2–2.5 h after the operation. (p.m.) — perpendicular microtubules, (p.v.) — phagoplasmic vesicles, (t.f.) — transverse fibers. Arrows indicate invaginations of the cell membrane. × 17,000

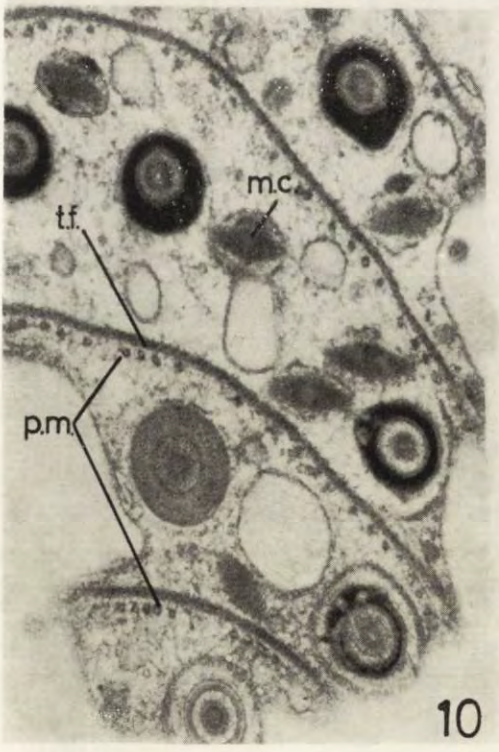
25: Tangential section of the middle part of ventral band 2–2.5 h after the operation. The right side of the micrograph is the gap deprived of transverse ribbons. (t.f.) — right transverse ribbon, (p.m.) — groups of perpendicular microtubules, (p.v.) — phagoplasmic vesicles. × 17,000

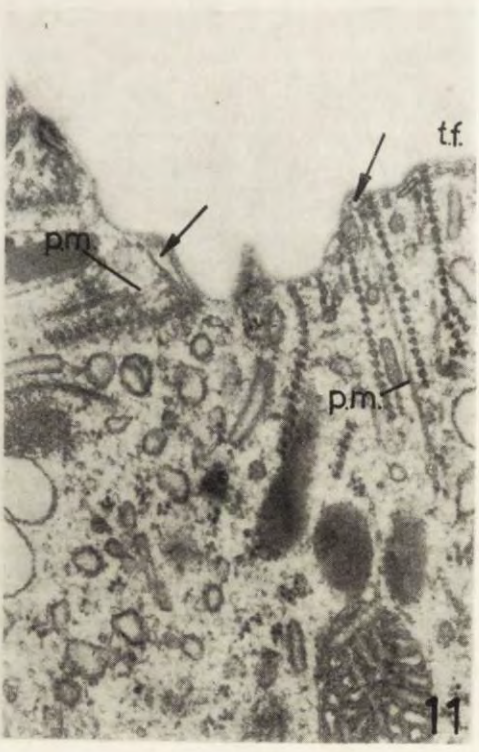
26: Tangential section of the posterior end of central fiber (c.f.) 3–3.5 h after the operation. It is encircled by cytostomal field. × 15,000

27: Tangential section of oral field 4–4.5 h after the operation. (c.k.) — circumcytostomal kinety, (t.f.) — transverse fibers, (n.) — nematodesma of the outer basket, („n") — nematodesma of the inner basket, (g.) — gap deprived of transverse fibres. × 13,500

28: Tangential section of oral field 6–6.5 h after the operation. (c.) — M cytostome, (c.k.) — circumcytostomal kinety, (n.) — nematodesmata of the outer basket, („n" — nematodesmata of the inner basket, (p.v.) — phagoplasmic vesicles. × 12,500







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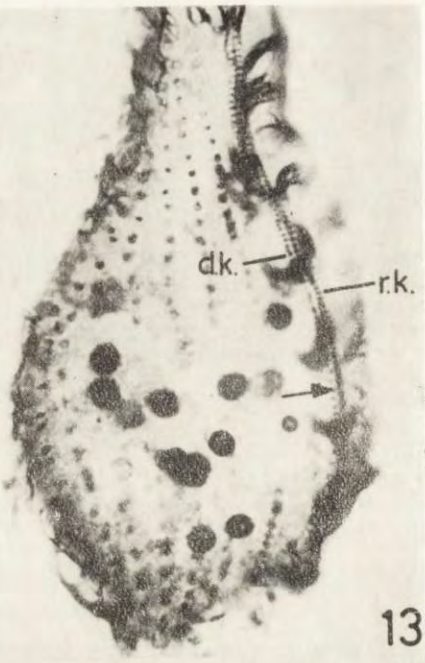

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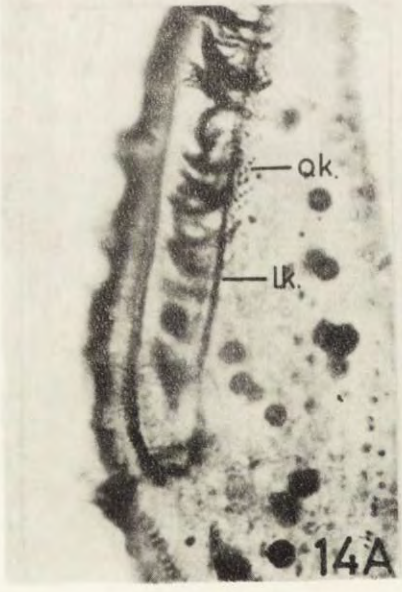

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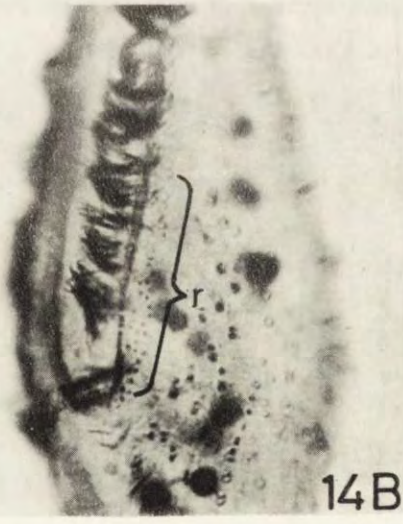

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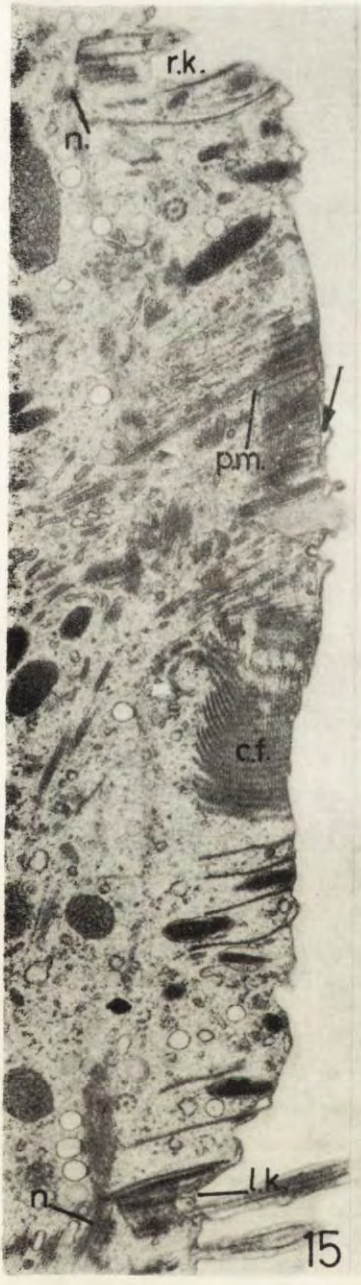

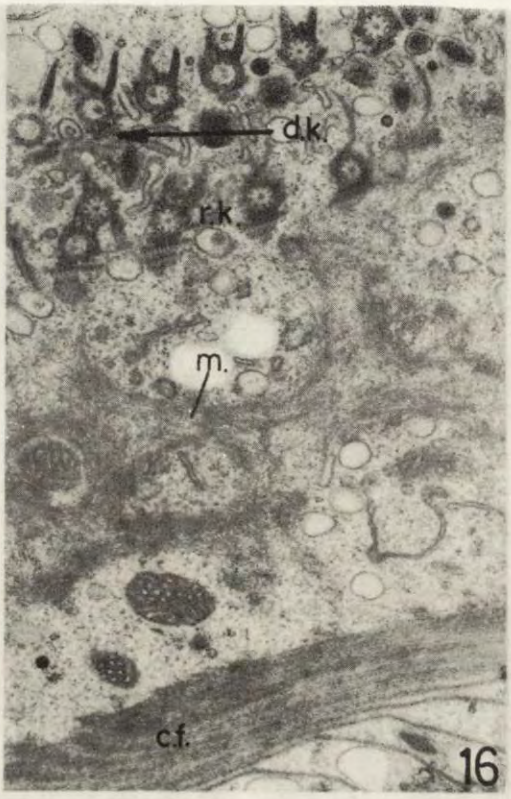

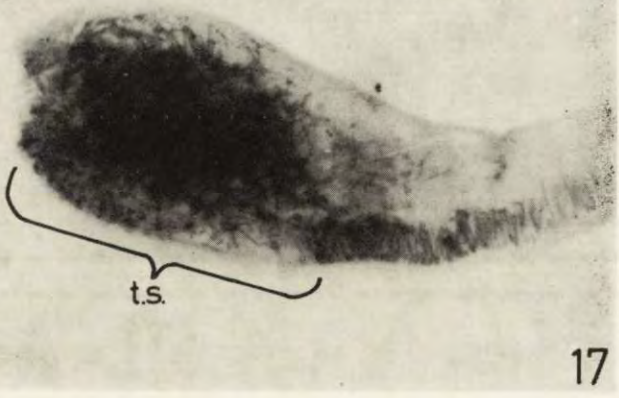

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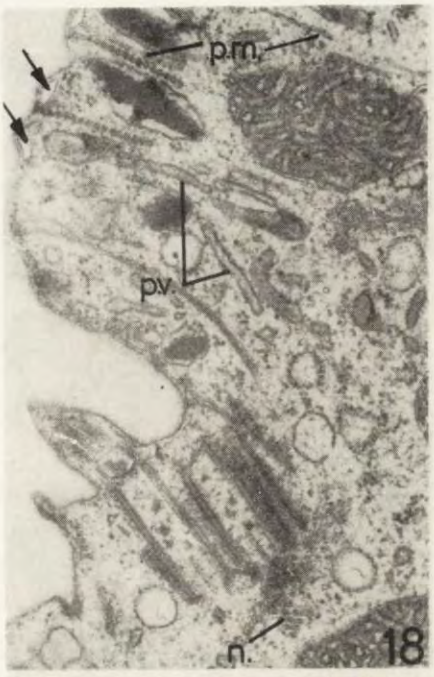

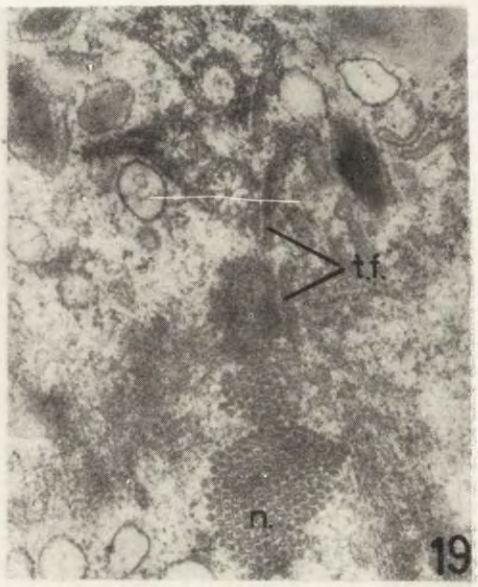

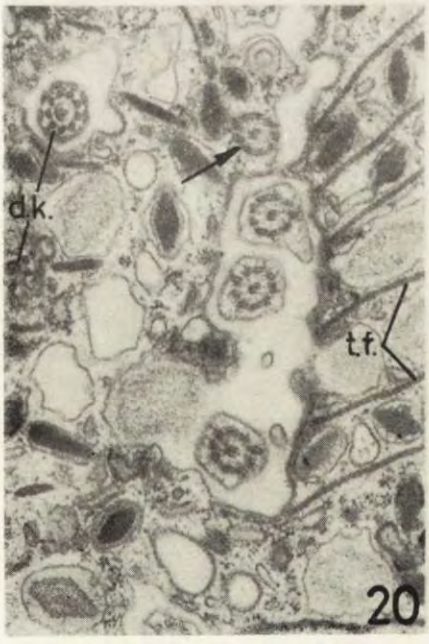

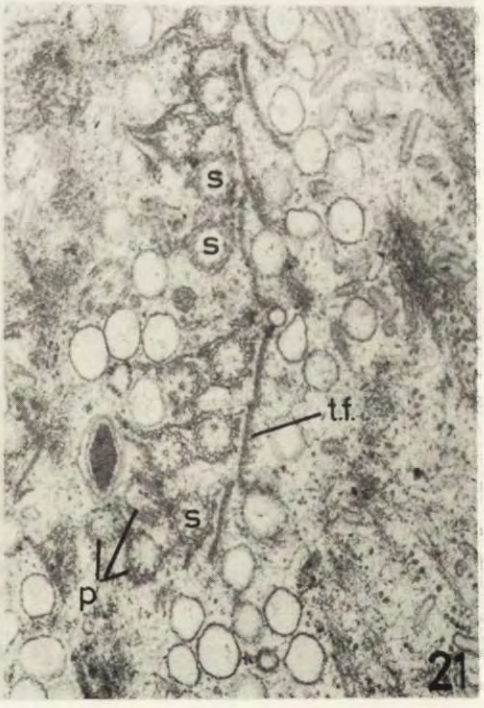

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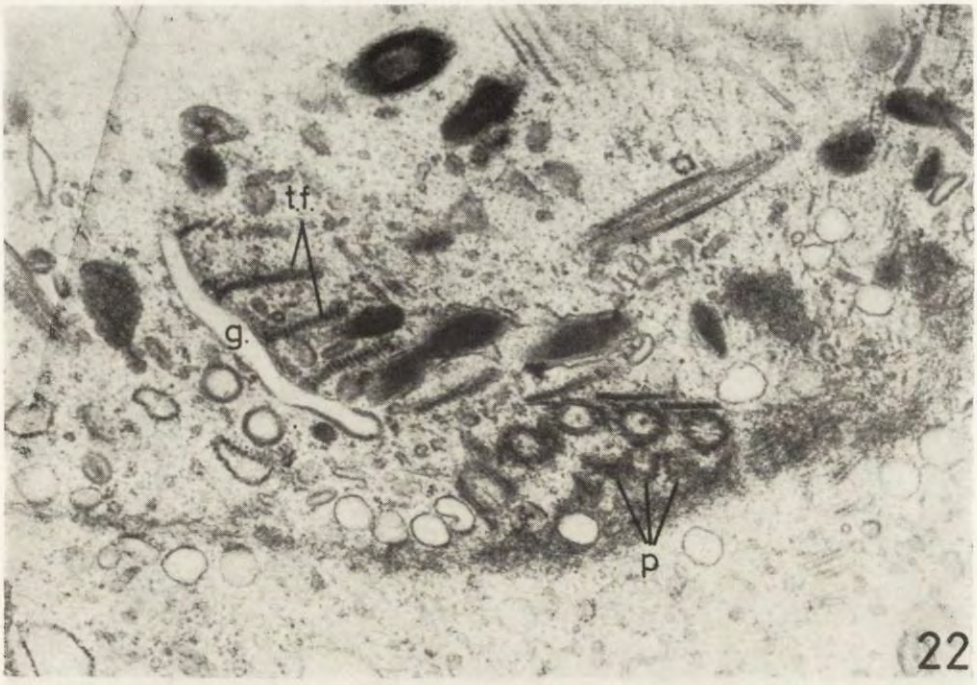

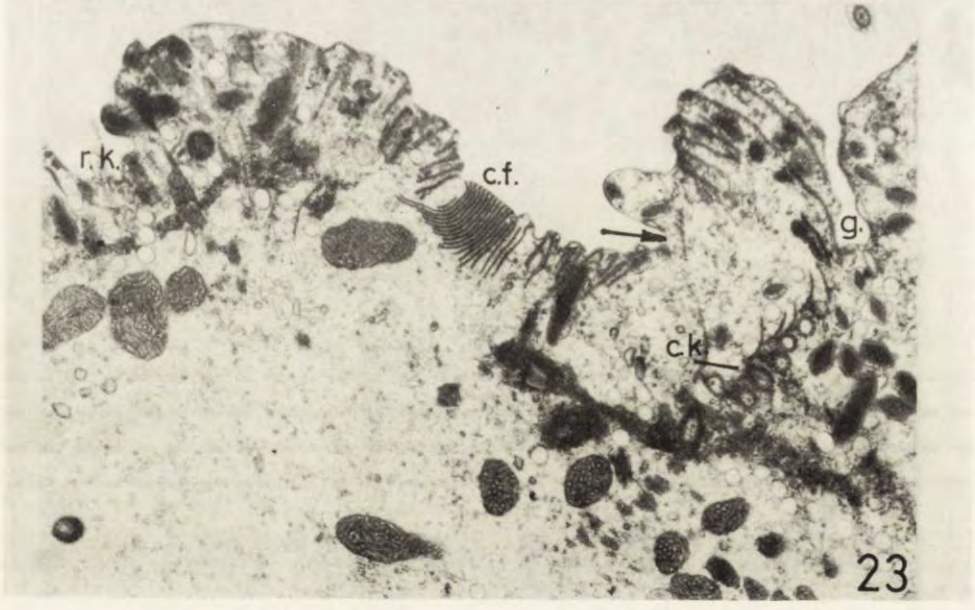

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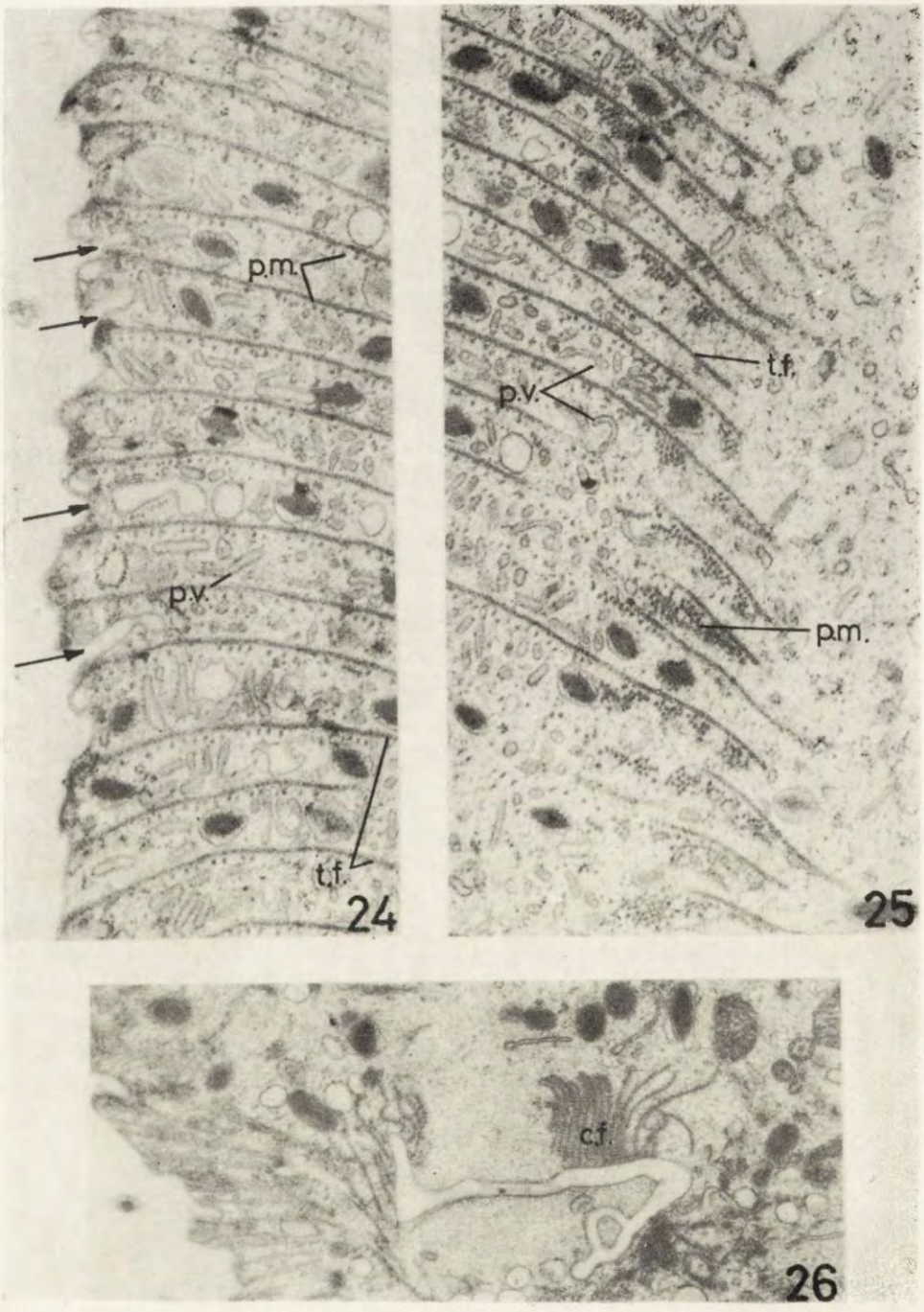

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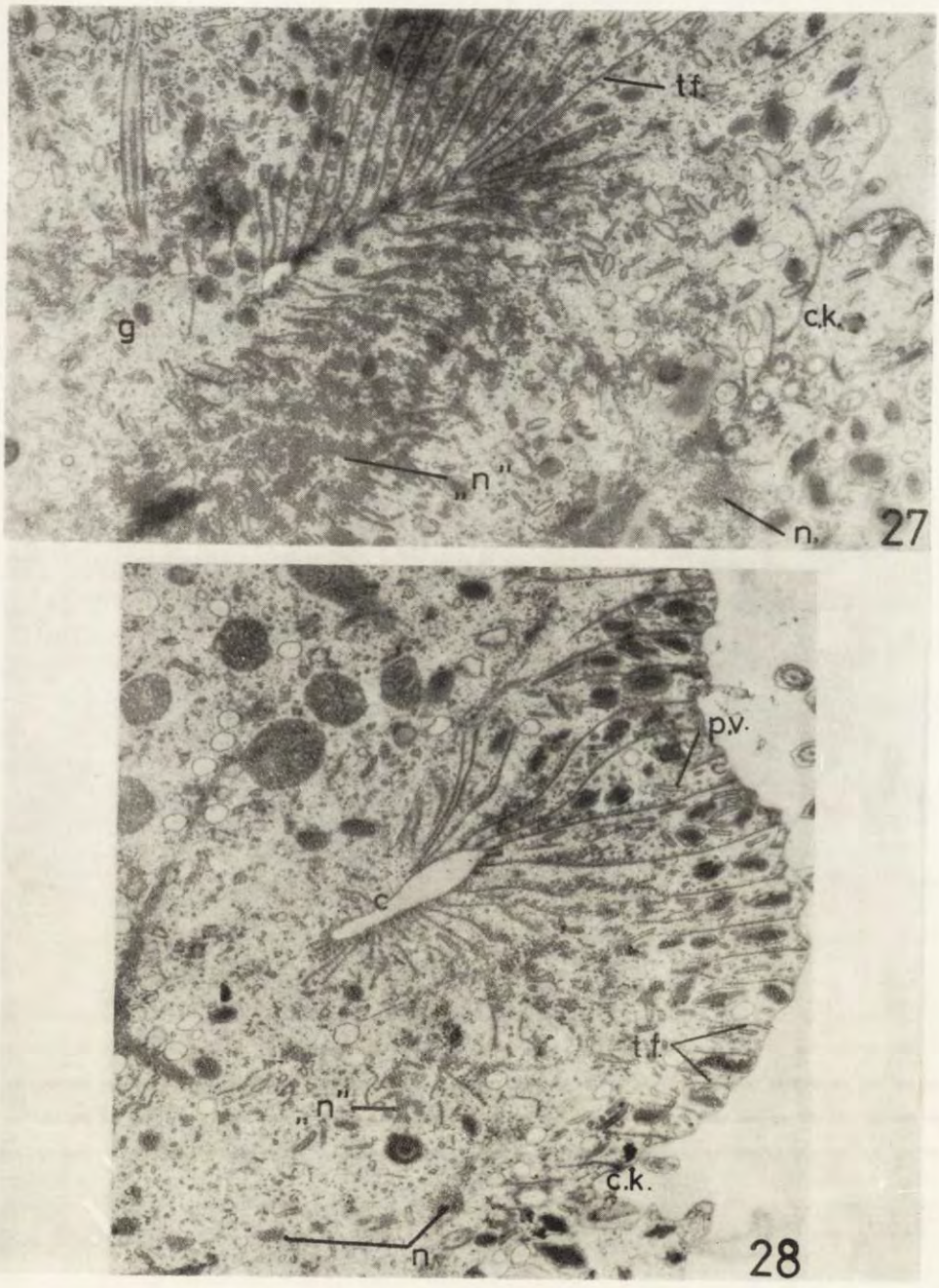

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Some Morphological and Cytological Anomalies in the Aged Cultures of *Stylonychia notophora* (Stokes)

Synopsis. Ageing clones of *Stylonychia notophora* were established and the different abnormal forms which appeared in the aged population were studied. Unlike the macronuclei of normal cells, the macronuclei of the ageing cells showed variations in their shape, size and number. The aberrant behaviour of the macronuclei of ageing clones during binary fission further resulted in the formation of different abnormal daughter cells. About 30% of the daughter cells were non-viable while the remaining daughter cells though divided but their progeny was not viable. In the aged culture, about a month old, doublets, cannibals and cysts were noticed. Doublets were non-viable, and showed L-shaped macronucleus extending along the longitudinal axis. Cysts formed in the aged culture were not viable and did not excyst.

Protozoans divide continuously by binary fission at a constant rate all through the period of immaturity, adolescence and maturity when they acquire the capacity to conjugate (Kimball 1964). However, at times due to unknown reasons (Grell 1973), the protozoans fail to conjugate and as a result senility or ageing sets in. Senility in a culture is manifested by decreasing fission rate of the animals and an increased appearance of abnormal forms (Sonneborn and Schneller 1955). These abnormal forms have been attributed to chromosomal imbalance brought about by continuous amitotic divisions of the macronucleus (Sonneborn and Schneller 1955). Synthesis of nucleic acids (Schwartz and Meister 1975, Klass and Sonneborn 1976) and proteins (Sundararaman and Cummings 1976) shows a progressing decline in the ageing cultures of *Paramecium aurelia*. Earlier Katashima (1971) reported that DNA synthesis gradually decreases to zero in *Euplotes* with the advance in ageing. This progressive fall in the biochemical activities of old cultures of *Euplotes* is also reflected by the appearance of certain abnormal forms. In the present paper some

abnormal forms in the aged cultures of *Stylonychia notophora* are described.

Material and Methods

Cultures of *Stylonychia notophora* were raised from cells collected from fresh water pools around Delhi. Cultures were maintained in sterilized hay infusion inoculated with the bacteria, *Klebsiella aerogenes* at 24 ± 1°C. Horlicks' malted milk was added periodically to facilitate the growth of bacteria. The old cultures were subcultured after 7 days. The ciliates did not show any sign of conjugation and started behaving erratically after 15 months. Cells were isolated from the aged cultures at random and stained with Feulgen-light green in order to study the general morphological and cytological variations.

The doublets present in the aged cultures were isolated and transferred individually to a multiple depression cavity block and allowed to divide. The daughter cells of the doublets were isolated and stained in order to study the pattern of macronuclear division.

The behaviour of aged abnormal cells during binary fission was studied by isolating cytokinetic forms which were allowed to complete division and the clones were raised from the resulting daughter cells. The cells produced after 72 h were counted. The mean generation time was determined by dividing the number of cells produced by the number of fission undergone during the 72 h interval.

Organisms which were likely to encyst assumed spherical shapes and exhibited sluggish circular movements. Such forms were isolated and the process of cyst formation was followed under the binocular microscope. A few forms were stained at varying intervals of time with Feulgen-light green in order to study the nuclear morphology during encystment.

Results

Stylonychia notophora has two cylindrical macronuclei placed close to one another in the center of the animal along its long axis. The S-phase of DNA synthesis is characterized by the appearance of a replication band (RB) in each macronucleus (Pl. I 1), which moves inwards along the length of the macronucleus during the course of DNA synthesis and finally disappears at the inner end. This marks the end of S-phase. In the G_2 phase, the two macronuclei fuse to form a composite body, which subsequently in the division (D) phase undergoes the primary and secondary nuclear divisions, thus resulting in the formation of four macronuclei. While the nuclear divisions are in progress, cytokinetic constriction appears, which gradually deepens and ultimately two daughter cells are formed.

Abnormal forms

In addition to the above mentioned normal forms, the aged cultures studied showed a number of abnormal forms. The abnormal forms could be broadly categorized into three types viz. the mononucleate, binucleate and trinucleate (Pl. I 2). Occasionally, the tetranucleate and the pentanucleate forms were also noticed (Pl. II 11). The mononucleate forms usualy possessed a single cylindrical macronucleus but in some animals the macronucleus was long, filamentous and beaded, the number of beads varying from 2–4 (Pl. I 3–4). The binucleate and the trinucleate abnormal forms possessed macronuclei of unequal size. They also exhibited variation in the shape of their macronuclei such as one of the macronuclei being beaded, oval or spherical while the remaining appeared normal i.e., cylindrical.

Stylonychia notophora normally possesses four micronuclei but in the aged culture as many as the macronuclei were noticed. The micronuclei did not show synchrony in their mitotic division.

Besides these abnormal forms, doublets (Pl. I 5) also appeared in the aged cultures and their macronuclei also showed morphological variations. When doublets were isolated and allowed to divide about 70% divided and the remaining cytolysed before division. Two types of daughter cells were produced by the irregular division of doublets, (i) animals with two normal sized macronuclei and an additional macronuclear fragment; new clones could be easily raised from these animals, (ii) Animals with a single normal macronucleus and an additional small fragment of the macronucleus. These forms were not viable and cytolysed within a few hours.

In the aged cultures a few cannibal giants were also noticed. These cannibals fed on the small spherical forms present usually at the bottom of the dish. All sublines of abnormal forms eventually died.

Division in abnormal forms

In the aged population, the cytokinetic forms also exhibited an abnormal macronuclear division. In many cases, the macronucleus extended across the cytokinetic furrow (Pl. I 6). When division occurred in such forms, it was an unequal division and the resulting daughter cells got fragmented macronucleus. Such daughter cells with fragmented macronucleus behaved abnormally during division. Cells having two normal macronuclei and an additional fragment of the macronucleus were able to develop into new clones. Replication bands appeared in the two normal macronuclei, which also fused to form a composite body; while the extra macronuclear fragment neither fused with the macronuclei nor developed its own RB. During cytokinesis, this fragment was distributed at random

(Pl. II 7, 8, 10). At times when this fragment was close to the fission line (Pl. II 7–8) the cytokinesis was delayed and on completion of the cytokinesis daughter cells with unequal macronuclear contents were formed. In the resulting daughter cells with one normal macronucleus and two macronuclear fragments RB appeared only in the normal macronucleus (Pl. I 2). Binucleate forms with RB appearing only in one of the two macronuclei were also noticed. In certain cases the failure of cytokinesis in the abnormal animals resulted in the formation of pentanucleate forms (Pl. II 11). However, the site at which such cells cleaved was as in the normal animals. In many aged cells tips of the macronuclei extending across the fission line were noticed (Pl. II 12), unlike the normal animals where the site of primary division of the macronucleus coincides with the fission line. The generation time of the aged cultures varied from 15 do 36 h and their rate of division also showed a decline with the increase in age. The generation time noticed in the normal culture was 9.5 h.

Encystment

The small spherical forms which were to encyst, became sluggish or immobile and were no longer translucent under the binocular microscope. Subsequently, a small ectocyst appeared which developed spiny ornamentation during the next 24 h. After the cyst was formed a small cytoplasmic protuberance extended out from its surface. The protuberance assumed a spherical shape within 20 min and ultimately got pinched off from the cyst and cytolysed immediately after its release. The cyst at this stage showed single compact spherical macronuclei. The cysts were not viable as no excystation was possible even at higher temperature (24–27°C) and in the presence of excess of food.

Discussion

In ciliates, an exconjugant usually undergoes about 1000 consecutive amitotic division before showing signs of senility (Katashima 1971), *Stylonychia* takes about 15 months to complete 1000 divisions after which senility sets in. The senility phase in *Stylonychia notophora* is characterized by increased number of cells with macronuclear abnormalities. Similar macronuclear abnormalities have been observed in aged clones of *Paramecium aurelia* (Sonneborn 1954), *P. caudatum* (Bovee 1960), *Tokophrya* (Rudzińska 1961) and *Euplotes* (Katashima 1971, Kosaka Toshikazu 1975). According to Katashima (1971) these abnormalities may be the result of irregular division of macronuclei during binary fission or due to their fragmentation. Such abnormalities may also occur due to the absence of autogamy or conjugation. This

leads to arrested growth and division and consequently abnormal forms appear.

Doublets which arise spontaneously in aged culture of *Stylonychia* differ from those produced by high temperature shocks (Totwen-Nowakowska 1965) or under the influence of chemical factors or operations (Tchang Tso-Sun et al. 1964), but closely resemble the doublets produced under low temperature conditions (Shivaji et al. 1976) in that they are unstable and resulting daughter cells exhibit a high rate of mortality. The abnormalities are perhaps the phenotypic expression of the changed genetic set up of the macronuclei which have undergone continuous reduction in their DNA content during repeated fissions. High mortality in the doublets may also be due to the aberrant behaviour of the macronucleus.

The abnormalities which appeared during DNA synthesis in the macronucleus are equally interesting. In normal *Stylonychia notophora* each macronucleus develops a RB during DNA synthesis. In the aged culture presence of a RB only in one of the two macronuclei and its absence in the fragmented macronucleus indicates a decline in DNA synthesis and other related biochemical activities. This was also observed by Katashima (1971) in *Euplotes*. Aged culture of *Stylonychia notophora* showed marked irregularities in macronuclear division. These irregularities, however, do not alter the site at which cell cleaved regardless of the position of the macronucleus in the cell. Thus ageing in *Stylonychia* does not seem to affect the site of cleavage in the cell. In this regard, it is interesting to note the results of studies on cleavage in eggs by Szollosi (1970) who concluded that the site of cleavage is determined by the spatial relationship within the cortex.

The micronuclear number is usually characteristic of a species. *Stylonychia notophora* generally possesses four micronuclei. However, in aged cultures cells with as many as ten micronuclei were noticed. This increased number may be a consequence of their irregular distribution at division.

Cannibalism was seen in the culture with the advance of ageing. Similar abnormality has been reported in an old culture of *Blepharisma* (Steinberg 1959) in which division was inhibited. Starvation does not appear to be the factor but the occurrence of small sized, spherical animals undergoing encystment in a dish appears to induce cannibalism.

A wide variety of abnormal conditions can cause encystment (Giese 1973) and ultimately arrest the biochemical activities of the organisms. The process of encystment in *Stylonychia* closely resembles that of other ciliates. However, a remarkable feature is the shedding of the cytoplasmic contents by the formation of a protuberance.

If encystment results from the failure of a particular metabolic enzyme owing to the lack of certain nutrients in the culture mediums, then one would expect excystment to be induced by the addition of these nutrients to the medium. In fact, experiments with *Blepharisma* indicate that the addition of nutrients will usually induce excystment (McLoughlin 1955). However, cysts from aged culture of *Stylonychia notophora* did not undergo excystation regardless of excess of nutrients in the medium. The factors which cause excystment in *Stylonychia* are still not known. It seems that ageing leads the macronucleus to a stage when its biochemical function is aither lost or altered and as a consequence it inhibits the synthesis of RNA and protein which are required for excystation (Lorenzo 1973).

All clones showed gradual decline in number of cells and eventually died. A number of hypotheses have been discussed to explain the decline in number and viability during the life cycle of a clone in ciliates (Sonneborn 1954, Kimball 1964, Siegel 1965). It seems that decline and death are the result of a cumulative fall in the biochemical activities of nucleus and cytoplasm.

ACKNOWLEDGEMENTS

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RÉSUMÉ

Des souches agées de *Stylonychia notophora* ont été cultivées. On a observé de différents formes anormales, qui sont apparues dans les populations vieillissantes. Différentes des macronuclei de cellules normales, les macronuclei de cellules agées montrainet des variations de forme, de dimensions et de nombre. Le comportment aberrant de macronuclei de souches vieillissantes durant la bipartition entraînait la formation d'anormales cellules descendantes. Environ 30% des cellules descendantes étaient non-viables, pendant que cellules subsistantes bien qu'elles se soint divisées ont produit les descendantes non-viables. Dans une culture vieillissante d'environ un mois, on recontrait des doublets, des canibales et des kystes. Les doublets étaient non-viables et avaient les macronuclei en forme de L, s'allongeant le long de l'axe longitudinal du corps. Les kystes formées les cultures agées étaient aussi non-viables et l'excystment ne se produsait pas.

REFERENCES

Bovee E. C. 1960: Morphological anomalies in a population of *Paramecium caudatum*. J. Protozool., 7, (Suppl.), 16.

Giese C. A. 1973: Encystment and excystment. In: *Blepharisma* (ed. C. A. Giese) Stanford, California, 247–265.

Grell K. G. 1973: Heredity. In: Protozoology, Springer Verlag, Berlin, 232–267.

Katashima R. 1971: Several features of aged cells in *Euplotes patella* Syngen 1. J. Sci. Hiroshima Univ. Ser. B, Div. 1 Zoology, 23, 59–92.

Kimball R. F. 1964: Physiological genetics of ciliates. In: Biochemistry and Physiology of Protozoa (ed. S. H. Hutner) 3, 244–296.

Klass M. R. and Sonneborn J. S. 1976: Studies on DNA content, RNA synthesis, and DNA template activity in aging cells of *Paramecium aurelia*. Expl. Cell Res., 98, 63–72.

Kosaka Toshikazu 1975: Age dependent monsters or macronuclear abnormalities, the length of life and a change in the fission rate with clonal aging in marine *Euplotes woodruffi*. J. Sci. Hiroshima Univ. Ser. B, Div. 1 (Zool.) 25, 173–190.

Lorenzo M. 1973: Physiological aspects of excystation in amoebe flagellate *Naegleria gruberi*. J. Protozool., 20, 81 (Abstr.).

McLoughlin D. C. 1955: Cystic phenomenon in ciliate *Blepharisma undulans*. J. Protozool., 2 (Suppl.), 11 (Abstr.).

Rudzińska M. A. 1961: The use of protozoans for studies on aging. Difference between young and old organisms of *Tokophrya infusorium* as revealed by light and electron microscopy. J. Geront, 16, 213–224.

Schwartz V. and Meister H. 1975: Aging in *Paramecium* several quantitative aspects. Arch. Protistenk, 117, 35–101.

Shivaji S., Saxena D. M. and Pillai M. K. K. 1976: Temperature induced abnormal forms in *Stylonychia notophora*. Acta Protozool., 15, 333–337.

Siegel R. W. 1965: Genetic control of the life cycle in ciliates. Progress in Protozoology, Abstr. Second Int. Conf. Protozool., London 1965, Excerpta Med. Int. Congr. Ser. No. 91, 59.

Sonneborn T. M. 1954: The relation of autogamy to senescence and rejuvenescence in *Paramecium aurelia*. J. Protozool., 1, 38–53.

Sonneborn T. M. and Schneller M. V. 1955: The basis of aging in variety 4 of *Paramecium aurelia*. J. Protozool., 2, 32 (Abstr.).

Steinberg P. 1959: The cause of giantism and cannibalism in *Blepharisma undulans*. Biol. Rev. City. College of New York, 21, 4–10.

Sundaraman V. and Cummings D. J. 1976: Macronuclear change in aging *Paramecium aurelia*. J. Protozool., 23, 21 (Abstract).

Szollosi D. 1970: Cortical cytoplasmic filaments of cleaving eggs: A structural element corresponding to the contractile ring. J. Cell Biol., 44, 192–209.

Tchang Tso-Sun N., Shi Xin-bai and Pang Yan-bin 1964: An induced monster ciliate transmitted through three hundred and more generations. Sci. Sin. 13, 850–853.

Totwen-Nowakowska I. 1965: Doublets of *Stylonychia mytilus* (O. F. M.) evoked by action of thermic shocks. Acta Protozool., 3, 335–361.

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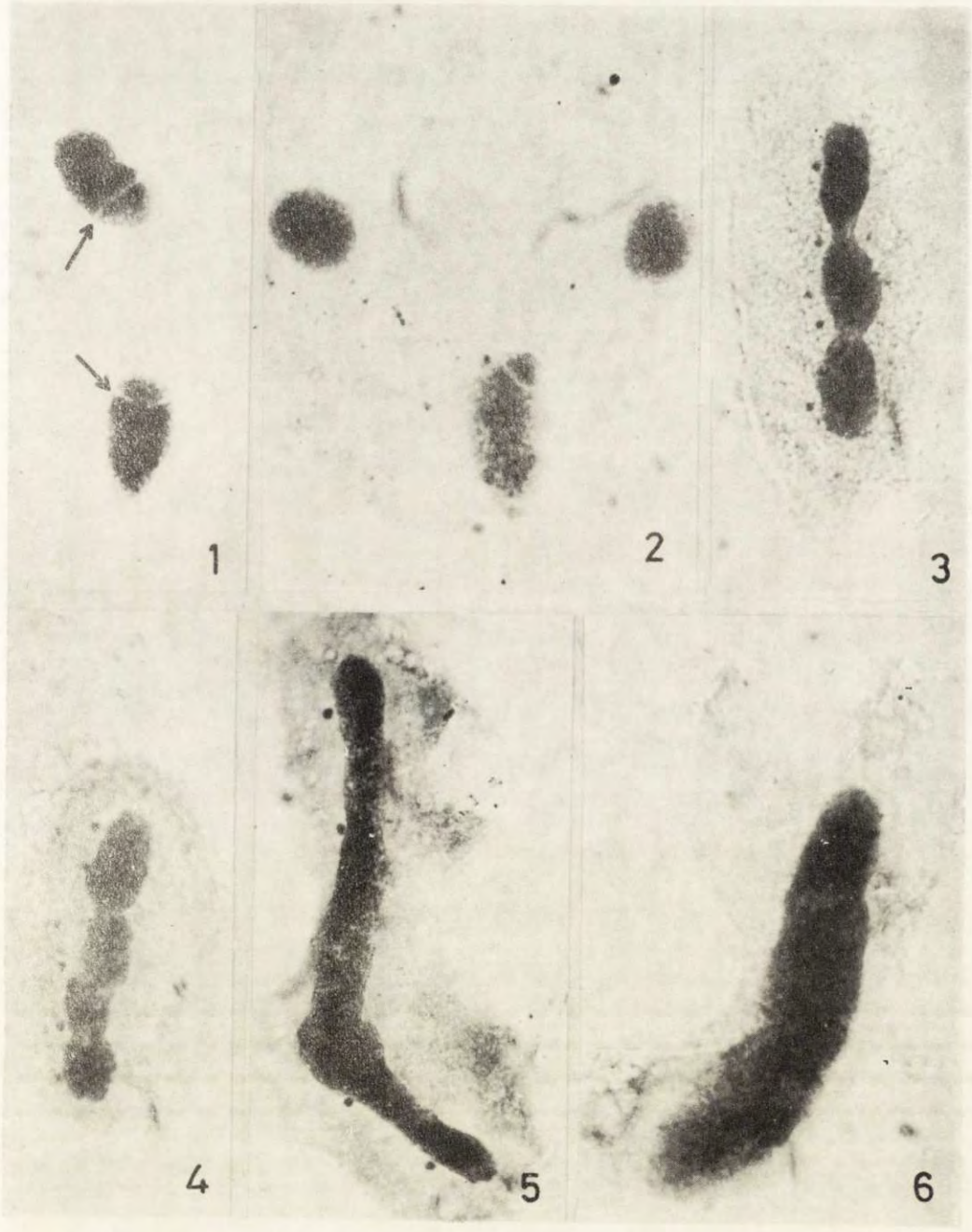
EXPLANATION OF PLATES I–II

Stylonychia notophora (Stokes)

1: A normal animal in S-phase showing replication bands (arrows) in the macronuclei. × 400
2: An aged form showing two abnormal spherical macronuclei and one normal macronucleus with replication band. × 400
3: An aged animal showing tribeaded macronucleus. × 400
4: An aged form showing tetrabeaded macronucleus. × 400
5: A doublet form showing single elonged L-shaped macronucleus. × 400
6: An aged cytokinetic animal showing elongated macronucleus extended across the fission line. × 400
7: An aged cytokinetic animal showing abnormal macronucleus across the fission line and two normal macronuclei undergoing secondary division. × 400
8: An aged cytokinetic animal showing abnormal macronucleus still extending across the fission line while the normal macronuclei have completed socondary division. × 400
9: An aged binucleate form showing replication band (arrow) only in one of the two macronuclei. × 400
10: An aged animal showing abnormal macronucleus at the basal end and two normal macronuclei under going secondary division. × 400
11: A pentanucleate form
12: An aged cytokinetic animal showing a tip of the macronucleus extending across the fission line. × 400.



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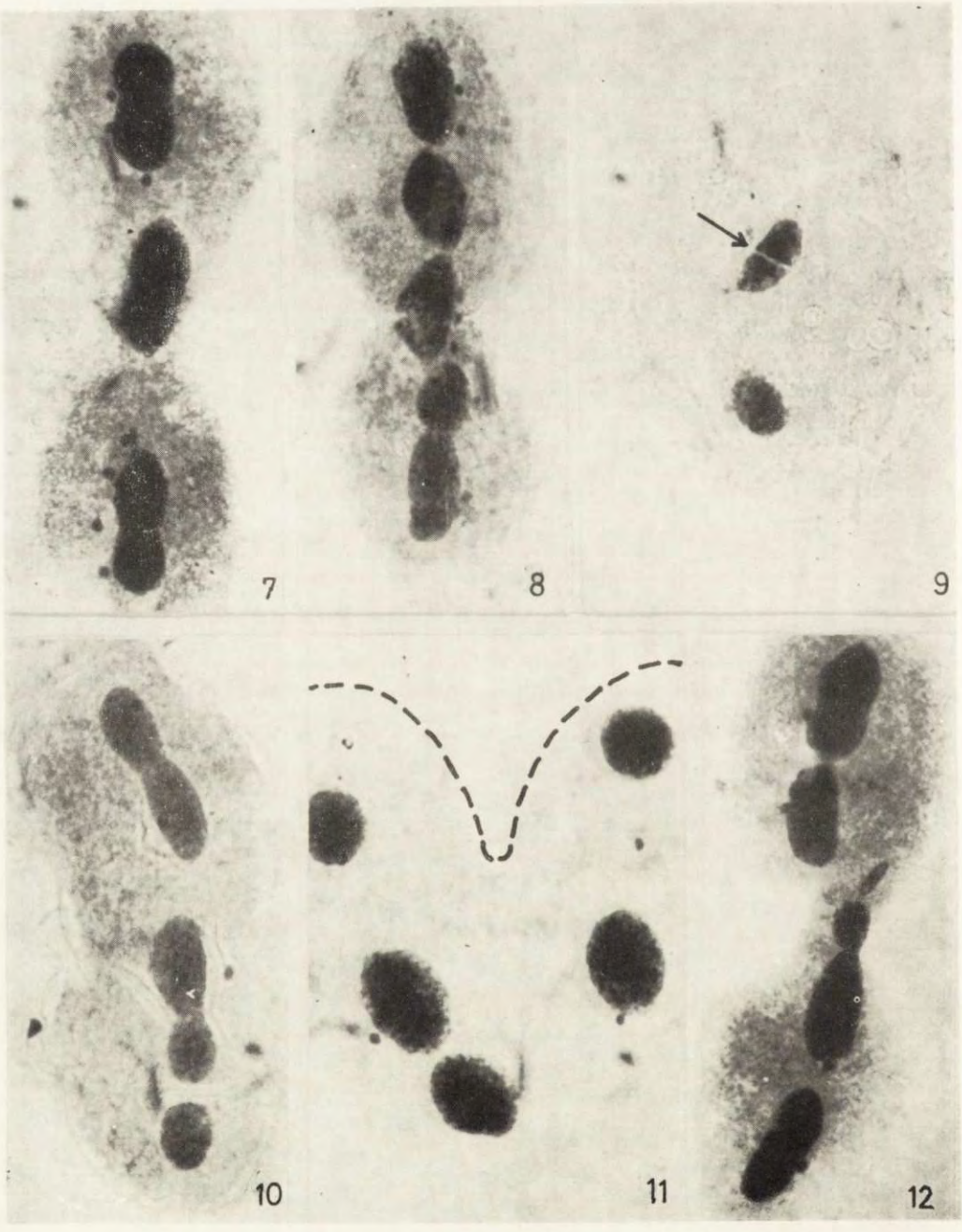

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Изменения наследственных характеристик, выявленные у амеб при их длительном культивировании

Changes of Hereditary Properties Observed during Prolonged Cultivation of Amoebas

Синопсис. В работе суммированы результаты 12-летнего изучения трех штаммов *Amoeba proteus* по признакам „спиртоустойчивость" и „метониноустойчивость". Полученные данные свидетельствуют о том, что при обычных лабораторных условиях культивирования у амеб возникли изменения штаммоспецифических характеристик. Один из штаммов изменился по обоим признакам, два других — лишь по признаку „метониноустойчивость". Высказывается предположение об эпигенотипической природе обнаруженных изменений. Приводятся данные других авторов относительно изменений ряда признаков у амеб, происходящих при их длительном культивировании в отсутствие экспериментальных воздействий.

В течение многих лет признаки „спиртоустойчивость" и „метониноустойчивость" служили маркерами в генетических исследованиях амеб, проводимых в Институте цитологии АН СССР. Это было обусловлено тем обстоятельством, что некоторые штаммы отличались друг от друга нормой реакции на определенные воздействия спирта и метионина. Применение метода пересадки ядер позволило установить, что наследование обоих признаков обусловлено ядром (Yudin and Sopina 1970). С помощью этих маркеров оказалось возможным подойти к решению таких проблем, как ядерно-ядерные и ядерно-цитоплазматические взаимоотношения, индукция наследственных изменений у амеб (см., например, Калинина и Юдин 1964, Калинина 1965 a, b, 1967, 1968, 1969, Калинина и др. 1967, Калинина и Горюнова 1975 a, b, Юдин и др. 1966).

Продолжая работу с тремя штаммами амеб, мы обнаружили, что у них спонтанно изменились уровни устойчивости к спирту или метионину. Сходные данные были получены ранее рядом [illegible] дова елей при работе с другими признаками и штаммами амеб (Jeon and Danielli 1971, Jeon and Lorch 1973). Jeon and Danielli пришли к заключению, что "некоторые, кажущиеся стабильными, признаки амеб подвергаются поразительным изменениям, если за культурами проводить наблюдения на протяжении длительного времени".

В настоящей работе подводятся итоги многолетнего изучения трех штаммов *Amoeba proteus* разного географического происхождения по признакам "спиртоустойчивость" и "метониноустойчивость" и демонстрируется характер изменений штаммов по этим признакам, выявленных при длительном культивировании[1].

Материал и методика

(1) Методы культивирования

Работа выполнена на штаммах L, C и B *A. proteus* разного географического происхождения (Yudin and Sopina 1970). Амеб выращивали по модифицированному методу Prescott and James (Prescott and James 1955, Оленов и др. 1961, Prescott and Carrier 1964). Культуральной средой являлся раствор неорганических солей — NaCl, KCl, $CaCl_2$, $CaHPO_4$, $MgCl_2$ — в бидистиллированной воде (среда Прескотта). Амеб кормили три раза в неделю *Tetrahymena pyriformis*, штамм GL. Среду в культурах меняли ежедневно. Амеб содержали при температуре 18–22°C.

(2) Методика тестирования культур по признакам „спиртоустойчивость" и „метониноустойчивость".

Спиртоустойчивость культур определяли двумя способами: во-первых, по ответу амеб на 5-минутное действие 7% этанола (Сопина и Юдин 1965); во-вторых, на 15-минутное действие 7% этанола[2].

Метониноустойчивость определяли по реакции клеток на 18-часовое действие 0.15 М метионина (Юдин 1961).

Как при определении спиртоустойчивости, так и метониноустойчивости штаммов для опыта брали выборки в 50 амеб из массовых культур и подсчитывали количество клеток, выживавших после указанных воздействий. Опыты проводили раз в сутки, приблизительно в одно время.

В большинстве случаев распределения значений выживаемости, составленные по результатам повторного тестирования культур, нельзя было считать нормальными. В связи с этим статистическая обработка материала заключалась в построении гистограмм и вычислении средних арифметических значений признаков.

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[1] Подчеркнем, что результаты настоящей работы касаются лишь тех культур амеб, которые мы содержим в нашей лаборатории.

[2] Для краткости вместо выражений „5-минутное действие 7% этанола" и „15-минутное действие 7% этанола" мы будем употреблять 5'–7% этанол и 15'–7% этанол, соответственно.

Экспериментальная часть и обсуждение

Большая часть сведений о признаке "спиртоустойчивость" была получена при определении устойчивости штаммов к 5′–7% этанолу.

На протяжении всего времени изучения (1964–1975 гг.) наследственной характеристикой штаммов С и В являлась их высокая устойчивость к данному воздействию. Это отражено на Рис. 1, представляющем распределения значений выживаемости амеб этих штаммов в 5′–7% этаноле. Гистограммы обобщают результаты 1525 опытов, проведенных со штаммом С и 1070 опытов — со штаммом В. Границы варьирования этого признака для обоих штаммов — 48% и 100%. Средняя выживаемость амеб С и В составляла 93.8% и 84.6%, соответственно. Таким образом, штаммоспецифические характеристики у амеб могут сохраняться неизменными на протяжении более 10 лет.

Иную картину мы наблюдали у штамма L. С течением времени его спиртоустойчивость менялась, и мы условно выделили три периода с разной нормой реакции этого штамма на 5′–7% этанол. На протяжении 5 лет (560 опытов) — с 1964 по 1968 гг. — штамм L был высокочувствительным к этому воздей-

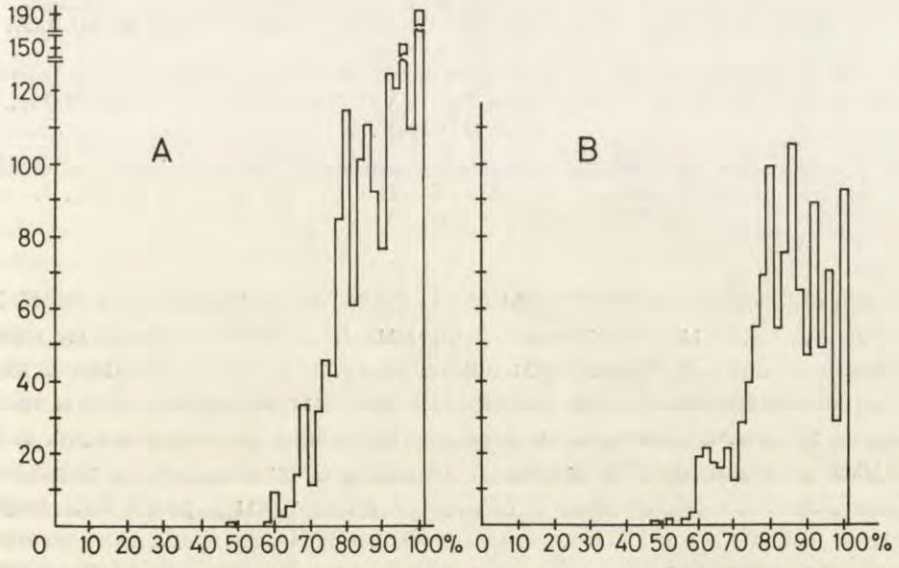

Рис. 1. Устойчивость штаммов С и В к 5-минутному действию 7% этанола на протяжении всего времени их изучения. По оси абсцисс — количество амеб, оставшихся живыми после отмены данного воздействия (число амеб в каждом опыте — 50 штук). По оси ординат — число опытов. А — штамм С в период с 1965 г. по 1975 г., В — штамм В — с 1964 г. по 1975 г.

Fig. 1. Resistance of strains C and B to 5-minute exposition to 7% ethanol during the whole period of their investigation. Abscissa — the number of survived amoebae (initial number of cells in each experiment — 50). Ordinate — the number of experiments. A — strain C within 1965–1975, B — strain B within 1964–1975

ствию: его средняя выживаемость равнялась 10.2% при границах варьирования признака от 0 до 28% (Рис. 2 А). В этот период между штаммами С и В, с одной стороны, и штаммом L, с другой, имелись значительные различия по реакции на 5′–7% этанол. Этот факт и явился отправным моментом для проведения рядом авторов генетических исследований, базировавшихся на наличии двух наследственно различающихся культур (Калинина 1965 b, Сопина и Юдин 1965, Юдин и др. 1966, Юдин и Николаева 1968 и др.).

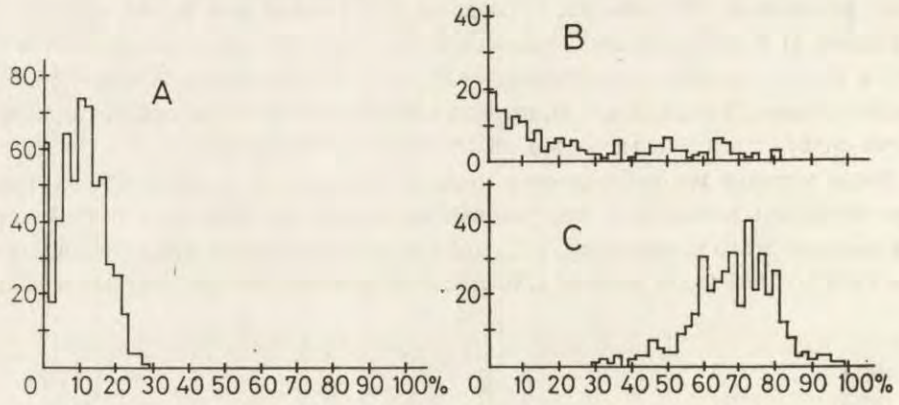

Рис. 2. Устойчивость штамма L к 5-минутному действию 7% этанола в разные периоды его изучения. По осям — то же, что на Рис. 1. А — 1964–1968 гг., В — 1969–1970 гг., С — 1970–1975 гг.

Fig. 2. Resistance of strain L to 5-minute exposition to 7% ethanol at various periods of investigation. Abscissa and ordinate — the same as on Fig. 1. A — 1964–1968, B — 1969–1970, C — 1970–1975

Однако, начиная с 1969 г., амебы L стали иначе реагировать на 5′–7% этанол. Однажды мы обнаружили у штамма L не свойственную ему выживаемость — 36%. В предыдущем опыте, за сутки до этого, штамм L имел обычную, низкую выживаемость. При дальнейшем последовательном тестировании мы стали отмечать чередование типичных и не типичных для этого штамма значений данного признака, то есть его вариабельность резко возросла. Для обозначения такого поведения культур амеб ранее был введен термин „нестабильность" (Калинина и Юдин 1964). Распределение значений выживаемости штамма L в этот период охватывало область от 0 до 80%, а средняя выживаемость стала составлять 23.8% (Рис. 2 В). Трудно сказать, когда и у скольких клеток данного штамма произошло описанное изменение по признаку „спиртоустойчивость". Тем не менее некоторые наблюдения могут помочь хотя бы частично ответить на этот вопрос. Уже в первые несколько дней периода нестабильности мы неоднократно отмечали у штамма L резкую (в пределах суток) смену высоких (70–80%) и низких (2–16%) зна-

чений исследуемого признака. Если при этом в опыт брали не одну, а несколько выборок одновременно, то для всех этих выборок получали сходные результаты — только высокие или только низкие значения. Это, по-видимому, свидетельствует о том, что штамм L был в значительной мере однородным в отношении нормы реакции составляющих его клеток на 5′–7% этанол. Иначе нужно допустить, что для нескольких выборок одного опыта были случайно отобраны преимущественно чувствительные особи, для другого опыта, проведенного спустя сутки — устойчивые. Это кажется маловероятным. Тот факт, что эта однородность штамма L была выявлена нами уже в самом начале периода нестабильности, вероятнее всего объясняется тем, что обсуждаемое изменение по признаку „спиртоустойчивость" произошло, по крайней мере, у большинства клеток этого штамма. Расширенная норма реакции на 5′–7% этанол была свойственна штамму в течение одного года (188 опытов). Поскольку за год у амеб, культивируемых по принятой методике, сменяется более 100 генераций, возникшее у штамма L изменение устойчивости к 5′–7% этанолу следует считать наследственным.

Еще большее отклонение от исходного фенотипа (1964–1968 гг.) было отмечено у штамма L в 1970 г.; исчезли низкие значения признака, характеризовавшие этот фенотип. День, когда было отмечено последнее низкое значение признака, мы условно считаем концом нестабильности. Следующий, третий период можно рассматривать как период стабильно высокой устойчивости штамма L к 5′–7% этанолу. Границы варьирования признака охватывали теперь область значений от 30 до 98% (Рис. 2 C). Средняя выживаемость, определенная по результатам 392 тестирований, стала равной 67.4%. По-видимому, как и в случае первого изменения штамма L по признаку „спиртоустойчивость", данное изменение, приведшее к новому высокому уровню устойчивости, произошло у всех или большей части клеток этого штамма. В пользу такого предположения говорит следующее. Опыт, когда было получено последнее низкое значение признака, отделен от начала периода высоких значений всего двумя неделями, в которые опытов не проводили. Таким образом, переход штамма L к новой норме реакции на 5′–7% этанол произошел довольно быстро, что и заставляет предположить массовый характер обсуждаемого изменения. Высокий уровень устойчивости к 5′–7% этанолу (однако, более низкий, чем у штаммов C и B) воспроизводится клетками L уже на протяжении 5 лет. Это позволяет считать его новой наследственной характеристикой данного штамма. Интересно отметить, что до сих пор в литературе не описаны случаи перехода культур амеб от нестабильного к стабильному состоянию по какому-либо признаку. Таким образом, за весь период наблюдения спиртоустойчивость штамма L менялась дважды: состояние стабильной чувствительности к 5′–7% этанолу сменилось нестабильностью, вслед за которой наступил период стабильной устойчивости к этому воздействию.

