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Circodinium minimum (Gassovsky, 1918), Electron-microscope Investigations *

Synopsis. The body of *Circodinium minimum* is coated with a single cellular membrane which adheres to a thin layer of epiplasma. Deeper on there are longitudinal microtubules grouped in clusters. A minute inconspicuous microfibrillar layer lies close to microtubules. In the cytoplasma underlying the microfibrillar layer, outside the ciliary zones, rare, unciliated kinetosomes are scattered. Such a structure of the cortex is much the same as that of *Ochoterenaia appendiculata.* Other similarities to the latter species may be recognized also in the structure of the vestibulum wall of *C. minimum.* As regards other studied characters *C. minimum* reveals certain features in common with *Entodiniomorphida,* and some others — with *Gymnostomata.*

The genus *Triadinium* was created by Fiorentini (1890) for the species *Triadinium caudatum* obtained from horse intestines. Later on, two other *Triadinium* species, namely *T. galea* and *T. minimum*, occurring in horse intestines were described by Gassovsky (1918). It appeared, however, that the attribution of *Triadinium* characters to *T. minimum* was based but on a superficial resemblance. Using the silver impregnation technique, Wolska (1969) recognized the buccal ciliature of *T. minimum* as being quite dissimilar to that of *T. caudatum*, so that she excluded this species from the genus *Triadinium* Florentini and transfered it, to a new-established genus *Circodinium* Wolska (Wolska 1971 b), which was included to the family *Blepharocorythidae*, because of the character of the mouth part.

Circodinium minimum (Gassovsky) was assumed to be most closely related with *Ochoterenaia appendiculata* Chavarria. The reasons were as follows: the presence of the caudal process with a bundle of cilia, the position of the pulsating vacuole and the cytopyge, and the situation of the nucleus in relation to the vestibulum. An origin of a rounded shape of the body, untypical of *Blepharocorythidae*, was discussed in Wolska

* This investigation was supported by Committee of Cytobiology of the Polish Academy of Sciences.

(1969). In brief, *Circodinium minimum* appears to be a *Ochoterenaia appendiculata* whose anterior end has been bent ventrally.

The electron-microscope studies on *Circodinium minimum* were undertaken with the aim of better learning of this very species as well as supporting the view mentioned above. In this paper, a structure of its cortex and buccal apparatus is reported.

Among *Blepharocorythidae*, only two *Blepharocorys* species (Grain 1966) and *Ochoterenaia appendiculata* (Wolska 1978 c) were examined under the electron-microscope. A possibility of comparison, especially with the latter-mentioned species, looks promising. Another reason of using the electron-microscope technique was a need of ascertaining the existence and the position of the so-called special cilia, the kinetosomes of which get silvered very weak and are hardly visible under the light microscope.

A morphological description of the species may be found elsewhere (e.g., Strelkov 1939), and the infraciliature was described in Wolska (1969, 1971 b). Only a scheme of ciliature distribution in *C. minimum* is offered in Fig. 1. Its body is 31–40 μm long and 22–36 μm wide.

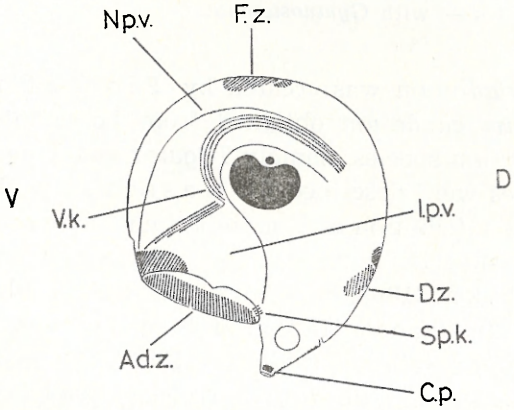

Fig. 1. *Circodinium minimum* (Gassovsky), scheme of ciliature distribution; left-side view, the protozoan is shown as transparent. Dorsal side (D), ventral side (V), adoral zone (Ad. z.), frontal zone (F. z.), dorsal zone (D. z.), caudal process (C. p.), special kinetosomes (Sp. k.), kinetics of the vestibulum (V. k), initial part of the vestibulum (I. p. v.), narrowed part of the vestibulum (N. p. v.), fibres are omitted

Material and Methods

Samples of contents of horse intestines were collected at the knackery at Rawicz immediately after slaughter and transported in vacuum flasks to the laboratory in Łódź. Only alive ciliates found in samples were selected and prepared to embedding in Epon according to Grain (1966). Sections cut on the ultramictorome III LKB

were collected on Formvar-coated grids and contrasted with uranyl acetate and lead citrate according to Reynolds. The majority of the grids was examined with the Tesla BS 513 A, and some other with the Tesla BS 500 microscope.

Results

The body of *Circodinium minimum* is covered with a single cell membrane — unit membrane which adheres to the underlying amorphous layer of epiplasm (Pl. I 1). Deeper on there is a layer of longitudinal microtubules, arranged in separate groups, each consisting of a few or about a dozen tubules lying at two levels (Pl. I 1). Between the groups single tubules happen to occur. A thin layer of the electron-dense substance isolates groups of microtubules from epiplasm (Pl. I 1). Just under the microtubular layer there is a inconspicuous coat of circular microfibrils (Pl. I 4, Pl. II 5). Therefrom, in some places, larger agglomerates of microfibrils come away deep into the cytoplasm (Pl. III 10), some of them being directed towards the wall of the vestibulum (Pl. III 11). In the cytoplasm underlying immediately the microfibrile layer, unciliated kinetosomes are sparsely spaced (Pl. I 2, 3, Pl. II 5, 6, 7, Pl. V 17). The discussed layers are usually separated from the inner cytoplasm by ergastoplasm (Pl. III 9).

Four long rows of cilia run obliquely along the wall of the vestibulum (Pl. IV 15, Pl. V 16, Pl. VI 20), the observation being concordant with that performed in light-microscope, silvered preparations. The rows of cilia are separated with cytoplasmatic crests. The unciliated wall of the vestibulum is overgrown with microtubules that form bands positioned almost perpendicularly to the wall surface (Pl. IV 12, 13, Pl. VI 21, 22). Deeper on in the vestibulum wall there are certain strands structured from material different in the initial broaden part of the vestibulum (Fig. 1) and in the narrowed tubular part. In the narrowed part of the vestibulum such strands, that have been described from silvered preparations as semicircular fibres, are regularly arranged and run almost perpendicularly to the long axis of the vestibulum. In the initial broaden part of the vestibulum, particular strands differ in thickness and run intricately. So much they are hardly interpretable that even ultracuttings were not helpful in the topographic and structural analyses of those "fibres". The electron photos seem to reveal only that in the initial part of the vestibulum the "fibres" look like strands built up of the amorphous substance, strongly extended in places (Pl. II 8, Pl. IV 12, 13, Pl. V 18). In the mid-vestibulum they differ in thickness and shape, and seem to be structured of the grainy-fibrilar material (Pl. IV 14, Pl. VI 21). In the narrowed part of the vestibulum they look like clusters of densely group-

ed microfibrils (Pl. V 12); these being joint with bands of microtubules (Pl. V 19), associates of kinetosomes of the vestibulum, probably transverse ones (Pl. VI 20).

The vestibulum ends with a cytostome. The cytopharynx is built of 25 bands of microtubules (rideaux de tubules). The same figure was observed in many sections through the cytopharynx (Pl. VII 23, 24, 26). Through the cytoplasm that surrounds the cytopharynx, here and there single microtubules or their small groups run irregularly (Pl. VII 23).

At the base of the caudal process, close to the adoral zone on the right body side, there is, in a deep pouch, a small group of short, deformed cilia (Pl. VII 27, Pl. VIII 28, 29), the so-called special cilia. Their kinetosomes are characterized by either a rudimental, or lacking at all axosomes, but there occur numerous tiny axial grains (Pl. VII 27, Pl. VIII 30). The cilia are swollen (Pl. VIII 30) and lacking in central fibres; their outlines in cross-sections are untypical: rectangular or square (Pl. VIII 31). The special cilia are groupped in several rows isolated from each other with folds of cytoplasm (Pl. VIII 31). The position of the special cilia is the same as that of the small kinetosome group observed in the silvered preparations.

In different regions of the *C. minimum* body there happen to occur certain corpuscles resembling those appearing in *Alloizona* (*Buetschliidae*) in concretion vacuole (G r a i n 1966) (Pl. VII 23, 25).

D i s c u s s i o n

The cortex of *Circodinium minimum* is, in general, similarly organized as that of other examined *Blepharocorythidae*, and it resembles most of all that of *Ochoterenaia appendiculata.*

In *Blepharocorys uncinata, B. jubata* (G r a i n 1966) and *Ochoterenaia oppendiculata* (W o l s k a 1978 c), a layer of epiplasm underlies the cell membrane and overlies a double layer of longitudinal microtubules which adheres to a layer of circular microfibrils. The microtubular layer is continuous in *B. uncinata* and *B. jubata*, whereas in *O. appendiculata* it is broken into clusters. A similar division of microtubules into clusters may be observed in *C. minimum*, too. The layer of epiplasm is very broad in *Blepharocorys* species, but it is thin in *O. appendiculata* and *C. minimum.* Sections through the cortex of *O. appendiculata* and *C. minimum* are so much resembling each other that their distinguishing would not be possible were it not that the cell membrane of *O. appendiculata* coheres to epiplasma but in some places, and preponderatingly it comes off epiplasm.

Another similarity between *Circodinium minimum* and *Ochoterenaia*

appendiculata appears to be the occurrence of unciliated kinetosomes scattered in cytoplasm just beneath the microfibrilar layer. Such kinetosomes have not been observed in the genus *Blepharocorys.*

The vestibulum wall of *C. minimum* is structured similarly as that of *O. appendiculata* and a bit differently from that of the genus *Blepharocorys.* In the latter, important role in rigidity of non ciliated wall of the vestibulum (Grain 1966) perform reticular fibres. In both *C. minimum* and *O. appendiculata* no reticular fibres have been observed, and their supporting semicircular fibres are in my opinion structured of the amorphous material and, of very densely packed microfibrils.

The cytopharynx of *C. minimum* is not surrounded by nemadesms, being much the same as those of *O. appendiculata* and *Blepharocorys.* The group of short deformed cilia situated in the open pouch at the base of the caudal process in *C. minimum* is similarly structured as cilia in the pouch of *O. appendiculata.* The presence of such a ciliature is not, however, a peculiarity of exclusively the two mentioned species. Isolated group of kinetosomes situated similarly as in *O. appendiculata* was reported from all the studied representatives of *Blepharocorythidae* from the horse or elephant intestines in light microscopy and named "special cilia" or "special kinetosomes" (Wolska 1971 a). It may be supposed that the very structure of this group is much the same in all the *Blepharocorythidae.* Moreover, this is not a peculiarity of *Blepharocorythidae* only. Similar cilia were observed under the electron microscope in *Ophryoscolecidae* (Bretschneider 1962, Roth and Shigenaka 1964), *Tripalmaria dogieli* (Wolska 1978 a), *Triadinium caudatum* (Wolska 1978 b), thus, in various *Entodiniomorphida.* Therefore, the feature appears to be common in *Blepharocorythidae* and *Entodiniomorphida.* These cilia are supposed to play a sensorial role, as it is the case of similarly structured cilia of several free-living *Gymnostomata,* which was discussed elsewhere (Wolska 1978 b, 1979).

Certain features in common appear to be possessed also by *Blepharocorythidae* and *Buetschliidae*; namely, in cytoplasm of both *C. minimum* and *O. appendiculata,* there occur some corpuscles resembling those described by Grain (1966) in the — concretion vacuole of *Alloizona.*

The obtained results of electron-microscope examinations show that the inclusion of *Circodinium minimum* into the *Blepharocorythidae* (Wolska 1971 b) was justifiable and that the species is more closely related with *O. appendiculata* than with the species of the genus *Blepharocorys.*

However, any more general conclusions may not be drawn from the fact that in naked parts of the *C. minimum* and *O. appendiculata* body some unciliated kinetosomes occur unless further, more detail studies are carried out.

RÉSUMÉ

Le corps de *Circodinium minimum* est couvert de membrane cellulaire simple qui adhère à une mince couche d'épiplasme. Les microtubules groupés en faisceaux longitudinaux se trouvent au dessous. Une couche exigue de microfibrilles adhère aux microtubules. Dans le cytoplasme juste au dessous des microfibrilles, et au delà des zones ciliés, on trouve des peu nombreux cinétosomes sans cils. Cette organisation du cortex ressemble étroitement celle du cortex de l'*Ochoterenaia appendiculata*. La ressemblance à l'*Ochoterenaia appendiculata* se manifeste également dans la structure des parois du vestibule. Pour ce qui concerne ses autres caractères étudiés, *C. minimum* ressemble d'un côté aux *Entodiniomorphida* et de l'autre aux *Gymnostomata*.

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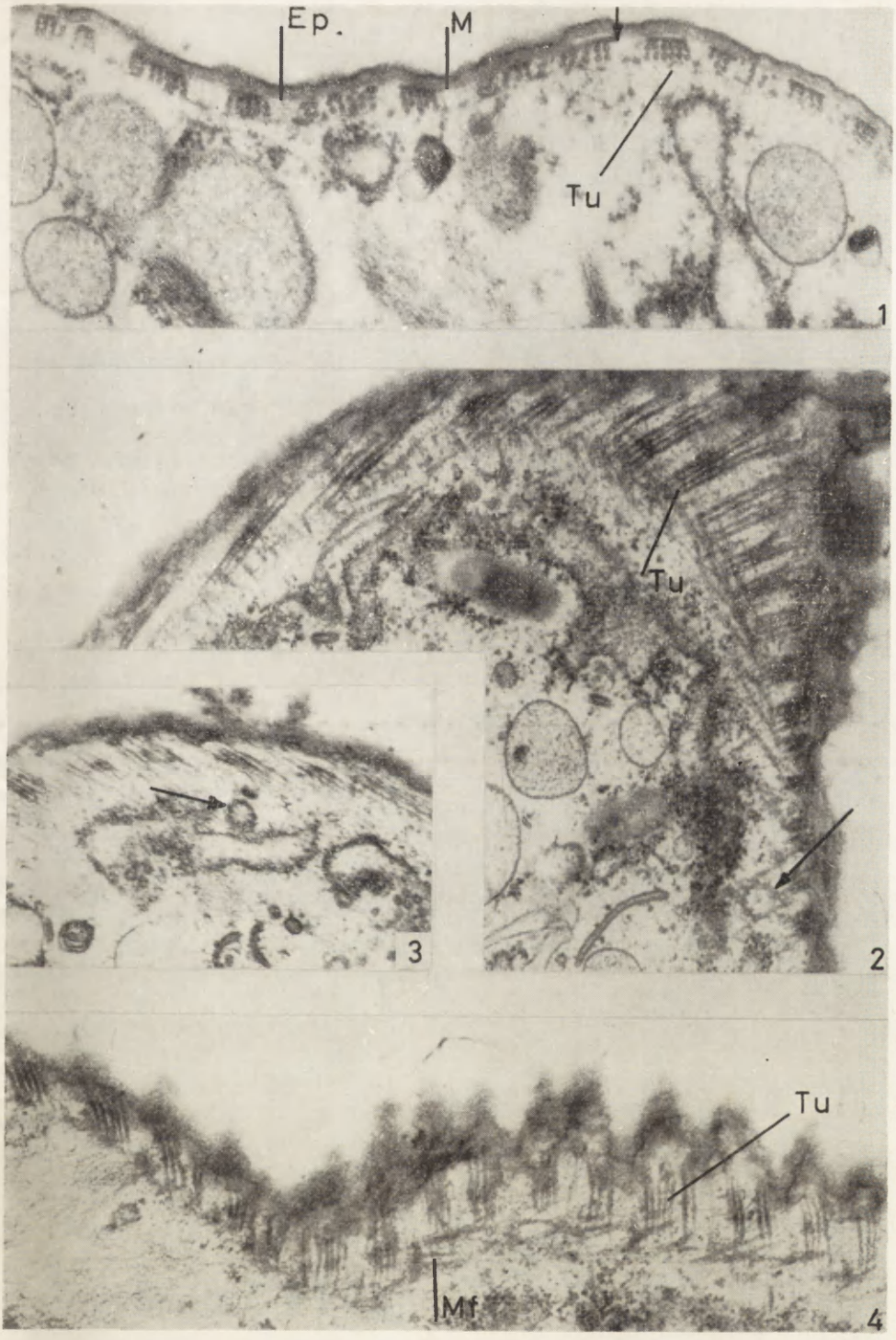
EXPLANATION OF PLATES I–VIII

Circodinium minimum

1: Section perpendicular to the surface. Cell membrane (M), epiplasma (Ep), microtubules cut transversally (Tu); arrowed is dense substance. × 39 600
2: Section tangential to the surface, slightly oblique. Microtubules (TU), arrowed is kinetosome. × 33 600
3: Same as 2. Arrowed is kinetosome, × 25 500
4: Oblique section. Microtubules (Tu), microfibrils (Mf), × 33 600
5: Oblique section. Microfibrils (Mf), arrowed is kinetosome, × 23 000
6: Section tangential to the surface, slightly oblique. Arrowed is kinetosome, × 48 700
7: Same as 6. Arrowed is kinetosome, × 48 700
8: Section through the initial part of the vestibulum. Arrowed is a strand of amorphous material, × 30 800
9: Oblique section, × 25 500
10: Slightly oblique section. Microfibrils (Mf), × 24 300
11: Slightly oblique section. Microfibrils (Mf), vestibulum (V), × 30 000
12: Section through the initial part of the vestibulum wall. Bands of microtubules (Tu). Arrowed is a strand of amorphous material, × 27 000
13: Section through the initial part of the vestibulum wall. Bands of microtubuls (Tu). Arrowed is a strand of amorphous material, × 14 000
14: Section through the vestibulum wall. Arrowed is a strand of grainy-fibrillar material, × 26 400
15: Section through the vestibulum, × 17 500
16: Section through the vestibulum, × 24 300
17: Oblique section, kinetosomes (arrowed), × 32 000
18: Section through the initial part of the vestibulum. Arrowed is a strand of amorphous material, × 23 000
19: Section through the narrowed part of the vestibulum. Semicircular fibres cut transversally, slightly obliquely (arrowed), × 27 800
20: Longitudinal section through the vestibulum, × 33 000
21: Section through the vestibulum. Bands of microtubules (Tu), strands of grainy-fibrillar material (arrowed), × 26 700
22: Oblique section through the vestibulum. Visible are kinetosomes of a single row of vestibular cilia, microfibrils on the opposite wall. Arrowed is a bore of the vestibulum, × 22 000
23: Longitudinal section through the terminal part of the vestibulum and the cytopharynx. Vestibulum (V), cytostome (Cy), bands of microtubules = rideaux de tubules (R), mineral concretions? (arrowed), × 16 100
24: Cross-section through the cytopharynx, × 12 900
25: Section through the mid part of the body, mineral concretions are visible, × 9 200
26: Cross-section through the cytopharynx, × 22 000
27: Section through the "pouch" and special cilia of a single row, × 20 700
28: Sagittal section through the basal part of the caudal process. Kinetosomes of special cilia (arrowed), pulsating vacuole (C.v.), × 10 000
29: A fragment of 28, × 33 600
30: Longitudinal section through a kinetosome and a special cilium. Arrowed is a rudimental axosome. Axial grains (G), × 26 400
31: Special cilia without central fibres, × 33 600



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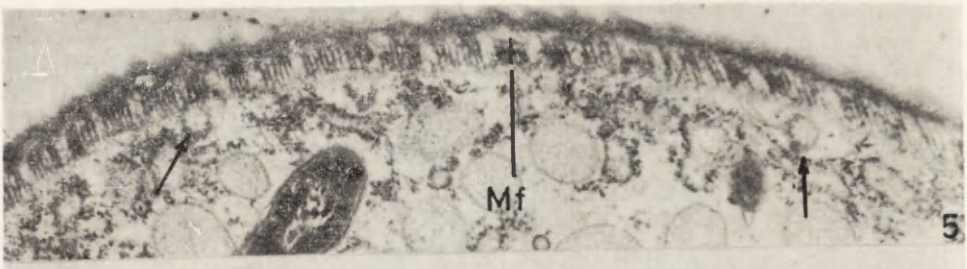

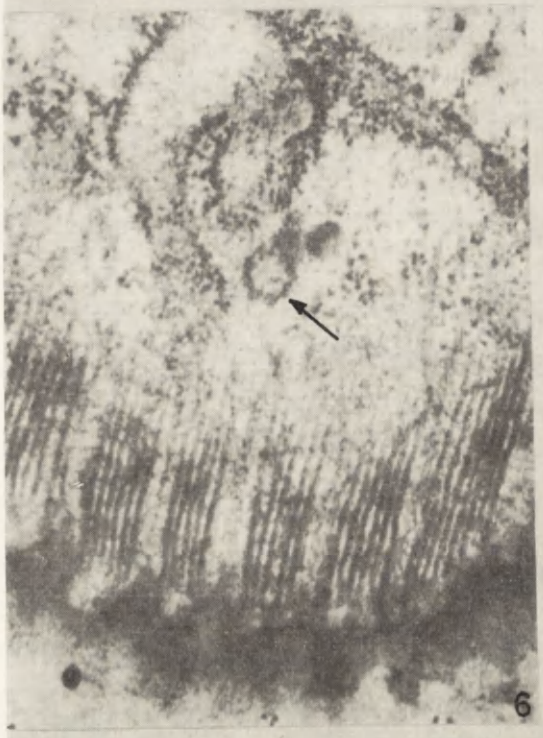

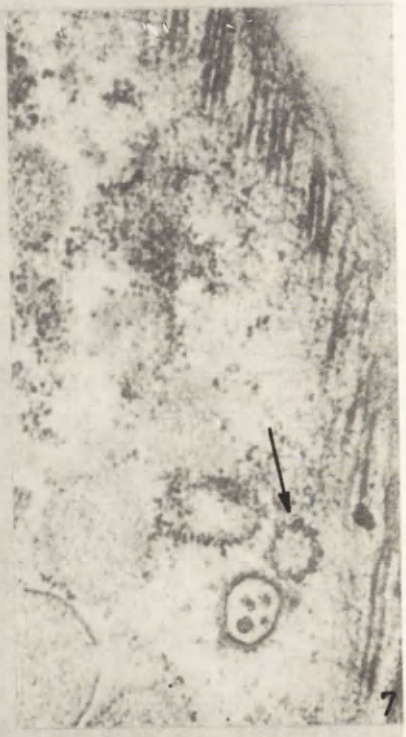

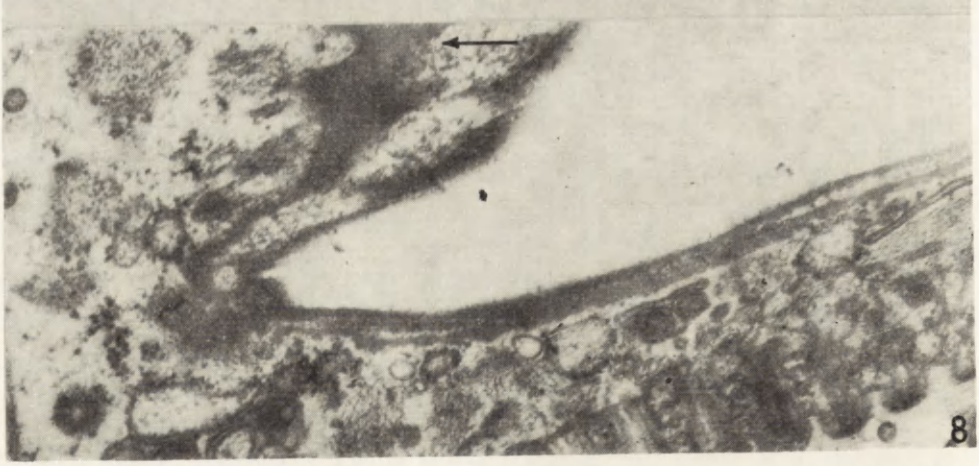

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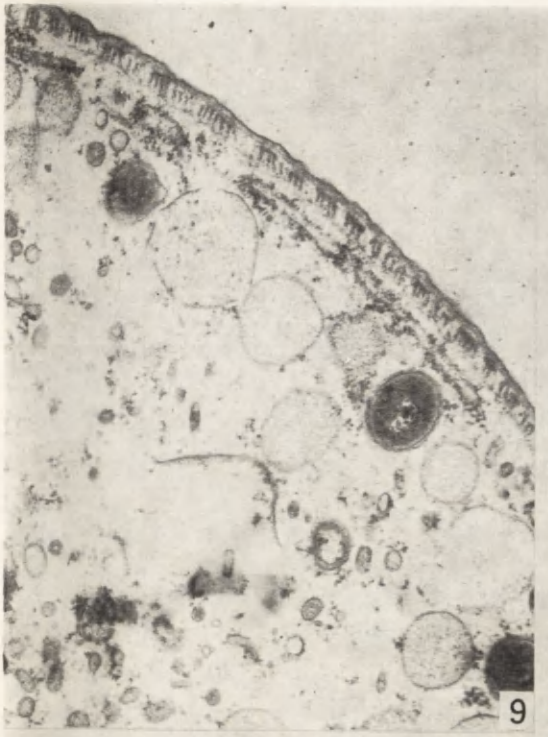

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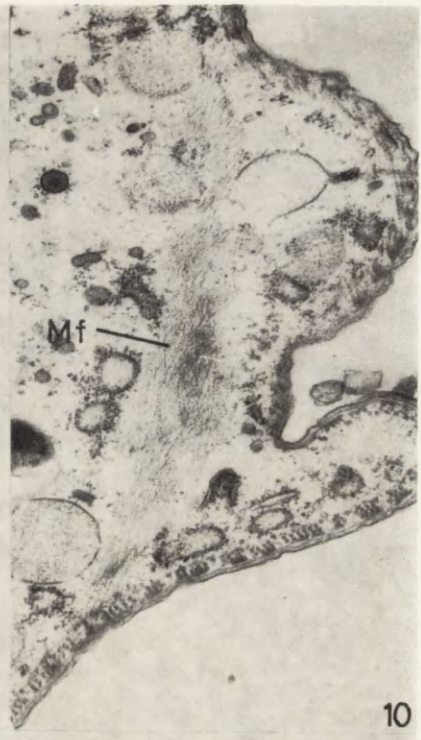

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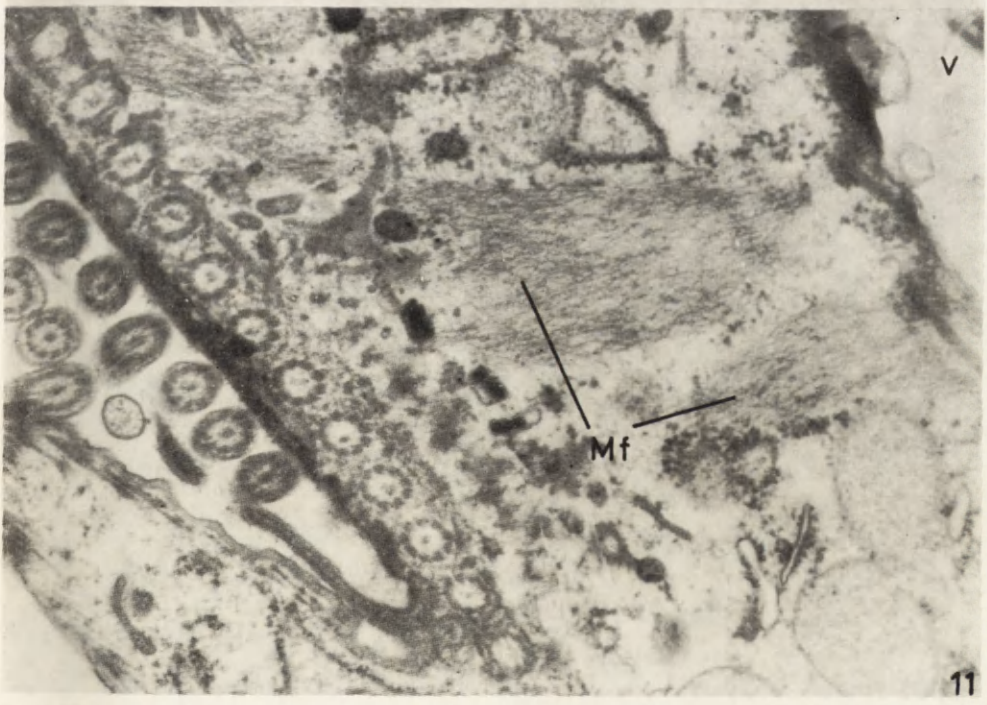

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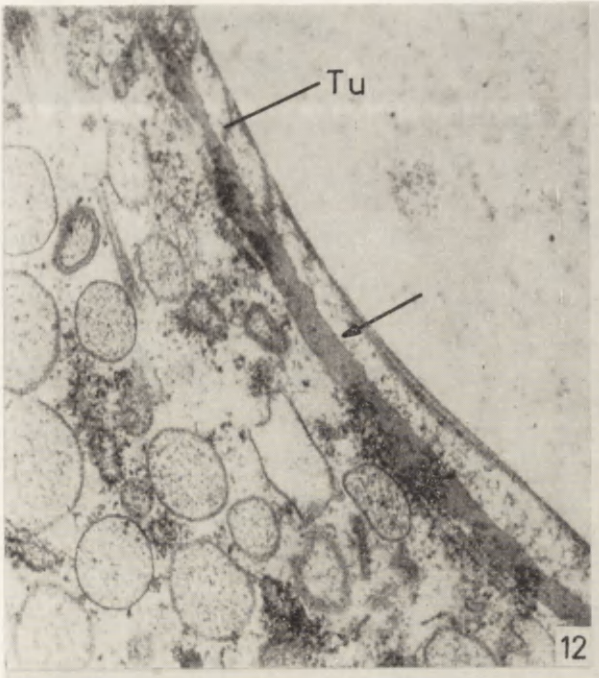

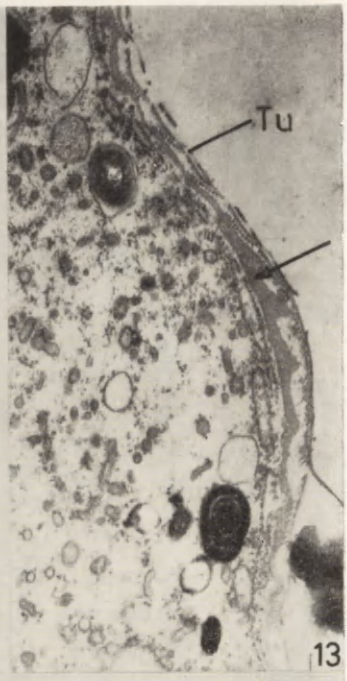

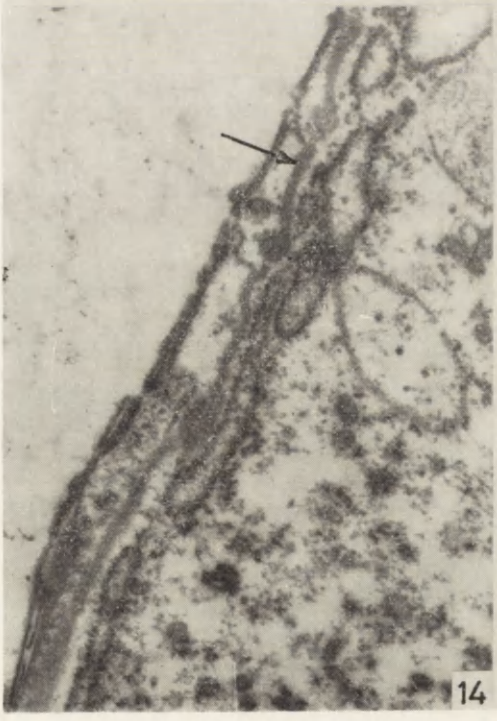

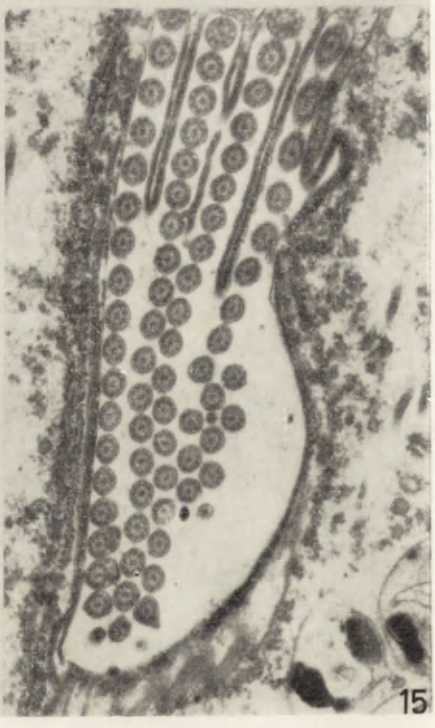

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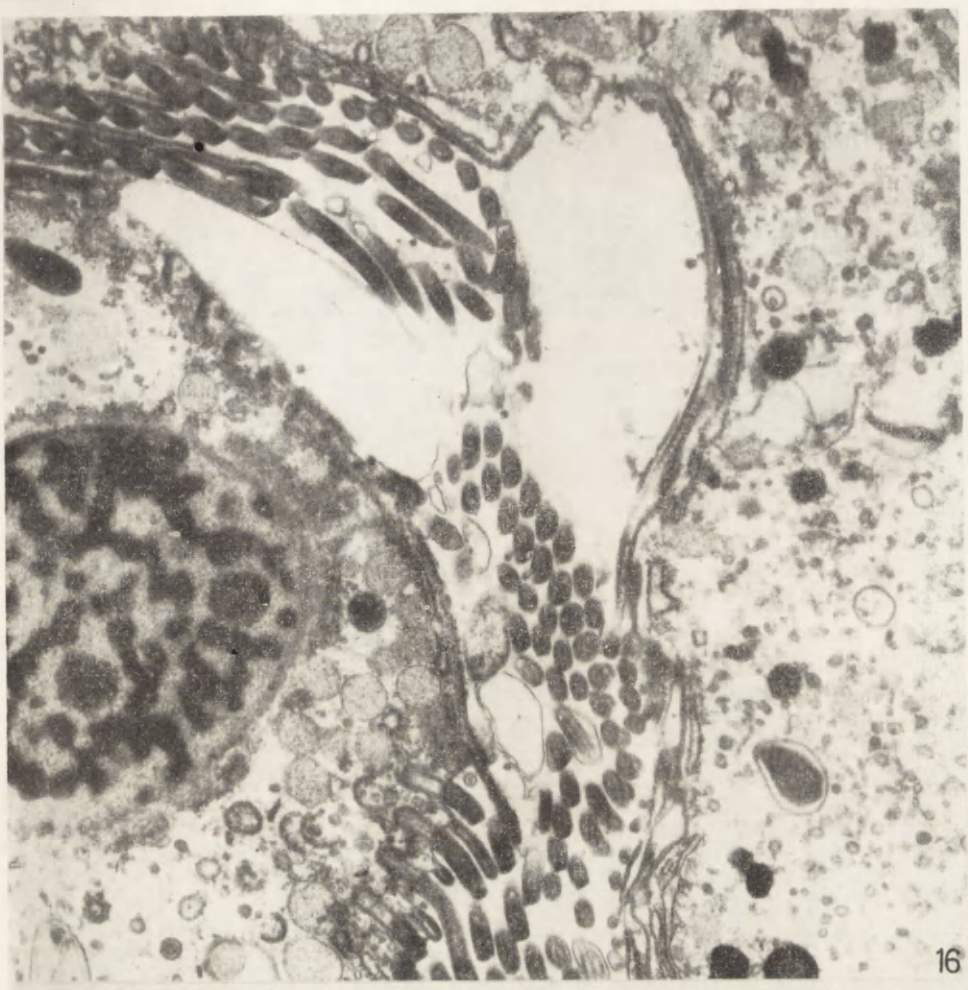

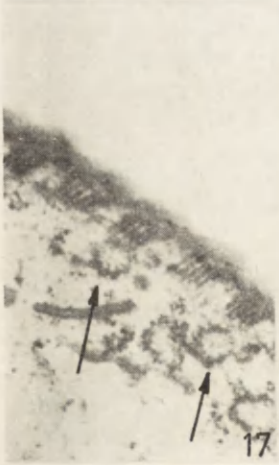

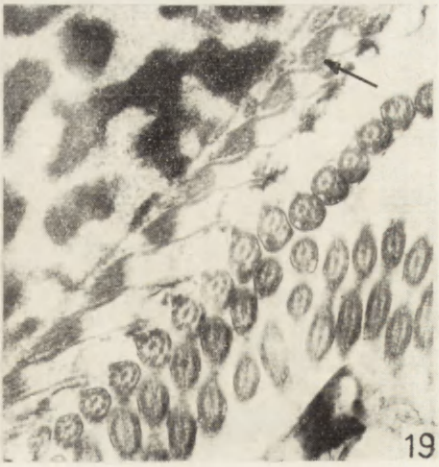

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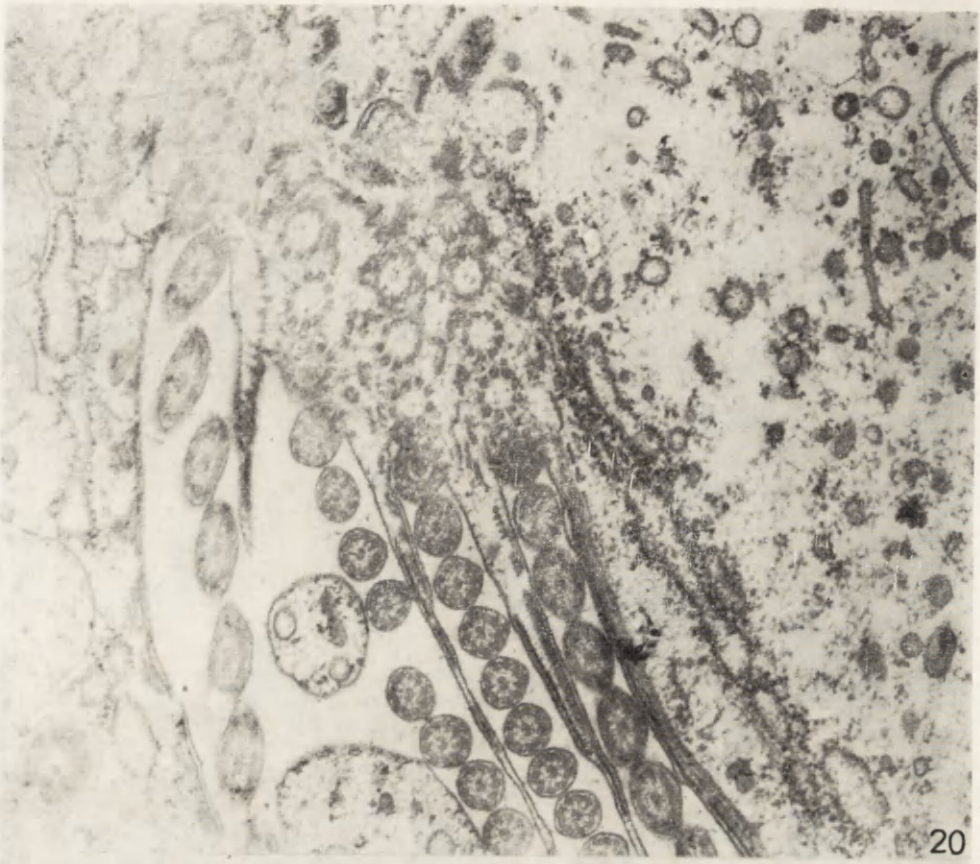

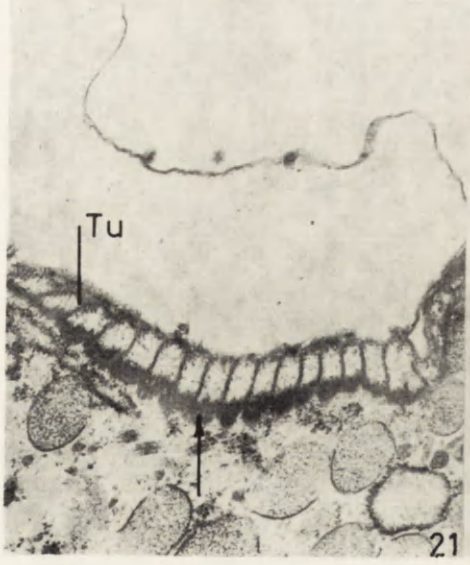

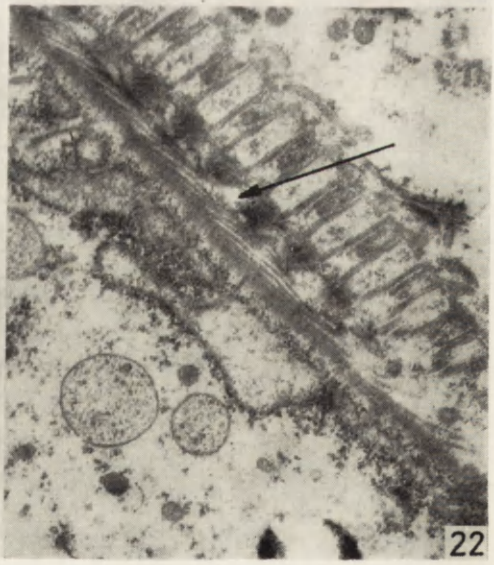

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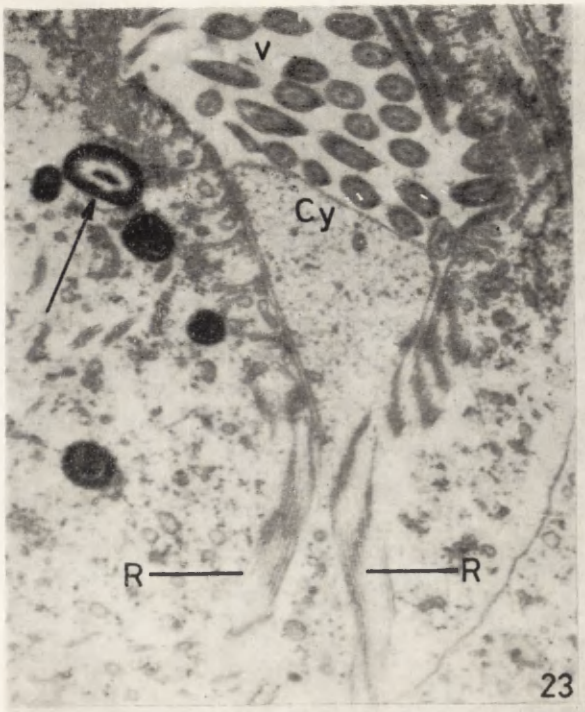

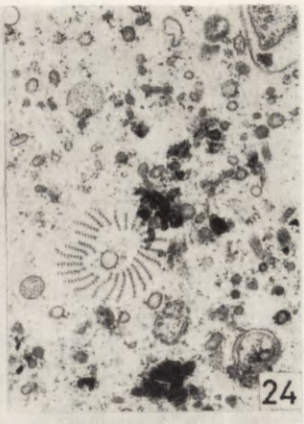

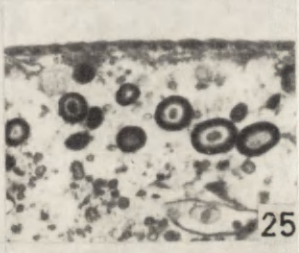

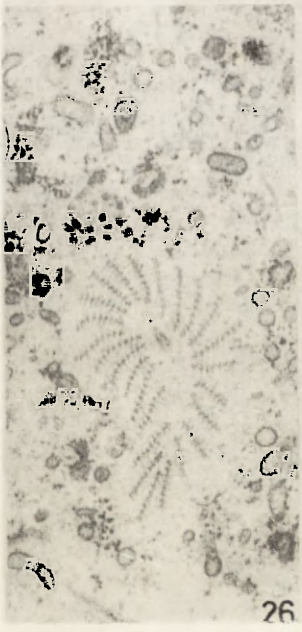

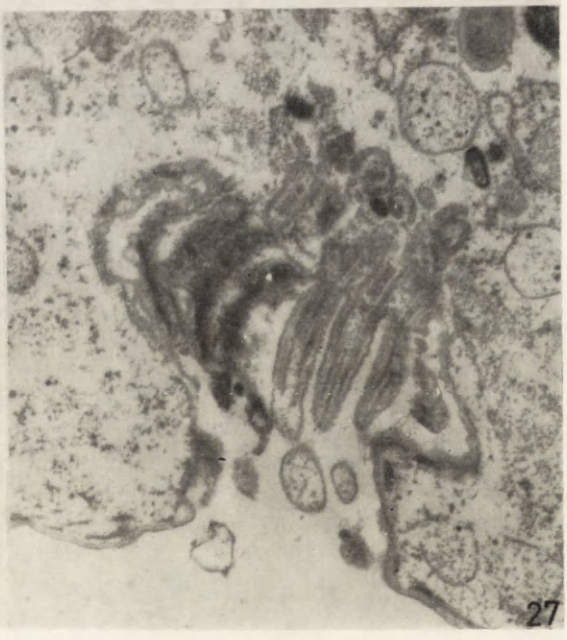

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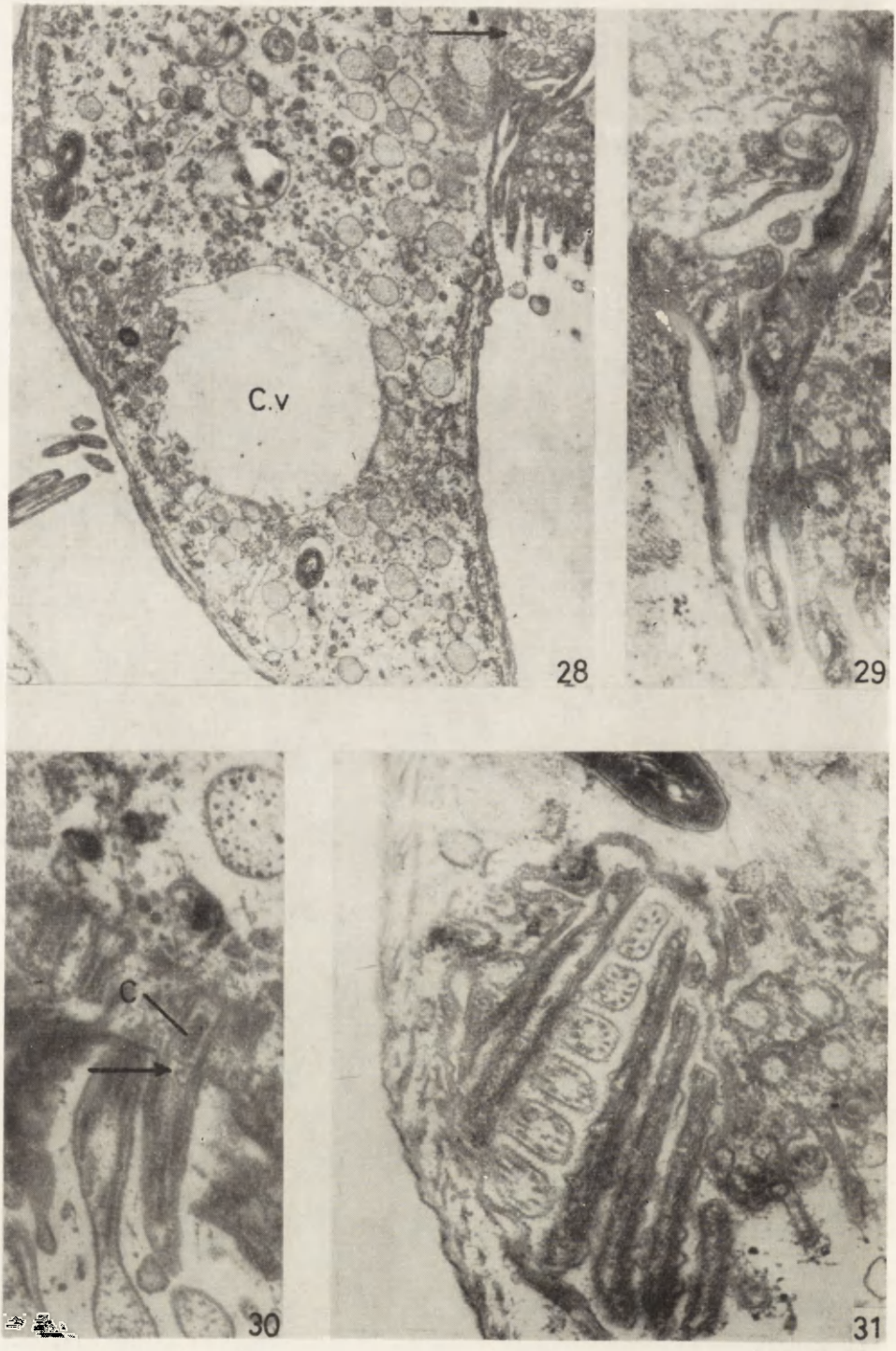

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Ultrastructure of Isolated *Paramecium aurelia* Macronuclei

Synopsis. In isolated macronuclei of *Paramecium aurelia* the general pattern of heterochromatin and nucleoli is well preserved. Contrary to that, nuclear matrix is changed, in the matrix of the majority of macronuclei hardly any euchromatin filaments and ribonucleoprotein structures may be observed.

Isolation of nuclei from various cells has been applied as a method supplying many possibilities of nuclear metabolism examination. The macronucleus of *Paramecium aurelia* seems to be a very interesting model for such study, because of its high degree of polyploidy, on the one hand, and on the other hand, of its very particular organization of heterochromatin into numerous, dense small bodies dispersed regularly through the whole macronucleus.

As shown by light microscope autoradiography, these nuclei could be used for the study of RNA synthesis (Skoczylas and Krawczyńska 1975). This problem is further investigated in our laboratory by way of RNA polymerase assay in macronuclei with modified chromatin at the ultrastructural level (in preparation). For the latter study, it proved, however, neccessary to know the fine organization of isolated macronuclei. This was the more important, since, as reported in the literature, each procedure of isolation changes the nuclear organization, both in mammalian and protozoan cells (Gorovsky 1970, Gorovsky et al. 1975, Cummings 1972, Cummings and Tait 1975, Cummings 1977, Isaacks et al. 1973, Laval and Bouteille 1973, Skoczylas and Soldo 1975).

Considering this, the aim of the present paper was to study the ultrastructural organization of isolated macronuclei in different samples obtained by the same procedure of isolation, and to compare it with

ultrastructure of macronuclei *in situ*. This problem was investigated by cytochemical methods at the electron microscope level using the routine procedure and the Bernhard's reaction allowing for localization of ribonucleoproteins.

Material and Methods

The macronuclei were isolated after Skoczylas and Soldo (1975) from the axenically cultured *Paramecium aurelia* 299 S, syngen 2.

The cells were collected at 200 g during 3 min on MSE oil centrifuge from the early logarithmic phase of growth, where 100% of vegetative cells has been detected, as estimated by means of methyl green staining of their macronuclei. The material was then homogenized in 20 vol. of 4% gum arabic supplemented with n-octanol (0.65 ml/100 ml of gum arabic). After 30 s of homogenization the homogenate was checked under the light microscope, and if any whole cell was seen, the homogenization was prolonged. Then the homogenate was centrifuged in Janetzki centrifuge at 0 — +4°C at 500 g during 15 min. The supernatant contaminated with cytoplasmic fragments, micronuclei and pellicle was discarded. The macronuclear pellet was twice washed in octanol-free gum arabic at 500 g and 250 g, respectively. Since the *Paramecium* population did not include cells with any autogamous changes, it was possible to stop the isolation procedure at the step of crude nuclear fraction.

The same preparation was carried out with sodium bisulfite added to the gum arabic to a final concentration of 50 mM, in order to inhibit the proteolytic enzymes activity (Panyim and Chalkley 1969).

The crude nuclear fractions prepared with and without sodium bisulfite were used for ultrastructural study.

For electron microscope examination both whole cells and the isolated macronuclei were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 during 1 h, postfixed for 30 min in 1% osmium tetraoxide, buffered as above, processed through series of ethanols, propylenoxide and embedded in Epon 812. The ultrathin sections were contrasted with uranyl acetate followed by lead citrate.

For ribonucleoprotein (RNP) staining the samples were fixed in buffered 2% glutaraldehyde only and embedded as before. The ultrathin sections were contrasted for 15 min in 4.5% uranyl acetate in 70% ethanol, then carefully dried and treated during 30–60 min with 0.2 M EDTA (adjusted to pH 7.0 with NaOH) and finally stained for 5 min with lead citrate (Bernhard 1969).

All sections were examined in JEM 100 B electron microscope.

Results

Plate I 1 shows the typical ultrastructure of the *Paramecium aurelia* macronucleus, *in situ*. Its main characteristics (Jurand et al. 1962), are heterochromatin organized in numerous small bodies (chromatin bodies) and nucleoli dispersed between them. These structures are embedded in the nuclear matrix, that means nucleoplasm with euchromatin seen as delicate fibrils. Plate I 2 reveals the localization of ribonucleoproteins

(RNP), heavily contrasted according to Bernhard's procedure, in the macronucleus *in situ*. This material is localized at the border of nucleoli, it is moreover found in the nuclear matrix. Heterochromatin remains bleached after this procedure, indicating a high content of deoxyribonucleoproteins (DNP). The DNP material is also seen in the nucleoli.

The macronucleus isolated in gum arabic is presented in Pl. II 3. The general pattern of its organization is well preserved. The nucleoli are easily distinguishable showing their fibrogranular structure. However, some differences in the appearance of chromatin bodies can be observed. Their borders seem to be not so sharply outlined as in the nuclei *in situ* (Pl. I 1). But the latter is not a common feature of all isolated macronuclei, Pl. III 6 presents another macronucleus with clearly delineated small bodies, obtained in the same preparation. The next notable dissimilarity in the nuclei under study is observed in the electron density of chromatin bodies and nucleoli. The latter are less contrasted (after the routine procedure) than the chromatin bodies. In isolated macronuclei the relative electron density of these two structures may be quite opposite (Pl. II 3). The most striking differences between particular isolated macronuclei are noted in the preservation of nuclear matrix structures. The ultrastructure of the macronucleus with quite well preserved nuclear matrix material is seen in Pl. III 6. On the contrary, in Pl. III 5 we can observe another isolated macronucleus in which hardly any filaments and other particulate structures can be distinguished in the nuclear matrix. Both these nuclei were isolated from the same preparation in gum arabic.

Plate II 4 reveals the localization of RNP in the isolated macronucleus of *P. aurelia*. The high content of RNP is seen in the nucleoli, not only at their border, but also in the central part. Futhermore, some ribonucleoproteins are seen accumulated around the small bodies, and very few, if any RNP was localized in the nuclear matrix (compare with Pl. I 2).

As mentioned above in some experiments for the isolation of *P. aurelia* macronuclei, gum arabic supplemented with sodium bisulfite was used. The ultrastructure of the macronucleus isolated in such medium is presented in Pl. III 7. The internal organization of this macronucleus resembles that, presented in Pl. II 3, III 5, 6. The only difference concerns the very rich cytoplasmic contamination with ribosomes and cytoplasmic membranes attached to the external nuclear envelope.

Discussion

The ultrastructure of isolated macronuclei, as presented in this paper, when observed at low magnification is similar to that observed in

macronuclei *in situ*: the characteristic small and large bodies are preserved. However, at higher magnification some differences are noted. The most sensitive to the isolation procedure proved to be the nuclear matrix. This is independent of the usage of sodium bisulfite, thus indicating that the changes are not evoked by the action of proteolytic enzymes.

As already mentioned, both euchromatin fibrils and ribonucleoprotein structures are easily lost during the isolation procedure. This loss seems to be different in various preparations as well as in individual nuclei obtained in the same batch. As the cultures used did not divide synchronously, it is suggested, that the different preservation of nuclear matrix may be the result of some delicate changes of nuclear components occurring during various phases of the cell cycle.

It has been reported, that the content of RNA in isolated macronuclei of *Paramecium* (descending from the same stock, and cultured axenically) may vary from about 7–24% (Skoczylas and Soldo 1975). The various preservation of RNP material in the nuclear matrix seems to be the illustration of this findings. As concerns the DNA content in the pure fraction of *Paramecium aurelia* macronuclei, it amounts to only 50–60% of that in intact cells (Skoczylas and Soldo 1975). It cannot be excluded, that this low value is caused by loss of DNA during the isolation procedure. Thus, it seems probable, that these changes are manifested at the ultrastructural level not only by the different preservation of nuclear matrix material, but also by changes of relative electron density of chromatin bodies as well as of nucleoli.

Cummings (1977) using the same isolation procedure as ours, and another one, based on detergents, reported a similar general pattern of isolated macronuclei organization. However, when cells were harvested from the exautogamous cultures, he managed to demonstrate two kinds of macronuclei; those observed by us, and others with homogenous appearance of their ultrastructure, which he called "immature macronuclei". In our experiments, in the crude nuclear fraction a number of such nuclei could be also observed, if the *Paramecium* population containing 1–2% of cells undergoing autogamous changes was collected.

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RÉSUMÉ

Dans les macronoyaux isolés du *Paramecium aurelia* l'organisation de la hétérochromatine et des nucleoles sont bien préservées. Par contre, le matrix du noyau

est altéré, dans le matrix des macronoyaux on ne trouve presque pas des filaments d'euchromatine ni des structures ribonucléoproteiques.

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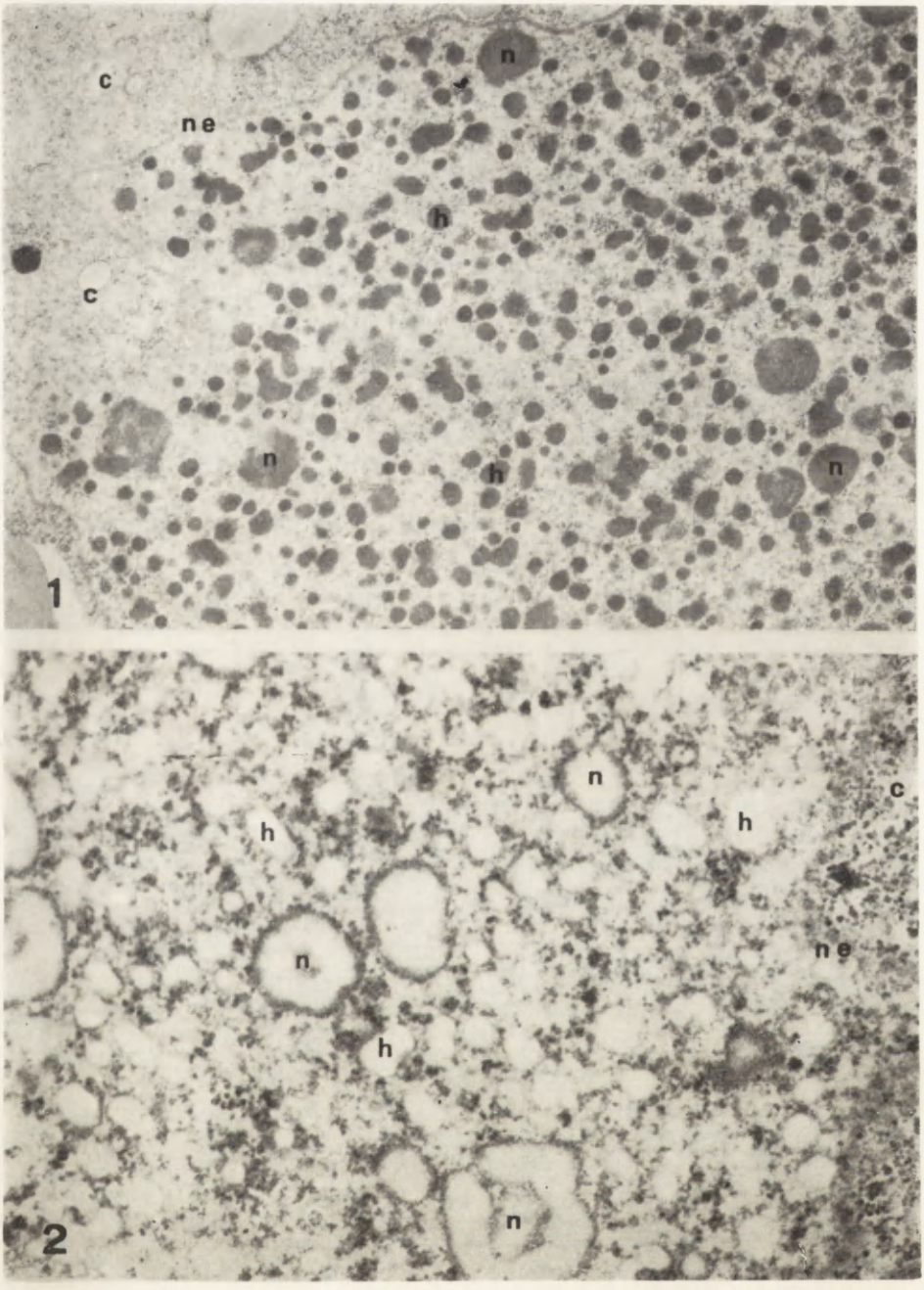
EXPLANATION OF PLATES I–III

1: A section through the *Paramecium aurelia* macronucleus *in situ* (routine procedure). × 12 000
2: Localization of ribonucleoproteins in *P. aurelia* macronucleus *in situ* (Bernhard's staining). × 27 000
3: Isolated macronucleus (routine procedure). × 12 000
4: Localization of ribonucleoproteins in isolated macronucleus (Bernhard's staining). × 27 000
5: Macronucleus isolated in gum arabic with poor preservation of nuclear matrix material (arrow) — routine procedure. × 24 000
6: Macronucleus isolated in the same preparation as before with well preserved nuclear matrix material (arrow), routine procedure. × 24 000
7: Macronucleus isolated in gum arabic supplemented with sodium bisulfite, routine procedure. × 24 000
Notice cytoplasmic contamination attached to the external nuclear membrane, (asterisk)

Abbreviations: ne — nuclear envelope, h — heterochromatin (chromatin bodies), n — nucleoli, c — cytoplasm



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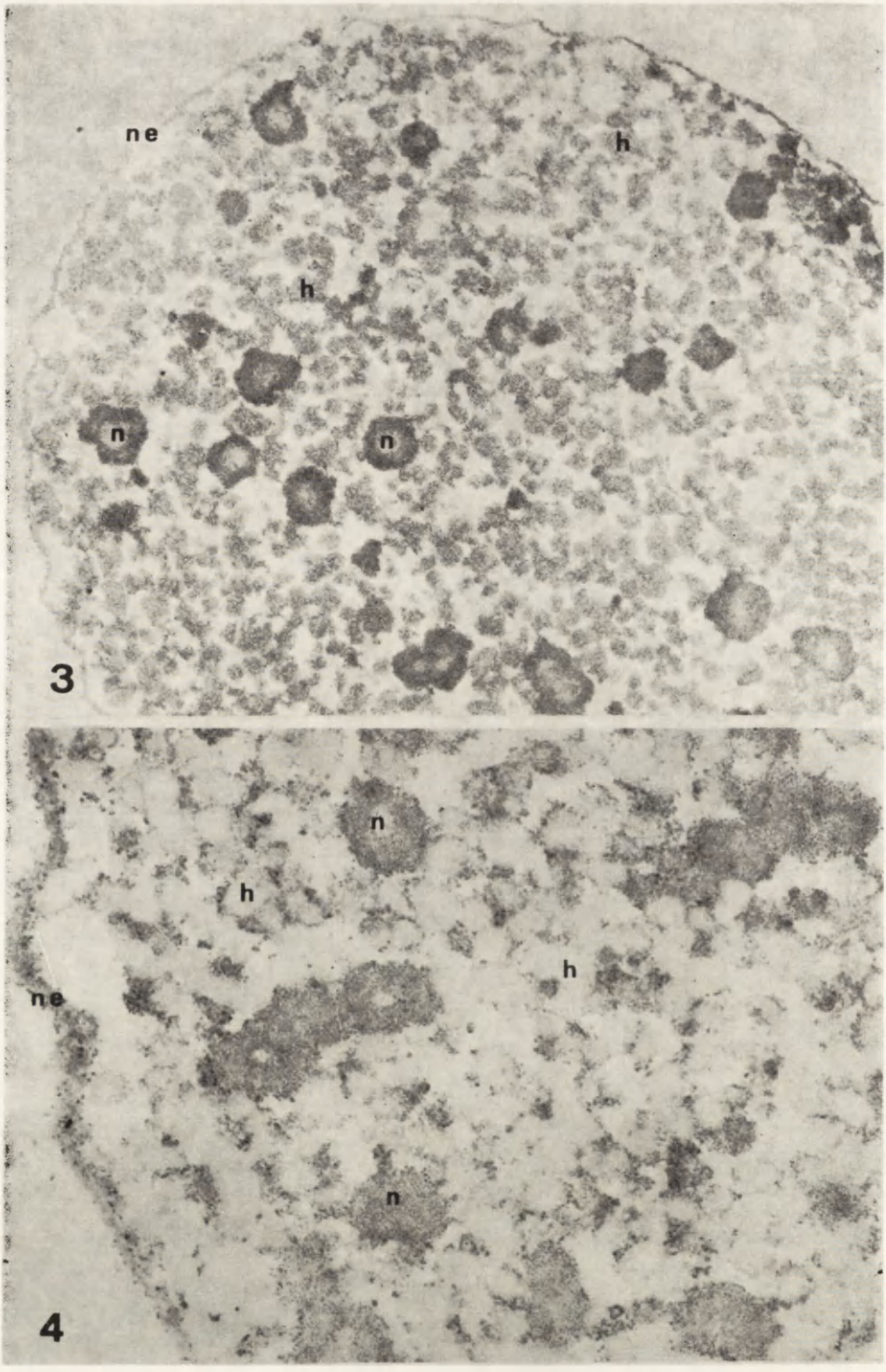

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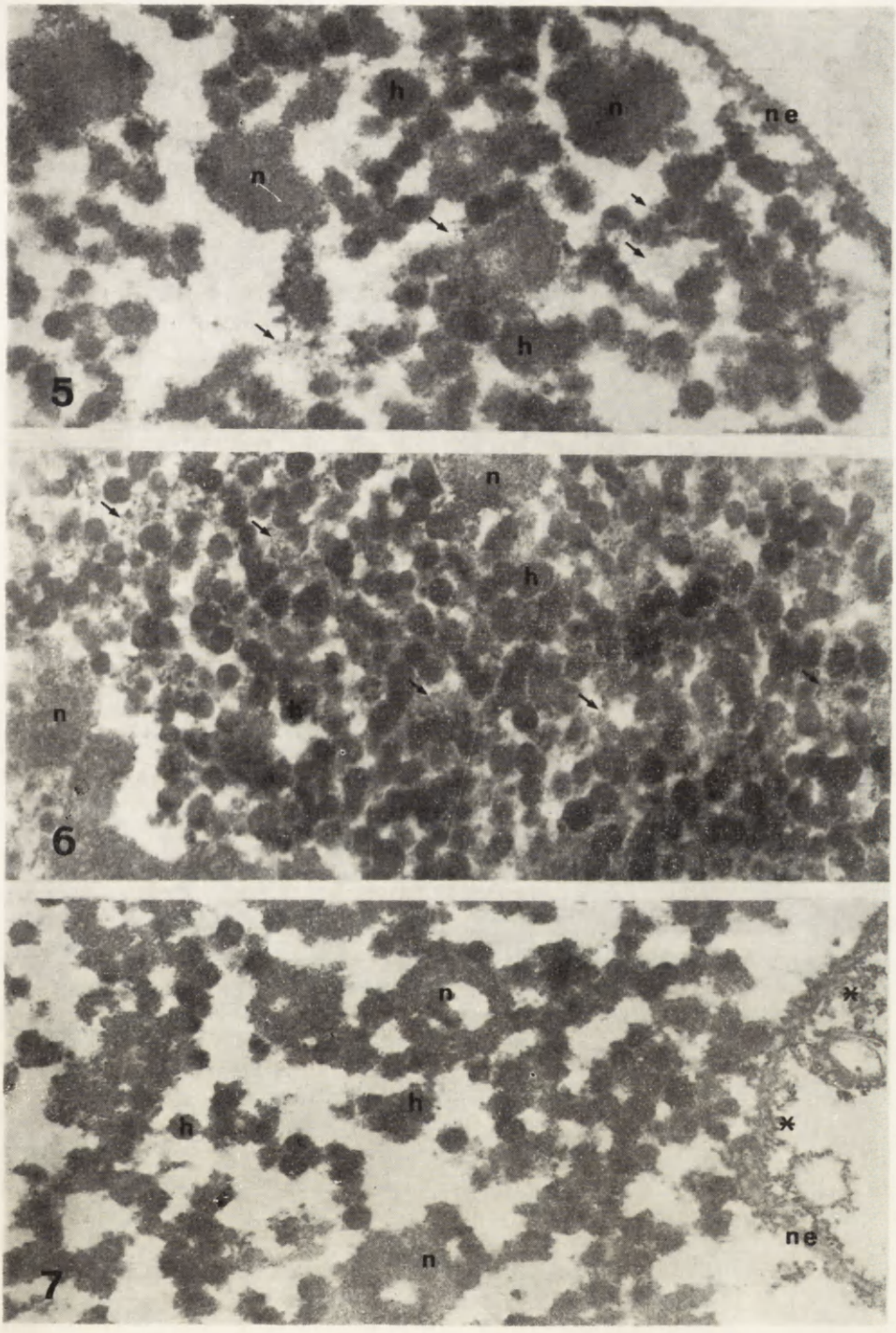

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Flagellates *Spironucleus anguillae* sp. n. Parasites of Eel (*Anguilla anguilla* L.)

Synopsis. A new species of flagellates, *Spironucleus anguillae* sp. n. is described. Those flagellates were found in visceral organs (liver, kidney, and spleen), in peripheral blood and in necrotic muscles of eel, *Anguilla anguilla* L., affected by *pestis anguillarum* (*morbus anguillarum*). The location of the invasion is indicate of a parasitic nature of the flagellates.

Flagellates of the genus *Spironucleus* were being found, i.a., in intestine of *Ctenopharyngodon idella, Barbus barbus,* more seldom in carp (*Cyprinus carpio*), *Hypophthalmichthys nobilis,* and *Chondrostoma nasus* (Chen-Chiu-Leu 1956, Molnar 1974) as well as in several species of aquarium cichlids (Schäperclaus 1954, Kulda and Lom 1964 a, b). Furthermore, *Hexamita* sp. were found to live in reproductive organs of trematodes *Deropristis inflanta,* the trematodes themselves being parasitic to eel *Anguilla rostrata* (Hunninen and Wichtermann 1938).

The present account describes the *Spironucleus* flagellates found in eel (*Anguilla anguilla* L.) affected by acute *pestis anguillarum.* The parasites morphology as well as their manner of parasitism and location in a host set them apart as a new species; consequently, they are described under the name of *Spironucleus anguillae* sp. n.

Material and Methods

Fishes showing symptoms of *pestis anguillarum* were caught in the northern Szczecin Firth and obtained for studies from the Karsibór and Przytor Fishing Bases. The samples consisted of 5-15 individuals each; the size and weight of the

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fishes ranged within 5-80 cm and 250-850 g, respectively. The parasitological, bacteriological and hematological analyses were performed in May, June, July and September 1974, at the same time, clinical and anatomopathological changes were recorded and a cytological assessment was made in order to describe the symptoms of the disease (Einszporn-Orecka 1976 a, b) and to detect alterations related to the flagellates invasion (Einszporn-Orecka, in press).

The fishes, except for the first batch of 11 individuals, were examined to detect the presence of the protozoans by checking the fishes' peripheral blood, gall bladder and intestine contents. Additionally, smears were made of peripheral blood taken from caudal vein and of cardiac blood. Tissue preparations of necrotic skin as well as of parenchyma of liver, spleen, pro-, meso-, and metanephros were also made. Dried blood smears and tissue preparations were stained using May-Grünwald, Giemsa and Wright techniques.

Description

Spironucleus anguillae sp. n.

Observations on morphology. The protozoans are almost exactly egg-shaped (Pl. I 1, 2) with the anterior end distinctly protruding. Cell length and width vary from 9.6 to 12.8 μm (± 0.86) and from 7.2 to 9.6 μm ± 0.7), respectively, the mean values calculated from measurements of 50 individuals being, respectively, 8.2 and 11.0 μm for length and width.

Two compact, well-staining sausage-like nuclei are situated near the anterior end of the cell and extend, on the average, to one-third of the cell length. Proximal ends of the nuclei are set apart from each other. The nuclei length as measured along a straight line from the anterior to posterior end ranges within 3.2–3.6 μm with a maximum at 4.0 μm. Between the nuclei and close to the central part of the nuclear membrane lie two oval, non-staining bodies resembling vacuoles, of rather stable dimensions, their length and width ranging within 2.0–2.8 μm and 1.6–1.8 μm, respectively (Pl. I 1, 2).

Six anterior flagellae are attached to basal bodies arranged in two Λ-form triangular groups of three. The basal bodies are connected by thin filaments (Pl. I 2 c). Each group is placed above the proximal end of the nucleus in the convex part of the cell, very close to its anterior margin. The anterior flagellae, measuring 16–18 μm, are 1.5 times longer than the cell itself (Pl. I 2 b).

Large, well-staining basal bodies of the posterior flagellae are occupying a central position (Pl. I 2 a, b, c). Twisting posterior grooves are seen as thickened double lines. In the cell's posterior part those flagellae are released outwards in slightly concave places which are not, however, caudal funnels (Pl. I 2 b). Free parts of the posterior flagellae measure approximately 16–18 μm.

Spironucleus anguillae sp. n. were found in one sample only, in 10

eel individuals out of the 11 examined. Seven fishes contained the protozoans in their peripheral and cardiac blood; the preparations made of liver, spleen, and kidney parenchyma of 3, 4 and 4 individuals, respectively, revealed the presence of the parasites, too. The protozoans were very numerous in necrotically changed skin of all the fishes infested. The protozoans' abundance varied from single to numerous cells in spleen and from single to very numerous cells in peripheral blood, liver, and kidney. In a single instance, the flagellates were present in smears of all organs checked, the peripheral blood and liver being most severely affected. Very large amounts of the flagellates were observed in kidney smears (Pl. II 3) and in necrotically changed skin (Pl. II 4).

Discussion

Certain morphological features separate the flagellates *Spironucleus anguillae* sp. n. present in eel (*Anguilla anguilla* L.) from *S. elegans* Lavier, 1936 recorded from *Pterophyllum scalare* and amphibians.

Thus *Spironucleus anguillae* sp. n. are much larger and broader than *S. elegans* (cf. 9.6–12.8 μm length and 7.2–9.6 μm width ranges in the first against the respective ranges of 7–8 μm and 3–4 μm in the latter). Further comparison shows the first species to be more closely related, in terms of shape and size, to *Hexamita salmonis* with its length and width ranges of 10–12 μm and 6–8 μm, respectively (Kulda and Lom 1964 b). *Spironucleus anguillae* sp. n. are also similar to *Hexamita* (= *Spironucleus* sp. according to Kulda and Lom 1964 b) found in trematodes *Deropristis inflata* dwelling in intestines of eel *Anguilla rostrata*. Cells of the latter species being of a length (7.7–14.3 μm), they are, however, more slender (3.3–6.7 μm width range) and pointed posteriorly.

Furthermore, different is the situation of nuclei in the two species under comparison: the *Spironucleus anguillae* nuclei are situated more centrally and set rather far apart, while the proximal ends of the *S. elegans* nuclei almost contact each other to form a characteristic S-pattern.

Consequently, both the anterior and posterior basal bodies are positioned differently in the two species, those elements being closer to the protruding end in the *S. anguillae* cell compared with *S. elegans* whose basal bodies lie close to the anterior ends of the two nuclei. Additionally, cells of *Spironucleus anguillae* sp. n. exhibit the presence of vacuole-like non-staining spaces between the nuclei, which has not been observed in the remaining protozoans of the genera known to date.

Considering the morphological differences described above, the author

feel justified to erect a new species, *Spironucleus anguillae* sp. n., to hold the flagellates parasitising eel *Anguilla anguilla*.

The literature available as well as the author's own observations contravene the common belief that flagellates occurring in fishes are parasites specific of a repective organ they are found in. The *Hexamita* parasites were regarded primarily as commensals, or intestinal (Schmidt 1920, Davis 1923, 1956, Duijn 1956, Uzmann et al. 1963, 1965) and gall bladder (Duijn 1965) parasites. On one occasion, however, they were found in blood (Schäperclaus 1954) and in liver of a trout (Duijn 1965). It was Amlacher (1961, 1972) who found *Hexamita symphysodonis* to have invaded intestine, gall blader, liver, and heart of *Symphysodon discus*. Also Molnar (1974) documented a mass occurrence of *Spironucleus* sp. in *Barbus barbus*, the protozoans being present in necrotic part of liver and in intestine along with a strong invasion of *Pomphorhynchus laevis*. To quote the same author, dead aquarium fishes contained the flagellates in their injured muscles, viscera, gall bladder, and intestine, an extensive inflammation and invasion of *Capillaria* sp. nematodes being simultaneously recorded.

Spironucleus anguillae sp. n. were present in all parenchymal organs (liver, spleen, kidney), in peripheral and cardiac blood as well as in necrotic muscles of eel *Anguilla anguilla*, neither the intestine nor gall bladder having been examined. The infestation in eels, similarly to the cases described by Molnar (1974) is an accompanying invasion following the progressive necrotic changes that develop under certain conditions such as pest, septicaemia, lesions caused by acanthocephalans etc.

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RÉSUMÉ

Une espèce nouvelle de flagellé, *Spironucleus anguillae* sp. n., est décrite. Elle a été trouvée chez les anguilles, *Anguilla anguilla* L., touchées par *Pestis anguillarum* (*morbus anguillarum*), dans des organes viscéraux (le foie, les reins, la rate), dans la circulation sanguine péripherique et dans les muscles nécrotiques. Ces localisations et l'apparition invasive de cette espèce indiquent qu'il s'agit d'une forme parasitaire.