С 1970 г. штаммы C, B и L стали обнаруживать в значительной мере сходную реакцию на действие 5′–7% этанола. Это сделало затруднительным использование ответа исследуемых штаммов на данное воздействие для их генетического маркирования (имеется в виду постановка опытов, для которых требуется сочетание штаммов C–L или B–L).

Решено было выяснить, в какой мере сближение норм реакций на 5′–7% этанол у штаммов C–L и B–L свидетельствует о существенном уменьшении разницы между этими штаммами по спиртоустойчивости вообще. Иными словами, можно ли подобрать такую экспозицию или концентрацию этанола, при которых обнаружились бы четкие различия между названными штаммами. Оказалось, что это осуществимо: увеличение продолжительности воздействия 7% этанола до 15 минут привело к выявлению таких различий. На Рис. 3 изображены гистограммы, характеризующие выживаемость исследуемых штаммов в 15′–7% этаноле. В то время как для штаммов C и B средние величины признаков равняются соответственно 85.8% и 73.4%, для штамма L она составляет 6.8%. Эти соотношения средних величин выживаемостей сопоставимы с теми, которые характеризовали штаммы в начале исследования (1964–1968 гг.). Так же, как и ранее, различия между штаммами C и B, с одной стороны, и L, с другой, столь существенны, что соответствующие этим штаммам распределения не трансгрессируют. Выживаемость штамма C в 15′–7% этаноле варьирует от 64 до 100%, штамма B — от 54 до 100%, штамма L — от 0 до 22%. Выявленные различия сохраняются уже на протяжении более

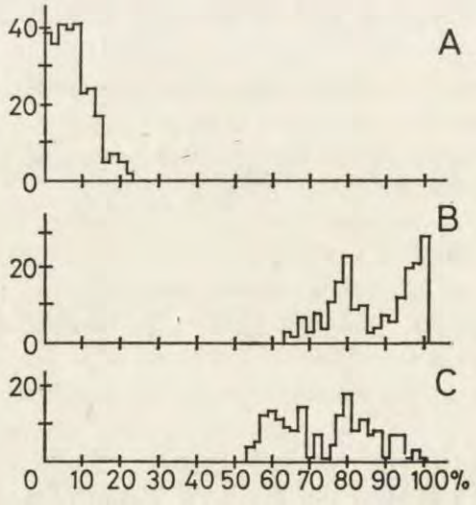

Рис. 3 Устойчивость штаммов L, C и B к 15-минутному действию 7% этанола в период с 1974 по 1976 гг. По осям — то же, что на Рис. 1. A — штамм L, B — штамм C, C — штамм B

Fig. 3. Resistance of strains L, C, and B to 15-minute exposition to 7% ethanol during 1974–1976. Abscissa and ordinate — the same as on Fig. 1. A — strain L, B — strain C, C — strain B

2 лет (проведено 200 опытов со штаммом C, 172 — со штаммом B и 280 — со штаммом L). Таким образом, представленные данные показывают, что, хотя уровни устойчивости к 5′–7% этанолу у штаммов в большой мере сблизились, между штаммами C–L и B–L еще сохраняются довольно значительные наследственные различия по спиртоустойчивости вообще. Так как устойчивость к 15′–7% этанолу дает возможность выявлять вновь возникшую наследственную особенность у штамма L по отношению к штаммам C и B, эта устойчивость может быть использована в качестве нового генетического маркера.

Одновременно с изучением спиртоустойчивости штаммов C, B и L мы определяли их метиониноустойчивость.

С 1965 по 1973 гг. штамм C и с 1964 по 1973 гг. штамм B были высокоустойчивыми к воздействию метионина: средняя выживаемость первого штамма составляла 72.2%, при границах варьирования признака от 28 до 100%; второго — 67.6%, при границах варьирования — 28% и 100% (Рис. 4 C, E). Эти данные основаны на 257 тестированиях амеб C и 412 — амеб B. Но в 1973 г. у этих штаммов в один и тот же день мы отметили изменение их реакции на метионин: они стали так называемыми нестабильными. Значения выживаемостей у них теперь варьировали от 0 до 100% (Рис. 4 D, F). В этот период средняя выживаемость штамма C, определенная по результатам 144 опытов,

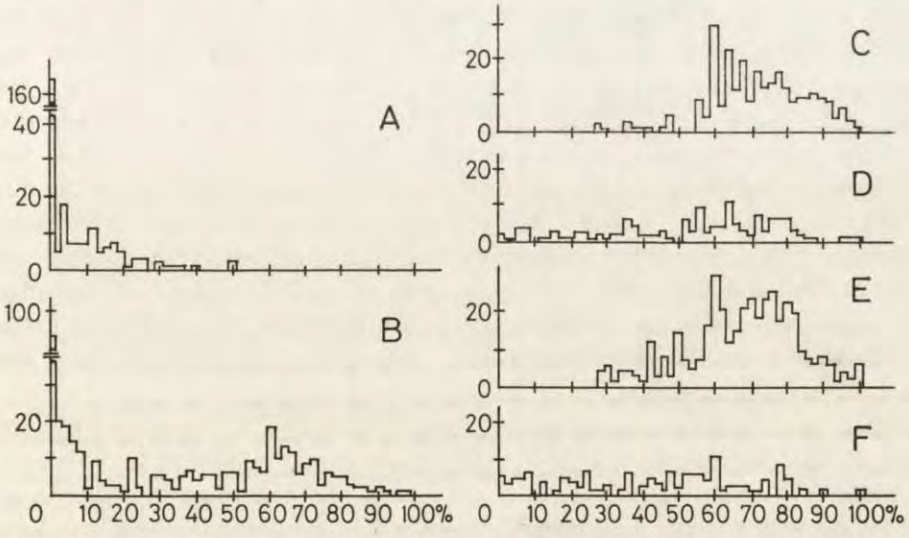

Рис. 4. Устойчивость штаммов L, C и B к 18-часовому действию 0.15 M метионина в разные периоды их изучения. По осям — то же, что на Рис. 1. A — штамм в период с 1965 по 1969 гг., B — он же — с 1970 по 1976 гг., C — штамм C в период с 1965 по 1973 гг., D — он же — с 1973 по 1976 гг., E — штамм B в период с 1964 по 1973 гг., F — он же — с 1973 по 1976 гг.

Fig. 4. Resistance of strains L, C and B to 18-hour exposition to 0.15 M methionine at various periods of investigation. Abscissa and ordinate — the same as on Fig. 1. A — strain L in 1964–1969, B — strain L in 1970–1976, C — strain C in 1965–1973, D — strain C in 1973–1976, E — strain B in 1964–1973, F — strain B in 1973–1976

была равной 51.8%; штамма В, вычисленная по результатам 125 опытов — 42.6%. Наши наблюдения показали, что особенности перехода штаммов С и В к периоду нестабильности по признаку „метониноустойчивость" сходны с характером перехода штамма L к нестабильности по признаку „спиртоустойчивость". В рассматриваемом случае нетипичные, низкие значения признака (они равнялись для двух одновременно взятых выборок штамма С 2% и 0%, штамма В — 4% и 6%) были зарегистрированы через сутки после типичных значений (для штамма С — 74% и 72%, для штамма В — 48% и 44%). Путем рассуждений, аналогичных тем, которые были сделаны нами при обсуждении нестабильности у штамма L по спиртоустойчивости (стр. 82,83), мы пришли к выводу, что, по-видимому, изменение по признаку „метониноустойчивость" произошло, по крайней мере, у большей части клеток штаммов С и В. Эти изменения сохраняются у данных штаммов на протяжении уже трех лет, что дает основание отнести их к категории наследственных.

С 1964 по 1969 гг. наследственной особенностью штамма L являлась его высокая чувствительность к метионину (Рис. 4А). Распределение, характеризующее выживаемость амеб L при действии этого агента, составлено по результатам 275 опытов. В этот период средняя выживаемость данного штамма была равной 4.8%; границы варьирования признака — 0% и 50%.

Однако с 1970 г. штамм L стал нестабильным в отношении данного признака. Значения выживаемости находились теперь в пределах 0—98% (Рис. 4В). Среднее значение признака, определенное по результатам 379 опытов, возросло до 31.4%. Общая картина перехода штамма L к нестабильности в данном случае была подобна тем, которые мы описали выше для штаммов С, В и L. В одном из опытов штамм L оказался необычно устойчивым к действию метионина — для двух одновременно взятых выборок мы получили 82% и 78% выживаемости. За сутки до этого для двух выборок значения признака равнялись 0%. Наблюдавшееся явление, как и в предыдущих случаях возникновения нестабильности, мы объяснили тем, что измененными по признаку „метониноустойчивость" оказались все или большая часть клеток штамма L. Эта новая штаммоспецифическая характеристика сохраняется у него уже на протяжении 6 лет.

Итак, изменения по признаку „метониноустойчивость" произошли у всех трех штаммов при более чем 10-летнем их ведении по обычной методике.

Представленные выше данные показывают, что в течение нескольких лет (с 1964 по 1969 гг.) между штаммами С–L и В–L существовали значительные различия по ответу на действие метионина. В это время признак „метониноустойчивость" использовался в качестве маркера в генетических экспериментах, для которых требовалось наличие наследственно различающихся культур (Калинина и Юдин 1964, Калинина 1965а, Юдин 1961, Юдин и др. 1966, Юдин и Николаева 1968). Естественно, что после того, как эти межштаммовые различия сильно уменьшились, использование устойчивости к 18-ча-

совсму действию 0.15 М метионина для маркирования данных культур в такого рода исследованиях стало малоприемлемым.

Данные, полученные при многолетнем изучении трех штаммов амеб, подтверждают высказанное ранее предположение о различной генетической детерминированности признаков „спиртоустойчивость" и „метониноустойчивость" (Yudin and Sopina 1970). Об этом свидетельствует тот факт, что признаки вели себя независимо друг от друга. У штаммов С и В изменения были зарегистрированы лишь по одному из них; у штамма L изменения по этим признакам появились неодновременно и носили неодинаковый характер.

Как мы уже отмечали, имеются достаточные основания считать каждое из рассмотренных изменений наследственным. Возникает вопрос о природе этих изменений: связаны ли они с изменениями самого генетического материала или с изменениями активности генов. Нам кажется, что следующие факты свидетельствуют в пользу второго предположения. Как было сказано выше, для всех случаев описанных изменений — два раза по признаку „спиртоустойчивость" у штамма L и один раз по признаку „метониноустойчивость" у штаммов С, В и L — мы имели основание считать, что эти изменения возникли у всех или большей части клеток этих штаммов. Далее, изменения по метониноустойчивости были выявлены одновременно у двух штаммов — С и В. Большая частота произошедших изменений делает маловероятным предположение об их мутационной природе. В основе данных изменений не лежат, по-видимому, и рекомбинационные процессы, так как у амеб не обнаружены ни половой, ни парасексуальный процессы.

К обсуждению представленных в нашей работе данных следует привлечь данные других исследований, выполненных с использованием признаков „спиртоустойчивость" и „метониноустойчивость". Наследственные изменения по этим признакам возникали у амеб при следующих условиях: совмещении в одной цитоплазме генетически различающихся ядер (Калинина и Юдин 1964, Калинина 1965 a, b, Юдин и др. 1971); рентгеновском облучении (Калинина 1967); обработке рибонуклеазой (Калинина 1969), актиномицином Д (Калинина 1968), эритромицином (Юдин 1976); инъекции амебной гетерологичной РНК (Калинина и др. 1967); микрургическом вмешательстве в делящиеся клетки (Калинина и Горюнова 1973, 1975 a, b). Принимая во внимание высокую частоту наследственных изменений и специфику некоторых воздействий, авторы предположили, что эти изменения носят эпигенотипический характер, то есть возникли на уровне механизмов регуляции генной активности. Во всех случаях у опытных культур изменения проявлялись в расширении границ варьирования признаков — наряду с типичными для исходных штаммов значениями появлялись не свойственные им ранее (то есть такие культуры вели себя как нестабильные). По своему фенотипическому проявлению некоторые изменения, выявленные нами при длительном наблюдении за штаммами, сходны с вышеуказанными индуцированными — нестабильность по

метониноустойчивости у штаммов C, B и L и по спиртоустойчивости у штамма L.

Все изложенные факты привели нас к предположению, что изменения штаммоспецифических характеристик у амеб, выявленные нами при их длительном культивировании, имеют эпигенотипическую природу[3].

Приведенные нами данные об изменениях признаков „спиртоустойчивость" и „метониноустойчивость" у амеб трех штаммов — далеко не единичный пример изменений, выявленных при продолжительной работе с этим объектом.

Ряд исследователей наблюдали изменения морфологических и физиологических штаммоспецифических признаков у амеб как при длительном содержании культур в одной и той же лаборатории, так и при ведении этих же культур в других лабораториях. Изменения происходили и с контрольными, и с экспериментально полученными культурами (Jeon and Danielli 1971, Jeon and Lorch 1973)[4].

Jeon and Danielli сообщили о многочисленных примерах такого рода изменений. Приведем некоторые из них. В 1963 г. между амебами P и D существовало шестикратное различие по устойчивости к стрептомицину, а в 1965 г. при ведении их в той же лаборатории эти штаммы отличались лишь вдвое. Другой пример. За время 15-летнего изучения гибридов между штаммами P и D были отмечены изменения таких наследственных характеристик, как диаметр ядра, форма клетки, реакция на антисыворотку, устойчивость к стрептомицину, ядерно-цитоплазматическая совместимость. В частности, через три года после получения, гибрид $P^я$ $D^ц$ (я — ядро, ц — цитоплазма), давал 40% жизнеспособных комбинаций со штаммом D; спустя следующие два года такие же сочетания ядра и цитоплазмы были жизнеспособными не более чем в 1% случаев.

Обсуждая возможные механизмы изменений, обнаруженных при длительном культивировании амеб, Jeon and Lorch (1973) высказали предположение, согласно которому „наблюдаемые фенотипические вариации были обусловлены вначале эпигенотипически, хотя в дальнейшем мог бы оказаться затронутым и ядерный геном". Это высказывание авторов об эпигенотипической природе данных изменений в значительной степени сходно с интерпретацией, которую мы дали нашим результатам. Однако трудно представить, какая зависимость могла бы существовать между изменениями в эпигенотипе и изменениями в ядерном геноме.

Причины появления изменений у амеб при их длительном ведении в лабораторных условиях неясны. Jeon and Danielli предположили, что эти изменения могут возникать под влиянием таких внешних факторов, как химический

[3] Следует отметить, что мы не исключаем возможности возникновения в ходе длительного культивирования амеб изменений иной природы (например, мутационной).

[4] Все изменения такого рода авторы назвали „фенотипическим дрейфом". Мы не используем это определение, так как в генетике термин „дрейф" обычно употребляется в определенном смысле — для обозначения случайных колебаний частот генов.

и микрофлоральный состав воздуха, методика культивирования, лабораторное оборудование, работающий с культурами исследователь. Рассматривая с этой точки зрения наши данные, мы должны отметить, что изученные штаммы культивировались в одной и той же лаборатории, по одной и той же методике, одними и теми же экспериментаторами. Однако, общепринятая методика культивирования амеб — не в аксеничных условиях и не на синтетических средах — не настолько стандартна, чтобы исключить влияние многих неконтролируемых факторов.

Длительное наблюдение заразличными штаммами амеб позволяет расширить наши представления о резервах наследственной изменчивости этого организма, раскрывающихся в нормальных условиях, без экспериментального воздействия. Так как наследственные характеристики амеб меняются даже при их культивировании по сравнительно стандартизированным методикам, то, очевидно, это происходит и в природе (возможно, и в большем масштабе). Если окажутся верными предположения об эпигенотипической природе обсуждаемых в настоящей работе изменений (как обнаруженных при длительном ведении культур, так и индуцированных), это будет свидетельствовать о большом значении эпигенотипических механизмов для наследственной изменчивости у амеб. Вероятно, что у амеб — агамных и, скорее всего, полиплоидных (полигеномных) простейших — такие механизмы могли бы поставлять материал для деятельности естественного отбора и обеспечивать этому организму пластичность в приспособлении к условиям внешней среды.

SUMMARY

Investigation of 3 strains of *Amoeba proteus* within 12 years revealed significant changes of their strain-specific characters, i.e., the alcoholresistance and methionineresistance. One of the strains changed with respect to both markers, two other ones – only with respect to methionineresistance. The observed shifts persisted within several years. Possible mechanisms of hereditary changes in amoebas during their prolonged cultivation under normal conditions are discussed.

ЛИТЕРАТУРА

Jeon K. W. and Danielli J. F. 1971: Micrurgical studies with large free-living amebas. Int. Rev. Cytol., 30, 49–89.

Jeon K. W. and Lorch I. J. 1973: Strain specificity in *Amoeba proteus*. In: The Biology of *Amoeba*. N. Y.–London, Acad. Press, 549–567.

Калинина Л. В. 1965 a: О генетическом взаимодействии ядер в гетерокарионах у амеб. Цитология, 7, 66–72.

Калинина Л. В. 1965 b: Новые данные о генетическом взаимодействии ядер в гетерокарионах у амеб. Цитология, 7, 401–404.

Калинина Л. В. 1967: Наследственные изменения, вызываемые у амеб рентгеновским облучением. Цитология, 9, 1543–1549.

Калинина Л. В. 1968: Наследуемые изменения у амеб, вызываемые действием актиномицина Д. Цитология, 10, 1588–1597.

Калинина Л. В. 1969: Наследственные изменения у амеб, вызываемые рибонуклеазой. Журнал общ., биол. 30, 80–86.

Калинина Л. В. и Юдин А. Л. 1964: Генетическое взаимодействие ядер в гетерокарионах у амеб. Цитология, 6, 695–709.

Калинина Л. В., Юдин А. Л., Подгаецкая Д. Я., Фридлянская И. И., Воробьев В. И. и Оленов Ю. М. 1967: Действие препаратов РНК, извлеченных из амеб штамма В на амеб штамма L. Матер. науч. конф. Ин-та цитологии АН СССР, посвященной 50-летию Великой Октябрьской Социалистической революции. Ленинград, 50–51.

Калинина Л. В. и Горюнова Л. Б. 1973: Микрургическое разделение амеб во время митоза. В: Структура, функция и реактивность клеток. Ленинград, 58–60.

Калинина Л. В. и Горюнова Л. Б. 1975 a: Изменения наследственных свойств амеб при микрургическом воздействии во время деления (метафаза-ранняя анафаза). Цитология, 17, 474–477.

Калинина Л. В. и Горюнова Л. Б. 1975 b: Изменения наследственных свойств амеб при минрургическом воздействии во время деления (анафаза — ранняя телофаза). Цитология, 17, 580-582.

Оленов Ю. М., Юдин А. Л. и Подгаецкая Д. Я. 1961: О чувствительности амеб к действию больших концентраций аминокислот. Цитология, 3, 281–292.

Prescott D. M. and James T. W. 1955: Culturing of *Amoeba proteus on Tetrahymena*. Exp. Cell Res., 8, 256–258.

Prescott D. M. and Carrier R. F. 1964: Experimental procedures and cultural methods for *Euplotes eurystomus* and *Amoeba proteus*. In: Methods in Cell Physiology, N. Y., 85–95.

Yudin A. L. and Sopina V. A. 1970: On the role of nucleus and cytoplasm in the inheritance of some characters in *Amoeba* (nuclear transfer experiments). Acta Protozool., 8, 1–39.

Сопина В. А. и Юдин А. Л. 1965: О наследовании устойчивости к этиловому спирту у амеб. Цитология, 7, 334–340.

Юдин А. Л. 1961: О роли ядра и цитоплазмы в наследовании некоторых признаков у амеб Цитология, 3, 569–576.

Юдин А. Л. 1976: Влияние некоторых ингибиторов биосинтезов на дестабилизирующую активность ядер в гетерокарионах у амеб. Матер. 2-го Всесоюзного съезда протозоологов, Киев, 164–165,

Юдин А. Л. и Николаева Г. В. 1968: Дополнительные данные о времени, необходимом для генетического взаимодействия ядер в гетерокарионах у амеб. Цитология, 10, 1008–1023.

Юдин А. Л., Калинина Л. В. и Николаева Г. В. 1966: О времени, необходимом для генетического взаимодействия ядер в гетерокарионах у амеб. В: Клеточная наследственность и злокачественный рост. Москва–Ленинград, 41–51.

Юдин А. Л., Махлин Е. Е. и Николаева Г. В. 1971: Наследственная дестабилизация ядер в нежизнеспособных гетерокарионах у амеб. Генетика, 7, 49-57.

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Морфология и жизнеспособность амикронуклеарных клонов инфузорий *Paramecium bursaria*

Morphology and Viability of Amicronucleate Clones of the Ciliate *Paramecium bursaria*

Синопсис. Проведено сравнение ряда биометрических показателей 25 амикронуклеарных и 147 нормальных клонов *Paramecium bursaria* в разные сроки культивирования на протяжении двух — девяти лет. Оказалось, что изменения этих показателей у амикронуклеарных клонов соответствуют тем, которые происходят при старении нормальных клонов: уменьшение размеров инфузорий, округление клеток и макронуклеуса, фрагментация макронуклеуса по типу гемиксиса. Старение амикронуклеарных клонов выражено ярче и наступает скорее чем нормальных. Скорость размножения амикронуклеарных клеток ниже, чем нормальных, старые амикронуклеарные клоны нежизнеспособны при субклонировании. Микронуклеус играет активную роль в вегетативный период жизненного цикла *P. bursaria*, причем роль его тем больше, чем старше клоны. Амикронуклеарные *P. bursaria* имеют нормальную реакцию спаривания.

Наличие двух типов ядер в клетке, различных морфологически и функционально, выделяет инфузорий в особую, своеобразную группу одноклеточных организмов. Если без макронуклеуса — вегетативного ядра, инфузории не способны к сколько-нибудь длительному существованию, то отсутствие микронуклеуса — генеративного ядра, в ряде случаев не приводит к гибели клетки. Более того, существует ряд видов инфузорий, для которых амикронуклеарные линии являются обычными.

Особи приблизительно 20 видов инфузорий, разнообразных в систематическом отношении, были найдены без микронуклеусов в естественных популяциях, причем большинство из них оказались способными положить начало жизнеспособным амикронуклеарным клонам (Обзоры: Beers 1946, Wichterman 1953, Борхсениус 1975). Описаны также случаи спонтанной утраты микронуклеусов в процессе культивирования либо в результате их измель-

Таблица 1

Влияние удаления микронуклеуса на жизнеспособность клонов некоторых инфузорий

Table 1

The influence of micronucleus removal on the viability of clones of some ciliates

Виды инфузорий Species of ciliates	Природные жизнеспособные клоны без микронуклеуса Natural viable clones without micronucleus	Клоны, спонтанно утратившие микронуклеус Clones with spontaneously lost micronucleus	Экспериментальные клоны Experimental clones					
			Способ уничтожения микронуклеуса Way of destroying of the micronucleus	Скорость размножения Reproduction rate	Жизнеспособность Viability	Способность к спариванию Capability to mating	Способность к регенерации и стоматогенезу Capability to regeneration and stomatogenesis	Литература References
Tetrahymena pyriformis	+	+	X-лучи X-rays	Замедлена Slow	Гибель сразу или через 2 деления Death immediate or after two divisions	—	?	Wells 1961
Paramecium caudatum	+	+	Ультрацентрифугирование Микрургия Мочевина X-лучи Ultracentrifuging Micrurgy, Urea X-rays	Замедлена Slow	Снижена Reduced	+	—	King and Beams 1937 Lewin 1910, Schwartz 1934 Miyake 1956 Wichterman 1960

Paramecium bursaria	+	+	Микургия Micrurgy	Замедлена Slow	Снижена Reduced	+	–	Schwartz 1939, 1947 Tartar and Chen 1941
Stentor coeruleus	–	–	Микургия Micrurgy	Нормальна Normal	Нормальна Normal	?	+	Schwartz 1934, 1935
Stylonychia mytilus	–	–	Уф, X и β лучи UV, X and β-rays	Замедлена Slow	Снижена Reduced	+	–	Ammermann 1965, 1970, 1971, Frick 1967
Euplotes patella	–	+	Микургия Micrurgy	Замедлена Slow	Снижена Reduced	+	–	Kimball 1941, Reynolds 1932
Euplotes eurystomus	–	–	Микургия Micrurgy	Замедлена Slow	Гибель через 1–2 дня Death after 1 or 2 days	?	–	Taylor and Farber 1924

чения и нарушения митоза у всех особей клона (Diller 1959, Rosenbaum et al.1966, Allen and Weremiuk 1971, Katashima 1973 a, b), либо вследствие нарушения распределения дочерних микронуклеусов при вегетативном делении у части инфузорий клона (Woodruff 1931, Diller 1940, Wichterman 1954). При спонтанной утрате микронуклеуса в ряде случаев наблюдалось снижение жизнеспособности инфузорий (Allen and Weremiuk 1971).

Несмотря на существование природных и спонтанно возникших амикронуклеарных клонов, экспериментальное удаление микронуклеуса, как правило, приводило к гибели инфузорий или резкому снижению их жизнеспособности (Табл. 1). Лишь часть амикронуклеарных клеток могут приспособиться к отсутствию микронуклеуса и дать начало клону (Ammermann 1970, 1971). Исключением являются лишь *Stentor polymorphus* и *Oxytricha fallax*, у которых уничтожение микронуклеуса не сказывается на жизнедеятельности вегетативной клетки (Reynolds 1932, Schwartz 1934, 1935). В ряде случаев (у *Tetrahymena*) отсутствие микронуклеуса сказывается в ослаблении или отсутствии реакции спаривания (Elliott and Hayes 1953). В амикронуклеарных клонах, как правило, нарушены или невозможны процессы регенерации и стоматогенеза у эксконъюгантов (Табл. 1 и Diller 1962). В последние годы появились данные об увеличении значения микронуклеуса в жизнедеятельности инфузорий с возрастом клона, вплоть до гибели амикронуклеарных клеток в старых линиях (Weindurch und Doerder 1975).

Нами проведено исследование природных и спонтанно возникших амикронуклеарных клонов *Paramecium bursaria* — вида, у которого в естественных популяциях нередко встречаются особи без микронуклеуса.

Материал

Материалом для настоящего исследования послужили 174 клона инфузорий *Paramecium bursaria* (Focke). Особи, которые положили начало клонам, изолированы из природных популяций водоемов Ленинграда и его пригородов в 1966–1971 г. Один клон получен из Дальних Зеленцов (Кольский полуостров) и 9 клонов — из Якутии. 25 клонов (14%) оказались амикронуклеарными. Все инфузории содержали в цитоплазме зеленые симбиотические водоросли — *Chlorella*, характерные для *P. bursaria*. Клоны содержались в одинаковых условиях на свету при комнатной температуре. Инфузории культивировались на салатной среде по стандартной методике, в качестве подкормки использовалась культура *Aerobacter aerogenes*.

Для морфологической характеристики клонов служили стандартные препараты, изготовленные из инфузорий всех клонов в разные периоды культивирования. Фиксатором служила жидкость Ниссенбаума, окрашивались препараты по Фельгену с предварительным гидролизом в 1N HCl при 60°C в течение 6 минут.

Результаты и обсуждение

Генеративное ядро ряда видов рода *Paramecium*, в отличие от микронуклеусов большинства инфузорий, как правило, является полиплоидным. Такие

данные существуют для *P. caudatum* (Борхсениус et al. 1968, Борхсениус и Осипов 1971 a, b, Осипов и Борхсениус 1973), *P. putrinum* (Янковский 1972), *P. bursaria* (Chen 1940 a, b, c, Wichterman 1946, Овчинникова 1970, Голикова 1972, 1974). Результатом полиплоидности микронуклеуса *P. bursaria*, по-видимому, является широкий полиморфизм всех признаков, в том числе и ядер, этих инфузорий, а возможно, и значительный процент амикронуклеарных особей в природных популяциях (как следствие нарушения митоза в полиплоидных ядрах).

Встречаемость амикронуклеарных инфузорий

Сведения об использованных в работе амикронуклеарных клонах *P. bursaria* представлены в Табл. 2. В нашем материале 12 клонов были амикронуклеарными с начала культивирования. Количество амикронуклеарных клеток в естественных популяциях обычно больше, но многие из них обладают пониженной жизнеспособностью и не клонируются или образуют слабые, быстро гибнущие клоны. В 10 клонах утрата микронуклеуса произошла спонтанно в процессе культивирования. Наконец, в 3 клонах исчезновению микронуклеуса предшествовало поражение его бактериями, морфологически похожими на омега-частицы микронуклеуса *P. caudatum* (Осипов и Ивахнюк 1972). Данные об этих паразитах будут опубликованы отдельно. Разные амикронуклеарные клоны были жизнеспособными от 0,5 года до 6 лет, в среднем около 2 лет.

Морфологические изменения в амикронуклеарных клонах

Сравнение некоторых морфологических признаков нормальных и амикронуклеарных клонов показало, что по суммарным данным, в среднем амикронуклеарные клетки мало отличаются от нормальных (Табл. 3). Достоверно различаются лишь показатели формы тела (отношение длины к ширине) и формы макронуклеуса. В клетках безмикронуклеусных клонов оба показателя, как правило, ниже, чем в нормальных, то есть и сами инфузории и их макронуклеусы менее вытянуты.

Более детальный биометрический анализ влияния спонтанной утраты микронуклеуса на размер тела и величину макронуклеуса был проведен на 7 клонах, которые в начале четырехлетнего периода культивирования были нормальны, а в конце стали амикронуклеарными. Результаты представлены в Таблице 4. В большинстве клонов утеря микронуклеуса сопровождалась уменьшением средней длины клеток (особенно резким в клонах $БН_{16}$ и $ДС_{12}$), в то время как средняя ширина клеток в одних клонах снижалась, а в других, напротив, возрастала. Макронуклеус к концу четырехлетнего периода (то есть в амикронуклеарном состоянии клонов), как правило, становился несколько короче (за исключением клона $БН_{20}$) и шире (кроме клонов $ДС_{12}$ и $ДС_{22}$).

Таблица 2

Амикронуклеарные клоны *Paramecium bursaria*

Table 2

The amicronucleate clones of *Paramecium bursaria*

Название клона Clone name	Время изоляции Isolation time	Состояние после изоляции Condition after isolation	Время обнаружения амикронуклеарных инфузорий Date of detection of amicronucleate ciliates	Судьба клона Fate of clone	Появление структур, напоминающих микронуклеус Appearance of structures, resembling micronucleus	Время пребывания без Ми в годах Longevity of amicronucleate clones in years
1	2	3	4	5	6	7
OP_{13} OR_{13}	V, 1967	нормальные normal	I, 1974	+ в начале 1976 + in the beginning of 1976	—	2
OP_{24} OR_{24}	V, 1967	нормальные normal	I, 1974	+ в конце 1975 + in the end of 1975	—	2
OP_{25} OR_{25}	V, 1967	нормальные normal	II, 1970	жив alive	III, 1972	2
$ПП_3$ PP_3	V, 1967	нормальные normal	IV, 1974	+ в конце 1975 + in the end of 1975	IV, 1975	1
$БС_2$ BS_2	VI, 1967	амикронуклеарные amicronucleate	III, 1968	+ в II, 1973 + in II, 1973	—	5
$БС_8$ BS_8	VI, 1967	амикронуклеарные amicronucleate	III, 1968	+ в IX, 1974 + in IX, 1974	—	6
$БС_{12}$ BS_{12}	VI, 1967	амикронуклеарные amicronucleate	III, 1968	+ в XII, 1971 + in XII, 1971	—	4
$БС_{33}$ BS_{33}	VI, 1967	амикронуклеарные amicronucleate	III, 1968	+ в X, 1971 + in X, 1971	—	3.5
$БН_1$ BN_1	X, 1971	амикронуклеарные amicronucleate	XII, 1971	+ в VII, 1974 + in VII, 1974	—	2.5
$БН_{12}$ BN_{12}	X, 1971	амикронуклеарные amicronucleate	XII, 1971	жив alive	XII, 1974	3

1	2	3	4	5	6	7
$БН_{16}$	X, 1971	нормальные	XII, 1974	+ в III, 1976	—	1
BN_{16}		normal		+ in III, 1976		
$БН_{19}$	X, 1971	нормальные	XII, 1974	жив	—	2
BN_{19}		normal		alive		
$БН_{20}$	X, 1971	нормальные	XII, 1974	+ в III, 1975	—	0.3
BN_{20}		normal		+ in III, 1975		
$БН_{22}$	X, 1971	бактерии в Ми	III, 1972	+ в II, 1975	XII, 1974	2.5
BN_{22}		bacteria in Mi		+ in II, 1975		
$БН_{24}$	X, 1971	бактерии в Ми	XII, 1974	+ в VII, 1974	—	0.5
BN_{24}		bacteria in Mi		+ in VII, 1974		
$БН_{27}$	X, 1971	амикронуклеарные	III, 1972	+ в II, 1976	—	3
BN_{27}		amicronucleate		+ in II, 1976		
$БН_{28}$	X, 1971	амикронуклеарные	I, 1972	+ в II, 1976	XII, 1974	3
BN_{28}		amicronucleate		+ in II, 1976		
$БН_{31}$	X, 1971	бактерии в Ми	XII, 1974	жив	—	2
BN_{31}		bacteria in Mi		alive		
$БН_{39}$	X, 1971	амикронуклеарные	XI, 1971	+ в XII, 1975	XII, 1974	3
BN_{39}		amicronucleate		+ in XII, 1975		
$Д_2$	IX, 1971	амикронуклеарные	X, 1971	+ в II, 1976	XII, 1973	2
D_2		amicronucleate		+ in II, 1976		
$Д_{2\text{-}1}$	XI, 1971	амикронуклеарные	XII, 1974	+ в V, 1976	—	4.5
$D_{2\text{-}1}$		amicronucleate		+ in V, 1976		
$Д_3$	IX, 1971	нормальные	XII, 1974	+ в X, 1976	—	2
D_3		normal		+ in X, 1976		
$ДС_{12}$	X, 1971	нормальные	XII, 1974	жив	—	2
DS_{12}		normal		alive		
$ДС_{22}$	X, 1971	нормальные	XII, 1974	+ в VIII, 1976	—	1.5
DS_{22}		normal		+ in VIII, 1976		
$ДС_{34}$	X, 1971	амикронуклеарные	X, 1971	+ в XI, 1976	—	5
DS_{34}		amicronucleate		+ in XI, 1976		

Сокращения: Ми — микронуклеус, + — гибель клона.
Abbreviations: Mu — micronucleus, + death of clone.

Таблица 3

Средняя величина клеток и ядер нормальных и амикронуклеарных *Paramecium bursaria* (в микронах). Суммарные данные по 147 нормальным и 25 амикронуклеарным клонам за весь период их культивирования

Table 3

The average size of cells and nuclei of normal and amicronucleate *Paramecium bursaria* (in microns). Total data on 147 normal and 25 amicronucleate clones during the whole period of their cultivation

	Параметры Parameters	Амикронуклеарные клоны Amicronucleate clones	Нормальные клоны Normal clones
Клетка Cell	Длина Length	92,8±1.6 (79—108)	89.8±0.7 (73–114)
	Ширина Width	41.4±1.0 (34—52)	37.3±0.4 (27–53)
	Длина / Ширина Length / Width	2.2 (1.9–2.7)	2.4 (1.8–3.1)
Макронуклеус Macronucleus	Длина Length	29.8±0.7 (25—38)	30.0±0.3 (20–41)
	Ширина Width	15.4±0.5 (12—25)	14.2±0.2 (11—19)
	Длина / Ширина Length / Width	1.9 (1.5–2.1)	2.1 (1.5–2.8)
Микронуклеус Micronucleus	Длина Length	—	13.0±0.1 (6.5–19.5)
	Ширина Width	—	5.2±0.1 (1.6–8.4)
	Длина / Ширина Length / Width	—	2.5 (1.6–6.8)

В усредненном виде данные по этим семи клонам представлены на Рис. 1 А. На рисунке видно отчетливое уменьшение средней абсолютной длины клеток и макронуклеуса при некотором увеличении ширины последних, а также соответственное снижение показателей как формы клеток, так и макронуклеуса. Такого рода изменения отмечались у амикронуклеарных *P. bursaria* и раньше (Schwartz 1947). Возникает, однако, вопрос, не являются ли они просто результатом старения клонов. Для решения этого вопроса мы провели анализ тех же показателей в начале и конце четырехлетнего периода культивирования для 7 нормальных клонов (микронуклеарных), имеющих то же происхождение и тот же срок жизни, что и спонтанно возникшие амикронуклеарные. Результаты представлены на Рис. 1 В, из которых видно, что в нормальных клонах при длительном культивировании происходят изменения такого же типа, как и в утративших микронуклеус: снижение длины клеток и макронуклеуса при почти неизменной ширине тех и других. Следовательно, изменение формы

Таблица 4

Изменение биометрических показателей в 7 клонах *P. bursaria* после утери ими микронуклеуса на протяжении четырех лет

Table 4

Changes of biometrical indices in 7 clones of *P. bursaria* after the loss of the micronucleus during four years

Клон Clone	Состояние клона Clone condition	Клетка — Cell			Макронуклеус — Macronucleus		
		Длина Length	Ширина Width	Длина / Ширина Length / Width	Длина Length	Ширина Width	Длина / Ширина Length / Width
OP_{13}	Нормальный Normal	87.9±1.3	29.4±0.6	3.0	33.2±0.7	11.9±0.2	2.8
OR_{13}	Без микронуклеуса Without Mi	87.5±1.9	34.9±1.1	2.5	28.4±0.9	13.9±0.4	2.0
$ПП_3$ PP_3	Нормальный Normal	88.0±1.7	35.3±1.0	2.5	31.9±0.4	13.3±0.3	2.4
	Без микронуклеуса Without Mi	82.4±2.3	37.3+1.0	2.2	31.5±0.7	15.7+0.3	2.0
$БН_{16}$ BN_{16}	Нормальный Normal	97.0±1.5	44.1±1.3	2.2	33.1±0.8	15.2±0.5	2.2
	Без микронуклеуса Without Mi	82.4±4.2	40.1±2.0	2.0	29.2±0.7	15.8±0.6	1.8
$БН_{19}$ BN_{19}	Нормальный Normal	87.3±2.9	31.7±1.9	2.8	36.0±1.0	13.6±0.8	2.6
	Без микронуклеуса Without Mi	86.1±2.2	37.5±1.2	2.3	31.7±0.7	14.8±0.4	2.1
$БН_{20}$ BN_{20}	Нормальный Normal	93.4±1.6	43.3±1.0	2.2	26.4±0.5	11.9±0.3	2.2
	Без микронуклеуса Without Mi	90.7±1.7	40.5±1.0	2.2	30.4±0.6	15.6±0.4	2.0
$ДС_{12}$ DS_{12}	Нормальный Normal	104.6±2.4	48.5±1.6	2.2	28.6±0.8	17.2±0.5	1.7
	Без микронуклеуса Without Mi	81.9±1.7	35.7±1.0	2.3	25.4±0.7	12.3±0.4	2.1
$ДС_{22}$ DS_{22}	Нормальный Normal	95.4±2.2	43.0±1.8	2.2	34.8±0.8	14.3±0.4	2.4
	Без микронуклеуса Without Mi	92.8±2.1	38.8±1.1	2.4	27.8±1.0	13.9±0.5	2.0

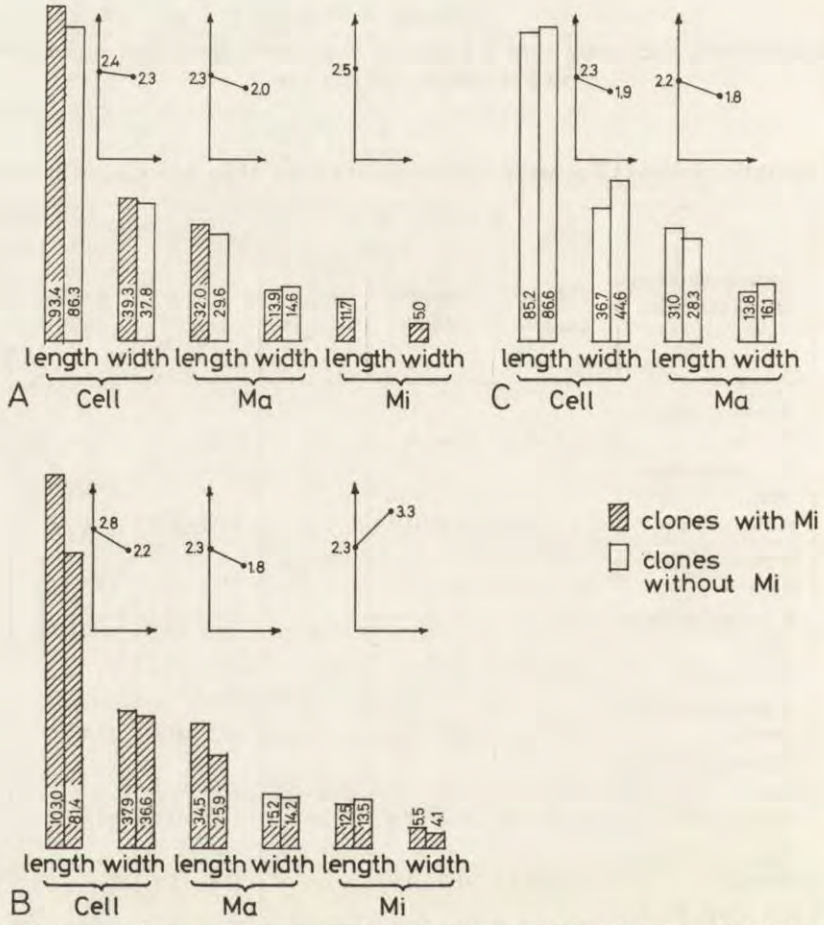

Рис. 1. Изменения биометрических показателей амикронуклеарных и нормальных клонов *Paramecium bursaria* в разные сроки культивирования. А — Средние данные по 7 клонам, утратившим микронуклеус на протяжении 4 лет ($ОР_{13}$, $ПП_3$, $БН_{16}$, $БН_{19}$, $БН_{20}$, $ДС_{12}$, $ДС_{22}$). В — Средние данные по 7 нормальным клонам на протяжении 4 лет ($ОР_9$, $БС_{10}$, $БС_{31}$, $БН_{26}$, $БН_{44}$, $БН_{47}$, $ДС_{17}$). С — Средние данные по 3 амикронуклеарным клонам за 2 года ($БС_2$, $БС_8$, $БС_{33}$). Высота столбиков пропорциональна длине (в микронах). На графиках над гистограммами приведены изменения показателя формы клетки, макро- и микронуклеуса (отношение длины к ширине).

Fig. 1. Changes of biometrical indices of amicronucleate and normal clones of *Paramecium bursaria* in different periods of cultivation. A — Average data of 7 clones, which lost their micronuclei during 4 years (OR_{13}, PP_3, BN_{16}, BN_{19}, BN_{20}, DS_{12}, DS_{22}). B — Average data of 7 normal clones in the course of 4 years (OR_9, BS_{10}, BS_{31}, BN_{26}, BN_{44}, BN_{47}, DS_{17}). C — Average data of 3 amicronucleate clones for 2 years (BS_2, BS_8, BS_{33}). The height of columns is proportional to the length (in micrones). Changes of the cell shape, expressed by length to width ratio, are given above the histograms. Designations: Mi — micronucleus, Ma — macronucleus

клеток и макронуклеуса — скорее всего следствие именно старения клонов, а не утраты микронуклеуса.

Ранее было показано (Голикова 1972), что на относительно небольших отрезках времени (1–2 года) в нормальных клонах в процессе культивирования

P. bursaria не происходит закономерных изменений биометрических показателей. Клоны *P. bursaria*, прожившие более двух лет, очевидно, уже должны считаться старыми (Jennings 1944 a, b, Wichterman 1953, Schwartz und Meister 1973), и изменения, происходящие в них, скорее всего определяются именно старением.

В трех амикронуклеарных клонах (Рис. 1 С) на протяжении двух лет культивирования изменения размеров клетки носили скорее обратный характер — инфузории сохранили прежнюю длину и стали заметно шире. Напротив, макронуклеус изменился в том же направлении, что и в первых двух категориях особей — то есть стал короче и шире.

В микронуклеарных клонах практически все особи имеют нормальный макронуклеус (Pl. I 1). Напротив, в амикронуклеарных клонах часто встречаются аномалии строения макронуклеуса (Pl. I 2–4, Pl. II 5, 7–9). На нем нередко образуются выросты, либо он расчленяется на 2 или более частей без деления самой клетки (Pl. I 2–4). Иногда от него отделяются один или несколько фрагментов (Pl. II 7–9). Все эти явления напоминают так называемый гемиксис (Diller 1936, Райков 1967, 1968). Частота гемиксиса значительно выше в амикронуклеарных клонах, чем в нормальных: только в четырех из 147 клонов с микронуклеусами ($БН_{48}$, $Б_3П_3$ и $ДС_{22}$) и только в 4% клеток макронуклеус был фрагментирован на две части, в то время как в 6 из 25 амикронуклеарных клонов ($БН_{19}$, $БН_{20}$, $Д_2$, $Д_3$, $ДС_{12}$, $ДС_{22}$) от 5 до 44% клеток имели подобные аномалии. Фрагментацию макронуклеуса в амикронуклеарных клонах наблюдал Wenzel (1955) у *Spathidium ascendens* и Schwartz (1957) при конъюгации амикронуклеарных *P. bursaria*, у которых в норме фрагментации макронуклеуса у экскоңъюгантов не происходит. Наряду с гемиксисом, в амикронуклеарных клонах *P. bursaria* гораздо чаще (7 случаев), чем в нормальных (1 случай) наблюдается аномальное деление особей, при котором макронуклеус не делится и отходит целиком в одну из дочерних особей (Pl. II 5). В результате, в клоне появляются полностью безъядерные клетки (Pl. II 6). Сходные аномалии макронуклеуса описаны для стареющих клонов *Paramecium multimicronucleatum* (Ganapathy and Rao 1959).

Для амикронуклеарных *P. bursaria* описано вторичное образование „микронуклеусов", способных к делению, из фрагментов макронуклеуса (Schwartz 1958). Сходное явление — возникновение „псевдомикронуклеусов" из фрагментов макронуклеуса после уничтожения микронуклеуса обнаружено также у *Stylonychia mytilus* (Ammermann 1965, 1970, 1971). Поскольку известно, что гемиксис характерен прежде всего для стареющих клонов, можно полагать, что его большая частота в амикронуклеарных клонах обусловлена их преждевременным старением.

В нашем материале в 7 клонах ($ОР_{25}$, $ПП_3$, $БН_{12}$, $БН_{22}$, $БН_{28}$, $БН_{39}$ и $Д_2$ — см. Табл. 2), считавшихся нами на основании просмотра препаратов амикронуклеарными, также наблюдалось появление структур, которые по своему местоположению в клетке, характеру окраски и морфологии неотличимы от

микронуклеуса (Pl. II 9, 10). Такие „микронуклеусы" всегда были вытянутыми в длину, „хвостатыми", похожими на микронуклеус в телофазе (Борхсениус et al. 1968). Такую форму принимает также микронуклеус в старых нормальных клонах *P. bursaria* (Pl. II 10) и в клонах, содержащихся при низкой температуре (Pl. II 12). Появление этих структур в амикронуклеарных клонах совпадает, как правило, с некоторым уменьшением размеров клеток и макронуклеуса (Рис. 2 A, B, C). Из числа 7 клонов, где они были обнаружены, 4 клона ($БН_{12}$,

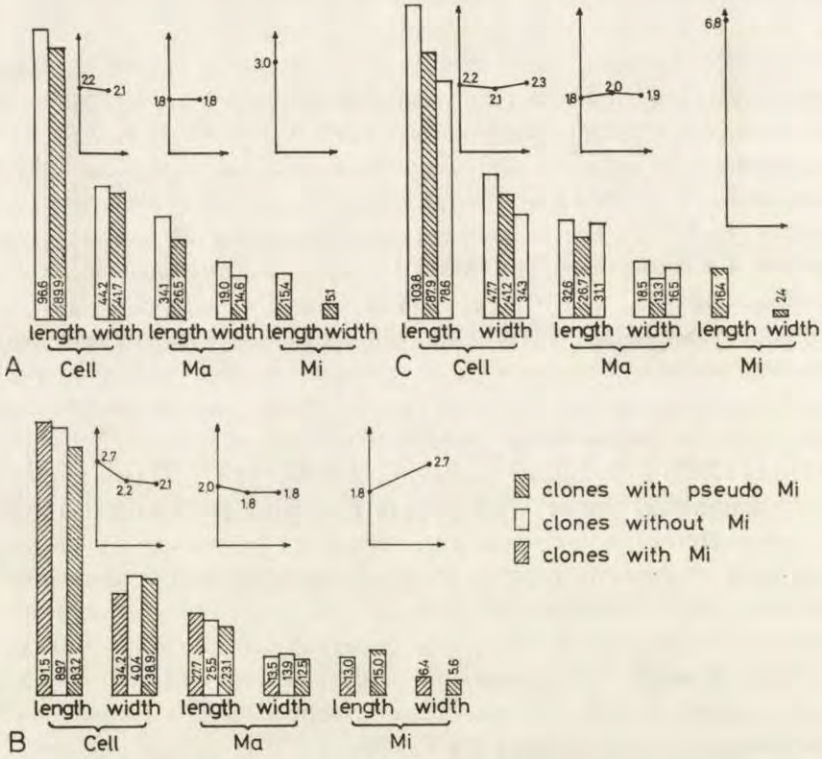

Рис. 2. Изменения биометрических показателей амикронуклеарных клонов в процессе приобретения ими и повторной утраты микронуклеусоподобных образований. А — Средние данные по 3 амикронуклеарным клонам в процессе появления в них микронуклеусоподобных образований ($БН_{12}$, $БН_{39}$, $Д_2$) на протяжении двух лет. В — Изменения в клоне $ОР_{25}$ в процессе утраты микронуклеуса и приобретения микронуклеусоподобных образований на протяжении 5 лет. С — Изменения в клоне $БН_{28}$ с появлением микронуклеусоподобных образований и их последующим исчезновением (на протяжении 3 лет). Построение гистограмм и графиков и обозначения — такие же, как на Рис. 1.

Fig. 2. Changes of biometrical indices of amicronucleate clones in the course of appearance and repeated loss of micronucleus-like structures. A — Average data of 3 amicronucleate clones in the course of appearing of micronucleus-like structures (BN_{12}, BN_{39}, D_2) during 2 years. B — Changes in the clone OR_{25} in the course of losing their micronucleus and appearing of micronucleus-like structures (during 5 years). C — Changes in the clone BN_{28} in the course of appearing of micronucleus-like structures and their following disappearance (during 3 years). Diagrams and designation are the same as in Fig. 1

$БН_{28}$, $БМ_{39}$ и $Д_2$) были без микронуклеусов с начала культивирования, а остальные утратили микронуклеус позже. Иногда наблюдается повторное исчезновение микронуклеусоподобных структур. В клоне $БН_{28}$ это сопровождалось продолжающимся уменьшением размеров тела инфузорий, но одновременно некоторым увеличением размеров их макронуклеусов (Рис. 2 С).

Поскольку контроль за состоянием клонов на разных этапах культивирования проводился только по тотальным препаратам, на которых представлена произвольная выборка особей, трудно сказать, за счет каких структур возникли эти микронуклеусоподобные образования. В принципе, можно допустить одну из трех возможностей: а) Сохранение в „амикронуклеарном" клоне отдельных микронуклеарных особей, которые в определенный момент могли размножиться. б) Утрату микронуклеуса в большинстве особей клона в результате их поражения бактериями и спонтанное освобождение микронуклеуса отдельных особей от паразитов. Такие освобожденные от бактерий микронуклеусы *P. bursaria* имеют, как правило, своеобразную вытянутую „кометовидную" форму. Фокин и Осипов (1975) отмечают, что при восстановлении микронуклеуса *P. caudatum* после заражения его смега-частицами он также имеет вытянутую „кометовидную" форму. в) Возникновение „псевдомикронуклеусов", способных к митозу и выполняющих в вегетативной клетке функции микронуклеуса из фрагментов макронуклеуса.

Физиологические особенности амикронуклеарных клонов

При многолетних наблюдениях над *P. bursaria* в условиях культивирования нами отмечалось снижение жизнеспособности и скорости размножения амикронуклеарных клонов. Прирост численности инфузорий в нескольких субклонах амикронуклеарных клонов, в сравнении с субклонами нормальных клонов, был определен путем подсчета численности потомства каждой из 25 особей данного клона в течение двух недель (Рис. 3). Для этого особи — родоначальницы субклонов были рассажены в отдельные микроаквариумы и получали ежедневную подкормку. Среда не менялась. На Рис. 3 А показан прирост численности инфузорий в среднем по 25 субклонам для двух нормальных клонов ($БС_{16}$ и $БС_{19}$), на Рис. 3 В и С — прирост численности в среднем по 25 субклонам для каждого из пяти амикронуклеарных клонов ($БН_{12}$, $БН_{19}$, $ОР_9$, $ОР_{25}$ и $БС_6$). Видно, что скорость размножения первых двух амикронуклеарных клонов (Рис. 3 В) была ниже, чем у нормальных клонов. В клонах $ОР_9$ и $БС_6$ размножение в субклонах было неустойчивым, в некоторых субклонах отсутствовало вовсе и в целом прекращалось к 7–10 дню. Наконец, в субклонах клона $ОР_{25}$ (Рис. 3 С) размножение практически отсутствовало.

Разница в ходе кривых на Рис. 3 В и 3 С объясняется, по-видимому, возрастом клонов. Клоны $БН_{12}$ и $БН_{19}$ моложе клонов $ОР_9$, $ОР_{25}$ и $БС_6$ на 4,5 года. Кроме того, нельзя не отметить тот факт, что утрата микронуклеуса в клонах

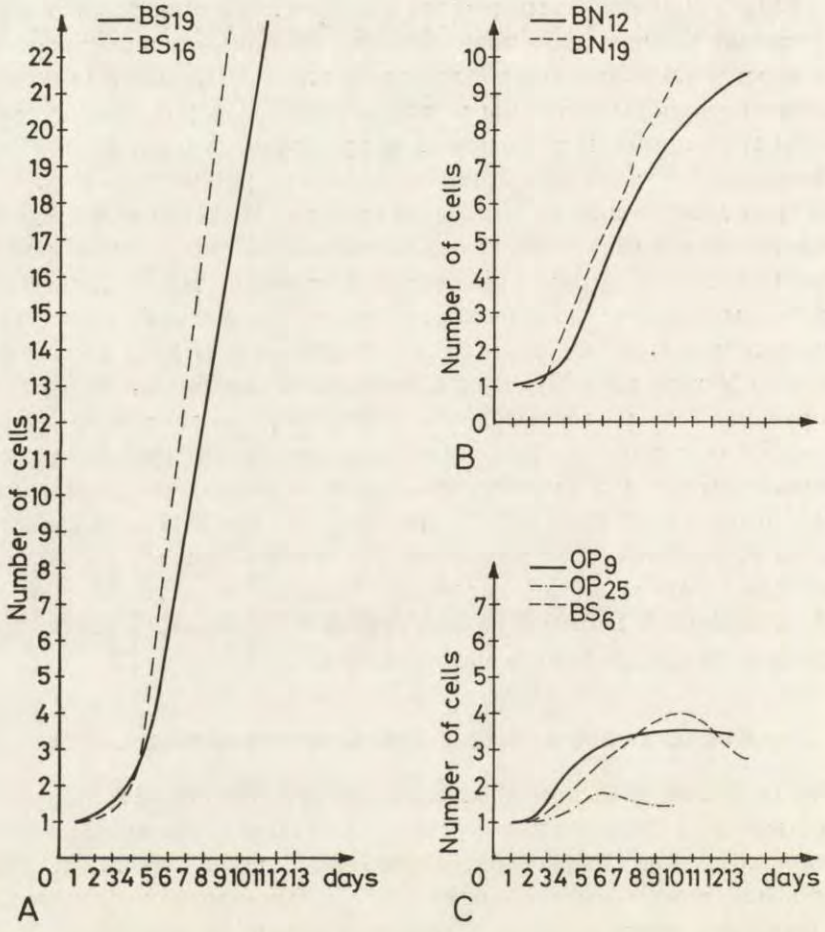

Рис. 3. Увеличение количества инфузорий некоторых нормальных и амикронуклеарных клонов *Paramecium bursaria* при субклонировании. Каждая точка на графике представляет собой среднее количество инфузорий в субклоне в данное время (среднее по 25 субклонам данного клона). А — нормальные клоны; В, С — амикронуклеарные клоны.

Fig. 3. The increasing amount of ciliates of some normal and amicronucleate clones of *Paramecium bursaria*. Each point represents the average amount of ciliates in a subclone in a given moment (average of 25 subclones of each clone). A — normal clones, B, C — amicronucleate clones

OP_9 и $БС_6$ произошла недавно и эти клоны могли быть плохо приспособлены к амикронуклеарному существованию. Последняя группа клонов (Рис. 3 С) еще жизнеспособна в массовых пробирочных культурах, но уже не поддается субклонированию (в микроаквариумах субклоны через некоторое время гибнут).

В последнее время появляется все больше данных о связи возраста клона и функционального состояния ядер. Frick (1967) показал, что эксперименталь-

ное удаление микронуклеуса в старых клонах *Stylonychia mytilus* приводит к гибели клеток через 2–3 деления вследствие нарушения стоматогенеза, тогда как инфузории из молодых клонов этого вида могут дать жизнеспособные клоны после уничтожения генеративного ядра (Ammermann 1965, 1970, 1971). Спонтанно возникшие амикронуклеарные клетки старых клонов *Tetrahymena pyriformis* нежизнеспособны (Weindurch and Doerder 1975), хотя как раз для этого вида существуют данные о многолетнем нормальном существовании амикронуклеарных клонов (Corliss 1953, Clark and Elliott 1956, McDonald 1958). Значение возраста клона для его жизнеспособности уже давно не вызывает сомнений (Jennings 1944 a, b, Sonneborn 1947, Wichterman 1953, Sonneborn and Dippel 1960, Nanney 1974, Sundararman and Cummings 1976). В последнее время появляются данные об изменении количества ДНК в ядрах инфузорий в зависимости от возраста клонов, замедлении репликации ДНК с возрастом, возникновении генетической несбалансированности в макронуклеусе при многократных делениях (Schwartz and Meister 1973, 1975, Smith-Sonneborn and Klass 1974). Для амикронуклеарного клона *Tetrahymena pyriformis* также приводятся данные о замедлении синтеза ДНК в макронуклеусе (Cameron et. al. 1965) по сравнению с нормальными клонами.

„Старые" амикронуклеарные клетки теряют жизнеспособность чаще всего из-за нарушений в ходе стоматогенеза при делении (Frick 1967), и гибель эксконъюгантов при конъюгации амикронуклеарных партнеров объясняется невозможностью восстановления ротового аппарата у эксконъюгантов (Diller 1962, Осипов и Тавровская 1969). Конъюгация старых микронуклеарных клонов также обычно летальна (Jennings 1944 b, Chen 1951). Вполне вероятно, что противоречивые данные о влиянии амикронуклеарности на морфологию, функциональное состояние и морфогенез инфузорий отчасти вызваны тем, что разные авторы имели дело с клонами, разными по возрасту и функциональному состоянию. Если роль генеративного ядра в обновлении макронуклеуса в ходе полового процесса несомненна, то активность его в вегетативный период цикла инфузорий долгое время подвергалась сомнению. Оно считалось более или менее инертным. Однако, в последние годы было доказано наличие синтеза ДНК в микронуклеусе (обзор Борхсениус, 1975). Литературные и собственные данные склоняют нас в сторону признания за микронуклеусом несомненного значения в жизнедеятельности вегетативных инфузорий, тем большего, чем больше возраст клона.

Амикронуклеарные клоны *P. bursaria* имеют нормальную реакцию спаривания. Нами были проведены опыты по скрещиванию клона OP_9, еще не утратившего тогда микронуклеус, с 25 амикронуклеарными клонами. Оказалось, что 11 из них были комплементарными по типу спаривания клону OP_9 и между ними происходила конъюгация. В процессе конъюгации или вскоре после нее погибли все конъюганты и эксконъюганты в 5 сочетаниях клонов. Из выживших эксконъюгантов в остальных 6 сочетаниях клонов было

получено 60 эксконъюгантных клонов, из которых 13 погибли в течение первых недель. Из оставшихся эксконъюгантных клонов большинство (30) оказались безмикронуклеусными, остальные имели микронуклеусы в виде „теней" или „телофазного" типа. Такие миронуклеусы имеют тенденцию исчезать в процессе культивирования.

SUMMARY

Some biometrical indices of 25 amicronucleate clones of *Paramecium bursaria* were compared with the same of 147 normal clones. All the clones were tested in various cultivating periods in the course of 2–9 years. It has been found, that changes of these indices in amicronucleate clones are the same as in the course of aging of normal clones. These changes are as follows: decreasing of the ciliate dimensions, rounding of the cell and macronucleus, and the hemixis type of fragmentation of the macronuclei. Aging of the amicronucleate clones is more strikingly expressed and appears earlier than in normal clones. The rate of asexual reproduction is lower in amicronucleate cells than in normal ones. Old amicronuclate clones are lacking of vitality under subcloning. The micronucleus play an active role in the vegetative period of *P. bursaria* life cycle, its role increases with the aging of clone. Amicronucleate *P. bursaria* have normal mating reactions.

ЛИТЕРАТУРА

Allen S. L. and Weremiuk S. L. 1971: Defective micronuclei and genomic exclusion in selected C+ subclones of *Tetrahymena*. J. Protozool., 18, 3, 509–515.

Ammermann D. 1965: Cytologische und genetische Untersuchungen an dem Ciliaten *Stylonychia mytilus* Ehrenberg. Arch. Protistenk., 108, 109–152.

Ammermann D. 1970: The micronucleus of the ciliate *Stylonychia mytilus*, its nucleic acid synthesis and its function. Exp. Cell. Res., 61, 1, 6–12.

Ammermann D. 1971: Morphology and development of the macronuclei of the ciliates *Stylonychia mytilus* and *Euplotes aediculatus*. Chromosoma, 33, 209–221.

Beers C. D. 1946: *Tillina magna*: micronucleus number, encystment and vitality in diverse clones; capabilities of amicronucleate races. Biol. Bull., 9, 3, 256–271.

Борхсениус О. Н. 1975: К вопросу об активности генеративного ядра инфузорий. Вестн. ЛГУ, 21 Биол., вып. 4), 7–16.

Борхсениус О. Н., Скобло И. И. и Осипов Д. В. 1968: Полиморфизм микронуклеусов *Paramecium caudatum* Ehrb. I. Морфологический тип интерфазных ядер. Цитология, 18, 2, 227–235.

Борхсениус О. Н. и Осипов Д. В. 1971 a. Полиморфизм микронуклеусов *Paramecium caudatum*, III. Цитофлуорометрическое исследование количества ДНК. Цитология, 13, 8, 1041–1044.

Борхсениус О. Н. и Осипов Д. В. 1971 b: Полиморфизм микронуклеусов *Paramecium caudatum*. IV. Сравнительный анализ ядерной реорганизации в процессе деления созревания у четырех морфологических типов. Цитология, 13, 10, 1289–1298.

Cameron I. L., Padilla G. M. and Wysinger B. B. 1965: Regulation of the DNA — synthetic period within the cell cycle of *Tetrahymena pyriformis*.

Progress in Protozoology, Abstr. Second Int. Conf. Protozool. London 1965, Excerpta Med. Int. Congr., ser. No. 91, 236–237.
Chen T. T. 1940 a: Polyploidy in *Paramecium bursaria*. Proceed. Nat. Acad. Sci. USA, 26, 4, 239–240.
Chen T. T. 1940 b: Polyploidy and its origin in *Paramecium*. J. Hered., 31, 4, 175–176.
Chen T. T. 1940 c: A further study on polyploidy in *Paramecium*. J. Hered., 31, 249–250.
Chen T. T. 1951: Conjugation in *Paramecium bursaria*. IV. Nuclear behavior in conjugation between old and young clones. J. Morph., 88, 293–360.
Clark G. and Elliot A. M. 1956: Nuclear behavior in haploid clones of *Tetrahymena pyriformis*. J. Protozool., 3, Suppl., 3.
Corliss J. O. 1953: Comparative studies on holotrichous ciliates in the *Colpidium — Glaucoma — Leucophrys — Tetrahymena* group. Parasitology, 43, 1–2, 49–87.
Diller W. F. 1936: Nuclear reorganization prozess in *Paramecium aurelia*, with discription of an autogamy and "hemixis". J. Morph., 59, 1, 11–67.
Diller W. F. 1940: Nuclear variation in *Paramecium caudatum*. J. Morph., 66, 605–633.
Diller W. F. 1959: Possible origin of hypoploidy in *Paramecium trichium*. J. Protozool., 6 (Suppl.), 19.
Diller W. F. 1962: The relationship of the micronucleus to stomatogenesis in some ciliates. J. Protozool., 9, Suppl., 15–16.
Elliott A. M. and Hayes R. E. 1953. Mating types in *Tetrahymena*. Biol. Bull., 105, 269–284.
Фокин С. И. и Осипов Д. В. 1975: Влияние УФ микрооблучения на ядерный аппарат и цитоплазму инфузорий *Paramecium caudatum*. Цитология, 17, 9, 1073–1080.
Frick D. 1967: Organellbildung und Kernverhältnisse bei *Stylonychia mytilus*. Biol. Zbl., 86, 5, 629–655.
Ganapathy P. N. and Narasimha Rao M. N. 1959: Macronuclear reorganisation in *Paramecium multimicronucleatum* (Powers and Mitchell). J. Zool. Soc., India, 1959, 11, 1, 26–29.
Голикова М. Н. 1972: Полиморфизм микронуклеуса *Paramecium bursaria* Focke. Цитология, 14, 5, 637–645.
Голикова М. Н. 1974: Цитофотометрическое исследование количества ДНК в микронуклеусе *Paramecium bursaria*. Acta Protozool., 13, 8, 109–120.
Янковский А. В. 1972: Ядерный аппарат *Ciliophora*. Сообщение I. Полиплоидия микронуклеуса у инфузорий как видовой признак. Генетика, 8, 4, 78–84.
Jennings H. S. 1944 a: *Paramecium bursaria*: life history. I. Immaturity, maturity and age. Biol. Bull., 86, 131–145.
Jennings H. S. 1944 b: *Paramecium bursaria*: life history. II. Age and death of clones in relation to the results of conjugation. J. Exp. Zool., 96, 27–52.
Katashima R. 1973 a: Nuclear transfer and haploidy resulted from mating of normals with amicronucleates in *Euplotes patella*, syngen 1. J. Sci. Hirosh. Univ., B, 1, 24, 2, 51–83.
Katashima R. 1973 b: Behavior of haploid and minute micronuclei at cell division and conjugation in *Euplotes patella*, syngen 1. J. Sci. Hirosh. Univ., B, 1, 24, 2, 83–109.
Kimball R. F. 1941. Double animals and amicronucleate animals in *Euplotes patella* with particular reference to their conjugation. J. Exp. Zool., 86, 1–33.
King R. and Beams H. 1937: The effect of ultracentrifuging on *Paramecium*, with special reference to recovery and macronuclear reorganization. J. Morph. 61, 27–49.
Lewin K. R. 1910: Nuclear relations of *Paramecium caudatum* during the asexual period. Camb. Phil. Soc. Proc., 16, 39–41.
Mc Donald B. B. 1958: Quantitative aspects of deoxyribose nucleic acid (DNA) metabolism in an amicronucleate strain of *Tetrahymena*. Biol. Bull., 114, 71–94.
Miyake A. 1956: Artificially induced micronuclear variation in *Paramecium caudatum*. J. Inst. Polyt. Osaka Univ., D, Biol., 7, 147–161.
Nanney D. L. 1974: Aging and long-term temporal regulation in ciliated protozoa. A critical rewiew. Mechanisms of Aging and Development, 3, 81–105.

Ovchinnikova L. P. 1970: Variability of DNA content in micronuclei of *Paramecium bursaria*. Acta Protozool., 7, 211–220.
Осипов Д. В. и Борхсениус О. Н. 1973: Особенности полиплоидии микронуклеусов у инфузорий. Цитология, 15, 3, 243–253.
Осипов Д. В. и Ивахнюк И. С. 1972: Омега-частицы – симбиотические бактерии микронуклеуса инфузории *Paramecium caudatum* клона MI—48. Цитология, 14, 11, 1414–1418.
Осипов Д. В. и Тавровская М. В. 1969: Микронуклеус и морфогенез кортекса у *Paramecium caudatum*. В кн. Успехи протозоол., Ленинград, Наука, 103–104.
Райков И. Б. 1967: Кариология простейших. Л. Наука.
Raikov I. 1968: The macronucleus of ciliates. In: Research in Protozool., Pergamon Press., New York, 3, 1.
Reynolds M. E. 1932: Regeneration in an amicronucleate infusorian. J. Exp. Zool., 62, 327–361.
Rosenbaum N., Erwin J., Beach D. and Holz G. L., Jr. 1966: The induction of phospholipid requirement and morphological abnormalities in *Tetrahymena pyriformis* by growth at supraoptimal temperatures. J. Protozool., 13, 4, 535–546.
Smith-Sonneborn J. and Klass M. 1974: Changes in the DNA synthesis pattern of *Paramecium* with increased clonal age and interfission time. J. Cell Biol. 61, 3, 591–598.
Sundararman V. and Cummings D. J. 1976: Morphological changes in aging cell lines of *Paramecium aurelia*. I. Alterations in cytoplasm. Mechanisms of Aging and Development, 5, 139–154.
Schwartz V. 1934: Versuche über Regeneration und Kerndimorphismus der Ciliaten. Nachr. Acad. Wiss. Göttingen, M.-ph., 6, 1, 143–155.
Schwartz V. 1935: Versuche über Regeneration und Kerndimorphismus bei *Stentor coeruleus* Ehrbg. Arch. Protistenk., 85, 100–139.
Schwartz V. 1939: Konjugation micronukleusloser Paramecien. Naturwiss., 27, 724.
Schwartz V. 1947: Über die Physiologie des Kerndimorphismus bei *Paramecium bursaria*. Z. Naturforsch., 2b, 369–381.
Schwartz V. 1957: Über den Formwechsel achromatischer Substanz in der Teilung des Makronucleus von *Paramecium bursaria*. Biol. Zbl., 76, 1, 1–23.
Schwartz V. 1958: Chromosomen im Makronucleus von *Paramecium bursaria*. Biol. Zbl., 77, 3, 347–364.
Schwartz V. und Meister H. 1973: Eine Altersveränderung des Makronucleus von *Paramecium*. Z. Naturforsch., 28c, 3/4, 232.
Schwartz V. und Meister H. 1975: Einige quantative Daten zum Problem des Alterns bei *Paramecium*. Arch. Protistenk., 117, 85–109.
Sonneborn T. M. 1947: Recent advances in the genetics of *Paramecium* and *Euplotes*. Adv. Genet., 1, 263–358.
Sonneborn T. M. and Dippel R. V. 1960: Cellular changes with age in *Paramecium*. Am. Inst. Biol. Sci. Symp., 6, 285.
Tartar V. and Chen T. T. 1941: Mating reactions of enucleate fragments in *Paramecium bursaria*. Biol. Bull., 80, 1, 130–138.
Taylor C. V. and Farber W. P. 1924: Fatal effects of the removal of the micronucleus in *Euplotes*. Univ. Calif. Publ. Zool., 26, 131–144.
Weindurch R. H. and Doerder F. P. 1975: Age — dependent micronuclear deterioration in *Tetrahymena pyriformis*, syngen 1. Mechanisms of Ageing and Development, 4 (3–4), 263–279.
Wells C. 1961: Evidence for micronuclear function during vegetative growth and reproduction of the ciliate *Tetrahymena pyriformis*. J. Protozool., 8, 284–290.
Wenzel F. 1955: Über eine Artentstehung innerhalb der Gattung *Spathidium* (*Holotricha, Ciliata*). Arch. Protistenk., 100, 4, 515–540.
Wichterman R. 1946: Further evidence of polyploidy in the conjugation of green and colorless *Paramecium bursaria*. Biol. Bull., 91, 234.
Wichterman R. 1953: The Biology of *Paramecium*.
Wichterman R. 1954: The common occurrence of micronuclear variation during binary fission in an unusual race of *Paramecium caudatum*. J. Protozool., 1, 54–59.

Wichterman R. 1960: Production of viable races of *Paramecium caudatum* after micronuclear elimination with X-rays. Biol. Bull., 119, 3, 348.
Woodruff L. L. 1931: Micronuclear variation in *Paramecium bursaria*. Q. J. Micros. Sci., 74, 573–545.

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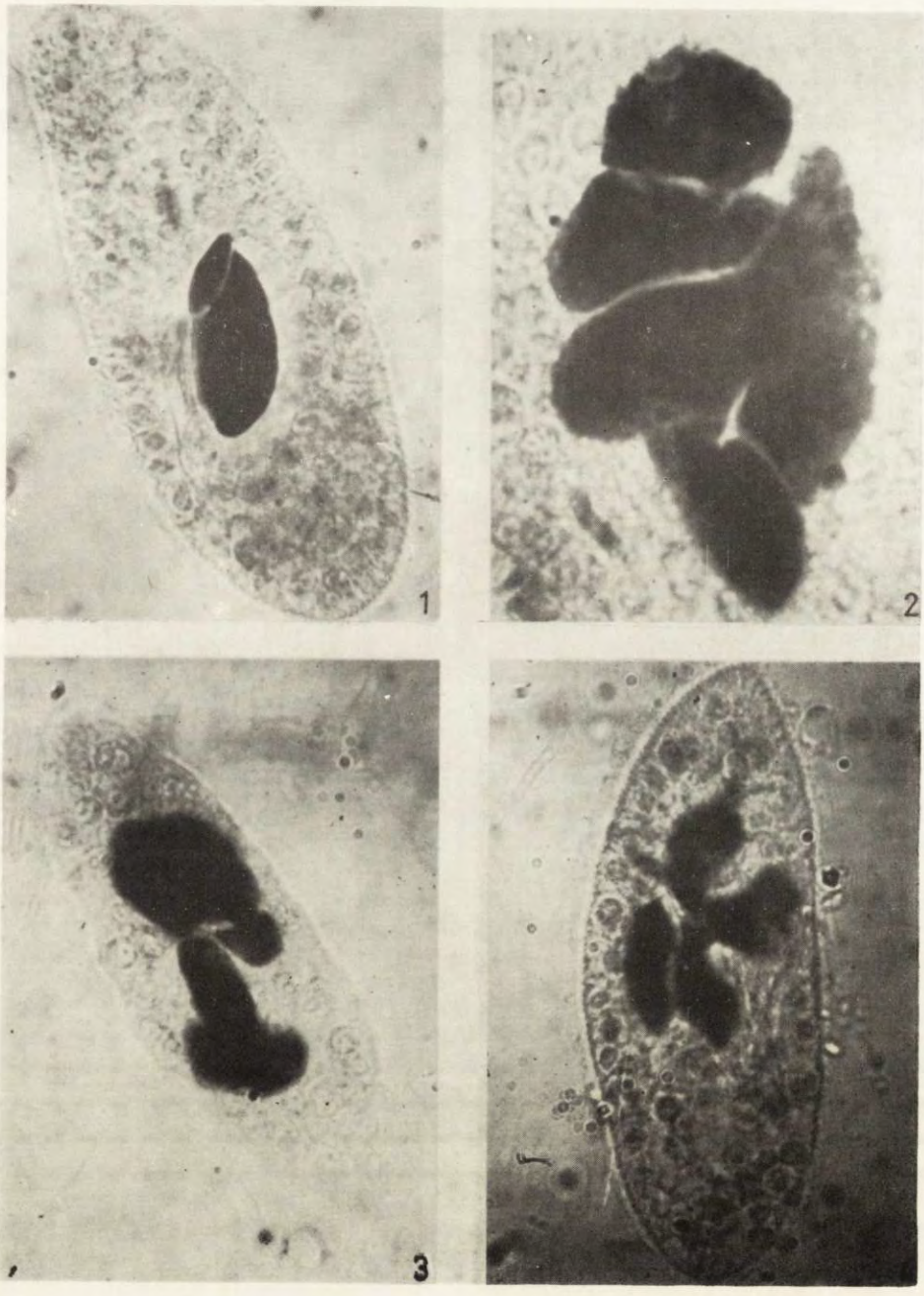
ПОДПИСИ К ТАБЛИЦАМ I–II

1–4: Изменение морфологии макронуклеуса в амикронуклеарных клонах *Paramecium bursaria*.
1 — нормальная особь; 2, 3 и 4 — фрагментация макронуклеуса. Увеличение: 1, 3, 4 — 500×, 2 — 1800×.
5–12: Морфология ядерного аппарата в амикронуклеарных, „старых" и „холодных" клонах *Paramecium bursaria*.
5 — деление амикронуклеарной инфузории без деления ее макронуклеуса; 6 — безъядерная особь; 7, 8 — отделение мелких фрагментов от макронуклеуса; 9 — фрагменты и микронуклеусоподобное образование около макронуклеуса; 10 — микронуклеус „старой" инфузории; 11 — микронуклеусоподобное образование; 12 — микронуклеус инфузорий, содержащихся при низкой температуре.
Увеличение: 5, 6 — 500×; 7–12 — 1800 ×.

EXPLANATION OF PLATES I–II

1–4: Change of the shape of macronucleus in amicronucleate clones of *Paramecium bursaria*. 1 — Normal cell, 2, 3, 4 — fragmentation of macronucleus. Magnification: 1, 3, 4 — 500 ×, 2 — 1800 ×

5–12: Morphology of the nuclear apparatus in amicronucleate, "old" and "cold" clones of *Paramecium bursaria*. 5 — division of an amicronucleate ciliate without division of its macronucleus, 6 — cell without nucleus, 7, 8 — separation of small fragments of macronucleus, 9 — fragments and micronucleus-like structure near macronucleus, 10 — micronucleus of an "old" ciliate, 11 — micronucleus-like structure, 12 — the micronucleus of ciliates living in low temperature. Magnification: 5,6 — 500 ×, 7–12 — 1800 ×.



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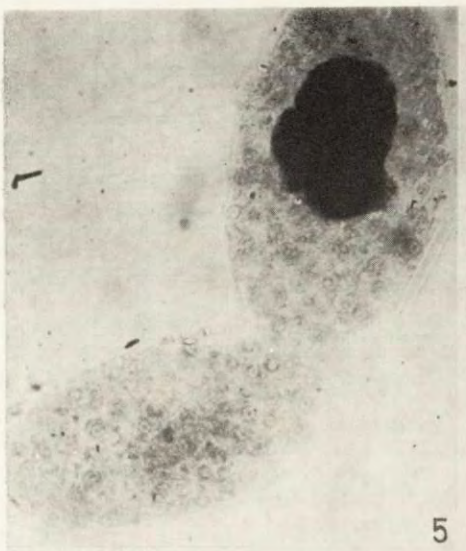

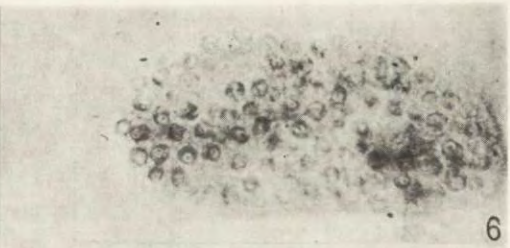

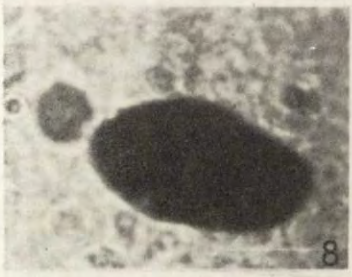

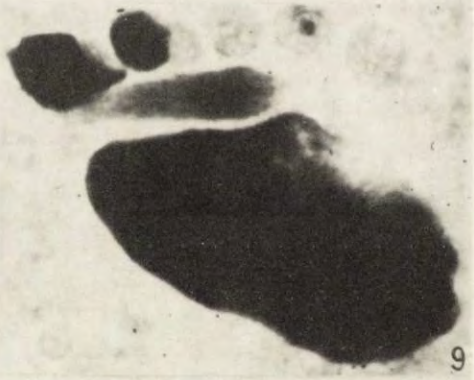

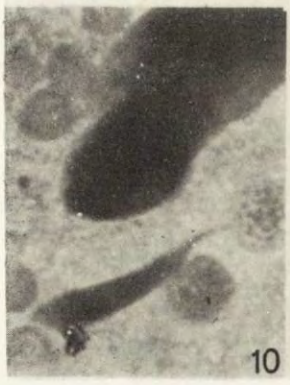

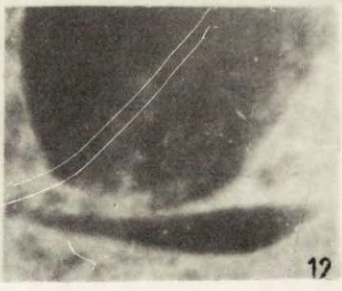

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The Nature of the Iodinophilous Vacuole in *Myxosporidia*

Synopsis. Different methods of iodinophilous vacuole detection are considered. It is shown that phase-constrast microscopy does not resolve the vacuole until some time after glycerine-gelatine preparations have been made. The iodinophilous vacuole was observed in the spores of *Myxobolus mülleri* under an electron microscope. Spores of *Myxobolus mülleri, M. bramae, M. dispar, M. albovi, M. exiguus, M. nemeczeki, Thelohanellus oculi-leucisci, Henneguya psorospermica, Myxosoma anurus, Myxidium rhodei, Kudoa* sp. were stained with Lugol's iodine and treated with the help of PAS and Shabadash's (1949) techniques. The results of these experiments are discussed. The polysaccharide dynamics in the spore during its formation is described. When the spore is outside the host, the vacuole content is gradually utilized, the expenditure rate being dependent on the ambient temperature. The iodinophilous, vacuole is regarded as a depot of reserve nutrients (of glycogen type). The opinion is shared that the iodinophilous vacuole is a genuine morphological formation in the spores of *Myxobolidae.*

Over a hundred years ago Müller (1841 a, b, c) and later Bütschli (1881) discovered that the sporoplasm of multicellular spores contained a vacuole which they initially took for a nucleus. The vacuole that stained easily with iodine was called iodinophilous (Thélohan 1889). It was further discovered that the vacuole was filled with some polysaccharide (Kudo 1918, 1921), most probably, glycogen. This discovery was corroborated by many investigators who chiefly used two methods of vacuole identification — carmine staining according to Best (Petruschevsky 1932, Bond 1949, Walliker 1968, Galinsky and Meglitsch 1970) and different modifications of the Bauer-Felgen, or PAS reaction (Bond 1937, 1940, Walliker 1968, Galinsky and Meglitsch 1970). Many authors point out, however, that the spore's

shell displays low permeability to the chemicals applied, which necessitates a non-standard treatment and, thus, makes the histochemical reaction less reliable.

The iodinophilous vacuole is usually easily detectible and, therefore, most of the workers studying *Myxosporidia* used its presence or absence as a taxonomic trait in discriminating families and, sometimes, even taxa of a higher order (Thélohan 1892, Gurley 1893, Labbe 1899, Doflein 1899, Auerbach 1910, Poche 1913, Davis 1917, Kudo 1919, 1933 Tripathi 1948, Meglitsch 1960, Schulman 1959, 1966). Recently, some of the authors have developed a basically different approach — they completely reject the importance of the iodinophilous vacuole as a taxonomic character (Akhmerov 1960, Walliker 1966, 1968, Lom 1969 a, b). So, it is necessary to study the iodinophilous vacuole in greater detail. Particularly important is the study of methods of the vacuole identification as well as of its changes in the course of formation of the spores and their existence outside the host. These problems make the subject of the present paper.

Material and Methods

The following myxosporidian species were used as material for our investigations: *Kudoa* sp.[1] (order *Multivalvulea*, family *Tetracapsulidae*); *Myxidium rhodei*[2] (order *Bivalvulea*, suborder *Bipolaria*, family *Myxidiidae*); *Myxosoma anurus*[3] (order *Bivalvulea*, suborder *Platysporea*, family *Myxosomatida*); *Henneguya psorospermica*[3], *Thelohanellus oculi-leucisci*[4], *Myxobolus bramae*[5], *M. dispar*[4], *M. albovi*[2], *M. exiguus*[5], *M. nemeczeki*[4], *M. mülleri*[4,5] (order *Bivalvulea*, suborder *Platysporea*, family *Myxobolidae*). Living spores were examined with the help of a phase-contrast device. Glycerine-gelatine was used for making preparations from fresh material. Carbolic acid was sometimes added for preservation purposes.

Spores of all the above species were stained with Lugol's iodine. The iodinophilous vacuole was discovered in the *Myxobolidae* family alone. The fullest staining of the above spore sample could be achieved within 10 to 15 min. This should be taken into account in quantitative studies. The entire material (usually cysts) was treated histochemically. Fixation took place immediately upon dissection of the fish. Bouin's fixative on neutral formaline Carnoy's fixative, a mixture of nine parts of absolute alcohol with one part of formaline, as well as Shabadash's fixative (1949) were used for the purpose. Upon fixation, the material was embedded in paraffin either according to a standard technique or with the help of methyl-

[1] From *Micromesistius poutassou* (Risso)
[2] From *Rutilus rutilus* (Linne)
[3] From *Esox lucius* (Linne)
[4] From *Leuciscus idus* (Linne)
[5] From *Abramis bramae* (Linne)

benzoate and benzene treatment. Control was effected by applying saliva to deparaffined sections at 37°C during 30 to 45 min. Then the PAS staining (Pearse 1960) and the treatment as introduced by Shabadash (1949) were used. Both methods proved to be satisfactory and yielded identical results.

For electron microscopic investigations spores were fixed according to Caulfield with subsequent embedding in araldite. Ultrathin sections were studied with the YEM-5 G electron microscope.

For studying the changes of the iodinophilous vacuole in the spores outside the host, a spore water suspension was made from cysts sampled on gills. Normally, one cyst was sufficient for preparation of the suspension. When cysts were too small, which was rather an exception, several of them were used after being identified as belonging to the same species and the same age stage. Gill lamellae and other foreign bodies were carefully removed. The spores were stored at a temperature of −5°C, +3°C and +18°C. In some instances, the spore suspension was divided into two portions placed under different temperature conditions either from the very beginning or some time after the start of the experiment. The spores were stained by Lugol's iodine.

Results and Discussion

Phase contrast is now widely used, in the USSR in particular, for studying myxosporidian spores (Donec and Schulman 1973). Their identification and description, however, are seldom based on living material. The routine procedure makes use of glycerine-gelatine preparations which are studied microscopically some time after they have been made. In this case the iodinophilous vacuole looks like a light-coloured homogeneous spot.

Our investigations have shown that living spores of *M. mülleri* almost never display an iodinophilous vacuole when subject to phase contrast, though staining with Lugol's iodine can make this vacuole quite distinct. The glycerine-gelatine preparations made from the same material exhibit, if not very clearly, a vacuole in many spores after 10 do 15 days. Still later, after 2 to 4 months, almost each of the spores on the slides has a perfectly visible iodinophilous vacuole.

So, the phase-contrast device does not allow to detect the iodinophilous vacuole until some time after the preparation was made. But in the spores of *Henneguya psorospermica* the vacuole is often hardly visible even after a long storage in glycerine-gelatine.

Preparations stored in glycerine-gelatine proper start displaying the vacuole much later than those, which are additionally treated with carbolic acid. The spores of *Myxobolus* acted upon with 1 N carbolic acid prior to being placed in glycerine-gelatine, will display a distinct iodinophilous vacuole no later than in some 10 to 15 day, no matter

whether examined with the help of the phase-contrast device or without it.

The effect of the iodinophilous vacuole staining with Lugol's iodine disappears under an acid influence. The staining may reappear, if much less intensively, on the spores' exposure to repeated treatment with Lugol's solution.

All the above allows to suggest that addition of acids, the carbolic acid included, to the glycerine-gelatine preparation results in accelerating polysaccharide hydrolysis in the iodinophilous vacuole ("washing-out"), which makes it visible under the phase-contrast device or, sometimes, without it — under an ordinary microscope. Fermentative hydrolysis of glycogen is also possible, since glycerine-gelatine preparations usually do not involve any special fixation of spores (Shabadash 1949).

One could expect that the fairly large iodinophilous vacuole shall be easily seen with the electron microscope, the more so that most of the ultrastructural studies dealt with specimens of the vacuole-possessing species (Kheisin et al. 1961, Lom et Puytorac 1965). It was not so, until Schubert (1968) described an iodinophilous vacuole discovered with the electron microscope in the spores of *Henneguya pinae*. According to him, the vacuole is enveloped with a layer of densely packed granules. The iodinophilous vacuole was also scoped by Uspenskaya (1971) in her electron microscope investigations of the spores of *Myxobolus disparoides*.

We have succeeded in observing the iodinophilous vacuole in the spores of *M. mülleri*. Occupying the middle and upper part of the sporoplasm it is filled with granulated material and can be well seen in both longitudinal and oblique sections through the spore (Pl. I 1,2). The vacuole localization as observed with the electron microscope is in good agreement with the results of light microscopy.

Our histochemical reactions have shown that the spores of the myxosporidian species under study — the *Myxobolidae* family — contain polysaccharides of at least two kinds: (1) those which are chiefly confirmed to the sporoplasm can be easily dissolved by saliva, and, thus, are close to glycogene, and (2) those which are concentrated as small granules near the polar capsules, which do not disappear when acted upon with saliva and, thus, are different from glycogen. The fact that pericapsular granules are located outside the sporoplasm, the only cell in the multicellular spores which functions normally, is evidence of their being insignificant in the organism's metabolism. Judging by their location, those granules are the remains of polysaccharide inclusions into the cytoplasm of a capsulogenic cell.

When staining the spores of *Myxobolus pfeiferi* with Lugol's iodine

known to reveal the iodinophilous vacuole alone, Keysselitz (1908) observed a small stained area of irregular shape between the polar capsules. We have recorded a similar formation in the spores of *M. ellipsoides* sampled from *Tinca tinca*. This area may be related with pericapsular granules, though they can usually be found closer to distal and central parts of the polar capsules, while the area revealed by Lugol's iodine is situated nearer the anterior portion of the spore.

The presence of pericapsular granules is a regular feature of the spores in the species of the *Myxobolidae* family under study. They also show very little variation in their distribution. So, in our discussion of the polysaccharides of the sporoplasm we shall make no mention of those granules unless it is necessary to emphasize especially their absence.

The techniques used in glycogen identification being of an adequate specificity and yielding similar results, one can safely suggest that the sporoplasm contains glycogen or some other glycogenous polysaccharide.

Polysaccharides were observed in the spores of not only the iodinophilous-vacuole-possessing forms (the *Myxobolidae* family), but also in the organisms lacking that organelle (*Kudoa, Myxidium, Myxosoma*). In *Kudoa* sp., small polysaccharide pellets are confined to the upper part of the sporoplasm and around the polar capsules (Fig. 1 A). *Myxidium rhodei* which belongs to a more primitive suborder of the order *Bivalvulea-Bipolaria* also displays an intensive polysaccharide reaction (Fig. 1 B). The polysaccharides in the sporoplasm of *Myxosoma anurus* have the form of small grains (Fig. 1 C).

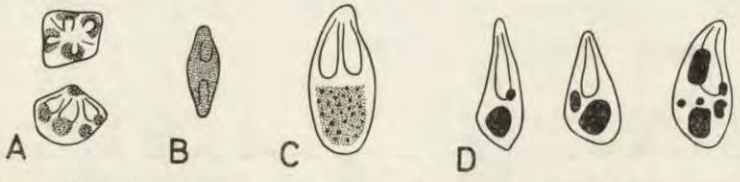

Fig. 1. Polysaccharides in spores (PAS): A — *Kudoa* sp., B — *Myxidium rhodei*, C — *Myxosoma anurus*, D — *Thelohanellus oculi-leucisci*

Of particular interest is the *Myxobolidae* family, for which the presence of the iodinophilous vacuole is the most characteristic trait. In sporoplasms of the spores of *Henneguya psorospermica*, the polysaccharides are either shaped into fairly large granules or spread diffusely (Fig. 3 A). In some instances a distinct, brightly stained vacuole can be seen. The spores of *Thelohanellus oculi-leucisci* usually display a vacuole, though there are cases when the sporoplasm would contain several large pellets of polysaccharides (Fig. 1 D).

There is an evident similarity in the polysaccharides distribution in the spores of the six representatives of the genus *Myxobolus* that have been investigated. The spores of *M. müller*i which were subjected to a more detailed study displayed polysaccharide staining of the following types:

(1) Polysaccharides are confined to the iodinophilous vacuole alone. No pericapsular granules are observed (Fig. 2 A).

(2) Polysaccharides appear as a big uniformly stained iodinophilous vacuole (Fig. 2 B).

(3) Polysaccharides are distributed similarly to 2, but the vacuole displays granulation (Fig. 2 C).

(4) Polysaccharides are concentrated in the iodinophilous vacuole, but some stained inclusions can be also frequently seen along the periphery of the sporoplasm (Fig. 2 D).

(5) Polysaccharides occur not only in the iodinophilous vacuole but also around the basal part of polar capsules (Fig. 2 E).

(6) Polysaccharides are distributed fairly uniformly over the whole sporoplasm, but the iodinophilous vacuole stains brighter (Fig. 2 F).

(7) Polysaccharides occur in pellets. The iodinophilous vacuole is lacking (Fig. 2 G).

(8) Polysaccharides are diffused to form a background for small granules. No trace of the iodinophilous vacuole (Fig. 2 H).

(9) Polysaccharides show a uniform distribution throughout the sporoplasm. The iodinophilous vacuole is lacking (Fig. 2 I).

The spores of one of the cysts in *M. müller*i displayed an iodinophilous vacuole where lighter spots could be seen against a brightly coloured background. In other cases, the vacuole was paler as compared with the

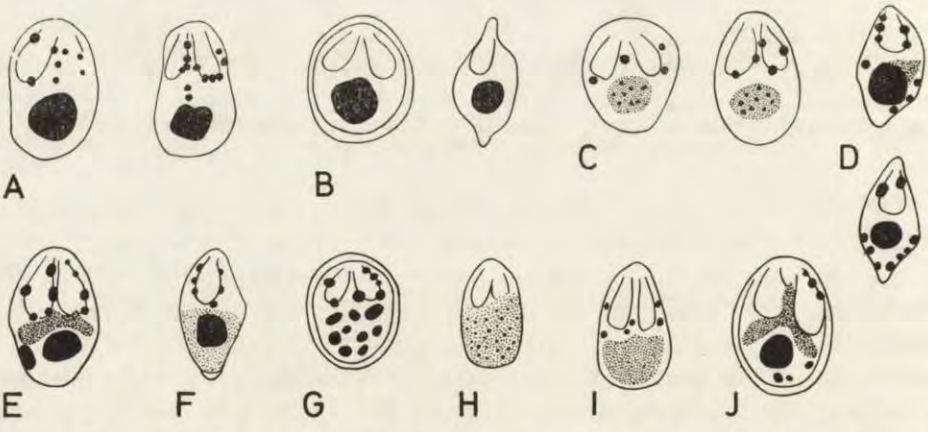

Fig. 2. Polysaccharides in *Myxobolus müller*i spores (PAS) (explanations in text)

sporoplasm. A similar picture can be observed in the spores of *Henneguya psorospermica.*

The proportion of spores with different content and distribution of polysaccharides varies considerably from cyst to cyst. In the adult cyst, the bulk of spores (80 to 90 per cent) would have a well stained iodinophilous vacuole. The rest of the spores chiefly has polysaccharides in pellet form.

The polysaccharide distribution in the spores of other species of the genus *Myxobolus* under study has very much in common with that in *M. müller i.* In *M. dispar,* polysaccharides are concentrated around the basal parts of polar capsules in the shape of large elongated inclusions which envelope their bases and sometimes enter the intercapsular space. *M. nemeczeki* is distinguished by a large number of spores with a medium-size granulation and a smaller number — with a distinct iodinophilous vacuole. Notice that *M. nemeczeki* is one of those species of the genus *Myxobolus* which have a very small and poorly discernible vacuole. This accounted for its being at one time attributed to the genus *Myxosoma* (*M. lobatum* Nemeczek, 1911).

It would be of interest to compare different methods of iodinophilous vacuole detection as applied to the spores of one and the same cyst of *Myxobolus müller i.* In living spores the phase-contrast device revealed no iodinophilous vacuoles. Meanwhile, Lugol's iodine and the PAS technique exposed them in 54.5 and 85.2 per cent of the spores respectively. And finally, glycerine-gelatine preparations examined with a phase-contrast microscope after a six months' preservation displayed the iodinophilous vacuole in 92 per cent of the spores.

It is likely that Lugol's iodine stains only vacuoles which are filled with polysaccharides, while the PAS reaction exposes polysaccharides both in the vacuole and in the cytoplasm of the sporoplasm. In the latter case, large closely spaced pellets of polysaccharides might be taken for the iodinophilous vacuole. One cannot rule out the possibility that Lugol's iodine and the PAS reaction expose polysaccharides of slightly different nature. As to the phase-contrast microscopy, it probably allows to reveal iodinophilous vacuoles devoid of their usual content.

Difficulties which arise in studying the iodinophilous vacuole may be due to complex changes it undergoes during the spore's lifetime. The state and quantity of polysaccharides in the spore are undoubtedly interrelated with the degree of its maturity. As far back as 1908, K e y s s e l i t z indicated that the iodinophilous vacuole did not appear until the spore reached a certain stage of development, B o n d (1940) mentions casually that larger glycogen pellets are to be found in more mature spores.

We have also made an attempt at establishing connection between the quantity and state of polysaccharides and the spore maturity. This task is facilitated by variations in the spores' position — mature myxosporidian spores usually take the central part of the cyst, while the younger ones, not unlike pansporoblasts, are confirmed to the periphery.

The number of polysaccharide inclusions in the sporoblasts increases with time. As the spore grows more mature the polysaccharides form ever larger pellets in it and gradually concentrate in the iodinophilous vacuole. This is very well exemplified by *Henneguya psorospermica* (Fig. 3 A), *Myxobolus mülleri* (Fig. 3 B), *Thelohanellus oculi-leucisci* (Fig. 3 C). Immature spores of *Kudoa* sp. which occur in sporoblasts, possess hardly any polysaccharide inclusions, and in case they do, these inclusions are far less numerous than in mature spores.

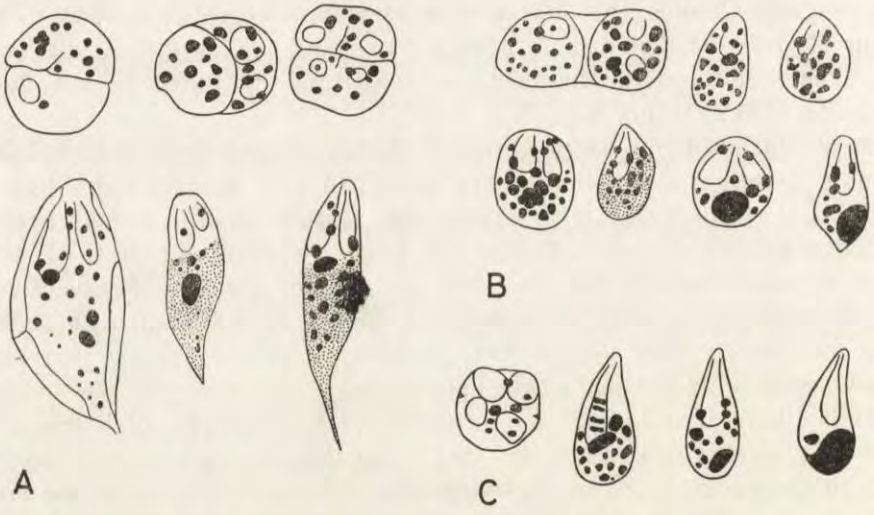

Fig. 3. Polysaccharides in developing spores (PAS); A — *Henneguya psorospermica*, B — *Myxobolus mülleri*, C — *Thelohanellus oculi-leucisci*

In discussions of the effect of Lugol's iodine on myxosporidian spores, it was a common practice to note just the presence or absence of the iodinophilous vacuole. Galinsky and Meglitsch (1970), however, paid attention to variations in the staining of different spores of *Henneguya exilis*. They experimented with spores preserved in a chilled or frozen state, or at room temperature. Their observations showed that with the lapse of time Lugol's iodine would stain fewer distinct iodinophilous vacuoles, while more spores would expose a uniformly stained sporoplasm.

By the degree of staining with Lugol's iodine, fresh spores can be

subdivided into three groups: (1) spores possessing an iodinophilous vacuole which stains well; (2) spores, in which the iodinophilous vacuole does not stain, but the whole sporoplasm stains diffusely; (3) spores which do not stain at all.

Examination of preparations shows that eventually the number of spores with a distinct vacuole is reduced and the spores with diffusely stained sporoplasm grow more numerous. The PAS reaction reveals similar differences in staining of fresh spores and those which have been kept for some time outside the host. Besides, there is a gradual increase in the number of non-staining spores, the bulk of which has a dark granular sporoplasm. The shell-valves of the spores are often half open and capsule dislocated, so that they look dead. In the end, most of the spores stop staining and decompose. Changes in the spores preserved at different temperatures have been traced for *Myxobolus albovi* (Table 1, Fig. 4), *M. cyprinicola* (Table 2) and *M. mülleri* (Table 3, Fig. 5).

Table 1

Variations in the number of spores with the different type of staining by Lugol's iodine from the young cysts of *Myxobolus albovi* (+18°C and −5°C)

Number of days	Temperature °C							
	+18°C				+3°C			
	total number of spores	number of spores (%)			total number	number of spores (%)		
		with clear vacuole	diffusely stained	not stained		with clear vacuole	diffusely stained	not stained
0	388	82.0±1.94	17.4±1.92	0.6±0.38	388	82.0±1.94	17.4±1.92	0.6±0.3
1	352	71.0±2.41	27.8±2.38	1.2±0.34	202	76.5±3.02	23.5±2.8	1.0±0.7
3	154	9.8±2.39	85.5±2.85	5.2±1.75	162	52.0±3.82	47.5±3.9	0.5±0.5
5	107	3.0±1.65	90.5±2.38	6.5±2.4	123	74.0±3.95	26.0±3.95	–

During the first three or four days of the experiment, the increase in the number of spores with a diffusely stained sporoplasm is associated with a proportional reduction of the number of spores displaying a distinct iodinophilous vacuole. This relationship is only slightly upset by negligible number of non-staining spores. So at the early stages of the experiment, variation in the number of spores with a distinct iodinophilous vacuole is a sufficiently characteristic feature of the process. This accounts for the choice of this single parameter to be shown, in Table 4 and Fig. 6 depicting *M. mülleri*. On the fifth or sixth day of the experiment, the curve of non-staining spores ascends steeply,

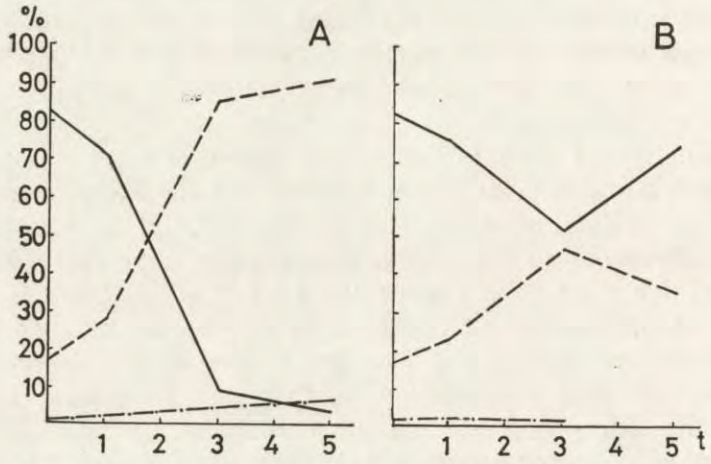

Fig. 4. Changes in the number of spores, representing various staining properties in Lugol's iodine, originating from young cyst of *Myxobolus albovi*. A — +18°C, B — −5°C. ——— number of spores with iodinophilous vacuole, — — — number of spores with diffuse type of staining of sporoplasm, —·—·—· number of not stained spores

Table 2

Variations in the number of spores with different type of staining by Lugol's iodine from the cysts *Myxobolus cyprinicola* (+18°C and +3°C)

Number of days	Temperature °C							
	+18°C				+3°C			
	total number of spores	number of spores (%)			total number of spores	number of spores (%)		
		with clear vacuole	diffusely stained	not stained		with clear vacuole	diffusely stained	not stained
0	313	65.0±2.7	35.0±2.7	—	—	—	—	—
1	236	57.0±3.2	42.0±3.2	1.0±0.72	—	—	—	—
3	223	9.4±1.95	90.6±1.95	—	—	—	—	—

which evidences that most of the spores have perished and, eventually, decomposed (Fig. 5).

The above data indicate that glycogenous polysaccharides tend to pass gradually from the iodinophilous vacuole into the cytoplasm of the sporoplasm. This is most probably due to the transport of reserve nutrients, which get involved into metabolism of the sporoplasm and are utilized there. A similar interpretation was suggested by Galinsky and Meglitsch (1970). This suggestion is also corroborated by the fact that mass dying out of spores does not begin until a full transfer of glycogenous substances from the iodinophilous vacuole into the sporo-

Table 3

Variations in the number of spores with different type of staining by Lugol's iodine from the young cysts *Myxobolus mülleri* (−18°C)

Number of days	Total number of spores	Number of spores (%)		
		with clear vacuole	diffusely stained	not stained
0	279	86.0±2.08	11.1±1.89	2.9±1.01
3	135	5.9±2.01	89.0±2.69	5.1±1,89
4	139	3.6±1.58	88.5±2.71	7.9±2.31
6	125	—	69.5±4.09	30.5±4.09

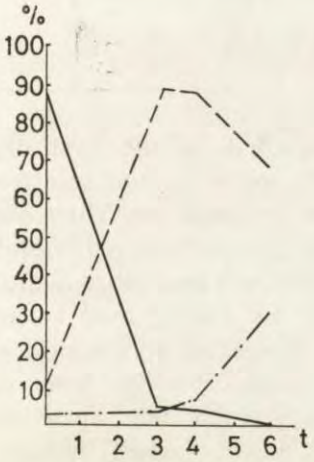

Fig. 5. Changes in the number of spores, representing various staining properties in Lugol's iodine, originating from young cyst of *Myxobolus mülleri* (+18°). ——— number of spores with iodinophilous vacuole, — — — number of spores with diffuse type of staining of sporoplasm, —·—·—· number of not stained spores

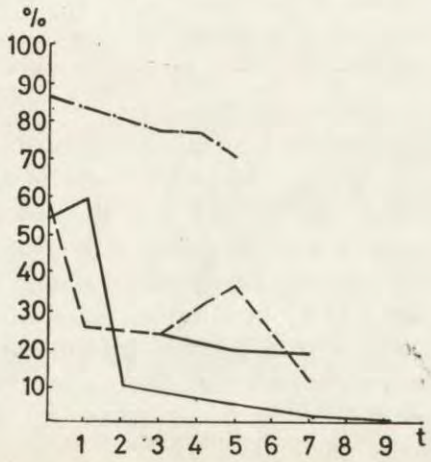

Fig. 6. Changes in the number of spores with intensively stained iodinophilous vacuole, originating from cysts of *Myxobolus mülleri*. ——— mature cyst (+18°C), — — — mature cyst (+3°C), —·—·—· young cyst (+3°C)

plasm. A temperature decrease radically slows down the process of polysaccharides transfer owing to reduced metabolic intensity.

Some of the samples which were kept at a low temperature displayed an increase in the number of spores with distinct iodinophilous vacuoles on the fifth day of the experiment. In one sample preserved at room

Table 4

Variation in the number of spores with iodinophilous vacuole staining perfectly by Lugol's iodine from the cysts of *Myxobolus mülleri* (18°C and + 3°C)

Number of days	Mature cysts T = +3°C		Mature cysts T = +18°C		Mature cysts T = +18°C			Young cysts T = +3°C		
	total number of spores	number of spores with vacuole (%)	total number of spores	number of spores with vacuole (%)	number of days	total number of spores	number of spores with vacuole (%)	number of days	total number of spores	number of spores with vacuole (%)
0	203	59.0±3.55	—	—	0	202	54.5±3.50	0	279	86.0±2.08
1	217	26.0±2.95	—	—	1	349	59.0±2.63	3	101	77.2±4.07
3	269	24.0±2.60	—	—	2	206	10.2±2.11	4	146	77.0±3.48
4	240	31.0±2.98	—	—	5	342	5.8±1.28	5	120	71.5±4.17
5	231	37.0±3.17	301	20.0±2.30	6	64	—	—	—	—
7	212	12.5±2.20	262	19.5±2.44	7	114	1.76±1.22	—	—	—
—	—	—	—	—	9	171	—	—	—	—

temperature, such an increase was already recorded on the first day (Table 4). Galinsky and Meglitsch (1970) report a similar effect after the first 24 h in the spores kept at room temperature. They also observed an increase in the number of spores with a distinct iodinophilous vacuole among those spores which were first frozen and subsequently kept at room temperature during a week. This peculiarity may be accounted for by nonuniform maturing of the spores in myxosporidian cysts. So, in the course of the experiment, polysaccharides keep on accumulating in the immature spores. It is thus possible that by a certain moment there will be more spores which have reached maturity than those in which the polysaccharides have passed from the iodinophilous vacuole into the cytoplasm of the sporoplasm. Quantitative data expressed by the curve rise show the difference between the number of spores just forming a vacuole and those already losing it. Spores' maturing is radically slowed down by cooling and therefore is displayed at 24 hours' intervals. At room temperature this is a much faster process, so that much smaller time intervals should be used in measurements.

The expenditure of polysaccharides of the iodinophilous vacuole does not seem to begin at the moment when the spore gets into the water or the sporoplasm enters the digestive tract of the host. It probably starts earlier, yet in the cyst. This is born out by a larger proportion of spores with a distinctly stained vacuole in the juvenile cysts than in the mature ones (82–86 per cent and 54–59 per cent respectively). A grown-up spore loses contact with the pansporoblast which actually ceases to exist, and begins to utilize its own polysaccharides.

Summing up the above information, we could suggest a diagram illustrating the general trend of the polysaccharide dynamics in the amoeboid embryo of myxobolidian spores (Fig. 7).

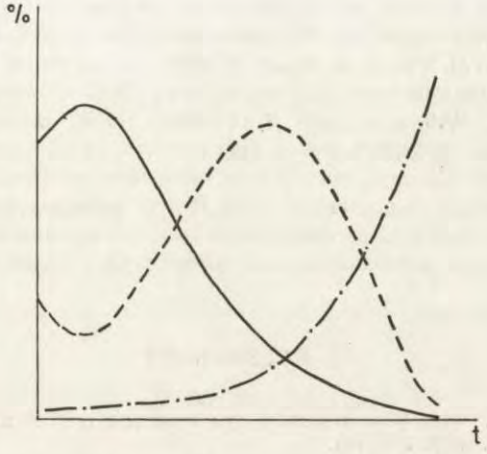

Fig. 7. Dynamics of polysaccharide content in sporoplasm, of *Myxobolidae* spores (after treating with Lugol's iodine). ——— number of spores with iodinophilous vacuole, — — — number of spores with diffuse type of staining of sporoplasm, —·—·—· number of not stained spores

Conclusions

(1) The iodinophilous vacuole is not an artefact but a genuine organelle of the sporoplasm in *Myxobolidae* and, most probably, *Myxobilatidae* and *Neomyxobolidae*. It can be detected with an electron microscope. To display an iodinophilous vacuole, living spores should be stained with Lugol's iodine. The iodinophilous vacuole can be easily exposed in the spores treated with various fixatives and acids and, in particular, examined with the help of the phase-contrast device. The PAS reaction also has a good staining effect on the iodinophilous vacuole.

(2) The data obtained with the help of various techniques are evidence of a polysaccharide, probably glycogenous, nature of the iodinophilous vacuole content.

(3) As the spore matures, the amount of polysaccharides in the sporoplasm increases. At first, they are chiefly confined to the iodinophilous vacuole, but later gradually pass into the sporoplasm. This corroborates the belief that the iodinophilous vacuole is a depot of reserve nutrients for the spores of *Myxobolidae*.

РЕЗЮМЕ

Рассмотрены методы обнаружения йодофильной вакуоли. Показано, что фазово-контрастная микроскопия выявляет вакуоль лишь через некоторое время после изготовления глицерин-желатиновых препаратов. Йодофильная вакуоль обнаружена при электронно-микроскопическом исследовании спор *Myxobolus mülleri*. Обсуждаются результаты обработки спор *Myxobolus mülleri, M. bramae, M. dispar, M. albovi, M. exiguus, M. nemeczeki, Thelohanellus oculi-leucisci, Henneguya psorospermica, Myxosoma anurus, Myxidium rhodei, Kudoa* sp. жидкостью Люголя, по реакции PAS и методом Шабадаша (1949). Описывается динамика полисахаридов споры при ее формировании. При пребывании спор вне хозяина имеет место постепенное расходование содержимого йодофильной вакуоли, скорость которого находится в зависимости от температуры внешней среды. Йодофильная вакуоль рассматривается как депо запасных питательных веществ (типа гликогена). Поддерживается мнение о реальности йодофильной вакуоли как морфологического образования в спорах *Myxobolidae*.

REFERENCES

Akhmerov A. H. 1960: Mikrosporidii ryb bassejna reki Amur. Rybn. hoz. Vnutr. vodoemov Lat. SSR, 240–307.

Auerbach M. 1910: Die Cnidosporidien (Myxosporidien, Actynomyxidien, Microsporidien). Eine Monographische Studie. Leipzig; VII, 261.

Bond F. F. 1937: A probable constituent of spore coat of Myxosporidian spores. J. Parasitol., 23, 542–543.

Bond F. F. 1940: Glycogenous material in Myxosporidian spores. J. Parasitol., 26, 29.

Bütschli O. 1881: Myxosporidien. Zool. Jb. 1, 162–164.

Davis H. S. 1917: *Myxosporidia* of the Beaufort Region. A systematic and biological study. Bull. U.S. Bur. Fish., 35, 203–243.

Doflein F. 1899: Fortschritte aus dem Gebiete der Myxosporidienkunde. Zusammenfassende Uebersicht. Zool. Zentbl., 6, 361–379.

Donec Z. S. and Schulman S. S. 1973: O metodach issledovanija *Myxosporidia* (*Protozoa, Cnidosporidia*). Parazitologija 7, 191–193.

Galinsky G. and Meglitsch P. 1970: Notes on the effect of pretreatment of spores on the iodinophile vacuole of *Henneguya exilis*. Proc. Iova Acad. Sci., 76, 431–435.

Gurley R. R. 1893: On the classification of the *Myxosporidia* a group of protozoon parasites infesting fishes. Bull. U.S. Fish. Comm. 11, 402–407.

Kheissin E. M., Schulman S. S. and Vinnichenko L. P. 1961: Stroenije spor *Myxobolus*. Citologija 3, 6, 662–667.

Keysselitz G. 1908: Die Entwicklung von *Myxobolus pfeifferi*. Th. Teil I u. II. Arch. Protistenk., 11, 252–308.

Kudo R. 1918: Contributions to the study of parasitic *Protozoa*. IV. Note on some *Myxosporidia* from certain fish in the vicinity of Woods Hole. J. Parasitol., 5, 11–16.

Kudo R. 1919: (1920): Studies on *Myxosporidia*. A synopsis on genera and species of *Myxosporidia*. Biol. Monogr., 5, 3–4, 1–265.

Kudo R. 1921: On the nature of structure characteristic of cnidosporidian spores. Trans. Am. Microsc. Soc., 11, 2, 59–74.

Labbe A. 1899: *Sporozoa*. In: Das Tierreich, 5, 20, 180.

Lom J. 1969 a: On a new taxonomic character in *Myxosporidia*, as demonstrated in descriptions of two new species of *Myxobolus*. Folia Parasit., 16, 97–103.

Lom J. 1969 b: *Myxobolus undulatus* sp. n. a new myxosporidian from minows (*Phoxinus phoxinus*). Folia Parasit., 16, 4–296.

Lom J. and Puytorac P. 1965: Studies on the myxosporidian ultrastructure and polar capsule development. Acta Protozool., 1, 53–65.

Meglitsch P. 1960: Some coelozoic myxosporidia from New Zeeland fishes. I. General and family *Ceratomyxidae*. Trans. R. Soc. N. Z. 88, 2, 265–365.
Müller J. 1841 a: Krankhafter Hautsusschlag mit spezifisch organisierten Korperchen (Psorospermien). Ber. Preuss. Acad. Wiss., 212–221.
Müller J. 1841 b: Ueber eine eigentumliche Krankhafte parasitische Bildung mit spezifisch organisierten Samenkorperchen. Ber. Preus. Acad. Wiss., 246–250.
Müller J. 1841 c: Ueber Psorospermien. Arch. Physiol. Wiss. Med., 5, 477–496.
Petruschevsky G. K. 1932: Zur Systematik und Cytologie der *Myxosporidia* aus einigen Fischen des Weissen Meers. Arch. Protistenk., 78, 3, 543–556.
Pearse E. 1960: Histochemistry. London.
Poche F. 1913: Das System der Protozoen. Arch. Protistenk., 30, 125–321.
Shabadash A. L. 1949: Problemy gistohimičeskogo issledovanija glikogena normalnoj nervnoj sistemy. Opyt harakteristiki biologičeskih svojstv i različij nejronov. Medgiz, Moskva, 1–332.
Schubert G. 1968: Elektronen mikroskopische Untersuchungen zur Sporenentwicklung von *Henneguya pinnae* Schubert (*Sporozoa, Myxosporidia, Myxobolidae*), Z. Parasitenk., 30, 57–77.
Schulman S. S. 1959: Novaja sistema mikrosporidij. Tr. Karelsk. Fil. A. N. SSSR, 14, Voprosy parazit. Karelii, 33–47.
Schulman S. S. 1966: Miksosporidii fauny SSSR. "Nauka" Moskva-Leningrad 1, 507.
Thélohan P. 1889: Sur la constitution des spores des myxosporidies. C. r. Acad. Sci., 109, 919–922.
Thélohan P. 1892: Observation sur les myxosporidies et essai de classification des ces organismes. Bull. Soc. Philom., 4, 165–178.
Tripathi Y. R. 1948: Some new *Myxosporidia* from Plymouth with a proposed new classification of the order. Parasitology, 39, 1–2, 110–118.
Uspenskaja A. V. 1971: Ultratonkoe stroenie nekotoryh vidov miksosporidij. Materialy 1 sezda Vses. obšč. protozoologov oktjabr. 1971, g. Baku, izd. ELM, 93.
Walliker D. 1966: Some protozoan parasites of the roach (*Rutilus rutilus*). Trans. R. Trop. Med. Hyg. 60, 13.
Walliker D. 1968: The nature of the iodinophilous vacuole of myxosporidien spores and a proposal synonymize the genus *Myxosoma* Thelohan, 1892 with the genus *Myxobolus* Bütschli, 1882, J. Protozool., 15, 571–575.

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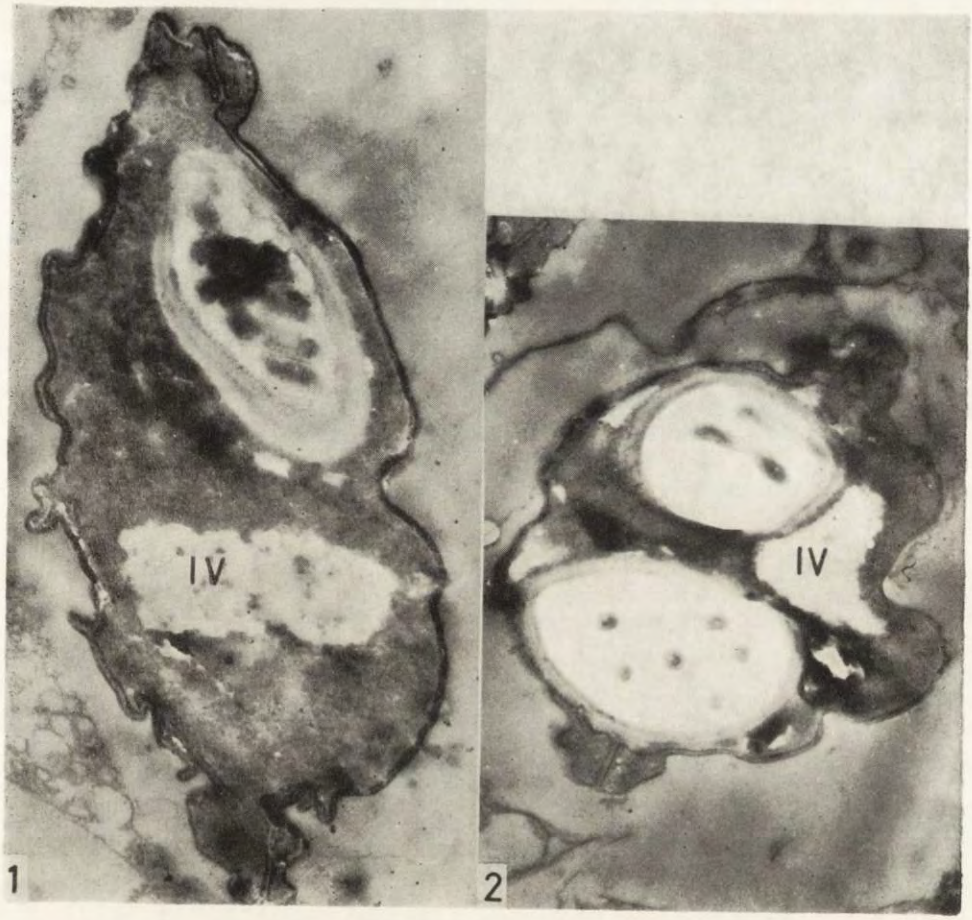
EXPLANATION OF PLATE I

Sections through *Myxobolus mülleri* spore. A — 19500 ×, B — 25000 ×, IV — iodinophilous vacuole



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Iodinophilous Vacuole and Ecology of Freshwater *Myxosporidia*

Synopsis. It has been shown experimentally that the iodinophilous vacuole in the spores of *Myxobolus ellipsoides* Thélohan, 1892 has a greater specific weight than the sporoplasm. Consequently, it makes the spores heavier increasing their immersion rate in water. In this connection, some alterations have been introduced in the distribution of the species *Myxobolus* and *Thelohanellus* among ecological groups depending on the immersion rate of the spores in water. A great number of the *Myxobolus* species have been attributed to a group with rapidly immersing spores. The appearence of the iodinophilous vacuole in *Myxobolidae* is associated with adaptation of freshwater *Myxosporidia* to bottom feeding fishes — benthophages and phytophages. Special devices for floating in the genera *Henneguya, Neohenneguya, Phlogospora, Hoferellus* are found to be secondary. Lability of the shape, size and state of the iodinophilous vacuole, along with adaptive changes favoured wide dispersal of *Myxobolidae* among freshwater fishes of most diverse ecology. Thus the iodinophilous vacuole as a spore hydrostatic apparatus is ascribed the major role in the evolution of *Myxobolidae.*

The ecological analysis of freshwater *Myxosporidia* (Donec 1964) revealed the uneven distribution of various groups of these parasites among fish-hosts, which is closely related to some ecological peculiarities of the latter, i.e., with their feeding habits, once in bottom-feeding fishes — benthophages and phytophages — there occur specimens of nearly all *Myxosporidia* genera; in fishes taking food in the depth of water — planctophages and predators — we find mainly representatives of the genera *Henneguya, Myxobilatus, Myxidium, Zschokkella, Chloromyxum, Sphaerospora, Hoferellus.* It was suggested that the spores of these species have adaptive devices for floating or slow immersion in the depth of water. Accordingly the freshwater Myxosporidia were divid-

ed in two ecological groups: I — species with rapidly immersing spores (*Myxobolus, Myxosoma, Thelohanellus*); II — species with slowly immersing spores (*Henneguya, Myxobilatus, Myxidium, Chloromyxum, Sphaerospora, Hoferellus*).