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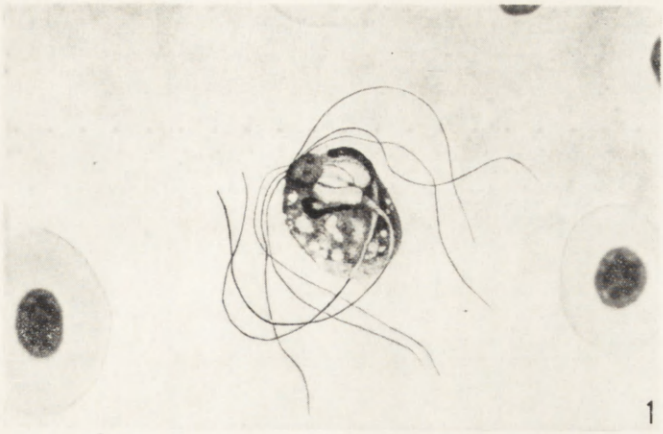
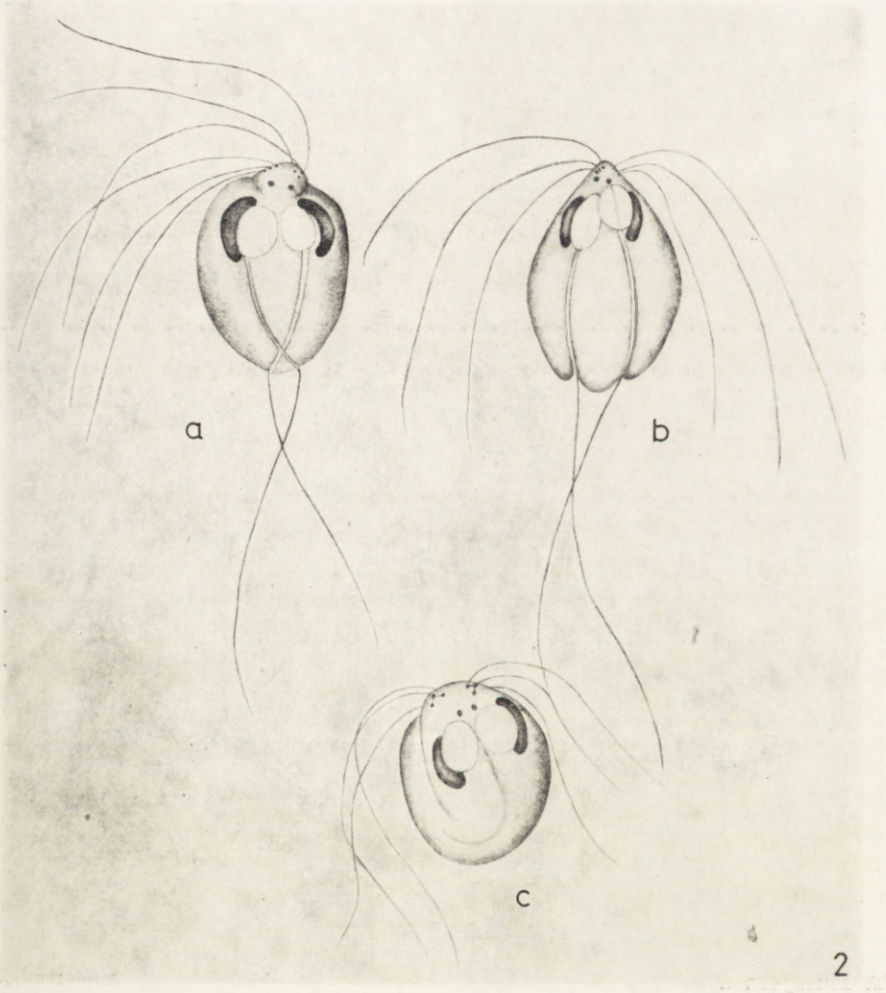
EXPLANATION OF PLATES I–II

1: A flagellate *Spironucleus anguillae* in eel's peripheral blood
2: *Spironucleus anguillae* — schematic diagram of a cell a, b, c
3: A flagellate from eel's kidney
4: *Spironucleus anguillae* from necrotic muscles in *pestis anguillarum*





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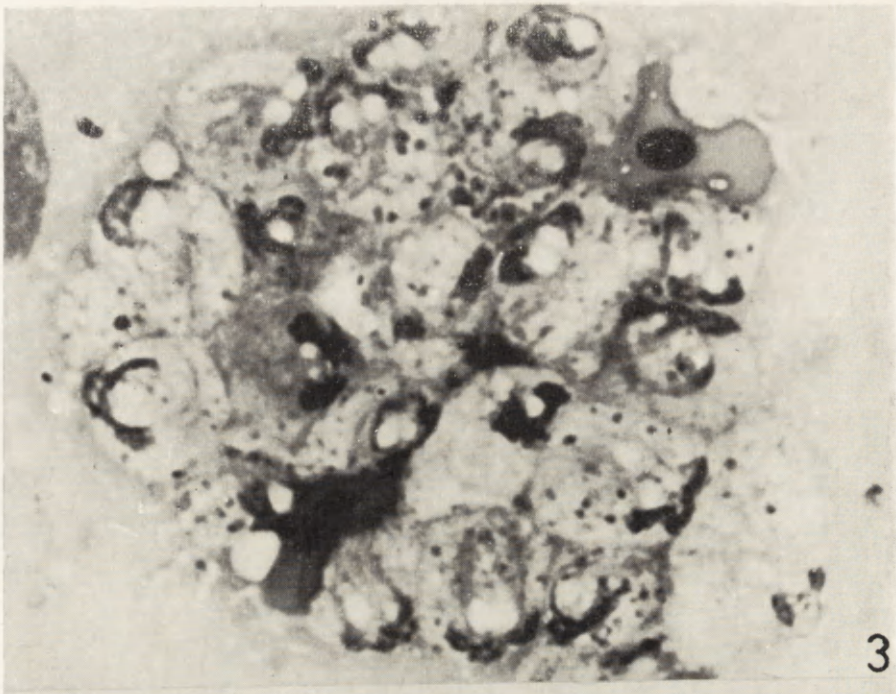

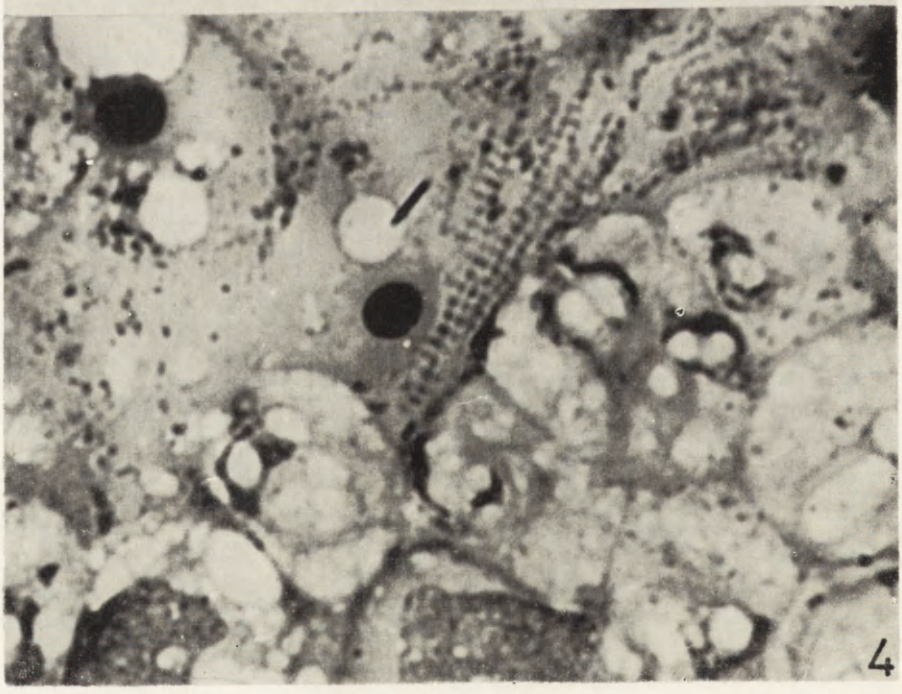

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Two Species of Flagellates of the Genus *Chilomastix* Alexeieff, 1912 (*Mastigophora: Retortamonadida*) from Amphibians and Reptiles in India

Synopsis. Chilomastix hemidactyli sp. n. and *C. qadrii* Krishnamurthy, 1970 are described from the rectal contents of *Hemidactylus giganteus* Stoliczka, 1872 and *Rana cyanophlyctis* Schneider, 1799 respectively. *C. hemidactyli* is distinguished by three granules in the blepharoplastic complex, one giving rise to 2 anterior flagella, the second to the third anterior flagellum and the third to the cytostomal flagellum and cytostomal fibril. The nucleus is either at the extreme anterior end or slightly behind and has scattered chromatin granules and a small endosome. *C. qadrii* is recorded from a new host, with slight variations.

Flagellates of the genus *Chilomastix* were first recorded from amphibians by Alexeieff (1909) under the name *Macrostoma*. Later, realizing that the name was preoccupied, the same author transferred it to the genus *Tetramitus* Perty, 1852. In 1912, he differentiated these organisms and erected the genus *Chilomastix* to accommodate them. Many species of the genus have been described since then from a variety of vertebrates. The present communication gives an account of the morphology of two species from frogs and lizards in India.

Material and Methods

The flagellates were found in the rectal contents of the two hosts namely *Hemidactylus giganteus* Stoliczka, 1872 and *Rana cyanophlyctis* Schneider, 1799 from around Aurangabad. Permanent smears of the material were stained with Heidenhain's iron haematoxylin or phospho-tungstic haematoxylin (after Baker 1969)

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after fixation in Schaudinn's fluid or with Giemsa's stain after exposure to osmic vapours and fixation in methanol. The drawings were made with a Camera Lucida at a magnification of 2000 ×.

Results

Chilomastix qadrii Krishnamurthy, 1970 (Fig. 1)

Morphology

The organism has a somewhat elongated (Fig. 1 *5*) or ovoidal (Fig. 1 *3*, *4*) body. The posterior end suddenly tapers into a narrow (Fig. 1 *2*, *3*, *5*) spike-like structure, which is conspicuous in the living condition and reaches a maximum length of 7.7 μm. In some of the stained preparations, the posterior end appears rounded (Fig. 1 *1*, *8*). The cytoplasm is vacuolated and contains bacteria and other granules (Fig. 1 *2*, *4*, *7*). The pellicle is thin. The nucleus is close to the anterior end of the body and is rounded (Fig. 1 *1*, *2*, *8*) or triangular (Fig. 1 *5*, *6*) in shape.

The flagella arise from a blepharoplastic complex at the extreme anterior end of the body, often obscured by the nucleus. In some, where they can be seen the complex appears to be of two (Fig. 1 *4*) or three (Fig. 1 *1*, *7*) granules. Two of the granules placed close together give rise to three free flagella, one from one and two from the other granule (Fig. 1 *7*). The third granule, a little away from the first two, gives origin to the cytostomal flagellum. The flagella are unequal, the shortest being a little more than half body length and the longest about as long as the body. The cytostomal flagellum is generally the shortest.

The cytostome extends upto about the middle of the body and is more or less uniform in width. Its outer edge is bordered by a cytostomal fibril, which recurves along the base of the pouch. The origin of the fibril is not clear, but it appears to arise from one of the two granules giving rise to the anterior flagella (Fig. 1 *4*).

Measurements (range with average in brackets)

Length of the body	7.7–20.6 μm (13.7 μm)
Width of the body	5.7–13.3 μm (8.8 μm)
Length of spike	1.5–7.7 μm (5.4 μm)
Length of flagellum I	3.6–12.9 μm (7.8 μm)
Length of flagellum II	4.1–13.9 μm (9.5 μm)
Length of flagellum III	4.6–20.6 μm (11.8 μm)
Length of cytostomal flagellum	3.6–10.3 μm (6.6 μm)
Size of nucleus	2.6–5.7 μm × 3.1–7.7 μm (3.8 × 4.3 μm)

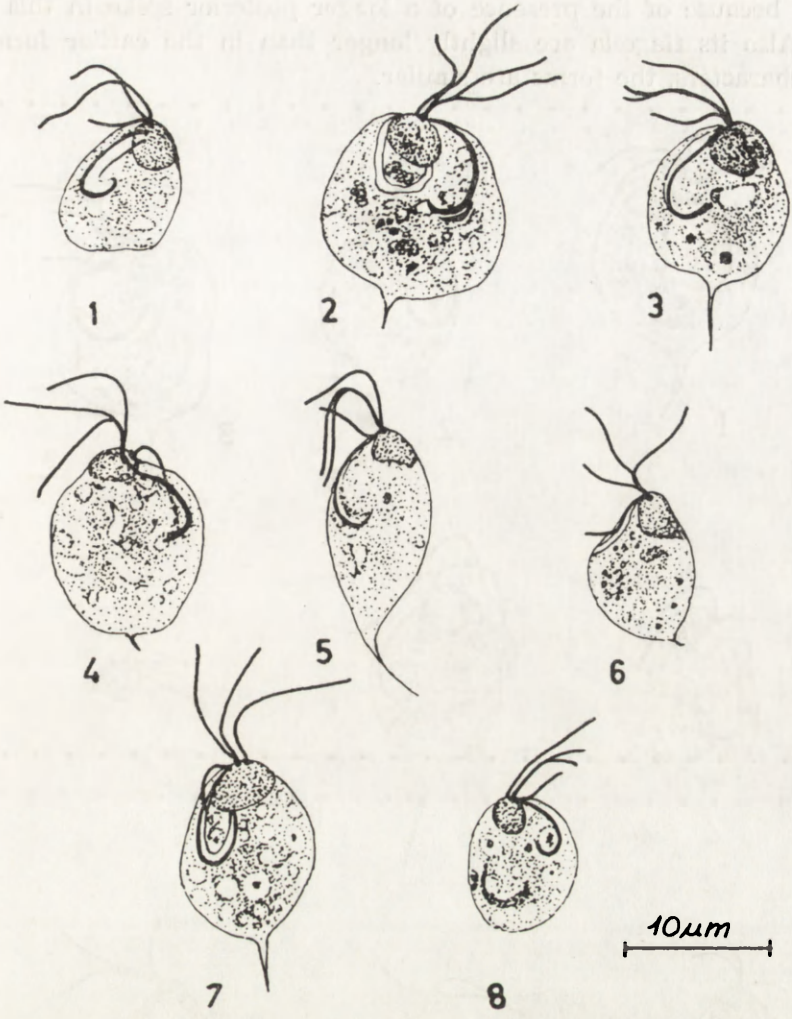

Fig. 1. *Chilomastix qadrii* Krishnamurthy, 1970 (All figures from smears exposed to osmic vapours, fixed in methyl alcohol and stained with Giemsa stain) *1*, *7* — showing three blepharoplasts, origin of flagella and fibril, *2* — showing vacuoles and granules in cytoplasm, *3*, *5* — showing the cytostome and a posterior spike, *4* — showing two blepharoplasts and origin of flagella, *6*, *8* — showing general structure

Comments

This species was described by K r i s h n a m u r t h y (1970) from the rectum of *Rana tigrina* Daudin, 1802 and is now being recorded from *R. cyanophlyctis*, Schneider, 1799 with minor variations. The present organism is longer than the one described earlier, being 7.7–20.6 µm (13.7 µm) as against 7.7–13.4 µm (10.6 µm) long. This appears to be

mainly because of the presence of a longer posterior spike in this organism. Also its flagella are slightly longer than in the earlier forms. In other characters, the forms are similar.

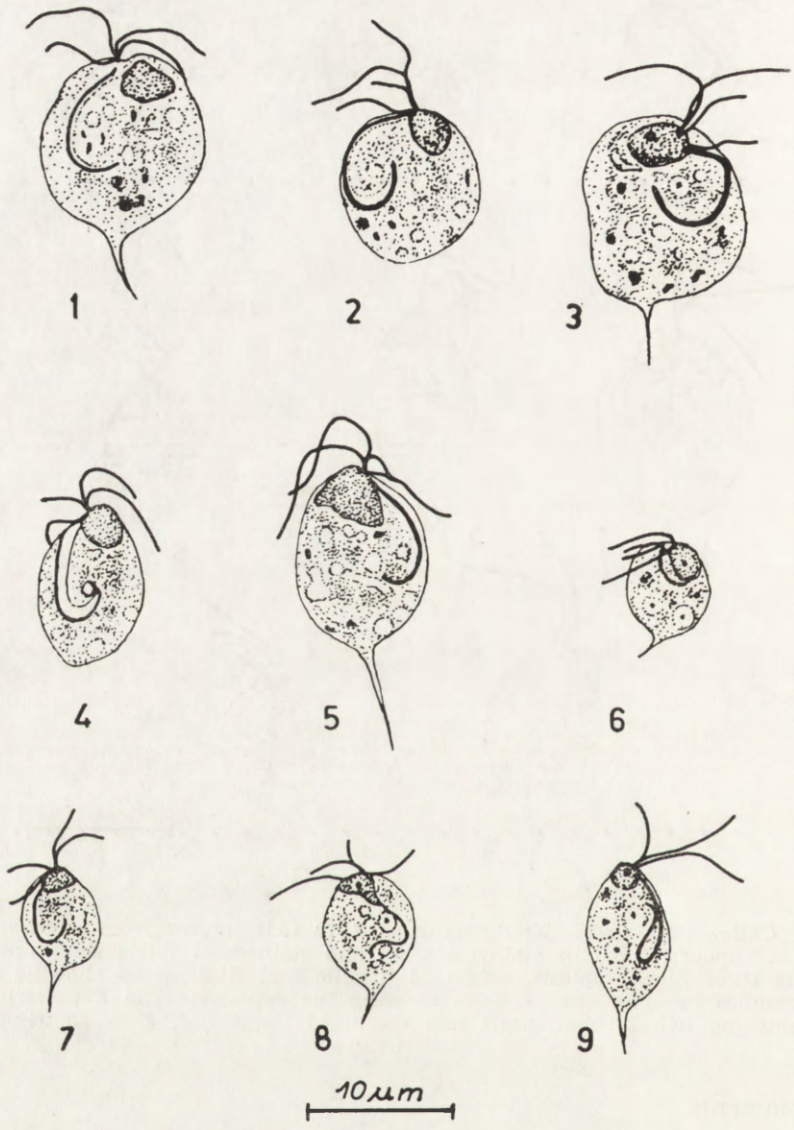

Fig. 2. *Chilomastix hemidactyli* sp. n. (*1–5* from smears exposed to osmic vapours, fixed in methyl alcohol and stained with Giemsa stain. *6* to *9* from smears fixed in Schaudinn's fluid and stained with tungsto-phosphoric acid haematoxylin) *1*, *7* — showing general structure, *2* — a rounded form showing a conspicuous cytostome, *3* — showing the blepharoplast and origin of flagella and fibril, *4* — showing two cytostomal fibrils, *5* — showing a long posterior spike, *6*, *8* — showing nuclear structure, *9* — showing a long and narrow cytostome

Species: *Chilomastix qadrii* Krishnamurthy, 1970
Host: *Rana cyanophlyctis* Schneider, 1799
Habitat: Rectum
Locality: Aurangabad, Maharashtra, India, Sept. 11, 1972.

Chilomastix hemidactyli sp. n.
(Fig. 2)

Morphology

The body of the parasite is ovoidal (Fig. 2 *1*, *4*, *5*) or spherical (Fig. 2 *2*), with a posterior spike which is conspicuous in the living condition and reaches a maximum length of 8.7 μm. In most of the stained preparations it is long and prominent.

The cytoplasm is vacuolated and contains bacteria and granules. The pellicle is thin but stains well.

The nucleus is situated at the extreme anterior end of the body (Fig. 2 *2*, *4*, *5*) or slightly behind (Fig. 2 *1*, *3*). It is rounded or ovoid (Fig. 2 *2*, *3*, *6*) or irregular in shape (Fig. 2 *1*, *5*, *8*). It has a distinct nuclear memebrane enclosing scattered chromatin granules and a small central endosome (Fig. 2 *6*, *8*, *9*).

A blepharoplastic complex of three granules is situated between the nucleus and the anterior end of the body (Fig. 2 *3*). Two of the three granules are close to one another and often stain together as a single mass (Fig. 2 *6*) while the third is separated from this (Fig. 2 *3*). The first of these granules gives rise to two anterior flagella, the second to the third anterior flagellum and the third to the recurrent flagellum and cytostomal fibrils (Fig. 2 *3*).

The three flagella are unequal in their length, the shortest measuring about half to three-fourths of the body and the longest about as long as the body. The fourth flagellum which extends into the cytostome is slightly longer than the shortest anterior flagellum. In several cases this comes out of the cytostome and trails on the side of the body (Fig. 2 *1*, *3*, *7*).

The cytostome is conspicuous extending upto the middle (Fig. 2 *3*, *5*) or the junction of the middle and posterior thirds of the body (Fig. 2 *4*, *7*). It is broad and sac-like (Fig. 2 *2*, to *4*, *7*), being somewhat narrower anteriorly. Along its outer border it has a conspicuously staining cytostomal fibril. This runs along its outer border upto the posterior end of the sac and recurves anteriorly (Fig. 2 *2* to *4*, *7*, *9*). It is slightly thicker than the flagella. In well differentiated specimens, a second fibril could be seen running parallel to first one (Fig. 2 *4*). It is comparatively thinner and shorter than the first fibril.

Measurements (range and average within brackets; all measurements are in microns)

Length of the body	7.70–12.85 (10.30)
Breadth of the body	5.14–11.31 (7.74)
Length of the posterior projection	1.03–8.73 (4.70)
Length of the anterior flagellum I	3.08–9.77 (5.20)
Length of the anterior flagellum II	4.11–12.85 (8.30)
Length of the anterior flagellum III	5.65–15.42 (10.60)
Length of the recurrent flagellum	4.11–10.80 (7.00)
Length of the cytostome	5.65–13.40 (9.14)
Length of the nucleus	2.05–4.11 (3.70)
Width of the nucleus	1.54–5.14 (3.10)

Comments

Spiecies of this genus occur in several vertebrates, particularly in mammals. They have been reported from reptilian hosts, only a few cases. W e n y o n (1920) was the first to report the occurrence of *Chilomastix* in lizards, but he gave no description. Subsequently two species have been described from reptiles, *C. bursa* Moskowitz, 1951 and *C. wenyoni* Janakidevi, 1961, both from lizards.

The organism described above comes close to these species in its general features. However, there are several differences distinguishing it from the two earlier species. This species is about the same length as *C. bursa* but is relatively broader (5.14–11.31 μm as against 4.00–6.00 μm). It also differs in size from *C. wenyoni*, measuring 7.70 to 12.85 × 5.14 to 11.31 μm (10.33 × 7.74 μm) as against 8.00 to 20.50 × 2.50 to 10.50 μm (18.00 × 6.00 μm). *C. bursa* has a single blepharoplast giving origin to all the flagella and the fibrils. *C. wenyoni* has four granules, three giving rise to the three anterior flagella and the fourth to the cytostomal flagellum and the right cytostomal fibril. On the other hand, the present species has a set of three granules, the first giving rise to two anterior flagella, the second to the third anterior flagellum and the third to the cytostomal flagellum and right cytostomal fibril. The nucleus of *C. wenyoni* is about 2 μm behind the anterior end, while that of *C. bursa* is at the extreme anterior end of the body. In this organism it is either at the extreme anterior end or about 1.0 μm behind. The endosomal substance is distributed in the form of one to several plaques along the under surface of the nuclear membrane in the two earlier described species. However, the parasite under discussion has scattered chromatin granules and a small central endosome.

In view of these differences the organism is considered distinct and named *Chilomastix hemidactyli* sp. n. after the generic name of the host.

Species: *Chilomastix hemidactyli* sp. n.
Host: *Hemidactylus giganteus* Stoliczka, 1872
Habitat: Rectum
Locality: Aurangabad, Maharashtra, India, March 27, 1972.

The type material of the species are deposited in the protozoology section, Department of Zoology, Marathwada University, Aurangabad, Maharashtra, India.

ACKNOWLEDGEMENT

Author is indebted to Dr R. Krishnamurthy, Reader in Zoology, Marathwada University, Aurangabad for his help and encouragement. Thanks are due to Dr S. Mehdi Ali and Dr R. Nagabhushanam for providing laboratory and library facilities.

RÉSUMÉ

Chilomastix hemidactyli sp. n. et *C. qadri* Krishnamurthy, 1975 ont été trouvés, respectivement dans le contenu du rectum de *Hemidactylus giganteus* Stoliczka, 1872 et de *Rana cyanophlyctis* Schneider, 1799. *C. hemidactyli* est caractérisé par la présence de trois granules dans son complexe blépharoplastique, dont une se trouve à la base de deux flagelles antérieures, la seconde à la base de la troisième flagelle antérieure, et la troisième à la base de la flagelle cytostomale et du fibre cytostomal. Le noyau est situé soit à l'extrémité antérieure du corps, soit légèrement derrière, et il est pourvu des granules dispersées de chromatine et d'un petit endosome. *C. qadri* est dérit, avec des legères modifications, chez un hôte nouveau.

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Studies on Two Flagellates of the Genus *Monocercomonoides* Travis, 1932 (*Mastigophora* : *Polymastigina*) from Amphibians and Reptiles in India

Synopsis. Monocercomonoides dobelli sp. n., is described from the rectal contents of the toad, *Bufo melanostictus* Schneider, 1799. It is distinguished by its unequal anterior flagella and uneven thickness of the axoneme. It measures 6.7–14.4 μm × 5.1–12.9 μm (average: 9.2 × 7.7 μm). *Monocercomonoides mehdii* Krishnamurthy, 1967 is recorded from a new lizard host, with slight variations in the nature of axoneme.

Grassi (1879) was the first to describe a flagellate of this genus from *Gryllotalpa*. He placed it in the subgenus *Retortamonas* under the genus *Monocercomonas*. He later transferred it to the subgenus *Schedoacercomonas* under the same genus. In 1881, he synonymised *S. gryllotalpae* with *S. melolonthae* and renamed it as *M. insectorum*. Since the generic name was preoccupied, Travis (1932) erected the genus *Monocercomonoides* to accommodate these flagellates. Since then similar flagellates have been recorded from a variety of hosts. The present communication gives an acount of two species of the genus — a new species from the toad and a new host record for *M. mehdii* Krishnamurthy, 1967.

Material and Methods

In the course of the present survey 31 toads (*Bufo melanostictus* Schneider, 1799) were examined for intestinal flagellates, of which 26 were positive. Of these species described herein was found in two of the toads. The infection was moderate in both cases and the parasite was found along with flagellates of the genus *Monocercomonas*. *M. mehdii* was found in two lizards (*Calotes* sp.) examined, along with parasites of the genus *Proteromonas*.

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The flagellates were studied in the live condition with the help of vital stains like toluidene blue and methylene blue. Permanent preparations were made for detailed study by both dry and wet methods. For the former, the smears were exposed to osmic vapours, fixed in methanol and stained with Giemsa's stain. The wet preparations were made by fixation in Schaudinn's fluid, followed by staining either with Heidenhain's iron haematoxylin or Phospho-tungstic haematoxylin (as per the procedure outlined by Baker 1969). The drawings were made with a Leitz Camera Lucida, at a magnification of 2000 ×.

Results

Monocercomonoides dobelli sp. n.
(Fig. 1)

Morphology

The flagellates are spherical (Fig. 1 *2*, *6*, *7*) or slightly elongated and ovoidal (Fig. 1 *1*, *8*) or somewhat irregular (Fig. 1 *3*, *5*) in shape. Some of the organisms are broader than long (Fig. 1 *4*).

There are two blepharoplasts placed close to the anterior end of the body, about 1.6 μm apart and connected to each other by a delicate rhizoplast (Fig. 1 *3*, *5*). The granules lie at the same level (Fig. 1 *1*, *2*) or slightly at different levels, the dorsal being slightly above the ventral (Fig. 1 *4*, *5*, *6*). The two anterior flagella arising from the ventral blepharoplast are unequal (Fig. 1 *2*, *4*, *8*), the shorter one being about one and one-fourth times and the longer one about one and three-fourth times the body length. The third anterior flagellum arising from the dorsal blepharoplast is as long as the shorter flagellum of the other side. The trailing flagellum is relatively longer (Fig. 1 *2*, *6*, *7*) and about two to two and a half times as long as the body. Some of the flagella are acronematic (Fig. 1 *2*, *3*, *5*, *6*). No funis was seen.

The axoneme arises from the dorsal blepharoplast and extends through the length of the body. It is thin as it arises and curves around the nucleus (Fig. 1 *1*, *4–6*) but becomes thicker distally and appears to be flexible Fig. 1 *2*, *4*, *5*). The posterior end either curves inwards (Fig. 1 *1*) or terminates near the posterior end of the body (Fig. 1 *2*, *5*).

The nucleus is placed close to the anterior end, with its upper border abutting against the blepharoplasts (Fig. 1 *1*, *2*, *4*). It is slightly broader than long and has its anterior surface flattened in the region between the blepharoplasts (Fig. 1 *2*, *4*, *6*). It has a central (Fig. 1 *8*) or an eccentric (Fig. 1 *7*) endosome surrounded by scattered chromatin granules. The cytoplasm is vacuolated (Fig. 1 *4*, *6*) and has a few deeply staining granules (Fig. 1 *2*, *3*, *5*).

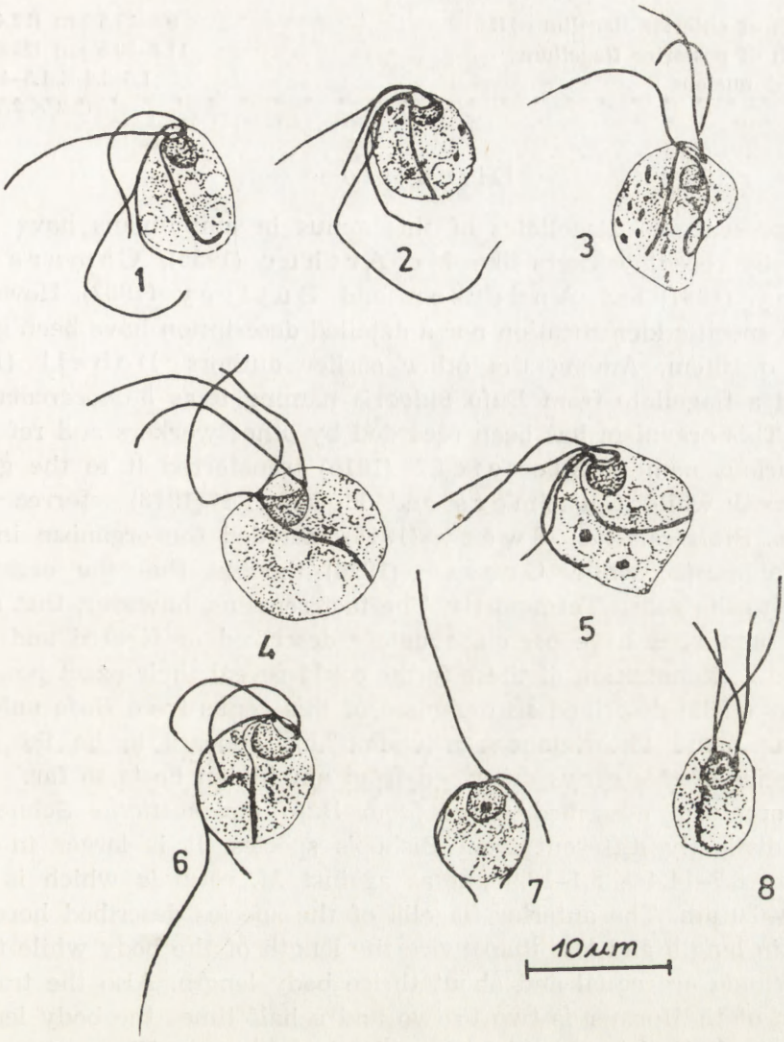

Fig. 1. *Monocercomonoides dobelli* sp. n. (*1–6* from smears exposed to osmic vapours, fixed in methyl alcohol and stained with Giemsa stain, *7*, *8* from smears fixed in Schaudinn's fluid and stained with tungsto-phosphoric acid haematoxylin), *1* — ovoidal form showing general structure, *2* — rounded form showing the blepharoplasts, origin of flagella and structure of axoneme, *3* — showing rhizoplast and acronematic flagella, *4* — showing blepharoplasts, origin of flagella and axoneme, *5* — showing rhizoplast, structure of axoneme and origin of flagella, *6* — showing structure of axoneme and acronematic flagella, *7*, *8* — showing nuclear structure

Measurements (range and average within brackets)

Length of the body	6.7–14.4 µm (9.2 µm)
Maximum breadth of body	5.1–12.9 µm (7.7 µm)
Length of anterior flagellum I	8.7–16.5 µm (12.3 µm)
Length of anterior flagellum II	10.8–24.7 µm (15.3 µm)

Length of anterior flagellum III	9.3–17.5 μm (12.4 μm)
Length of posterior flagellum	11.8–30.5 μm (22.6 μm)
Size of nucleus	1.5–3.6 × 1.5–4.1 μm (2.2 × 2.7 μm)

Discussion

The presence of flagellates of this genus in amphibians have been recorded by recent workers like Mc Arthur (1955), Camara and Buttrey (1961) and Anderson and Buttrey (1962). However, neither a specific identification nor a detailed description have been given by any of them. Among the other earlier authors Dobell (1909) described a flagellate from *Bufo vulgaris* naming it as *Monocercomonas bufonis*. This organism has been recorded by other workers and referred to by various names. Alexeieff (1916) transferred it to the genus *Trichomastix* while Chalmers and Pekkola (1918) referred it to the genus *Protetramitus*. Swezy (1916) included the organism in the genus *Polymastix* while Grassé (1926) thought that the organism belonged to the genus *Tetramastix*. The fact remains, however, that none of these organisms have been adequately described or figured and only a detailed reexamination of these forms could reveal their exact position. Bishop (1932) described an organism of this genus from *Bufo vulgaris* under the name *Retortamonas rotunda*. This appears to be the only valid species of this genus described from amphibian hosts so far.

The organism described above from *Bufo melanostictus* Schneider, 1799 is distinctly different from Bishop's species. It is larger in size, measuring 6.7–14.4 × 5.1–12.9 μm as against *M. rotunda* which is 6.0–8.0 × 5.0–6.0 μm. The anterior flagella of the species described here are unequal in length and less than twice the length of the body while those of *M. rotunda* are equal and about thrice body length. Also the trailing flagellum of the former is two to two and a half times the body length, while that of the latter is six times the body length. The axoneme of *M. rotunda* is a homogenous rod projecting slightly at the posterior end but that of the species under discussion is thinner near its origin and thicker in the trunk region.

In view of its distinctness, the species is considered to be a new one and named *Monocercomonoides dobelli* sp. n.

Species: *Monocercomonoides dobelli* sp. n.
Host: *Bufo melanostictus* Schneider, 1799
Habitat: Rectum
Locality: Aurangabad, Maharashtra, India September 10, 1971

Monocercomonoides mehdii Krishnamurthy, 1967
(Fig. 2)

Morphology

The parasite appears round or ovoid in the living condition and moves about with a zig-zag motion, at moderate speed. In fixed preparations, the shape is maintained in most cases (Fig. 2 *1*, *3*, *4*, *6*) but some become irregular (Fig. 2 *5*).

The two blepharoplasts lying just above the nucleus are 1.4 μm apart and connected by a rhizoplast running along the upper border of the nucleus (Fig. 2 *1*, *2*) or above it (Fig. 2 *4*, *5*). The two anterior flagella

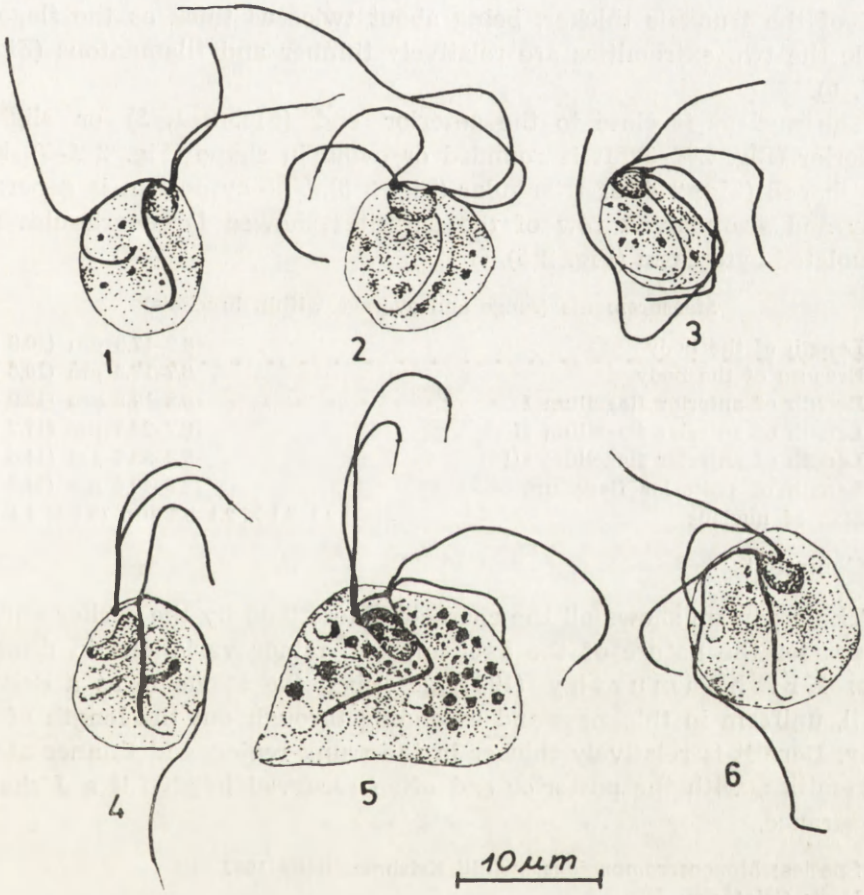

Fig. 2. *Monocercomonoides mehdii* Krishnamurthy, 1967. (All figures from smears exposed to osmic vapours, fixed in methyl alcohol and stained with Giemsa stain), *1* — showing blepharoplasts, rhizoplast and J-shaped axoneme, *2* — showing the origin of the flagella, *3*, *6* — showing general structure, *4* — showing the blepharoplasts, rhizoplast, the origin of mastigont elements and kidney-shaped nucleus, *5* — a large irregular form showing cytoplasmic granules

arising from the ventral blepharoplast are unequal, one being about as long as the body and the other about one and a half times the body length. The third anterior flagellum, arising from the dorsal blepharoplast, is slightly shorter than the longest anterior flagellum. The trailing flagellum is one and a half to two and a half times the body length. Some or all the flagella end in distinct acronemes (Fig. 2 *1, 2, 4, 5*). No funis was seen.

The axoneme arises from the dorsal blepharoplast and, passing around the nucleus (Fig. 2, *1, 6*) or beside the nucleus (Fig. 2 *4*), extends up to the posterior end of the body. It never projects outside, but recurves at the posterior end (Fig. 2 *1, 2, 6*) being somewhat J-shaped. The major part of the trunk is thicker, being about twice as thick as the flagella, while the two extremities are relatively thinner and filamentous (Fig. 2 *1, 4, 6*).

The nucleus is close to the anterior end (Fig. 2 *1, 2*) or slightly posterior (Fig. 2 *4–6*). It is rounded or ovoid in shape (Fig. 2 *1–3*), kidney shaped (Fig. 2 *4*) or triangular (Fig. 2 *6*). The cytoplasm is generally clear and granular. A few of the parasites showed large granules and vacuolated cytoplasm (Fig. 2 *5*).

Measurements (range and average within brackets)

Length of the body	8.2–17.5 µm (10.8 µm)
Breadth of the body	6.7–17.5 µm (10.6 µm)
Length of anterior flagellum I	6.2–19.5 µm (12.9 µm)
Length of anterior flagellum II	8.7–26.7 µm (17.7 µm)
Length of anterior flagellum III	8.8–23.6 µm (14.6 µm)
Length of posterior flagellum	12.9–40.6 µm (24.7 µm)
Size of nucleus	2.1–5.1 × 2.1–5.1 µm (3.1 × 3.4 µm)

Comments

The organism shows all the characters described by the earlier author, except for the nature of the axoneme and minor variations in dimensions. Krishnamurthy (1967) described the axoneme as a delicate fibril, uniform in thickness and extending through out the length of the body. Here it is relatively thicker in the trunk region and thinner at the extremities, with the posterior end often recurved to give it a J shaped appearance.

Species: *Monocercomonoides mehdii* Krishnamurthy 1967
Host: *Calotes* sp.
Habitat: Rectum
Locality: Aurangabad, Maharashtra, India August 23, 1972

The slides of the type material are deposited in the Protozoology Section, Department of Zoology, Marathwada University, Aurangabad.

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RÉSUMÉ

Monocercomonoides dobelli sp. n. a été trouvé dans le contenu du rectum du crapaud, *Bufo melanostictus* Schneider, 1799. Il est caractérisé par ses flagelles antérieures inégales, et par l'épaisseur inégale de son axonème. Ses dimensions se situent entre 6.7–14.4 μm × 5.1–12.9 μm (moyenne: 9.2 × 7.7 μm). *Monocercomonoides mehdii* Krishnamurthy, 1967 est décrit, avec des légères modifications de la nature de son axonème, chez un lézard qui est un hôte nouveau peu cette espèce.

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A New Aseptate Gregarine, *Monocystis pontodrili* sp. n. from the Littoral Oligochaete, *Pontodrilus bermudensis* Beddard [1]

Synopsis. The morphology and life-history of a new aseptate gregarine, *Monocystis pontodrili* sp. n. developing in the coelomic fluid and occurring between the body muscles is described. The trophozoites are solitary and an anterior mucron is clearly seen. The gametes are isogamous and spherical. Spores are boat-shaped and octozoic.

While studying the histology of the littoral oligochaete, *Pontodrilus bermudensis* Beddard we came across an aseptate gregarine belonging to the genus *Monocystis* in the coelomic fluid and between the body muscles. Several species of *Monocystis* have been reported from a variety of oligochaetes from different parts of the world but a perusal of the literature showed that no aseptate gregarine belonging to the genus *Monocystis* has so far been reported from the present host either from India or from any other part of the world. For reasons discussed elsewhere, the present form is considered a new species and the name *Monocystis pontodrili* sp. n. is proposed for the same.

Material and Methods

Pontodrilus bermudensis Beddard, a littoral oligochaete occurs in large numbers along with other polychaetes like *Lycastis indica*, *Marphysa gravelyi* and a sipunculid worm, *Siphonosoma australe* in the southern lighter channel at Visakhapatnam harbour (Latitude 17°41' Longitude 83°17') where salinity fluctuations are large (6–33‰). The worms occur buried about 10–12" deep in the sediment occurring in the midtide level. They also occur underneath the stones in the same area. The worms after collection were maintained in the laboratory, singly in glass finger

[1] Abstract presented at the IX International Colloqium on Invertebrate Pathology held at Kingston, Ontario, Canada. August-September 1976.

bowls containing brackish water (Salinity 20–25‰). There are no external indications of infection and hence all of them were opened to detect infection. Smears from the infected hosts were fixed in Schaudinn's fluid and stained with Delafield haematoxylin. Material for sectioning was fixed in alcoholic Bouin's fluid for 1 h at 60°C and at room temperature for 24 h. Sections were cut at 8 μm thickness and stained with Heidenhain's iron haematoxylin. All the measurements are given in microns and the drawings are made with the aid of a camera lucida.

Observations

70% of the worms ranging in size from 8–12 cm (mature forms) collected during November 1974–May 1975 were found infected with an aseptate gregarine belonging to the genus *Monocystis*. The percentage infection in smaller forms measuring less than 6 cm (immature forms) was only about 20.

The smaller trophozoites, the bigger forms and the cysts were found in the coelomic fluid. Some of the cysts were found attached to the body muscles. The smallest trophozoite observed was opaque white and measured 200 × 65 μm. The body does not show any differentiation and the cytoplasm is uniformly hyaline. The nucleus is oval and contains a single deeply stained eccentrically placed endosome (Fig. 1 *1*).

The fully grown trophozoites as seen in smears are elongated and almost cylindrical measuring 350 × 150 μm. Both the ends are broadly rounded. A crescent-shaped portion of the trophozoite at one end has deeply stained protoplasm without any inclusions while the rest of the trophozoite has alveolated protoplasm containing fine granular inclusions. The deeply stained portion of the trophozoite probably represents the mucron. The nucleus is oval and is placed either at the anterior, middle or the posterior end of the body. The single endosome seen in earlier stages is broken up and shows two large and about eight small chromatin bodies. The body wall is very thin and bursts even under the slightest pressure (Fig. 2). The fully grown trophozoites and the cysts were seen in worms collected during November-May period which is the breeding period of the worms. It has not been possible to ascertain if there is any relationship between the life-history of the parasite and the breeding period of the host.

The earliest cyst observed was binucleate with a thin wall. It measures 300 × 160 μm inclusive of an outer mucoidal ectocyst which is about 5 μm thick. The cytoplasm in both the gametocytes is finely alveolated and does not show any differential staining. The nucleus is spherical and contains about 10–15 chromatin granules interconnected by a fine net-work (Fig. 1 *3*). During the later stages of development of the cyst the number of nuclei have increased and each gametocyte shows

10–15 nuclei. Each nucleus shows a single deeply stained eccentrically placed endosome surrounded by a clear nuclear membrane. The cytoplasm is coarsely alveolated. The mucoidal ectocyst has increased in thickness and measures 10–12 μm. (Fig. 1 *4*). When the gametes are formed, they are arranged along the periphery in several rows (Fig. 1 *5*). The gametes are spherical and isogamous measuring 4.0–4.5 μm in diameter. The cytoplasm is hyaline and the nucleus which is deeply stained

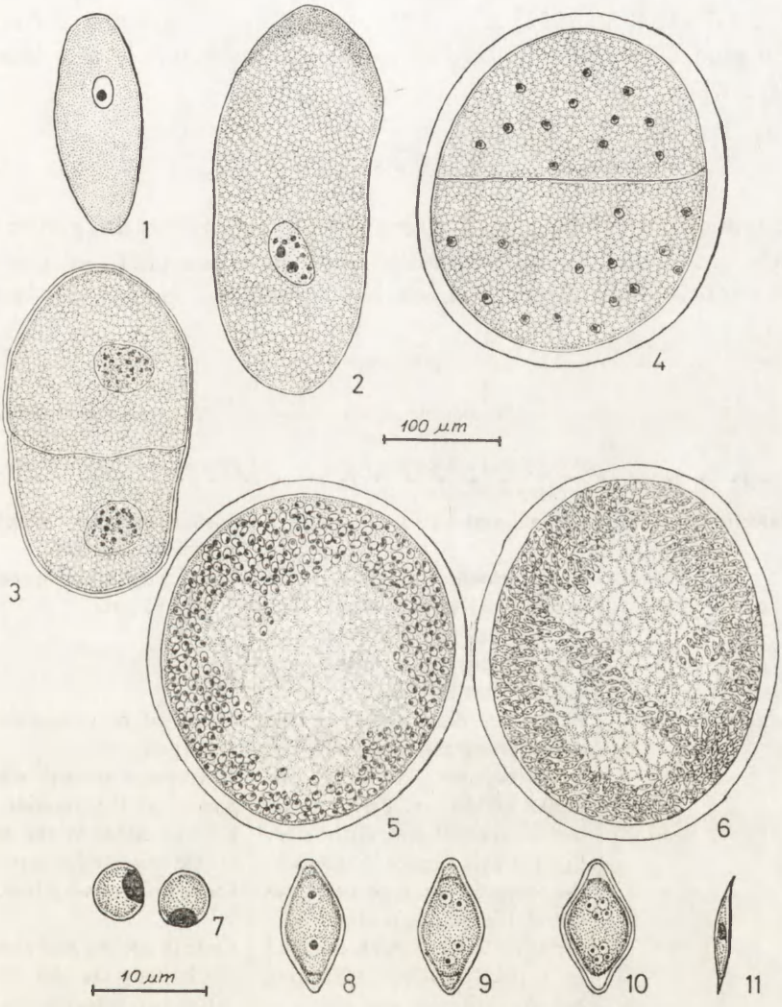

Fig. 1. *Monocystic pontodrili* sp. n. *1* — Young trophozoite (from smear), *2* — Mature trophozoite (from smear), *3* — T. S. Early cyst showing two nuclei, *4* — T. S. Cyst showing several nuclei in each gametocyte, *5* — T. S. Cyst showing the fully formed gametes — Note the peripheral arrangement of gametes, *6* — T. S. Cyst showing spores — Note peripheral arrangment, *7* — Isogamous gametes, *8* — Nucleate sporoblast, *9* — 4 Nucleate sporoblast, *10* — 8 Nucleate spore, *11* — A single sporozoite

is eccentrically placed and is in close contact with the wall (Fig. 1 7). When the spores are formed they are arranged along the periphery of the cyst (Fig. 1 6). The spores are typically boat-shaped and measure 8.0 × 3.2 μm. The binucleate and tetranucleate spores alone were seen in sections of the cysts (Fig. 1 *8*, *9*) and the octonucleate spores were seen free in the coelomic fluid (Fig. 1 *10*). Apparently the final nuclear division in the spores takes place after the cyst bursts and releases the spores into the coelomic fluid. The sporozoites are single-shaped measuring 7.2 × 1.6 μm (Fig. 1 *11*) and are arranged in two groups of four each at either pole. A small quantity of sporocyst residuum is left behind in the centre when the sporozoites are fully formed.

Discussion

Numerous species of aseptate gregarines belonging to the genus *Monocystis* are reported from oligochaetes from different parts of the world. Levine (1977) has given a check list of all the species. *M. nidata* is

Table 1

	Monocystis nidata	*Monocystis pontodrili* sp. n.
Host:	*Pontodrilus ephippiger* Rosa (*Megascilicidae*)	*Pontodrilus bermudensis* Beddard
Site of infection:	Seminal vesicles	Coelom and body muscles and seminal vesicles
Young trophozoite:	Intrablastophorical, appears like a small cell with a conspicuous nucleus and cytoplasm eosinophilic. Sometimes seen with spermatids attached to it.	Young trophozoite in coelom, 200 × 65 μm.
Sporadins:	Elongated, drawn close at the centre giving the shape of a silk worm coccoon. Measures 260–290 × 90–120 μm. Paraglycogen bodies unequal and distributed. Epicyte well marked. Nucleus in the centre 50 μm in diameter, never in the bulged portion.	Elongated or cylindrical, 350 × × 150 μm. A crescent shaped mucron is present at the anterior end. Nucleus either in the anterior, middle or posterior portion of the body. Epicyte very thin.
Cyst:	More or less spherical, enclosed in a thick envelope measuring 3–4 μm. Diameter not given.	Cysts in coelom and also embedded in body muscles. Size 350 × × 160 μm, ectocyst 10–12 μm.
Gametes:	Not described	Isogamous, 4.0–4.5 μm in diameter
Spores:	Navicular, 9 μm in length	Boat-shaped, 8.0 × 3.2 μm
Sporozoites:	Not described	Spindle shaped, 7.2 × 1.6 μm
Locality:	Asia and Indo China	South India.

the only species which is reported from hosts related to the present host (Boisson 1957). Four species of *Monocystis* are reported from India. They are *M. bengalensis, M. llyodi* (Ghosh 1923) and *M. pheritimi* (Bhatia and Chatterjee 1925) all from *Pheritima postuma. M. mathaii* is reported from the seminal vesicless of *Megascolex trilobatus* (Bhatia and Setna 1926). The present form is the first report of an aseptate gregarine from the brackish water oligochaetes from India. It differs from the only species, *M. nidata* reported from related hosts in several respects (Table 1) and hence the present form is considered a new species and the name *Monocystis pontodrili* sp. n. after the name of the host is proposed.

Diagnosis

Trophozoites elongated, solitary reaching a maximum size of 350 × 150 μm; cysts spherical or oval with an outer ectocyst reaching a maximum size of 350 × 160 μm, sometimes attached to body muscles. Gametes isogamous, spherical, 4.0–4.5 μm in diameter. Spores typically boat-shaped measuring 8.0 × 3.2 μm; sporozoites spindle-shaped measuring 7.2 × 1.6 μm. Sporocysts residuum present.

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RÉSUMÉ

La morphologie et la vie d'un nouveau aseptate gregarine, *Monocystis pontodrili* sp. n. qui atteint le fluide coelomique les vésicules séminales et qui survient entre les muscles du corps sont decrités. Les trophozoites sont solitaires et un mucron antérreur se voit clarement. Les gamétes sont isogamous et sphériques. Les spores sont en forme baten et octozoiques.

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Псаммофильные инфузории Болгарского побережья Черного моря

Psammobiotic Ciliates of the Bulgarian Coast of the Black Sea

Синопсис. В песке верхней литорали болгарского побережья Черного моря обнаружено 49 видов и 3 разновидности псаммофильных инфузорий. Из них один вид — *Tracheloraphis totevi* sp. n. описывается как новый, а 9 видов впервые отмечаются в составе псаммофильной фауны Черного моря: *Tracheloraphis discolor*, *Tr. striatus*, *Kentrophoros flavum*, *Geleia tenuis*, *Helicoprorodon multinucleatus*, *Pseudoprorodon arenicola*, *Chaenea psammophila*, *Loxophilum vermiforme* и *Litonotus elongatus*.

По составу фауна занимает промежуточное положение между микро- и мезопоральной. Найбольшее число специфических псаммофильных форм населяет умеренно загрязненный, мелкий гетерогенный песок с преобладающим размером песчинок от 0.25 до 0.3 mm. Многие микро- и эврипоральные виды образуют здесь массовые популяции.

Проанализировано распределение обнаруженных видов по исследованным географическим районам Мирового океана. Показано, что неспецифические псаммофильные инфузорий имеют большую степень общности, чем типичные псаммобионтные формы.

За последние два–три десетилетия были проведены многочисленные разносторонные исследования (фаунистические, экологические, морфологические и т. д.), посвященные изучению псаммофильных инфузорий. Интерес к ним обьясняется следующими причинами: (1) инфузорий — важнейшие индикаторы чистоты водоемов; (2) инфузорий играют существенную роль в биологическом самоочищении воды, как промежуточное звено в пищевой цепи между бактериями и бентическими и планктонными беспозвоночными; (3) многие морские инфузорий (отр. *Karyorelictida*) интересны в цитологическом отношении в связи с особенностями строения их ядерного апарата.

Результатом фаунистических исследований было опубликование списков видов псаммофильных инфузорий разных районов Мирового океана (Fauré-Fremiet 1950, 1951, Dragesco 1960, 1963 a, b, 1965, Borror 1962, Petran 1963, 1967, 1971, 1976, Hartwig 1973 a, b, 1977, Райков 1960, 1962, 1963, Агамалиев 1967, 1968, 1971, Бурковский 1970, Ковалева 1966, 1967 и мн. др., см. также примечание к Табл. 1) Сравнение этих списков обнаружило, что некаторые инфузории (как специфические, так и неспецифические псаммобионты) имеют повсеместное распрсстранение и, вероятно, их можно считат космополитами.

В работах экологического направления изучаются факторы, влияющие на состав псаммофильной фауны инфузорий: размер зерен песка, температура и соленость морской (интерстициальнсй) воды, содержание в ней кислорода, степен загрязнения грунта, его подвижность и др. (Fauré-Fremiet 1950, 1951, Swedmark 1964, Borror 1968, Fenchel and Jansson 1966, Fenchel 1969 и др.), а также суточная и сезонная ритмика в вертикальных и горизонтальных миграциях псаммофильных инфузорий (Агамалиев 1970, 1971 а, Агамалиев и Багиров 1975).

Имеющийся фаунистический материал по псаммофильным инфузориям в основном касается районов Атлантики, Средиземного и Балтийского морей, а также Каспийского моря, в котором обнаружено 305 видов инфузорий из примерно 500 известных в настоящему времени. Найменее исследованным из европейских морей остается Баренцово море. Псаммофильная фауна инфузории Черного моря подробно изучалась только на побережье Румынии (Petran 1963, 1967, 1971, 1976) и в Крыму (Ковалева 1966). Побережье Болгарии до сих пор оставалось мало исследованным. Краткие сведения о составе псаммофильных инфузорий береговой полосы имеются в работах Czapik (1952), Вълканов (1954) и Detcheva (1977). В грунтовых водах песчанного пляжа Варненского залива Czapik (1952) обнаружила 21 вид инфузорий, из каторых 12 видов являются облигатными псаммобионтами. В том же самом биотопе Вълканов (1954) отмечает еще 2 вида инфузорий, которые принадлежат к эвритопным формам.

Материал и методы исследования

Сбор материала проводился в сентябре–октябре 1976 года во время экспедиционной поездки, организованной Институтом зоологии Болгарской Академии Наук. Пользуемся случаем выразить сердечную благодарность биологу Института зоологии Димитеру Тотеву за неоценимую помощь, оказанную во время сбора материала в экспедиционных условиях.

Всего было изучено 135 проб песчанного грунта, взятых из литоральной зоны южных районов (Приморско — устье реки Ропотамо–Аркутино–Бургас) и северных районов (Варна–Албена–Балчик). Пробы песка брали с глубины 20–50 cm путем соскабливания поверхностных слоев грунта; при этом выбирали песчанные участки береговой зоны, защищенные

от постоянного действия прибоя и находящиеся в стороне от культурных пляжей. Для изучения пробы небольшие порции песка помещали в чашку Петри с морской водой, содержащей 2 ml 12% хлористого магния, который частично анестезирует инфузорий, уменшая их тигмотаксис (Faure-Fremiet 1950). Извлеченных из песка инфузорий вылавливали с помощью тонкой пипетки и помещали в солонки с чистой морской водой для дальнейших наблюдений или фиксации. В качествие фиксатора, одновременно приклеивающего инфузорий к покровному стеклу, использовали модифицированную смесь Ниссенбаума (с удвоенным содержанием третичного бутилвого спирта).

Для изучения ядерного апарата применяли временную окраску нефиксированных инфузорий метиловым зеленым — пиронином. Фиксированных инфузорий окрашивали по Фельгену.

Результаты и обсуждение

Найболее богатыми как по видовому составу, так и по численности отдельных типичных псаммофильных форм, оказались пробы песка, взятые из литорали песчаного пляжа, расположенного на расстоянии примерно 1 km к югу от устья р. Ропотамо. С этого пляжа был взят песок для гранулометрического анализа по методике, примененной Fauré-Fremiet (1950). Результат выражен в виде кумулятивной кривой (Рис. 1). Видно, что песок этого найболее показа-

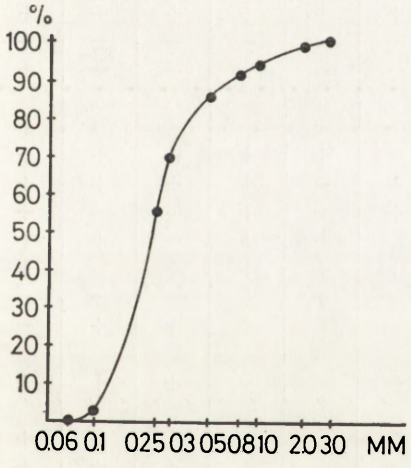

Рис. 1. Кривая гранулометрии песка. По оси абсцисс — размер песчинок (логарифмическая шкала); по оси ординат — кумулятивный процент веса песка

Fig. 1. Sand granulometry. Abscissa — sand granule diameter (in logarithmic scale). Ordinate — cumulative weight of sand in per cents

тельного района побережья мелкий, но довольно гетерогенный: 54% (по весу) песчинок мельче 0.25 mm, 32% — крупнее 0.3 mm, 16% — крупнее 0.5 mm, 8% — крупнее 1 mm. Модальный размер песчинок, определяемый местом максимальной крутизны кривой, лежит вблизи 0.25–0.3 mm. По нашим наблюдениям большинство облигатных псаммофильных инфузорий являются микропоральными и эврипоральними формами, развивающимися в особености в мелком песке или песке с переходным размером гранул, в котором они при благоприятной степени загрязнения и малой подвижности грунта чаще всего

Таблица 1

Table 1

Псаммофильные инфузории болгарского побережья Черного моря и их географическое распространение

Psammobiotic ciliates of the Bulgarian coast of the Black Sea and their geographic distribution

Виды Species	Северная Атлантика North Atlantic	Экваториальная Атлантика Equatorial Atlantic	Средиземное море Mediterranean Sea	Балтика Baltic Sea	Северное море North Sea	Белое и Баренцево моря Barentz and White Sea	Японское море Japan Sea	Каспийское море Caspian Sea	Черное море Black Sea
1	2	3	4	5	6	7	8	9	10
Fam. *Trachelocercidae* Kent									
(1) *Trachelocerca coluber* Kahl	–	–	+	+	+	+	+	–	+
(2) *T. variabilis* Kovaleva	–	–	–	–	–	–	–	–	+
(3) *T. geopetiti* Dragesco	–	–	+	–	–	–	–	–	+
(4) *Tracheloraphis totevi* sp. n.	–	–	–	–	–	–	–	–	+
(5) *Tr. prenanti* Dragesco	+	–	+	–	+	+	+	+	+
(6) *Tr. incaudatus* (Kahl)	+	+	+	+	–	+	+	+	+
(7) *Tr. margaritatus* (Kahl)	+	–	–	+	–	+	–	–	+
(8) *Tr. teissieri* Dragesco	+	+	–	–	–	+	–	+	+
(9) *Tr. kahli* Raikov	+	–	+	+	+	+	–	–	+
(10) *Tr. discolor* Raikov	–	–	–	–	+	+	–	–	–
(11) *Tr. striatus* Raikov	–	–	–	–	–	+	+	+	–
(12) *Tr. drachi* f. *bimicromultinucleata* Raikov	–	–	–	–	–	–	+	+	+
(13) *Trachelonema sulcata* Kovaleva	–	–	–	–	–	–	+	–	+
(14) *Tr. longicollis* Dragesco	+	+	–	–	–	+	+	+	+
Fam. *Loxodidae* But.									
(15) *Kentrophoros fistulosum* F.-Fr.	+	–	+	–	–	–	–	–	+
(16) *K. tubiformis* Raikov and Kovaleva	–	–	–	–	–	–	+	–	+
(17) *K. flavum* Raikov and Kovaleva	–	–	–	–	–	–	+	–	–
(18) *Remanella multinucleata* Kahl	+	+	+	+	+	+	–	–	+

1	2	3	4	5	6	7	8	9	10
(19) *R. rugosa* Kahl	+	+	+	+	+	+	+	+	+
(20) *R. granulosa* Kahl	+	−	−	+	−	+	+	+	+
Fam. *Geleiidae* Kahl									
(21) *Geleia orbis* F.-Fr.	+	+	+	+	−	+	+	−	+
(22) *G. fossata* Kahl	+	+	+	+	−	+	+	−	+
(23) *G. tenius* Dragesco	+	−	+	−	−	−	−	−	−
(24) *G. nigriceps* Kahl	+	+	−	+	+	+	+	−	+
Fam. *Prorodontidae* Kent									
(25) *Helicoprorodon gigas* Kahl	+	−	+	+	+	+	+	−	+
(26) *H. multinucleatus* Dragesco	+	+	−	−	−	+	−	−	−
(27) *H. minutus* Bock	+	+	−	+	+	+	+	−	+
(28) *Pseudoprorodon arenicola* Kahl	+	−	+	+	+	+	−	+	−
(29) *Prorodon* sp.									
Fam. *Colepidae* Ehr.									
(30) *Coleps* sp.									
Fam. *Enchelyidae* Ehr.									
(31) *Chaenea psammophila* Drag.	+	−	−	−	−	−	+	−	−
Fam. *Didiniidae* Poche									
(32) *Mesodinium pupula* Kahl	+	+	+	+	−	+	+	+	+
Fam. *Amphileptidae* But.									
(33) *Loxophyllum setigerum* Guenn.	+	+	+	+	−	+	+	+	+
(34) *L. vermiforme* Sauerbrey	+	−	−	+	+	+	−	−	−
(35) *Litonotus elongatus* Dragesco	+	+	−	−	−	−	−	−	−
(36) *L. cygnus* O.F.M.	−	−	−	+	−	−	−	+	+
Fam. *Coelosomididae* Corl.									
(37) *Paraspathidium fuscum* (Kahl)	+	+	+	+	+	+	+	+	+
Fam. *Frontoniidae* Kahl.									
(38) *Frontonia marina* F.-Dom.	+	+	+	+	+	+	+	+	+
Fam. *Uronematidae* Thomp.									

1	2	3	4	5	6	7	8	9	10
(39) *Uronema marina* Dujardin	−	+	−	+	+	+	−	−	+
Fam. *Spirostomidae* St.									
(40) *Anigsteinia clarissima* (Anigstein)	+	−	+	+	+	+	+	+	+
(41) *A. clarissima* f. *arenicola* (Kahl)	+	−	+	+	+	+	+	+	+
(42) *Gruberia uninucleata* Kahl	+	+	+	+	−	+	−	−	+
(43) *G. calkinsi* Beltran	+	−	−	−	−	−	−	−	+
Fam. *Condylostomatidae* Kahl									
(44) *Condylostoma arenarium* Spiegel	+	+	+	+	+	+	+	+	+
(45) *C. remanei* Spiegel	+	+	+	+	+	+	+	+	+
(46) *C. remanei* var. *oxyoura* Drag.	+	−	−	−	−	−	−	−	+
Fam. *Holostichidae* F.-Fr.									
(47) *Keronopsis rubra* (Ehr.)	−	+	+	−	−	+	+	+	+
(48) *Trachelostyla caudata* Kahl	+	−	+	+	+	+	+	+	+
Fam. *Oxytrichidae* Ehr.									
(49) *Oxytricha discifera* Kahl.	−	−	−	+	−	+	+	+	+
Fam. *Euplotidae* Ehr.									
(50) *Diophrys scutum* Dujardin	+	+	+	+	+	+	+	+	+
(51) *Euplotes* sp.									
(52) *Uronychia transfuga* (O.F.M.)	+	+	+	+	+	+	+	+	+

Примечание: В графу "Северная Атлантика" включены данные по районам биологических станций в Роскове, Конкарно (Франция) и Вудс Холле (США) (Dragesco 1960, 1963 a, b; Fauré-Fremiet 1950, 1951); в графу "Экваториальная Атлантика" — данные оп Мексиканскому заливу, бразильскому и африканскому побережьям Атлантического океана, а также по Бермудским островам (Borror 1962, Kattar 1970, Dragesco 1965, Hartwig 1977), в графу "Средиземное море" — данные по районам Баньюльса (Франция), Марселя и Неаполя (Fauré-Fremiet 1950, 1951, Dragesco 1954 a, 1960, 1963 a, b, Vacelet 1961 a, b, Nobili 1957), в графу "Балтика" — данные по Кильской бухте (Kahl 1930-1935, 1933; Bock 1952 a, b) Гданскому заливу Czapik et Jordan 1976), в графу "Северное море" — данные по острову Сильт, Гельголандскому заливу и Осло-Фиорду (Kahl 1933, Feld 1955, Fenchel and Jansson 1966, Fenchel 1969, Hartwig 1973 a, b), по Белому и Баренцеву морям использованы данные Райкова (1960, 1962), Бурковского (1970) и Ковалевой (1967,) по Японскому морю — данные Райкова (1963) и Райкова и Ковалевой (1968), по Каспийскому морю — данные Агамалиева (1967, 1968, 1970, 1971 a, b, c), в графу "Черное море" включены данные по составу фауны псаммофильных инфузорий румынского побережья (Tucolesco 1961, Petran 1963, 1967, 1971, 1976), а также учтены списки видов инфузорий, обитающих в Крыму (Ковалева, 1966) и в мезопсаммоне болгарского побережья Черного моря (Czapik 1952).

The column "North Atlantic" comprises the data from biological stations in Roscoff, Concarneau and Woods Hole (Dragesco 1960, 1963 a, b, Fauré-Fremiet 1950, 1951);

образуют массовые популяции. Эти данные хорошо согласуются с данными Petran (1976), которая также отмечает видовое (качественное) и количественное богатство проб, взятых с пляжей, где грунт имеет средний размер песчинок 0.3–0.35 mm.

В составе псаммофильной фауны исследованных районов болгарского побережья Черного моря отмечено 49 видов и 3 разновидности инфузорий, список которых приводится в Таблице 1.[1] Одновременно указывается географическое распространение каждого вида на основании имеющихся в литературе данных (см. примечание к Табл. 1).

Обращает на себя внимание тот факт, что почти половина (24 вида из 49) обнаруженных нами видов инфузорий относится к отр. *Karyorelictida*, который включает три семейства инфузорий, обладающих ядерным аппаратом необычного строения („кариологические реликты"). Все представители этого отряда инфузорий — типичные псаммобионты, имеющие целый комплекс приспособлений к жизни в капиллярных пространствах: сильно вытянутую или уплощенную форму тела (*Tracheloraphis prenanti*, *Tr. teissieri*, *Kentrophoros fistulosum*, *Geleia orbis*, *Remanella multinucleata* и др.), высокую сократимость (*Tracheloraphis totevi* sp. n., *Kentrophoros tubiformis*, *Geleia nigriceps* и др.), сильный тигмотаксис (*Trachelonema sulcata*, *Remanella multinucleata*, *Kentrophoros flavum* и др.) и т.д. Кариореликтиды — преимущественно эврипоральные мезосапробные формы. Единичные экземпляры почти каждого вида *Karyorelictida* можно встретить как в сильно загрязненном (заиленном) песке, так и в очень чистом песчаном грунте. Однако массовое развитие видов происходит только при некой оптимальной степени сапробности, которая для разных видов различна. Определено полисапробными формами, образующими массовые популяции в сильно загрязненном песке, являются виды рода *Geleia*, особенно *Geleia orbis*.

Из 24 видов отр. *Karyorelictida*, отмеченных нами, 8 видов (*Trachelocerca geopetiti*, *Tracheloraphis totevi* sp. n., *Tr. kahli*, *Tr. prenanti*, *Trachelonema sulcata*, *Kentrophoros flavum*, *Geleia orbis* и *Remanella multinucleata*) обнаружены почти

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the column "Equatorial Atlantic" comprises the data from the Gulf of Mexico, Brasilian and African coasts of the Atlantic and from the Bermuda islands (Berror 1962, Kattar 1970, Dragesco 1965, Hartwig 1977); the column "Mediterranean Sea" — the data from Banyuls sur Mer, Marseille and Naples (Fauré-Fremiet 1950, 1951, Dragesco 1954 a, 1960, 1963 a, b, Vacelet 1961 a, b, Nobili 1957); in the column "Baltic Sea" — the data from Kieler Bucht (Kahl 1930-1935, 1933, Bock 1952 a, b, Gdańsk Bay Czapik and Jordan 1976); in the column "North Sea" — the data from Sylt island, Helgolander Bucht and from Oslofjord (Kahl 1933, Field 1955, Fenchel and Jansson 1966, Fenchel 1969, Hartwig 1973 a, b) in the column "Barentz and White Sea" the data by Raikov 1963 and Raikov and Kovaleva 1968 are used; in the colum "Caspian Sea" the data by Agamaliev (1967, 1968, 1970, 1971 a, b, c); in the column "Black Sea" — the data from Roumanian coast (Tucolesco 1961, Petran 1963, 1967, 1971, 1976), from Crimea (Kovaleva 1966) and from the mesopsammon of Bulgarian coast (Czapik 1952).

[1] При составлении списка видов была принята новая система (Corliss 1975).

Таблица 2
Table 2

Видовой состав псаммофильной фауны инфузорий Черного моря
Species composition of the fauna of psammobiotic ciliates of the Black Sea

Виды Species	Распространение на побережьях Distribution on coasts		
	Румынии Roumanian	СССР (Крым) USSR (Crimea)	Болгарии Bulgarian
1	2	3	4
Fam. *Trachelocercidae* Kent			
(1) *Trachelocerca* coluber Kahl	−	+	+
(2) *T. variabilis* Kovaleva	−	+	+
(3) *T. geopetiti* Dragesco	−	+	+
(4) *T. entzi* Kahl	+	−	+
(5) *T. multinucleata* Dragesco	+	+	−
(6) *T. tenuicollis* Quenn.	+	−	−
(7) **Tracheloraphis totevi* sp. n.	−	−	+
(8) *Tr. prenanti* Dragesco	+	+	+
(9) *Tr. incaudatus* (Kahl)	+	+	+
(10) *Tr. margaritatus* (Kahl)	+	−	+
(11) *Tr. teissieri* Dragesco	−	+	+
(12) *Tr. kahli* Raikov	+	−	+
(13) **Tr. discolor* Raikov	−	−	+
(14) **Tr. striatus* Raikov	−	−	+
(15) *Tr. drachi* Dragesco	+	+	+
(16) *Tr. drachi* f. *bimicronucleata* Raikov	−	+	+
(17) *Tr. phoenicopterus* Cohn	+	−	+
(18) *Tr. dogieli* Raikov	+	+	−
(19) *Tracheloraphis swedmarki* Dragesco	+	+	−
(20) *Tr. crassus* Raikov	−	+	−
(21) *Tr. vermiformis* Raikov	−	+	−
(22) *Trachelonema sulcata* Kovaleva	+	+	+
(23) *Tn. longicollis* Dragesco	+	+	+
(24) *Tn. minima* Dragesco	+	−	−
Fam. *Loxodidae* But.			
(25) *Kentrophoros fistulosum* F.-Fr.	−	+	+
(26) *K. tubiformis* Raikov et Kovaleva	−	+	+
(27) **K. flavum* Raikov et Kovaleva	−	−	+
(28) *K. fasciolatum* Dragesco	+	−	−
(29) *K. gracile* Raikov	+	−	−
(30) *K. ponticum* Kovaleva	−	+	−
(31) *Remanella multinucleata* Kahl	+	+	+

1	2	3	4
(32) *R. rugosa* Kahl	+	+	+
(33) *R. granulosa* Kahl	+	+	+
(34) *R. rugosa* var. *unicorpusculata* Kahl	–	+	+
(35) *R. faurei* Dragesco	+	–	–
(36) *R. margaritifera* Kahl	+	–	–
(37) *R. minuta* Dragesco	+	–	–
Fam. *Geleiidae* Kahl			
(38) *Geleia orbis* F.-Fr.	+	+	+
(39) *G. fossata* Kahl	+	+	+
(40) **G. tenuis* Dragesco	–	–	+
(41) *G. nigriceps* Kahl	+	+	+
(42) *G. decolor* Kahl	–	+	+
Fam. *Holophryidae* Perty			
(43) *Holophrya oblonga* (Maupas)	+	–	+
(44) *H. coronata* Morgan	–	–	+
Fam. *Prorodontidae* Kent			
(45) *Helicoprorodon gigas* Kahl	+	+	+
(46) **H. multinucleatus* Dragesco	–	–	+
(47) *H. minutus* Bock	–	+	+
(48) *H. orientalis* Raikov	–	+	–
(49) **Pseudoprorodon arenicola* Kahl	–	–	+
(50) *Prorodon marinus* Cl. et L.	+	–	–
(51) *P. multinucleatus* Dragesco	+	–	–
(52) *Placus ovum* Kahl	–	–	+
(53) *P. striatus* Cohn	–	–	+
Fam. *Colepidae* Ehr.			
(54) *Coleps tesselata* Kahl	+	–	–
(55) *C. pulcher* Spiegel	+	–	–
(56) *C. similis* Kahl	+	–	–
Fam. *Enchelyidae* Ehr.			
(57) **Chaenea psammophila* Dragesco	–	–	+
(58) *C. vorax* Quenn.	–	–	+
(59) *Lacrymaria coronata* Cl. et L.	+	–	–
(60) *L. caudata* Kahl	+	–	–
(61) *L. lagenula* Cl. et L.	+	–	–
Fam. *Didiniidae* Poche			
(62) *Mesodinum pupula* Kahl	+	–	+
Fam. *Amphileptidae*			
(63) *Loxophyllum setigerum* Quenn.	+	+	+
(64) **L. vermiforme Sauerbrey*	–	–	+

1	2	3	4
(65) *L. helus* Stokes	+	−	−
(66) *L. laevigatum* Dragesco	+	+	−
(67) *L. kahli* Dragesco	−	+	−
(68) *L. multiplicatum* Kahl	−	−	+
(69) **Litonotus elongatus* Dragesco	−	−	+
(70) *L. cygnus* O.F.M.	−	+	+
(71) *L. lamella* Ehr.	+	−	−
(72) *Heminotus caudatus* Kahl	+	−	−
Fam. *Coelosomididae* Corl.			
(73) *Paraspathidium fuscum* (Kahl)	+	+	+
(74) *Coelosomides marina* Anigstein	+	−	−
Fam. *Plagiopylidae* Schew.			
(75) *Plagiopyla ovata* Kahl	+	−	−
Fam. *Chlamydodontidae* St.			
(76) *Chlamydodon cyclops* Entz.	−	−	+
(77) *Cryptopharynx setigerus* Kahl	+	−	−
Fam. *Frontoniidae* Kahl.			
(78) *Frontonia marina* Fab.-Dom.	+	+	+
(79) *F. arenaria* Kahl	+	+	−
Fam. *Uronematidae* Thomp.			
(80) *Uronema marina* Dujardin	+	−	+
Fam. *Pleuronematidae* Kent			
(81) *Pleuronema marinum* Dujardin	+	−	−
(82) *P. chrysalis* Ehr.	+	−	−
(83) *P. coronatum* Kent	+	−	−
Fam. *Spirostomidae* St.			
(84) *Anigsteinia clarissima* (Anigstein)	+	+	+
(85) *A. clarissima* f. *arenicola* (Kahl)	+	+	+
(86) *Gruberia uninucleata* Kahl	+	+	+
(87) *G. calkinsi* Beltran	−	+	+
Fam. *Metopidae* Kahl			
88) *Metopus contortus* Quenn.	+	−	−
Fam. *Condylostomatidae* Kahl			
(89) *Condylostoma arenarium* Spiegel	+	+	+
(90) *C. remanei* Spiegel	+	+	+
(91) *C. remanei* var. *oxyoura* Dragesco	−	+	+

1	2	3	4
Fam. *Peritromidae* St.			
(92) *Peritromus faurei* Kahl	+	–	–
Fam. *Strombidiidae* F.-Fr.			
(93) *Strombidium sauerbreyae* Kahl	+	+	–
(94) *Strombidium arenicola* Dragesco	+	–	–
Fam. *Holostichidae* F.-Fr.			
(95) *Keronopsis rubra* (Ehr.)	+	+	+
(96) *K. arenivorus* Dragesco	+	–	–
(97) *Trachelostyla caudata* Kahl	+	+	+
(98) *T. dubia* Dragesco	+	–	–
(99) *T. pediculiformis* (Cohn)	–	–	+
Fam. *Oxytrichidae* Ehr.			
(100) *Oxytricha discifera* Kahl	–	+	+
(101) *O. dubia* O.F.M.	+	–	–
Fam. *Keronidae* Dujardin			
(102) *Epiclintes ambiguus* O.F.M.	+	–	–
Fem. *Aspidiscidae* Ehr.			
(103) *Aspidisca lincaster* Stein	+	–	–
Fam. *Euplotidae* Ehr.			
(104) *Diophrys acutum* Dujardin	+	+	+
(105) *D. appendiculata* (Ehr.)	–	–	+
(106) *Euplotes vanuus* O.F.M.	+	–	–
(107) *E. charon* O.F.M.	+	–	–
(108) *E. harpa* Stein	+	–	–
(109) *Uronychia transfuga* O.F.M.	+	+	+

во всех пробах, взятых в разных районах болгарского побережья, причем во многих пробах они оказались массовыми формами.