Morphological analysis of myxosporidian spores carried out by Schulman (1966) on a whole support this conclusion. In fact, the spores of *Henneguya, Myxobilatus, Hoferellus* and of some *Sphaerospora* species are equipped with special processes increasing their surface, which aids in slow immersion of the spores. The spores of *Myxidium* and *Zschokkella* have a spindle-like shape enhancing their relative surface. At the same time a number of corrections were introduced. It was found that the globular form typical of the *Sphaerospora* and *Chloromyxum* spores is less beneficial for floating since the ball has the smallest relative surface. In some representatives of these genera the floating ability of the spores is increased to a considerable reduction in the spore size, leading to essential increase of the relative surface of the spore. Therefore, these two genera may be attributed to a group with slowly immersing spores. On the other hand, species with large spores should be placed in a group with rapidly immersing spores or in an intermediate group supplemented by Schulman (1966). Moreover, Schulman stressed out that the spores of *Myxobolus* and *Myxosoma* have the lens-like shape, which is more beneficial for floating than the globular form since it has a large relative surface. Hence he transferred most specimens of these genera with comparatively small spores to the group with slowly immersing spores or to the intermediate group. These corrections are rather in good conformity with the laws of *Myxosporidia* distribution among fish ecological groups.

The only exception is the genus *Myxobolus*. In accordance with the scheme proposed by Schulman (1966) the majority of species from this genus belong to the groups with slowly immersing spores and to the intermediate group [1]. *Myxobolus*, however, is associated largely with bottom-feeding fishes and is less frequently met in fishes feeding in the depth of water. Consequently, it may be assumed that the immersion rate of spores is influenced, along with their size and shape, by some unknown factor. The distinguishing feature of the spores of the family

[1] According to Schulman (1966) the group with slowly immersing spores includes species having globular spores with the diameter no greater than 8 μm as well as species with lens-like spores the length and width of which do not exceed 12 μm and the thickness 6 μm. The intermediate group comprises species possesing globular spores with the diameter 8–9 μm and the lens-like spores with the length and width — 12–14 μm and the thickness 6–7 μm. The groups with rapidly immersing spore comprises species with larger spores. When one of the spore poles is narrowed or there occur various kind of protrusions or processes these species are classed accordingly with groups containing spores of greater buoyancy.

Myxobolidae is the presence in the sporoplasm of an iodinophilous vacuole containing glycogen or a substance relative to glycogen. Naturally, it may be suggested to have the molecular weight different from that of the sporoplasm. The question is whether the iodinophilous vacuole increases or decreases the specific weight of the spore. Although this question was put by Schulman even in 1966, there was no sufficient evidence for its solution.

To define relationships between the specific weight of the iodinophilous vacuole and that of the sarcoplasm a set of experiments were performed.

As the *Myxosporidia* spore as a rule do not exceed in size 15–20 μm this question can be answered only with the aid of centrifugation. It was necessary first of all to determine how spores would be oriented during centrifugation, i.e., which of the spore poles is heavier. For this purpose the spores were examined under a microscope in vertically oriented, flat-parallel capillaries filled with water. The spores in some specimens of the genera *Myxobolus, Henneguya* and *Myxosoma* were found to immerse their front poles with the polar capsules down. Thus it became clear that after centrifugation the spores will be oriented with the front ends to the periphery.

Later on a suspension of the spores of *Myxobolus ellipsoides* Thelohan, 1892 (species with a large well oriented iodinophilous vacuole) was centrifuged in water fom 15 min to 3 h at 500–5000 rev/min. The optimum results were obtained at centrifugation for 40–50 min at 3500–4500 rev/min. After centrifugation the spores exactly in the same manner as the control spores were stained with Lugol's iodine and examined under a microscope. By the position of the iodinophilous vacuole in the sporoplasm the spores were divided in three groups: (1) the spores with the iodinophilous vacuole adjacent to the anterior part of the sporoplasm; (2) the spores with the iodinophilous vacuole occupying the median position; (3) spores with the iodinophilous vacuole in the posterior position. It was found that at centrifugation the iodinophilous vacuole tends to shift toward the polar capsule, which is indicative of higher specific weight of the vacuole as compared with that of the surrounding cytoplasm (Table 1).

The results of the experiments permit the following conclusions:

Since the specific weight of the iodinophilous vacuole proved to be higher than that of sporoplasm the iodinophilous vacuole increases the total specific weight of the spore, and accordingly, its immersion rate. On Schulman's list (1966) 17 *Myxobolus* species were included into the intermediate group. Taking into account all the above we must

Table 1

The iodinophilous vacuole location in the sporoplasm of *Myxobolus ellipsoides* spores

Experiment	Number of spores		Location of iodinophilous vacuole	Number of spores with the different location of the iodinophilous vacuole (%)	
	in control	in experiment		in control	in experiment
I	130	120	anterior	33.8	60.0
			central	30.8	16.7
			posterior	35.4	23.3
II	180	180	anterior	33.3	51.15
			central	20.6	7.78
			posterior	46.1	41.07
III	230	145	anterior	30.4	48.3
			central	16.5	16.6
			posterior	53.1	35.1
IV	169	120	anterior	36.1	50.8
			central	17.2	17.5
			posterior	46.7	31.7
Total	709	656	anterior	31.8	52.0
			central	21.2	14.0
			posterior	47.0	34.0

attribute these species presumably to the group with rapidly immersing spores.

The question is more complicated with representatives of the genus *Myxobolus* placed by Schulman into the group with slowly immersing spores. The majority of them will be transferred to the intermediate group and only species with small spores or very small iodinophilous vacuoles will remain in this group.

Issuing from this we reconsidered all species of the genus *Myxobolus* found in freshwater bodies of the Soviet Union. The experiments show that the polar capsules are much heavier than the sporoplasm. We took into account even such factor as the relative volume occupied by polar capsules in the spore-cavity. As a result of this revision nearly all representatives of the genus *Myxobolus* previously attributed to the intermediate groups were placed in the group with rapidly immersing spores. The exceptions were three species — *M. sprostoni, M. gigi, M. spatulatus,* which although not exceeding 14 μm in size, have a small iodinophilous vacuole. Moreover, *M. spatulatus* has a process, enhancing the spore surface, and *M. gigi* is characterized by the elongated and narrowed anterior spore end, which also increases the relative surface. In addition to these 3 species 15 species with a comparatively large iodinophilous vacuole as well as *M. artus* and *M. neurobius* with the

polar capsules occupying the major part of the spore cavity were transfered from the group with slowly immersing spores to the intermediate group. Species with small spores, small iodinophilous vacuoles and also spores possessing special floating devices presented as various kind of process, ridges etc. were left in the group with slowly immersing spore.

It should be also pointed out that representatives of another genus of the family *Myxobolidae* — *Thelohanellus* should be attributed to the group with rapidly immersing spores since their spores are comparatively bigger in size and have large iodinophilous vacuoles.

Table 2

Myxobolus and *Thelohanellus* species with fast-sinking spores

(1) *M. kawabatae*	(28) *M. lussi*
(2) *M. thelohanellus*	(29) *M. oviformis*
(3) *M. chondrostomi*	(30) *M. achmerovi*
(4) *M. anisocapsularis*	(31) *M. pfeifferi*
(5) *M.* sp. Donec	(32) *M. elegans*
(6) *M. orientalisi*	(33) *M. albovi*
(7) *M. schulmanr*	(34) *M. isakovi*
(8) *M. grandiinte capsularis*	(35) *M. baueri*
(9) *M. permagnus*	(36) *M. crassus*
(10) *M. kubanicum*	(37) *M. koi*
(11) *M. cyprini*	(38) *M. dispar*
(12) *M. gigas*	(39) *M. pseudorasborae*
(13) *M. ellipsoides*	(40) *M. obpyriformis*
(14) *M. hemibarbi*	(41) *M. mülleri*
(15) *M. magnus*	(42) *M. squamae*
(16) *M. bramae*	(43) *M. amurensis*
(17) *M. carassii*	(44) *M. lotae*
(18) *M. spaerocapsularis*	(45) *M. diversicapsularis*
(19) *M. petruschewskyi*	(46) *M. donecae*
(20) *M. solidus*	(47) *M. rachmani*
(21) *M. dogieli*	(48) *Thelohanellus fuhrmanni*
(22) *M. talievi*	(49) *T. pyriformis*
(23) *M. krokhini*	(50) *T. oculi-leucisci*
(24) *M. toyamai*	(51) *T. misgurni*
(25) *M. infundibulatus*	(52) *T. dogieli*
(26) *M. drjagini*	(53) *T. catlae*
(27) *M. poljanskii*	

Below is given a list of distribution of the *Myxobolus* and *Thelohanellus* species by three ecological groups (Table 2, 3 and 4). It includes species not mentioned for some or other reason by Schulman in his Table.

Such distribution of *Myxosporidia* among the three groups must not be regarded as immutable or absolute. Some factors may change this

Table 3

Myxobolus species with moderate speed of sinking

(1) *M. cordis*	(13) *M. alienus*
(2) *M. lomi*	(14) *M. spatulatus*
(3) *M. sandrae*	(15) *M. improvisus*
(4) *M. disparoides*	(16) *M. artus*
(5) *M. musculi*	(17) *M. pseudodispar*
(6) *M. macrocapsularis*	(18) *M. neurobius*
(7) *M. obesus*	(19) *M. suturalis*
(8) *M. cyprinicola*	(20) *M. exiguus*
(9) *M. lobatus*	(21) *M. rotundatus*
(10) *M. karelicus*	(22) *M. gigi*
(11) *M. nemachili*	(23) *M. sprostoni*
(12) *M. thymalli*	

Table 4

Myxobolus species with slow-sinking spores

(1) *M. miyairii*	(12) *M. phylloides*
(2) *M. yini*	(13) *M. follius*
(3) *M. cristatus*	(14) *M. rotundus*
(4) *M. nemeczeki*	(15) *M. latus*
(5) *M. pavlovskii*	(16) *M. cheisini*
(6) *M. problematicus*	(17) *M. intimus*
(7) *M. niei*	(18) *M. kovali*
(8) *M. parvus*	(19) *M. brevicauda*
(9) *M. minutus*	(20) *M. longicordis*
(10) *M. cniporus*	(21) *M. pseudocordis*
(11) *M. uheni*	

ration. Thus, turbulent current may provoke floating and asscend of even rapidly immersing spores. Since the rise of water temperature is accompanied by a notable decrease of its viscosity, which in turn involves an increase in the immersion rate of the spore one can expect that one and the same species in the north would belong to one ecological group, and in the south, to another. Thus, according to some authors (Schulman 1966, Schaova et al. 1967, Schaova 1969) in the south, in conditions of the Kuban' river, *Myxobolus musculi* and *M. pseudodispar* were transferred to the group with rapidly immersing spores. Furthermore, in different seasons one and the same species may be classed with different ecological groups. So in the north *Lota lota* is strongly infested with *M. lotae* and *M. mülleri* attributed to the group with rapidly immersing spores. This phenomenon although inexplicable

at first sight is accounted for by the fact that *Lota lota* feed actively in the cold season when the water temperature is low. As at that time the viscosity of water increases about two times the immersion rate of the spores decreases drastically. The above species, therefore are transferred to the intermediate group and possibly even to the group with slowly immersing spores. This increases considerably the possibility for ingestion of the *M. lotae* and *M. mülleri* spores in the depth of water. All this provide conditions for widening the range of *Myxosporidia* hosts most of which exhibit wide specificity.

Enlargement of the host range is also promoted by variability of the spore size and shape strongly pronounced in *Myxosporidia*. As has been already mentioned, it determines the immersion rate of the spores. All these changes, however, are related either to the environmental conditions or to constant morphological character of the spore. The iodinophilous vacuole exhibits great individual variability (in different spores it has different dimensions) and even changes during ontogenesis.

According to Galinsky and Meglitsch (1970) and Podlipaev (1974) the glycogen content alters considerably in the iodinophilous vacuole. Consequently one and the same spore can change its immersion rate in water at different developmental stages.

Such lability of the iodinophilous vacuole by far permits the parasites to occupy various ecological niches and enlarge their range of hosts. Since the majority of the representatives of the most numerous genus *Myxobolus* and also of the genus *Thelohanellus* belong to the group with rapidly immersing spores it may be suggested that the main trend of the *Myxobolidae* evolution was directed to adaptation to parasitizing in bottom-feeding fishes — benthophages and phytophages. Their feeding habits in combination with rather a small depth of a water body created favourable conditions for ingestion of spores accumulated at the bottom. The appearence of the iodinophilous vacuole undoubtedly stimulated this process. Adaptation of some representatives of *Myxobolus* to slow immersion is by far the secondary phenomenon, which did not spread widely within the genus.

The thing is different with the representatives of other genera of the family *Myxobolidae: Henneguya, Neohenneguya, Phlogospora, Hoferellus*. Among these genera only the first contains a comparatively large number of species. In this group the evolution was directed towards adaptation of spores to floating. This was favoured by appropriate change in the spore shape, the appearence of processes and even increase in the number and size of fat drops inside the spore (Podlipaev 1972). At the same time in the species of this group the iodinophilous vacuole

is diminished notably, which testifies in favour of the secondary process of adaptation to floating in the family *Myxobolidae*.

Thus the iodinophilous vacuole should be regarded as a particular organoid promoting adaptation of some *Myxosporidia* families to freshwater fishes, primarily to benthophages and phytophages. Its lability helped a wide distribution of *Myxosporidia* among freshwater fishes of most diverse ecology.

РЕЗЮМЕ

Экспериментально установлено, что йодофильная вакуоль в спорах *Myxobolus ellipsoides* Thélohan, 1892 имеет больший удельный вес, чем цитоплазма амебоидного зародыша и, следовательно, утяжеляет споры, тем самым увеличивая скорость их погружения в воде. В связи с этим внесены изменения в распределение видов *Myxobolus и Thelohanellus* по экологическим группам в зависимости от скорости опускания спор в воде. Значительное число видов *Myxobolus* отнесены к группе с быстро опускающимися спорами. Появление йодофильной вакуоли у *Myxobolidae* связывается с адаптацией пресноводных миксоспоридий к рыбам, берущим пищу со дна — бентофагам и фитофагам. Приспособления к парению в родах *Henneguya, Neohenneguya, Phlogospora, Hoferellus* носят вторичный характер. Лабильность формы, размеров и состояния йодофильной вакуоли в совокупности с адаптивными изменениями формы спор способствовали широкому распространению *Myxobolidae* среди пресноводных рыб с самой разнообразной экологией. Таким образом, йодофильной вакуоли как гидростаточескому аппарату споры придается важная роль в эволюции *Myxobolidae*.

REFERENCES

Donec Z. S. 1964: Slizistyje sporoviki (*Myxosporidia*) presnovodnyh ryb USSR. Avtoreferat kand. diss. Vsesojuzn. naučno-issled. institut ozern i rečn. rybn. hoz., 1–17.

Donec Z. S. and Podlipaev S. A. 1974: Rol iodofilnoj vakuoli v ekologii presnovodnyh miksosporidii. VI Vsesojuzn. soveščanie po boleznjam i parazitam ryb. Tez. dokl. Moskva, 77–81.

Galinsky D. and Meglitsch P. 1970: Notes on the effect of pretreatment of spores on the iodinophilous vacuole of *Henneguya exilis*. Proc. Iowa Acad. Sci., 76, 431–435.

Podlipaev S. A. 1972: Žir v sporah i cistah nekotoryh slizistyh sporovikov. Parazitologija, 6, 506–508.

Podlipaev S. A. 1974: Polisaharidy v sporah slizistyh sporovikov *Myxobolidae*. Parazitologija, 8, 535–542.

Schaova N. D. 1969: Miksosporidii nižnego i srednego tečenija reki Kubani., Parazitologija, 3, 92–96.

Schaova N. D., Kabanova R. M. and Loseva T. C. 1967: Miksosporidii ryb srednego učastka reki Kubani. Materialy tretej zoologič. konferencii pedagogičn. inst. RSFSR. Volgograd.

Schulman S. S. 1966: Miksosporidii fauny SSSR. Izd. "Nauka" Moskva-Leningrad, 1–507.

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The Use of Iodinophilous Vacuole in Taxonomy of *Myxosporidia*

Synopsis. The iodinophilous vacuole played a very important role in the evolution of freshwater *Myxosporidia*, especially in the fluorishing of *Myxobolidae* family. Since the existence of the iodinophilous vacuole has been confirmed both morphologically and physiologically, it should be taken into account in achieving a natural system of *Myxosporidia*.

Myxosporidia is a remarkable group of parasitic protozoa which has reached a multicellular level of organization independently of *Metazoa*. This is most evident in the unique structure of myxosporidian spores consisting of 4 to 13 highly specialized cells. Strangely enough, the least studied of those cells is the sporoplasm, only cell in the spore which is poorly specialized, normally built and normally functioning. It is this cell that is responsible for continuation of the life cycle in *Myxosporidia*.

Very little is known about the iodinophilous vacuole occurring in the sporoplasm of many myxosporidian species. The earliest attempts to study it date back to the middle of the nineteenth century. The iodinophilous vacuole was first discovered by Müller (1841 a, b, c), but only Thélohan (1889) approached a reasonable explanation of its nature. The results of staining the spores with various chemical agents allowed him to suggest that there was a glycogenous substance in iodinophilous vacuoles. His data were later corroborated by Kudo (1921) and other researchers.

Most of the authors (Gurley 1894, Dogiel 1932, Bond 1940, Schulman 1966, Akhmerov 1960, Galinsky et Meglitsch 1970 et al.) tend to believe that the iodinophilous vacuole is a depot of reserve nutrients for the sporoplasm. Hence, some of the scientists attach great importance to the vacuole in the evolution of *Myxosporidia*

(Gurley 1894, Tripathi 1948). It is clear that systematics of *Myxosporidia* could not do without taking that organelle into account.

In his earliest classification Thélohan (1892) considered the presence or absence of iodinophilous vacuole as an important taxonomic character sufficient for isolating two superfamilies, one of which is composed of *Myxobolidae* and the other — of *Myxidiidae* and *Chloromyxidae*. On this basis the genera *Myxosoma* and *Myxobolus* which are morphologically alike, except for the absence of iodinophilous vacuole in the former, were placed in two different families, *Myxosoma* being referred to *Myxidiidae*.

The overwhelming majority of the authors of later classifications considered the iodinophilous vacuole as an important taxonomic character allowing to recognize taxa at the family level (Gurley 1893, Labbe 1899, Doflein 1899, Auerbach 1910, Poche 1913, Davis 1917, Kudo 1919, 1933, Meglitsch 1960) Poche (1913) and later observers placed *Myxosoma* and other related genera in the family *Myxosomatidae*. Tripathi (1948), placing an even stronger emphasis on that character, subdivided the suborder *Unipolaria* into two superfamilies: *Myxoboloidea* possessing the iodinophilous vacuole and *Ceratomyxoidea* lacking it.

Schulman's (1959, 1966) classification of *Myxosporidia* based on spore structure also accepts the iodinophilous vacuole as a family character. On the strength of this consideration, Schulman distinguishes one iodinophilous-vacuole-possessing family in each suborder *Bivalvulea*. But he points out that this is one of the least reliable taxonomic characters, stressing the vagueness of the iodinophilous vacuole problem. Such a wide application of the vacuole as a key character of large taxa is chiefly due to its being fairly conspicuous under the microscope. On the other hand, its observation may be not always that easy. Firstly, it cannot be usually seen under a light microscope without a special preliminary treatment. Experience also shows that it is distinguished by no means in all cases or in every spore.

As far back as 1893, Gurley pointed out the vague contours of the iodinophilous vacuole in *Myxobolus globosus*. The same peculiarity of that species was indicated by Galinsky and Meglitsch (1960), while Lewis (1968) observed it in *M. argenteus*. Davis (1968) failed to find an iodinophilous vacuole in *Henneguya psorospermica* and *H. oviperda*. According to Akhmerov (1960), in certain species not all the spores of the same preparation woud reveal an iodinophilous vacuole. Walliker (1966) observed no iodinophilous vacuole in *Myxobolus pseudodispar*.

All these difficulties made some of the observers doubt the possibility

of using the iodinophilous vacuole as a taxonomic character. Akhmerov was the first to deny the vacuole that right as part of his more general concept of rejecting all biochemical indices in taxonomic classification. So, while recognizing Tripathi's system as a whole, Akhmerov invalidates the superfamily *Myxosomatinae* as a synonym of the genus *Myxobolus*. Walliker (1968) shares Akhmerov's view as to the iodinophilous vacuole being unsuitable for taxonomic purposes. He substantiates his opinion by pointing out that vacuoles display great instability when myxosporidian spores are stained with the PAS technique. He also indicates that in *Myxosoma heterospora* stained with carmine according to Best only a few spores exhibit iodinophilous vacuoles.

Lom (1969 a, b) is also against the use of the iodinophilous vacuole in classification of *Myxosporidia*. He finds a proof for his opinion in the great size variation of the vacuole in *Myxobolus cycloides* together with the lack of an iodinophilous vacuole in some spores of *M. intimus*. Moreover, Lom maintains that spores of some cysts in *M. mülleri* and *M. ellipsoides* lack such vacuoles altogether. As a result, Lom concludes that the genera *Myxosoma* and *Myxobolus* should be regarded as synonyms, while the family *Myxosomatidae* must be invalidated.

This innovation does not make the classification of *Myxosporidia* a bit easier. To begin with, the genus *Myxobolus*, embracing a great number of species as it is, acquires a still wider range, which involves further complications in identification of its representatives. On the other hand, it becomes difficult to identify some well-differentiated species which have every reason to be recognized as independent. Thus, it is the presence of iodinophilous vacuole that allows *Myxobolus aeglifini* parasitizing the cartilage of sea-water codfish to be easily distinguished from *Myxosoma cerebralis* which invades the cartilage of freshwater salmoniid fish (Kabata 1957). Even Lom, rejecting the significance of iodinophilous vacuole for classification, had to stop halfway: he invalidated the family *Myxosomatidae*, but included the genus *Myxosoma* into the family *Myxobolidae* on the sole basis of the absence of the above vacuole (Ergens and Lom 1970).

So, we were facing an unusual situation when neither the advocates nor the opponents of the iodinophilous vacuole as a systematic character had arguments strong enough to substantiate their point of view. The problem required a thorough investigation into the iodinophilous vacuole and its functional role. To find a solution a number of species has been studied both possessing the iodinophilous vacuole — *Myxobolus exiguus, M. bramae, M. dispar, M. albovi, M. nemeczeki, M. mülleri, Henneguya psorospermica, Thelohanellus oculi-leucisci,* and lacking it — *Myxosoma anurus, Myxidium rhodei, Kudoa sp.* (Donec et Podlipaev 1974).

The results of these investigations together with information of some other authors allowed certain conclusions to be drawn. The existence of iodinophilous vacuole has been proved alongside the polysaccharide, possibly glycogenous nature of its contents. The spores display a complex dynamics of storing and utilizing those polysaccharides as part of their vital functions. This also accounts for the difficulties that one may have in recognizing the iodinophilous vacuole at some stages of the spore development when sticking to one particular method. A complete lack of the vacuole at early stages of the spore ontogeny is no obstacle yet for identifying it in the species under study, since the cysts usually contain spores varying in their development from juvenile to mature ones. Total lack of mature spores makes identification of the species unreliable due to possible differences in their shape and size. When examining the living material, one should keep in mind the polysaccharide expenditure. This factor may be responsible for Walliker's data on a low number of iodinophilous-vacuole-possessing spores. So, it is necessary to use absolutely fresh material for iodinophilous vacuole identification. Polysaccharides are known to occur also in sporoplasm of the spores of all myxosporidian species studied. But in the spores of the species lacking an iodinophilous vacuole polysaccharide globules are not confined to any particular organelle, they can be observed anywhere in the sporoplasm's cytoplasm. When recognizing iodinophilous vacuoles in some spores of *Myxosoma heterospora* Walliker must have dealt with individual large polysaccharide globuls. It should be also noted that Best's carmine staining he used is not specific enough for glycogen identification (Shabadash 1949).

The stock of glycogenous polysaccharides is suggestive of a possible role of iodinophilous vacuoles as an energy resources depot for the sporoplasm. This is also proved by the pattern of storing and utilizing the polysaccharides (Galinsky et Meglitsch 1970, Podlipaev 1974 a, b). Had, however, the role of the iodinophilous vacuole been limited to energy storage, a similar trend of evolution would have been observed in other myxosporidian groups. But iodinophilous vacuoles occur in no more than 63 per cent of all known species of *Myxosporidae* which belong to three out of the fourteen existing families, namely, to *Myxobolidae* from the suborder *Platysporea* (278 species), *Myxobilatidae* from the suborder *Eurysporea* (16 species) and *Neomyxobolidae* from the suborder *Bipolaria* (1 species). About 98 per cent of the species possessing an iodinophilous vacuole occur in fresh water, the remaining two per cent having passed into the sea secondarily (Fig. 1, 2). Though the greatest variety of myxosporidian forms are to be found in the sea, the typicaly marine species are lacking the iodinophilous vacuole. On the other hand, the bulk of the freshwater *Myxosporidia*

is formed by the three vacuole-possessing families. This is indicative *a priori* of some supplementary significant function of the iodinophilous vacuole in fresh water.

Donec and Podlipaev (1974) proved that the iodinophilous vacuole makes the spore heavier. This character might have been very important in the freshwater *Myxosporidia* evolution. *Myxosporidia* originated in sea water, as is evidenced both by their great variety and the occurrence of most primitive forms in the sea (Schulman 1966). After invading fresh water they faced radically new conditions. Fairly small depths, great density of fish population with predominant benthophages and phytophages made it easier for the spores to invade the host. Under such circumstances, the spores found it more favourable to sink down and accumulate at the bottom of the basin. This accounts for the benthophagous and phytophagous fish to be the most infected with *Myxosporidia* (Donec 1964, Schulman 1966, Donec and Podlipaev 1974). On the other hand, faster sinking reduced the transport of the spores by the current.

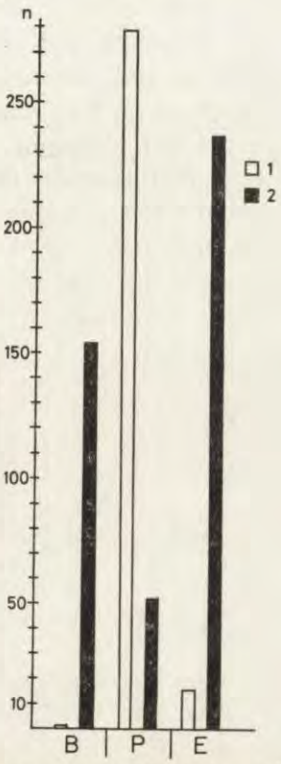

Fig. 1. Distribution of myxosporidian species with iodinophilous vacuole in various orders of *Bivulvalea*: B — *Bipolaria*, P — *Platysporea*, E — *Eurysporea*. 1 — species with iodinophilous vacuole, 2 — species without iodinophilous vacuole, n —

The absolute majority of freshwater *Myxosporidia* (94 per cent) belong to the family *Myxobolidae*, in the first place to the genera *Myxobolus* and *Henneguya* — 61.5 and 26 per cent respectively. According to Schulman (1966), the evolution of the relatively young family *Myxobolidae* is connected with cyprinid fish that usually pick their food from the bottom or behave as phytophages. The best proof of this assumption is the record-breaking number of 170 myxobolidan species that invade the cyprinid fishes, whereas the remaining 20 orders of fish harbour no more than 116 species of *Myxobolidae*.

The above evolutionary pattern made the presence of an iodinophilous vacuole particularly advantageous as it speeded up the spore's sinking to the bottom. The burst of speciation that followed, resulted in an adaptive radiation of *Myxosporidia* and in a growing number of their hosts. The parasites spread into predators, planktophages and the like, often outside the cyprinid family. This, in turn, necessitated slowing

down the speed of spore's sinking, even though without a complete disappearance of the iodinophilous vacuole. It just grew smaller in size, which was associated with accumulation of more fat within the spore and with certain other changes: (P o d l i p a e v 1972) the spore itself also grew smaller, changed its shape so as to produce a bigger relative surface, and developed tail outgrowths in such species as *Henneguya, Phlogospora, Neohenneguya, Hoferellus.*

As the glycogen content varies even within the spores of one and the same species, their sinking speed also show variation. This may account for a wider range of hosts which is so characteristic of most *Myxobolidae.*

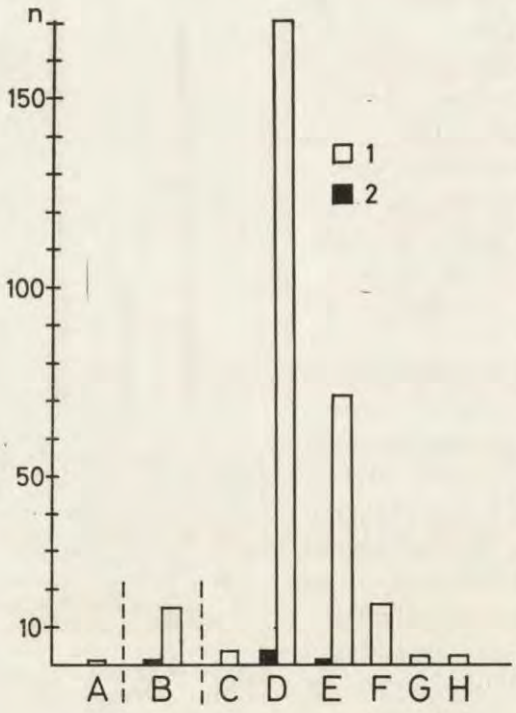

Fig. 2. Distribution of marine and fresh-water myxosporidian species in genera having iodinophilous vacuole: *Bipolaria*: A — *Neomyxobolus*; *Eurysporea*: B — *Myxobilatus*; *Platysporea*: C — *Hoferellus*, D — *Myxobolus*, E — *Henneguya*, F — *Thelohanellus*, G — *Phlogosporea*, H — *Neohenneguya*. 1 — Fresh-water species, 2 — marine species, n — number of species

Out of 300 species of the *Myxobolidae* family only three are coelozoic parasites, which is due to the general evolutionary trend towards histozoic parasitism in the suborder *Platysporea.* This trend is another factor contributing to flourishing of the family discussed.

Formation of an iodinophilous vacuole in some representatives of the suborders *Bipolaria* (*Neomyxobolidae*) and *Eurysporea* (*Myxobilatidae*) also occurred in fresh water and is, most likely an example of a later convergent development. *Myxobilatus* (16 species) and *Neomyxobolus* (1 species) are primitive genera, with oligosporous plasmodia initially confined to urinary and gall bladders of fishes, therefore they are more limited in occurrence than *Myxobolidae.*

So, the iodinophilous vacuole must have played an important role in the evolution of freshwater *Myxosporidia* with *Myxobolidae* as the most flourishing group.

All the above evidences that it would be unjustified to reject the iodinophilous vacuole as a taxonomic character in classifying *Myxosporidia*. This organelle is a morphological and physiological reality of an undoubtedly great adaptive significance. Its importance is not limited to species identification of the genus *Myxobolus*, as without it the organisms lose diagnostic distinctness. The role the iodinophilous vacuole played in the evolution of *Myxobolidae* makes it still more significant as a taxonomic character, since it is impossible to achieve a natural system of any animal group without taking into consideration phylogenetic relationships within it.

РЕЗЮМЕ

Йодофильная вакуоль сыграла большую роль в эволюции пресноводных миксоспоридий обеспечив бурный расцвет семейства *Myxobolidae*. Поскольку она реальна как морфологически, так и физиологически, имеет большое адаптивное значение, ее необходимо учитывать при построении естественной системы миксоспоридий.

REFERENCES

Akhmerov A. H. 1960: Miksosporidii ryb basseina reki Amur. Rybn. hoz. vnutr. vodoemov Lat SSR, 5, 240–307.
Auerbach M. 1910: Die Cnidosporidien (Myxosporidien, Actynomyxidien, Microsporidien). Eine Monographische Studie. Leipzig; VIII, 261.
Bond F. F. 1940: Glycogenous material in myxosporidian spores, J. Parasitol., 26, 29.
Davis E. H. 1968: Myxosporidian parasites from the fishes of the river Lugg. J. Protozool., 15, 3, 471–480.
Davis H. S. 1917: Myxosporidia of the Beaufort Region. A systematic and biological study. Bull. U. S. Bur. Fisch., 35, 203–249.
Doflein F. 1899: Fortschritte auf dem Gebiete der Myxosporidienkunde. Zusammenfassende Uebersicht. Zool. Zentrbl., 6, 361–379.
Dogiel V. A. 1932: Presnovodnye *Myxosporidia* SSSR. Opredeliteli organizmov presnyh vod SSSR, 4, Lenshabtehizdat, 1–70.
Donec Z. S. 1964: Slizistye sporoviki *Myxosporidia* presnovodnyh ryb USSR. Avtoref. kand. diss. Vsesojuznogo naučno-issled. inst. ozern. i rečn. rybn. hoz., 1–17.
Donec Z. S. and Podlipaev S. A. 1974: Rol iodofilnoj vakuoli v ekologii presnovodnyh miksosporidii. UL vsesojuzn. soveščane po boleznjam i parazitam ryb. Tez. dokl. M., 77–81.
Ergens R. and Lom I. 1970: Puvodei parasitarnih nemoči ryb. Academia, Praha, 1–383.
Galinsky D. and Meglitsch P. 1970: Notes on the effect of pretreatment of spores on the iodinophillous vacuole of *Henneguya exilis*. Proc. Iowa Acad. Sci., 76, 431–435.
Gurley R. H. 1893: On the classification of the *Myxosporidia* a group of protozoon parasites infesting Fishes. Bull. U. S. Fish. Comm., 11, 402–407.
Gurley R. R. 1894: The *Myxosporidia* or psorosperms of fishes and the epidemies produced by them. Rep. U. S. Fish. Comm., 26, 65–304.
Kabata Z. 1957: Note on a new host *Myxobolus aeglefini*. Parasitology, 47, 165–168.

Kudo R. 1919: (1920): Studies on *Myxosporidia*. A synopsis on genera and species of *Myxosporidia*. Ill. Biol. Monogr., 5, 3–4, 1–265.
Kudo R. 1921: On the nature of structure characteristic of cnidosporidian spores. Trans. Am. Microsc. Soc., 11, 2, 59–74.
Kudo R. 1933: A taxonomic consideration of *Myxosporidia*. Trans. Am. Microsc. Soc., 52, 192–216.
Labbé A. 1899: *Sporozoa*. In das Tierreich, 5, 20, 180.
Lewis D. 1968: *Myxobolus argenteus* sp. n. (*Protozoa, Myxosporidia*) a parasite of the golden shiner. J. Parasitol., 54, 5, 1034–1037.
Lom J. 1969 a: On a new taxonomic character in *Myxosporidia*, as demonstrated in descriptions of two new species of *Myxobolus*. Folia Parasitol., 16, 97–103.
Lom J. 1969 b: *Myxobolus undulatus* sp. n. a new myxosporidian from minows (*Phoxinus phoxinus*). Folia Parasitol., 16, 4, 296.
Meglitsch P. 1960: Some coelozoic *Myxosporidia* from new Zeeland Fishes. I. General and Family *Ceratomyxidae*. Trans. R. Soc. N. Z. 88, 2, 265–365.
Müller J. 1841 a: Krankhafter Hautsusschlag mit spezifich organisierten Korperchen (Psorospermien). Ber. Preuss. Acad. Wiss., 212–221.
Müller J. 1841 b: Ueber eine eigentümliche Krankhafte parasitische Bildung mit spezifich organisierten Samenkorperchen. Ber. Preus. Acad. Wiss., 246–250.
Müller J. 1841 c: Ueber Psorospermien. Arch. Anat. Physiol. Wiss., Med., 5, 477–496.
Poche F. 1913: Das System der Protozoen. Arch. Protistenk., 30, 125–321.
Podlipaev S. A. 1972: Žir v sporah i cistah nekotoryh slizistyh sporovikov. Parazitologija, 6, 506–508.
Podlipaev S. A. 1974 a: Polisaharidy v sporah nekotoryh miksosporidij. Ul. Vsesojuzn. soveščanie po boleznjam i parazitam ryb. Tez. dokl. M., 185–187.
Podlipaev S. A. 1974 b: Polisaharidy v sporah slizistyh sporovikov sem. *Myxobolidae*. Parazitologija, 8, 535–542.
Shabadash A. L. 1949: Problemy gistohimičeskogo issledovanija glikogena normalnoj nervnoj sistemy. Opyt harakteristiki biologičeskih svojstv i različij nejronov. Medgiz, M., 1–322.
Schulman S. S. 1959: Novaja sistema miksosporidij. Tr. Karelsk. fil. A. N. SSSR, 14. Vopr. parazitol. Karelin, 33–47.
Schulman S. S. 1966: Miksosporidii fauny SSSR. Nauka, Moskva–Leningrad, 1–507.
Thélohan P. 1889: Sur la constitution des spores des myxosporidies. C. r. Acad. Sci., 109, 919–922.
Thélohan P. 1892: Observation sur les myxosporidies et essai de classification de ces organismes. Bull. Soc. Philom., 4, 165–178.
Tripathi Y. R. 1948: Some new *Myxosporidia* from Plymouth with a proposed new classification of the order. Parasitology, 39, 1–2, 110–118.
Walliker D. 1966: Some protozoan parasites of the roach (*Rutilus rutilus*). Trans. R. Soc. Med. Hyg. 60, 13.
Walliker D. 1968: The nature of the iodinophilous vacuole of myxosporidian spores and a proposal to synonymize the genus *Myxosoma* Thélohan, 1882 with the genus *Myxobolus* Bütchli, 1882. J. Protozool., 15, 571–575.

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Adaptations morphologiques des thécamoebiens psammobiontes du psammal supralittoral des mers

Synopsis. Dans la partie générale du travail sont présentées en bref les conditions écologiques dans le psammal supralittoral de mers, qui est habité par une assotiation spécifique des thécamoebiens psammobiontes et psammophiles. Les adaptations morphologiques les plus importantes des thécamoebiens psammobiontes sont les suivant:

— Dimensions des thèques relativement plus petites par rapport à celles des thécamoebiens dulçaquicoles;
— Thèques latéralement ou dorso-ventralement aplaties;
— Thèques fines chitineuses;
— Présence d'excroissances latérales ou caudales des thèques;
— Filopodes longues et délicats, penètrant entre les grains du sable;
— Présence d'un évasement caractéristique de la thèque dans la région du pseudostome, ayant des fonctions fixatives.

Le nombre des thécamoebiens connus jusqu'à présent est de 1500 espèces et sous-espèces environs, groupées en 18 familles. Suivant le milieu qu'elles habitent, elles peuvent être divisées en quatre catégories écologiques, à voire:

(a) Dulçaquicoles — qui habitent les lacs, les étangs, les sources, les cours, les flaques et les bassins provisoires (phytothelmes, lithothelmes) et les autres nappes d'eau douce. Dans la catégories des thécamoebiens dulçaquicoles se rapportent plus de 50% des taxons connus jusqu'à présent.

(b) Muscicoles — qui habitent les tourbières sphaigneux et les marécages de la haute montagne ainsi que les mousses humides, rocheuses et épiphytes. A cette catégorie écologique se rapportent 35% environs des thécamoebiens connus.

(c) Terricoles — qui habitent les différents types de sol y compris la couche des feuilles mortes humides des forêts et les matières végétales couvrant le sol des forêts de la haute montagne. A cette catégories se rapportent 10% environs des thécamoebiens décrits.

(d) Marines — qui habitent les eaux salées à différent degré de salinité. Cette catégorie comprend les thécamoebiens trouvés dans les eaux soumâtres du littoral ou du continent ainsi que les thécamoebiens psammobiontes habitant les espaces interstitiels du psammal littoral et supralittoral des mers et des océans. A cette catégorie se rapportent 4% environs des thécamoebiens connus actuelement dont la plupart sont découverts il n'y a que 8–10 ans.

Entre les différents catégories écologiques n'existe pas une limite nette de telle façon que certaines espèces, par exemple des eaux douces, peuvent être trouvées aussi dans certains biotopes muscicoles et versa — des espèces connues des biotopes muscicoles peuvent être trouvées aussi dans certains types du sol ou dans les biotope dulçaquicoles.

Les thécamoebiens des différents milieux écologiques présentent plusieurs adaptations morphologiques et biologiques spéciphiques et dues à des différentes conditions écologiques du milieu. Certains de ces adaptations morphologiques et biologiques des thécamoebiens des trois premières catégories ont été déjà objet de plusieurs études (Deflandre 1953, Thomas 1959, Bonnet 1959, 1960, 1961, 1964, 1975, Chardez 1967, 1968, Schönborn 1962 a, 1962 b, 1964, 1971, Coûteaux 1975 etc.).

Dans la littérature il n'y a pas d'études spéciales sur les adaptations des thécamoebiens interstitiels psammobiontes. Golemansky (1969) attire pour la première fois l'attention sur certaines adaptations morphologiques des thécamoebiens interstitiels du psammal supralittoral des mers en indiquant deux nouveaux types morphologiques de leur thèques. Chardez (1972) indique aussi que les thécamoebiens des biotopes interstitiels présentent certaines adaptations spécifiques parmi lesquelles il note la forme évasée du col autour du pseudostome, l'absence des cornes ou d'autres excroissances des théques, présence d'une enveloppe chitinoide et leur flexibilité relative. Bonnet (1975) analysant d'une manière comparative les types morphologiques des thèques des thécamoebiens considère une partie des thécamoebiens psammobiontes comme un type morphologique isolé qu'il réunie dans un type nouveau — cotylostome.

Le psammal supralittoral en tant que milieu écologique des thécamoebiens interstitiels du psammal supralittoral des mers afain de montrer leurs adaptations morphologiques spécifiques à la vie dans le milieu écologique indiqué.

Le psammal supralittoral en tant que milieu écologique

Le psammal supralittoral des mers représente un milieu écologique spécifique qui se distingue des biotopes continentaux typique ainsi que

des biotopes marines. Ce phénomène s'explique par le fait que le psammal supralittoral est la zone limite entre la mer et la terre ferme dans laquelle s'effectue le mélange et l'influence mutuelle incessante entre les eaux salées de la mer et les eaux superficielles et souterraines du continent. Dans cette zone de melange continu, la salinité des eaux en tant que facteur écologique est très différente et varie de 0.1‰ dans les régions les plus éloignées du littoral jusqu'à 35‰ dans la bande de sable lavée sans cesse par les vagues de la mer (Fig. 1). Dans les mers et les océans où les flux et les reflux sont bien marqués on constate souvent une élevation sensible de la salinité des eaux dans le psammal supralittoral après que la mer se retire. Cette élevation est dûe au fait que la bande de sable, restée sur le littoral pendant le reflux, subit une évaporation d'une partie des eaux souterraines pendant les jours secs, chauds et ensoleillés et la salinité monte jusqu'au 37–40‰. Nous avons constaté une élevation similaire sensible par exemple sur le littoral du Golfe de Guinée (Océan Atlantique) où la température de l'air est relativement élevée et l'affluence des eaux douces continentales dans le sable supralittorale est in signifiant (G o l e m a n s k y 1976).

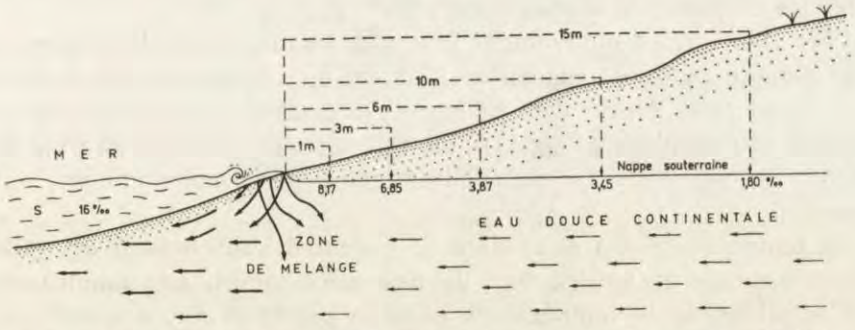

Fig. 1. Profil schématique de la plage de Côte du Soleil (Mer Noire)

Le niveau des eaux souterraines dans le psammal supralittoral des mers varie aussi sensiblement qu'en fonction de l'inclinaison du littoral et qu'en fonction de plusieurs facteurs climatiques, saisonniers, granulométriques etc. Comme un regle général le niveau des eaux souterraines dans une même plage sableuse pendant ou après les pluis est plus élevé par rapport aux mois secs et chauds de l'année. La composition granulométrique et la forme du sable, dont dépend la grandeur des espaces interstitiels, ont aussi une influence remarquable sur la quantité et le niveau des eaux souterraines. Les sables à grains fins homogènes (Mo = 0.1–0.4 mm) contiennent une quantité plus élevée des eaux interstitielles qui maintiennent par voie capillaire un niveau plus élevé par rapport

aux sables à gros grains hétérogènes (Mo = 0.5–1.6 mm). Les espaces interstitiels entre les grains de sable dans les sables à gros grains hétérogènes sont souvent remplis des grains plus fins ou des particules des matières organique qui font réduire la quantité des eaux libres et les espaces dont les habitants psammobiontes ont besoin.

Dans les zones de sable plus éloignées de la mer les espaces interstitiels entre les grains de sable sont souvent remplis des particules argileuses, minérales et organiques apportées par les eaux souterraines du continent. Ces zones ne sont pas habitées d'habitude de thécamoebiens psammobiontes; elles hebergent très souvent des thécamoebiens pédobiontes ou dulçaquicoles, apportés par les eaux continentales.

Les eaux souterraines dans le psammal supralittoral sont soumises à l'action active de plusieurs courants d'eau. Dans les zones les plus rapprochées de la mer dans lesquelles s'effectue le débordement incessant des vagues sur la bande de sable se manifeste une infiltration continue des eaux de la surface vers la profondeur et de la terre ferme vers la mer. Pendant la période des houles fortes ce mouvement gagne des régions plus étendues et souvent la surface entière de la plage sableuse. En résultat s'effectue une lavage et une refoulement de la faune psammobionte du psammal supralittoral vers la mer.

Parallèlement au mouvement étudié des eaux salées de la mer, penetrant dans le psammal supralitoral, existe un second courant à direction fixée de la terre ferme vers la mer — le courant des eaux douces souterraines du continent (Fig. 1). La force de ce courant varie aussi et dépend de plusieurs facteurs climatiques, géomorphologiques, saisonniers etc.

La température des eaux dans le psammal supralittoral est relativement constante et voisine à celle des eaux salées. Les amplitudes les plus sensibles de la température dans le psammal supralittoral ont été constaté pendant le printemps et l'été (Jansson 1967, 1968, Golemansky 1970 a). En général, les amplitudes des températures le jour et la nuit sont relativement petites et surtout dans les mers et les océans des latitudes géographiques modérées et chaudes et ne provoquent pas une influence sensible à la faune psammobionte. D'après Jansson (1967, 1968) la température des eaux souterraines dans le psammal, supralittoral de la Mer du Nord pendant l'hiver ne dépasse pas — 2–3°C.

Les eaux souterraines interstitielles dans le psammal supralittoral des mers ont une réaction neutre ou faiblement alcaline (pH = 7.2–8.4). Leur acidité augmente au fur et au mésure vers le continent et raison directe de renouvellement des eaux souterraines et de leur enrichissement en matiere organique ou particules argileuses, apportées par les eaux douces

du continent. Les variations de pH, établies par nous dans le psammal supralittoral des mers (Golemansky 1970 a, 1970 b) indiquent les mêmes tendances, montrées plus haut et confirment les recherches de Brucé (1928), Pennak (1951), Jansson (1968) etc. sur les changements de l'acidité dans les plages sableuses des mers.

Les quantités de l'oxygène dans les eaux souterraines du psammal supralittoral varient sensiblement, mais présentent toujours une tendance de réduction de la mer vers le continent parallèlement à l'élevation de la profondeur de la nappe d'eau. Dans la zone du mouvement actif des eaux à proximité immédiate du littoral, l'aération des eaux est la plus forte et la quantité d'oxygène dissou est la plus élevée. Dans les régions plus éloignées où il y a une augmentation des matières argileuses et organiques, la quantité de l'oxygène se réduit et dans certains cas atteint des valeurs nulles. Dans ces cas on peut constater la présence d'une couche foncée de sable contenant de l'hydrogène sulfuré et l'absence totale de thécamoebiens psammobiontes vivants (Tableau 1 et Tableau 2).

Tableau 1

Distribution de l'oxygène (mg/l) dans six plages de littoral bulgare de la Mer Noire

Distance de la mer (m) / Profondeur (m) / Stations	1	3	4	6	10	12	15	20	40
	0.25	0.40	0.50	0.80	0.95	1.10	1.15	1.25	1.50
Primorsko	4.18	4.32	—*	2.05	—	1.15	—	0.72	—
Arkutino	4.95	3.85	—	3.02	0.82	—	—	—	—
Nessebar	4.80	—	2.92	1.60	0.42	—	0.25	—	—
Côte du soleil	4.20	3.05	—	2.12	—	0.75	—	0.43	0.10
Sables d'or	—	4.62	—	3.35	1.15	—	0.40	—	—
Chabla	5.23	—	1.82	1.05	—	0.33	—	—	—

* Les tirets montrent qu'il manquent des mesurages dans les stations indiquées.

De cette analyse très brève on peut se rendre compte que le psammal supralittoral des mers présente des conditions écologiques assez spécifiques à la faune psammobionte. De son côte les habitants de ce milieu écologique ont acquis plusieurs adaptations (morphologiques, biologiques, écologiques), leur permettant d'habiter ce biotope particulier. Les thécamoebiens psammobiontes obligatoires ont acquis aussi au cours de l'évolution un grand nombre des adaptations morphologiques, biologiques, et écologiques à la vie dans ce milieu, dont une partie fait objet de la présente étude.

Tableau 2

Distribution de l'oxygène (mg/l) dans quatre plages de littoral roumain de la Mer Noire

Distance de la mer (m) / Profondeur (m) / Stations	1	2	4	6	8	10	15	20
	0.25	0.30	0.90	1	1.20	1.30	1.25	1.35
Agigea	2.59	—*	2.78	—	1.69	—	0.82	—
Eforie Nord	—	4.68	—	4.04	—	1.85	—	—
Saturn	—	2.25	—	2.19	—	2.24	—	0.70
Vama Vecce	—	5.34	—	4.36	—	2.52	—	—

* Les tirets montrent qu'il manquent des mesurages dans les stations indiquées.

Adaptations morphologiques des thécamoebiens psammobiontes

(1) Dimensions des théques relativement plus petites des thécamoebiens psammobiontes par rapport à celles des thécamoebiens dulçaquicoles. Du Tableau 3 on constate que des 33 espèces des thécamoebiens psammobiontes obligatoires connus jusqu'à présent, la plupart (plus de 75%) ont des dimensions des thèques de 15 à 60 μm et 7 espèces seulement ont des dimensions de 60 à 100 μm. Une seule espèce — *Lagenidiopsis elegans* (Gruber) à des dimensions au dessus de 100 μm.

Une pareille régularité est constatée aussi dans les cas des thécamobiens psammobiontes facultatifs (p. ex. *Difflugia lucida* Penard, *Cyclopyxis kahli* Deflandre, *Hyalosphaenia cuneata* Stein etc.). Les dimensions des espèces citées des biotopes interstitiels sont plus réduites par rapport à celles d'eau douce et sont voisines à celles trouvées dans les différents types du sol. Chardez (1972) attire aussi l'attention sur cette particularité des thécamoebiens psammobiontes. Les diemensions plus réduites des thèques des thécamoebiens psammobiontes sont, sans doute, une adaptation au milieu particulier de vie — le espaces interstitiels réduits entre les grains de sable dans le psammal supralittoral.

(2) Thèques latéralement ou dorso-ventralement aplaties. Ce caractère morphologique se manifeste chez la plupart des thécamoebiens psammobiontes obligatoires et facultatifs. Une thèque applatie possedent 22 espèces des thécamoebiens psammobiontes obligatoires connues jusqu'à présent. Prèsque tous les espèces connues des genres *Psammonobiotus* et *Centropyxiella* ont son thèque latéralement ou dorso-ventralement applatie. Une partie des thécamoebiens psammobiontes facultatifs, p. ex. *Difflugia lucida* Penard, *Hyalosphaenia cuneata* Stein, *Centropyxis constricta* (Ehrenberg) etc. ont également une thèque applatie latéralement ou dorso- ventralement. Des thèques à séction circulaire possedent

seulement les espèces dont les dimensions de la thèque sont moins de 60 μm. comme p. ex. *Difflugiella psamophila* Gol., *Micramphora pontica* Valkanov, *Amphorellopsis lucida* Gol. etc. (Tableau 3).

Un intérêt particulier présente la découverte dans le psammal supralittoral des mers de l'espèce *Cyphoderia ampulla* (Ehrenberg) — une ubiquiste typique aux dimensions relativements grandes (au-dessus de 100 μm), trouvée jusqu'à maintenant dans la plupart des biotopes dulçaquicolés, terricoles et marins. Mais, parallèlement à la forme typique à section transversale ronde de la thèque, dans le psammal supralittoral des mers nous avons trouvé aussi des populations avec une thèque latéralement applatie dont la position taxonomique reste pour l'instant inconnue.

(3) Thèques fines chitineuses aux xenozomes uniques disposés en général dans la région du col et du pseudostome. Certaines espèces ont des thèques entièrement chitineuses sans structure visible (*Chardezia caudata* Gol., *Amphorellopsis lucida* Gol., *Psammonobiotus minutus* Gol. etc.). D'autres ont des thèques formées par des écailles (ellipsoidales, ovales, rectangulaires ou polygonales), pareilles aux thèques de certains espèces de la fam. *Euglyphidae* (*Corythion, Euglypha, Cyphoderia*). Quelques espèces seulement des genres *Centropyxiella, Micropsammella, Alepiella* et *Amphorellopsis* ont des thèques formées et par des éléments exogènes en taille réduite, semblables aux thèques semi-transparentes des

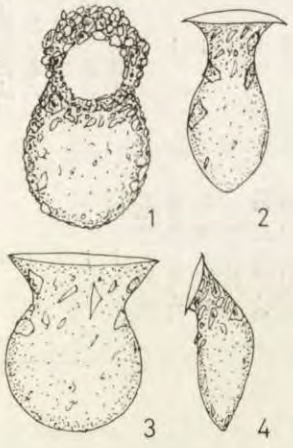

Fig. 2. 1 — *Centropyxiella arenaria* Valkanov (× 1000), 2 — *Amphorellopsis taschevi* Gol. (× 1800), 3 — *Micramphora pontica* Valkanov (× 2500), 4 — *Micropsammella retorta* Gol. (× 1200)

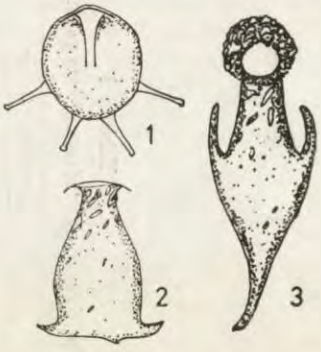

Fig. 3. 1 — *Lagenidiopsis valkanovi* Gol. (× 700), 2 — *Amphorellopsis elegans* Gol. (× 800), 3 — *Alepiella tricornuta* Gol. (× 900)

Tableau 3

Dimensions des thèques des thécamoebiens psammobiontes obligatoires

Espèces	Longueur totale des thèques				Comprimées latéralement ou dorsoventralement
	15–30 μm	30–60 μm	60–100 μm	Au dessus de 100 μm	
Micramphora hellebauti Chardez	14–18× 8–12	–	–	–	–
Micramphora pontica Valkanov	15–30×13–20	–	–	–	–
Amphorellopsis lucida Gol.	17–26× 8–12	–	–	–	–
Cryptodifflugia lanceolata Gol.	18–25×11–17	–	–	–	+
Diflugiella psammophila Gol.	20–24×12–13	–	–	–	–
Amphorellopsis taschevi Gol.	20–35×10–15	–	–	–	–
Psammonobiotus balticus Gol.	20–22×18–20	–	–	–	+
Psammonobiotus plana Chardez	20–26× 5–8	–	–	–	+
Psammonobiotus golemanskyi Chardez	20–35×13–18	–	–	–	+
Psammonobiotus linearis Gol.	21–24×10–20	–	–	–	–
Psammonobiotus minutus Gol.	23–30×14–19	–	–	–	+
Psammonobiotus communis Gol.	–	23–52×16–31	–	–	+
Rhumbleriella filosa Gol.	–	28–40×28–38	–	–	+
Lagenidiopsis valkanovi Gol.	–	30–61×20–45	–	–	+
Chardezia caudata Gol.	–	33–48×13–16	–	–	–
Centropyxiella arenaria Valkanov	–	33–74×25–59	–	–	+
Amphorellopsis elegans Gol.	–	35–63×17–28	–	–	+

c. d. tableau 3

Cyphoderia littoralis Gol.	–	38–51×18–26	–	–	+
Micropsammella retorta Gol.	–	38–56×16–23	–	–	–
Pseudocorythion mannei Chardez	–	40–45×10–12	–	–	–
Corythionella acolla Gol.	–	40–73×27–38	–	–	+
Pseudocorythion wailesi Gol.	–	43–58×20–34	–	–	+
Messemvriella filosa Gol.	–	46–58×18–20	–	–	–
Pomoriella valkanovi Gol.	–	47–53×20–25	–	–	–
Corythionella minima Gol.	–	50–54×23–34	–	–	+
Pseudocorythion acutum (Wailes) Valkanov	–	–	55–90×23–26	–	+
Centropyxiella gibbosa Valkanov	–	–	60×40	–	+
Amphorellopsis maximus Gol.	–	–	63–77×33–54	–	+
Centropyxiella elegans Gol.	–	–	70×80	–	+
Centropyxiella lucida Gol.	–	–	70–81×38–42	–	+
Corythionella pontica Gol.	–	–	77–88×33–40	–	+
Alepiella tricornuta Gol.	–	–	85–100×40–45	–	+
Lagenidiopsis elegans Gol.	–	–	–	112×50	+

psammobiontes facultatifs comme *Difflugia lucida* Penard, *D. subterranea* Gol., *Pseudodifflugia gracilis* Schlumberger etc. (Fig. 2). Dans les cas des thèques, possedant de xenosomes, ceux-ci sont placés surtout dans la moitié antérieur de la thèque et autour du col et du pseudostome, tandis que la partie postérieure est entièrement chitineuse. Etant formées de matière chitineuse les thèques des thécamoebiens psammobiontes sont incolores et transparentes. Les thèques plus légères de ces organismes facilitent sans doute leurs mouvements dans les espaces interstitiels.

(4) La présence d'excroissances latérales ou caudales des thèques qui empechent l'emportation des thècamoebiens psammobiontes par les courants d'eau. Il faut d'ailleur noter que les excroissances morphologiques indiquées sont relativement rares chez les thécamoebiens psammobiontes. Des cornes latérales sont observées p. ex. chez les espèces *Lagenidiopsis valkanovi* Gol., *Alepiella tricornuta* Gol. et parfois chez *Amphorellopsis elegans* Gol. (Fig. 3). Chez l'espece *Alepiella tricornuta* les deux cornes latérales sont disposées contre le sens du mouvement des animaux. Nous considérons ces cornes latérales comme une adaptation de résistance contre les courants d'eau permanents dans le psammal supralittoral des mers. Des cornes caudales de différente grandeur sont observées chez les espèces *Pseudocorythion acutum* (Wailes), *Chardezia caudata* Gol., *Corythionella minima* Gol., *Micropsammella retorta* Gol., parfois chez *Amphorellopsis lucida* Gol. etc.

(5) Filopodes très longs et délicats qui penètrent entre les grains du sable et facilitent le mouvement et la prise de nourriture. Très caractéristiques de ce point de vue sont les espèces du genre *Lagenidiopsis* qui n'ont qu'un seul filopode assez long et pareil à flagellum. Sa longueur dépasse parfois 3–4 fois la longueur des thèques et au cours du nouvement tâte d'avance le chemin et choisit le sens du mouvement dans le système complexe d'espaces interstitiels entre les grains du sable. Parmi les thécamoebiens psammobiontes obligatoires seulement une espèce, notamment *Pomeriella valkanovi* Gol. appartient au groupe des thécamoebiens à lobopodes.

(6) La présence d'un évasement caractéristique de la thèque dans la région du pseudostome, formé surtout de la matiere chitineuse et plus rarement des éléments exogènes. Cette évasement agit comme une ventouse et permet aux organismes de s'attacher plus solidement sur le substrat — dans le cas c'est la surface des grains de sable. L'importance adaptive de cet évasement spécifique peut être résumée en deux points:

(a) d'une part il permet une fixation plus solide des organismes sur le substrat et empêche leur évacuation et déplacement par les courants d'eau dans le psammal supralittoral des mers;

(b) d'autre part, la forme évasée du col de la thèque dans la règion

du pseudostome, qui chez les individus actifs est rempli de l'ectoplasme hyaline, facilite aussi la prise de la nourriture des thècamoebiens psammobiontes. Ils se nourrissent des bactéries, des algues et d'autres organismes unicellulaires, qu'il "lèchent" avec ses pseudopodes de la surface des grains de sable. L'évasement de la thèque est formé avant tout d'une matière délicate chitineuse et il est assez souple et flexible, surtout à sa périphérie, ce qui permet une adhérence relativement solide et résistante au substrat. Chardez (1972) attire également l'attention sur cette adaptation morphologique des thècamoebiens psammobiontes et il écrit notamment: "La forme généralement très évasée du col et la tendance vers la formation d'un sol ventral permettent une bonne adhérance aux grains formant le substrat solide".

Ce caractère morphologique et spécifique seulement aux thécamoebiens psammobiontes obligatoire nous donne la raison de les grouper en deux types morphologiques différents. A la base de la classification des types morphologiques des thécamoebiens, proposée par Bonnet (1964) et Chardez (1968) nous avons ajouté en 1969 deux types morphologiques nouveaux, à savoir:

Type acrostome à évasement, caractérisé par une symmétrie axiale ou monaxone de la thèque et un pseudostome terminal, ayant en vue apicale la forme d'un disque entourant l'ouverture buccale. A ce type appartiennent les thécamoebiens de genre *Micramphora* et *Amphorellopsis*.

Type plagiostome à évasement, caractérisé par une symmétrie bilatérale de la thèque et un pseudostome excentré et placé sur une sole ventrale plus ou moins plane. En vue ventrale d'évasement de la thèque a l'aspect d'un disque entourant l'orifice buccale. A ce type morphologique apartiennent les genres *Psammonobiotus, Corythionella, Pseudocorythion, Alepiella, Centropyxiella, Micropsammella, Chardezia* et *Messemvriella*.

SUMMARY

The specific living conditions in the inhabited by relatively rich associations of psammobiont and psammophylous testate amoebas supralittoral psammal of seas are briefly reviewed. The more important morphological adaptations of the psammobiont testate amoebas to the specific environment are as follows:

Relatively smaller shells of psammobiont testate amoebas compared with fresh water species;

Lateral or dorso-ventral flattened shells;

Light, chitinoid shells;

Side processes of shells on some obligatory psammobiont;

Long and fine filopods, penetrating among the sand particles;

Infundibular extension of shells arround the pseudostome with mainly fixing functions.

BIBLIOGRAPHIE

Bonnet L. 1959: Quelques aspects des populations thécamoebienes endogées. Bull. Soc. Hist. Nat. Toulouse, 94, 413–428.
Bonnet L. 1960: Kystes et trophophase chez *Plagiopyxis minuta* Bonnet (Thècamoebiens). Bull. Soc. Zool. Fr., 85, 1, 43–52.
Bonnet L. 1961: Caractères généraux des populations thécamoebiennes endogées. Pedobiologia, 1, 1, 6–24.
Bonnet L. 1964: Le peuplement thécamoebien des sols. Rev. Ecol. et Biol. Sol, 1, 2, 123–408.
Bonnet L. 1975: Types morphologiques, écologie et évolution de la thèque chez les thécamoebiens. Protistologica, 11, 3, 363–378.
Bruce J. R. 1928: Physical factors on the sandy beach. 11. Chemical changes. J. Mar. Biol. Assoc. U.K., 15, 533–565.
Chardez D. 1967: Histoire Naturelle des Protozoaires Thécamoebiens. (Ed. Boubée), Paris, 100 pp.
Chardez D. 1968: Etude statistique sur l'écologie et la morphologie des Thécamoebiens. Hydrobiologia, 32, 1/2, 271–287.
Chardez D. 1972: Etude sur les thécamoebiens des biotopes interstitiels, psammons littoraux et zones marginales souterraines des eaux douces. Bull. Rech. Agron. Gembloux, 6, 3/4, 257–268.
Coûteaux M.-M. 1975: Quelques aspects des relations entre les Thécamoebiens et les sols. Rev. Ecol. et Biol. Sol., 12, 1, 45–55.
Deflandre G. 1953: Thécamoebiens. In: Traité de Zoologie, 1, 2, Ed. Masson, Paris, 97–148.
Golemansky V. 1969: Sur une biocénose thécamoebienne peu connue des eaux souterraines littorales des mers. Progress in Protozoology, Abstr. Third. Int. Congr. Protozool., Leningrad 1969, "Nauka", 194.
Golemansky V. 1970 a: Rhizopodes nouveaux du psammon littoral de la Mer Noire. Protistologica, 6, 4, 365–371.
Golemansky V. 1970 b: Contribution à la connaissance des thécamoebiens (*Rhizopoda, Testacea*) des eaux souterraines littorales du golf de Gdansk (Pologne). Bull. Inst. Zool. Mus. Sofia, 32, 77–87.
Golemansky V. 1976: Rhizopodes psammobiontes (*Protozoa, Rhizopoda*) du psammal supralittoral des côtes guinéennes de l'Atlantique. Acta Zool. Bulgarica, 4, 23–29.
Jansson B.-O. 1967: Diurnal and annual variations of temperature and salinity of interstitial water in sandy beaches. Ophelia, 4, 173–201.
Jansson B.-O. 1968: Studies on the ecology of the insterstitial fauna of Marine Sandy beaches. Thesis, Stockholm, 1–16.
Pennak R. W. 1951: Comparative ecology of the interstitial fauna of fresh-water and Marine beaches. Ann. Biol., 27, 449–479.
Schönborn W. 1962 a: Die Ökologie der Testaceen im oligotrophen See, dargestellt am Beispiel des Grossen Stechlinsees. Limnologica (Berlin), 1, 2, 111–182.
Schönborn W. 1962 b: Zur Ökologie der Sphagnicolen, bryocolen und terricolen Testaceen. Limnologica (Berlin), 1, 3, 231–254.
Schönborn W. 1964: Lebensformtypen und Lebensraumwechsel der Testaceen. Limnologica (Berlin), 2, 3, 321–335.
Schönborn W. 1971: A study of evolution exemplified on Testacea. J. Gen. Biol., 32, 5, 530–540.
Thomas R. 1959: Les thécamoebiens muscicoles et terricoles: notion d'écologie générale et comparative. P.-V. Soc. Linn. de Bordeaux, 97, 1–27.

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Ciliary Activity of Stationary *Opalina*

Synopsis. High-speed cinemicrographic documentations on the locomotion of *Opalina*, freely slowing down from swimming to stationary, reveal conclusively that the ciliary beat patterns in both locomotory states differ. Instead of exhibiting the continuous helical pattern documented previously for swimming *Opalina* (Cheung 1973, Cheung and Jahn 1973, 1975 a) or the classic discontinuous forward-and-return pattern typical of some other cilitiates (Boggs et al. 1970; Cheung and Boggs 1977; Cheung and Winet 1975; Parducz 1967), the cilia of stationary *Opalina* beat with a three-dimensional rotational pattern.

Comparison of available data on the ciliary beat of *Opalina* in the literature reveals that the patterns described by various investigators differ significantly (Cheung 1973; Cheung and Jahn 1973, 1975 a; Parducz 1967; Sleigh 1960, 1968, 1974; Tamm and Horridge 1969, 1970). Taking into account the fact that such varying data were obtained under different experimental as well as locomotory conditions (hanging-drop, arresting micro-electrodes, quieting agents, rapid-fixation, free-swimming, slowing, stationary etc.) dependent on the technique utilized, it is only logical to assume that the discrepancy in the literature is probably due to the varying experimental or locomotory conditions and visual misinterpretations (Cheung 1973; Cheung and Jahn 1975 a).

It is generally assumed that the ciliary beat pattern is the same in swimming and stationary ciliates and, consequently, not much research has been conducted to investigate the ciliary activities of *Opalina* in the various locomotory states.

This report describes an extensive study on the ciliary activities of *Opalina*, freely slowing down from swimming to stationary. Slow motion (2–24 fps) and frame-by-frame analyses of the available high-speed cinemicrographs (taken at 400–500 fps) under Nomarski optics can serve to reveal the detailed ciliary activities of *Opalina* under various

locomotory conditions, so as to provide a better understanding of the precise nature of the ciliary activities typical of *Opalina*.

Materials and Methods

Opalina obtrigonoidea were obtained in the usual manner (Cheung 1973; Cheung and Jahn 1975 a). High-speed cinemicrographs on the ciliary activities of *Opalina*, freely slowing down from swimming to stationary, were made and analyzed in the standardized methods as described in detail in previous reports (Cheung 1973; Cheung and Jahn 1975 a, b, 1976; Cheung and Winet 1975). The high-speed cinemicrographic system is set up as shown in Fig. 1.

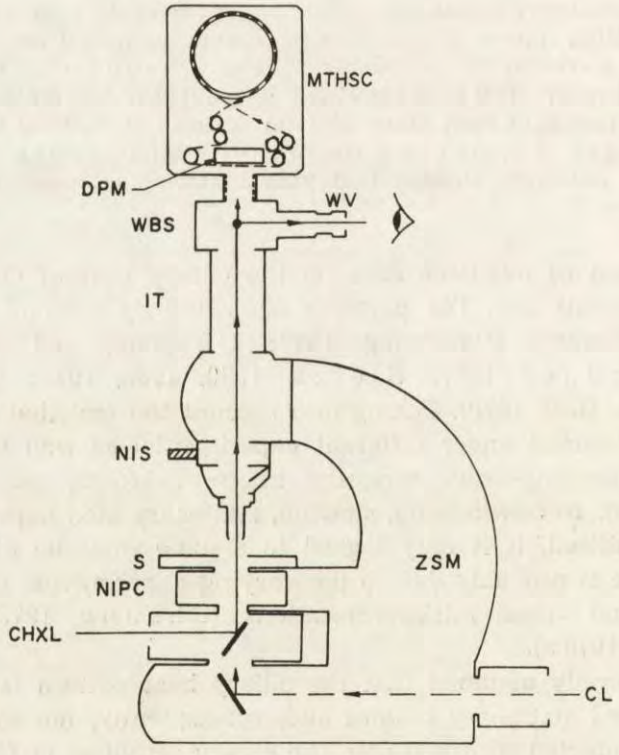

Fig. 1. Optical arrangement of the high-speed cinemicrographic set-up at Caltech. The much simplified light path is represented by lines with arrows. MTHSC, Milliken Teledyne high-speed camera (Model DBM-55); DPM, pin registry module; WV, Wild viewer; WBS, Wild beam-splitter; IT, intermediate tube; NIS, Nomarski interference contrast slide III; S, stage; NIPC, Nomarski interference-phase (Achromatic-Aplanatic) 1·4 N. A. condenser; ZSM, Zeiss standard WL research microscope; CHXL, Chadwick–Helmuth xenon point light source (Strobex); CL, standard Zeiss tungsten light source

Results and Analyses

It is revealed in the analyses of the available high-speed cinemicrographs that the ciliary beat pattern of swimming *Opalina* is continuous and without any differentiation into forward-and-return strokes. At first glance (and even with tracings in one plane), the cilia of swimming *Opalina* appear to undulate in a planar manner similar to that of sea-urchin sperms (Cheung 1973; Cheung and Jahn 1975 a; Gray 1955) or the posterior flagellum of *Ceratium* (Cheung 1977; Jahn et al. 1963). Such a phenomenon is shown clearly in Pl. I (two successive frames of a selected sequence of a 16 mm high-speed cinemicrograph taken at 400 fps at 645× under Nomarski interference contrast optics). Careful analyses of the cilia on both surface and profile views indicate that the ciliary beat is actually three-dimensional; consisting of two individual waves traveling at right angles to one another, and 90° out of phase (Cheung 1973; Cheung and Jahn 1975 a). This phenomenon, geometrically, constitutes a continuous traveling helix (The actual movies from which this conclusion is drawn

Table 1

Physical Characteristics	Quantitative Measurements	Locomotory State
Length of cilia (μm)	13.5±1.0	
Frequency of beat (Hz)	26 ± 2	free swimming
	18 ± 2	at start of free slowing
	8 ± 1	at conclusion of slowing; rotational beat starts, but ciliary orientation is still posterior as shown in Fig. 2 b.
	5 ± 2	stationary; rotational beat is at right angles to cell surface, as shown in Fig. 2 c.
Metachrony	Symplectic	free swimming, as shown in Pl. 1.
	Symplectic	at start of free slowing
	Symplectic	during slowing, but before rotational beat starts
	Symplectic	right before rotational beat starts
	none	right after beat change
	none	stationary
Velocity of metachronal waves (μm per sec)	180 ± 25	free swimming
	120 ± 35	at start of free slowing
	85 ± 25	during slowing, before rotational beat starts
	none	right after beat change
	none	stationary

and the actual analyses were presented at the AIBS meeting at New Orleans, 1976). Further analyses from other cinemicrographic segments all confirmed that the cilia of swimming *Opalina* beat with such a traveling three-dimensional (helical) pattern, propagating from base to tip along the entire length of the cilia. Whether the three-dimensional wave shows a perfect helix depends on experimental conditions; but the continuous propagatory nature of the ciliary beat is evident (as shown in Pl. I). Such traveling waves are distally oriented and undulate in perfect metachrony.

Metachronal waves can easily be seen in swimming *Opalina*. The wave crests form a right-wound spiral on the uniform surface of the rather long cilia (13.5 ± 1.0 μm). High-speed cinemicrographs (400 fps) viewed at 16–24 fps enable one to see such metachronal waves clearly. The metachrony conforms to the Symplectic type of the Knight-Jones convention in that the propagation of the continuous helical ciliary beat progresses in the direction of the metachronal waves. Such metachronal waves have been documented to travel with a velocity of up to 200 μm per second. The cilia of *Opalina*, typical of ciliates with Symplectic metachrony, are packed very closely together. Actually, they are so tightly packed that it appears as if they bunch together to form a continuous undulating ciliary envelope. Besides illustrating the continuous undulatory movement of the ciliary beat as well as the close-packed nature of the ciliary arrangement, Plate I also shows the existence and the type of the metachronal waves clearly. In a free swimming *Opalina*, the surface of the organism literally behaves like a progressive wavy envelope, with the peak of the metachronal waves appearing as transverse lines propagating from the anterior and progressing regularly toward the posterior end of the organism.

In slowing freely from swimming to stationary, the directional orientation of the cilia starts to change as the angular inclination of the cilia increases. Upon reaching the stationary state, the angular inclination has increased so much that the cilia are directed upward (basically at right angles to the cell surface) and not posteriorly oriented. The continuous helical bet pattern, which is typical of swimming *Opalina*, is not exhibited in the stationary state. Instead, the cilia beat in a rotational manner (as shown in Pl. II and III and as illustrated in Fig. 2b and c). In the stationary state, the cilia either behave disoriented and simply wriggle about or exhibit the rotational (spiralling) pattern with the cilia rotating (or gyrating) about the base and, at the same time, describing the wall of a conical envelope (as illustrated in Fig. 2 b and c). A similar pattern has been described by P a r d u c z for ciliates under chemically-induced hazardous conditions (P a r d u c z 1967). However, it should be

emphasized that the rotational beat pattern described in this report is observed and documented under normal conditions, without the presence of irritants and the organisms are freely slowing down from swimming to stationary without applied restrictions.

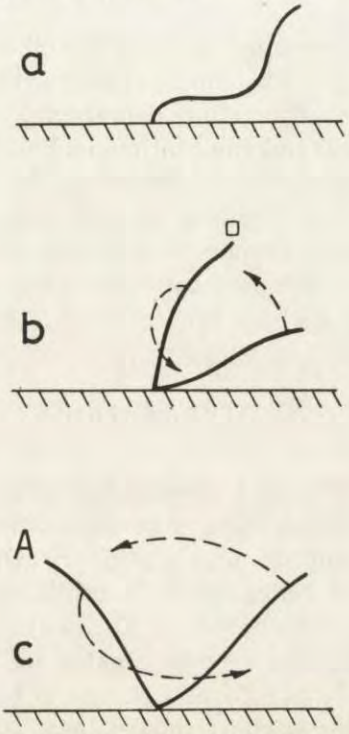

Fig. 2. (a) A tracing of one cilium in Pl. I. Note the posterior ciliary orientation and the undulatory (wavy) beat pattern. (b) At random tracings of two cilia at the time when the organism has just acquired the stationary state. The cilium at the left side does not conform to any specific shape and its rotational movement describes an irregular cone-shaped envelope. Note the slight posterior orientation of the cilia. (c) Tracings of 2 cilia in Pl. II. Note the curved configuration of the cilia and the upward ciliary orientation. Gyration of the curved cilia (as indicated by the arrows) will describe a conicoidal envelope. Comparison of (a) with (b) and (c) is similar to a comparison of Plates I, II, and III as Figure 2 (a, b and c) is traced out to show the ciliary characteristics of Plates I, II and III

At a glance, the cilia of stationary *Opalina* in profile are in sharp focus only at the left and right edges of the cone (as illustrated in Fig. 2 b and c). This phenomenon produces an optical illusion of an apparent discontinuous back-and-forth movement and is quite similar to the forward-and-return pattern described in the literature (P a r d u c z 1967; S l e i g h 1960, 1968, 1974). However, frame-by-frame analyses of available high-speed cinemicrographs reveal that this is only an illusion and that the cilia are actually beating in a continuous rotational (spiralling) manner.

As shown clearly in Pl. II and III, the angular orientation of the cilia of stationary *Opalina* is directed away from the cell surface (at right angles to the cell surface) and there is a distinct absence of ciliary coordination as compared to the perfect Symplectic metachrony shown in Pl. I.

In returning to the swimming state, the cilia that are exhibiting the rotational or conical pattern simply realign their activities; with the angular inclination of the cilia decreasing, the ciliary orientation posteriorly directed again and the continuous helical beat pattern resumed. The realignement of the cilia in resuming the continuous helical beat pattern (characteristic of *Opalina* and a few other ciliates in their swimming state) is very similar to the last stage of a normal ciliary reversal, as described previously by Cheung and Jahn (Cheung 1973; Cheung and Jahn 1975a; Jahn 1975).

Discussion

Analyses of the high-speed cinemicrographs on the ciliary activities of *Opalina* (free swimming, free slowing, stationary) confirm that the beat patterns of swimming and stationary *Opalina* differ. A three-dimensional continuous helical beat is exhibited in free swimming (as documented in Pl. I and shown in Fig. 2 a) and a three-dimensional rotational spiral beat pattern predominates in the stationary state (as documented in Plates II and III and shown in Fig. 2 b and c).

The beat pattern described above as "rotational", "conical" or "spiralling" is basically the same form designated as "conicoidal" "cone-shaped" or "funnel-shaped" in previous reports (Cheung 1973); Kuźnicki 1970; Kuźnicki et al. 1970; Parducz 1967). Minor variations in characteristics may appear in a detailed comparison — the geometric difference of the straight edge of a cone as compared with the curved edge of a conicoid. However, the basic configuration can be considered similar. The shape of the cilia of a stationary *Opalina* is not definite — they can be curved, bent slightly inward at the tip or even appear to be without any definite shape, as can be seen in Pl. II and III. Consider a cilium slightly curved in shape as shown in Fig. 2 c; upon gyration of the curved cilium about its base, a three-dimensional envelope with a slightly curved edge is formed. This shape is termed a conicoid, meaning a figure which differs from a cone in that the sides are curved instead of straight (see Kuźnicki et al. 1970). The terms "conical", "cone-shaped", "conicoidal" and "funnel-shaped" refer to the shape of the envelope of beat, whereas the terms "rotational" or "spiralling"

describe the beat itself. A more precise description of the ciliary beat of stationary *Opalina* would then be a three-dimensional spiral (rotational) beat with a cone-shaped envelope.

Such a spiralling movement of cilia has been described in the literature for the cilia of *Paramecium* and *Colpidium* under various abnormal and hazardous conditions (Parducz 1967). However, the spiralling conical beat pattern typical of stationary *Opalina* is described under ideal physiological conditions, with the organisms locomoting normally without any applied restrictions in the well-defined Naitoh's medium and in the absence of any irritants or hazardous chemicals, as applied by some investigators (Parducz 1967).

At a glance (under the microscope or in normal-speed cinemicrographs), the cilia of stationary *Opalina* in profile are in sharp focus only at the left and right edges of the cone-shaped (or conicoidal) envelope. Regrettably, this phenomenon creates an illusion of a back-and-forth movement which resembles the perfectly correct classic forward-and-return strokes described by Sir James Gray (1928) for *Mytilus*. Figure 2 b and c illustrates this misconception clearly. However, frame-by-frame tracings of high-speed cinemicrographs on the locomotion of *Opalina* reveal that the cilia are actually beating in a rotational (spiralling manner).

It is described in the literature that the beat frequency of *Opalina* cilia is about 4 Hz with the cilia executing a forward-and-return beat pattern (Sleigh 1960, 1968, 1974). Careful cinemicrographic analyses reveal that of all the locomotory states of *Opalina,* only the rotational ciliary beat of stationary *Opalina* has a beat frequency of about 4 Hz (5 ± 2 Hz). Beat frequency of swimming organisms are of much higher values (26 ± 2 Hz). Apparently, the beat description of *Opalina* in the literature is actually a description of a misconceived view of the edges of the spiral cone-shaped envelope of stationary *Opalina*. Such an incidence is not surprising as *Opalina* is extremely efficient in swimming and it is simply impossible for previous investigators to observe the ciliary activities directly under the microscope or even using cinemicrographic methods with a less than high-speed set-up. Quieting agents, hanging-drop preparations and arresting micro-electrodes are often used and the ciliary activities described are basically those of (or close to) the stationary state. A simple comparison of the beat frequencies can illustrate this point well. The back-and-forth beat pattern described in the literature is actually a description of the edges of the cone-shaped envelope of the rotational beat in its profile view.

It is now documented and confirmed that the cilia of *Opalina* beat in more than one pattern in various locomotory states (as clearly shown in

Pl. I, II and III) and the same situation also occurs in *Paramecium* (Kuźnicki et al. 1970). The general concept and practice of generalizing ciliary beat patterns is not well founded and scientists should be more precise in describing ciliary activities, taking into account the locomotory states as well as the technique utilized. For studying LIVE ciliary activities in detail, the more dependable tool is high-speed cinemicrography under differential interference contrast optics (Cheung 1973; Cheung and Jahn 1975 a, b, 1976; Cheung and Winet 1975). Such a special set-up-can overcome most optical as well as technical problems encountered in direct observation and in microscopic documentation of ciliary activities as due to the small dimensions of cilia, their high angular velocity, high frequency of beat, fast forward velocity of locomotion, density of distribution and their unique optical properties which are almost identical to those of the cytoplasm as well as those of the surrounding fluid. Continuous utilization of this established technique may help to cast some light on this intriguing phenomenon of ciliary activities.

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RÉSUMÉ

L'enrégistrement cinématographique à cadence accélérée de la locomotion des *Opalina* qui ralentissaient leur mouvement jusqu'à l'arrêt complet, prouve avec certitude que le pattern du battement ciliaire est différent dans les deux types du comportement locomotorique. Les cils de l'*Opalina* à l'arrêt manifestent des battements rotatifs en trois dimensions, au lieu lide de présenter le pattern helicoïdal continu démontre antérieurement chez les *Opalina* pendant la nage (Cheung 1973, Cheung et Jahn 1973, 1975 a) ou bien le pattern discontinu caractérisé par le phases progressives et regressives, classique chez des différents autres ciliés (Boggs et al. 1970, Cheung et Boggs 1977, Cheung et Winet 1975, Parducz 1967).

REFERENCES

Boggs N., Jahn T. L. and Fonseca J. R. 1970: Ciliary activity of *Spirostomum*. J. Protozool., 17, 9 (suppl.).

Cheung A. T. W. 1973: Doctoral Thesis. Determination of ciliary beat in *Opalina obtrigonoidea* and rabbit tracheal explants. University of California, Los Angeles.

Cheung A. T. W. 1977: Experimental investigations of the movement of fluid by *Ceratium* (in preparation).

Cheung A. T. W. and Boggs N. 1977: Ciliary activities of *Spirostomum ambiguum* (in preparation).

Cheung A. T. W. and Jahn T. L. 1973: Continuous traveling helical ciliary beat of *Opalina*. J. Protozool., 29, 499.

Cheung A. T. W. and Jahn T. L. 1975 a. Helical nature of the continuous ciliary beat of *Opalina*. Acta Protozool., 14, 111–124.

Cheung A. T. W. and Jahn T. L. 1975 b. Determination of the movement pattern of the epithelial cilia of rabbit trachea and clearance mechanism of the tracheal muco-ciliary clearance system. In: Swimming and Flying in Nature (edis Wu, Brokaw and Brennen). Vol. 1, pp. 289–300, Plenum Press, New York.

Cheung A. T. W. and Jahn T. L. (1976). High speed cinemicrographic studies on rabbit tracheal (ciliated) epithelia: determination of the beat pattern of tracheal cilia. Pediat. Res., 10, 140–144.

Cheung A. T. W. and Winet H. 1975: Flow Velocity Profile over a Ciliated Surface. In: Swimming and Flying in Nature (eds. Wu, Brokaw and Brennen). Vol. I, pp. 223–234. Plenum Press, New York.

Gray J. 1955. The movement of sea-urchin spermatozoan. J. exp. Biol., 32, 775–801.

Jahn T. L. 1975. New problems in propulsion of microorganisms. In: Swimming and Flying in Nature (eds. Wu, Brokaw and Brennen). Vol. I, pp. 325–338. Plenum Press, New York.

Jahn T. L., Harmon W. M. and Landman M. (1963). Mechanism of locomotion in flagellates. I. *Ceratium*. J. Protozool., 10, 358–363.

Kuźnicki L. 1970: Mechanism of the motor responses of *Paramecium*. Acta Protozool., 8, 83–118.

Kuźnicki L., Jahn T. L. and Fonseca J. R. 1970. Helical nature of the ciliary beat of *Paramecium multimicronucleatum*. J. Protozool., 17, 16–24.

Parducz B. 1967: Ciliary movement and coordination in ciliates. Int. Rev. Cytol., 91–128.

Sleigh M. A. (1960). The Form of beat of *Stentor* and *Opalina*. J. Exp. Biol., 37, 1–10.

Sleigh M. A. 1968: Patterns of ciliary beating. In: Aspects of Cell Motility (XXII Sym. Soc. Exp. Biol.). 22, 131–150. Cambridge University Press, London.

Sleigh M. A. ed. 1974. The Biology of Cilia and Flagella. Academic Press, London.

Tamm S. L. and Horridge G. A. 1969. Critical point drying for scanning electron microscopic study of ciliary motion. Science, 163, 817–819.

Tamm S. L. and Horridge G. A. (1970). The relation between the orientation of the central fibers and the direction of beat of *Opalina*. Proc. R. Soc. B175, 219–233.

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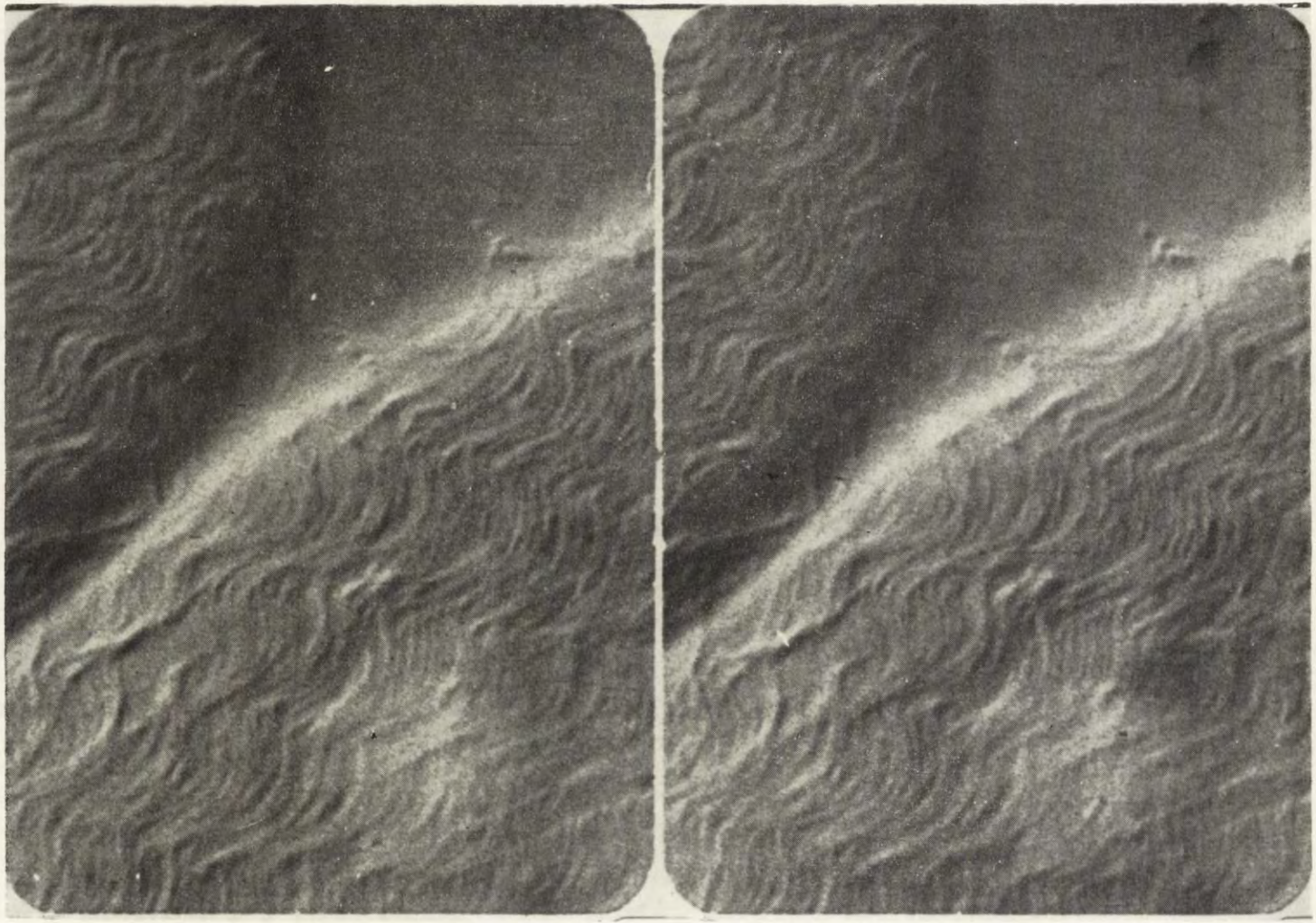
EXPLANATIONS OF PLATES I–III

Plate I. A print of two successive frames on the surface view of *Opalina* (2 organisms freely swimming side by side). Note the continuity of the propagatory wave pattern, the absence of the return stroke, the perfect Symplectic metachrony the close-packed nature of the ciliary configuration. The overall magnification is about 645 × and the print is made from a selected sequence of 16 mm high-speed cinemicrograph, taken at 400 fps under Nomarski optics.

Plate II. A print of two successive frames on the surface view of *Opalina* that has just acquired the stationary state from free slowing. Note the absence of the undulatory ciliary waves and the apparent lack of ciliary coordination. All the cilia are not directed posterior (as in the free swimming state) and they are either not beating in any specific pattern or are exhibiting a rotational pattern. Marker "A" denotes cilia that are curved as shown in Figure 2 c and are exhibiting a rotational (spiralling) beat which describes a conicoidal envelope. Marker "B" denotes cilia that are relatively straight in postural configuration and are exhibiting a rotational (spiralling) beat which describes a conical envelope. Marker "□" denotes cilia that are not in any definite shape as shown in Figure 2b. However, their beat pattern is either rotational and describes an irregular cone-shape envelope or they simply wriggle about without shaving any definite beat pattern. (625 × taken at 400 fps under Nomarski optics).

Plate III. A print of two successive frames on a profile view of the edge of a stationary *Opalina*. Indications of TOP and BOTTOM refer to the surfaces of the organism with the side edge of the body in between the dotted markers. The print serves to show the "optical-section" focusing characteristic of Nomarski optics. The focus of the optical set-up is centered on the right top surface with the cilia at this region in sharp focus; however, organelles at any other level are consequently out of sharp focus because of the negligible depth of the optics. The notations of markers and specifications are the same as in Plate II.



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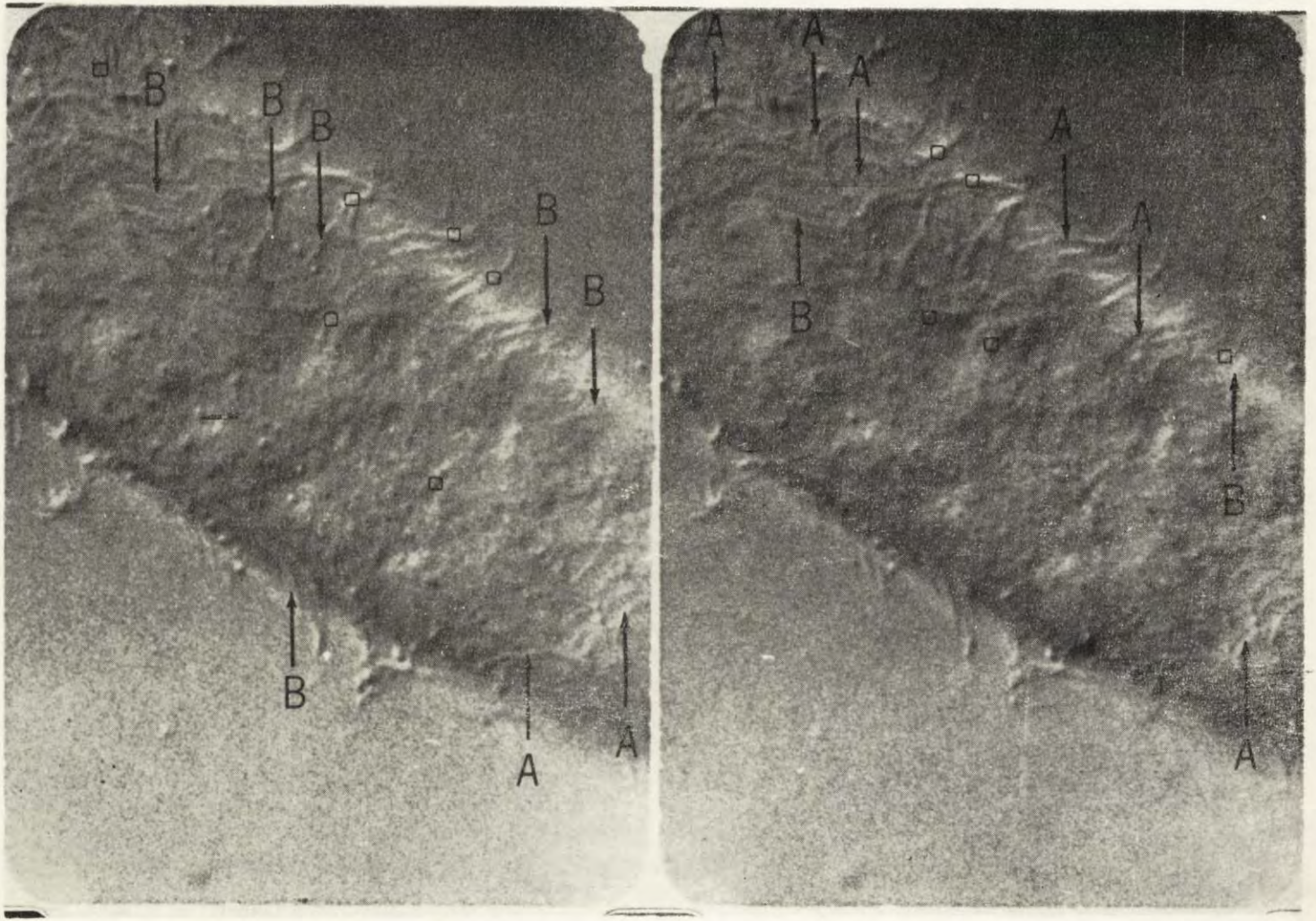

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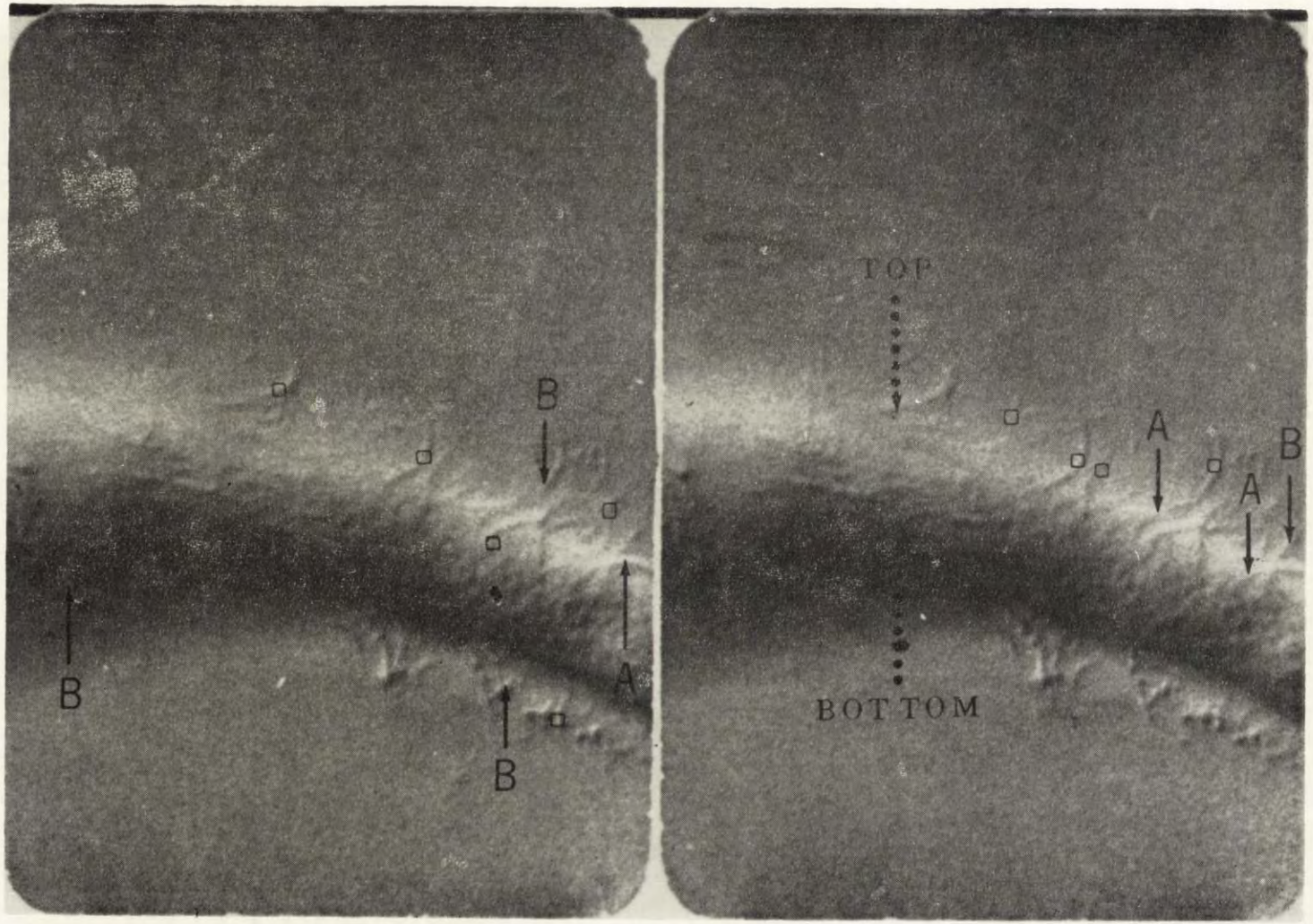

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Effects of Various Substances on Cell Shape, Motile Activity and Membrane Potential in *Amoeba proteus*

Synopsis. Cells of *Amoeba proteus* ceased their normal locomotion and changed cell shape after incubation with various Ca^{2+} antagonists (Mn^{2+}, La^{3+}, UO_2^{2+}, Concanavalin A, and Verapamil). Changes in the cell shape were accompanied by a membrane depolarization. By increasing the external Ca^{2+}-concentration these effects were reversible. Treatment with an anesthetic (benzamide) and with substances which probably affect cytoplasmic structures (citric acid and ruthenium red) also caused inhibition of cytoplasmic streaming. The effect of benzamide was, in contrast to that of citric acid, reversible by calcium. Incubation with ruthenium red resulted in a hyperpolarization of the cell membrane although the amebae became stationary. Substances which had no recognizable influence on shape and motility of amebae (acetylcholine, PCMB, cysteine, histamine) apparently had no effect on the bioelectrical properties of the cells. Electrophysiological investigations on the action of pinocytosis inducers (alcian blue, ATP, NaCl) indicate a similar controlling mechanism for ameboid movement and andocytotic processes. The central role of calcium ions in permeability changes of the cell membrane is discussed.

Several authors have demonstrated potential differences (PD) in free living cells such as amebae (Batueva 1964, Batueva and Lev 1967, Bingley 1964, 1966, Bingley and Thompson 1962) or ciliates (Dryl 1970, Machemer 1970). Results of Bingley (1966) and Batueva (1965 a, b) first indicated a relationship between motility and membrane potential in *Amoeba proteus*.

According to previous results (Braatz-Schade and Haberey 1975, Braatz-Schade et al. 1973) the membrane potential in *Amoeba proteus* is directly correlated to the morphology of cell shape and motile activity of the cells. Depending on the actual form and locomotion rate of the amebae, bioelectrical potentials from −5 mV to −90 mV were measured. Amebae with high locomotive activity are always characterized by high potential values while low PDs were measured in stationary forms. This correlation was found in amebae

taken from cultures with a physiological pH of 6.5 as well as in amebae from cultures with various H-ion-concentrations in the external medium (Braatz-Schade and Stockem 1972).

The importance of the external calcium ion concentration on cell shape and motility has been pointed out by many investigators (Cuthbert 1970, Koketsu 1965, Loewenstein 1966). In regeneration experiments (Braatz-Schade and Haberey 1975) calcium was found to suppress the influence of changes in the external H-ion-concentration. Hence changes in the calcium concentrations may effect the permeability of the membrane in two different ways: by changing the structure of the membrane itself or by an influence on the surface charges which may form an electrical barrier for the transport of ions.

Motility, cell shape and membrane potential differences were analysed in amebae subjected to substances known to change cell membrane properties.

Material and Methods

Amoeba proteus (dark strain, Princeton collection) was cultured in Chalkley's medium (Haberey and Stockem 1971). For test series, only polypodial, normal locomotive forms of amebae were chosen. After washing, the cells were transfered to the test solutions (see Table 1).

Table 1

Summary of the tested substances

Agent	Solvent	Concentration	pH-value
manganese chloride	Chalkley medium	3 mM	7.0
lanthanum nitrate	Chalkley medium	3 mM	7.0
Concanavalin A	Chalkley medium	0.05%	7.0
uranyl acetate	Tris-maleate-buffer	10^{-6} M	6.9
Verapamil (Isoptin, Iproveratril; Fa. Knoll, Ludwigshafen)	Chalkley medium	10^{-3} M	7.0
benzamide	Chalkley medium without Ca^{2+}	50 mM	7.0
ruthenium red	Chalkley medium	0.001% 0.0001%	6.5
citric acid	Chalkley medium	1 mM	6.0
acetylcholine	Chalkley medium	10^{-4} M	6.5
histamine		10^{-4} M	6.5
PCMB		10^{-4} M	6.5
cysteine		10^{-3} M	6.5
alcian blue	Chalkley medium	0.01%	6.5
NaCl	Phosphate buffer 0.01 M	0.125 M	6.0
ATP	ion — solution	5 mM	7.0

The concentration of the test compounds were taken from Korohoda[1] who had shown their effectiveness on the motile activity of amebae.

Micromanipulation of the glass microelectrodes was described in previous papers (Braatz-Schade 1974, Braatz-Schade and Haberey 1975, Braatz-Schade et al. 1973).

Electrophysiological measurements were made using the method of Bingley (1964).

Results

Polypodial amebae show an average potential difference of −50 mV ±12 (Braatz-Schade and Haberey 1975, Braatz-Schade et al. 1973). Treatment of such cells with various test media (Table 1) resulted in general in changes of shape, motile activity and bioelectrical potentials. Since the rate and the characteristics of these alterations depended on the individual substance applied, it is necessary to report the effects in detail. A summary of the results is found in Table 2.

(a) Effect of manganese chloride

Shortly after exchange of the culture medium with a 3 mM solution

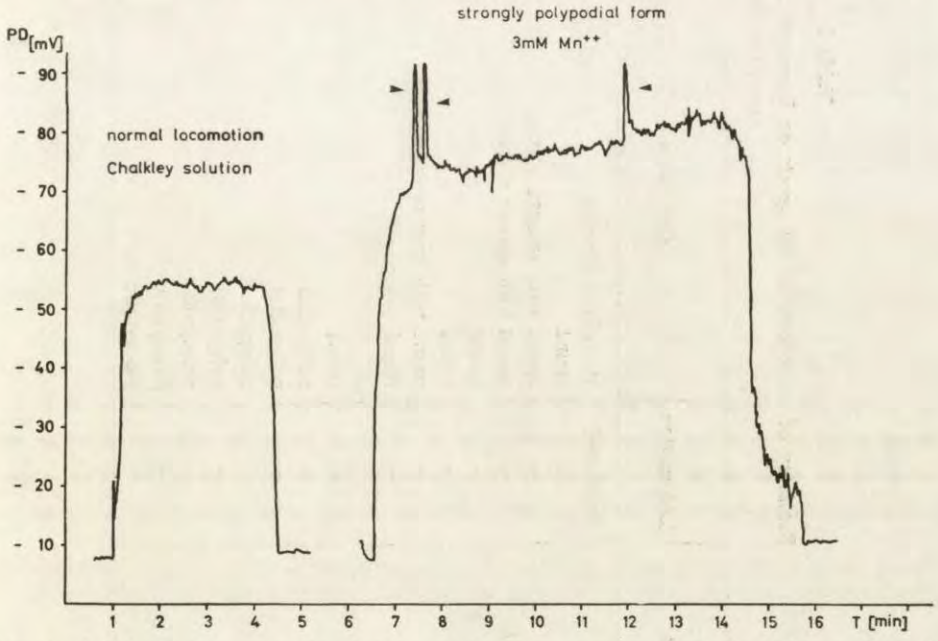

Fig. 1. Original PD recording before and after exposure of *Amoeba proteus* to 3 mM $MnCl_2$. The second curve clearly shows the increase of the membrane potential shortly after addition of Mn^{2+} while the ameba became strongly polypodial

[1] I wish to thank Dr W. Korohoda for the determination of the active substances.

Table 2

Summary of the effects of the agents on locomotion, cell shape and membrane potential in *Amoeba proteus*

Agent	Locomotion	Cell shape	PD±SD (mV)	Time (min)	Reversibility by	
					Chalkley medium	Ca^{2+}
	intensified	strongly polypodial	−60±10	15	/	/
$MnCl_2$	−	round	−32± 5	30	+	+
$La(NO_3)_3$	−	round with smooth surface	−23±10	15–30	−	+
Concanavalin A	−	round with smooth surface	−16± 9	15–30	−	(+)
UO_2-acetate	−	round	−11± 3	15–30	−	+
Verapamil	−	round	−20± 9	30	−	+
benzamide	−	round with long protuberances	−24± 9	30	−	+
ruthenium red	−	round	−64±12	30–60	PD dropped to values which corresponded to the round forms	
citric acid	−	round	− 3± 2	30–60	−	−
acetylcholine	+	polypodial	−41± 2	30–60	/	/
histamine	+	polypodial	−47± 8	30–60	/	/
PCMB	+	polypodial	−40± 4	30–60	/	/
cysteine	+	polypodial	−50± 3	30–60	/	/
alcian blue	−	pinocytotic	−27± 4	10–30	−	−
NaCl	−	pinocytotic	− 3±1.5	10–30	−	(+)
ATP	−	pinocytotic	+ 4± 1	10–20	−	(+)

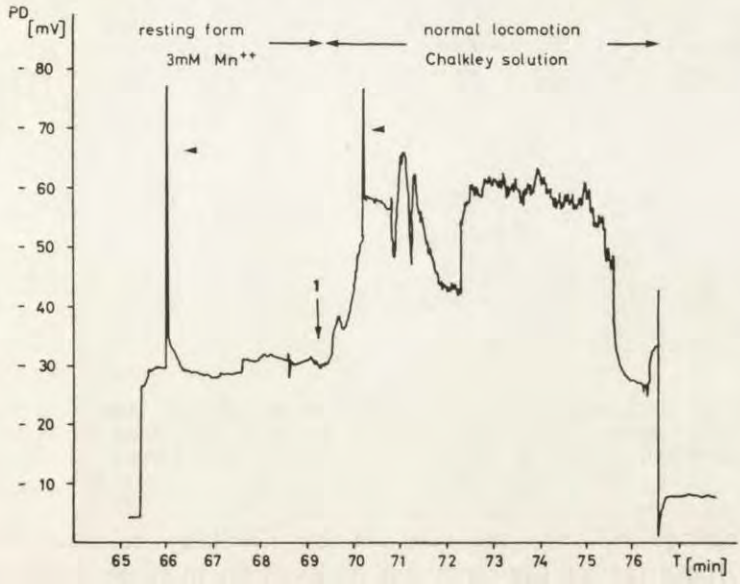

Fig. 2. Original PD recording of a motionless ameba incubated with 3 mM $MnCl_2$ for more than one hour. After washing with normal culture medium (arrow 1) the membrane potential increased to original values (compare with Fig. 1, first curve). The triangles indicate the discharging of the vacuole

of manganese chloride, the amebae became strongly polypodial and attached to the substratum. Simultaneously, they showed a high motile activity while the membrane potential raised to an average potential of −60 mV ±10 (Fig. 1). After a 30 min, treatment with Mn^{2+}, however, locomotion became sluggish and the membrane potential decreased to −32 mV ±5 (Fig. 2). The effect of Mn^{2+} was reversible either by washing with Chalkley solution (pH 6.5) (Fig. 2) or by increasing the Ca^{2+} — concentration in the test medium.

(b) Effect of lanthanum nitrate

About 5 min after transfering to a 3 mM lanthanum solution the amebae stopped moving and became spherical with a smooth surface. These cells showed a low average potential of −23 mV ±10 (Fig. 3). The effect of La^{3+} was not reversible by washing with Chalkley solution. Increasing the Ca^{2+}-concentration from 0.04 mM up to 0.5 mM, however, led to an increase of the PD to the original values (Fig. 3).

(c) Effect of Concanavalin A

Like lanthanum nitrate, 0.05% Concanavalin A forced the amebae to cease locomotion and round off. Simultaneously, the average potential fell to −16 mV ±9. In comparison to measurements on similar forms under physiological conditions, bioelectrical potentials in these cells were

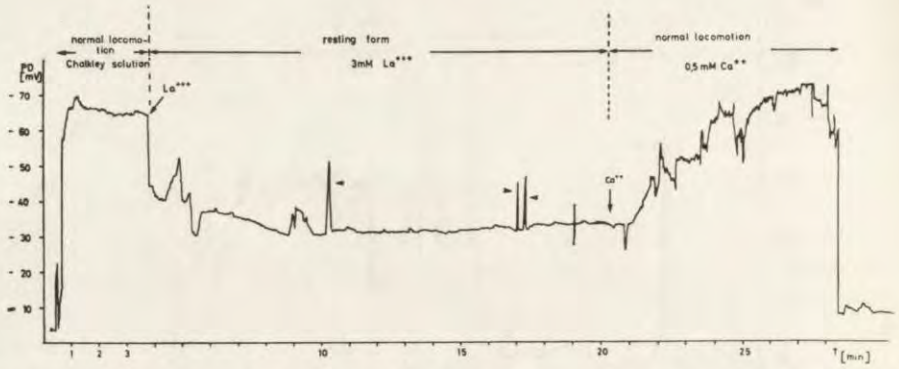

Fig. 3. Effect of 3 mM lanthanum nitrate on membrane potential of *Amoeba proteus*: Shortly after addition of La^{3+} the potential decreased while the ameba stopped locomotion. Increasing the Ca^{2+} — concentration had the antagonizing effect. The triangles indicate the discharging of the vacuole

high. Exchange of the test medium by Chalkley solution failed in reversing the effect of Concanavalin A. Even an increase of the Ca^{2+} — concentration caused only a small rise of PD. The amebae did not recover completely.

(d) Effect of uranyl acetate

Shortly after treatment with 10^{-6} M uranyl acetate, the amebae stopped moving and gradually rounded up. The bioelectrical potentials dropped to −11 mV ±3 during these morphological changes. Cells returned to their normal locomotion rates after increasing the Ca-ion-concentration in the medium. Simultaneously the PD rose to values of about −45 mV ±3. By washing with normal culture medium, however, the effect of uranyl acetate was not abolished.

(e) Effect of Verapamil (Iproveratril)

Verapamil is known for its coronal dilating effect. Application to *Amoeba proteus* inhibited movement of the cells. The simultaneous measurement of membrane potentials showed values of −20 mV ±9 (Fig. 4). Reversal of Verapamil dependent effects could only be reached by increasing the Ca^{2+} — concentration in the test solution (Fig. 5).

(f) Effect of benzamide

After treatment with 50 mM benzamide — a relaxing agent (Korohoda and Wohlfarth-Bottermann 1976) — *Amoeba proteus* formed long pseupodia in all directions without any change of position. The cells showed only cytoplasmic streaming. Electrophysiological measurements in these amebae gave values of −24 mV ±9.

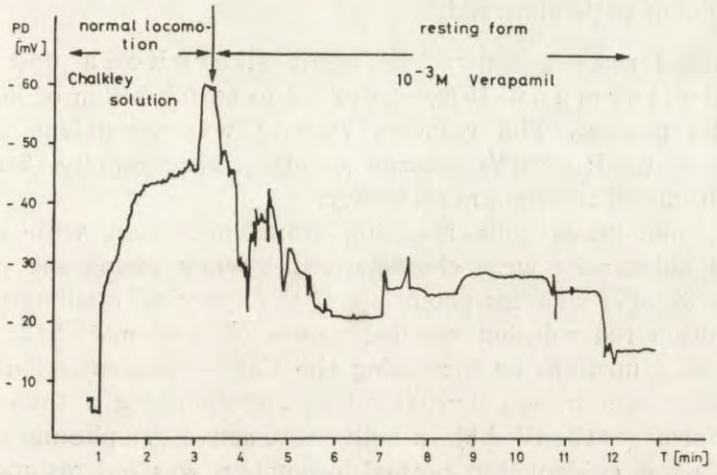

Fig. 4. PD recording during treatment of *Amoeba proteus* with 10^{-3} M Verapamil: The ameba stopped locomotion and the membrane potential was diminished

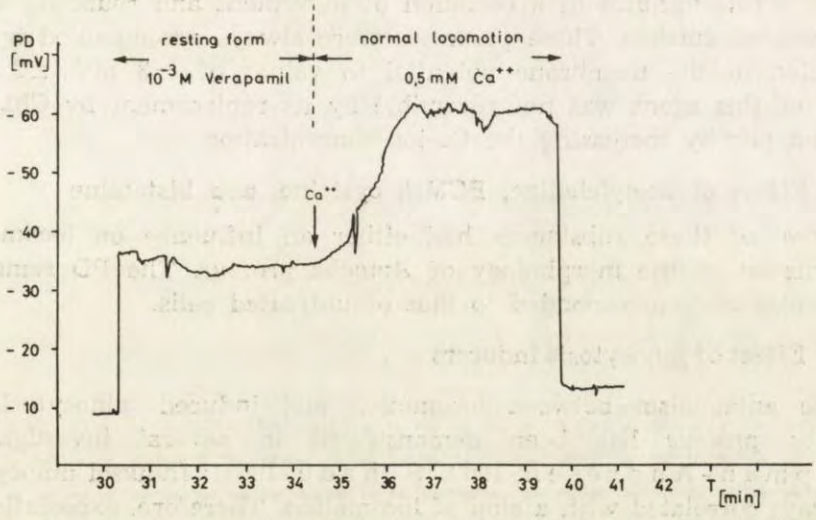

Fig. 5. After application of Ca^{2+} to the test medium with 10^{-3} M Verapamil *Amoeba proteus* began to stream normally and the PD increased to original values

Occasionally positive potentials were found in cells which had even ceased cytoplasmic streaming.

The replacement of benzamide by culture solution had no antagonizing effect. But after increasing the Ca-ion-concentration in Chalkley medium all treated amebae resumed locomotion. At the same time, the PD reached values typical for polypodial moving cells.

(g) Effect of ruthenium red

Ruthenium red — a contracting agent (Korohoda and Wohlfarth-Bottermann 1976) — also led to an inhibition of locomotion in *Amoeba proteus*. The reaction velocity was dependent upon the concentration used: 0.001% induced an effect very rapidly (3 min) and 0.0001% produced an effect more slowly.

Round, motionless cells resulting from incubation with all other mentioned substances were characterized by low membrane potentials (−20 to −30 mV) whereas potentials of the spherical stationary cells in the ruthenium red solution reached values of −64 mV ±12. Washing with Chalkley medium or increasing the Ca^{2+} — concentration resulted in diminished membrane potential values corresponding to those of other spherical forms (−17 mV ±8). In both cases, active cytoplasmic streaming of the cells was evident, but normal locomotion was not resumed.

(h) Effect of citric acid

The addition of citric acid to a final concentration of 1 mM resulted within a few minutes in a cessation of movement and rounding up of all observed amebae. These processes were always accompanied by the reduction of the membrane potential to values of −3 mV ±2. The effect of this agent was not reversible by its replacement by Chalkley medium nor by increasing the Ca-ion-concentration.

(i) Effect of acetylcholine, PCMB, cysteine, and histamine

None of these substances had either an influence on locomotion patterns or on the morphology of *Amoeba proteus*. The PD remained unchanged and corresponded to that of untreated cells.

(j) Effect of pinocytosis inducers

The antagonism between locomotion and induced pinocytosis in *Amoeba proteus* has been demonstrated in several investigations (Chapman-Andresen 1962, Schade 1971): Induced pinocytosis is always correlated with a stop of locomotion. Therefore, exploration of the influence of these inducers on membrane potential in *Amoeba proteus* seemed to be appropriate.

(1) Effect of alcian blue

0.01% alcian blue was used to induce pinocytosis. After treatment cells immediately stopped locomotion. The first pinocytotic channels could be recognized after 5 min in regions of the former uroid (Chapman-Andresen 1962, Stockem 1966). The simultaneous potential measurement demonstrated that PD decreased to values which were

clearly lower than those of locomoting cells. It was difficult to obtain an exact value because the potential was very unstable and oscillated between −23 mV and −31 mV in all measurements. The effect of alcian blue was not reversible.

(2) Effect of NaCl

0.125 M NaCl had a stronger effect than alcian blue on both pinocytotic activity and membrane depolarization. PD reached values of −3 mV ±1.5 (measured in stationary pinocytotic active amebae). Increasing the calcium-ion-concentration had no totally reversible effect on shape and locomotive streaming of cells and caused only a small increase of membrane potential.

(3) Effect of ATP

5 mM ATP also induced endocytotic activity in *Amoeba proteus*. Again the cells stopped locomotion immediately after application of ATP and showed pinocytotic channels within 5 min. Electrophysiological investigations in such amebae demonstrated positive potentials of +4 mV ±1. Subsequent addition of Ca^{2+} resulted in a rise of PD to values of −15 mV ±2 without remarkable changes in the shape and locomotion rate of the amebae.

Discussion

The electrophysiological investigations on *Amoeba proteus* reported here in this paper clearly demonstrate that the membrane potential can be influenced by numerous agents. Nearly all tested substances (see Table 2) have an effect on locomotion, cell shape, and bioelectrical membrane potentials. After incubation with various media cells stopped locomotive streaming and the electrical activity of cell membrane was altered.

The theory that cations, and especially Ca-ions, can be bound to the external negative charges of the mucous layer and thereby modulate the permeability of the plasmalemma has been discussed by several authors (for further reference see: Braatz-Schade 1974, Komnick et al. 1973). Changes in the surface charge are directly responsible for modifications in electrical potentials and influence the barrier function of the membrane.

The significance of Mn^{2+} and La^{3+} as antagonists to Ca^{2+} was confirmed in many experiments (see: Braatz-Schade 1974). Both ions have a strong affinity to calcium binding sites on the membrane and,

therefore, can displace or substitute the Ca-ions. This caused a change in permeability of the membrane and effects the membrane potentials (Fig. 1–3). Mn-ions change membrane properties slower than La-ions. Furthermore, Mn^{2+} can replace Ca-ions for a certain period of time (Fig. 1). This phenomenon corresponds to results from Yanaga and Holland (1969). The effect of La^{3+} and Mn^{2+} can be abolished by increasing the external Ca-ion-concentration. This confirms the calcium antagonistic influence described by several authors (for further reference see: Braatz-Schade 1974).

With the results presented here a similar mechanism might be proposed for Concanavalin A and UO_2^{2+} as calcium reverses the action which these substances have on membrane permeability. Verapamil, a Ca^{2+} antagonistic drug (Golenhofen and Lammel 1972, Golenhofen and Petranyi 1969) caused the same locomotion pattern and membrane depolarization (Fig. 4) as the other Ca^{2+} antagonistic substances. These results indicate that calcium plays an important role for stimulus reception at the plasmalemma.