Анализируя распределение черноморских псаммофильных инфузорий отр. *Karyorelictida* по разным географическим районам, нельзя не отметить, что многие обнаруженные нами представители сем. *Trachelocercidae* не имеют всесветного распространения. Так, из 14 отмеченных видов этого семейства повсеместно распространены только 4 вида — *Tracheloraphis incaudatus*, *Tr. prenanti*, *Tr. kahli* и *Trachelonema longicollis*. Два вида — *Tracheloraphis geopetiti* и *Trachelonema sulcata* — обнаружены только в умеренно теплых морях — первый в Черном и Средиземном, второй — в Черном и Японском. И, наконец,

один вид — *Trachelocerca variabilis* — пока отмечается только в Черном море (в районе Крыма и на болгарском побережье). Таким образом, судить о том, являются ли космополитами эти типичные псаммобионты, пока еще преждевременно. Этот вывод можно отнести и к видам *Kentrophoros* (сем. *Loxodidae*). В то же время обнаруженные нами виды *Remanella* (сем. *Loxodidae*), представители сем. *Geleiidae*, а также псаммофильные формы из отрядов *Prostomatida* и *Haptorida* имеют гораздо более широкое распространение. И, наконеце самую большую степень общности обнаруживают те виды, которые не явля, ются специфическими псаммофильными формами (т. е. встречаются такж- и в других биотопах). К ним относятся *Paraspathidium fuscum*, *Frontonia marina*, *Anigsteinia clarissima* и ее разновидность *f. arenicola*, виды рода *Condylostoma*, *Diophrys scutum*, *Uronychia transfuga* и некоторые другие. Эти эврипоральные и эврисапробные виды обнаружены в псаммоне всех исследованных районов Мирового океана. Однако ни один из этих видов инфузорий в изученных нами пробах песчаного грунта не встречался в массовых количествах. В наших пробах, взятых из верхней сублиторали в разных районах побережья, всегда преобладали типичные псаммобионты из семейств *Trachelocercidae*, *Loxodidae*, *Geleiidae*; наряду с ними, чаще других неспецифических псаммофильных форм встречались виды рода *Condylostoma*, *Paraspathidium fuscum*, *Keronopsis rubra* и *Trachelostyla caudata*.

Таким образом, несмотря на то, что из обнаруженных нами видов только один оказался новым, а все остальные уже были отмечены разными авторами в тех или иных районах, мы не можем безоговорочно присоединиться к гипотезе Faure-Fremiet (1951) о космополитном характере псаммофильной фауны в целом. Не исключено, что все же существует приуроченность отдельных видов или групп видов ко вполне определенным водоемам. Эта точка зрения, высказанная Бурковским (1970), так же, как и гипотеза Faure-Fremiet (1951), нуждается в дополнительном подтверждении.

В Таблице 2 приводится список видов псаммофильных инфузорий Черного моря и указывается их распространение на побережьях Румынии, Болгарии и Советского Союза. В Таблице учтены данные Czapik (1952), Petran (1963, 1967, 1971, 1976), Ковалевой (1966) и результаты настоящего исследования. Составленный таким образом общий список видов включает 109 форм (из них 1 новый вид). 24 вида являются общими для всех исследованных районов черноморского побережья; 38 видов обнаружены только на румынском побережье;[2] 6 видов указываются только для Крыма; наконец, 17 видов пока найдены только на болгарском побережье, из них 9 — впервые отмечаются в составе черноморской псаммофильной фауны вообще (отмечены в Таблице 2 звездочками).

[2] Такое число видов, характерных только для румынского побережья, происходит в основном за счет неспецифических псаммофильных форм, которые обнаружила Petran в ходе многолетних исследований разных горизонтов песчаной литорали береговой зоны Румынии.

Анализ Таблицы 2 показывает, что почти 75% фауны (72 вида из 109) — это типичные псаммобионты, больше половины которых относится к отр. *Karyorelictida* (24 вида к сем. *Trachelocercidae*, 13 — к сем. *Loxodidae* и 5 — к сем. *Geleiidae*). Приведенные цифры позволяют нам сделать вывод, что общий характер черноморский псаммофильной фауны инфузорий определяется специфическими для псаммона видами, которые, будучи массовыми формами несомнено играют основную роль в процессах, связанных с биологическим самоочищением морской воды и песчаного грунта.

Описание видов

Несмотря на то, что морфология большинства видов, перечисленных в Таблице 1, более или менее подробно освещена в литературе, мы сочли целесообразным, кроме описания нового вида *Tracheloraphis totevi* sp. n. дополнить описания еще двух видов инфузорий (*Trachelocerca geopetiti* и *Trachelonema sulcata*), каторые являются самыми характерными в псаммоне болгарского побережья Черного моря и в то же время практически не отмечены в других географических районах.

Tracheloraphis totevi sp. n. Рис. 2a, b

Очен крупная инфузория, размер каторой при максимальном вытягивании достигает 2000–3000 μm. При механическом раздражении и при фиксации сильно сокращается, укорачиваяс при этом почти вчетверо.

Передний конец тела *Tr. totevi* sp. n. заканчивается ротовой воронкой, по краю которой расположены длинные реснички. Задний конец тела заострен и вытянут в длинный тонкий „хвост", как правило, загнутый на конце латерально (Рис. 2a).

Число ресничных рядов колеблется от 42 до 60 (чаще всего 50–55). Характерной чертой этого вида является наличие узкой безресничной зоны, примерно равной 2–3 ресничным рядам (Рис. 2a).

Сократительная вакуоль отсутствует.

Питается диатомовыми водорослями.

Ядерный аппарат состоит из 16–22 макронуклеусов и 2 микронуклеусов, расположенных в виде компактной группы в центре тела инфузории. Макронуклеусы содержат 1–3 нуклеолы и хроматиновые гранулы. Микронуклеусы компактные, фельген-положительные (Рис. 2b).

По строению ядерного аппарата эта инфузория очень напоминает *Tracheloraphis prenanti* forma *multicineta*, обнаруженную Райковым и Ковалевой в Японском море (Raikov and Kovaleva 1968), но существенно отличается от нее числом ресничных рядов (у *Tracheloraphis totevi* — 42–60; у *Tr. prenanti*

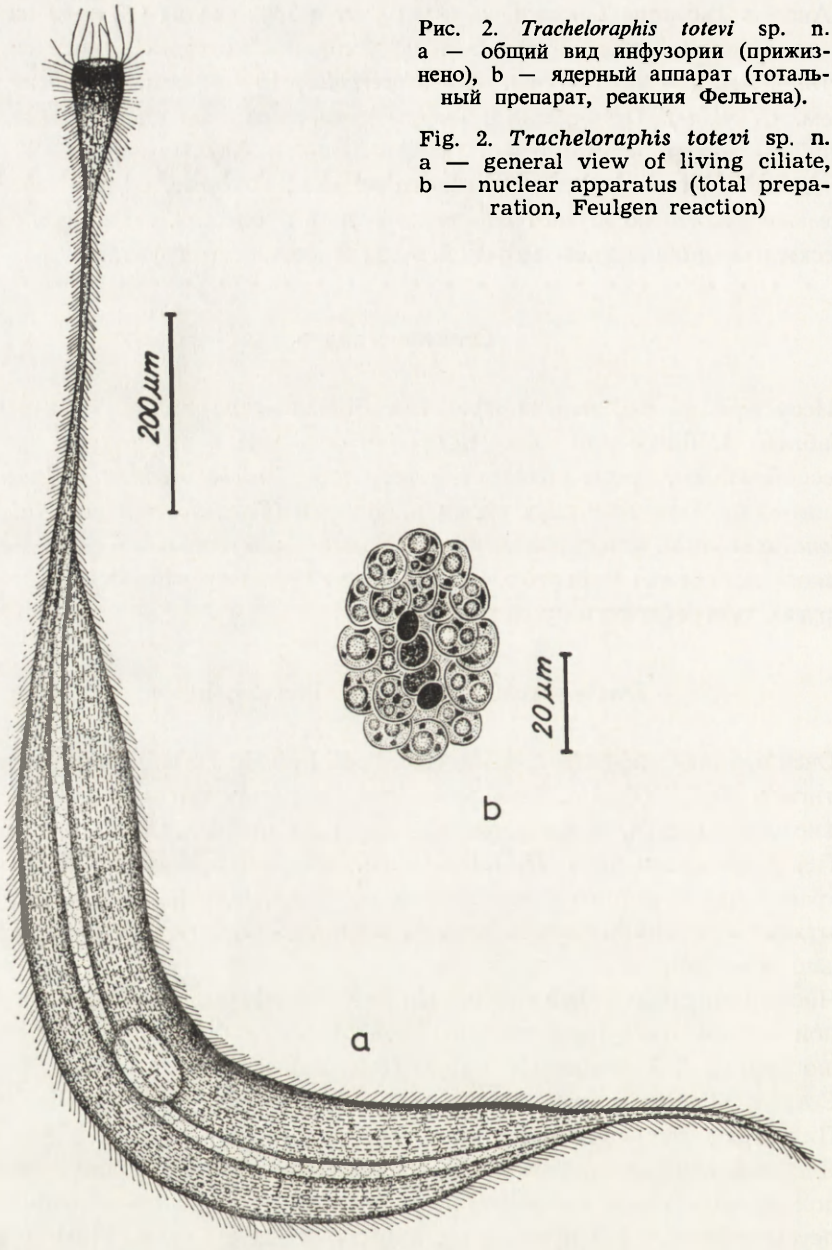

Рис. 2. *Tracheloraphis totevi* sp. n. a — общий вид инфузории (прижизненно), b — ядерный аппарат (тотальный препарат, реакция Фельгена).

Fig. 2. *Tracheloraphis totevi* sp. n. a — general view of living ciliate, b — nuclear apparatus (total preparation, Feulgen reaction)

f. *multicineta* — 20–26) и шириной „голой" полоски, равной у *Tr. totevi* 2–3 ресничным рядам, а у *Tr. prenanti* f. *multicineta* — 6–8. Больше ни один из известных видов рода *Tracheloraphis* не имеет подобного ядерного аппарата (с двумя микронуклеусами и более чем десятью макронуклеусами в составе единого комплекса).

Tracheloraphis totevi — один из самых распространенных видов болгарского псаммона, обитающий преимущественно в мелком гетерогенном песке умеренной степени сапробности.

Trachelocerca geopetiti Dragesco, 1954. Рис. 3a, b, c

Эта инфузория впервые обнаружена Dragesco 1954b в мелком песке пляжа Баньюльса (Средиземное море, Франция). Крупная форма, с длиной тела 1000–1800 μm.

Число ресничных рядов, по данным Dragesco, равняется 50. Однако у черноморской формы чаще встречаются особи с количеством ресничных рядов больше 60. Между рядами ресниц располагаются протрихоцисты двух типов — крупные овальные и мелкие сферические (Рис. 3c).

Передний конец тела имеет вид отчетливо выраженной „головки", заполненной минеральными гранулами. Ротовая воронка, занимающая терминальное положение, окружена венчиком длинных ресничек и лишена трихоцист и видимых немадесм.

Задний конец тела инфузории закруглен (Рис. 3a).

Макро- и микронуклеусы *T. geopetiti* собраны в компактную группу, расположенную в центре тела (Рис. 3a, b). Макронуклеусы *T. geopetiti* (4) довольно крупные и содержат большое количество мелких нуклеол, среди которых распределены хроматиновые элементы (Рис. 3b). Микронуклеусы (2) компактные, фельген-положительные.

Кроме побережья Средиземного моря, вид отмечен в Черном море в Крыму (единичные экземпляры), в болгарском псаммоне встречается в большом количестве в мелком гетерогенном песке средней степени сапробности.

Trachelonema sulcata Kovaleva, 1966. Рис. 4a, b, c, d

Тело инфузории ланцетовидное, сильно сплющенное в дорзовентральном направлении. Спереди оно плавно переходит в „шейку", коротая заканчивается слегка расширенной „головкой", заполненной светопреломляющими минеральными гранулами (Рис. 4a). Рот расположен терминально и имеет вид простой воронки без трихоцист и видимых немадесм. Ротовая воронка, по краю которой расположен венчик удлиненных ресничек, загнутых внутрь, снабжена продольной щелью, проходящей по дорзальной стороне (Рис. 4c) Иногда наблюдается выпячивание из ротового отверстия участка голой цитоплазмы

Задний конец тела вытянут в короткий „хвост", слегка загнутый на конце.

Общая длина инфузории варьирует от 800 до 1200 μm (в вытянутом состоянии).

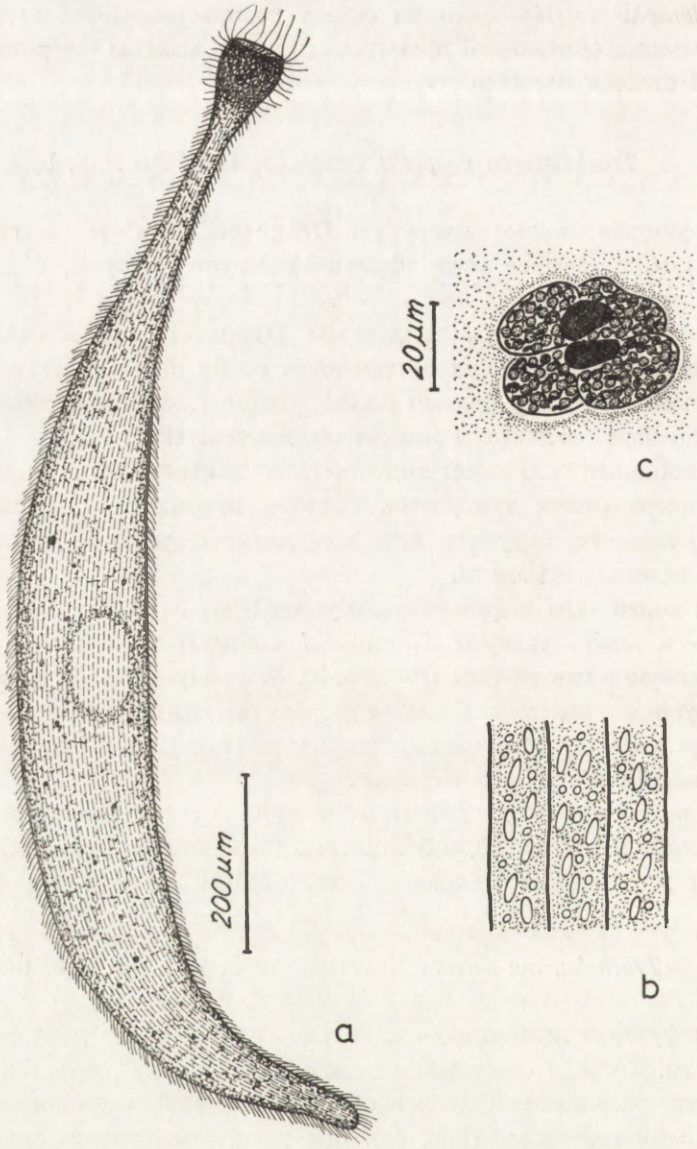

Рис. 3. *Trachelocerca geopetiti* Dragesco, 1954. a — общий вид инфузории (прижизнено), b — протрихоцисты (прижизнено), c — ядерный аппарат (тотальный препарат, реакция Фельгена)

Fig. 3. *Trachelocerca geopetiti* Dragesco, 1954. a — general view of living ciliate, b — protrichocysts (in living specimen), c — nuclear apparatus (total preparation, Feulgen reaction).

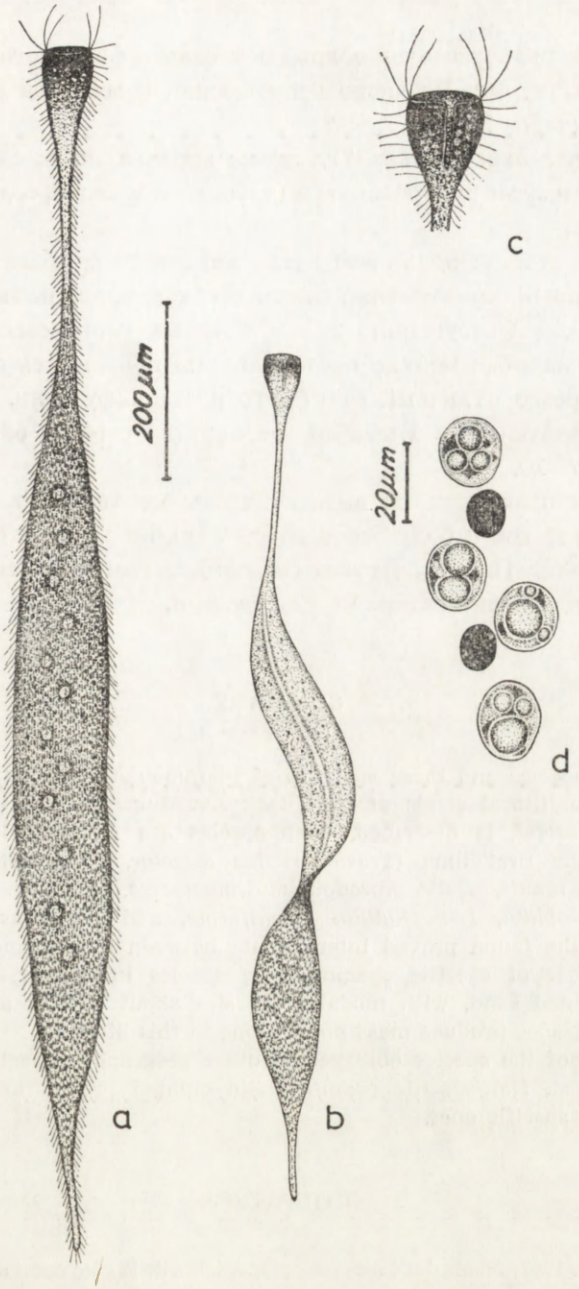

Рис. 4. *Trachelonema sulcata* Kovaleva, 1966. a — общий вид инфузории, прижизнено, b — то же, видны уплощенная форма тела, односторонний ресничный покров, узкая продолная бороздка, c — "головка" инфузории, b — фрагмент ядерного аппарата (тотальный препарат, реакция Фельгена)

Fig. 4. *Trachelonema sulcata* Kovaleva, 1966. a — general view of living ciliate, b — the same, flattened body shape, unilateral ciliary covering and narrow longitudinal furrow are seen, c — "head" of the ciliate, d — a phragment of nuclear apparatus (total preparation, Feulgen reaction)

Продольные ряды ресничек покрывают только физиологически брюшную сторону тела (Рис. 4b). Их число непостоянно, колеблется у разных особей от 16 до 24 (чаще 20).

Прижизненная окраска тела *Tr. sulcata* коричневая, ее определяют находящиеся в цитоплазме протрихоцисты (крупные и мелкие), содержащие коричневый пигмент.

По спинной стороне вдоль всего тела животного проходит узкий желобок (Рис. 4b), который прижизненно выглядит как продольная более светлая полоска, благодаря отсутствию в нем крупных коричневых протрихоцист. Имеющиеся в желобке мелкие протрихоцисты придают ему светлокоричневый оттенок, резко отличный от общего цвета инфузории.

Число макронуклеусов *Tn. sulcata* варьирует от 14 до 68, а микронуклеусов — от 3 до 20.

Массовые популяции этого вида отмечены не только в псаммоне Черного моря (советское и болгарское побережья), но также в псаммоне Японского моря (залив Посьет). Других сведений о географическом распространении этого вида в литературе не обнаружено.

SUMMARY

Forty-nine species and three varieties of psammobiotic ciliates have been found in the upper sublittoral sands of the Black sea Bulgarian coast. A new species, *Tracheloraphis totevi*, is described. Nine species are recorded in the Black sea psammon for the first time (*Tracheloraphis discolor*, *Tr. striatus*, *Kentrophoros flavum*, *Geleia tenuis*, *Helicoprorodon multinucleatus*, *Pseudoprorodon arenicoli*, *Chaenea psammophila*, *Loxophyllum vermiforme*, and *Litonotus elongatus*). The composition of the fauna proved intermediate between micro- and mesoporal. The maximum number of specific psammobiotic species inhabits fine, heterogeneous, moderately polluted sand, with modal grain size about 0.25–0.3 mm. Many micro- and euryporal species produce mass populations in this biotop.

Distribution of the species observed in other geographical regions of the World ocean is analyzed. Non-specific psammobiotic ciliated proved to be more widely distributed than specific ones.

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Thécamoebiens psammobiontes du supralittoral vietnamien de la Mer Chinoise et description de *Cryptodifflugia brevicolla* sp. n. (*Rhizopoda* : *Arcellinida*)

Synopsis. Dans le psammal supralittoral vietnamien de la Mer Chinoise 22 espèces de thécamoebiens interstitiels étaient trouvées, dont 21 espèces sont des psammobiontes stricts. L'espèce *Cryptodifflugia brevicolla* sp. n. est décrite. Pour d'autres espèces rares ou mal connues des nouveaux renseignements sont donnés sur leur morphologie et répartition géographique. On a mis en évidence aussi que les thécamoebiens interstitiels sont presque également répandus dans les différentes zones des plages des mers avec des flux et des reflux bien marqués.

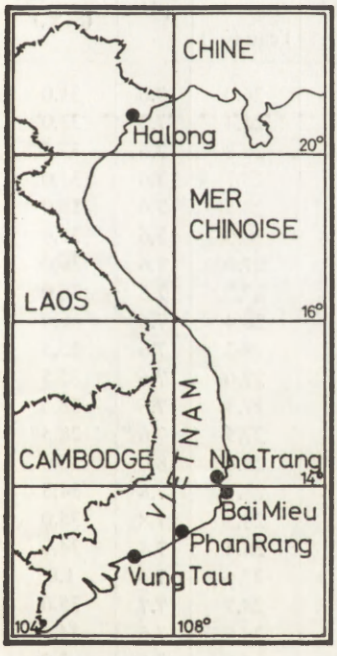

Fig. 1. Carte schématique du littoral vietnamien de la Mer Chinoise avec les localités explorées

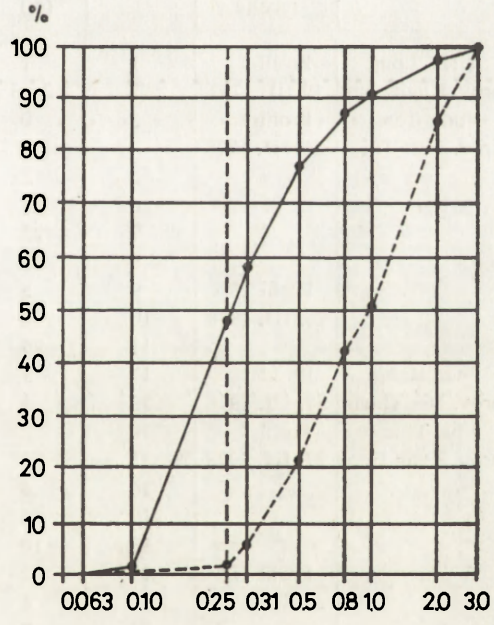

Fig. 2. Caractéristiques granulomètriques du sable de deux plages explorées (Courbes sémilogarithmiques)
——— plage de v. Vung Tau
..... plage de v. Nha Trang

L'étude des thécamoebiens psammobiontes du supralittoral vietnamien de la Mer Chinoise a été effectuée au mois de Mars 1978. Sur la Fig. 1 sont montrées plus exactement les localités de provenance du matériel recolté. Au total, 35 échantillons de psammon, ont été explorées, provenant de 24 stations sur le supralittoral vietnamien. Les localités, et les stations respectives, le temps de recolte et les paramètres écologiques des matériaux étudiés sont présentés au Tableau 1. Les stations explorées étaient placées sur les profils transversales des plage sableuses. Pour les plages les plus riches en thécamoebiens psammobiontes nous avons aussi procédé aux analyses granulomètriques du sable, dont les caractéristiques sont illustrées à la Fig. 2.

Tableau 1

Localités, temps de recolte at paramètres écologiques des stations explorées.

Localités	Profils Temps de recolte	Stations	Distance de la Mer (m)	Profondeur dans le sable (m)	Tempera-ture de l'eau (C°)	pH	Salinité (S ‰)
Ha Long	Profil I	1	1	0.20	26.0	7.6	31.0
prov. Kuang Nin	19. III. 1978	2	2—3	0.10	26.4	7.3	32.0
Vung Tau	Profil I	3	0	0.05	28.8	7.6	37.5
prov. Fok Tui	26. III. 1978	4	2	0.10	28.7	7.6	35.0
		5	5	0.15	28.5	7.6	36.0
		6	15	0.15	28.5	7.6	35.0
		7	25	0.30	27.00	7.6	36.0
		8	35	0.35	27.2	7.5	36.0
	Profil II	9	5	0.15	28.4	7.6	36.0
	26. III. 1978	10	15	0.25	28.2	7.6	35.5
		11	25	0.40	27.0	7.6	35.5
Phan Rang	Profil I	12	3	0.70	27.5	7.6	38.5
prov. Nin Thuan	27. III. 1978	13	5	0.80	27.5	7.6	28.5
Nha Trang	Profil I	14	2	0.30	28.8	8.0	36.0
prov. Kuan Hoa	27. III. 1978	15	3	0.30	28.7	7.8	34.5
		16	4	0.80	28.5	7.8	35.0
		17	6	0.90	28.4	7.4	34.0
		18	10	1.20	27.6	7.4	1.0
	Profil II	19	2	0.25	28.7	7.7	35.0
	28. III. 1978	20	4	0.60	28.5	7.6	34.0
		21	7	1.0	27.5	7.6	3.0
ile Bai Mieu	Profil I						
prov. Kuan Hoa	28. III. 1978	22	2	0.20	28.9	7.9	35.0
		23	3	0.30	28.7	7.6	36.0
		24	4	0.50	28.0	7.6	25.5

L'etude a permis de trouver et d'identifier au total 22 espèces de thécamoebiens, apartenant à 11 genres. La liste complète des espèces trouvées dans les plages explorées est présentèe dans Tableau 2. Parmi les thécamoebiens observés une espèce est décrite pour la première fois dans le présent article. Ci-dessous nous donnons sa description complète.

Tableau 2

Liste des espèces trouvées et leur répartition dans les plages explorées

Espèces trouvées	Ha Long	Vung Tau	Phan Rang	Nha Trang	Bai Mieu	Catégorie écologique
Psammonobiotus communis Gol.	+	+	+	+	–	
Psammonobiotus minutus Gol.	+	–	+	+	+	
Psammonobiotus golemanskyi Chardez	–	+	+	–	–	
Psammonobiotus balticus Gol.	–	+	–	+	–	
Centropyxiella arenaria Valkanov	–	–	–	+	–	
Corythionella minima Gol.	+	–	–	+	–	
Corythionella sudzukii Chardez	–	–	+	+	–	psammobionte
Micramphora pontica Valkanov	–	+	–	+	+	
Micramphora tokioensis Sudzuki	–	+	–	+	+	
Micropsammella retorta Gol.	+	+	–	+	–	
Amphorellopsis taschevi Gol.	+	+	–	+	–	
Amphorellopsis carinata Chardez	–	–	–	+	–	
Amphorellopsis lucida Gol.	–	–	+	+	–	
Cyphoderia littoralis (Valk.) Gol.	–	+	+	+	–	
Cyphoderia ampulla (Ehrenb.)	+	–	–	–	–	
Cryptodifflugia lanceolata Gol.	–	+	–	+	–	eurybionte
Cryptodifflugia brevicolla sp. n.	–	–	–	+	–	psammobionte
Difflugiella psammophila Gol.	+	+	+	+	–	
Pseudodifflugia andreevi Gol.	–	–	–	+	–	
Pseudodifflugia sp.	+	+	+	+	–	?
Lagenidiopsis valkanovi Gol.	–	–	–	+	+	
Lagenidiopsis elegans (Gruber) Gol.	–	–	–	+	+	
Au total	8	11	8	20	5	

Cryptodifflugia brevicolla sp. n. Fig. 3 a, b

Description: Thèque de contour régulier, ovoïde et latéralement comprimée. Le fond de la thèque est arrondi. La section transversale dans la région de la panse est elliptique. La thèque est incolore et transparente.

Le pseudostome est circulaire. Dans la région du pseudostome la thèque forme une petite collerette hyaline et transparente.

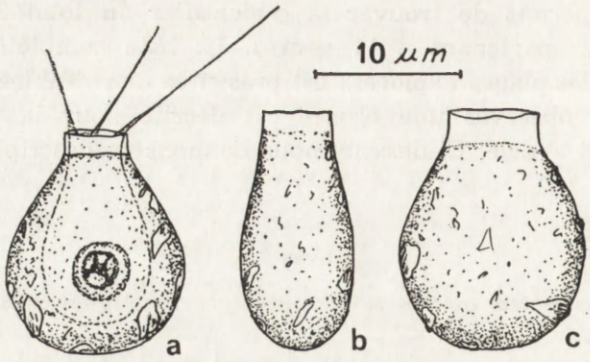

Fig. 3. *Cryptodifflugia brevicolla* sp. n. a — exemplaire vivant, b — vue de profil, c — vue latérale

Les dimensions de la thèque varient dans les limites suivantes: hauteur: 14–18 µm; largeur: 12–15 µm; épaisseur: 5–11 µm, pseudostome: 4–7 µm, collerette: 5–8 × 1.5–3.0 µm.

Le cytoplasme est hyaline, transparent, sans epipodes et avec un seul noyau.

Localité: *Cryptodifflugia brevicolla* sp. n. été trouvée dans la plage sableuse de la ville Nha Trang (28. III. 1978). Distance de la mer: 7 m, profondeur dans le sable; 1 m, température de l'eau 27.5°C, salinité 3.0‰, pH 7.6.

Discussion: Par la présence d'une collerette chitineuse claire autour du pseudostome l'espèce *Cryptodifflugia brevicolla* sp. n. diffère des autres espèces connues du genre. Nous considérons la présence de cette collerette, qui semble d'être flexible dans sa périférie, comme une adaptation morphologique de l'animal à la vie dans le milieu interstitiel du supralittoral marin.

Parmi les autres thécamoebiens observés, un intérêt plus particulier présentent les epèces *Micramphora tokioensis* Sudzuki, *Corythionella sudzukii* Chardez et *Amphorellopsis carinata* Chardez, rétrouvées au cours de l'etude présente pour la deuxième fois lors de leur découverte.

Micramphora tokioensis Sudzuki (Fig. 4 a, b) était décrite pour le première fois dans les eaux interstitielles de la plage de Tokyo (Pacifique). D'après Sudzuki les dimensions de la thèque varient de 31–40 µm de longueur et de 22–30 µm de diamètre. En plus, le diamètre de l'évasement du pseudostome est supérier de celui de la thèque. L'espèce a été trouvée à une salinité de 12.77‰.

Nous avons rétrouvé *M. tokioensis* dans plusieurs stations des plages de Voung Tau, Nha Trang et de l'ile Bai Mieu, dans les conditions d'une salinité beaucoup plus élevée: 36‰ (stations 8, 14, 15, 22). La forme de

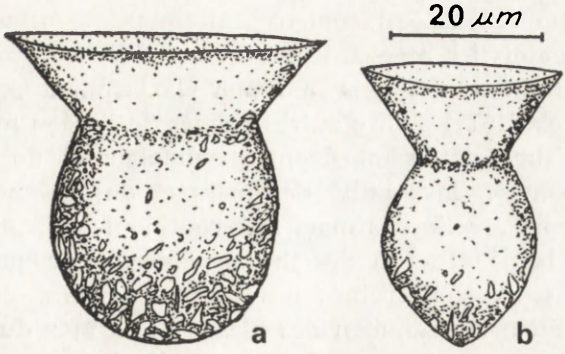

Fig. 4. *Micramphora tokioensis* Sudzuki a — thèque arrondie; b — thèque ogivale

la thèque est allongée et la panse est ovale ou ogivale. Le revêtement de la thèque est plus solide que celui des espèces *M. pontica* et *M. hellebauti*, et on observe souvent des éléments exogènes, incrustés sur la base chitineuse. Les dimensions, observées par nous, sont proches de celles, citées par Sudzuki: hauteur 33–40 μm, diamètre de la thèque 21–31 μm, col. 11–24 μm, diamètre de l'évasement 24–44 μm.

Corythionella sudzukii Chardez est connue seulement de la Mer du Nord. D'apres C h a r d e z (1977) la thèque a une collerette assez réduite et la panse est légèrement comprimée dorso-ventralement. Les dimensions varient de 26 à 30 μm de longueur, 10 à 15 μm, de largeur, et de 8 à 10 μm d'epaisseur. Le pseudostome circulaire est de 4 a 7 μm. Dans nos matériaux *C. sudzukii* a été observée dans deux stations (12 et 16) à une salinité de 38.5‰ et de 35‰. Il est intéressant de noter, que nous avons souvent observé dans une même population des exemplaires avec séction transversale soit circulaire soit élliptique. En plus, les dimensions de la thèque sont, d'après nos observations, supérieures à celles indiquées par Chardez, et notamment: longueur: 30–48 μm, diamètre: 15–24 μm, pseudostome: 8–11 μm. Le cytoplasme des animaux vivants ne remplit pas la thèque complètement et il contient un seul noyau. Les autres caractères taxonomiques correspondent à la description originale.

Amphorellopsis carinata Chardez a été observé une seule fois dans la station 16. Cette espèce, très délicate de point de vue morphologique a, d'après nos observations, les dimensions suivantes: hauteur: 16–19 μm, diamètre: 9–12.8 μm, col. 6 μm, évasement du col. 6–11 μm. L'espèce est connue jusqu'à present seulement de la Mer du Nord.

La liste des espèces trouvées (Tableau 2) permet de constater que la plupart des thécamoebiens interstitiels du supralittoral vietnamien de la Mer Chinoise sont des psammobiontes strictes, à l'exception de l'espèce *Cyphoderia ampulla*, considérée comme eurybionte à distribution cosmo-

polite. Il manque des thécamoebiens dulçaquicoles ou terricoles qu'on trouve toujours dans les mers intercontinentales à la salinité plus faible. Une telle constatation à été déjà faite par Golemansky (1976, 1978) et par Chardez (1977) pour d'autres parties de l'océan mondial. D'après nos recherches sur les thécamoebiens psammobiontes de l'Atlantique et de la Mer Japonaise, la salinité des mers et des océans ouverts, plus élevée par rapport à celle des mers intercontinentales, constitue une barière limitante la pénétration des thécamoebiens dulçaquicoles et terricoles dans les eaux souterraines des plages marines. En étudiant les thécamoebiens du mésopsammon des plages de la Mer du Nord Chardez (1977) arrive aussi à la conclusion que les longues plages à faibles déclivités ont des zones de balancement des marées assez importantes et constituent un biotope mésopsammique ou la salinité est à peu près

Tableau 3

Répartition horizontale des thécamoebiens psammobiontes dans les plages explorées

Espèces trouvées	Distance de la mer			
	0–3 m Stations: 1, 2, 3, 4, 12, 14, 15, 19, 23.	4–6 m Stations: 5, 9, 13, 16, 17, 20, 24	7–10 m Stations: 18, 21	11–35 m. Stations: 6, 7, 8, 10 11
Psammonobiotus communis Gol.	+	+	+	+
Psammonobiotus minutus Gol.	+	+	+	–
Psammonobiotus golemanskyi Chardez	+	–	–	+
Psammonobiotus balticus Gol.	–	+	+	+
Centropyxiella arenaria Valkanov	–	+	+	–
Corythionella minima Gol.	+	–	–	–
Corythionella sudzukii Chardez	+	+	–	–
Micramphora pontica Valkanov	+	+	+	+
Micramphora tokioensis Sudzuki	+	–	–	+
Micropsammella retorta Gol.	+	+	–	+
Amphorellopsis taschevi Gol.	+	+	+	+
Amphorellopsis carinata Chardez	–	+	–	–
Amphorellopsis lucida Gol.	+	–	+	–
Cyphoderia littoralis (Valkanov) Gol.	+	+	+	+
Cyphoderia ampulla (Ehrenberg)	+	–	–	–
Cryptodifflugia lanceolata Gol.	+	–	–	+
Cryptodifflugia brevicolla sp. n.	–	–	+	–
Difflugiella psammophila Gol.	+	+	+	+
Pseudodifflugia andreevi Gol.	–	–	+	–
Pseudodifflugia sp.	+	+	+	+
Lagenidiopsis valkanovi Gol.	+	+	+	–
Lagenidiopsis elegans (Gruber) Gol.	+	–	–	–
Au total	17	13	13	11

égale à celle des eaux marines de surface. L'etude présente encore une fois démontre le rôle limitant de la salinité dans la répartition des thécamoebiens dans le psammal supralittoral.

Dans une étude sur la distribution horizontale des thécamoebiens dans les eaux interstitielles de la Mer Baltique et de la Mer Noire nous avons constaté que les zones de la plage, situées entre 3 et 10–12 m de distance de la mer sont plus riches en thécamoebiens psammobiontes, que les zones situées a la proximité immédiate de la mer (0–3 m), ou à une distance plus grande (plus de 15 m), dont la faune est moins riche et heterogène (Golemansky 1973, 1974). Nous avons essayé, en se basant sur le matériel présent, de verifier la validité de cette conclusion pour le supralittoral de la Mer Chinoise, qui est une mer ouverte typique avec des flux et des reflux bien marqués. Les resultats présentés au Tableau 3 montrent que les thécamoebiens interstitiels sont presque également répartis sur toute la largeur des plages étudiées, de 0 à 35 m. Ces résultats, bien que préliminaires, indiquent que dans les plages des mers avec de balancement de marées assez importantes l'association des thécamoebiens interstitiels est presque également répartie sur toute la largeur de la plage avec une faible tendence de diminution du côté de la mer vers le continent.

SUMMARY

Interstitial testaceans belonging to 22 species were found in the supralittoral psammal of the Vietnamese coast of the Chinese Sea. The majority of them (21) are obligatory psammobionts. A new species *Cryptodifflugia brevicolla* sp. n. is described. New data are given on the morphology and distribution of some little known species. It was established that the interstitial testaceans are distributed almost uniformly in the different beach zones of the seas with typical high and low tides.

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la Mer Japonaise et description de deux nouvelles espèces: *Rhumbleriella coreana* n. sp. et *Amphorellopsis conica* n. sp. (*Rhizopoda, Testacea*). Acta Zool. Bulgarica, 10, (sous presse).

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Psammobiont *Rhizopoda* and *Actinopoda* from Marine Beaches of Japan [1]

Synopsis. Some preliminary investigations on the marine interstitial fauna in four sandy beaches on the Japanese coast have revealed the occurrence of several *Testacea* species previously unknown to Japan. The present paper aims, as the first step, at reporting the appearance of some 40 taxa of *Testacea*, 15 of *Amoebida* and 1 species of *Actinopoda*.

Since 1975, the present writer has had an opportunity to investigate the fauna of beach-line habitats entrusted by the Kankyō-Chō (Environmental Agency), Tōkyō-To (Tokyo Metropolitan Government) and Nōjūi-Gakubu (College of Agricultural and Veterinary Medicine) of the writer's University. The marine beaches and stations investigated are as follows (Fig. 1):

(A) Tokyo region (35.6°N, 139.8°E): Two stations, st. 2 and st. 4, were selected on the sandy island created in the bay of Tokyo in 1975 by reclamation from the sea (Sudzuki 1977 a, c). The former station is located on the beach whose sand was brought from the bottom of the bay of Tokyo itself, the latter being located on the beach whose sand was brought from the Kashima region in Ibaragi Prefecture, facing the Pacific Ocean, some 80 km northeast of Tokyo. The distance between both stations is about 500 m. The beach sands were almost all collected between 11^{00} and 13^{30} o'clock on the following days: 19/VI, 16/VII, 8/IX, 30/X, 6/XI, 24/XII in 1976 and 18/I, 4/II, 7/III in 1977. The salinity of station 2 in November 1976 was 12.772‰.