Surface Ca^{2+} controls the membrane permeability to ions. By displacing the calcium from its extracellular binding sites by drugs, the stability of membrane is lowered and thereby the permeability for cations increases (Brandt and Freeman 1967, Brandt and Hendil 1970, Josefsson et al. 1975b). The resulting change in the ionic composition of the intracellular milieu can be followed by measuring the depolarization of the membrane and the change in membrane resistance. The depolarization of the membrane is described in the presented paper. Results of other authors confirm the relationship between membrane potential and resistance (Josefsson et al. 1975a).

A similar mechanism about extracellular calcium is discussed for nerve membranes (Singer and Tasaki 1968). In nerve cells the sodium pump is responsible for regenerating the ionic distribution across the membrane. In amebae there is no evidence for active transport across the plasmalemma (Batueva and Lev 1967, Bruce and Marshall 1965). The pulsating vacuole would possibly undertake this function of ion regulation. This theory is supported by results of Chapman-Andresen and Dick (1962) and by own observations that hyperpolarization of the membrane occurs when the vacuole is discharged (Fig. 1, 3, triangles). Perhaps amebae restore the normal potential by regulating the internal ionic composition with help of the vacuole.

It is an interesting fact that amebae can normally move for a certain length of time without any extracellular calcium (Schade 1971). Therefore, the motility controlling mechanism does not seem to be

necessarily related to direct influx of Ca-ions but to the presence of bound Ca^{2+} at the fixed negative charges on the membrane surface. Consequently, Ca^{2+} antagonists (such as La^{3+}, Mn^{2+}, UO_2^{2+} etc.) do not block a calcium influx but in fact interfere with binding processes at the membrane.

Although the exact influence of benzamide on membrane properties is yet unknown, this substance seems to alter the structure of the membrane in such a way that the plasmalemma loses its function as a selective barrier. Ca-ions, however, are able to antagonize this distruction by restoring the membrane, normal locomotion and the physiological PD in *Amoeba proteus*.

Ruthenium red was the only agent which caused a hyperpolarization of membrane but simultaneously stopped locomotion in the cells. It is demonstrated that ruthenium red inhibits the Ca^{2+} pump of isolated ER and mitochondria (Madeira and Antunes-Madeira 1974, Moore 1971). Although it is yet unknown whether ruthenium red penetrates the cell membrane of amebae, the presented results suggest that this substance affects cytoplasmic structures by an unknown trigger mechanism which could release intracellular Ca^{2+} and hence induce a contracted state. The results with ruthenium red indicate that bioelectrical potentials are not only correlated with shape and motility of amebae but are also an indicator for the activity of the contractile apparatus of the cell. In this respect the results of Bingley and Thompson (1962) are of interest. They measured a different PD in uroid (−70 mV) and front pseudopodia (−30 mV) of amebae. That would mean that the uroid is an area of contraction whereas the pseudopodia are areas of relaxation.

Citric acid caused irreversible effects. It probably does not only alter membrane properties but may react directly on cytoplasmic structures.

From the results of others and those presented here, it seems to be justified to arrange the tested agents into 3 groups:

(1) Ca^{2+} antagonists which displace or substitute extracellular calcium at the membrane surface: Mn^{2+}, La^{3+}, UO_2^{2+}, Concanavalin A, Verapamil.

(2) Anesthetics which alter the structure of the membrane: benzamide.

(3) Substances which directly or indirectly affect cytoplasmic structures: citric acid, ruthenium red.

Even induction of pinocytosis in *Amoeba proteus* with alcian blue, NaCl and ATP results in a membrane depolarization. The rate of depolarization depends on the specific inductor. These results correspond to recent investigations of Josefsson et al. (1975a). They found that the membrane potential decreases almost proportionally to the number of channels when pinocytosis was induced by alkali metal ions. Inhibition

of pinocytosis by Ca^{2+} was accompanied by an increase of membrane potential and of input resistance (see: Josefsson 1975). Similar results were earlier described by Brandt and Freeman (1967). They observed that Ca^{2+} increased the effective membrane resistance of *Amoeba proteus* and thereby had an inhibitory effect on pinocytosis.

The fact that the influence of the examined agents is not totally reversible by calcium could be explained by an irreversible binding of these substances to the membrane or by an irrecoverable damage of the mucous layer (Braatz-Schade and Stockem 1973). In the results reported here all substances which influence motility or endocytotic phenomena at the same time affect the permeability of membrane. Changes of the selective permeability of the cell membrane could therefore be the mechanism which controls ameboid movement as well as endocytotic processes by electrical phenomena.

A conductance from plasmalemma to the contractile apparatus is yet unknown. The Ca^{2+}pump located in the vesicle membrane (Reinold and Stockem 1972) may form the connecting link. The mechanism of endocytosis discussed by Allison (1973) is very similar to this theory.

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ZUSAMMENFASSUNG

Nach Inkubation mit verschiedenen Ca^{2+} — Antagonisten (Mn^{2+}, La^{3+}, UO_2^{2+}, Concanavalin A und Verapamil) stellen Bewegungsstadien von *Amoeba proteus* ihre Fortbewegung ein und verändern ihre Zellform. Diese Phänomene werden von einer Depolarisation der Membran begleitet. Durch Erhöhung der extrazellulären Ca^{2+} — Konzentration sind die genannten Effekte reversibel. Das Anaesthetikum Benzamid und Substanzen, die wahrscheinlich an cytoplasmatischen Strukturen angreifen (Zitronensäure und Ruthenium Rot), bewirken ebenfalls eine Hemmung der amöboiden Bewegung. Die bei Anwendung von Benzamid und Zitronensäure gleichzeitig auftretende Depolarisation der Zellmembran ist nur bei Benzamid durch Calcium reversibel. Nach Inkubation mit Ruthenium Rot kann man eine Hyperpolarisation der Membran feststellen. Substanzen, die weder einen erkennbaren Einfluss auf Gestalt noch Bewegungsaktivität der Amöben haben (Acetylcholin, PCMB, Cystein, Histamin) verändern auch die bioelektrischen Eigenschaften der Zellen nicht. Elektrophysiologische Untersuchungen zur Wirkung von Pinocytose-Induktoren (Alcian Blau, ATP, NaCl) deuten auf einen ähnlichen Kon-

trollmechanismus für amöboide Bewegung und endocytotische Prozesse hin. Die zentrale Bedeutung der Ca-Ionen für Permeabilitätsänderungen der Zellmembran wird diskutiert.

REFERENCES

Allison A. C. 1973: The role of microfilaments and microtubules in cell movement, endocytosis and exocytosis. In: Locomotion of Tissue Cells, Ciba Foundation Symp., 14, 109–148.

Batueva I. V. 1964: On the value of membrane potential in *Amoeba proteus*. Cytologija, 6, 209–213.

Batueva I. V. 1965 a: The electrical parameter of *Amoeba proteus* I. Motionles amoebae. Cytologija, 7, 188–196.

Batueva I. V. 1965 b: The electrical parameters of *Amoeba proteus*. II. The motile amoebae. Cytologija, 7, 553–561.

Batueva I. V. and Lev A. A. 1967: Electrochemical properties of the plasmalemma of *Amoeba proteus*. I. The dependence of membrane potential on concentration of potassium and sodium chloride and sulfate solutions. Cytologija, 9, 680–691.

Bingley M. S. 1964: Methods for the measurements of bioelectric potentials in amoebae. Exp. Cell Res., 34, 266–282.

Bingley M. S. 1966: Membrane potentials in *Amoeba proteus*. J. exp. Biol., 45, 251–267.

Bingley M. S. and Thompson C. M. 1962: Bioelectric potentials in relation to movement in amoebae. J. Theor. Biol., 2, 16–32.

Braatz-Schade K. 1974: Elektrophysiologische Untersuchungen zur Steuerung des Bewegungsverhaltens von *Amoeba proteus*. PhD Thesis, Bonn.

Braatz-Schade K. and Haberey M. 1975: Bioelectrical potentials and motile activity in *Amoeba proteus*. Cytobiologie, 11, 87–94.

Braatz-Schade K. and Stockem W. 1972: Pinocytose und Bewegung von Amöben. IX. Der Einfluss von verschiedenen Kationen auf das Bewegungsverhalten von *Amoeba proteus*. Arch. Protistenk., 114, 272–290.

Braatz-Schade K. and Stockem W. 1973: Pinocytose und Bewegung von Amöben. IX. Der Einfluss von verschiedenen Kationen auf das Bewegungszierten Endocytose bei *Amoeba proteus*. Z. Zellforsch., 137, 97–109.

Braatz-Schade K., Haberey M. and Stockem W. 1973: On the correlation between membrane potential, cell shape and motile activity in *Amoeba proteus*. Exp. Cell Res., 80, 456–458.

Brandt P. W. and Freeman A. R. 1967: Plasma membrane: Substructural changes correlated with electrical resistant and pinocytosis. Science, 3, 582–585.

Brandt P. W. and Hendil K. B. 1970: Properties of the plasma membrane of amoeba. Surface Chemistry of biol. Systems., 323–335.

Bruce D. L. and Marshall J. M. 1965: Some ionic and bioelectrical properties of the ameba *Chaos chaos*. J. gen. Physiol., 49, 151–178.

Chapman-Andresen C. 1962: Studies on pinocytosis in amoeba. C. r. Trav. Lab. Carlsberg, 33, 73–264.

Chapman-Andresen C. and Dick D. A. T. 1962: Sodium and bromine fluxes in the amoeba *Chaos chaos* L. C. r. Trav. Lab. Carlsberg, 32, 445.

Cuthbert A. W. 1970: A symposium on calcium and cellular function. Publ. by Macmillan and Co., Ltd.

Dryl S. 1970: Response of ciliate protozoa to external stimuli. Acta Protozool., 7, 325–333.

Golenhofen K. and Lammel E. 1972: Selective suppression of some components of spontaneous activity in various types of smooth muscle by Iproveratril (Verapamil). Pflügers Arch., 331, 233–243.

Golenhofen K. and Petranyi P. 1969: Spikes of smooth muscle in calcium--free solution (isolated taenia coli of the guinea-pig). Experientia, 25, 271–273.

Haberey M. and Stockem W. 1971: *Amoeba proteus*: Morphologie, Zucht und Verhalten. Mikrokosmos, 60, 33–42.

Josefsson J. O. 1975: Studies on the mechanism of induction of pinocytosis in *Amoeba proteus*. Acta physiol. scand. suppl., 432, 5–65.
Josefsson J. O., Holmer N. G. and Hansson S. E. 1975: Membrane potential and conductance during pinocytosis induced in *Amoeba proteus* with alkali metal ions. Acta physiol. scand., 94, 278–288.
Josefsson J. O., Johansson G. and Hansson S. E. 1975: Inhibition of induced pinocytosis in *Amoeba proteus* by membrane stabilizing drugs. Acta physiol. scand., 95, 270–285.
Koketsu K. 1965: The role of calcium in membrane excitation. Proc. 23rd Int. Congr. Physiol. Sci., 4, 521–540.
Komnick H., Stockem W. and Wohlfarth-Bottermann K. E. 1973: Cell motility: Mechanisms in protoplasmic streaming and amoeboid movement. Int. Rev. Cytol., 34, 169–249.
Korohoda W. and Wohlfarth-Bottermann K. E. 1976: Effects of relaxation and contraction stimulating solutions on the structure of cytoplasmic fibrils in plasmodia of *Physarum polycephalum*. Acta Protozool., 15, 195–202.
Loewenstein W. R. 1966: Permeability of membrane junctions. Ann. N. Y. Acad. Sci., 137, 441–472.
Machemer H. 1970: Korrelation zwischen Membranpotential und Fortbewegung bei *Stylonychia* (*Hypotricha*). Naturwissenschaften, 8, 389–399.
Madeira V. M. C. and Antunes-Madeira M. C. 1974: Interaction of ruthenium red with isolated sarcolemma. J. Membr. Biol., 17, 41–50.
Moore C. L. 1971: Specific inhibition of mitochondrial Ca^{2+} transport by ruthenium red. Biochem. Biophys. Res. Commun., 42, 298.
Reinold M. and Stockem W. 1972: Darstellung eines ATP-sensitiven Membransystems mit Ca^{2+} — transportierender Funktion bei Amöben. Cytobiologie, 6, 182–194.
Schade K. 1971: Der Einfluss von Ionen auf das Verhalten von *Amoeba proteus*. Hausarbeit der Fachprüfung f.d. Lehramt am Gymnasium, Bonn.
Singer I. and Tasaki I. 1968: Nerve excitability and membrane macromolecules. In: Biological Membranes, 347–410.
Stockem W. 1966: Pinocytose und Bewegung bei Amöben. I. Die Reaktion von *Amoeba proteus* auf verschiedene Markierungssubstanzen. Z. Zellforsch., 74, 372–400.
Yanaga T. and Holland W. C. 1969: Effects of Mn on transmembrane potential and contractility of atrial muscle. Am. J. Physiol., 127, 1280–1284.

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Effects of Ionic Ratios vs. Osmotic Pressure on the Rate of the Water - expelling Vesicle of *Tetrahymena pyriformis*

Synopsis. An analysis of the effects of solutions of single non-toxic metallic cations (Li^+, Na^+, K^+, Ba^{++}, Ca^{++} and Mg^{++}) shows that increases of each slow the rate at which the water-expelling vesicle of *Tetrahymena pyriformis* expels water except for Ba^{++}. When both a monovalent ion and a divalent one, especially K^+ and Ca^{++}, are present, the rate of the WEV varies, whether or not the osmotic pressure of the surrounding fluid varies. As the monovalent (Na^+)/divalent (Ca^{++}) ratio is changed, the rate of the WEV increases from 3.6/1 to 32.3/1, then remains nearly the same from 37.3/1 to 132.1/1, then decreases again above 132.1/1. Ba^{++}, which acts monovalently at membrane sites, with Ca^{++} increases the rate in solutions up to 1.51/1, decreasing it above 1.51/1, although there is no change of osmotic pressure since both Ba and Ca are divalent. When the $K^+/\sqrt{Ca^{++}}$ (Gibbs–Donnan) ratio in the solution is changed, the rate of the WEV is altered when osmotic pressure is maintained by non-ionic sucrose. Changes in pH alter the rate of the WEV and also alter the Gibbs–Donnan ratio. Statistical analyses of data show that significant alterations are due to monovalent/divalent changes, pH changes, additions of distilled water with sucrose, all of which change the Gibbs–Donnan ratio of the surrounding solution. Changes of osmotic pressure do not alter the rate of the WEV (by adding non-ionic sucrose) unless the Gibbs–Donnan ratio is concomitantly changed. It is concluded that water is imbibed (adsorbed onto protoplasmic proteins) and extruded by active syneresis as ions are exchanged, rather than the water being moved passively in relation to changes of osmotic pressure.

The water expelling vesicles (WEV or "contractile Vacuoles") of freshwater protozoa have long been assumed to fill with and expel water

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by direct response to changes in osmotic pressure as concentrations of dissolved substances change in the surrounding water, e.g., decreasing the rate of expulsion with the increase of concentration of a nonionizing solute (Czarska 1964) or an ionizing one (Herfs 1922; Eisenberg 1925, Kitching 1934, 1951, review, 1967, Yocum 1934, Gaw 1936, Czarska 1964). There is no agreement on how this presumed "osmoregulation" is achieved (Jahn and Boggs 1971).

Some researchers, however, say that even if influx of water into the cell were to be osmotic, the collection of excess water from the cytoplasm and its transfer to and expulsion from the water expulsion vesicle need not be. They assert that relative concentrations of metabolically-active and normally-present cations are involved, regardless of, or interacting with, osmotic pressure (Czarska 1964, Jahn and Boggs 1971, Organ and Bovee 1972).

Physical forces also alter the rate at which the WEV expels water, including temperature (Kitching 1948) increased hydrostatic pressure (Kitching 1951, 1957), direct electric current (Czarska 1964, and alternating current (Friend et al. 1975). So do certain chemical conditions, such as pH changes (Gaw 1936), added deuterium (Kitching and Padfield 1960), some inert gases (Sears and Gittelson 1964), externally added anesthetics (Czarska 1964) or adenosine-triphosphate (Organ et al. 1968). Such effects are not explainable solely in terms of osmotic pressures.

When balances of cations are altered, the rate of the WEV is also affected. Czarska (loc. cit.) found that at a steady external Gibbs-Donnan equilibrium the rate of the WEV does not change significantly, even though external osmotic pressures are changed. Jahn and Boggs (1971) and Organ and Bovee (1973) gave similar reports.

Our studies suggest that the osmotic pressures in the classic sense are not directly involved in the expulsion of the WEV; but that the balance of cations in the external solution (the "Jahn ratio") is related to the rate thereof and that the change in rate is related to the changes in the Gibbs-Donnan equilibrium.

Materials and Methods

Clonal *Tetrahymena pyriformis* were grown axenically in 2% peptone, 0.1% Na-acetate, 0.1% Na_2HPO_4 (Sims 1968) at pH 6.8, ~21°C. Log-phasic cells were immobilized on 1% agar over glass (Organ et al. 1968) and viewed at 400 × by Zeiss–Nomarski variable-phase contrast and interference microscopy.

Expulsive rates of the WEV were timed electrically to tenths of seconds. Each cycle was measured from collapse of the vesicle against its pore to the next collapse, only after a steady rhythm was seen 30–60 s after observations

began. Experiments lasted 5 to 20 min. Control-rate was established for organisms in Chalkley's solution (Chalkley 1930), which has an osmotic pressure of 0.9 atm.

Organisms were centrifuged for 1 min at 3000 rpm, washed once in the solution to be tested, recentrifuged and suspended in fresh test-solution. Experimental organisms were pipetted onto the agar films, covered with a glass cover slip and observed microscopically.

Solutions of single cations (Li^+, Na^+, K^+, Ba^{++}, Ca^{++} and Mg^{++}) were made as 1 M stock solutions of their respective chlorides. Dilutions were made to yield solutions of 0.48 to 4.8 atm for monovalent cations and of 0.72 to 7.2 atm for divalent cations. Osmotic pressures were calculated by the corrected gas-law equation (Glasstone 1960). $\pi = C \times G)$ RT; where π = osmotic pressure in atmospheres, C = molar concentration, G = cryoscopic coefficient, R = gas constant and T = absolute temperature.

Solutions of monovalent plus divalent ions were made up according to the Gibbs–Donnan ratio, i.e., the ratio of (monovalent cations) to (divalent cations) such that these varied from 0 to ∞, e.g., 0/1 or 0 = all Ca^{++} ions; while ∞ = all monovalently-active cations, either K^+, Na^+ or Ba^{++} (see Czarska 1964, for explanation of Ba^{++} and Jahn 1962, for explanation of why Ba^{++} acts monovalently). Solutions were made from 1.0 M stock solutions of the chlorides of those ions. The pH of these unbuffered solutions was 7.0 at 21°C.

Sucrose solutions of 0.12 to 4.9 atm osmotic pressure were made, both in doubly glass-distilled water and in Chalkley's solution, the latter being diluted to vary both ionic ratios and concentrations of sucrose. Also, Chalkley's solution was diluted to vary ionic ratios with 0.005 M sucrose added to give constant osmolarity. The pH was kept at 7.0 at 21°C.

All stock solutions were stored in special plastic containers to minimize adsorption of cations in the container.

The pH was varied from 6.0 to 8.3 with NaOH and HCl and solutions were buffered with either BES (N, N-Bis (2-hydroxyethyl 1)-2-amino-ethane sulfonic acid) at pH's below 7.0, or with Tris-(hydroxymethyl-amino methane)-HCl at pH's above 7.0. All such solutions were made in Chalkley's solution at constant ionic ratio. All pH measurements were at 21°C.

Each experiment was analyzed by single classification analysis of variance; groups of experiments by two-way analyses of variance; to determine significance (Sokal and Rohlf 1969). Each point on the graphs is the mean of 100 readings (10 readings for ten organisms) ± 1.96 S.E. Computations were done with either a Cogito 412 or Victor 1800 electronic calculator.

Results

(1) In solutions of the increased chlorides of single ions of Li, K, Na, Ca or Mg, the rate of expulsions of the WEV per min decreased, i.e., the time in s between expulsions was longer. Barium ions (Ba^{++}) had little effect (Fig. 1).

(2) In varied solutions containing both a monovalent cation (or monovalently-active one, e.g., Ba^{++}) and a divalent cation the rate of

expulsions per min (or time in s between expulsions) varies, whether or not the osmotic pressure of the fluid is varied.

(A) The monovalent/divalent ratio of K^+/Ca^{++} when varied by dilution alone from 1 M to 0.01 M caused a continuing decrease in osmotic pressure (calculated by the van't Hoff equation for electrolytic solutions) as the monovalent/divalent ratio was altered from 3.6/1 to 132.1/1. The rate of the WEV, however, increased at ratios of 3.6/1 to 32.3/1, remained nearly the same from ratios of 37.3/1 to 132.1/1 and increased again at ratios over 132.1/1 (Fig. 2).

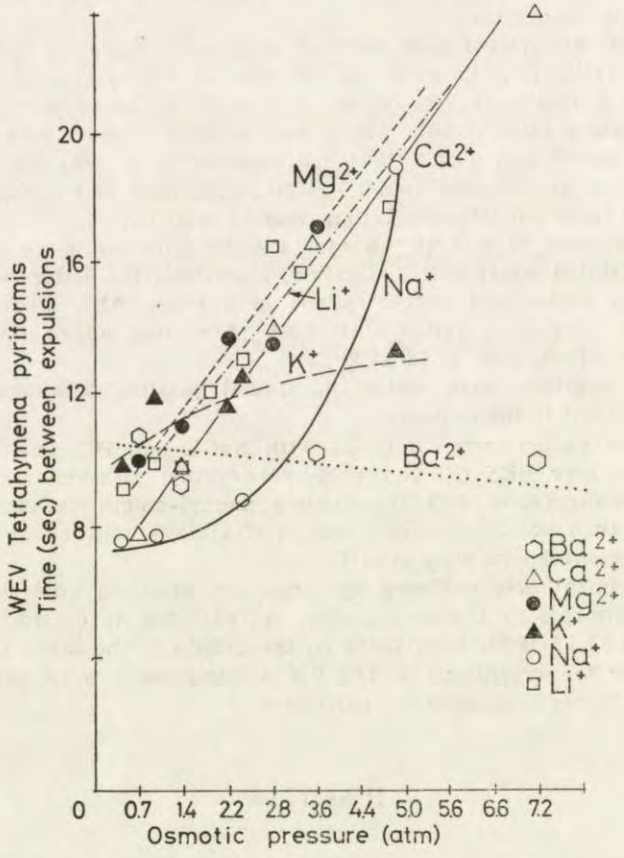

Fig. 1. Rate of the Water Expulsion Vesicle when exposed to osmotic pressures increased by addition of single monovalent or divalent cations

(B) The figures for Na^+/Ca^{++} dilutions also show an increase in rate of the WEV at lower ratios (1.5/1 to 3.6/1) and a relatively steady rate at ratios of 3.6/1 to 21.2/1, despite a continuous decrease of osmotic pressure at the Na^+/Ca^{++} ratios increase (Fig. 3).

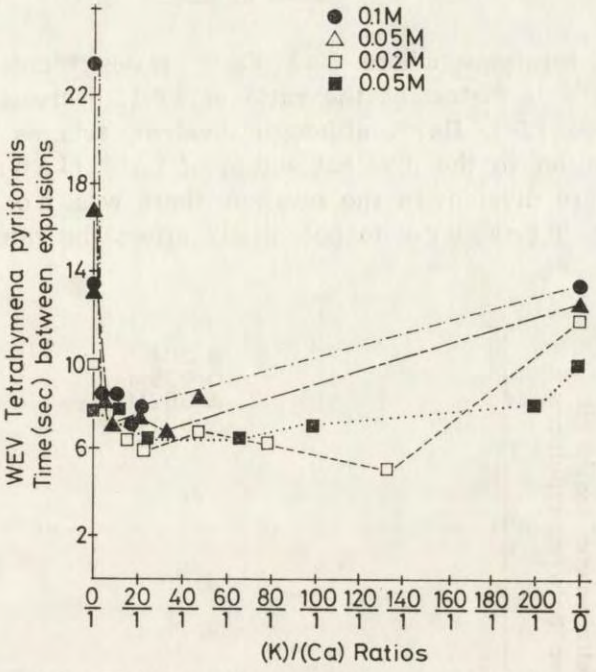

Fig. 2. The rate of the Water Expulsion Vesicle as exposed to different K^+/Ca^{++} ratios

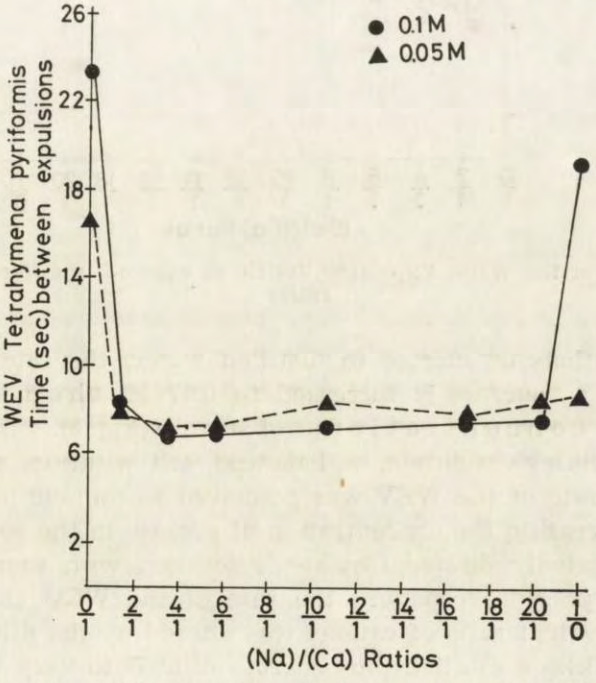

Fig. 3. The rate of the Water Expulsion Vesicle as exposed to different Na^+/Ca^{++} ratios

(C) In solutions where Ba^{++}/Ca^{++} ratios increased, the rate of the WEV is fastest at the ratio of 1.5/1, decreasing slowly for ratios above 1.5/1. Ba^{++}, although divalent, acts as a monovalent ion in relation to the divalent action of Ca^{++} (Jahn 1962). Since both ions are divalent in the solution, there was no change of osmotic pressure to potentially affect the rate of the WEV (Fig. 4).

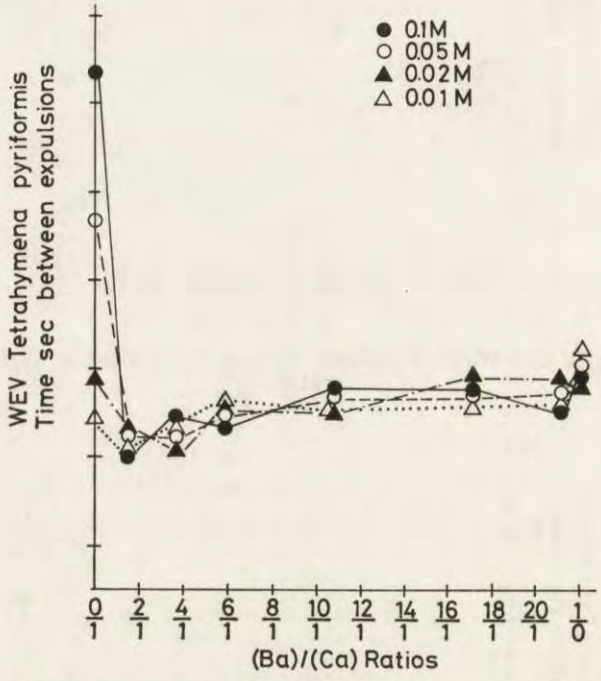

Fig. 4. The rate of the Water Expulsion Vesicle as exposed to different Ba^{++}/Ca^{++} ratios

(3) In solutions of sucrose in distilled water, the rate of the WEV decreases as sucrose is increased to 0.07 M, then increases in higher concentrations of sucrose (Fig. 5).

(4) In Chalkley's solution, a balanced salt solution, no significant effect on the rate of the WEV was perceived as osmotic pressures were altered by increasing the concentration of sucrose in the solution (Fig. 5).

(5) In repeatedly diluted Chalkley's solution with sucrose added to keep osmotic pressure constant, the rate of the WEV changed as the monovalent/divalent ratio of cations was altered by the dilutions (Fig. 6).

(6) In Chalkley's solution plus sucrose diluted to vary both monovalent/divalent cations and sucrose, the rate of the WEV changed as the

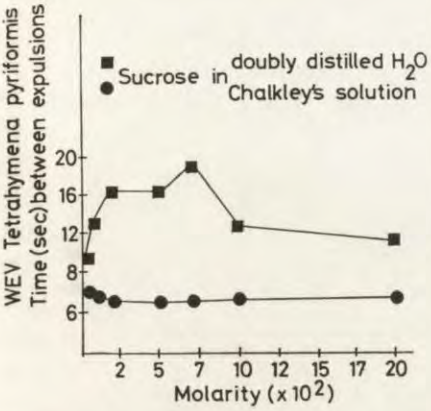

Fig. 5. The rate of the Water Expulsion Vesicle as exposed to various concentrations of sucrose

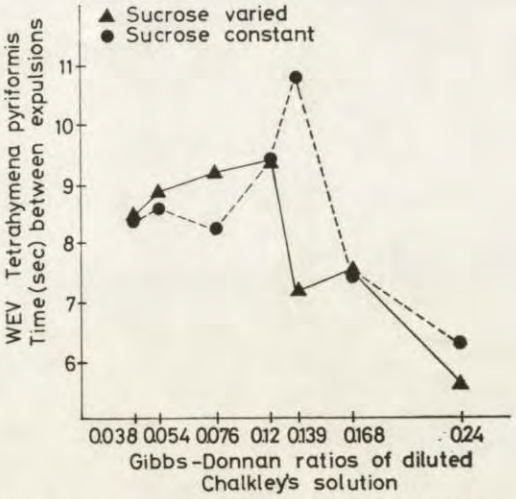

Fig. 6. The rate of the Water Expulsion Vesicle as exposed to the varied and constant sucrose concentrations, accompanied by changes in the Gibbs–Donnan ratio of cations

Gibbs-Donnan ratio ($K^+/\sqrt{Ca^{++}}$) changed, decreasing as the ratio decreased from 0.24 to 0.139, but increasing as the ratio further decreased from 0.139 to 0.038, despite continual decrease of osmotic pressure due to dilution of sucrose (Fig. 6).

(7) When Gibbs–Donnan ratios are calculated for the data of the experiments, the rate of the WEV varies at lower and higher Gibbs-Donnan ratios, rather than unidirectionally with osmotic pressures.

(8) Alterations of pH alter somewhat the rate of the WEV; both

Table 1

Two-way analysis of variance, testing the effect of sucrose treatment (sucrose in Chalkley's medium vs. sucrose in distilled H_2O) vs. sucrose concentration on the rate of the water expulsion vesicle. The variable measured was the time between expulsions of the WEV in seconds

Source of variation	df	SS	MS	F_s
Subgroups	13	3035.88	233.53	
Treatment: sucrose in Chalkley's medium vs. sucrose in distilled H_2O	1	2344.88	2344.88	177.64**
Sucrose concentrations	6	312.75	52.13	3.95*
Interaction of treatment vs. sucrose concentrations	6	378.25	63.04	4.78**
Within subgroups (error)	126	1662.60	13.20	
Total: Subgroups and error within subgroups	139	4698.48		

* $p < .005$, ** $p < 0.001$.

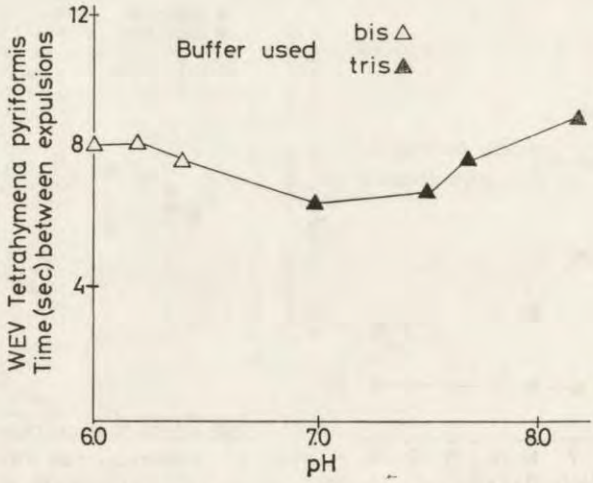

Fig. 7. The rate of the Water Expulsion Vesicle as exposed to changes of pH in Chalkley's solution

lower (acid) and higher (alkaline) pH's decreasing the rate as compared to pH's nearer 7.0 (neutral) (Fig. 7).

(9) Statistical analyses of variance for the data of the above experiments show that: (A) alterations of the rate of the WEV due to different treatments of sucrose concentration in distilled water are significant ($p < 001$) (Table 1). (B) Distilled water and sucrose concentrations significantly alter the rate of the WEV by altering Gibbs–Donnan ratios of

Table 2

Two-way analysis of variance, testing the effect of sucrose treatment (sucrose in diluted Chalkley's medium — sucrose concentration varying vs. sucrose in diluted Chalkley's medium — sucrose concentrations constant) vs. Gibbs–Donnan ratio of cations in diluted Chalkley's solutions on the rate of the water expulsion vesicle. The variable measured was the time between expulsions of the WEV in seconds

Source of variation	df	SS	MS	F_s
Subgroups	13	242.42	18.65	
Treatment: sucrose in diluted Chalkley's medium — sucrose concentration varying vs. sucrose in diluted Chalkley's medium — sucrose concentration constant	1	2.53	2.53	0.71ns
Gibbs–Donnan ratios (of cations in diluted Chalkley's medium)	6	181.27	30.21	8.53**
Interaction of treatment vs. Gibbs–Donnan ratios	6	58.62	9.77	2.76*
Within subgroups (error)	126	446.46	3.54	
Total: Subgroups and error within subgroups	139	688.88		

* $p < 0.025$, ** $p < 0.001$

cations in some way ($p < 0.005$) (Table 1). (C) There is significant interaction between treatment and sucrose concentration ($p < 0.001$). (D) Two-way analyses of variance show there is no significant difference between the different treatments with sucrose on the rate of the WEV (Table 2).

(10) Statistical analyses of variance also show that in experiments where monovalent/divalent ratios are expressed as Gibbs–Donnan ratios: (A) The changes in treatment, i.e., total molarity vs. Gibbs–Donnan ratios, significantly affect the rate of the WEV ($p < 0.005$). (B) The Gibbs–Donnan ratios are significantly related to the rate of the WEV($p < 0.001$). (C) The interactions of molarity and the Gibbs-Donnan ratios are not significant (Table 3). (D) Treatments of Chalkley's solution vs. Chalkley's control-solutions are not significant. (E) Differing osmolarities with constant Gibbs–Donnan ratios are not significant (Table 4).

Table 3

Two-way analysis of variance, testing the effect of total molarity vs. Gibbs–Donnan ratios of $K^+ - Ca^{++}$ions. The variable measured was the time between expulsions of the WEV in seconds. Only Gibbs–Donnan ratios between 0.3 and 1.5 were used

Source of variation	df	SS	MS	F_s
Subgroups	23	774.82	33.69	
Treatment: total molarity vs. Gibbs–Donnan ratio	3	231.85	77.28	5.17*
Gibbs–Donnan ratios	5	424.87	84.97	5.69**
Interaction of molarity and Gibbs–Donnan ratio	15	118.10	7.87	0.53ns
Within subgroups (error)	216	3226.15	14.94	
Total: Subgroups and error within subgroups	239	4000.97		

* $p < 0.005$, ** $p < 0.001$.

Table 4

Analysis of variance of means of times between expulsions of the WEV at a constant Gibbs–Donnan ratio of 1.3 as a function of osmolarity. The control value was measured in Chalkley's medium at a pH of 7.0. The pH of the experimental solutions was also 7.0.

Source of variation	df	SS	MS	F_s
Treatments	4	4.78	1.20	
Chalkley's control vs. treatments	1	2.38	2.38	1.95ns
Among treatments (constant Gibbs–Donnan ratio at different osmolarities)	3	2.40	0.80	0.656ns
Within groups (error)	45	54.95	1.22	
Total: Treatments plus error within groups	49	59.73		

* $F_{0.25}(1,45) = 1.36$, $F_{0.75}(3,45) = 0.289$.

(11) Statistical analyses of pH experiments show that: (A) Differences in rate of the WEV between the control solution and the pH-altered solutions are significant ($p < 0.005$). (B) Differences between varied pH's are also significant ($p < 0.005$) (Table 5).

Table 5

Analysis of variance of means of times between expulsions of the WEV exposed to various pH's at a constant Gibbs–Donnan ratio. BES buffer was used for pH's < 7.0; Tris–HCl was used for pH's ⩾ 7.0. The control was Chalkley's Tris-HCl at a pH of 7.0

Source of variation	df	SS	MS	F_s
Treatments	6	46.83	7.81	
Control vs. different pH's	1	16.43	16.43	10.60*
Among pH's	5	30.40	6.08	3.92*
Within treatments (error)	63	106.92	1.55	
Total: Subgroups and error within subgroups	69	153.75		

* $p < 0.005$.

Discussion

There has always been a question as to the validity of applying osmotic theory to living, cellular membranes. As the concepts of osmotic equilibrium were originally developed by van't Hoff (1887) they are valid only for non-living systems with non-living membranes as the barrier to permeation. Living membranes only approximate van't Hoff's osmotic rules (Lucké and McCutcheon 1932). It is well-known that some solutes move against an osmotic gradient through cell membranes, e.g., accumulation of potassium in the cell of *Valonia macrophysica* (Osterhout 1931) or in vertebrate muscle (Boyle and Conway 1941), or ecllusion of sodium from various cells (e.g., Hokin and Hokin 1965). Mechanisms of energy-requiring, non-osmotic nature, usually considered "active transport" or "pumps", have accordingly been assumed for the transport through cellular membranes of non-ionic solutes (e.g., glucose, Wilson 1964), or ions (e.g., Rasmussen and Ogata 1967).

Some biochemists and biophysicists (e.g., Fischer and Hooker 1933, Ling 1962, Cope 1967) and physiologists (e.g., Teorell 1953, Jahn 1962, 1966) discard osmotic theory almost entirely for protoplasm. They treat the proteins therein as ion- and water-exchanging colloids (with water acting covalently), resembling the activities of ion-exchanging resins. They propose that ions and water are exchanged in relationship to balances of freely-permeating ions which affect the imbibi-

tion and syneresis of water by the protoplasmic colloids, with membranes playing a lesser role.

Cope (1967) completely discards osmosis for protoplasmic hydration and dehydration, asserting that Bradley-isotherm theory is applicable thereto (Bradley 1936), with support from work with proteins by Hoover and Mellon (1950) and from Ling (1962), whose research and arguments on adsorption of water to proteins in multiple, polarized layers are well-known. Cope (loc. cit.) presented an equation which successfully predicts that the volume of a living cell should be a linear function of the logarithm of concentration of the solute(s) in the surrounding water. He assumes that the solute decreases the vapor-pressure of the solution, thereby decreasing adsorption of water by cellular proteins in accord with the Bradley isotherm, an assumption also compatible with the permeability of the cellular membrane to certain solutes.

By osmotic theory, protozoa in hypotonic solutions, e.g., fresh-water, should absorb water and pump it out via the WEV at a rate calculated as maintaining a constant osmotic pressure in the cytoplasm (Kitching 1951). Our results show this is not true. When the ratio of monovalent K^+/divalent Ca^{++} is maintained at a given Gibbs–Donnan ratio, the rate of expulsion of water by the WEV is not significantly changed, despite increasing hypotonicity of the surrounding solute. Further, the rate of expulsion of the WEV does not change significantly if the Gibbs–Donnan ratio of the solution is constant while the solution is made osmotically hypertonic with non-ionic sucrose.

The rate of expulsion of water by the WEV does significantly change, however, either while the solution is made hypotonic by dilution, or hypertonic by addition of ionic solutes, providing that dilution or concentration of the solution also alters the Gibbs-Donnan ratio of cations in solution. Change of pH also changes the Gibbs–Donnan ratio of cations in the solution (see Jahn and Bovee 1967). The altered rate of the WEV by pH is related to the alteration of the Gibbs–Donnan ratio of cations in the water, since lowering or raising pH concomitantly adds or removes a competitive cation, H^+ (or more likely as the hydronium ion, H_3O^+), thereby affecting K^+/Ca^{++} ratios both in the solution and at the cell membrane.

These observations and data and the statistical relevance supporting them are counter to osmotic theory. Some other explanation must be sought.

Because the relative balance between K^+ and Ca^{++} in the cell is the one normally shifted, we suggest that external alterations of the concentration of K^+ or Ca^{++} cause more or less of either to be adsorbed by

protoplasmic proteins. This, in turn, alters the configuration of protoplasmic colloids.

More water is adsorbed onto the proteins as K^+ is increased, since K^+ displaces Ca^{++} from its divalent chelations to cardinal sites (c-sites; Ling 1962), with dipolar water molecules occupying c-sites freed as K^+ binds monovalently where Ca^{++} had bound divalently. This causes the well-known swelling and decrease of viscosity of protoplasm resulting from excess K^+ in the surrounding water. Conversely, as Ca^{++} is increased, both K^+ and water are displaced as Ca^{++} binds c-sites divalently. This causes shrinkage, increase of viscosity and syneresis of water from the cytoplasm.

We should expect, then, as our data show, that as cations are exchanged by protoplasmic colloids, imbibition and syneresis of water should occur. These cause the flux of water into the WEV to vary in relationship to the ionic shifts as expressed by the Gibbs–Donnan ratio of cations in solution rather than as a response to the osmotic pressures caused by the total solutes. In turn, the rate at which the WEV fills and expels is also altered, so long as the energy-using mechanisms and cyclosis which regulate its expulsions are intact.

A more extensive theoretical treatment of this concept will be the subject of a future paper.

ZUSAMMENFASSUNG

Von den Kationen Li^+, Na^+, K^+, Ba^{++}, Ca^{++} und Mg^{++}, verlangsamen alle bis auf Ba^{++} mit ansteigender Konzentration die Aktivität der wasserausscheidenden Vesikel (WV) von *Tetrahymena pyriformis*. In Gegenwart eines mono- und divalenten Kationen, besonders von K^+ und Ca^{++}, variiert die Aktivität der WV unabhängig vom osmotischen Druck des Mediums. Verschiedene Proportionen der mono- und di-valenten Kationen (Na^{++}/Ca^{++}) beeinflussen die Aktivität der WV: diese nimmt zu mit dem Anstieg der Ratio von 3.6/1 auf 3.23/1, bleibt annähernd gleich während des Anstiegs von 37.3/1 auf 132.2/1, und sinkt bei höheren Verhältnissen wieder ab. Ba verhält sich an Membranen monovalent; kombiniert mit im Verhältnis bis zu 1.51/1 steigert es die Aktivität der WV, bei höheren Proportionen hemmt Ba, obwohl die beiden divalenten Ionen keine Veränderung des osmotischen Drucks bewirken. Veränderung der Gibbs–Donnan–Ratio ($K^+/\sqrt{Ca^{++}}$) bewirken Aktivitätsveränderungen der WV, auch wenn der osmotische Druck mit Sucrose konstant gehalten wird. Alle Änderungen der Versuchs bedingungen, welche die Gibbs-Donnan–Ratio verändern, bewirken Aktivitätswechsel der WV (z.B., Konzentrationsänderung der mono- oder divalenten Ionen, pH veränderungen, Zugabe von Wasser mit Sucrose), aber Änderungen des osmotischen Drucks alleine sind wirkungslos. Daraus wird geschlossen, dass Wasser nicht als Folge von Veränderungen des osmotischen Drucks aufgenommen wird, sondern zussamen mit dem austausch von Ionen zunächst aufgesaugt (an protoplasmische Proteine adsorbiert) un dann durch aktive Synerese ausgescheiden wird.

REFERENCES

Boyle P. J. and Conway E. J. 1941: Potassium accumulation in muscle and associated changes. J. Physiol., Lond., 100, 1–63.

Bradley R. S. 1936: Polymolecular adsorbed films. Part 1. The adsorption of argon on salt crystals at low temperatures, and the determination of surface fields. J. Chem. Soc., 139, 1467–1474.

Chalkley H. W. 1930: Stock cultures of *Amoeba proteus*. Science, 71, 442.

Cope F. W. 1967: A theory of cell hydration governed by the adsorption of water on cell proteins rather than by osmotic pressure. Bull. Math. Biophys., 29, 583–596.

Czarska L. 1964: Roles of K^+ and Ca^{++} ions in the excitability of the protozoan cell. Chemical and electrical stimulation of the contractile vacuole. Acta Protozool., 2, 287–296.

Eisenberg E. 1925: Function of the contractile vacuole in *Paramecium*. Arch. Biol., 35, 441–464.

Friend A. W. Jr., Finch E. D. and Schwan H. P. 1975: Low frequency field induced changes in the shape and motility of amoebas. Science, 187, 357–359.

Fischer M. H. and Hooker M. O. 1933: The Lyophilic Colloids (Their Theory and Practice). Thomas Press, Springfield, Ohio.

Gaw H. Z. 1936: Physiology of the contractile vacuole in ciliates. I. Effects of osmotic pressure. II. Effects of H^+ concentration. III. Effects of temperature. IV. Effects of heavy water. Arch. Protistenk., 87, 185–224.

Glasstone S. and Lewis D. 1960: Elements of Physical Chemistry, 2nd Ed. D. van Nostrand Co., New York.

Herfs A. 1922: The role of contractile vacuole in acclimitization of protozoa to different salinities. Arch Protistenk., 44, 227–260.

Hokin L. E. and Hokin M. R. 1965: The chemistry of the cell membrane. Sci. Amer., 213, 78–86.

Hoover S. R. and Mellon E. F. 1950: Application of polarization theory to sorption of water vapor by high polymers. J. Am. Chem. Soc., 72, 2562–2566.

Jahn T. L. 1962: The mechanism of ciliary movement. II. Ion antagonism and ciliary reversal. J. Cell. Comp. Physiol., 60, 217–228.

Jahn T. L. 1966: Contraction of protoplasm. II. Theory: Anodal vs. cathodal in relation to Ca^{++}. J. Cell. Comp. Physiol., 68, 135–148.

Jahn T. L. and Bovee E. C. 1967: Motile behavior of protozoa. In: Research in Protozoology, (ed. Chen, T.-T.), vol. 1. Pergamon Press, Oxford, 41–200.

Jahn T. L. and Boggs, N. 1971: Water flux through protozoa. J. Protozool., 18 (Suppl.), 21.

Kitching J. A. 1934: Osmotic relations in peritrich ciliates. J. Exp. Biol., 11, 364–381.

Kitching J. A. 1948: The physiology of contractile vacuoles. V. The effects of short-term variations of temperature on a fresh-water peritrich ciliate. J. Exp. Biol., 25, 406–420.

Kitching J. A. 1951: The physiology of the contractile vacuole. VII. Osmotic regulation in a suctorian with special reference to the mechanism of control of vacuolar output. J. Exp. Biol., 28, 203–214.

Kitching J. A. 1957: Effects of high hydrostatic pressure on the activity of flagellates and ciliates. J. Exp. Biol., 34, 494–570.

Kitching J. A. 1967: Contractile vacuoles, ionic regulation and excretion of protozoa. In: Research in Protozoology, vol. 1. (ed. Chen T.-T.) Pergamon Press, Oxford, 306–337.

Kitching J. A. and Padfield J. E. 1960: The physiology of contractile vacuoles. XI. Effects of heavy water on the water balance of a suctorian. J. Exp. Biol., 37, 73–82.

Ling G. N. 1962: A Physical Theory of the Living State: The Association–Induction Hypothesis. Blaisdell, New York.

Lucké B. and McCutcheon M. 1932: The living cell as an osmotic system and its permeability to water. Physiol. Rev., 12, 68–139.

Organ A. E. and Bovee E. C. 1972: Water flux in *Tetrahymena pyriformis*: Are osmotic differentials important? J. Protozool., 19 (Suppl.), 27.

Organ A. E., Bovee E. C. and Jahn T. L. 1968: ATP-accelerated cycling of the nephridial apparatus of *Paramecium multimicronucleatum*. J. Protozool., 15, 173–176.

Osterhout W. J. V. 1931: Physiological studies of single plant cells. Biol. Rev., 6, 369–411.

Rasmussen H. and Ogata E. 1966: Parathyroid hormone and the reaction of mitochondria to cations. Biochemistry, 5, 733–745.

Sears D. F. and Gittleson S. M. 1964: Cellular narcosis of *Paramecium multimicronucleatum* by xenon and other chemically inert gases. J. Protozool., 11, 538–546.

Sims J. P. 1968: Isocitric lyase activity in *Tetrahymena* sp. Masters thesis, St. John's University, Jamaica, New York.

Sokal R. R. and Rohlf F. J. 1969: Biometry. Freeman, San Francisco.

Teorell T. 1953: Transport processes and electrical phenomena in ionic membranes. Prog. Biophys. Mol. Biol., 3, 305–369.

van't Hoff J. H. 1887: Zeitschrift für physikal chemie, i, 481. (Translated and edited by H. C. Jones in The Modern Theory of Solutions, 1899, New York).

Wilson T. H. 1964: Structure and function of the intestinal absorptive cell. In: The Cellular Functions of Membrane Transport. (ed. Hoffman J. F.) Prentice-Hall, Englewood Cliffs, New Jersey, 215–229.

Yocum H. B. 1934: Acclimitization of freshwater ciliates to seawater. Biol. Bull. Woods Hole, 67, 273–276.

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Frontal Cap Formation and Origin of Monotactic Forms of *Amoeba proteus* Under Culture Conditions

Synopsis. Monotactic forms of *A. proteus* originate under culture conditions from polytactic individuals with vacuolized cytoplasm. Their vesicular frontal cap develops from vacuoles transported by cytoplasmic stream to pseudopodial tip. The vacuoles fuse together, the resulting vesicle is pressed by the stream against the frontal cell wall, and becomes the active frontal cap when it locally interrupts the continuity of cell cortex. The pseudopodium with frontal cap becomes permanently dominating, the cytoplasm flows continuously toward the cap, and the polytactic amoeba is reshaped into monotactic one. A spontaneous resorption of vesicular frontal cap, and recovery of polytactic form by amoeba, present an inversed sequence of the same stages.

In cultures of *Amoeba proteus* one can currently observe the presence of peculiar forms with cylindrical smooth body and strongly folded tail region. In such specimens, not only the tail but also the front is permanent, and it is formed by a hemispherical cap filled with an agranular, optically empty material. The axial endoplasm streaming is always directed towards the cap, and the fountain-like streaming pattern appears in such forms more commonly than in other individuals. Such forms, when mentioned by earlier authors, were traditionally described as "monopodial", and their frontal structure was called "the hyaline cap".

However, this terminology turned out to be very confusing because many authors were using the same terms to define essentially different things. The name "hyaline cap" was given as well to the specific frontal cap of the form described above, as to the crescent often formed by the hyaline ectoplasm at the tips of pseudopodia of other forms of amoeba. Recently, these two types of caps were unequivocally distinguished from one another by Korohoda and Stockem (1975, 1976). Therefore, in this study the first structure will be called: vesicular

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frontal cap, and the second one: hyaline frontal cap. For the sake of convenience, the simpler general term: frontal cap, will be used when its meaning is enough clear from the context.

The term "monopodial" was used as well for the peculiar forms with vesicular frontal caps, as for amoebae moving unidirectionally without frontal vesicle. This terminological ambiguity perpetuated from classical studies (e.g., Mast 1928) up to the modern literature (e.g., Wohlfarth-Bottermann and Stockem 1966). In three papers only (Seravin 1966, Stockem et al. 1969, and Grębecki 1977) a clear distinction was made between both types of "monopodial" amoebae, but old equivocal names were maintained. In this study, the terminology proposed recently by Grębecki and Grębecka (1978) is adopted: cylindrical amoebae with stable polarity maintained by the vesicular frontal cap will be called monotactic, individuals moving unidirectionally without vesicular caps being temporarily polarized by external stimuli will be called orthotactic, and the most common form characterized by a succession of leading pseudopodia during locomotion will be defined as polytactic.

The origin of the vesicular frontal cap is the most interesting problem concerning monotactic amoebae, because it is probably responsible for all other peculiarities of this form (and it is absent in other forms of *A. proteus*). There is a general agreement, from long time ago, that the cap content is fluid with a low concentration of dissolved organic material. This statement is certainly true, if restricted to the vesicular frontal caps. If we may refer to the views of authors who did not discern between the vesicular and the hyaline frontal caps, nor between monotactic and orthotactic amoebae, we should state that past theories postulate that frontal caps originate by filtration of syneretic fluid produced either in the contracting tail (Mast 1926, Goldacre 1956), either in a hypothetical zone of frontal contraction (Allen 1961, Allen and Cowden 1962). Korohoda and Stockem (1976) on the contrary, after distinguishing two types of frontal caps, postulate a vacuolar origin of the vesicular cap and suppose that its content is separated by a membrane from the granuloplasm. They base these conclusions on the fact that endoplasmic granules fail to penetrate inside it, and on observation of resorption of such a cap induced by Ca-enriched medium.

It should be stressed however, that the actual transformation of polytactic amoeba into a monotactic one, including the development of its vesicular frontal cap, and the cap decline occurring as a natural process during an inverse transformation, have never been observed until now. Description of these phenomena under normal culture conditions, is the subject of present study.

Material and Methods

Amoeba proteus cultures maintained in the Nencki Institute in Warsaw from many years, deriving originally from the Princeton strain, were used. Twice per week the culture medium (Pringsheim solution) was replaced by fresh one, and *Colpidia* added as food. Separate cultures of *Colpidium* sp. grown in the same Pringsheim solution, were fed with dried yolk. The cultures of amoebae were kept in shade, at room temperature.

Individuals selected for observations were transferred, together with a drop of Pringsheim medium taken from the same culture, into an oil chamber similar to those of de Fonbrune's system. The differential interference contrast microscope MPI 3 of PZO was used. It was equipped with the MF-matic device of Zeiss Jena for automatic photomicrography, coupled with Robot photographic camera for taking serial pictures.

Results

Precursors of monotactic amoebae

The full cycle of events which result eventually in the development of a monotactic individual of *A. proteus*, characterized by a single one permanent leading pseudopodium with its specific vesicular frontal cap, takes probably several hours. This study is based on observations lasting only 3–4 h, but the author selected, from cultures in which monotactic forms were spontaneously appearing, such specimens which were already in some respects different from the most common type.

Initially, observations were undertaken on individuals which manifested a tendency to keep one leading pseudopodium (with scarce and short-living lateral pseudopodia), but lacking a vesicular frontal cap, i.e., on amoebae presently defined as orthotactic. The fact that monotactic cells bearing prominent frontal caps present the form of *A. proteus* which shows the strongest and most stable morphodynamic polarity, allowed to presume that they originate from the fairly well polarized orthotactic specimens. It was expected therefore, that the transformation consists in the development of distinct vesicular frontal cap by orthotactic amoebae. However, the observations gave negative results: formation of vesicular cap has never been found in any orthotactic specimen.

Then, the attention has been directed to those polytactic amoebae which deviate from the type in the opposite manner: their motory polarity is clearly deficient, they lack the semi-permanent tail and irregularly change the direction of their motory axis. It was found that the cytoplasm of such cells is vacuolized more than usually. Prolonged

individual observations of such specimens permitted to demonstrate that they are at the origin of monotactic forms, and to reveal the stages of formation of their characteristic vesicular frontal caps.

Frontal cap formation from small vacuoles

The Plates I and II–III show the final effective stages of vesicular cap formation, which last about 1 min. The transport and tight packing of vacuoles at the temporary front of amoeba, such as it is seen in the Pl. I 1–3 and II 7–9 may be, during several hours, repeatedly observed at different alternating sites in the same cell. If uneffective, it terminates by an inversion of cytoplasmic stream, transfer of vacuoles up to a new temporary front, further reversal of streaming, etc. The period of uneffective capping may last several hours, and during that time frequency of the phenomenon, degree of vacuolization of cytoplasm and streaming velocity, apparently increase. Eventually, the frontal cap is produced as typical regular hemisphere and, consequently, the whole amoeba gradually assumes the peculiar shape of monotactic form (Pl. I 4–6, II 10–12 and III 13–18).

It seems that for an effective and definitive formation of vesicular frontal cap, most crucial is the lapse of time between the pictures 4 and 5 (Pl. I) and 9 and 10 (Pl. II). Observations of amoebae at this brief stage, lasting only a few seconds, are most conclusive for revealing the mechanism of cap formation. After completion of this stage, the cap becomes a powerful "attractant" for the cytoplasm streaming and constitutes the permanent front of moving cell.

The whole process of transformation of polytactic form of *A. proteus* into monotactic one, may be divided in several phases:

(1) Progressive vacuolization of the cytoplasm (initial period and total duration of this phase are unknown, because the observations were started on individuals which manifested fairly advanced degree of vacuolization).

(2) Gradual pushing of small and medium size vacuoles, by cytoplasm streaming, toward the tip of an advancing pseudopodium and their tight aggregation there (Pl. I 1 and II 7); the phenomenon is reversible and frequently repeated.

(3) Fusion of vacuoles in larger vesicles at the pseudopodium tip (Pl. I 2–3 and II 8–9); phase reversible as well.

(4) Sudden rupture of the cortical ectoplasm at the front of the vesicle, which is pressed against the cell wall by the vigorous streaming (Pl. I 4 and II 9), entering of the vesicle in contact with the outer membrane and immediate assuming the perfect hemispherical shape; from this moment the vesicular frontal cap is definitively formed, the process

becomes irreversible and further stages result only in completion and stabilization of the new form of amoeba.

(5) Acceleration of the cytoplasm streaming towards the frontal cap, retraction of other pseudopodia and tail formation (Pl. II 10–12 and III 13–18), quickly modeling the cell into the cylindrical shape characteristic of monotactic form. Further vacuoles may still fuse with the frontal cap during this phase (Pl. I 5), and probably during the whole life of monotactic amoeba (Pl. IX 52–53). The unidirectional vigorous cytoplasmic stream, which role is so important during the stages of cap development, corroborates in keeping the frontal cap active and in maintaining the monotactic shape of amoeba.

This course of events is most typical and it was observed and recorded repeatedly. However, some deviations of the process were found in specific cases, as described below.

Frontal cap formation from huge vacuoles

Sometimes, the cytoplasm of deficiently polarized polytactic specimens contains several big vacuoles (Pl. IV 19). Their transfer, by the unstable cytoplasmic stream, inside an advancing pseudopodium is difficult because of their size. However, if it happens to occur, the cap formation is very prompt: the streaming intensifies and keeps constant direction, it pushes the vacuole forward (Pl. IV 20–21), presses it against the frontal cell wall (Pl. IX 22–24), and the vacuole transforms into the vesicular frontal cap (Pl. V 25). The intense streaming directed toward the cap carries from the middle body regions another huge vacuole which approaches the frontal cap (Pl. X 25–27) and fuses with it (Pl. V 28). The growing size of the frontal cap is clearly seen in the Pl. V 28–30, in parallel with the gradual change of the whole body shape from the polytactic into monotactic form.

It should be stressed that the vacuoles building the frontal cap are never contractile ones, but probably they arise by fusion of smaller vacuoles inside the cytoplasm, or more probably, at the pseudopodial tips during earlier uneffective "trials" of cap formation, as described in the precedent chapter. The contractile vacuoles, even when they are very large and inactive, and closely adhere to the outer membrane (Pl. V 31–33), never form frontal caps, i.e., they fail to provoke either the unidirectional cytoplasmic stream, or the transformation of the whole cell body into a monotactic form.

Competition between developing caps

Plates VI and VII present a case of competition between two pseudopodia in which parallel phenomena of frontal cap formation were simul-

taneously under way. The sequence shows as well an effective as an abortive cap development, and it demonstrates clearly the unsteady motory polarity so typical for amoebae giving origin to monotactic forms.

First picture shows an individual, irregular in shape, with two well developed pseudopodia, for the moment apparently inactive. The left pseudopodium (Pl. VI 34) is tightly stuffed, from its basis up to the tip, by one strongly elongated vacuole, whereas the right one (Pl. VI 35) which is nearly equivalent in size, appears to be filled up with the granuloplasm. At this stage the cytoplasm is flowing toward the tip of the right pseudopodium, and the left one is still resting. In the Pl. VI 36 the right pseudopodium already shows the vacuoles aggregated at the tip, and the cytoplasm starts also to flow into the left one, fills it up to 3/4 of its length and relegates the vacuole to the distal 1/4.

At the next stage (Pl. VI 37) the right pseudopodium formed definitely a vesicular frontal cap, but this failed to provoke immediately a withdrawal of the left pseudopodium which became meantime also "ready" to produce its own cap. The further sequence of pictures shows (Pl. VI 37–39 and VII 40–42) that a bigger volume of cytoplasm flows toward the frontal cap at the right, but a part of streaming feeds also the left pseudopodium which still manifests clear tendency to build up the second frontal cap in the same cell. It reaches the critical stage (Pl. VII 42) which in the right pseudopodium has been attained earlier (Pl. VI 36) and resulted in the immediate cap formation. But the existence of another frontal cap produced a few seconds earlier, prevents to accomplish the development of a second one. The cytoplasmic stream in the left pseudopodium reverses its direction, the vesicle formed at the tip undergoes fragmentation and resorption, the whole pseudopodium retracts and is gradually incorporated into the tail region (Pl. VII 43–45).

Attention should be drawn to the anteriority effect during competition between two developing vesicular frontal caps. Out of two pseudopodia, equivalent at the beginning, this one becomes dominating which earlier produced its frontal cap. The anteriority effect has been revealed first by the present author (G r ę b e c k a 1977 b) in the case of competition between two artificial oil caps equal in size, always resulting in domination of this oil droplet which was earlier injected.

The anteriority effect is related to the role of the cytoplasm streaming which distributes across the cell a nuclear relaxation factor maintaining the motory polarity (G r ę b e c k a 1977 a). The role of streaming is clearly put in evidence by experiment shown in the Pl. VIII. The pictures present a monotactic amoeba after injection of an oil droplet. Usually, without any further experimental intervention, the original vesicular frontal cap would dominate and the artificial oil cap would be shifted

toward the tail and eliminated (Grębecka 1977 b). In this experiment, however, immediately after oil injection, when the oil cap was still attracting a part of the cytoplasmic stream, the opposite streaming directed towards the original vesicular cap was blocked by gently pressing amoeba with micropipette. As a result, the cytoplasm flow became completely reversed from the natural frontal cap toward the artificial oil cap, the natural cap has been resorbed, and the former frontal region of monotactic amoeba transformed in a typical tail. The experiment stresses again the importance of cytoplasm streaming not only in the development of a frontal cap, but also in keeping it active during the whole life of monotactic amoeba.

Recovery of polytactic form by monotactic amoebae

The monotactic forms of *A. proteus* are very stable, but after a mechanical shock (even that resulting of transferring them from the culture to the observation chamber) some of them may produce lateral pseudopodia and resume the polytactic appearance (Seravin 1966). However, usually they preserve frontal caps, pseudopodia bearing them are not resorbed, they become soon dominating again and reshape the amoebae, which were temporarily polytactic, back into monotactic ones.

More interesting are the cases of more profound recovery of polytactic condition, correlated with disintegration of the vesicular frontal cap. The author found some examples of such transformation occurring spontaneously without experimental intervention. One of them is presented on the Pl. X–XI, and selected stages of another are shown under higher magnification on the Pl. XII.

First symptom of oncoming changs is the interruption of cytoplasmic stream and then its reversal out of the frontal cap, followed by development of lateral pseudopodia in the middle body regions (Pl. X 58 and XII 76). Thereafter, the changes start in the frontal cap. Its anterior edge clearly detaches from the outer membrane, the cortical hyaloplasm reappears in the interstice and produces hyaline pseudopodia in front of it (Pl. X 59–64 and XII 76–79). As a result, the frontal cap is drawn away from the tip of pseudopodium (Pl. X 64 and XII 78), changes its shape from hemispherical into irregularly ovoidal, and looks again like a big cytoplasmic vacuole. It may be transferred in this condition toward the body middle and disintegrated there. More commonly, however, its fragmentation begins already in the frontal zone (Pl. X 64 — XI 70) and its content is recycled into the cytoplasm in form of smaller vacuoles. Anyhow, the fragmentation of frontal cap into vacuoles seems to be the only way of its resorption; there is no indication of any direct leakage of its fluid content into the cytoplasm. In parallel with the disintegration

of frontal cap, amoeba resumes a typical polytactic shape (Pl. XI 75 and XII 81).

It should be stressed that the role of cytoplasmic stream in maintaining the frontal cap in the active state, is reflected again in the process of cap decline, because its resorption under natural conditions begins by arresting the cytoplasm flowing toward the cap and then by streaming reversal out of it. Similar changes of streaming intensity and direction precede the resorption of frontal cap provoked experimentally (Korohoda and Stockem 1976, Grębecka 1978).

In general, the stages of resorption of the vesicular frontal cap repeat almost exactly, but in reverse order, the stages of its development. This principle is illustrated by the Pl. IX 54–57. The declining of frontal cap experimentally induced by medium enriched in Ca^{2+} as described by Korohoda and Stockem (1976), is in its general features similar to that which in the present study is for the first time described as a natural phenomenon occurring under usual culture conditions.

Discussion

Results of the present study confirm in full extent the opinion of Korohoda and Stockem (1975 and 1976) that frontal caps of monotactic amoebae are of vacuolar character, and complete some hypothetical points in this concept by providing observational data collected under normal culture conditions.

There is no doubt that the sequence of events leading finally to the development of a vesicular frontal cap and to reshaping amoeba into the monotactic form, is initiated by the vacuolization of cytoplasm in a polytactic individual.

An osmotic origin of such vacuolization does not seem very probable, because a disturbance of osmotic balance would be reflected in the pulsation rate of contractile vacuole and even in blocking its action in diastole, and this has never been observed in amoebae giving rise to monotactic forms. Moreover, it was found by the present author in an earlier study (Czarska and Grębecki 1966) that, by changing the osmotic pressure, one can produce forms called now orthotactic, but not monotactic with vesicular caps.

The possibility remains of vacuolization and transformation provoked specifically by an exogenous or an endogenous agent. Two arguments speak against the environmental origin of the transforming agent: (1) appearing of monotactic amoebae is not clearly related to the age of culture, its feeding, concentration of metabolites, etc., they never

form a majority of population but may appear in low percentage in all cultures, and (2) all the attempts to induce exprimentally the development of monotactic amoebae by influence of physical or chemical factors were unsuccessful: authors reporting some procedures to obtain "monopodial" amoebae (e.g., Käppner 1961, Czarska and Grębecki 1966, Wohlfarth-Bottermann and Stockem 1966) produced in the reality the orthotactic forms. The present author is rather disposed to consider the tendency of some specimens to the cytoplasm vacuolization and to the subsequent transformation into monotactic amoebae, as their individual character depending on the intracellular background.

It is questionable should we adhere or not to the view expressed by Seravin (1966), and accepted by Stockem et al. (1969), that monotactic amoebae are pathological. The observation of Seravin (1966) that often they round up and die is correct, and it is also true that they are incapable of taking food. On the other hand, however, it is well established in the present study that they can recover the polytactic form. When polytactic again, they probably can feed, divide and return to monotactic type by building frontal caps from vacuoles persisting in their cytoplasm. This last statement is based on a pilot experiment run by the present author (unpublished) in which several samples, containing each 10 isolated monotactic amoebae and *Colpidia*, gave after 1 week populations of 12–17 individuals per sample. The final populations were mixed: they were composed of some monotactic amoebae and some polytactic which manifested the tendency to build up frontal caps.

Observation of further changes occurring in vacuolized cells with unsteady polarity, also confirms the view that the vesicular frontal cap is vacuolar in character: the cytoplasm streaming accumulates the vacuoles at pseudopodial tips where they fuse together, but initially the pressure exerted by the stream is unsufficient to finalize the cap formation.

It is seen on the records corresponding to this phase that, just before the definitive cap formation, the vesicles produced by fusion of vacuoles are separated from the external frontal membrane of pseudopodial tip by a thin cortical hyaline layer. The existence of a crescent-like layer of hyaline ectoplasm between the vesicle and the outer membrane during cap development is particularly well seen in the Pl. IX 54–55, and reappearance of this structure during cap resorption in the Pl. IX 56–57. The critical irreversible stage of transformation consists in the rupture of this layer and pushing it aside. At this moment, when there is no cortical ectoplasm more between the vesicle and the outer membrane,

it becomes an active and permanent frontal cap, and amoeba quickly assumes the monotactic form.

This critical moment of spontaneous development of a natural vesicular cap is strictly comparable with the artificial cap formation by injection of an oil droplet against the inner face of the cell membrane of *A. proteus* (Goldacre 1961, Grębecka 1977 b). In both cases the change of streaming pattern is immediate and irreversible, and it arrives when the vacuole or the oil droplet enters in direct contact with the cell membrane, i.e., when they locally disrupt the continuity of the cortical ectoplasm layer. This last statement derived here from observations in the interference contrast microscope, is also confirmed by electron microscopy (Hrebenda and Grębecka 1978).

The decisive role of such a local discontinuity of the cortical ectoplasm layer may be easily understood in connection with the concept developed recently by some authors (Korohoda and Stockem 1975, Kalisz and Korohoda 1976, Grębecki 1976 and 1977) that this layer forms a semi-rigid frame which is responsible for the contractility and motory activity of amoebae. One should conclude that the vesicular frontal cap, or the artificial oil cap, form in amoeba a locus which never can contract, and becomes therefore an obligatory and permanent pseudopodial tip.

This interpretation is constistent with the view of Korohoda and Stockem (1976) that the vesicular frontal cap is itself responsible for appearance of the typical streaming pattern in monotactic *Amoeba proteus*, however it is more specific and also broader. It explains how identical effects are exerted by oil injections, and it permits to suppose that the formation of frontal cap and of monotactic motory type of amoeba might be probably imitated as well by other unspecific bodies inserted between the streaming endoplasm and the peripheral cell layer, and then forming a local breach in the contractile cortex.

The mechanism suggested above can be operational in the case of natural vesicular frontal cap under one condition: there should be a membrane or another border structure separating the content of the cap from the cytoplasm, as it was postulated by Korohoda and Stockem (1975). This postulation is supported in the present research by demonstration of the vacuolar origin of the vesicular frontal cap, and of its fragmentation into smaller vacuoles during spontaneous resorption. Moreover, in parallel investigations the existence of such border structure is put in evidence by micrurgical methods (Grębecka 1978) and by electron microscopy (Hrebenda and Grębecka 1978).

Evidence given by Korohoda and Stockem (1975) of non-fluid character of the hyaline caps and demonstration by the present

research of the vacuolar origin of the vesicular cap, make groundless the earlier speculations on the self-regulation of motory activity in amoeba (Goldacre 1956) and on the frontal contraction zone (Allen 1961), as far as they were based on a hypothetical filtration of syneretic fluid into the frontal cap.

RÉSUMÉ

Les formes monotactiques de l'*Amoeba proteus* se developpent, dans les conditions de culture en partant des individus polytactiques dont le cytoplasme est vacuolisé. Leur calotte frontale vésiculaire est originaire des vacuoles transportées par le courant cytoplasmique vers l'extrémité d'un pseudopode. La fusion de ces vacuoles se manifeste, la vésicule plus large qui en résulte est pressée par le courant contre la paroi frontale de la céllule, et elle dévient une calotte frontale active lorsqu'elle rompe localement la continuité du cortex céllulaire. La résorption spontanée de la calotte frontale, et le retour de l'amibe à la forme polytactique, se manifestent dans une séquence inverse des mêmes étapes.

REFERENCES

Allen R. D. 1961: Ameboid movement. In: The Cell. Academic Press. 2, 135–216.

Allen R. D. and Cowden R. R. 1962: Syneresis in ameboid movement: its localization by interference microscopy and its significance. J. Cell Biol., 12, 185–189.

Czarska L. and Grębecki A. 1966: Membrane folding and plasma-membrane ratio in the movement and shape transformation in *Amoeba proteus*. Acta Protozool., 4, 201–239.

Goldacre R. J. 1956: The regulation of movement and polar organisation in amoeba by intracellular feedback. Proc. 1st Internat. Congr. Cybernetics, 715–725.

Goldacre R. J. 1961: The role of the cell membrane in the locomotion of amoeba, and the source of the motive force and its control by feedback. Expl. Cell Res., (Suppl.) 8, 1–16.

Grębecka L. 1977 a: Behaviour of anucleate anterior and posterior fragments of *Amoeba proteus*. Acta Protozool., 16, 87–105.

Grębecka L. 1977 b: Changes of motory polarization in *Amoeba proteus* as induced by oil injections. Acta Protozool., 16, 107–120.

Grębecka L. 1978: Micrurgical experiments on the frontal cap of monotactic forms of *Amoeba proteus*. Acta Protozool., 17, 205–214.

Grębecki A. 1976: Co-axial motion of the semi-rigid cell frame in *Amoeba proteus*. Acta Protozool., 15, 221–248.

Grębecki A. 1977: Non-axial cell frame movements and the locomotion of *Amoeba proteus*. Acta Protozool., 16, 53–85.

Grębecki A. and Grębecka L. 1978: Morphodynamic types of *Amoeba proteus*; a terminological proposal. Protistologica (in press).

Hrebenda B. and Grębecka L. 1978: Ultrastructure of the frontal cap of monotactic forms of *Amoeba proteus*. Cytobiologie (in press).

Kalisz B. and Korohoda W. 1976: Experimental study on locomotion of *Amoeba proteus*. I. Movements in the nucleated and anucleated fragments of the amoebae after removal of the part of their cytoplasm. Acta Protozool., 15, 345–361.

Käppner W. 1961: Bewegungsphysiologische Untersuchungen an der Amöbe *Chaos chaos* L. I. Der Einfluss des pH des Mediums auf das bewegungsphysiologische Verhalten von *Chaos chaos*. Protoplasma, 53, 81–105.
Korohoda W. and Stockem W. 1975: On the nature of hyaline zones in the cytoplasm of *Amoeba proteus*. Microsc. Acta, 77, 129–141.
Korohoda W. and Stockem W. 1976: Two types of hyaline caps, constricting rings and the significance of contact for the locomotion of *Amoeba proteus*. Acta Protozool., 15, 179–185.
Mast S. O. 1926: Structure, movement, locomotion and stimulation in amoeba. J. Morphol. Physiol., 41, 347–425.
Mast S. O. 1928: Factors involved in changes in form in amoeba. J. Exp. Zool., 51, 97–120.
Stockem W., Wohlfarth-Bottermann K. E. and Haberey M. 1969: Pinocytose und Bewegung von Amöben. V. Konturveränderungen und Faltungsgrad der Zelloberfläche von *Amoeba proteus*. Cytobiologie 1, 37–57.
Seravin L. N. 1966: Monopodialnye formy *Amoeba proteus*. Dokl. Akad. Nauk SSSR, 166, 1472–1475.
Wohlfarth-Bottermann K. E. und Stockem W. 1966: Pinocytose und Bewegung von Amöben. II. Permanente und induzierte Pinocytose bei *Amoeba proteus*. Zeitschr. Zellforsch., 73, 444–474.

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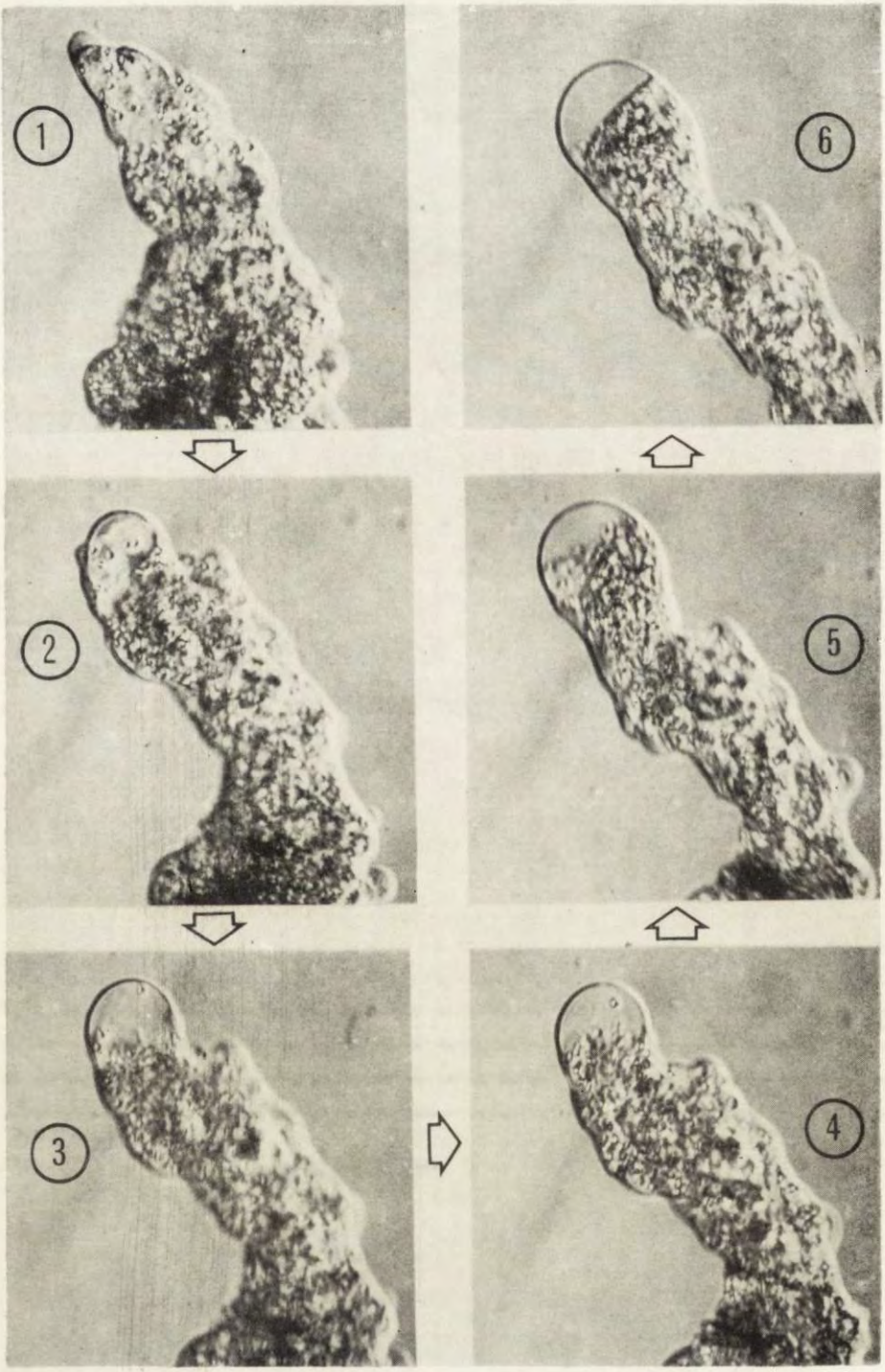
EXPLANATION OF PLATES I–XII

1–6: Selected stages of formation of the vesicular frontal cap from vacuoles accumulated at the tip of a pseudopodium of *Amoeba proteus*
7–18: Full cycle of transformation of amoeba into the monotactic form, including the development of vesicular frontal cap from small vacuoles
19–30: Development of monotactic form associated with frontal cap building from huge vacuoles
31–33: Examples of enlarged contractile vacuoles closely adhering to the cell walls which fail to transform into active frontal caps
34–45: Competition between two simultaneously developing frontal caps during transformation of a polytactic amoeba into monotactic one
46–51: Competition between the vesicular frontal cap of monotactic amoeba and an artificial oil cap, resulting in resorption of the original cap because of the blockade of cytoplasmic stream flowing in its direction
52–53: Accumulation of small cytoplasmic vacuoles at the borderline separating a well developed functional frontal cap from the endoplasm
54–57: Comparison of some stages of frontal cap formation (left) and of its resorption (right) in monotactic amoebae
58–75: Cycle of transformations leading from monotactic form of amoeba back to the polytactic one, including the decline of vesicular frontal cap
76–81: Selected stages of resorption of vesicular frontal cap in a monotactic amoeba recovering the polytactic form



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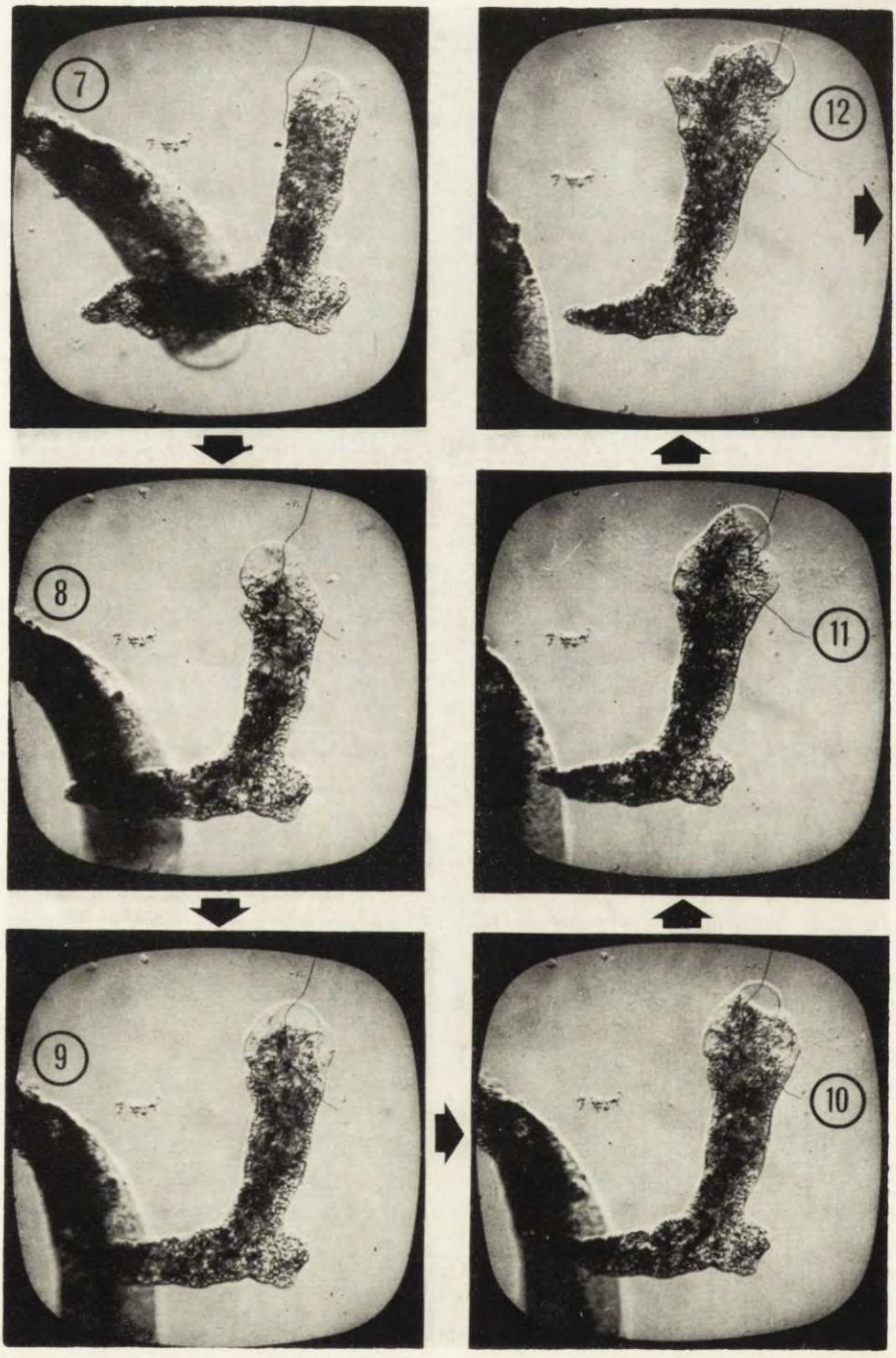

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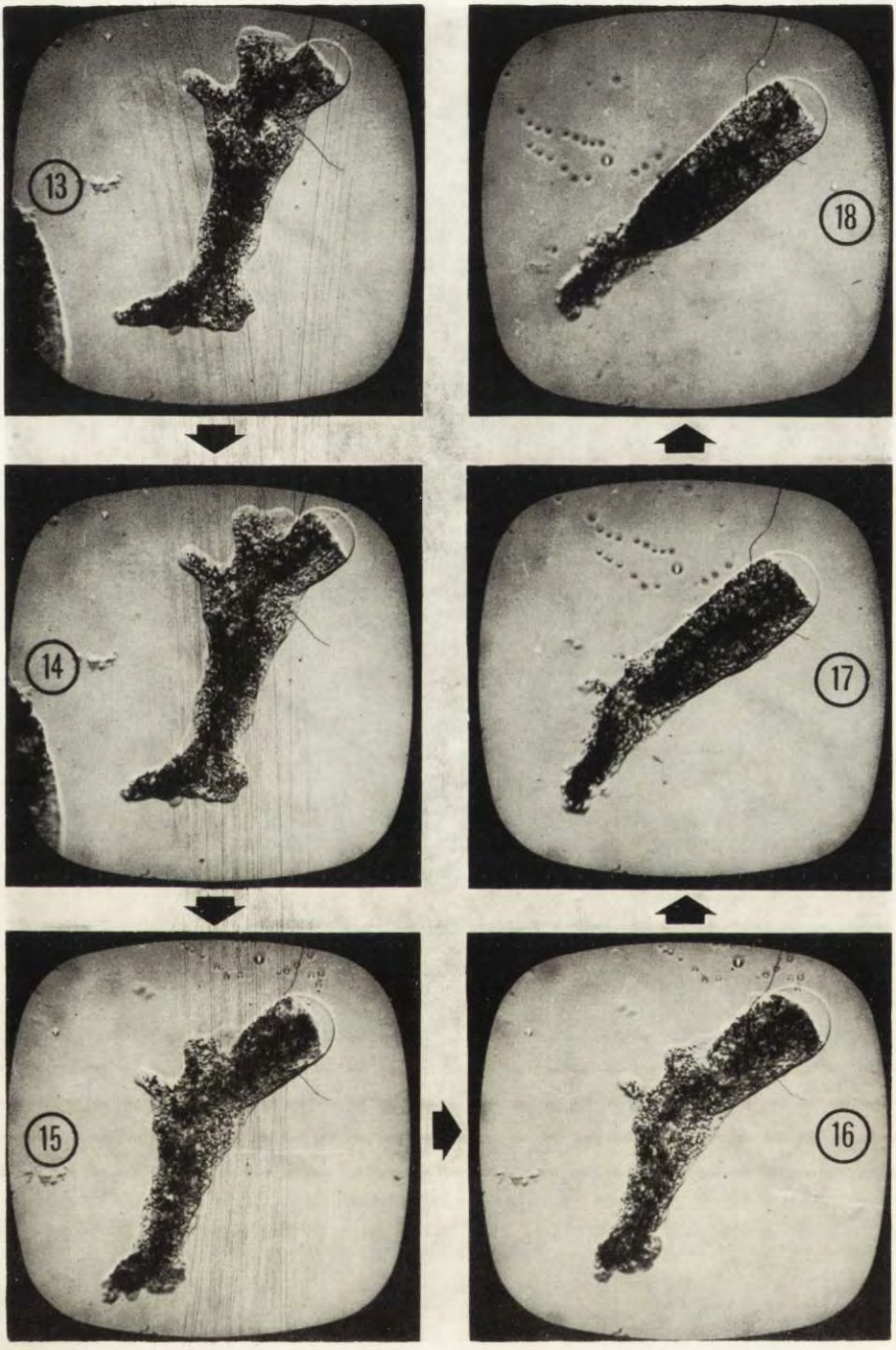

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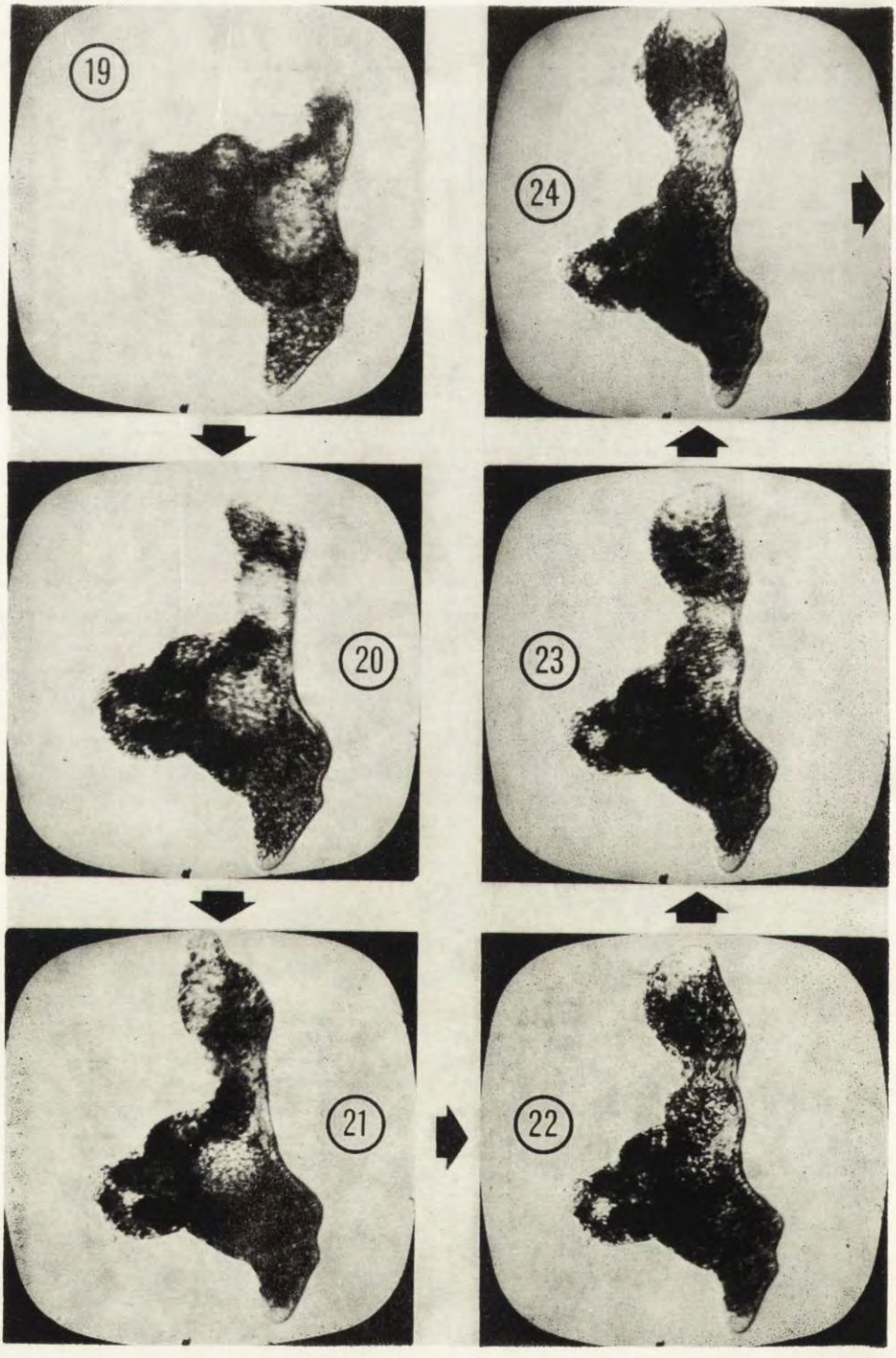

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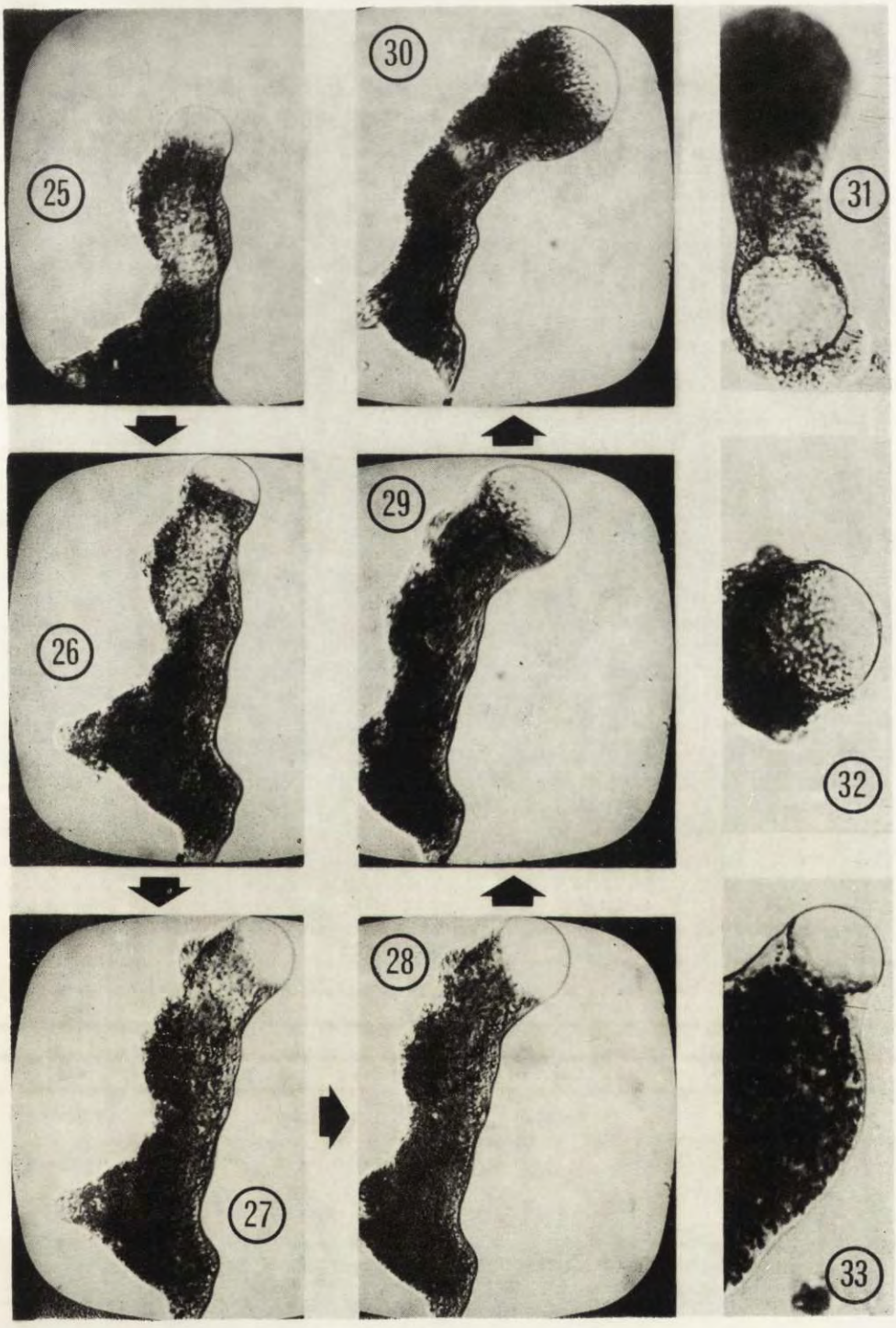

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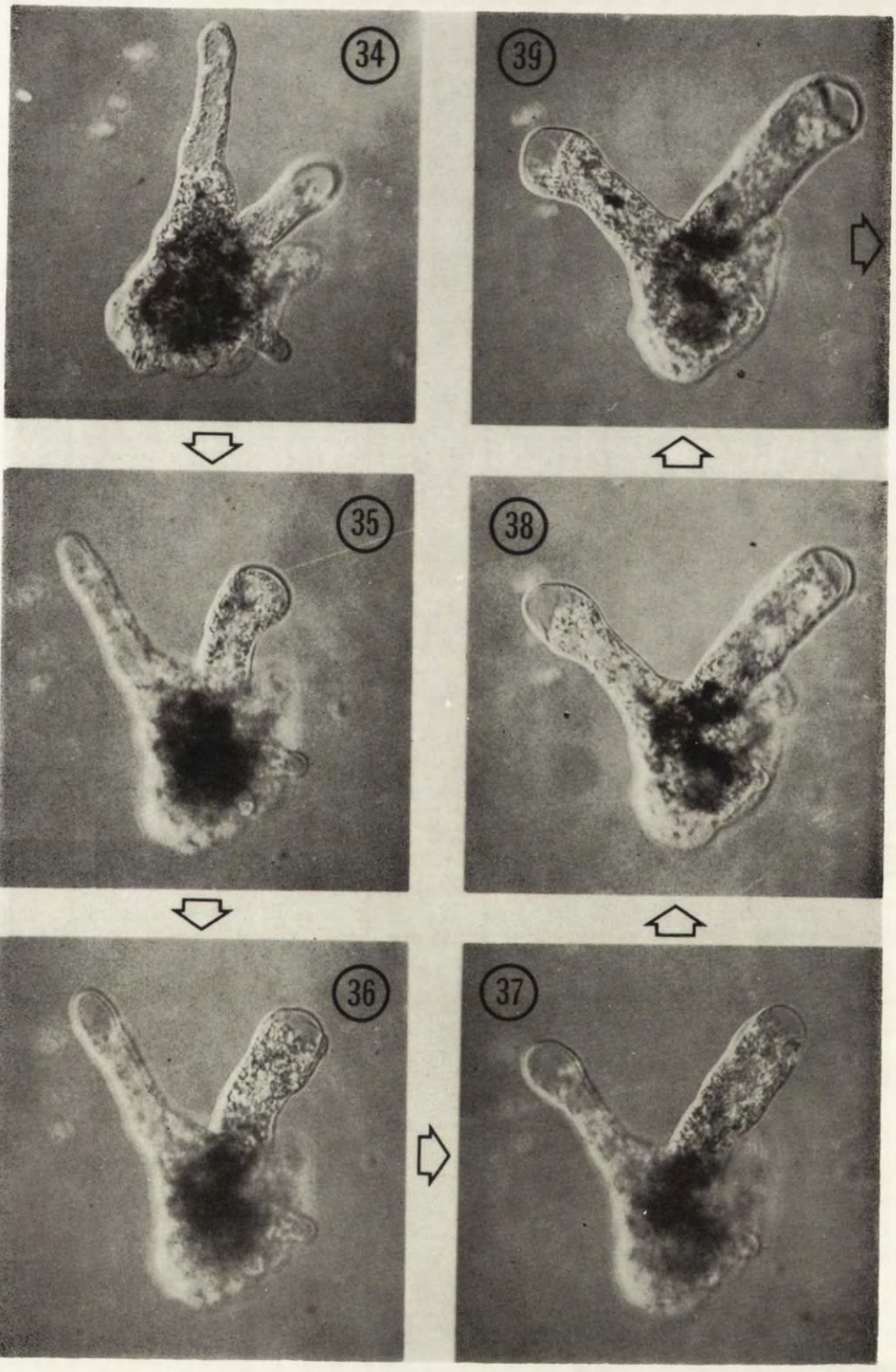

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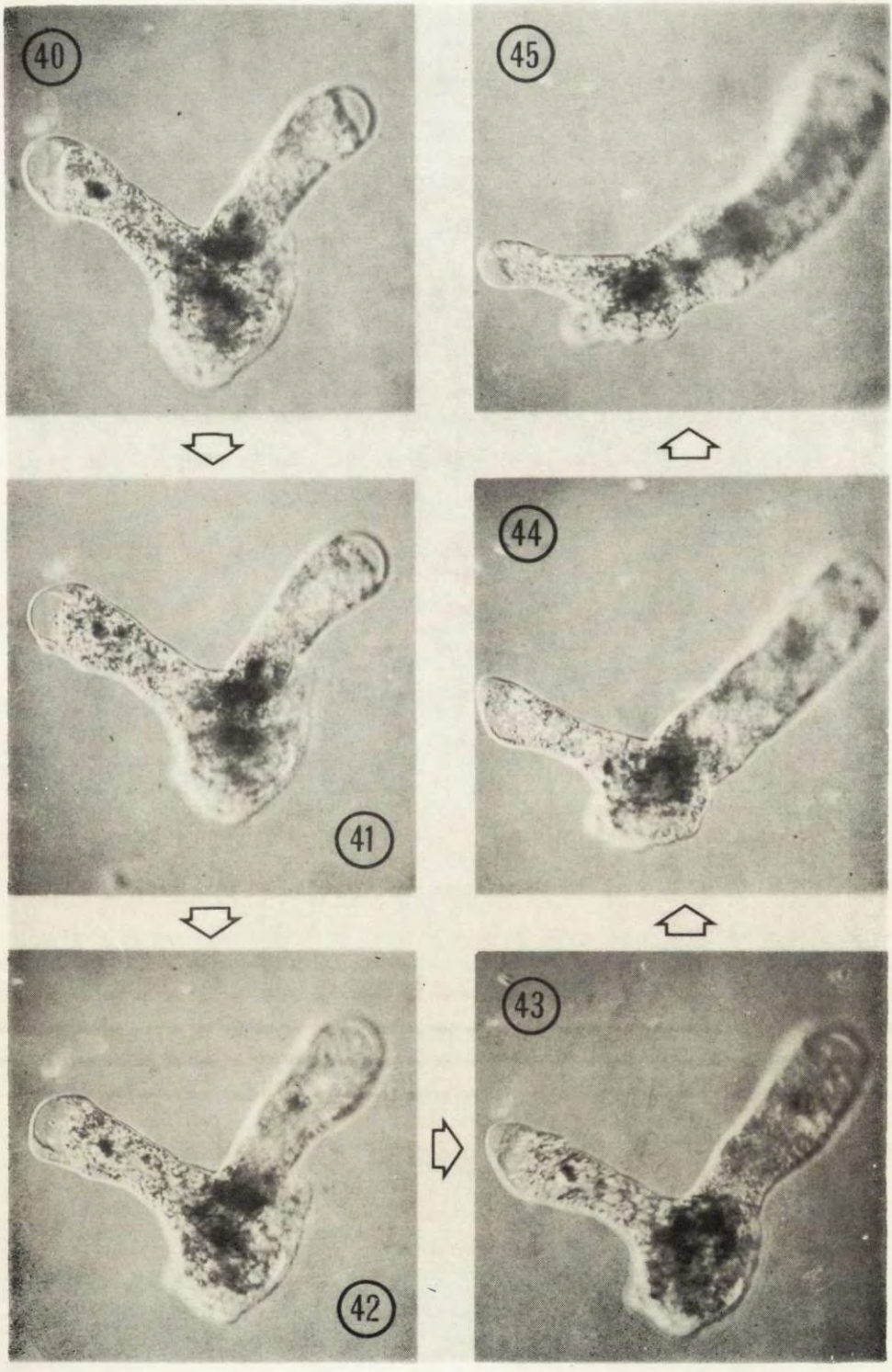

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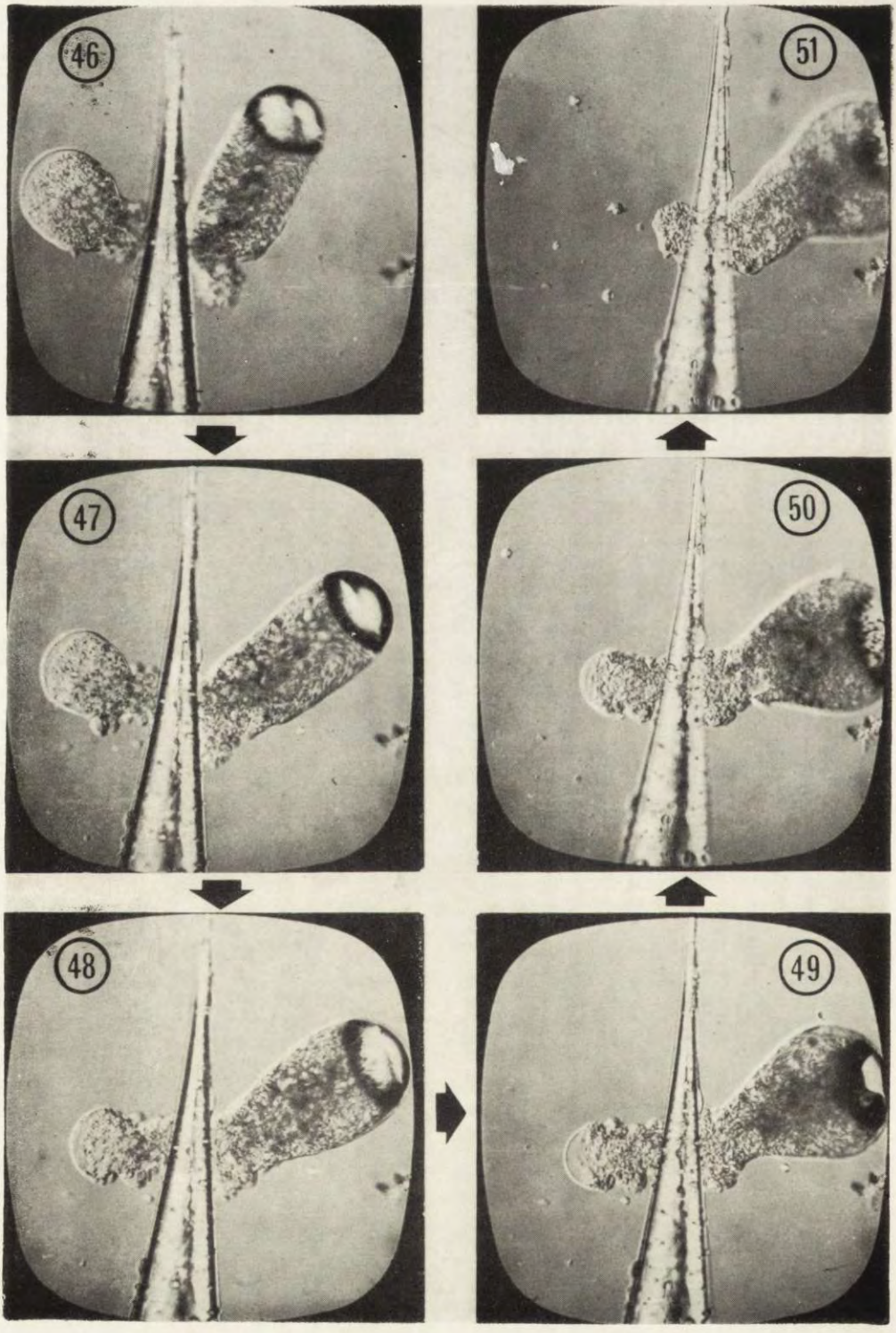

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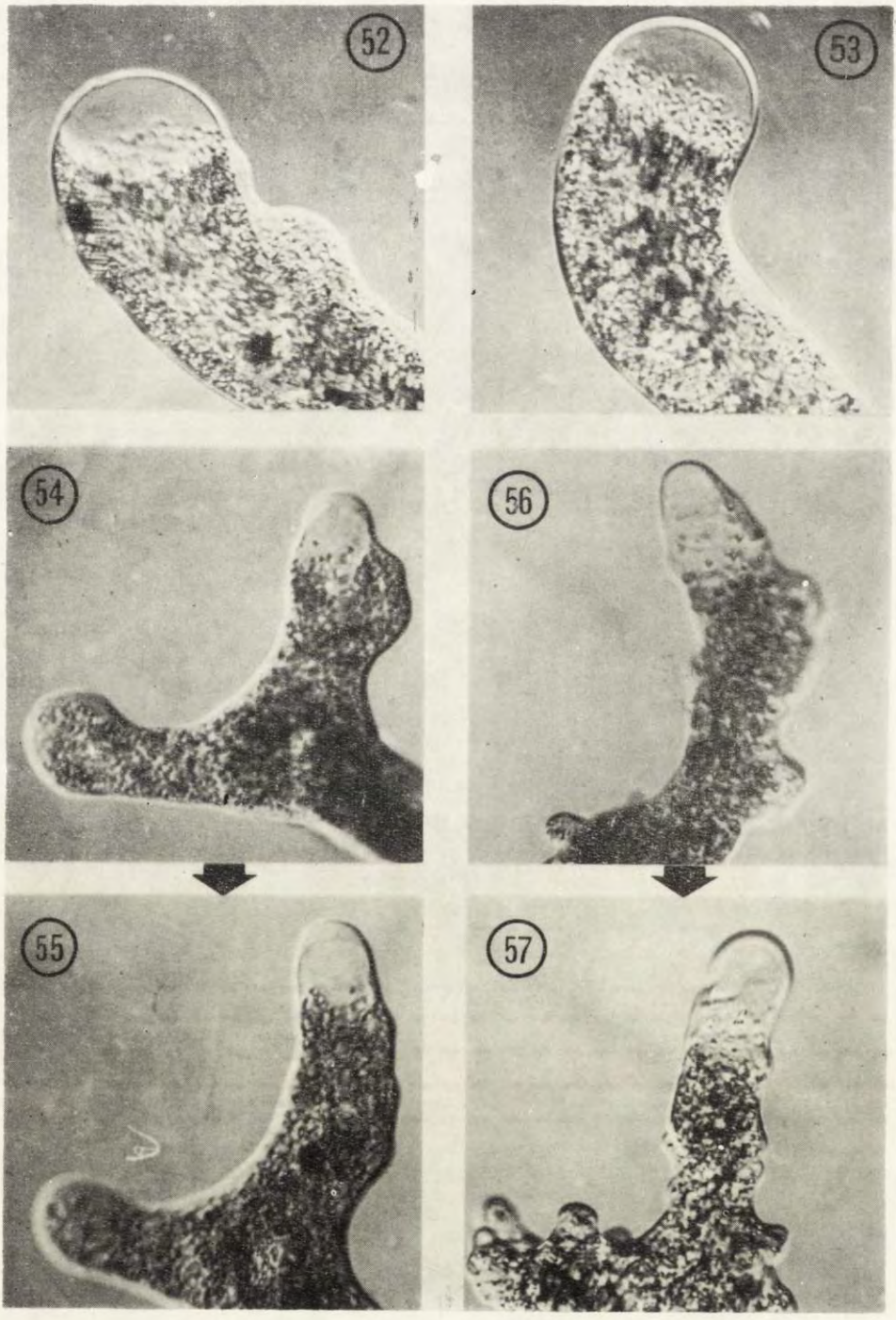

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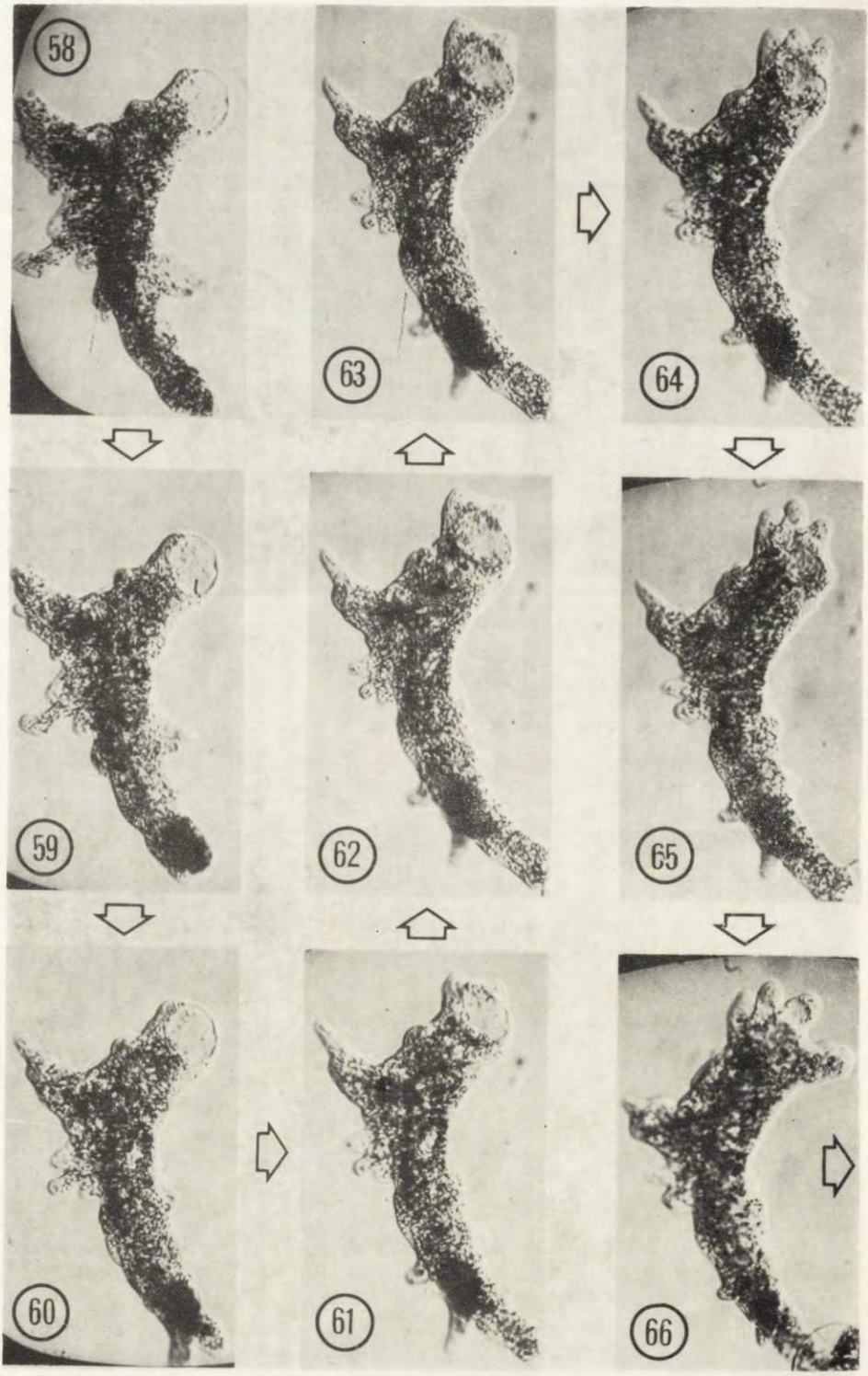

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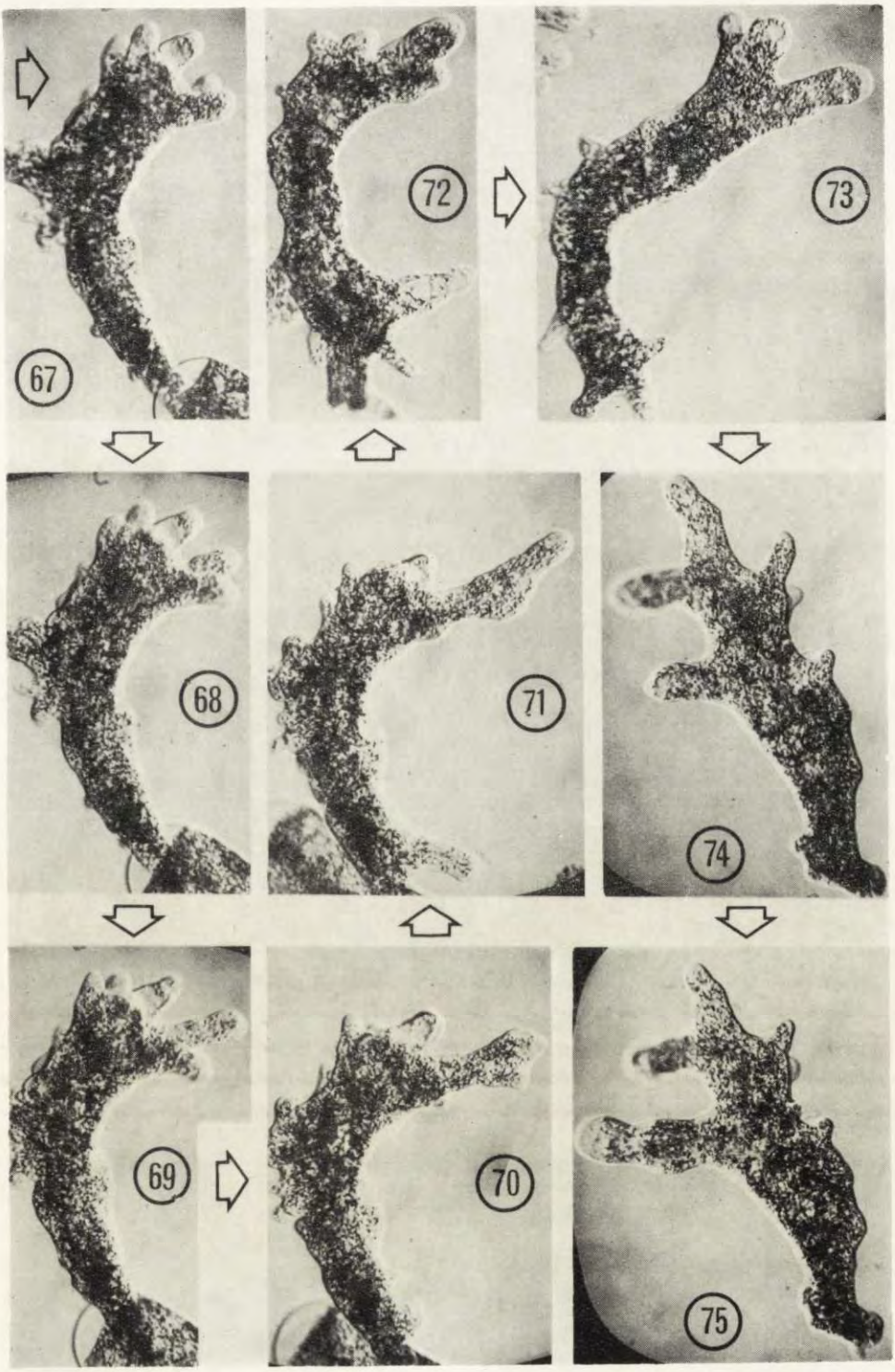

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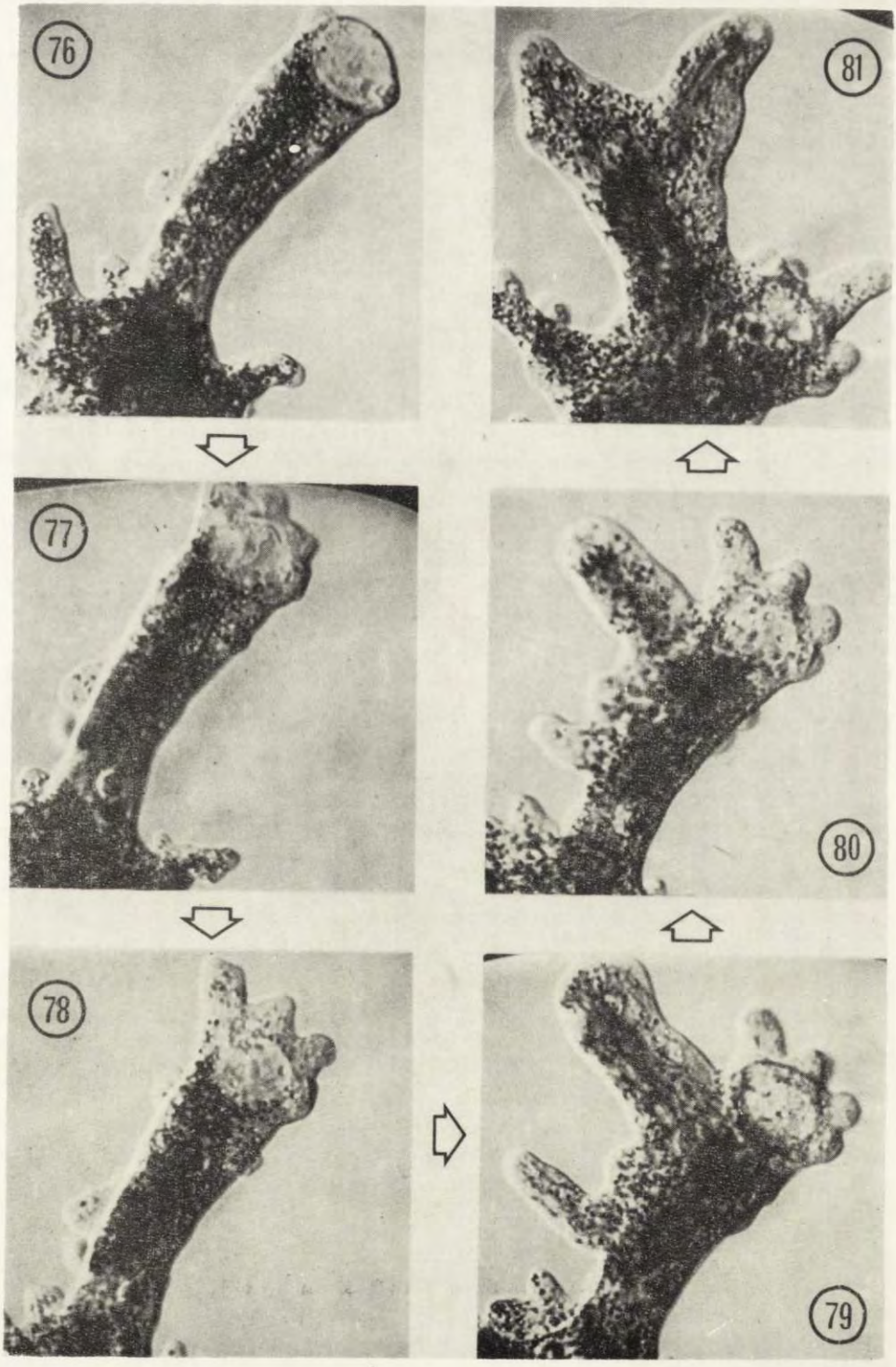

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Micrurgical Experiments on the Frontal Cap of Monotactic Forms of *Amoeba proteus*

Synopsis. Micrurgically damaged frontal caps of monotactic amoebae disintegrate into vacuoles and are resorbed, and they may be re-integrated from the residue vacuolar material. There exists a border structure separating the cap from the granuloplasm, and its mechanical injury results in disintegration of the cap. The existence of cap envelope is directly demonstrated in bicompartmental caps which are a stage of cap reintegration after longitudinal bisection. In a monotactic amoeba treated with ethanol, the vesicular frontal cap shows the same optical density as the contractile vacuole, but lower than hyaline ectoplasm and than a hyaline frontal cap. Conclusions are drawn concerning the vacuolar origin and vesicular character of such caps, and concerning the role they play in polarizing the streaming pattern and locomotion, by disrupting locally the continuity of the contractile cell cortex.

The present investigations were undertaken in the intention to complete and to support experimentally the data, concerning the nature and the origin of monotactic forms of *Amoeba proteus* and of their frontal caps, which in the precedent study (Grębecka 1978) were obtained by observation of developing and declining frontal caps, and of corresponding body shape transformations, under normal culture conditions. The major part of experiments consisted in various types of micrurgical injuring the active frontal caps in order to provoke different stages of their resorption, and in some cases, of their reconstitution. Special attention was paid to situations allowing to draw conclusions pertaining to the questions of frontal cap structure, its origin, the character of its content, the role of cell cortex and of cytoplasmic stream in cap formation and functions.

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The terminology recently proposed by Grębecki and Grębecka (1978) is used, as in the precedent article, to define the morphodynamic types of amoeba. According to it, the present study concerns the monotactic form (steadily "monopodial", with vesicular frontal cap, developing spontaneously, and not facultatively "monopodial", with hyaline frontal cap, obtained under unidirectional stimulation, which is called orthotactic), and its origin from the polytactic form (most common "polypodial" type, well attached and migrating, and not "radiate", with numerous very long poorly attached and non-migratory pseudopodia, which is called heterotactic).