(B) Shimoda region (34.4°N, 138.0°E): The following four stations have been investigated: 1. Shirahama, 2. Soto-ura, 3. Tsumekizaki (Marine Biological Station of Nihon Daigaku), 4. Nabeta (Marine Biological Research Center of Tsukuba Daigaku, formerly Marine Biological Station

[1] This paper was presented at the Ier Symposium International de Taxonomie et d'Écologie des Thécamoebiens held in Sofia on 29 September 1976.

Fig. 1. Map showing localities of sampling places

of Tokyo Kyoiku Daigaku). For stations 1 and 2, samplings were made on 27/X and 8/XII in 1976, further on 10/III, 19/VI and 30/VIII in 1977. For station 3 on 26/VI, 14/VII, 23/XII in 1976 and 8/I, 9/II in 1977 respectively. For station 4, only once a routine survey was made on 2/II in 1977. Some limnological data are shown below: For stations 1 and 2: The salinity value was between 17 and 18‰, pH was between 8.3–8.5. For station 3 (on 1/XI, 1976, 13^{30} O'clock): The water temp. = 16.2°C, pH = 7.82, Cl = 9.835‰, COD = 0.2 ppm, NH_4 — N = 0.016 ppm, NO_2 — N = 0.001 ppm, NO_3 — N = 4.238 ppm, PO_4 — P = 0.049 ppm.

(C) Tokuyama region (ca. 34.0°N, 131.8°E): The investigations were carried out at two stations, one saprobical, the other non-saprobical, both on a big sand beach of the Hikari Bay near Kasado Island in the Seto Inland Sea (S u d z u k i 1976). The sampling was made on 23/VII, 20/IX, 19/XI, 21/XII in 1975, 19/II, 14/V and 26/VII in 1976. The salinity ranged between 13 and 17‰, water temperature was 10.0–28.5°C, pH was between 8.1–8.3. The diameter of grain size was 1–2 mm on average.

(D) Ogasawara Island (27°1 N, 142°5E): The sand collection was made on 4th July 1976 by Dr. M. Takeda at three stations (Minami-bukuro, Miyano-hara and Kominato) on Chichi-jima (Island), some 1000 km south of Tokyo.

All the sand collections mentioned above were carried out, without exception, during low tide at four tidal levels, namely, low, mid, high and terrestrial hygrophilous zones, the last mentioned being 1 or 2 m landward from the high tidal line.

The sample was taken from the surface in the low tidal zone, from the layer 10–20 cm below the surface in the mid-tidal zone and from the layer 50–70 cm below the surface in the high tidal and terrestrial hygrophilous zones.

The faunological and zoozonological studies were made, based on the sand samples thus collected. For the zoozonological studies the number of individuals/cm^3/24 h were recorded, on the basis of the interstitial waters extracted from a 1 cm cube of a polyurethane foam buried in each sand sample (S u d z u k i 1977 b). But, for faunological studies the routine method by pipette was employed since the polyurethane method was not applicable to *Testacea* inhabiting a marine beach.

From these samples the present writer has thus far succeeded in identifying a total of some 50 species, together with other classes of animalcules, such as *Gastrotricha, Kinorhyncha, Archiannelida,* etc. (S u d z u k i 1976). Of these animal groups, the present paper deals with only *Testacea, Amoebida* and *Actinopoda.*

Before submitting this report, the writer would like to thank all members of participant in the Symposium, especially Doz. Dr. V. Golemansky (Sofia) for his generous help in the preparation of this paper.

Results

Results of investigations are summarized in Table 1. Some of species needs more comments.

Amphorellopsis (Pl. I 1–5): Very common in Shimoda beach. Several specimens have been collected from Tokuyama and even from Tokyo. Some morphologically different forms are recognized in the degree of constriction of the neck region, shape of caudal extremity and in the direction of pseudostome elongation. As to Japanese specimens, the neck is, in general, not so sharply constricted from the test contrary to other species. Consequently, some characteristics are found in the ratios of the diameter of neck region (DN) to that of widest part of the test (DT): — DN/DT × 100 = 38.2 in *A. lucida* (G o l e m a n s k y 1970, Fig. 7), 39.6

Table 1

Psammobiont Rhizopoda and Actinopoda from Japanese Beaches. L: Low tidal zone; M: Mid-tidal zone, H: High tidal zone, S: Terrestrial zone, *: Not a few, +: A few, x: Dead

Species Found / Localities	Tokyo				Shimoda				Tokuyama				Ogasawara
Tidal zones	L	M	H	S	L	M	H	S	L	M	H	S	M
Amphitrema?							x						
Amphorellopsis elegans (Pl. I 3-5)		*					*			+	*		
A. sp. (Pl. I 1-2)		+			+								
Assulina ? (Pl. VI 55)			x						x	x	x		
Campascus sp.											x		
Centropyxiella arenaria										x	+		
C. ? (Pl. VI 56)							x				x		
Centropyxis sp. (Pl. V 50)	x									x			
Corythionella acola?							x			x	*		+
C. minima var. *nipponica* (Pl. II 12-15)		x					+			*	x		*
C. pontica							x	+	+	*	x		x
Cryptodifflugia lanceorata												x	
Cyphoderia ampulla	+						x			x	*	x	x
C. littoralis (Pl. II 16-18)		*					x	*		x			
C. l. var. *simodensis* (Pl. II 19-20)					+								
Difflugia sp.	x								x	x			
Difflugiella ? (Pl. VI 57)	+						x				x	+	
Euglypha sp. (Pl. V 51)			x							x			
Lieberkuehnia ? (Pl. VII 71)	+		+		*								
Lagenidiopsis sp. f. *elegans* (Pl. III 21-23)					+					x			*
L. sp. f. *pyriforma* (Pl. III 24-26)							*						
L. sp. f. *rotunda* (Pl. III 27-29)							+			x			x
Micramphora pontica (Pl. IV 32-33)		+	+							x			x
M. tokioensis (Pl. IV 30-31)	x		+				*	x			x		+
M. ? (Pl. IV 34)													x
Micropsammella retorta var. *japonica* (Pl. I 9-11)							+				+		
M. retorta (Pl. I 6-8)							+						
Phryganella spp.								x			x		
Platoum ?	+												
Psammonobiotus balticus (Pl. IV 41-42)					x					x	x		
P. communis (Pl. IV 35)			+				*	+	+		x	x	x
P. golemanskyi-balticus (Pl. IV 36-39)			+				x	x		+	x	+	
P. minutus ? (Pl. IV 40)		+					+				x		
Pseudocorythion acutum (Pl. V 46)			+				*	+		x			
P. acutum var. *nipponicum* (Pl. V 47-48)											+		
P. wailesi (Pl. V 43-45)								+		x			x
P. sp. (Pl. V 49)										x			
Sphenoderia ? (Pl. VI 53-54)											x		+
Trinema enchelys (Pl. VI 52)		+							+	x	x	x	
T. lineare									x		x	x	
Vampyella sp.	+						+						

Volutella hemispiralis (Pl. VI 59)					x						
gen. ? 1 (Pl. VI 60)					+						
gen. ? 2 (Pl. VI 61 - 62)								x	x		
gen. ? 3 (Pl. VI 63)								x			
gen. ? 4 (Pl. VI 64)								x			
gen. ? 5 (Pl. VI 65, 66)								x			
gen. ? 6 (Pl. VI 67)					x						
gen. ? 7 (Pl. VI 68-9)											x
gen. ? 8 (Pl. VI 70)											x
Amoeba gorgonia										+	
A. granulosa	+				+	+					
A. sp. like *dumetosa*								+			
Astramoeba spp.					+		+		+		
A. stella			+								
Cochliopodium sp. like *tentaculatus*					+						
Dactylospherium ?								+			
Pelomyxa sp.					+	+					
Mayorella sp.	+										
Naegleria sp.		+	+								
Protamoeba grimmi								+			
Thecamoeba huminis						+		+			
Th. verrucosa (Pl. VI 58)	+					*			+		
Trichamoeba sp. like *schaefferi*	+					+					
T. pilosa?					+						
Vahlkampfia limax			+	*		*		+		+	+
Raphidiophrysopsis sessilis (Pl. VII 74)	*		+	+		+		*	+		

in *A. maximus* (Golemansky 1970, Fig. 5), 48.7 in *A. elegans* (Golemansky 1973, Fig. 8), 49.1 in *A. taschevi* (Golemansky 1976, Fig. 1), 46.5 and 48.9 in *Pseudodifflugia andreevi* (Golemansky 1976, Fig. 2), 53.3–60.4 in Japanese specimens.

In the size, general features of the test and the ratio of DN/DT, Japanese specimens shown in Pl. I 3–5 are allied with *A. elegans* Golemansky, 1970, while those shown in Pl. I 1 and 2 are rather very likely to the specimens of Golemansky (1976, p. 38, Figs. 2 a, b named as *Pseudodifflugia andreevi*). But, Japanese population of the species are easily distinguishable from the species mentioned above in the DN/DT ratio (cf. 59.1 in the specimens of Pl. I 3, 60.4 in Pl. I 1 and 53.3 in Pl. I 2). Other two species by Golemansky i.e., *A. maximus* and *A. lucida* have not yet been found from the Japanese coast.

Size: 35–39 μm long, 19–20 μm wide and 11 μm high.

Micropsammella (Pl. I 6–11): One species group apart from a type species *M. retorta* Valkanov, 1970 has been collected in the Japanese beaches. In Japan, a typical species *M. retorta* (Pl. I 6–8) was rather rare and only once has hitherto been found in the June samples from Shimoda with the size 31–38 μm long, 18–19 μm wide and 12 μm high.

Another species group which is shown in Pl. I 9–11 under the name of *M. v. japonica* was, on the contrary, often collected, not only from Shimoda, but also from Tokuyama. In the lateral view, this species is very much alike to *Chardezia* Golemansky, 1970 c, *Alepiella* Golemansky, 1970 a and the specimens by Golemansky (1976, p. 38, Figs. 1 a, b as *Amphorellopsis taschevi*). But *japonica* group differs from *Alepiella* in wanting lateral wing-like big projections, from *Chardezia* in possessing round plates on the test as the case of *Pseudocorythion,* recurvate elongation of pseudostome, further in wanting of long straight caudal projection. A *japonica* group is different from *Amphorellopsis taschevi* in the features of ornamentation of the test and those of caudal extremity. A *japonica* group is different from *M. retorta* in the shape of caudal extremity, ventral shape of the neck, features of pseudostome elongation and value of DN/DT × 100 (cf. 51.7 in *M. retorta,* 56.3 in *M. v. japonica* for the Japanese specimens, 57.1 and 62.2 in *M. retorta* for Golemansky's specimens (1970, Fig. 7 and 1973, Fig. 7)). A specimen by Golemansky (1973, p. 53, Fig. 7 b) has a caudal projection, but not dorsally curved. For this species group the present writer would like to give a name *Micropsammella retorta* var. *japonica* for convenience. Size of the type specimen: 41 μm long, 12 μm wide, 12 μm high. Pseudostome 10 μm.

Corythionella (Pl. II 12–15): Of three species described by Golemansky (1974 b) *pontica-minima* species group was very common in the Japanese beaches. The Japanese specimens were, however, more or less different from the original species in possessing (1) a number of scaly sculptures even on the elongated part of the pseudostome (see Pl. II 12–14), (2) relatively big pseudostome elongation, (3) intermediate shape of the caudal extremity between *C. pontica* and *C. minima.* For the reason, first, the ornamentations on the pseudostome elongation are very clear and visible under the normal powered microscope, second, the ratio of the width of pseudostome elongation to that of the test (WP/WT × 100) in Japanese specimen was about 110 (cf. for *C. minima* 96.6 in Golemansky (1973, Fig. 4), 119.2 in Golemansky (1974, p. 139, Fig. B), for *C. pontica* 94 in Golemansky (1974, p. 139, Fig. A)) and third, that of the LP/LT × 100 or the ratio of the length of pseudostome elongation to that of the test was 63.2–81.3 (cf. for *C. minima* 57.6 in Golemansky (1973, Fig. 4), 70.2 in Golemansky (1974, Fig. B), for *C. pontica* 75 in Golemansky (1974, Fig. A)) the present writer would like to propose the name *P. minima* var. *nipponica* for the Japanese population of the species.

Size: 48–55 μm long, 18–22 μm wide. Pseudostome 12–16 μm. Funnel-

like elongation of pseudostome 25–28 μm long, 23–20 μm wide. Pseudopodium 20 μm in length barely beyond the pseudostome elongation.

Cyphoderia (Pl. II 16–20): Judging from the size of the test, the most common species from Japan seems to belong to *C. littoralis*.

Size: 44–60 μm long, 18–21 μm in diameter. Pseudostome 8–9 μm in diameter. WP/WT × 100 = 58.0 (cf. 38.1 in Golemansky (1973, Fig. 6)), LP/LT × 100 = 25.9 (cf. 21.3 in Golemansky (1973, Fig. 6)).

Another species *C. ampulla* was rather uncommon.

Besides these two species, an aberrant specimen group with a weekly developed pseudostome elongation like a distinct genus *Campascus* Leidy has been found in the sample from Shimoda (Pl. II 19–20). For two reason followed, (1) the pattern of ornamentation of the test could belong to that of *Cyphoderia*, (2) pseudostome elongation was not so remarkable as in the case of *Campascus*, the present writer would like to treat this specimen group as *C. littoralis* var. *simodensis*.

Size of the specimen: 58 μm long, 21 μm wide and 23 μm high. Pseudostome 9 × 11 μm. Pseudopodia usually 5 in number, 62 μm in length. Deposited in the Biological Laboratory, Nihon Daigaku, Omiya.

Lagenidiopsis. (Pl. III 21–29): As to the Japanese species at least three forms are distinguishable. They are for convenience in this paper *L.* f. *rotunda* (Pl. III 27–29), *L.* f. *elegans* (Pl. III 21–23) and *L. pyriforma* (Pl. III 24–26). In *L.* f. *rotunda*, general forms remind us of *L. valkanovi* Golemansky 1974, but the caudal margin of the test is always very smooth and not a single specimen has appeared as the spined forms. Consequently, *L.* f. *rotunda* seems to the present writer at least to be different from *L. valkanovi* (Golemansky 1974 a) at the varieties level rather than polymorphic formae. The form *L. rotunda* has been collected from Shimoda, Tokuyama and Ogasawara.

Size: 31–65 μm long, 31–36 μm wide. Pseudopoda 110 μm long nearly three times the body length (cf. 30–61 μm long, 19–45 μm wide in Golemansky (1974, p. 3, 1976, p. 26)).

In *L.* f. *elegans*, no remarkable differences but the size are found between Japanese specimens and the original ones (Golemansky 1976 a).

Size: 48–86 μm long, 20–43 μm wide (cf. 112 μm long, 50 μm wide in Golemansky (1976, p. 41)). Collected from Shimoda, Tokuyama and Ogasawara.

The specimens shown in Pl. III 24 and 25 are neither discoidal (*rotunda* form) nor elliptical (*elegans* form) in type but egg-shaped. Hence the name *pyriforma*.

Size: 62–71 μm long, 42–46 μm wide. Collected from Shimoda. The

type specimen is deposited in the Biological Laboratory, Nihon Daigaku, Ōmiya. The length of buccal tube is too variable to be used for taxonomic characters. The behaviour of protoplasma in the pseudopodia was peculiar and granulated like *Granuloreticulosa* different from that of usual *Filosa*.

Micramphora (Pl. IV 30–33): Of two species described by Valkanov (1970) and Chardez (1973) *M. pontica* is the most common in the Japanese psammon.

Size: 16–19 μm long, 10–17 μm in diameter. The diameter of pseudostome elongation (DP) to that of the test (DT) × 100 = 110–111; cf. 105–111.7 in Valkanov (1970, Fig. 23), 118.5 in Golemansky (1976, Fig. 1 a), 88.2 in Golemansky (1973, Fig. 4).

Besides the above, bigger specimens shown in Pl. IV 30–31 have been collected from the bay of Tokyo. For convenience *M. tokioënsis* here.

Size: 31–40 μm long, 22–30 μm in diameter. The diameter of pseudostome elongation (DP) is always longer than that of the test (DT). DP/DT × 100 = 125–127.

The species *M.* ? as shown in Pl. IV 34 has been collected from Ogasawara island. This species is ellipsoid in form, 25 μm long 12 μm in diameter and DP/DT × 100 being 70.

M. hellebanti has not yet been found from the Japanese sandy beaches.

Psammonobiotus (Pl. IV 35–42): Of 6 species hitherto known from the marine beaches of the world, *P. communis, P. plena, P. golemanskyi* and *P. balticus* are very common in the Japanese coast. While *P. minuta* is not so common. *P. linearis* has not yet been discovered. For the classification of the species certain statistical approach might be necessary, since several intermediate forms have been found among *P. plena, P. golemanskyi* and *P. balticus* (see Pl. IV 36–42). If we compare the ratio of the length of pseudostome elongation (LP) to that of test (LT) and the height of the test (HT) to the length of test (LT) following numerical values would be obtainable. LP/LT × 100. For *P. linearis*: 47.4 (Golemansky 1970, Fig. 3), 39.9 (Golemansky 1973, Fig. 1), 53.4 (Golemansky 1974, Fig. E). For *P. communis*: 65.4 (Golemansky 1974, Fig. D). For *P. minutus*: 94.5 (Golemansky 1974, Fig. F.). For *P. golemanskyi*: 110.5 (Chardez 1971). For. *P. balticus*: 99.5 (Golemansky 1973, Fig. 2). For *P. plena*: 138.5 (Chardez 1971). HT/LT × 100 for *P. plena*: 38.5 (Chardez 1971). For *P. linearis*: 40.6 (Golemansky 1970, Fig. 4), 42.0 (Golemansky 1973, Fig. 1), 40.4 (Golemansky 1974, Fig. E). For *P. communis*: 50 (Golemansky 1974, Fig. D). For *P. minutus*: 61.1(?). For *P. golemanskyi*: 78.9(?). For *P. balticus*:

114.8 (Golemansky 1973, Fig. 2), 85.9 (Golemansky 1974, Fig. G), 68.6 (Golemansky 1974, Fig. 2 a).

If based on these values, each specimen shown in Pl. IV 35–42 would be classified in the following way:

Plate IV 35 LP/LT × 100 = 79.3 → *P. communis–minutus* group, Pl. IV 36: LP/LT × 100 = 128.6, Pl. IV 37: HT/LT × 100 = 70.6 → *P. golemanskyi–balticus* group, Pl. IV 38: LP/LT × 100 = 123.5, Pl. IV 39: HT/LT × 100 = 94.7 → *golemanskyi–balticus* group, Pl. IV 40: HT/LT × 100 = 68.2 → *minutus* group, Pl. IV 41: HT/LT × 100 = 95 → *balticus* group, Pl. IV 42: HT/LT × 100 = 88.9 → *balticus* group.

Pseudocorythion (Pl. V 43–49): Two cosmopolitan species, i. e., *P. acutum* Wailes (Valkanov 1970) and *P. wailesi* Golemansky 1971 have been commonly found in the Japanese coast. However, in Japanese specimen, both LP/LT and WP/WT values were more or less different. For *P. wailesi*: LP/LT × 100 = 72.1–78.3, cf. 53.6 in Golemansky (1974, Fig. 1 D), WP/WT × 100 = 102.9–115.6 cf. 87.1 in Golemansky (1974, p. 138, Fig. 1 D).

Size: 48–60 μm long, 19–22 μm wide.

For *P. acutum*: Japanese specimens are not so sharply different from the original specimen. LP/LT × 100 = 48.8 cf. 23.9 in Golemansky (1974, Fig. 1 A), 31.5 in Valkanov (1970, Fig. 17). WP/WT × 100 = 102.9 cf. 100 in Valkanov (1970, Fig. 17), 92.9 in Golemansky (1974, Fig. 1 A).

Size: 58 μm long, 20 μm wide.

Besides the above, the margin of pseudostome elongation in Japanese specimens is scalopped as in the case of *Corythionella* and never being smooth as in Golemansky's figures. A new variety here conveniently *P. acutum* var. *nipponicum* (Pl. V 47–48) has been recovered from the samples of Tokuyama. This variety is characterized by possessing an octagonal pseudostome elongation and by wanting caudal projection. The ornamentation of the test is similar to that of other species under this genus. The lateral features are like *P. acutum*. The value of LP/LT × 100 is 50, WP/WT × 100 being 100.

Size: (type specimen): 54 μm long, 20 μm wide and 19 μm high.

Deposited in the Biological Laboratory of Nihon Daigaku in Ōmiya. *P. mannei* Chardez has not yet been found.

RÉSUMÉ

L'étude préliminaire de la faune marine interstitiale sur quatre plages sablonneuses de la côte japonaise a révelé la présence de certaines espèces des

thécamoébiens nouvelles pour la Japon. L'article présent étant le prémier destiné à ce sujet, rend compte de l'occurrence d'environ 40 taxa des *Testacea*, 15 des *Amoebida*, et d'une espèce des *Actinopoda*.

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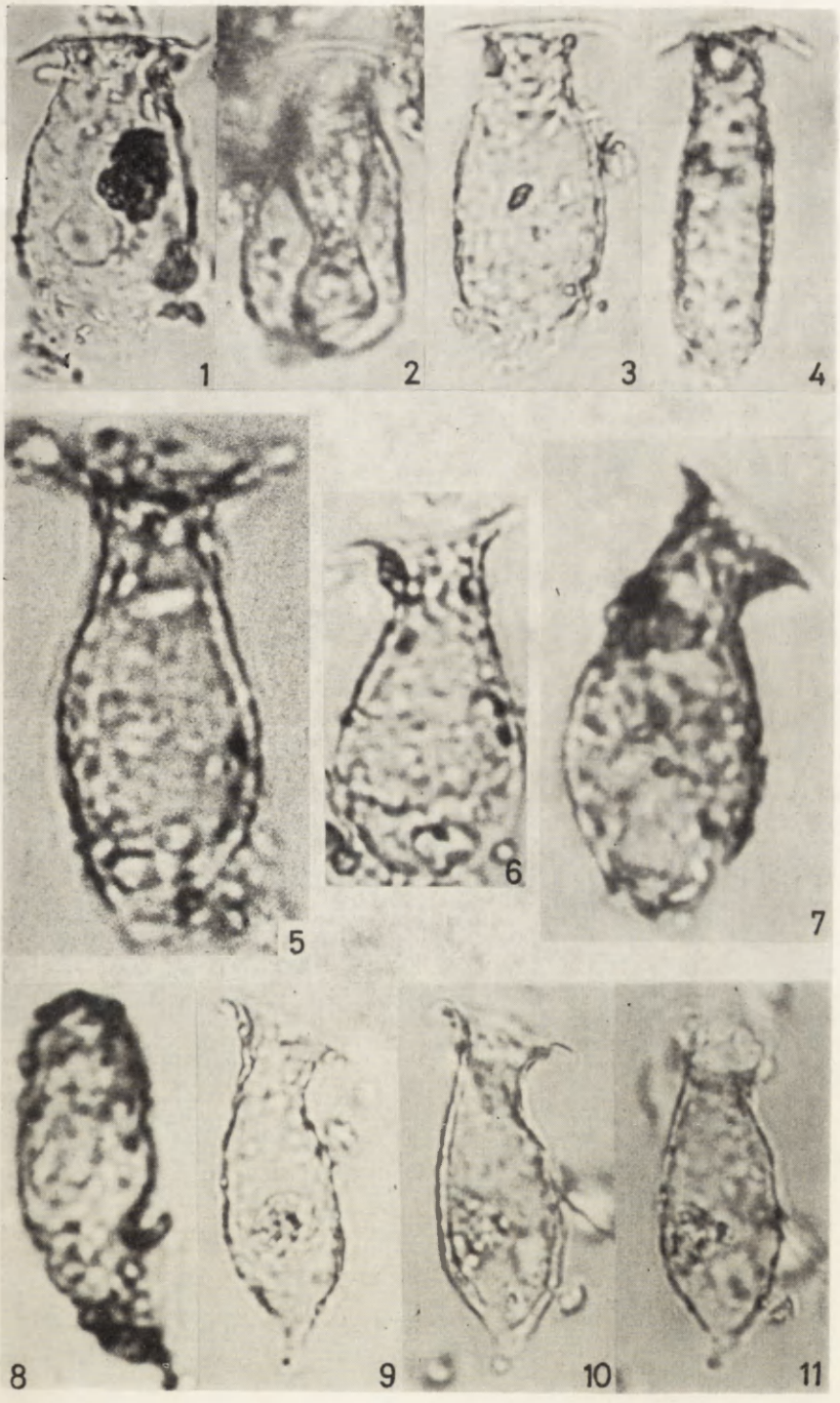
EXPLANATION OF PLATES I–VIII

1: *Amphorellopsis* sp. from Shimoda. 35 μm long, 20 μm wide
2: The same from Tokyo. Living material. 39 μm long, 19 μm wide
3: *A. elegans* from Shimoda. 39 μm long, 20 μm wide
4: The same (lateral view of the specimen of Fig. 3). 12 μm
5: The same from Shimoda. 34 μm long, 13 μm wide
6: *Micropsammella retorta* from Shimoda. 33 μm long, 18 μm wide
7: The same. 38 μm long, 19 μm wide
8: The same (lateral view of the specimen of Fig. 6). 12 μm high
9: *M. retorta* var. *japonica* from Tokuyama. Lateral view. 41 μm long, 12 μm high
10–11: The same (ventro-lateral view of the specimen of Fig. 9). 12 μm wide
12: *Corythionella minima* var. *nipponica* from Tokuyama. 55 μm long 20 μm wide
13: The same from Tokuyama. 54 μm long, 21 μm wide
14: The same from Tokuyama. 55 μm long, 22 μm wide
15: The same (lateral view) from Tokuyama. 48 μm long, 16 μm high
16: *Cyphoderia littoralis* (ventral) from Tokyo. 44 μm long, 18 μm wide
17: The same (ventro-lateral view) from Tokyo. 48 μm long, 20 μm wide
18: The same (lateral view) from Tokyo. 60 μm long, 21 μm high (magnification different)
19: *C. littoralis* var. *simodensis* from Shimoda. Dorsal view. 58 μm long, 21 μm wide
20: The same (lateral view of Fig. 19 but magnification is different) 23 μm high
21: *Lagenidiopsis* f. *elegans* from Tokuyama. 48 μm long, 20 μm wide
22: The same from Ogasawara. 70 μm long, 32 μm wide
23: The same from Shimoda. 86 μm in length, 43 μm in widest
24: *L.* f. *pyriforma* from Shimoda. 71 μm long, 46 μm wide
25: The same from Shimoda. 62 μm long, 42 μm wide
26: The same from Shimoda. 50 μm long, 36 μm wide
27: *L.* f. *rotunda* from Shimoda. 36 μm long, 31 μm wide
28: The same from Shimoda. 65 μm long, 60 μm wide
29: The same from Tokuyama. 38 μm long, 31 μm wide
30: *Micramphora tokioensis* from Tokyo. 40 μm long, 30 μm in diameter
31: The same from Tokyo. 31 μm long, 22 μm in diameter
32: *M. pontica* from Tokyo. 16 μm long, 10 μm in diameter
33: The same from Tokyo. Living material. 19 μm long, 13 μm in diameter
34: *M.* ? from Ogasawara. 25 μm long, 12 μm in diameter
35: *Psammonobiotus communis–minuta* group (ventral) from Tokuyama. 33 μm long, 30 μm wide
36: *P. golemanskyi–balticus* from Shimoda. 30 μm long, 20 μm wide
37: The same (lateral view of the specimen of Fig. 36). 10 μm high
38; The same from Tokyo. 20 μm long, 12 μm wide
39: The same (lateral view of the specimen, Fig. 38). 13 μm high
40: *P. minutus?* 12 μm high
41: *P. balticus* (lateral view) from Tokuyama. 20 μm long, 15 μm high
42: The same from Shimoda. 21 μm long, 18 μm high
43: *Pseudocorythion wailesi* from Tokuyama. 48 μm long, 22 μm wide
44: The same from Shimoda. 60 μm long, 19 μm wide
45: The same (lateral view) from Tokuyama. 50 μm long, 14 μm high
46: *P. acutum* from Tokuyama. 58 μm long, 20 μm wide
47: *P. acutum* var. *nipponicum* from Tokuyama. Ventral view. 54 μm long, 20 μm wide
48: The same (lateral view of the specimen, Fig. 47). 19 μm high
49: *P.* sp. from Tokuyama. 50 μm long, 20 μm high
50: *Centropyxis* sp. from Tokuyama. 80 μm long, 70 μm wide
51: *Euglypha* sp. from Tokuyama. 50 μm long, 30 μm wide
52: *Trinema enchelys* from Tokuyama. 50 μm long, 21 μm wide
53: gen. like *Paulinella* from Tokuyama. 33 μm long, 20 μm wide
54: The same (surface of the test of the specimen, Fig. 53)
55: gen. like *Assulina* from Shimoda. 39 μm long, 21 μm wide
56: gen. like *Centropyxiella* from Tokuyama. 36 μm long, 29 μm wide
57: gen. like *Difflugiella* from Tokuyama. 20 μm long, 10 μm in diameter
58: *Thecamoeba verrucosa?* from Tokuyama. 37 μm long, 22 μm wide

59: *Volutella hemispiralis* from Shimoda. 20 μm long, pseudostome 10 μm
60: gen.? 1 from Shimoda. 39 μm long, 21 μm wide
61: gen.? 2 from Tokuyama. Dorsal view. 39 μm long, 11 μm wide
62: The same (lateral view of the specimen, Fig. 61). 12 μm high
63: gen.? 3 from Tokuyama. 41 μm long, 19 μm wide
64: gen.? 4 from Tokuyama. 53 μm long, 21 μm wide
65: gen.? 5 from Tokuyama. Ventral view. 35 μm long, 21 μm wide
66: The same (lateral view of the specimen, Fig. 65). 20 μm high
67: gen.? 6 from Shimoda. 39 μm long, 21 μm wide
68: gen.? 7 from Ogasawara. Ventral view. 40 μm long, 10 μm wide
69: The same (lateral view of the specimen, Fig. 68). 11 μm high
70: gen.? 8 from Ogasawara. 40 μm long, 32 μm wide
71: gen. like *Lieberkuehnia* from Shimoda. 12 μm in diameter
72: gen. like *Messemvriella* from Shimoda (lateral view). 50 μm long, 20 μm high
73: The same (ventral view of the specimen, Fig. 72). 21 μm wide, pseudostome elongation 28 μm in diameter
74: *Raphidiophrysopsis sessilis* from Shimoda. 13 μm × 13 μm



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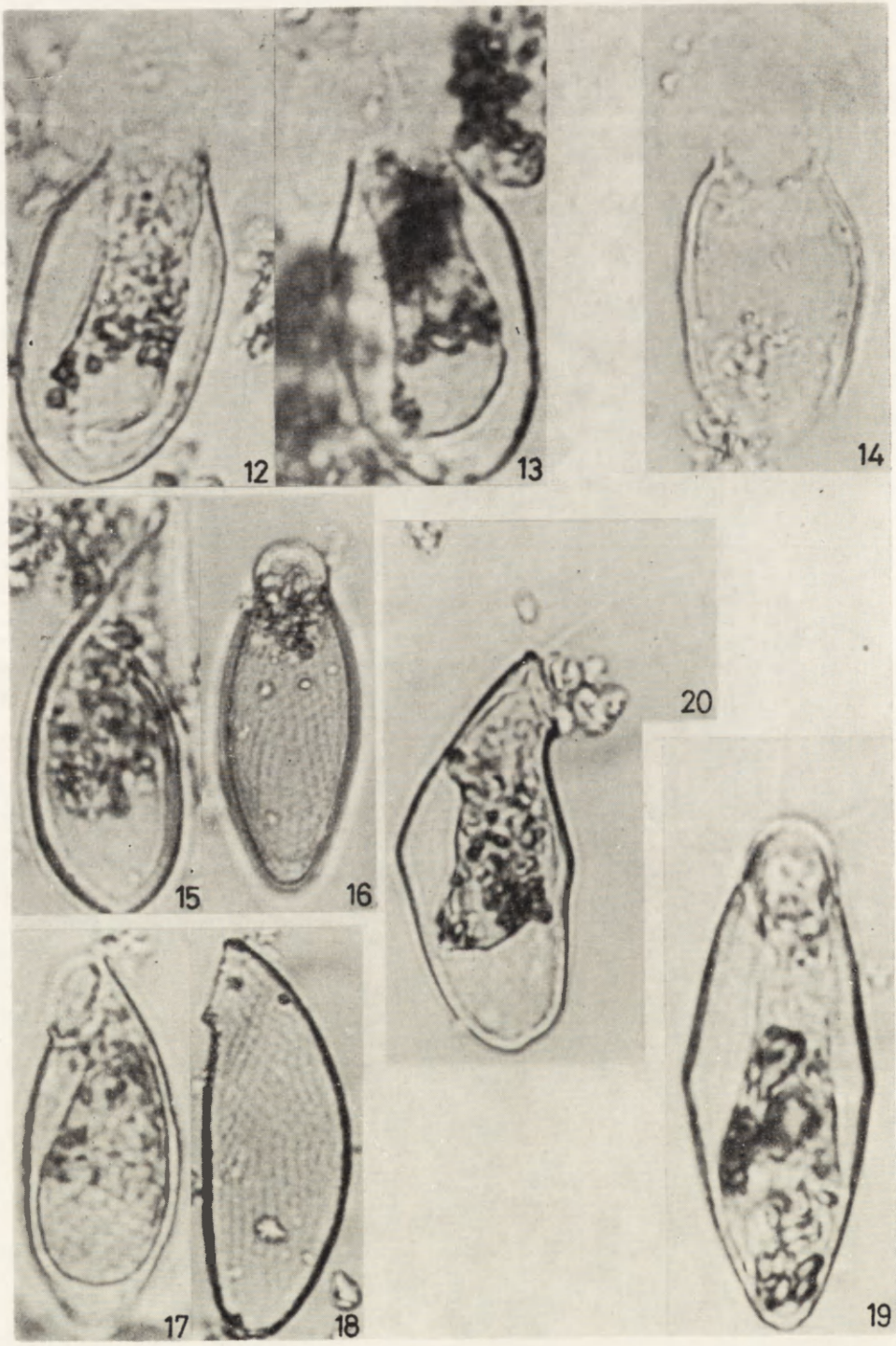

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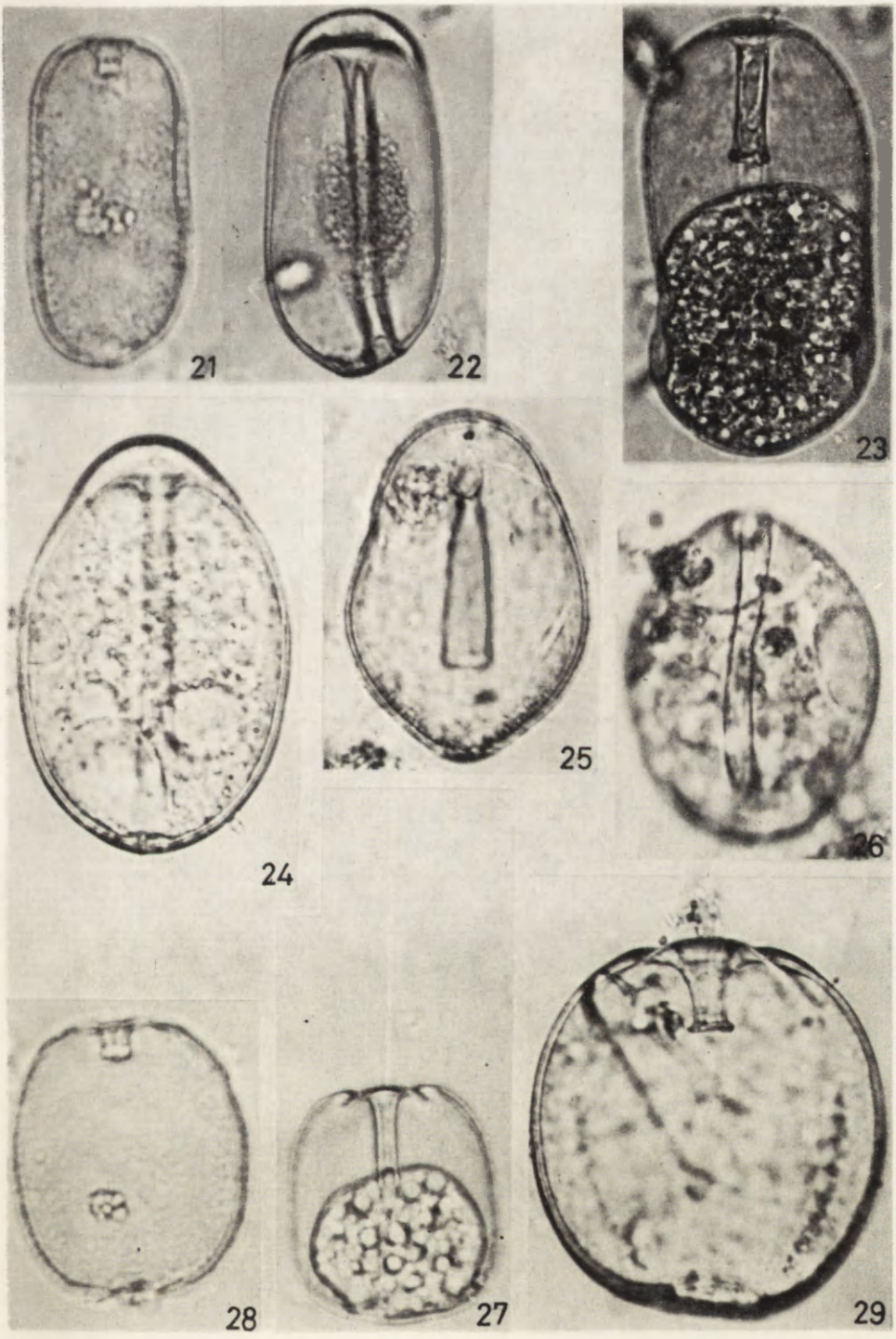