Material and Methods

Monotactic cells of *Amoeba proteus* (Princeton strain) were selected from cultures grown in the Pringsheim solution. Twice per week the culture medium was changed and the animals fed on *Colpidium* sp., kept in separate cultures. For growing *Colpidia,* the same Pringsheim solution was used as medium and the suspension of dried yolk as food.

The specimens selected for microoperations, observations and photomicrography, were put in drops of culture medium under paraffin oil, in chambers similar to those of de Fonbrune system. De Fonbrune micromanipulator served for operations and de Fonbrune microforge for making tools. The equipment for differential interference contrast microscopy and for semi-automatic taking serial pictures, were exactly the same as in the precedent study (Grębecka 1978).

Results

Effect of injuring the cap walls

The observation of Seravin (1966) that, after damaging the frontal cap with a microneedle, the monotactic amoeba becomes polytactic again, is confirmed. In the present study, either the frontal wall of the cap was perforated from outside, or its intracellular border from inside.

The Pl. I 1–7 presents the effects of injuring by microneedle the external cap wall. The operation is followed by extrusion of the cap content into the medium, where a part of it may be seen as a vacuole (Pl. I 3–6). The cap is cleared away together with an adjacent portion of cytoplasm (Pl. I 1–2). It allows to conclude that the extrusion is provoked by a strong local contraction of cell cortex behind the frontal cap. Such a contraction explains also why the subsequent change of the motory polarity of amoeba is so drastic, that the former front, after

elimination of its cap, becomes the new tail, the cytoplasm flows back up to the former uroid, blows it up and transforms it into the new front (Pl. I 4–6). Such a complete reversal of motory polarity is not obligatory but the most common result of this type of operation.

When the microneedle, introduced inside the monotactic amoeba, is used to damage the internal border structure separating the cap front the granuloplasm (Pl. II 9), the frontal cap is not extruded outside, but it becomes resorbed into the cytoplasm, in the manner similar to that of its spontaneous declining (Grębecka 1978). Injury of the internal wall of the cap is followed by invasion of granuloplasm to its interior (Pl. II 10–12), reappearance of cortical hyaloplasm layer between the frontal side of the cap and the outer cell membrane (Pl. II 10–14), and a shift of the former cap in vesicular form towards the cell interior (Pl. II 14–16). Amoeba assumes the polytactic body shape (Pl. II 16–17), but its motory polarity is unsteady and the former cap material persists in the cytoplasm in vacuolar form. In other words, the cell is in condition which usually precedes its natural transformation into a monotactic form (Grębecka 1978), and one could expect therefore that the recovery of polytactic character is not definitive. Anyhow, the resorption of vesicular frontal cap after penetration of microneedle to its interior from the cytoplasm side, puts in evidence the existence of a border structure separating the cap content from the granuloplasm.

Experimental alteration of the streaming pattern

Similar results are provided by microoperations which change the streaming pattern in monotactic amoebae. Two series of such experiments were carried out.

First type of intervention consisted in pressing with microneedle the cell region behind the frontal cap, in order to stop the afflux of endoplasm to the inner side of the cap. As a result, the first stages of cap resorption are produced, in particular its detachment from the outer membrane and reappearance of the cortical ectoplasm in the interstice (Pl. I 8).

Similar, although less advanced changes result from injection of a droplet of paraffin oil which, as described before (Grębecka 1977), forms initially a competitive artificial cap at the opposite cell pole. The oil cap reverses a part of the cytoplasmic stream in its own direction (Pl. III 18–19) which results in a temporary interruption of any flow in the vicinity of the original cap (Pl. III 20–21). In parallel, the borderline between the frontal cap and the granuloplasm becomes very distinct, and it incurves in the manner indicating that the peripheral cytoplasm starts to envelope the cap by circumfusing along the cell walls (Pl. III 20–22). In the meantime however, the influence of the competitive oil

drop falls down, and as a result, the cytoplasmic stream starts flowing again towards the original frontal cap, first stages of its resorption are reversed, and it gradually recovers its former shape (Pl. III 23–26).

The complete resorption of a vesicular frontal cap by combined effects of the competitive oil cap and of interrupting the cytoplasmic flow by external local pressure, is described in another paper (Grębecka 1978). All these experiments demonstrate that the cytoplasm streaming plays an important role in keeping the vesicular frontal cap active, and consequently in maintaining the stability of monotactic form of amoeba.

Exposure of monotactic amoebae to ethanol

The experiments reported above, and the description of analogous processes as they occur under normal culture conditions (Grębecka 1978), permit to suppose that frontal cap resorption is initiated when at least one of the following requirements is met: (a) destruction of the internal border structure of the cap, (b) arresting of the cytoplasmic flow behind it, (c) penetration of the cortical hyaloplasm between the cap membrane and the outer cell membrane. The (c) may be provoked by (a) and/or (b), or by other factors. Ethanol added to the medium may play the role of such a factor. Hüllsmann et al. (1976) obtained a considerable thickening of the peripheral hyaline ectoplasm layer in polytactic amoebae exposed to 2.5–5% ethanol solution in a three-compartment perfusion chamber.

The present experiment was more rough as to the methods, and more specific as to the subject: only monotactic amoebae with prominent frontal caps were treated with ethanol, but the concentration gradient was established by free diffusion of ethanol through the simple observation chamber, at room temperature. Monotactic amoebae were selected in the intention to follow the behaviour of vesicular frontal caps surrounded by a voluminous hyaline layer, to check whether they dissolve in it or keep their identity, and to look for a difference in optical density between the cap content and the hyaloplasm. The existence of such a difference has been shown by Korohoda and Stockem (1975), but not in one single cell with both media bordering each other.

In monotactic amoeba the changes provoked by ethanol start from the posterior body pole: the tail and the middle body region flatten and produce numerous broad and flat pseudopodia (Pl. VIII 67–69). Thereafter, the centripetal aggregation of granuloplasm begins, followed by rounding the body up, and in parallel the resorption of the frontal cap takes place, in the same way as described before under natural and other experimental conditions (Pl. VIII 70–74).

Even at very advanced stages of cap resorption one can see the

borders of the residual vesicle (Pl. IX 75) and of vacuoles resulting of its fragmentation (Pl. IX 76), as well as a distinct optical difference between their content and the hyaloplasm.

Particularly interesting is the case, shown in the Pl. IX 77, of a partly resorbed frontal cap adjacent to a contractile vacuole. Both bodies exhibit exactly the same optical density, distinctly lower than that of the surrounding cytoplasm, what confirms the vacuolar character and origin of the frontal cap of monotactic amoebae.

It was also possible, in this series of experiments, to take picture of a vacuole which is residue of resorbed frontal cap, co-existing within the same cell with a hyaline frontal cap at the tip of a new pseudopodium (Pl. IX 78). The optical difference of constituent material in both types of frontal caps appears unquestionable.

Bisection of frontal cap

Seravin (1966) wrote that "when a fragment of cap is preserved after operation, amoeba may recover the monopodial form after a certain time". This phenomenon was confirmed in the present research, and studied in more detailed manner, by dissecting vesicular frontal caps in two with microneedle applied along the longitudinal body axis (Pl. IV 27). Both distinctly separated halves (Pl. IV 28) behave often as an intact frontal cap during its spontaneous declining occurring under normal culture conditions (Grębecka 1978) and present the same stages of resorption (Pl. IV 28–33). The amoeba becomes temporarily polytactic with unstable motory polarity and keeps in the cytoplasm the cap material in vacuolar form (Pl. IV 33 — V 38). It rebuilds eventually from it a new frontal cap and becomes monotactic again (Pl. V 39–48). The whole sequence shown in the Plates IV–V repeats exactly, during 10–20 min, all the phases of cap development and of cap resorption which take several hours under usual culture conditions.

In some cases (Pl. VI and VII) the effects of bisection are more specific, and reveal particularly well the vacuolar nature of the frontal cap in monotactic amoeba. During first minutes after operation both halves fail to manifest any symptoms of resorption (Pl. VI 49–51). Then, one of them becomes clearly dominating and active, whereas the second (the right one in the pictures) shows first signs of resorption and is shifted to the body side (Pl. VI 52–57). Before its fragmentation could take place it is already carried away by the cytoplasm streaming and it approaches the functional half again, but from the inside (Pl. VII 58–60).

Suddenly, the granuloplasm is pushed aside and the membranes of the active frontal cap and of the approaching vacuole (which is nothing

else than the part of the same cap removed before) enter in close contact, and they become perfectly well visible in the light microscope against the background of agranular transparent cap fluid (Pl. VII 61–62). The pressure exerted by the cytoplasmic stream is so important that it produces a distinct flattening of both compartments of the cap. This most spectacular stage lasts a few seconds only, the membranes separating both compartments disrupt (Pl. VII 63), whereas the lateral membranes probably remain intact and fuse into one entity. After reintegration of frontal cap, the cytoplasmic stream intensifies and stabilizes again the monotactic form of amoeba (Pl. VII 64–66).

The critical stage, when for a few seconds the frontal cap is composed of two separate compartments, presents an unique situation when the membrane-like structure enveloping the vesicular frontal cap may be seen under light microscope *in vivo*, in an active monotactic individual, in usual culture medium, and under good optical conditions. The phenomenon was observed several times, but it may be correctly recorded on photomicrographs only exceptionally, when both compartments of the cap are by chance separated in a plane perpendicular to the plane of observation.

Intracellular injections of the culture medium

In the last series of experiments an attempt was made to provoke the development of a monotactic form of amoeba by injecting the Pringsheim culture medium into polytactic individuals. The results were not uniform and may be presented in three groups of effects produced:

(1) The cell body blows up, the limits of the injection site are not clearly cut but appear diffuse, the contractile vacuole accelerates its rate of pulsation. Probably it is a case when the injected fluid fails to be compartmentalized in vacuoles.

(2) The site of injection remains more transparent and the injected fluid apparently fails to diffuse freely in the surrounding cytoplasm (Pl. IX 79). Later on, aggregation of vacuoles begins at a point of cell periphery and their fusion is seen (Pl. IX 80–83). The picture is strictly similar to the first stages of frontal cap development.

(3) In one case only a normal and stable frontal cap was obtained, when the fluid has been injected very close to the outer cell membrane. The cap has been formed immediately, during the injection (as it happens regularly when the paraffin oil is injected in this manner). This unique experiment has not been photographically recorded. Probably it was a case of fortuitous injection inside the cortical layer which directly provoked its local disruption.

In general, the injections of culture medium confirm the necessity of vacuolization of cytoplasm, and of local breaking the continuity of cell cortex, for the effective formation of a vesicular frontal cap.

Discussion

The experiments on frontal caps of monotactic *A. proteus*, described in the present study, permit to state in general that:

(1) The vacuolar origin of frontal caps of this type (Grębecka 1978) is reflected in their fragmentation into vacuoles, during resorption induced experimentally by various microoperations and by exposure to ethanol. The fact that the culture liquid injected intracellularly may initiate the cap formation only when vacuolized, corroborates the same conclusion.

(2) Conclusion of Korohoda and Stockem (1975 and 1976) that such caps contain a watery fluid is confirmed by exposure of monotactic amoebae to ethanol, what permitted to demonstrate within the same single cell: a difference of optical density of vesicular frontal cap residue in respect to the peripheral hyaloplasm, its optical identity with contractile vacuole, and its optical difference in respect to a hyaline frontal cap.

(3) The presumption that vesicular frontal caps are surrounded by a membrane, is supported by their resorption as result of micrurgical injury of the border zone separating them from the granuloplasm, and in particular by demonstration in good optical conditions that two parts of bisected frontal cap may form temporarily, until they fuse again, a double cap composed of two compartments, each one cased within its own membrane-like envelope. The existence of structural cap walls is also demonstrated by electron microscopy, in a separate paper (Hrebenda and Grębecka 1978).

(4) The view expressed in the precedent study (Grębecka 1978) that the cytoplasmic stream plays a role in maintaining the vesicular frontal cap of monotactic amoeba in active state, is supported by the fact that a streaming recess precedes every case of cap resorption induced experimentally, and that its interruption by local mechanical pressure or by injection of a competitive oil cap provokes first stages of cap resorption.

(5) Another author's earlier conclusion that the vesicular frontal cap, as well as the artificial oil cap, exert their motory effects by disrupting

locally the continuity of cell cortex (motory functions of which are stressed by Korohoda and Stockem 1975, Kalisz and Korohoda 1976, and Grębecki 1977), is well corroborated by observations that cortical contraction behind the cap extrudes it out of the cell after a frontal puncture effected from outside, that during the cap resorption induced experimentally the cortical hyaloplasm circumfuses and penetrates again between the cap and the frontal wall of pseudopodium tip, and that a vigorous injection of culture liquid against the inner side of the cortex may result in an immediate cap formation.

RÉSUME

Chez les amibes monotactiques, des différentes lésions micrurgiques de la calotte frontale provoquent sa fragmentation en vacuoles, qui peuvent servir à une éventuelle réintégration de la calotte. Il existe une paroi entre la calotte et le granuloplasme, car l'endommagement mécanique de cette zone aboutit à la désintégration de toute la calotte. L'existence d'une telle enveloppe devient directement visible dans les calottes doubles obtenues en issue des dissections. Chez les amibes monotactiques exposées à l'action de l'étanol, leurs calottes frontales vésiculaires présentent une densité optique qui est la même que celle des vacuoles contractiles, mais inférieure à celle de l'ectoplasme hyaline et des calottes frontales hyalines. Les conclusions portent sur l'origine vacuolaire et la nature vésiculaire de telles calottes, ainsi que sur le rôle qu'elles jouent en polarisant le courant cytoplasmique et la locomotion par le fait qu'elles coupent localement la continuité du contractile cortex céllulaire.

REFERENCES

Grębecka L. 1977: Changes of motory polarization in *Amoeba proteus* as induced by oil injections. Acta Protozool., 16, 107–120.

Grębecka L. 1978: Frontal cap formation and origin of monotactic forms of *Amoeba proteus* under culture conditions. Acta Protozool., 17, 193–204.

Grębecki A. 1977: Non-axial cell frame movements and the locomotion of *Amoeba proteus*. Acta Protozool., 16, 53–85.

Grębecki A. and Grębecka L. 1978: Morphodynamic types of *Amoeba proteus*: a terminological proposal. Protistologica, (in press).

Hrebenda B. and Grębecka L. 1978: Ultrastructure of the frontal cap of monotactic forms of *Amoeba proteus*. Cytobiologie, (in press).

Hülsmann N., Korohoda W. and Stockem W. 1976: Reversible disorganization of motile activities in *Amoeba proteus* induced by general anaesthetics. Acta Protozool., 15, 187–194.

Kalisz B. and Korohoda W. 1976: Experimental study on locomotion of *Amoeba proteus*. I. Movements in the nucleated and anucleated fragments of the amoebae after removal of the part of their cytoplasm. Acta Protozool., 15, 345–361.

Korohoda W. and Stockem W. 1975: On the nature of hyaline zones in the cytoplasm of *Amoeba proteus*. Microsc. Acta, 77, 129–141.
Korohoda W. and Stockem W. 1976: Two types of hyaline caps, constricting rings and the significance of contact for the locomotion of *Amoeba proteus*. Acta Protozool., 15, 179–185.
Seravin L. N. 1966: Monopodialnye formy *Amoeba proteus*. Dokl. Akad. Nauk SSSR, 166, 1472–1475.

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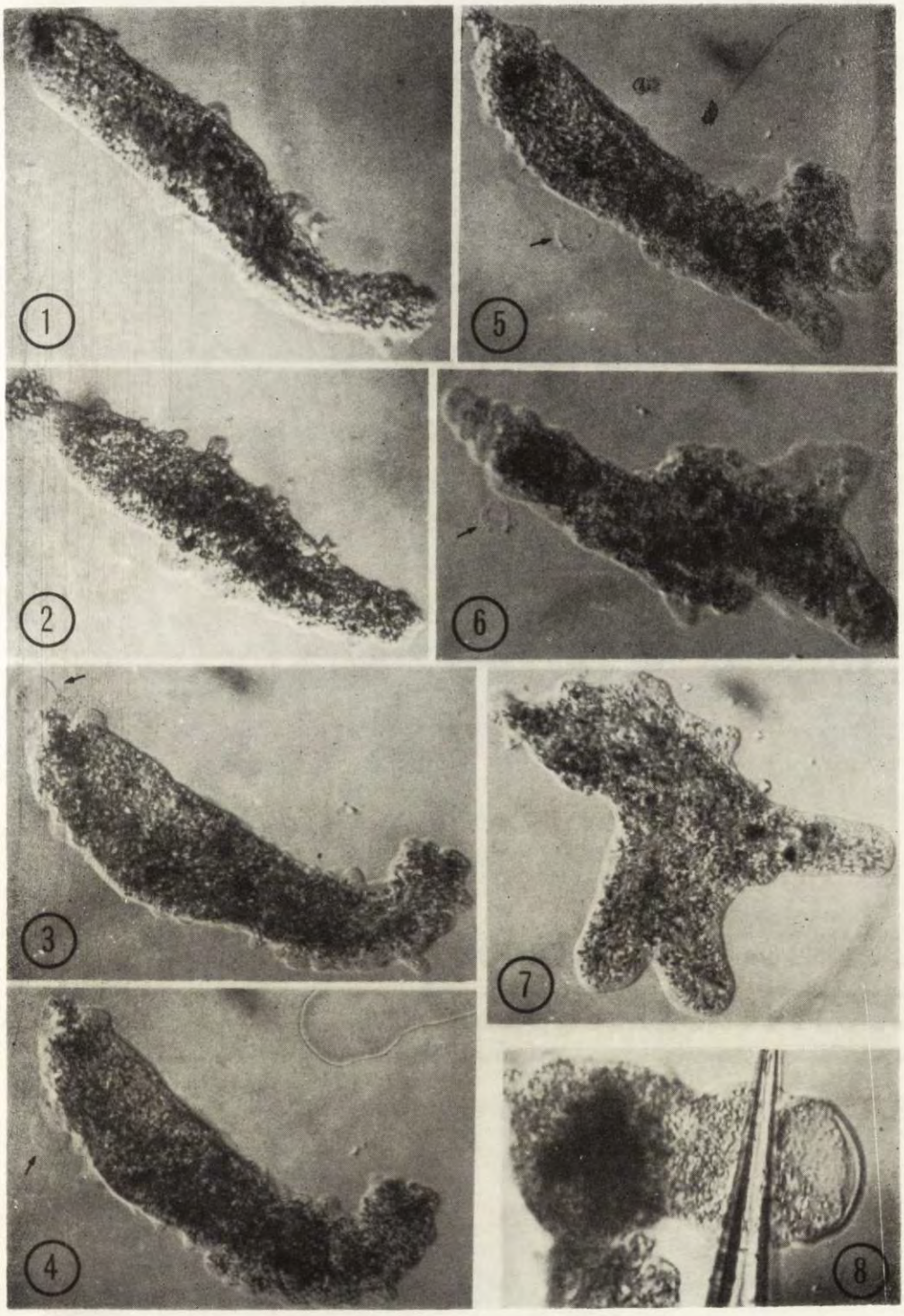
EXPLANATION OF PLATES I–IX

1–7: Extrusion of the frontal cap and inversion of motory polarity in monotactic amoeba after perforation of the external cap; wall arrows indicate the vesicle outside the cell which is a remnant of the extruded cap
8: An early stage of cap resorption obtained after interrupting the cytoplasmic stream by local mechanical pressure
9–17: Resorption of the frontal cap and transformation of monotactic amoeba into polytactic one after micrurgical injury of border zone between the cap and the granuloplasm
18–26: Initiation of first stages of frontal cap resorption, and recovery of its former shape and function, after injection of oil droplet which temporarily constitutes an opposite competitive cap
27–48: Full cycle of complete resorption of vesicular frontal cap and of its reconstitution from vacuoles (with corresponding changes of body shape), following a cap bisection in monotactic form of *Amoeba proteus*
49–66: A case of bisection not followed by cap fragmentation, when it reintegrates its entity from two large parts, which are temporarily arranged linearly as two compartments with well visible membrane-like structures enveloping each of them
67–74: Stages of resorption of vesicular frontal cap in monotactic amoeba under the influence of ethanol
75–76: Higher magnification of advanced stages of cap resorption in monotactic amoeba treated with ethanol; the cap residue presents an irregular vesicle (75) and then is fragmented into vacuoles (76), being still well distinct from the surrounding cytoplasm
77: Comparison of optical densities of frontal cap vesicular residue, of contractile vacuole, and of the surrounding cytoplasm, in an ethanol treated specimen
78: Difference of optical density between the frontal cap vesicular residue and a hyaline frontal cap, developed in a monotactic amoeba treated with ethanol
79–83: A case of intracellular injection of Pringsheim solution, when the injected fluid failed to mix freely with the cytoplasm, and subsequently the early stages of frontal cap formation from vacuoles were obtained



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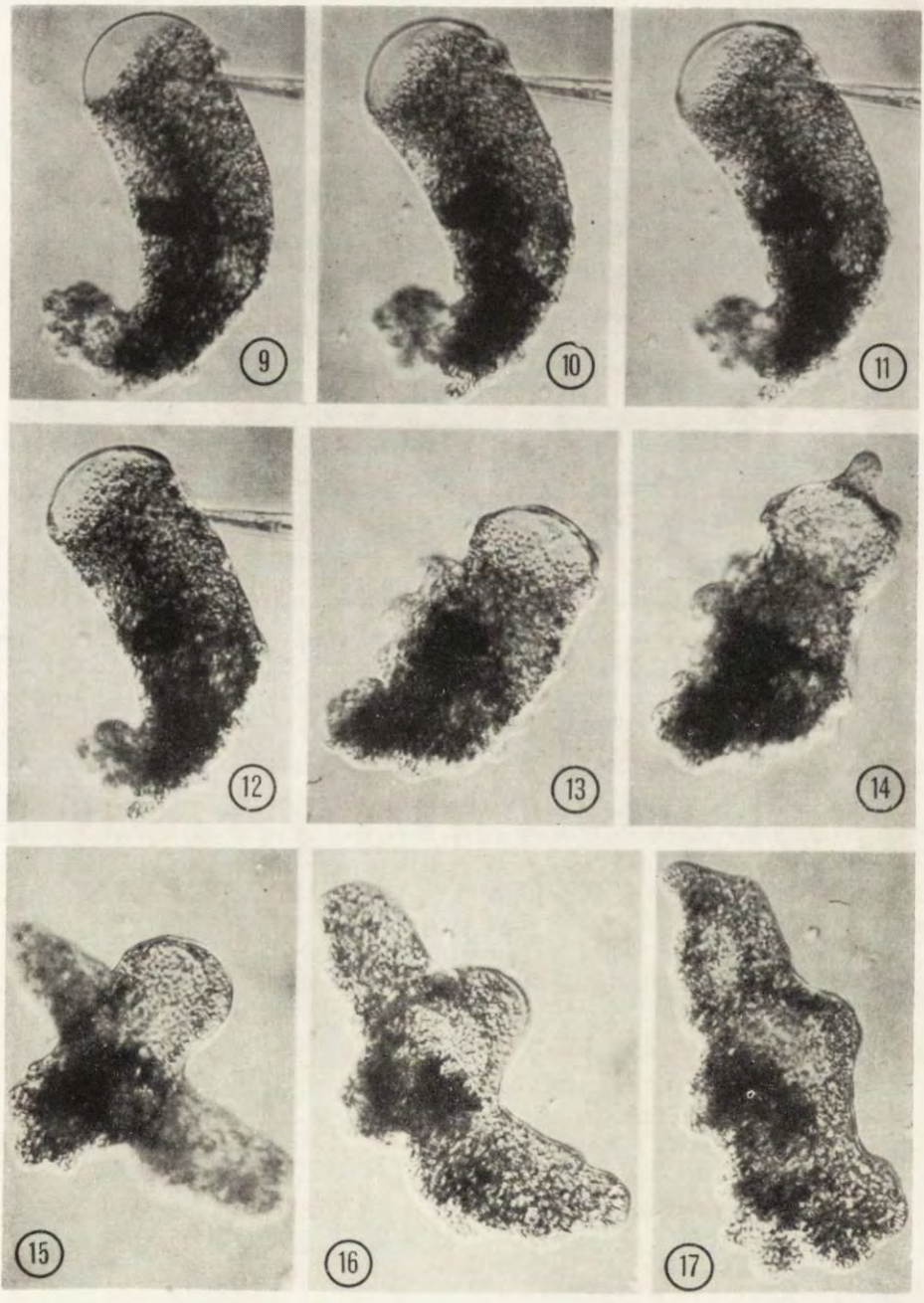

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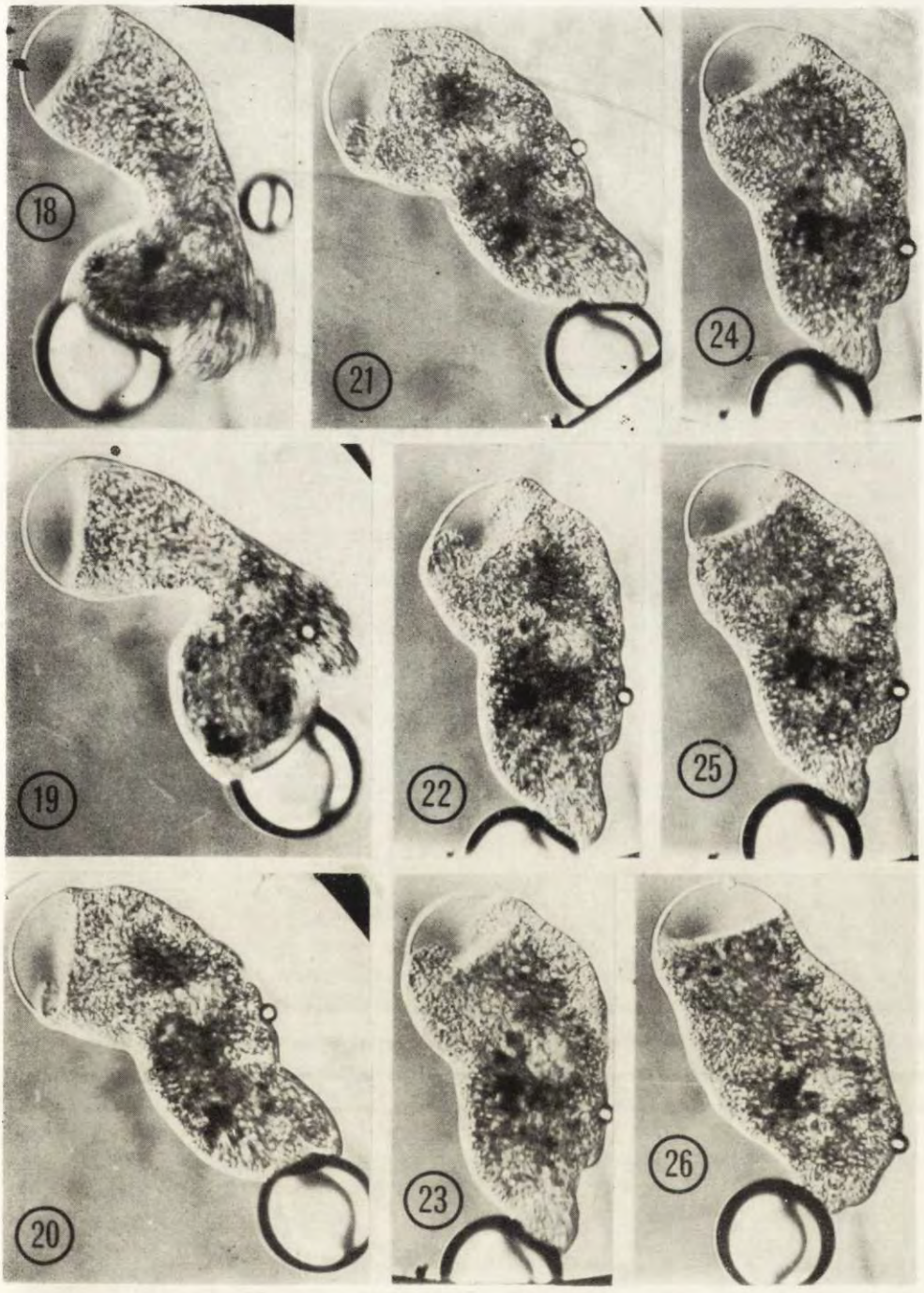

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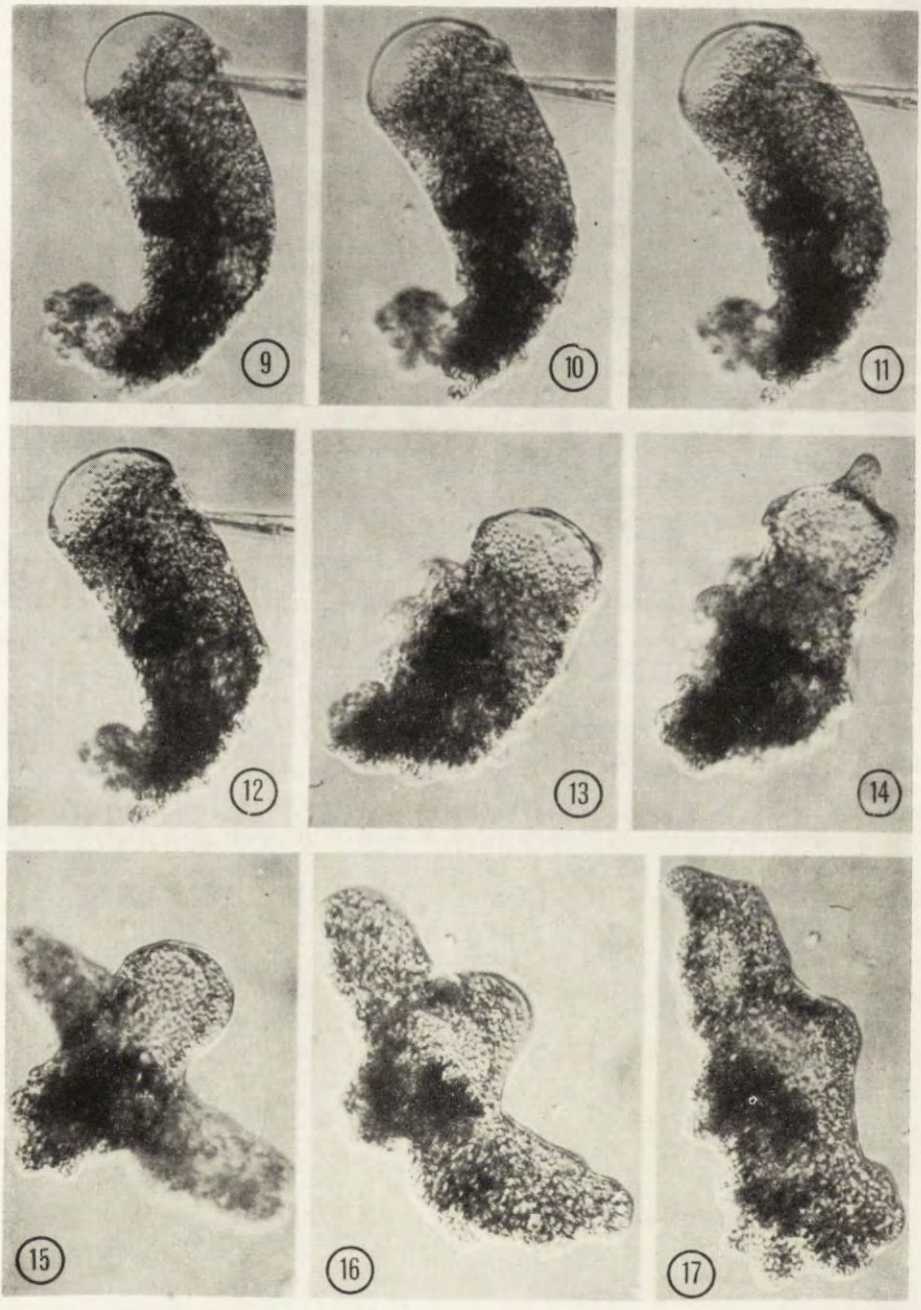

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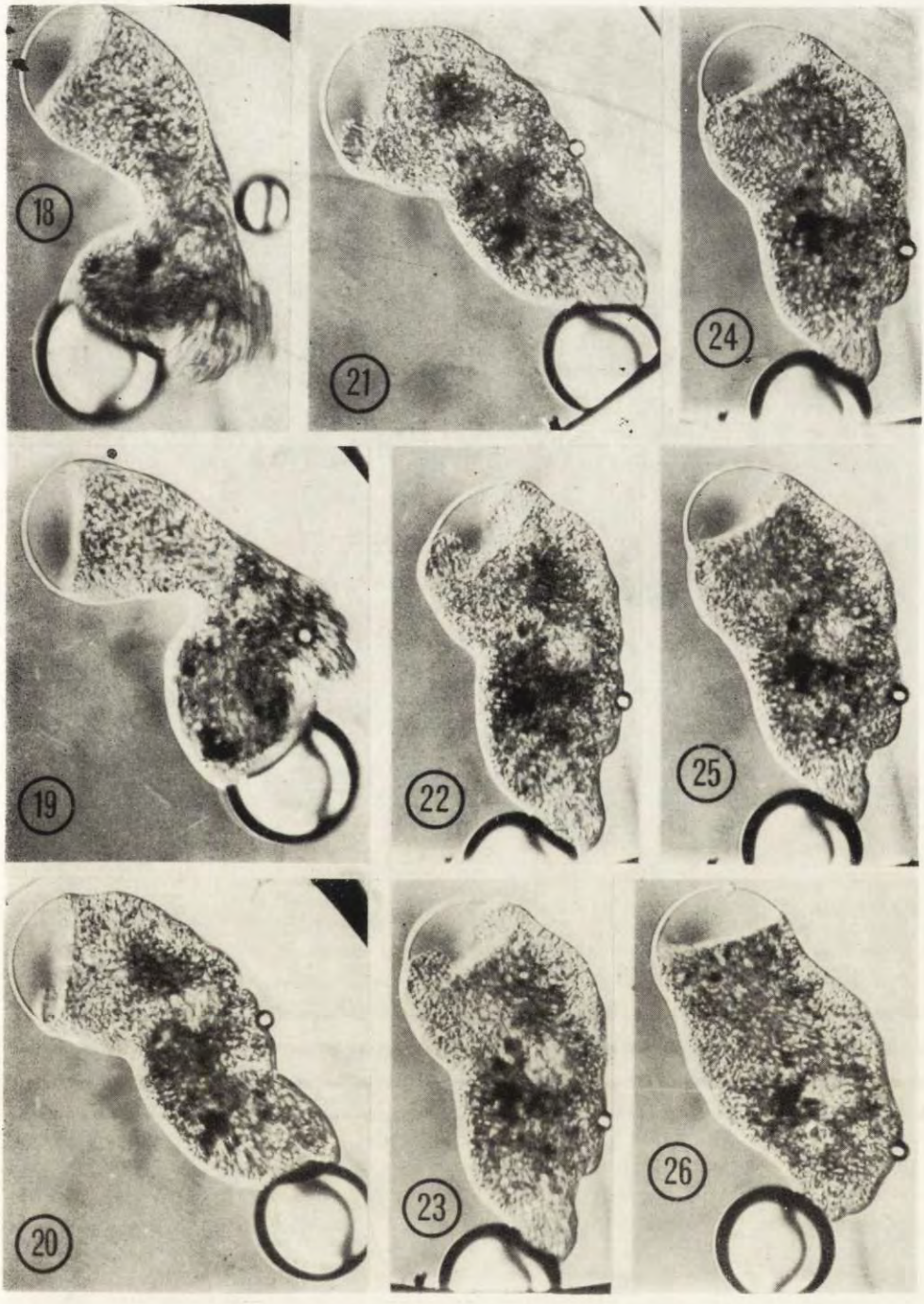

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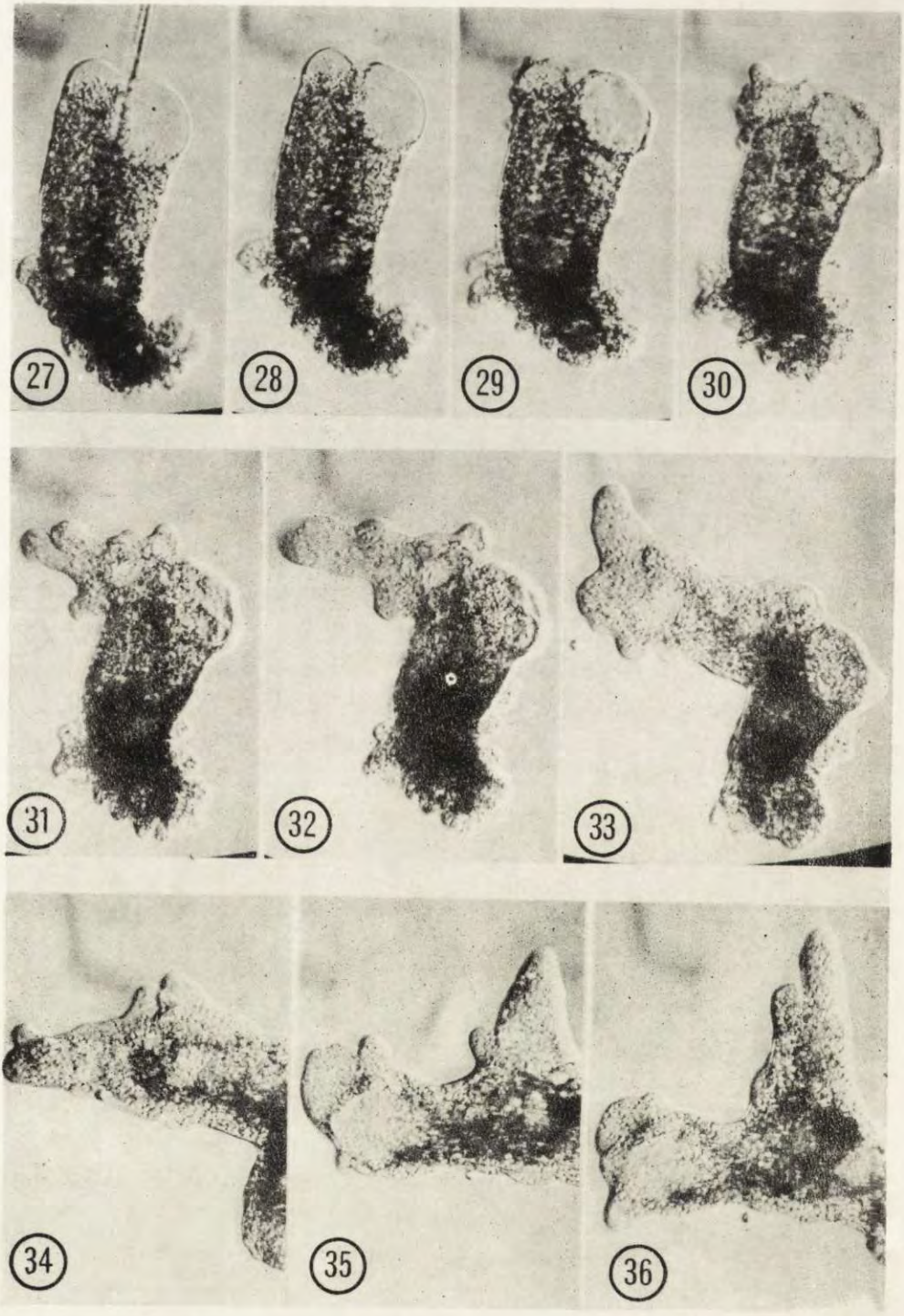

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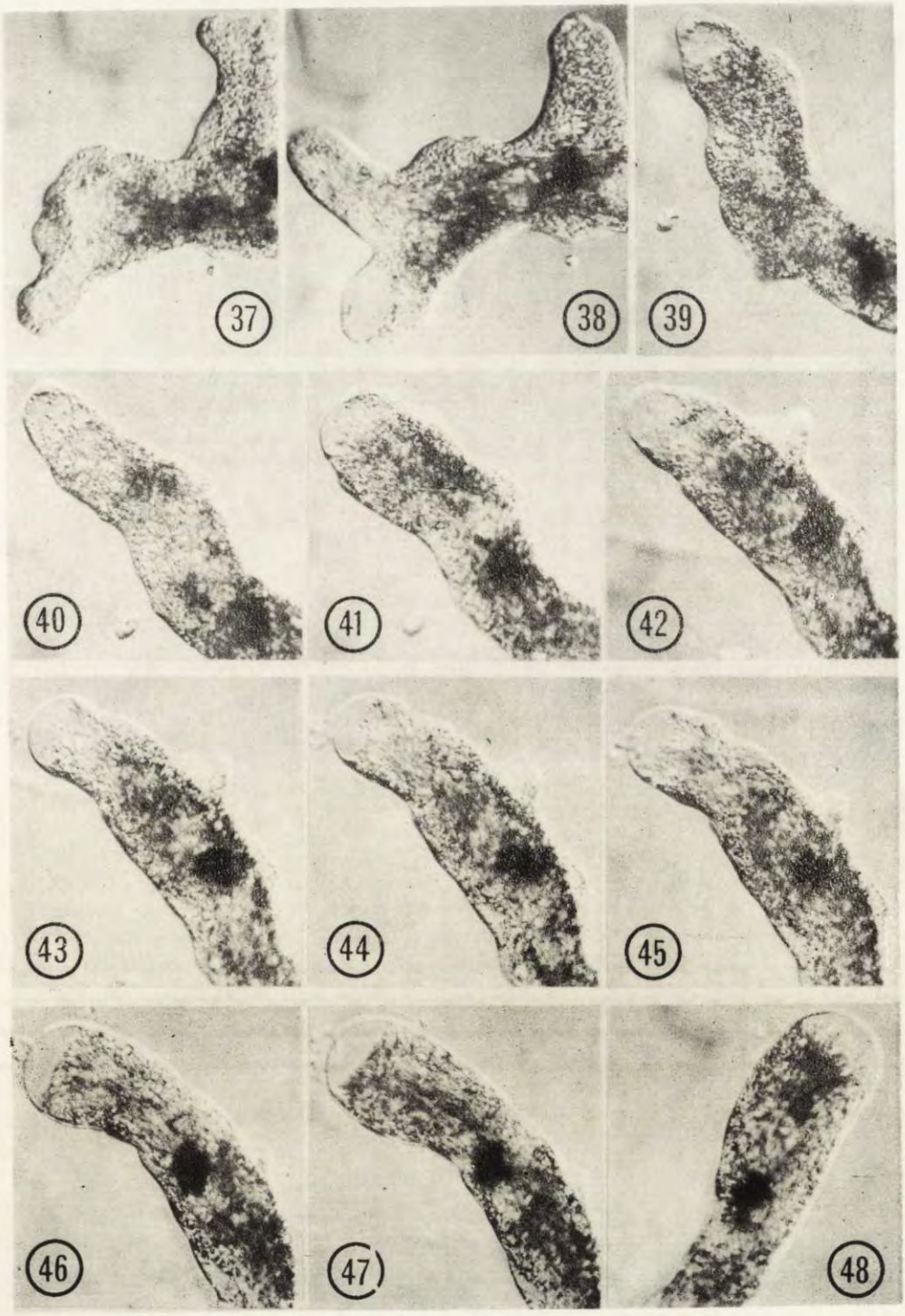

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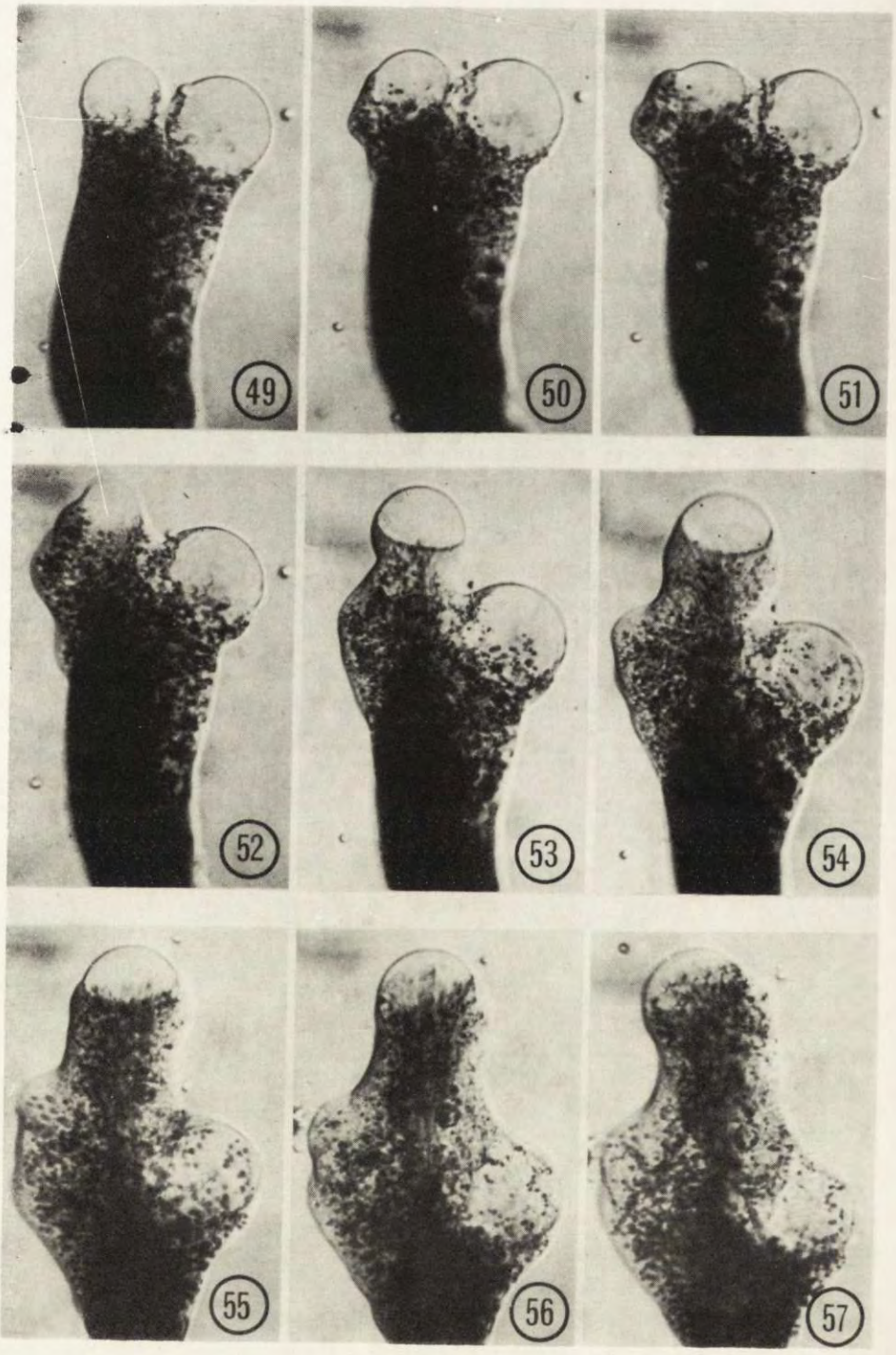

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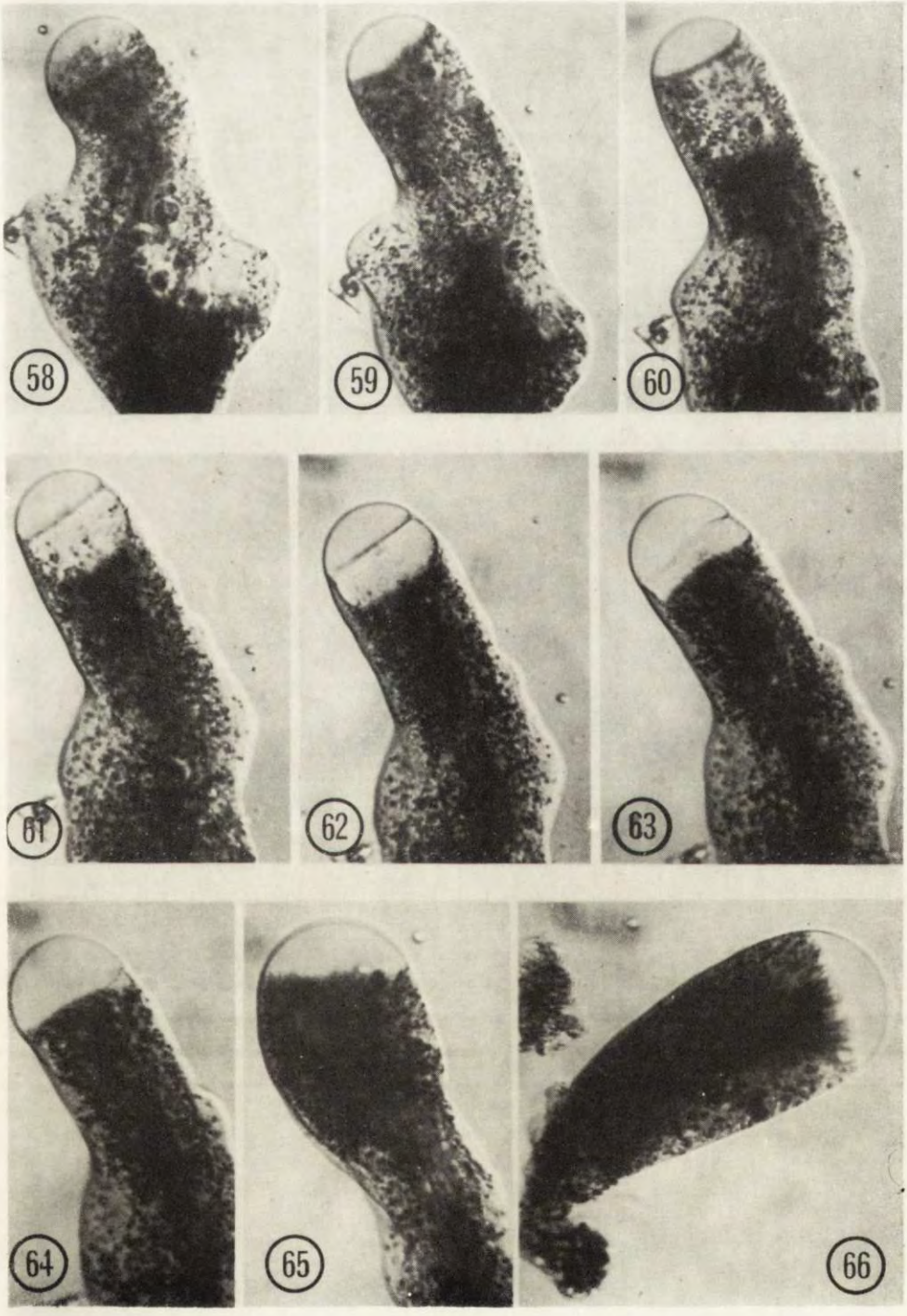

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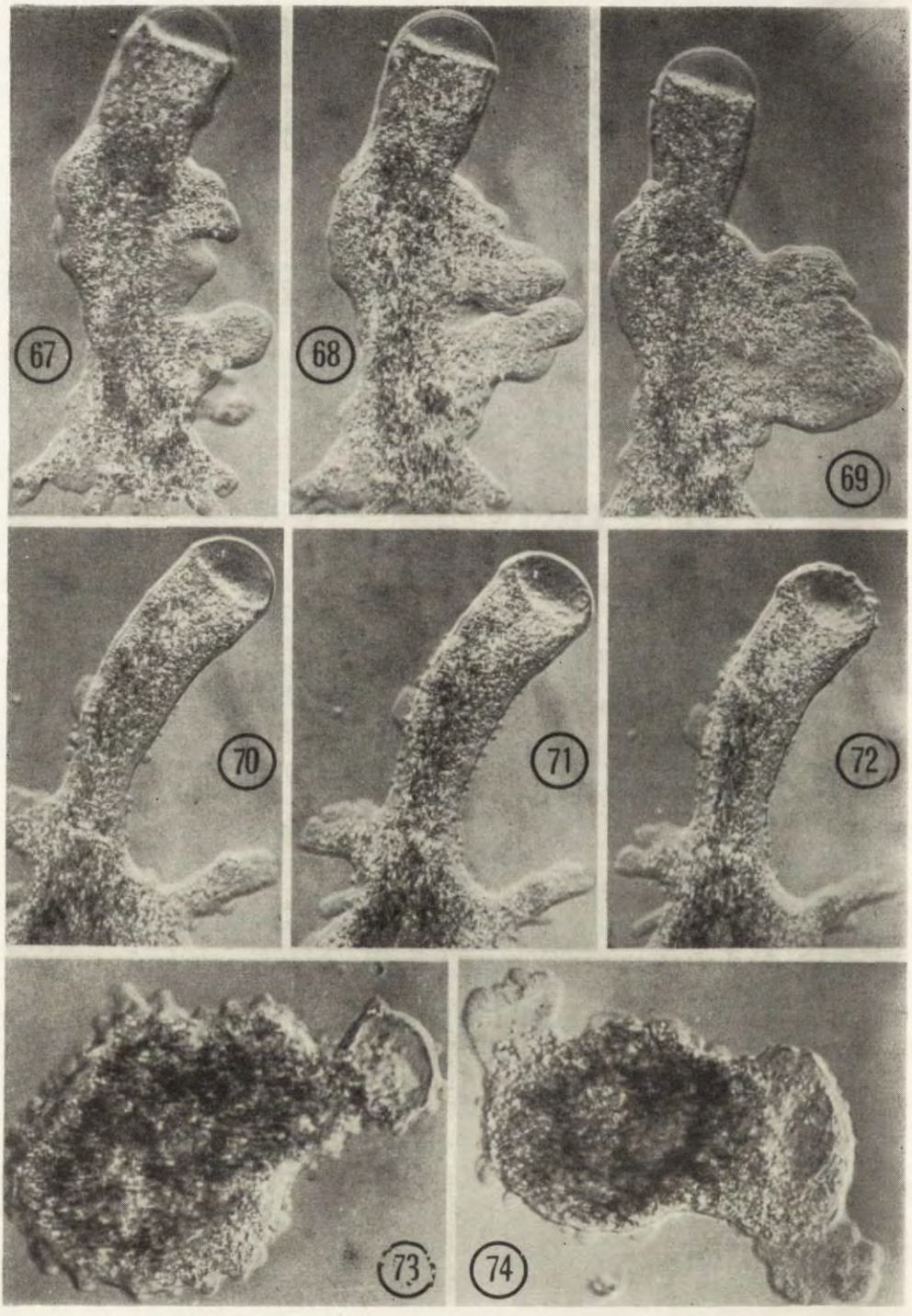

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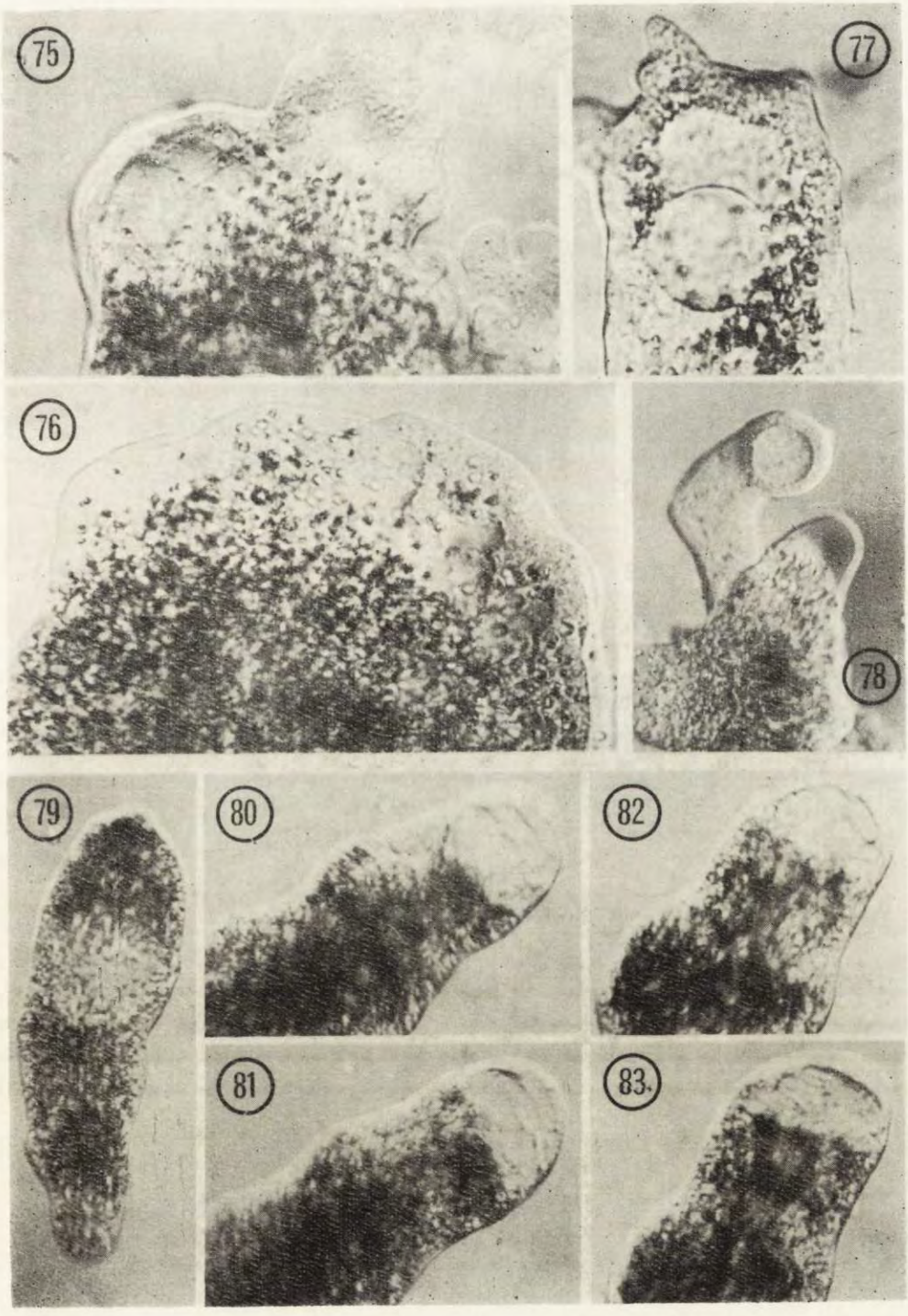

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Papers are accepted in English, French, German and Russian. Every paper should begin with the name and postal address of the laboratory, name and the surname of the author and title in the language of the text. The titles in German and French should be translated into English according to the demands of Current Contents. The paper should be accompanied by synopsis in the language of the text, not exceeding 100 words and by short summary in one of 4 languages accepted in the Journal. In the Russian text also the name and the postal address of the laboratory, legends of tables, plates and text-illustrations must be translated, the translation of the summary may be somewhat more extensive, and the name of the author should be given additionally also in the Latin characters.

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G e l e i J. von 1939: Das äussere Stützgerüstsystem des Parameciumkörpers. Arch. Protistenk., 92, 245–272.

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ERRATA

Acta Protozoologica, vol. 16, No. 3/4

Estela Sousa e Silva: Some ultrastructural variations of the nucleus in dinoflagellates throughout the life cycle.

page 277, line 26 from the top

instead of:

"1974 a, b, c) those "fragments" could be the chromosomal units, but"

should be:

"species through the life cycle and related not only to cell division"

In preparation:

W. Foissner: Das Silberliniensystem und die Infraciliatur der Gattungs *Platyophrya* Kahl, 1926, *Cyrtolophosis* Stokes, 1885 und *Colpoda* O. F. M., 1786: Ein Beitrag zur Systematik der *Colpodida* (*Ciliata, Vestibulifera*) — D. P. Haldar and N. Chakraborty: Observations on Three New Species of Cephaline Gregarines (*Protozoa*: *Sporozoa*) from Insects — M. J. Devdhar and S. Amoji: *Sciadiophora gagrellula* sp. n. from the Phalangid Arthropod, *Gagrellula saddlana* Roewer — Z. Žižka: Fine structure of the Neogregarine *Farinocystis tribolii* Weiser, 1953. Free Gametocytes — V. Golemansky: Description de neuf nouvelles espèces de Coccidies (*Coccidia, Eimeriidae*), parasites de Micromammifères en Bulgarie — J. D. Knell and G. E. Allen: Morphology and Ultrastructure of *Unikaryon minutum* sp. n. (*Microsporidia : Protozoa*), a Parasite of the Southern Pine Beetle, *Dendroctonus frontalis* — C. Kalavati and C. C. Narasimhamurti: A New Microsporidian Parasite *Toxoglugea tillargi* sp. n. from an Odonate, *Tholymis tillarga* — J. Bąkowska and M. Jerka-Dziadosz: Ultrastructural Analysis of the Infraciliature of the Oral Apparatus in *Paraurostyla weissei* (*Hypotricha*) — J. Kaczanowska: Shape Transformation, Contractility and Endocytose/Exocytose Cycle in *Paramecium* Genically Deprived of Excitability — И. И. Скобло, М. С. Раутиян и Д. В. Осипов: Потеря генеративных ядер у инфузории *Paramecium caudatum* вызываемая на ранных стадиях инфекции их симбиотическими бактериями — О. Н. Борхсениус и Д. В. Осипов: Влияние избирательной инактивации генеративных ядер инфузории *Paramecium caudatum* на вегетативные функции клеток — G. Nowakowska: Twisting of Suspended Monatactic Specimens of *Amoeba proteus* — G. Nowakowska and A. Grębecki: Attachment of *Amoeba proteus* to the Substrate during Upside-down Crawling — E. C. Bovee, R. A. Lindberg and R. E. Goddard: The Swimming Velocity of *Paramecium caudatum* — M. Opas, B. Hrebenda and B. Tołłoczko: Contractility of Glycerol-extracted Nuclei of *Amoeba proteus* — J. Sikora and A. Wasik: Cytoplasmic Streaming Within Ni^{2+} Immobilized *Paramecium aurelia* — Z. Baranowski: The Contraction-relaxation waves in *Physarum polycephalum* Plasmodia.

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