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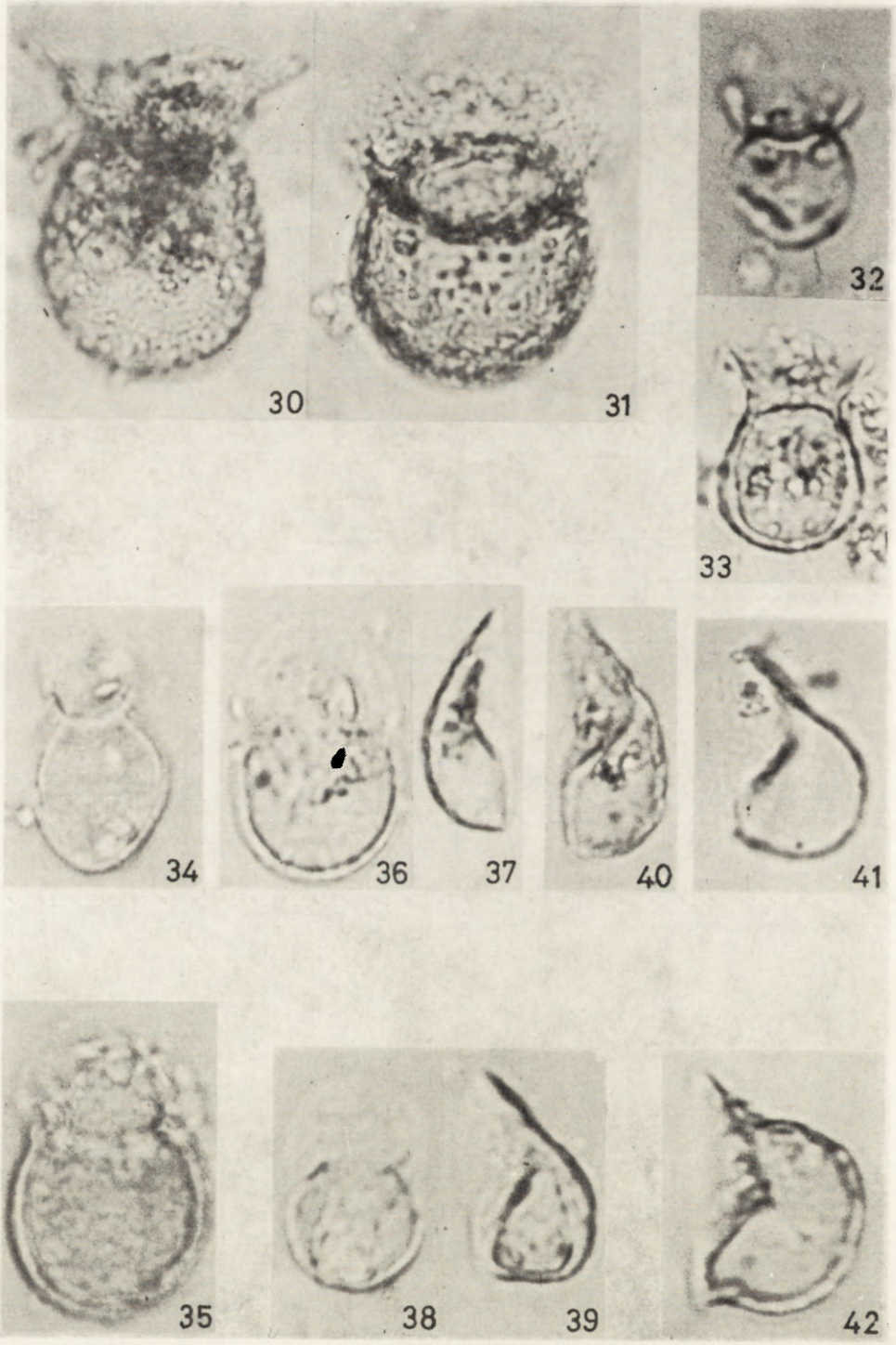

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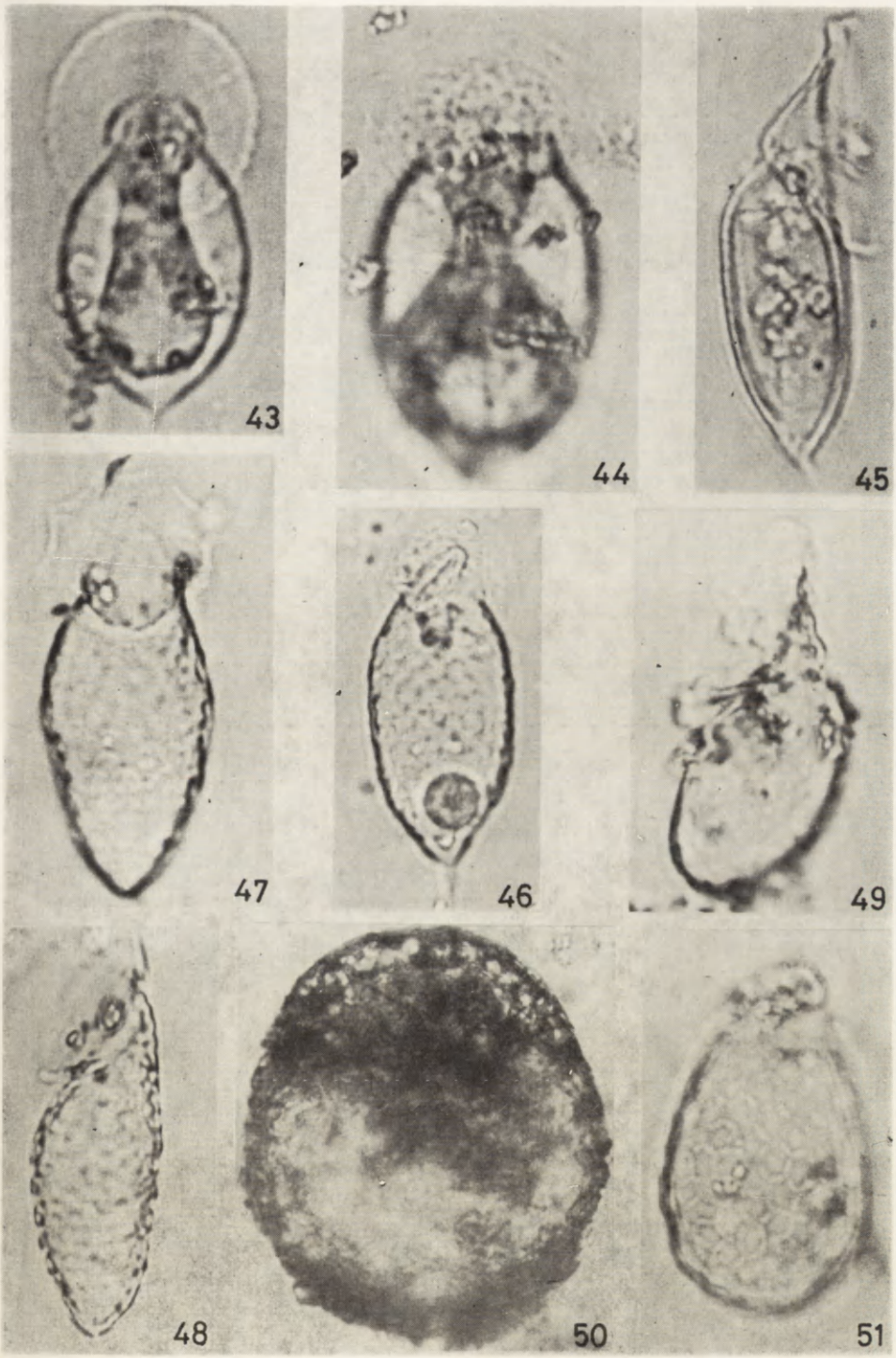

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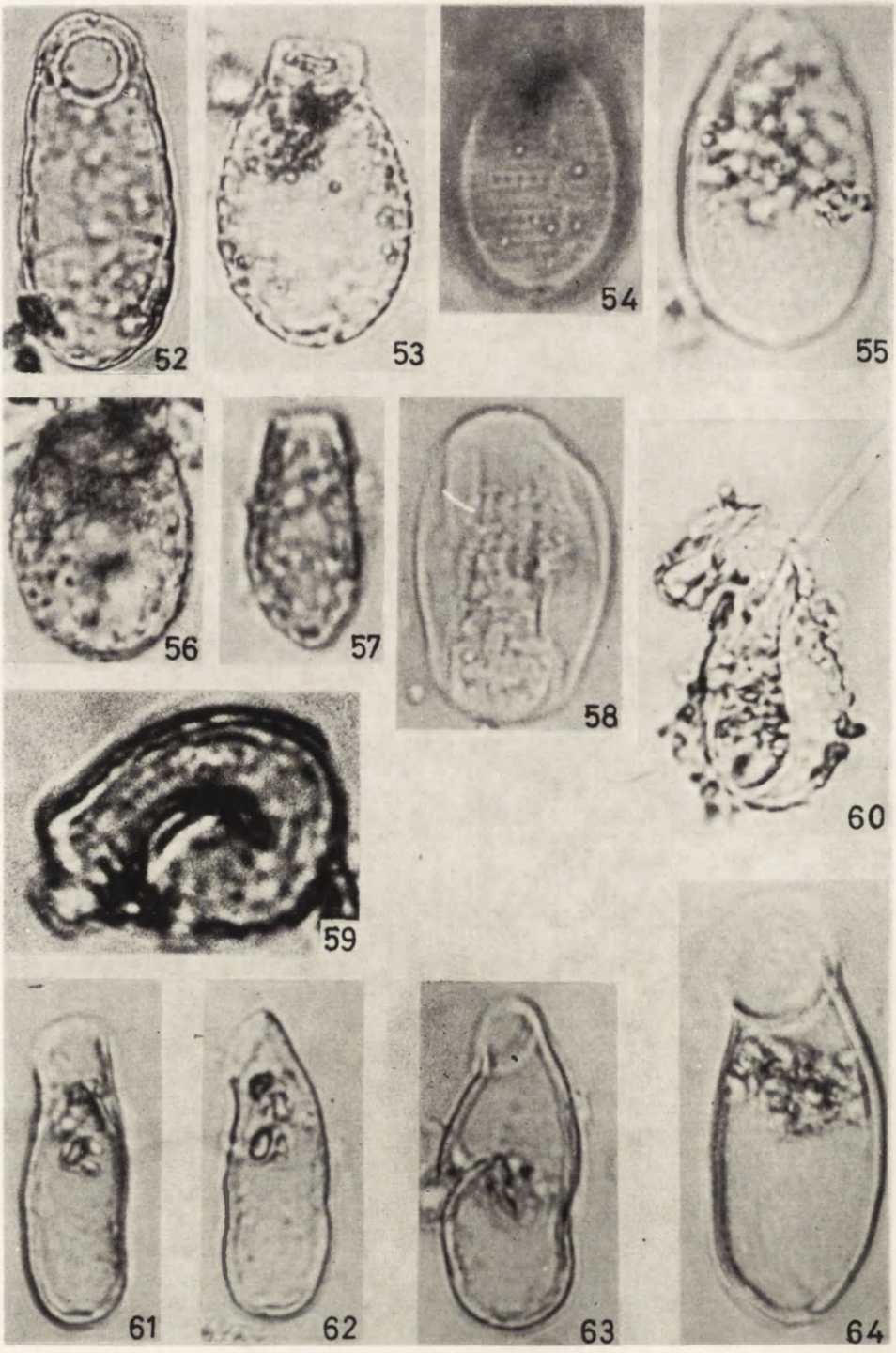

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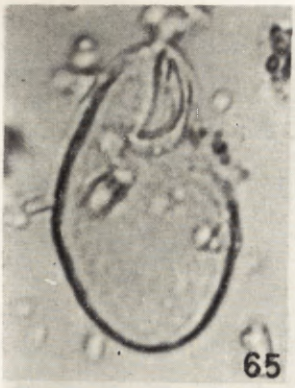

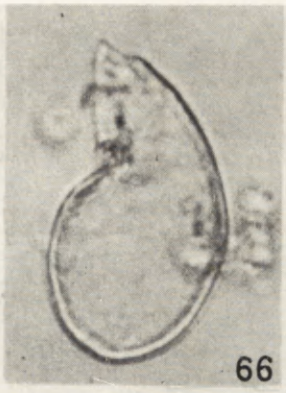

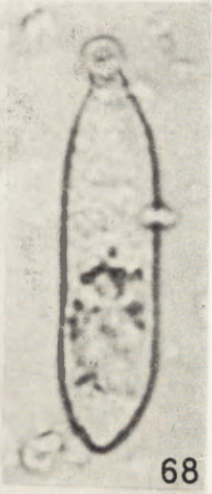

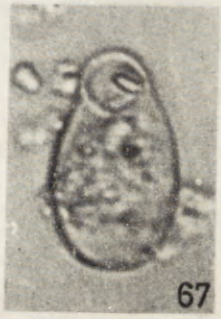

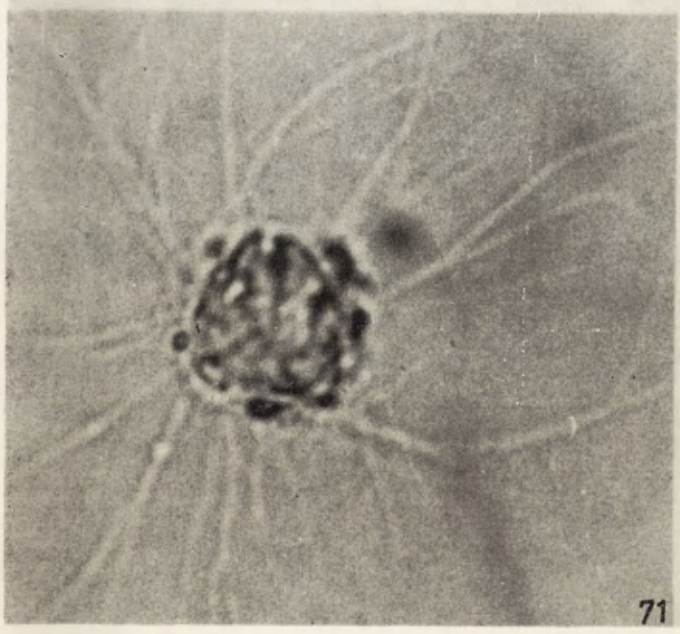

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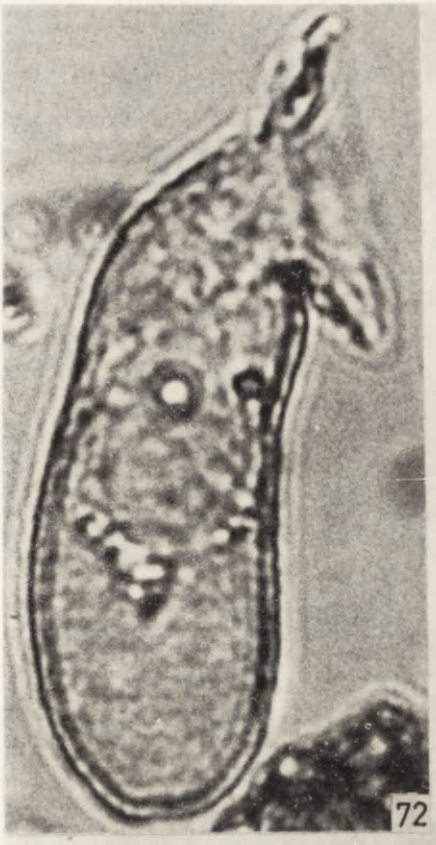

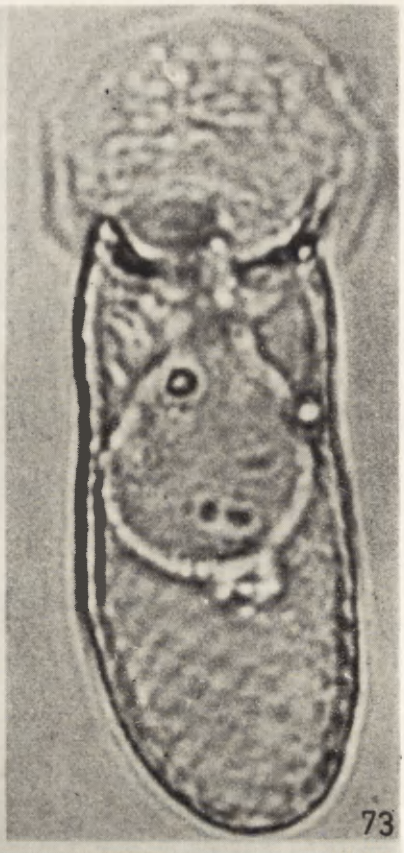

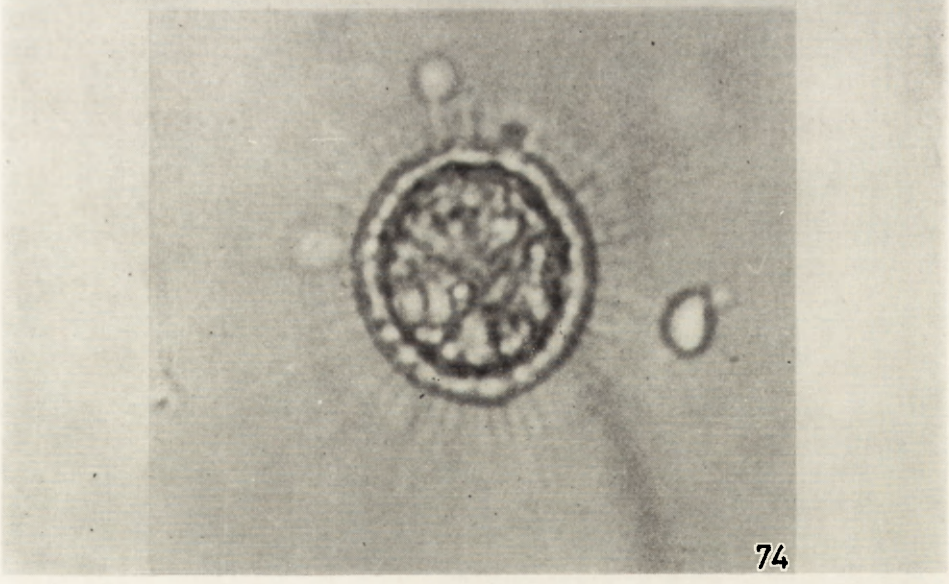

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ACTA PROTOZOOLOGICA

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Stephen F. NG

The Precise Site of Origin of the Contractile Vacuole Pore in *Tetrahymena* and its Morphogenetic Implications

Synopsis. The precise site of origin of the contractile vacuole pore (CVP) during cellular fission is investigated. New CVP arises in very close proximity to a basal body on the CVP meridian anterior to the fission zone. The new CVP is on the immediate left-posterior of the basal body on normal meridians. When the CVP meridian is rotated 180° the new CVP is found on the immediate right-anterior of the basal body. Thus, formation of the CVP is restricted by the heterogeneity of the microenvironment around the basal body; only a sector of this microenvironment allows CVP formation. The basal body serving as a local structural reference is, however, not the only decisive factor for CVP formation. In each cell cycle, on the average two basal bodies are selected for the purpose of CVP formation. Such selection is subjected to the influence of a specification which takes into consideration other structures or parameters of the cell.

The contractile vacuole pores (CVPs) of the Ciliate, *Tetrahymena,* offer a favorable system for the study of organellar development in unicellular organisms. The problem of CVP formation and positioning can be separated into two distinct, but not unrelated aspects: (i) 'General placement' refers to the positioning of the CVPs with respect to the organism as a whole; the CVPs are found posteriorly and about a quadrant to the right [1] of the mid-ventral region. (ii) 'Fine-positioning' refers to the positioning of the CVPs in relation to neighboring organelles; the CVPs are as a rule associated with the left side of particular ciliary rows (the CVP meridians) (Pl. I 1).

The problem of determination of the fine-positioning of the CVPs has been studied using 180°-rotated ('inverted') CVP meridians (Ng and

[1] "Right" and "left" as used with *Tetrahymena* in this report are defined with reference to the right and left of an observer imagined to be inside and lined up antero-posteriorly with the cell and facing the cortical region being examined.

Frankel 1977, Ng 1977). It has been found that inverted CVP meridians bear CVPs on their right side (Pl. I 2). Thus the two sides of the CVP meridian have different morphogenetic properties and such differences dictate the formation of the CVP almost invariably on one but not the other side of the CVP meridian. However, exactly which element of the CVP meridian is involved in such a determinative role is not evident from such studies. This is in part due to the fact that the previous report was based on non-dividing cells and, as evident from the present study, CVPs apparently can shift over short distance subsequent to their origination during fission. The present paper reports observations on dividers and shows that it is the microenvironment on the immediate left-posterior of the basal body on the CVP meridian just anterior to the fission zone which defines the precise site of origination of the CVP.

Materials and Methods

Stock

The cells belong to a mutant (mol^b/mol^b) of strain B of *Tetrahymena thermophila* (Frankel et al. 1976). Most of the cells possess in total 16 ciliary rows two of which (No. 4[2] and No. 5) are rotated 180°. The method of generation of the rotated rows is as previously reported (Ng and Frankel 1977).

Culture method

The animals are maintained in log phase in 1% proteose peptone — 0.1% yeast extract medium at 28°C for at least 4 days before staining.

Staining of CVPs and Identification of Inverted Ciliary Meridians

With an improved version of the protargol technique (Ng and Nelsen 1977) the CVP is seen as a ring which sometimes has a stellate appearance.

The inverted ciliary meridian can most easily be recognized by the position of the longitudinal microtubular band on one side of the basal bodies: in a normal ciliary meridian the band is on the right of the basal bodies whereas in an inverted meridian it is on the left (compare Pl. I 1 and 2). A fuller account based on other microtubular bands around basal bodies is given in Ng and Frankel (1977).

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[2] Numbering of ciliary meridians starts with the ventral meridian associated with oral primordium formation (No. 1) and proceeds clockwise toward the right of the cell.

Results

A preliminary study (N g, unpublished) shows that new CVPs make their first appearance at stage 5 of the fission cycle when the fission zone normally develops (for staging, see Frankel and Williams 1973).

Altogether 105 cells at stage 5 yielding 191 new CVPs are studied. The majority (85%) of them are found in association with meridians No. 5 (inverted) and No. 6 (normal). All (except 3, see below) are found in close proximity to, and off to one side of a basal body in mid-body region (Pl. I 3 and 4, Pl. II 5 and 6). This is in contrast to the location of some of the CVPs in the interfission period in non-dividers: over 10% of such CVPs may be found mid-way in between two ciliary meridians (Pl. II 7) or centrally on the CVP meridian (Pl. II 8) (N g 1977). In the present study, of the 86 new CVPs on normal meridians, 82 are on the left-posterior of the basal body and 4 on the left (Pl. I 3). Such basal bodies are all found anterior to the fission zone. Of the 105 new CVPs on inverted meridians, 86 are on the right-anterior of the basal body and 3 on the right (Pl. I 4). The remaining 16 cases cannot be assessed with certainty regarding their positional relationship with basal bodies because of two reasons: (i) In 3 cases the CVPs are not close to a basal body (Pl. III 9). (ii) The fission zone across an inverted meridian is frequently developed later than the appearance of the associating new CVP. Thus a new CVP on an inverted meridian may appear to be close to two basal bodies, one anterior and the other posterior to it (Pl. III 10). The positional relationship between the new CVP and the basal body in such cases is thus ambiguous; this applies to 13 new CVPs on inverted meridians. The morphogenetic significance of the relationship between CVP and fission zone is separately reported (N g 1979).

One-fourth of the cells indicate association of two new CVPs with one meridian (12 with normal and 17 with inverted meridians) (Pl. II 5 and 6). The pair of CVPs line up longitudinally adjacent to each other, each in close proximity to a basal body. The positional relationship between these CVPs and their associating basal bodies in both normal and inverted meridians is as outlined aboved.

There appears to be no correlation between presence of a cilium on the basal body and its association with a new CVP; about half of the basal bodies associated with new CVPs are ciliated (ciliated: not ciliated: = 45 : 41, 42 : 48 for normal and inverted meridians, respectively).

Discussion

The present study shows that the formation of new CVPs is almost invariably restricted to the immediate left-posterior of basal bodies in normal meridians and to the immediate right-anterior of basal bodies in inverted meridians. That the orientation of the basal body affects the site of origin of the new CVP suggests a positive influence of the basal body on CVP morphogenesis. One may envisage that the microenvironment around the basal body is highly heterogeneous; only a sector of the entire region allows formation of the CVP. Previous work have also indicated that the microenvironment around the basal body is heterogeneous and such heterogeneity determines the site of origin and orientation of newly-formed structures in the ciliary meridian, e.g., basal body, parasomal sac, microtubular bands and kinetodesmal fibre (Beisson and Sonneborn 1965, Ng and Frankel 1977).

The novel aspect of determination of the site of origin of the CVP by the basal body is that the CVP is not part of the ciliary meridian. Not every ciliary meridian of the cell is associated with a CVP but each potentially can be associated with one (Nanney 1967). Furthermore, each somatic basal body (except perhaps the most-anterior and most-posterior ones) along any ciliary meridian potentially can be involved in CVP morphogenesis by specifying the precise site of origin. This is obvious from the fact that those basal bodies not immediately anterior to the fission zone in a particular cell cycle would ultimately be brought to such a position because of relative shifting of their positions during binary fission. Such consideration indicate that almost all of the somatic basal bodies are endowed with the potential for specifying the precise site of origin of the CVP. However, during prefission morphogenesis two basal bodies on the average are singled out for CVP morphogenesis. The process of deciding which two basal bodies are involved (termed 'general placement') (Ng 1977) however resides not with the basal bodies themselves. A formal explanation regarding the general placement of the CVP has been advanced by Nanney (1968, 1972): the cell decides which ciliary meridians are associated with CVP formation by making use of the oral apparatus or the stomatogenic meridian (No. 1) as a reference. Along the CVP meridian the basal body immediately anterior to the fission zone is associated with the new CVP; this and other evidences strongly implicate the fission zone being involved in the process (Frankel et al. 1977). Ng, however, provides evidence which

argues to the contrary (Ng 1979) and the role of the fission zone in CVP morphogenesis presently remains obscure.

Nevertheless, the present study suggests that CVP formation must involve an interaction between the localized information vested with each basal body and some external general placement signal which specifies a particular basal body. Conceptually, the interaction may be thought of firstly as a specification of a region on the cell surface; CVP formation then takes place whenever a basal body falls within this region. An analogous demonstration of a similar type of phenomenon by surgical manipulations is found in *Oxytricha fallax*. In this organism the right marginal cirral row participates in the production of dorsal bristles during prefission morphogenesis whereas the left marginal cirral row normally does not (Grimes 1972). However, when the left marginal cirral row is shifted to the right body margin as a result of surgery and regeneration, then it participates in the formation of dorsal bristles (Grimes 1976). Moreover, even in the case of production of somatic basal bodies, the precise site of origin of which being defined with reference to a pre-existing basal body (e.g., in *Tetrahymena*, Ng and Frankel 1977), the decision as to whether or not a new basal body is to be formed at all is subjected to the influence of some other external structural reference or body parameters (e.g., in *Paramecium*, Sonneborn 1970; in *Tetrahymena*, Nanney 1971; in *Euplotes*, Frankel 1975 a, b). In all of the above cases, the common theme is clear. To form and position an organelle, the cell relies on two sets of information: a local structural reference in the immediate vicinity of the organelle that is to be formed and a specification which involves consideration of other often remote body structures or parameters.

ACKNOWLEDGEMENT

This work is supported by grant 158/359 from the University of Hong Kong.

RÉSUMÉ

La situation d'origine précise du pore vacuole contractile (CVP) pendant la fission des cellules est recherchée. Un nouveau CVP se leve tous proche au cinétosome sur le meridien du CVP à l'antérieur de la zone de fission. Sur un méridien normal le nouveau CVP est situé à gauche et postérieur du cinètosome. Quand on tourne par 180 degrees le méridien, le nouveau CVP se trouve à droit et antèrieur du cinétosome. La formation du CVP est ainsi limitée par l'irrégularité

du micromilieu autour du cinétosome; rien qu'un secteur de ce micromilieu permet la formation du CVP. Mais le cinétosome, qui sert comme renvoi de structure regional, n'est pas le seul agent d'importance en formation de CVP. En chaque cycle cellulaire on a choisi deux cinétosome en moyenne pour la formation du CVP. Ce choix est influencé par un autre agent qui a égard aux autres structures du cellule.

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EXPLANATION OF PLATES I–III

Tetrahymena thermophila: protargol staining; contractile vacuole pores (CVP) of interest are pointed out by arrows; in all cases the anterior end of the cell points towards the top of the page; inverted ciliary meridians are labelled i; 1m = longitudinal microtubular hand. × 2000

1: A non-divider showing the normal location of the two CVPs on the left side of CVP meridians

2: A non-divider with the two CVP meridians rotated 180°. The CVPs are found on the right of the CVP meridians

3: A divider showing a new CVP on the immediate left-posterior of the basal body anterior to the fission zone on a normal meridian (No. 6).

4: A divider showing a new CVP on the immediate right-anterior of the basal body on an inverted meridian (No. 5)

5: A divider having two new CVPs in association with the immediate left-posterior of two basal bodies on a normal meridian

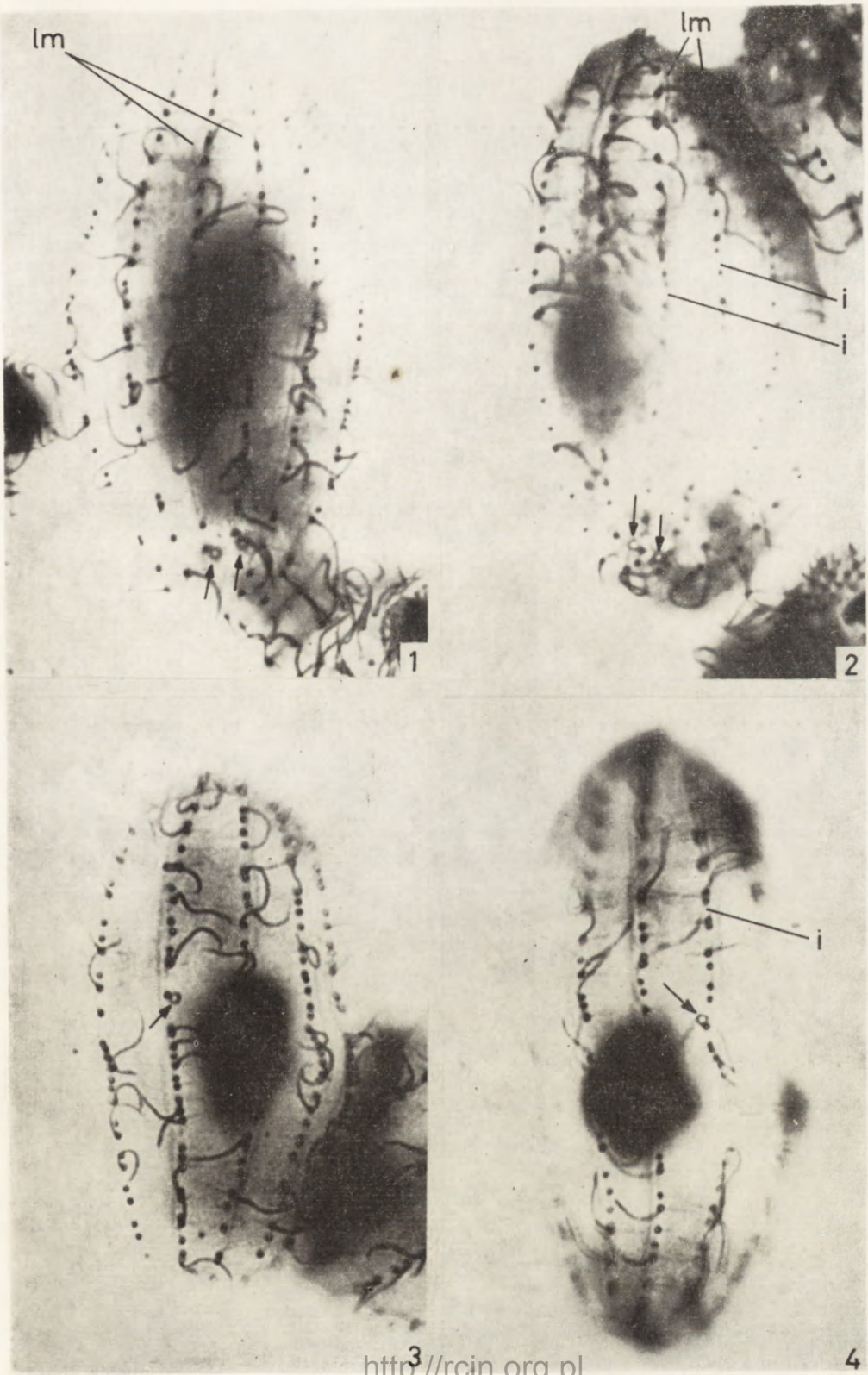
6: A divider having two new CVPs in association with the immediate right-anterior of two basal bodies on an inverted meridian

7: A non-divider showing a CVP midway in between two meridians

8: A non-divider showing a CVP lined up centrally with the basal bodies on the meridian

9: A divider showing a CVP not asociated with a basal body

10: A divider showing absence of a fission zone across an inverted meridian and illustrating the difficulty in assigning the associated new CVP to a particular basal body



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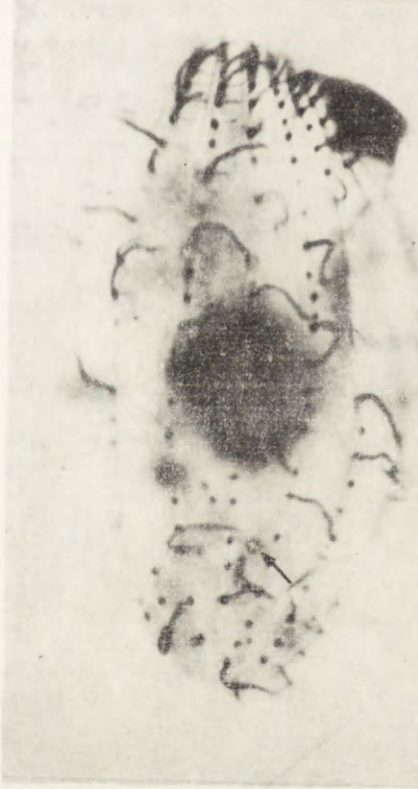

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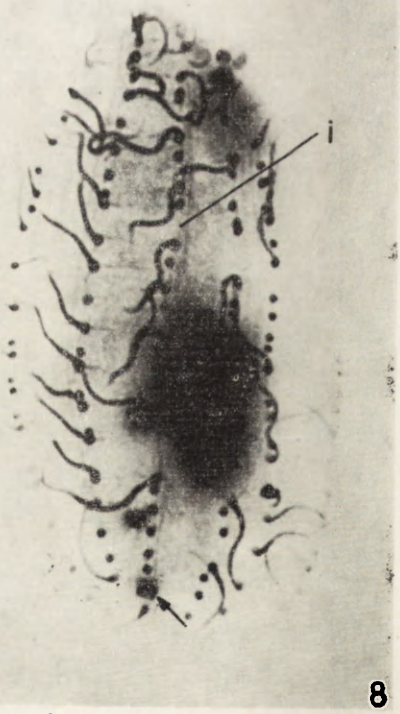

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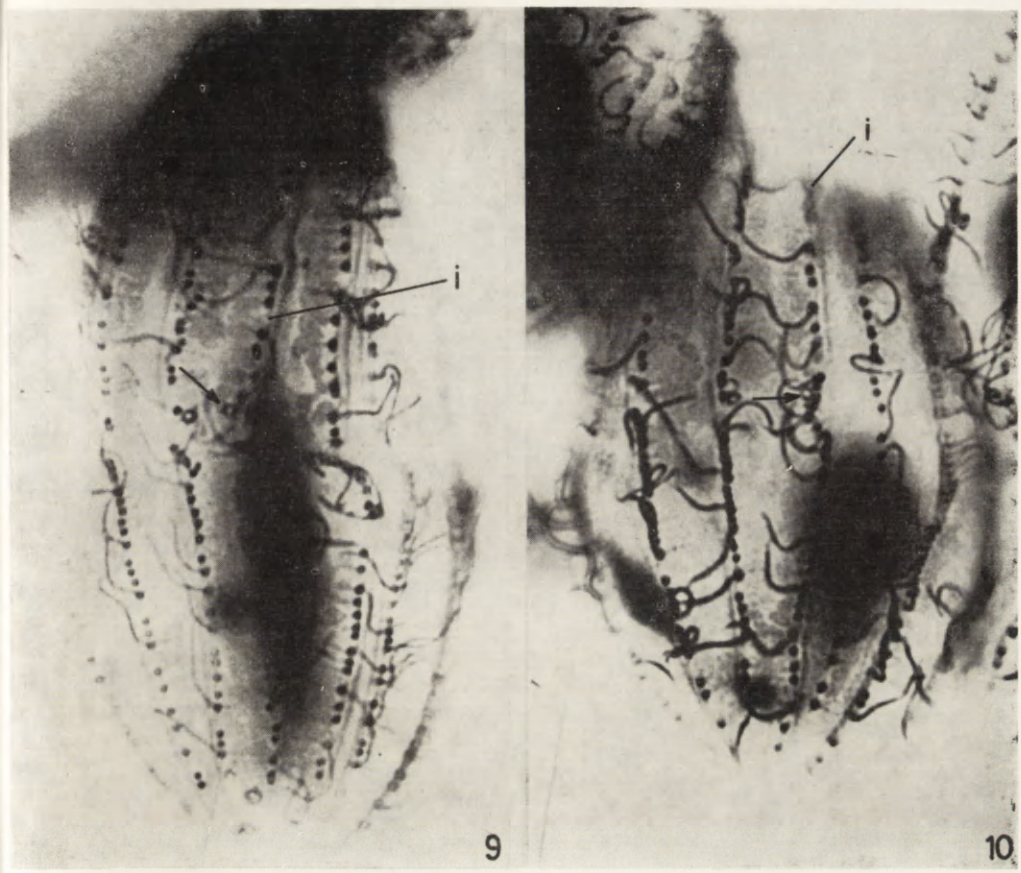

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Random Walk Analysis of Movement and Galvanotaxis of *Amoeba proteus*

Synopsis. The normal locomotion of *Amoeba proteus* can be represented by a two-dimensional random walk model. The mean diffusion coefficient is about 4×10^{-9} m^2 s^{-1}, the step length is approximately equal to the length of fully extended monopodal amoeba (about 400 μm), the traversal time for each step is about 3.0 min, the modulus of the mean intersegmental turning angle is about 30 degrees and the mean translational velocity is about 2.5 μm s^{-1}.

Amoebae exhibit both transient and long lasting responses when subjected to continuous uniform horizontal electric fields. The long term response, studied on populations of amoebae, consists of a net migration towards the cathode superimposed on the normal random walk. Both the mean cathodal migration velocity, a measure of this response, and the mean diffusion coefficient appear to increase linearly with the field strength up to 270 V m^{-1} where saturation occurs. Cathodal migration is caused by preferential pseudopod formation at the cathodal side of the cell.

The response of *Amoeba proteus* to a uniform externally applied electric field, seen as a change in the direction of cytoplasmic streaming such that the net flow is towards the cathode, has been studied by various investigators since it was first observed by Verworn (1896). Explanations for the response mechanism, based on different theories of amoeboid locomotion, have been given by Mast (1931), Hahnert (1932), Heilbrunn and Daugherty (1939), Goldacre (1964), Jahn (1966) and Korohoda and Kurowska (1970). No quantitative investigation of long term galvanotaxis (i.e., over several hours) has so far been attempted in *Amoeba*.

The random walk model is a method of representing the long term movements of cells or animals which move along straight paths for some specific time, and move off in a new direction chosen either completely at random, or within a restricted angular range (i.e., tending to persist in the original direction of movement). This model has been used to represent movements in mollusca (Coulson 1947), birds (Wilkinson 1952), fibroblasts (Gail and Boone 1970), spermatozoa (Brokaw

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1959, Roberts 1972), granulocytes (Peterson and Noble 1972) and in bacteria (Keller and Segel 1971 a, b, Lovely and Dahlquist 1975).

In this paper we show firstly that normal movements in *Amoeba proteus* can be represented by a two dimensional random walk model incorporating persistence of direction, and we have determined the parameters of the model. We then use a technique based on this representation to analyse the long term responses of *A. proteus* to constant electric fields. Finally we attempt to provide an explanation for the mechanism of the response.

Material and Methods

Culture Technique

Amoeba proteus was grown in a standard medium (Prescott and James 1955) containing rice grains. Cultures were kept in the dark at 20° C and the medium was changed every week. Before being used in an experiment, amoebae were washed three times with the experimental medium and then left in it for at least 30 min. The experimental medium was a modified Prescott-James solution, containing 0.15 mM $CaCl_2$ and 0.11 mM KCl. This medium increased adhesion of amoebae to the substrate during the electric field measurements, and also reduced electro-osmotic flow in the experimental vessel.

Apparatus and Experimental Technique

Random Walk Experiments

All experiments were performed in a perspex trough 52 cm long, 10 cm wide and 1 cm deep, with deeper reservoirs at either end to house stainless steel plate electrodes, each 3.5 cm × 5 cm. The amoebae were placed at a preassigned position (a circle of 3 mm diameter termed the origin) near the centre of the trough where the depth of medium was 0.5 cm. The movement of the amoebae was monitored against a background scale beneath. The experimental trough, together with a control, were placed in a thermostatically controlled metal water-jacket, the temperature of which was maintained at 20° C. The temperature of the medium in the troughs did not deviate from water-jacket temperature by more than 0.5° C, as determined in pilot experiments with a thermocouple. Observations and recordings were carried out using a low power Zeiss microscope placed above a window at the top of the box. Between readings this window was covered with an opaque shutter. Two techniques were employed to measure cell movement. In whole population experiments, a group of 20–30 amoebae were placed at the origin and left to move. Approximate coordinates of the centre of each amoeba were recorded every 30 min for a minimum of 2 h without any attempt to identify the amoebae from one time interval to the next. During observations (each taking about 3 min) amoebae were

illuminated from above with a lamp fitted with a heat filter. In the time-lapse experiments a Vinten Mk3 16 mm cine camera coupled to the Zeiss microscope with ×4 lens was used to photograph the amoebae from above at 1/2 min intervals. The amoebae were illuminated only during the exposure (~ 1 s). In these experiments movement of each amoeba in a group of 10 was followed by time lapse photography for a minimum of 30 min.

Electric Field Experiments

Continuous electric fields were applied between the electrodes for at least two hours.

Diffusion of any electrolytic products from the electrodes to the amoebae was minimized by the large electrode separation. Electric current flow through the trough was monitored during the experiments, the fall in current due to electrode polarisation being less than 2% in all cases. Each experiment was accompanied by a control experiment with a group of amoebae from the same culture, to provide a measure of the motility of that population. The movements of amoebae were recorded using both techniques described above. Further experiments were performed in which the experimental medium was prepared by making a solution of 0.3% methyl cellulose in the modified Prescott-James medium. During these experiments electro-osmotic flow was much reduced (1 nm s^{-1} per V m^{-1}) due to the high viscosity of the medium. It was observed that the adhesion of the amoebae to the substrate was enhanced in this medium at high field strengths, where they otherwise tend to detach and float away.

Results

Representation of Normal Amoeboid Movement

The displacement from the origin (R_i) of an amoeba at coordinates (x_i, y_i) is given by

$$R_i^2 = x_i^2 + y_i^2 \tag{1}$$

and the mean square displacement, $\bar{R}^2$, for a complete population is

$$\bar{R}^2 = \sum_{i=1}^{n} R_i^2/n \tag{2}$$

where n is the number of amoebae in the population. The dependence of $\bar{R}^2$ on time, t, for unstimulated amoebae in whole population experiments is shown in Fig. 1, and is represented by a straight line of the from

$$\bar{R}^2 = \alpha + \beta t \tag{3}$$

where the parameters (together with standard errors) are $\alpha = (-5.84 \pm 9.03)$ mm^2

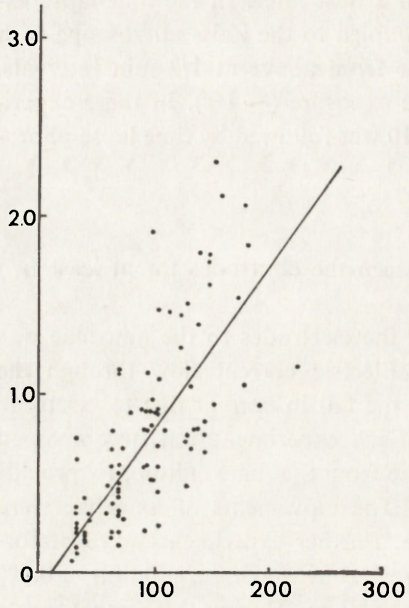

Fig. 1. Abscissa: time (min); ordinate: mean square distance (cm^2). Mean square displacement varies linearly with time. Each point represents at least 20 amoebae

and $\beta = (0.91 \pm 0.09)$ mm^2 min^{-1}, estimated using the method of linear regression (Draper and Smith 1966).

In a two dimensional random walk the mean square displacement is proportional to time and is given (Chandrasekhar 1943) by

$$\bar{R}^2 = 4Dt \tag{4}$$

where D is the diffusion coefficient. The straight line given by equation (3) does not pass through the origin as required by equation (4), but when the error in α is taken into account the origin is included in the confidence interval. Therefore the equivalent diffusion coefficient for a population of amoebae may be calculated from the slope (β) of Fig. 1; thus

$$D = \beta/4 = (3.8 \pm 0.4) \times 10^{-9} \, m^2 s^{-1}.$$

The parameters of the random walk are obtained from the analysis of the time-lapse recording experiments (Fig. 2 a, b). These are λ, the mean distance moved by the cell in a straight line without changing direction, τ, the mean time taken to traverse λ, θ, the modulus of the mean intersegmental turning angle; and u, the mean non-directional rate of locomotion which is given by λ/τ.

The values of these parameters for 10 amoebae were found to be

$\lambda = (440 \pm 20)$ μm
$\tau = (2.9 \pm 0.15)$ min
$\theta = (30 \pm 5)$ deg.
$u = (2.6 \pm 0.2)$ μm s^{-1}

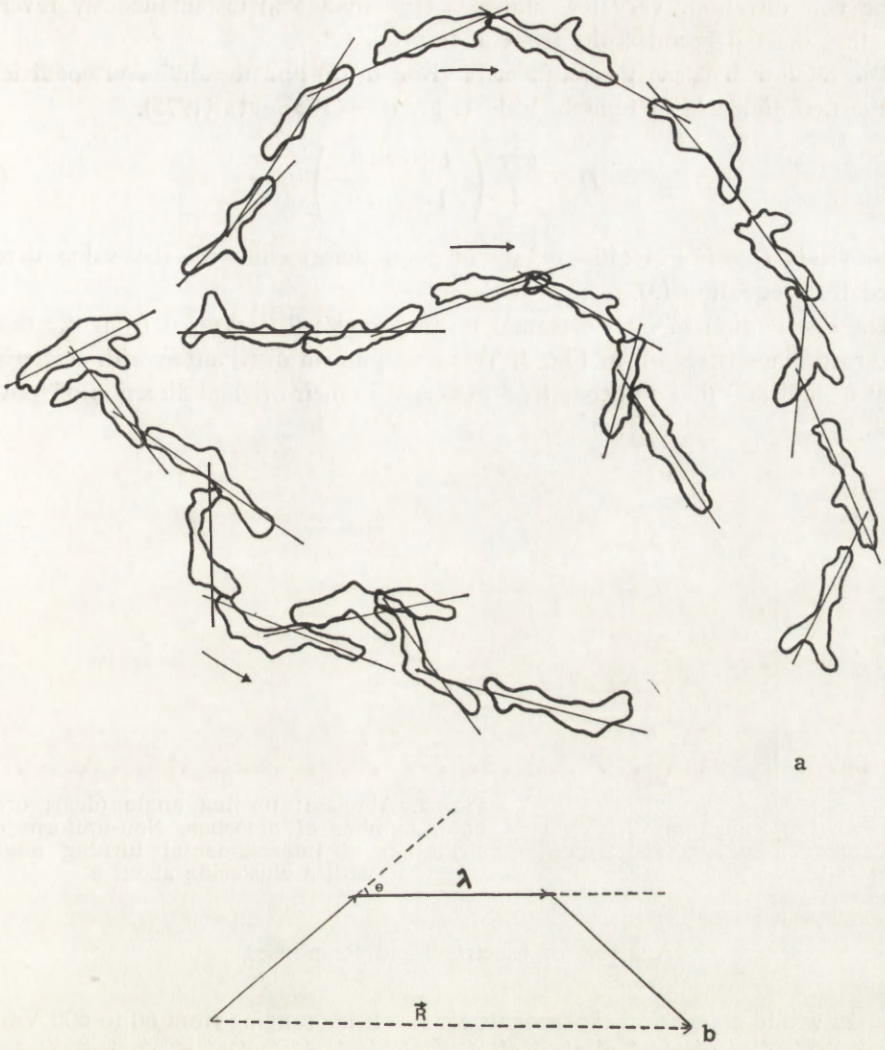

Fig. 2 a. Diagram demonstrating typical movement of *A. proteus* at 2–3 min intervals along its path; reconstructed from cinematographic single frame analysis. Note that changes in direction of movement take place over distances about 1–1.5 times the length of the cell, and anterior and posterior regions of the cell persist along the path

2 b. Parameters of the random walk model. λ (mean free path) = 0.44 ± 0.02 mm, Θ (modulus of mean turning angle) = $30.43° \pm 4.74°$, τ (periodic time to traverse λ) = 2.90 ± 0.15 min, u (non-directional rate of locomotion) = 2.57 ± 0.16 $\mu m \cdot s^{-1}$ $\vec{R}$ (position vector)

As illustrated in Fig. 2 (a), during locomotion λ remains almost constant and is approximated by the length of a fully extended monopodal amoeba. Changes in the intersegmental turning angle θ are performed by orienting pseudopodia

in the new direction. Very few amoebae (less than 5%) instantaneously reverse such that the tail becomes the front of the cell.

The relation between the parameters given in (5) and the diffusion coefficient for the two dimensional random walk is given by Roberts (1975).

$$D = \frac{u^2\tau}{4}\left(\frac{1+\cos\theta}{1-\cos\theta}\right) \tag{6}$$

which yields $D = (4\pm2)\times10^{-9}$ m^2 s^{-1} in good agreement with the value determined from equation (3).

The distribution of intersegmental turning angles θ, measured from the time-lapse recordings is shown in Fig. 3. This non-uniform distribution with clustering about 0° indicates that amoebae tend to persist in their original direction of movement.

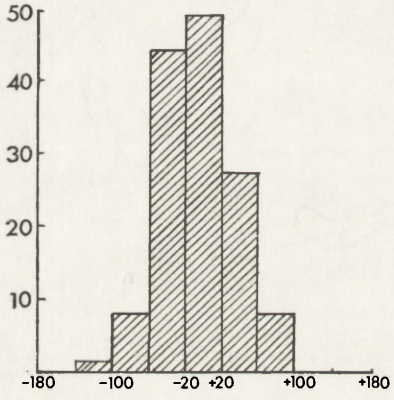

Fig. 3. Abscissa: turning angle (deg); ordinate: number of amoebae. Non-uniform distribution of intersegmental turning angles with a clustering about 0°

Analysis of Electric Field Responses

In 25 whole population experiments electric fields ranging from 60 to 600 Vm^{-1} were applied to groups of at least 20 amoebae, corresponding to observations on a total of about 600 amoebae. Results of these experiments were analysed in terms of the following parameters

(i) Diffusion coefficient in the field (D_E) calculated according to

$$D_E = \bar{R}_E^2/4t \tag{7}$$

where t is time and $\bar{R}_E^2$ is the mean square distance travelled from the starting circle.

(ii) Mean distance travelled by the population towards the cathode ($\bar{y}$) given by

$$\bar{y} = \frac{\sum_{i=1}^{n} y_i}{n} \tag{8}$$

where n is the number of amoebae in the sample.

(iii) Mean direction of movement (α) of the population at a given time, given by

$$\tan\alpha = \frac{\sum_{i=1}^{n} \sin\alpha_i}{\sum_{i=1}^{n} \cos\alpha_i} \tag{9}$$

where α_i is the instantaneous direction of travel of the i'th amoeba with respect to the cathode. For uniform motion towards the cathode $\alpha = 0$. Uniformity of α_i was tested using the Rayleigh test and α_i was compared with the cathode direction using the V-test at the 95% significance level (Schmidt- Koenig 1975).

(iv) Mean cathodal migration velocity (v), defined by

$$v = \frac{\bar{y}}{t} \tag{10}$$

(v) Percentage of the population that migrates towards the cathode in the field (n_R)

$$n_R = \frac{n_c}{n} \times 100\% \tag{11}$$

where n_c is the number of amoebae with a component of velocity towards the cathode at a given time and n is the total number of amoebae in the sample. n_R has a binomial distribution and for a sample of 25 amoebae the error in n_R is approximately $\pm 10\%$.

Table 1

Values of some parameters of locomotion for control and electrically stimulated populations; positive v towards the cathode, $\pm$ is the range of at least two experiments

E ($V \cdot m^{-1}$)	Controls		Field		
	$D(\times 10^9)$ ($m^2 \cdot s^{-1}$)	v ($\mu m \cdot s^{-1}$)	$D(\times 10^9)$ ($m^2 \cdot s^{-1}$)	v ($\mu m \cdot s^{-1}$)	n_R (%)
577	3.65±0.89	0.02±0.19	3.56±1.36	1.09±0.46	82
519	2.64±0.03	0.19±0.16	3.56±0.38	0.43±0.46	62
288	2.74±1.22	−0.95±0.01	6.63±3.24	1.07±0.49	80
166	1.96±0.25	0.24±0.25	3.92±1.10	0.81±0.16	67
144	5.19±0.81	0.12±0.13	6.80±1.82	1.56±0.57	80
135	2.25±1.04	−0.04±0.05	3.79±0.96	0.55±0.45	59
115	3.22±0.62	0.10±0.14	3.65±1.32	−0.11±0.20	45
93	2.57±0.40	−0.06±0.11	3.40±0.55	0.28±0.24	53
73	3.25±0.62	0.01±0.19	3.34±0.77	0.42±0.13	56
58	3.66±0.20	−0.03±0.35	5.09±2.87	0.60±0.28	62

The values of the above parameters at various field strengths and the corresponding control values are given in Table 1. It is seen that, although the response is very variable, amoebae respond to the electric fields with a net migration of the population towards the cathode. Choosing the mean cathodal migration velocity, v, as a measure of response to electric fields, we plot the variation of this parameter with field strength in Fig. 4 a. Error bars in this figure represent the range of values

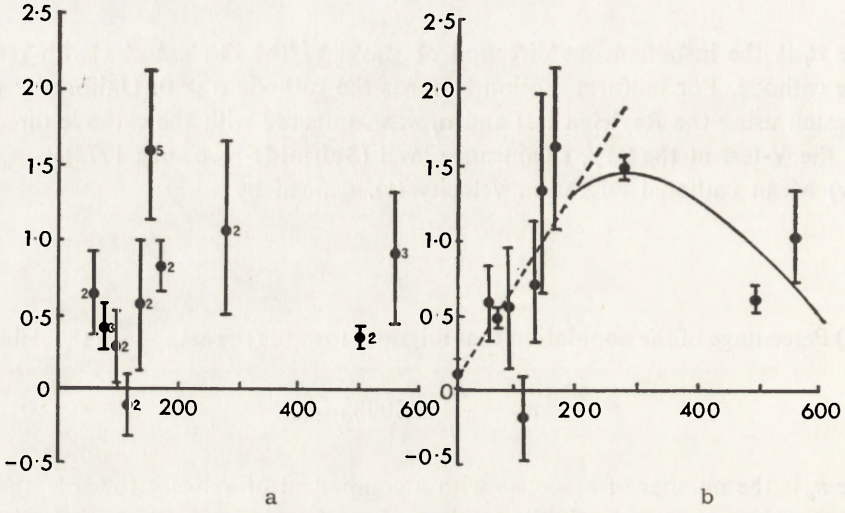

Fig. 4. Abscissa: electric field strength (V m^{-1}); ordinate: mean cathodal migration velocity ($\mu m\ s^{-1}$). Variation of the mean cathodal migration velocity with field strength. Error bars (symmetrical) represent the range of number of experiments indicated. Each point represents at least 40 amoebae. 4 (a) raw data, 4 (b) normalized

of v in different experiments. These large variations are partly due to the variability of the electric field response and partly due to different degrees of motile activity shown by different populations. Every electric field experiment is accompanied by a control experiment and the amoebae used in both experiments come from the same culture sample. The effects of this variation can be reduced if the diffusion coefficient of the control experiment is used to normalise the mean cathodal migration velocity of the accompanying electric field experiment as follows:

$$v^* = \frac{Dv}{D_c} \tag{12}$$

where D_c is the diffusion coefficient of the control experiment and D is the average diffusion coefficient calculated from the slope of equation (3). The variation of v^* with field strength is plotted in Fig. 4 b. v and v^* appear to increase linearly with field strength up to 280 V m^{-1} and then to fall off gradually. The cathodal migration velocity is at its maximum between 140 and 270 V m^{-1} and decreases as the field is further increased. Above about 600 V m^{-1} in the normal experimental medium

amoebae are detached and migrate passively towards the cathode at a rate of about 0.02 μm s^{-1} per V m^{-1} due to electrophoretic drift and electro-osmotic flow.

The random walk parameters calculated from the time-lapse analysis were found to be (at 200 V m^{-1})

$\lambda = (470 \pm 10)$ μm
$\tau = (2.5 \pm 0.1)$ min
$\theta = (26 \pm 3)$ deg
$u = (3.2 \pm 0.1)$ μm s^{-1}
$\alpha = 25$ deg

The rate of locomotion at this field strength is higher (by about 23%) than in controls, and D_E (determined from equation 6) is $(7.0 \pm 3.0) \times 10^{-9}$ m^2 s^{-1} (an increase of about 75%). The fractional increase in the diffusion coefficient $(D_E - D_c)/D_c$ is

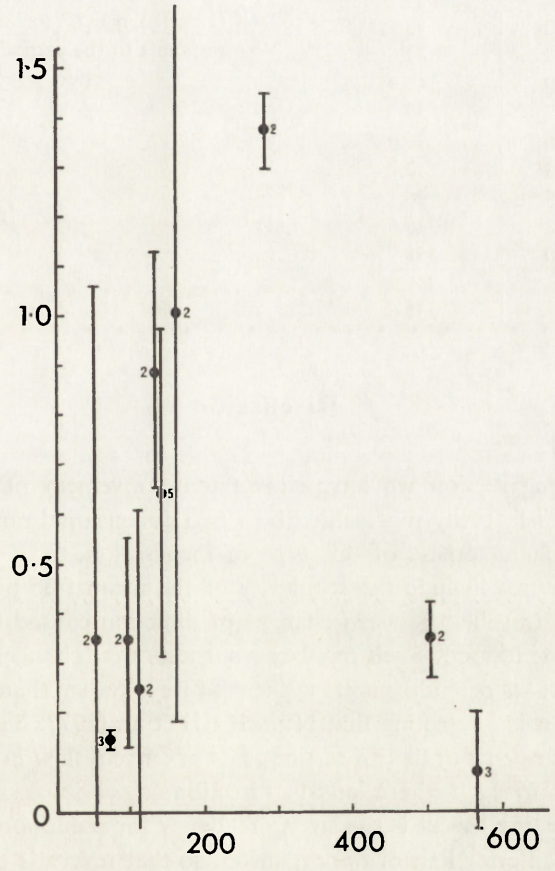

Fig. 5. Abscissa: electric field strength (V m^{-1}); ordinate: fractional increase in diffusion coefficient. Error bars (symmetrical) represent the range of number of experiments indicated. Each point represents at least 40 amoebae

shown in Fig. 5 as a function of field strength; the increase appears to be linear up to 250 V m^{-1} beyond which it decreases.

The values of the mean direction of movement (α) for a control population and for the corresponding electrically stimulated population (α_E) are given in Table 2. Statistical tests (Rayleigh and V-test) have shown that α_E is non-uniformly chosen by the electrically stimulated population and is clustered about the cathode direction (i.e., 0 deg), whereas for the control population it is uniformly chosen.

Table 2

Results from a typical experiment showing the mean direction of movement of amoebae in the control population (10 cells) and in the electric field (12 cells). $\alpha = 0^0$ (or 360^0) corresponds to the cathode direction

α (deg)	
Control	Electric field (200 V m^{-1})
199.4	57.7
16.2	73.7
294.2	42.6
284.9	46.9
146.7	37.3
182.6	76.0
319.3	2.7
335.3	26.5
309.3	336.6
56.0	351.9
	355.9
	331 6

Discussion

In the foregoing section we have shown that movement of an unstimulated amoeba can be satisfactorily represented by a two dimensional random walk model with parameters characteristic of this type of locomotion.

Interesting features include the regularity of the mean free path, which represents the distance travelled between changes of direction caused by the formation of a new major pseudopod. Such regularity suggests that changes in direction are induced by some sort of endogenous cyclical process rather than interaction with the environment; it is interesting that Nuccitelli et al. (1977) have found spontaneous, periodic transients in their measurements of current flow in *A. proteus* which might correspond to such alterations in direction.

Another interesting aspect is the strong tendency for pseudopodia to be formed in the previously anterior half of the organism, so that reversals of direction rarely occur. This observation suggests that there is some persisting feature in the membrane or other stable structure at the anterior (or the posterior) end which is in part responsible for the maintenance of the polarity.

The analysis of the movement of whole population of cells provides a sensitive assay of the effects of external stimuli, and we have used this method to analyse the electric field response in amoeba. The immediate effects of externally applied electric fields on cytoplasmic streaming of amoebae have already been extensively investigated. In agreement with Hahnert (1932) and Korohoda and Kurowska (1970) we found that when a field in the range 30–1000 V m^{-1} is first applied, cytoplasmic streaming ceases, then a pseudopod is extended towards the cathode and cathodal movement commences. This is a short term response, however, within minutes the cell accomodates to the field and then appears to ignore it. The present study shows that a small residual response continues to drive the amoebae towards the cathode, although the response is so weak and variable that this fact could probably never be elicited from observations on single amoebae.

There are two distinct aspects of this long term response, the non-directional rate of locomotion reflected in the diffusion coefficient D_E, and the rate of directional migration with respect to the electric field.

The non-directional rate of locomotion increases with the field strength in a manner consistent with a linear response up to 300 V m^{-1} (Fig. 5), and may be caused by an increased flux of ions through the cell membrane (see below). The directional response appears to depend upon the preferential formation of major pseudopodia towards the cathode.

The ionic fluxes associated with these two components are unclear, since there is no satisfactory model for ionic regulation of amoeboid movement in either normal or electrically stimulated organisms. It is, however, possible to suggest some of the effects of steady electric fields on the intracellular distribution of ions, in the light of recently published experiments. Nuccitelli et al. (1977) have shown by measurements taken with a vibrating electrode that in normal, moving *A. proteus*, there is an inward current at the tail end, (1–2 mA m^{-2}), probably carried by Ca^{++}, and an outward current at the advancing edge, perhaps consisting of K^+. Changes in this current pattern are followed by alterations in the direction of cytoplasmic streaming. The correlation between an inward flux of Ca^{++} and the formation of a rear end is in agreement with short term electrical stimulation.

Since calcium might be expected to be driven into the anodal (rear) end during galvanotaxis the events occurring at the front of the amoeba are more difficult to envisage, particularly as little is known about the ionic fluxes which induce pseudopod formation. Mechanical stimulation of the surface of the amoeba usually causes a momentary inward current followed by the formation of a pseudopod (Nuccitelli et al. 1977); this current is apparently not carried by Ca^{++} but some other species of ion. Electrical stimulation also results in the appearance of free Ca^{++} at the cathodal side of the cell which forms a pseudopod (Taylor et al. 1975). These observations could be explained if as a result of electrically induced depolarisation, which must occur at the cathodal end, there is an increase in membrane conductance to some species of ion, the influx of which results in internal Ca^{++} release

and pseudopod formation; in the highest field strengths used (500 V m^{-1}) the maximum depolarisation at the cathodal end is about 150 mV, and the current density through the membrane, assuming a specific membrane resistance of 10 KΩ cm^2 (Tasaki and Kamiya 1964; Prusch and Dunham 1972) is about 150 mA m^{-2}. It is interesting that despite the fact that this density is about a hundred times greater than the one recorded by Nuccitelli et al. (1977) the effect on the orientation is very small. These two effects, i.e., the increase in inward current at the rear end, and the depolarisation of the front, may also be responsible for the observed increase in the non-directional rate of locomotion in the long term galvanotactic responses.

Another problem that emerges is the marked accommodation to the electric field shown in the directional response when amoebae are observed more than a few minutes after the onset of the stimulus. Such an effect may result from active adjustments by the amoeba of the conductance of its membranes at the rear end to electrically driven ion currents, and at the front to depolarisation-dependent ion fluxes. Accommodation is not complete, however, and still allows a bias in pseudopod formation towards the cathode, and an increase in the non-directional rate of locomotion in the field.

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RÉSUMÉ

La locomotion normale de l'*Amoeba proteus* peut être présenteé en termes d'un modèle du mouvement fortuit sur la surface plate. Le coefficient moyen de diffusion s'élève à 4×10^{-9} m^2 s^{-1}, un seul "pas" du mouvement est à peu près égal á la longeur d'une amibe monopodiale entièrement étendue (ca. 400 μm) et il dure environ 3.0 min, l'angle de réorientation entre les "pas" étant d'environ 30° en moyenne, et la vitesse translatorique moyenne d'environ 2.5 μm s^{-1}.

Les amibes manifestent des réactions transitoires ainsi que des réactions de longue durée, lors de leur exposition continue à l'action des uniformes champs électriques horizontaux. La réponse de longue durée, étudieé dans les populations entières, consiste en migration nette vers la cathode, superposée à la locomotion normale fortuite. Ainsi la vitesse moyenne de la migration cathodique que le coefficient moyen de diffusion, augmentent de façon linéaire avec l'intensité du champ eléctrique jusqu'á 270 V m^{-1}, valeur qui représente la niveau de saturation. La migration cathodique est provoquée par la formation des pseudopodes de préférence du côté cathodique de la cellule.

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Effects of DMSO on the Motory Behaviour of *Amoeba proteus*

Synopsis. Solutions of 5% DMSO change the motory behaviour of amoebae during the 1st hour of incubation. In particular, they produce morphological and functional effects related to the raise of cell adhesiveness to the substratum, and they provoke fusions between different pseudopodia of the same individual. The prolonged action (24 h) of weaker concentrations (1–2%) results in promoting cell-to-cell contacts in the population of amoebae.

Dimethyl sulfoxide (DMSO) is one of the compounds having very extensive applications in different fields of biological science and medicine. For example, it is used as solvent of other active substances insoluble in water (e.g., the cytochalasin B), as component of some fixators in the electron microscopy and in the cryoscopy. The medical applications are based on its ability to facilitate the penetration of drugs through the cellular membranes; Wood and Wood (1975) state that "when mixed with DMSO, many drugs appear to be potentiated in their physiologic effect; thus smaller doses are required and less toxicity is demonstrated".

The present paper deals with the influence of DMSO on the cells of *Amoeba proteus*. The intention of the authors was, originally, to study the effects of the cytochalasin B on locomotion of amoeba, and the experiments with DMSO were undertaken as preliminary control test. It turned out that DMSO modifies the motory functions of amoeba within the same range of concentrations which are needed to dissolve the cytochalasin B. This would create so many difficulties in discrimination between effects produced by one or the other agent, and so evident danger of mutual potentiation of effects when both agents are acting together,

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that further experiments with the cytochalasin B were abandoned. However, the effects of DMSO alone proved to be enough interesting to merit a separate report.

Material and Methods

Cultures of *Amoeba proteus* were grown in Pringsheim's medium. *Colpidia*, serving as food for amoebae, were maintained in separate cultures fed on the dried yolk.

Several dozens of amoebae were transferred for experiments from the stock cultures to the Godet glass chambers filled with DMSO solution. For the microscopic control and photomicrography the samples taken from the Godet chamber were placed on the microscopic slides and protected from evaporation by paraffin oil. This precaution was necessary to enable observations for at least 1 h or longer.

The DMSO used in the majority of experiments was manufactured by Reachim (USSR). Control tests were carried out with the Serva and BDH products and the same results were obtained, except of the slight differences in the time of action.

In one series of experiments the immediate effects of 5% DMSO solution, and those produced during the 1st hour of incubation, were recorded. In another series, the prolonged action of DMSO was studied by exposing amoebae to 1% or 2% solutions of this compound for 24 h. All the experiments were carried out at 22–25°C.

Results

The first reaction of amoebae to the 5% DMSO solution is the same as to any other kind of non-directional stimulus acting uniformely upon the whole cell surface: polytactic specimens become heterotactic, with long and thin pseudopodia and deficient attachment to the substratum. But the recovery, which follows 20–60 min. later, is different than in the untreated specimens. While usually the heterotactic amoebae return to the migratory condition by producing initially only one huge pseudopodium of the orthotactic type (Grębecki and Grębecka 1978), the cells exposed to DMSO reestablish the firm contact with the substratum in many points simultaneously and produce several locomotory pseudopodia which are flat, strongly adhesive, and spread wide over the glass surface (Pl. II 7–12).

The other body regions shorten and shrink and gradually reconstitute the uroid which becomes nearly hemispherical and strongly wrinkled (Pl. I 3–6, and II–III 11–16). The most characteristic feature of conformation of the uroid in amoebae exposed to DMSO is shown in the Pl. III 17–18. It is the abundance of hyaline pseudopodia arranged in a fan-like

manner behind the posterior body edge, firmly adhering to the substratum and strongly stretched by the retraction of the tail of amoeba.

At the middle and anterior body regions the pseudopodia are not only flat and abnormally spread over the substratum, with the enlarged hyaline ectoplasm layer, but they manifest another trend not usual in the behaviour of untreated cells: a tendency to establish contact with the tips or lateral walls of neighbour pseudopodia. As a result, some areas empty and completely closed between pseudopodia are developing (Pl. I 1–4, and II–III 11–16). These empty areas in some extent resemble the food cups (however, the food cup formation is a three-dimensional phenomenon, not confined to the substratum plane). In some cases the phenomenon is probably limited to the simple contact between both membranes, and then the neighbour pseudopodia may disengage one from another during their contraction. But in other cases the fusion is complete, and the empty area forms a gap which becomes stable component of the contour of migrating amoeba, until it disappears in the uroidal zone.

In lower concentrations of DMSO (1–2%) which fail to exert any immediate influence on the cell shape and locomotion of amoebae, some peculiar behavioural changes appear after 24 h of incubation. Their manifestation is shown in the Pl. IV 19–24. The phenomenon of clumping, unknown in *A. proteus* under normal culture conditions, is produced. Amoebae form agglomerates, composed most often of 2–5 individuals, but sometimes larger. They move side-by-side and their cell membranes seem to adjoin closely one to another. The hyaline ectoplasm layer is enlarged as after the short exposure to the 5% DMSO solution. The adhesive pseudopodia may be observed to establish contact with the neighbouring cell (Pl. IV 20 and 24).

Many of the observed agglomerates were stable up to 15 min. During the stability period the cells move side-by-side in a common direction. The strength of intercellular contact was assessed by applying the photic stimulus to one cell (according to the light-shade method described by Grębecki et al. 1978) and checking its ability to leave the agglomeration. In some cases the stimulated amoeba moved freely away from the clump, but in the others amoebae were seen to drag, for a short time, the neighbouring individuals behind them.

Discussion

The shape and the behaviour of amoebae exposed to the influence of 5% DMSO solution indicate that this agent enhances their adhesion to the substratum. This is demonstrated by development of flat and

widely spread pseudopodia, and by flattening of all the lateral body margins with the parallel enlargement of the hyaline ectoplasm zone. The shape of the uroid, and in particular the multitude of small hyaline pseudopodia stretched behind the posterior body margin, lead to the same conclusion because the adhesive properties of such pseudopodia are well known from the literature (Bell and Jeon 1963, Haberey 1971, Nowakowska and Grębecki 1978).

Probably, the same unknown mechanism which favors the adhesion of the membrane of amoeba to the solid substratum, is also responsible for promoting the contacts between the different membranes in the same cell (fusion of pseudopodia and gap formation), and the contacts between membranes of different cells (the clumping phenomenon). It seems interesting to note that Norwood et al. (1976) described cell fusions in the culture of human skin epithelium exposed to the combined action of DMSO and polyethylene glycol.

The influence of DMSO on the free-living protozoan cells was not so extensively studied as its influence on the tissue cells. The most complete information concerns the DMSO effects on *Tetrahymena pyriformis*, and it was given in the series of papers by Nilsson (1974, 1976, 1977). It was demonstrated that this agent, in concentrations of 5–7.5%, inhibits the rate of pulsation of the contractile vacuoles, handicaps the food vacuoles formation, induces structural changes in helical formations of polyribosomes and inhibits the RNA synthesis. All these effects are reversible.

All the authors concerned with the DMSO effects on various animal cells agree as to its extremely high capability to penetrate through the membranes. Moreover, it is successfully used as vehiculum facilitating the penetration of other compounds, although "the integrity of most membranes is unaffected except where extremely concentrated (90–100%) doses of DMSO come into direct contact with the membrane" (Wood and Wood 1975). According to Szmant (1975) the high capability of DMSO to penetrate through the membranes without affecting their structure depends on "its relatively polar nature, its capacity to accept hydrogen bonds and its relatively small and compact structure". This combination of properties is also thought to explain the extreme ability of DMSO to combine with other molecules, and "its ability to replace some of the water molecules associated with the cellular constitutents or to affect the structure of the omnipresent water". This last statement of Szmant finds an experimental support in the study of Norwood et al. (1976).

These concepts might probably help to explain also the effects exerted by DMSO on the motory behaviour of amoeba. The idea of affecting the

properties of water adjacent to the cell membrane seems to be particularly attractive, since it could account for the increase of adhesiveness and the facilitation of contact between membranes. But the other possibility, that DMSO induces some molecular changes within the cell membrane of amoeba, can not be a priori discarded. The intracellular action of DMSO seems to be much less probable as factor producing the changes of motory behaviour of amoebae.

RÉSUMÉ

Le DMSO en concentration de 5% change le comportement motorique des amibes, lors de la première heure d'incubation. Les modifications de la forme et du mouvement indiquent que l'adhésion des cellules au substratum est favorisée. En outre, des fusions se produisent entre les différents pseudopodes du même individu. L'action des solutions de 1–2%, prolongée jusqu'à 24 h, se traduit en l'établissement des contacts entre les différentes cellules dans la population des amibes.

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EXPLANATION OF PLATES I–IV

Pl. I. Effects of 5% DMSO after 1 h

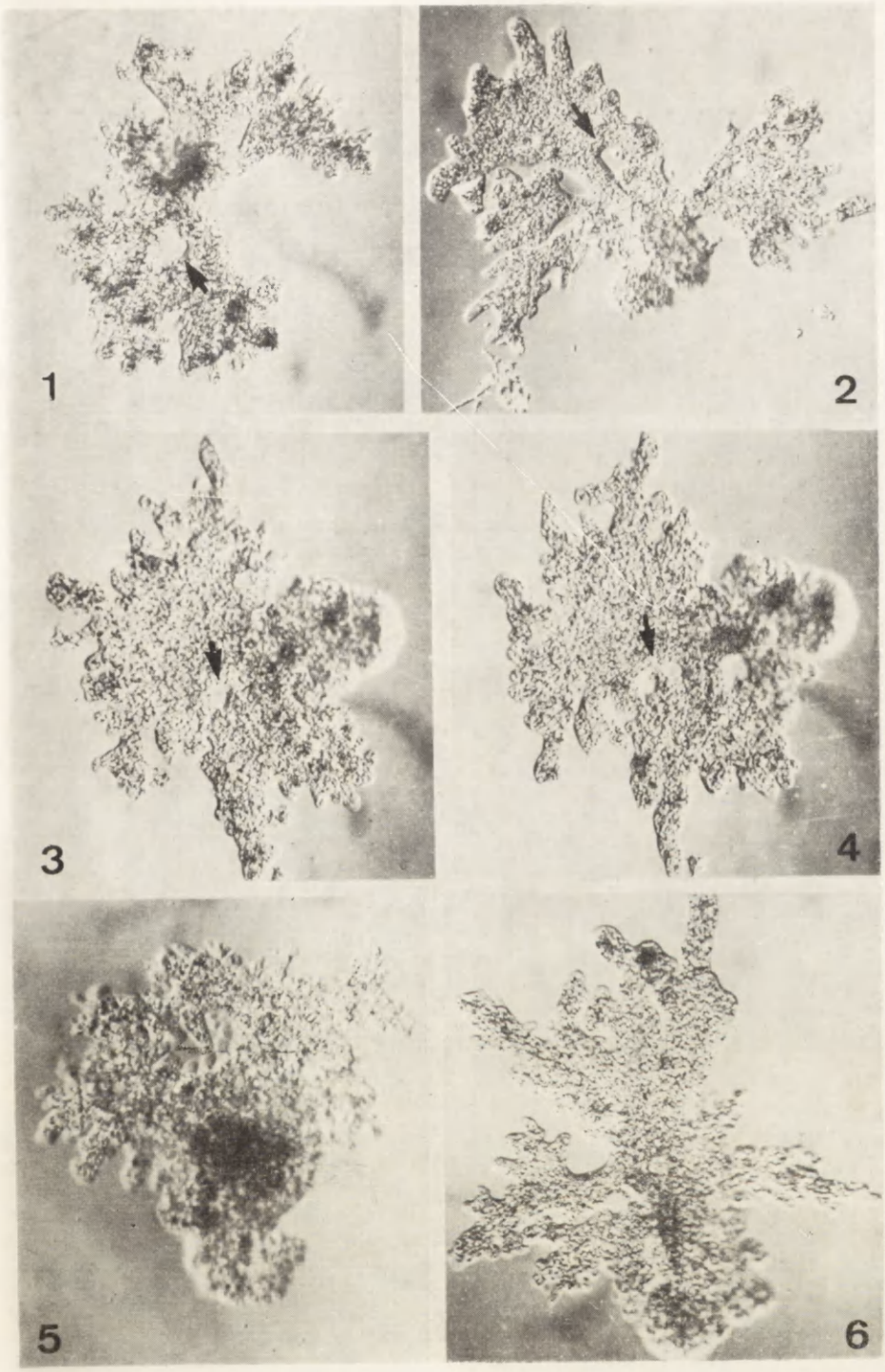
1–6: Examples of cells widely spread over the glass surface. Note the presence of gaps produced by earlier fusion of some pseudopodia (arrows)

Pl. II–III. Different phases of behaviour of an amoeba exposed for 1 h to the action of 5% DMSO

7–8: Heterotactic form obtained as immediate efect of DMSO, and the beginning of recovery
9–10: Re-establishing of firm adhesion to the substratum by means of several flat pseudopodia
11–16: Amoeba with pseudopodia widely spreading over the glass surface. Note the formation of closed empty areas between pseudopodia which come into contact by their anterior segments (arrows)
17–18: Later stages at which small adhesive pseudopodia are seen in large number behind the posterior body edge (arrows)

Pl. IV. Effect of 2% DMSO after 24 h

19–24: Examples of cell agglomerations. Note the flattened hyaline-rich body margins of amoebae (empty arrows), and the formation of short pseudopodia establishing contact between cells (plain arrows)



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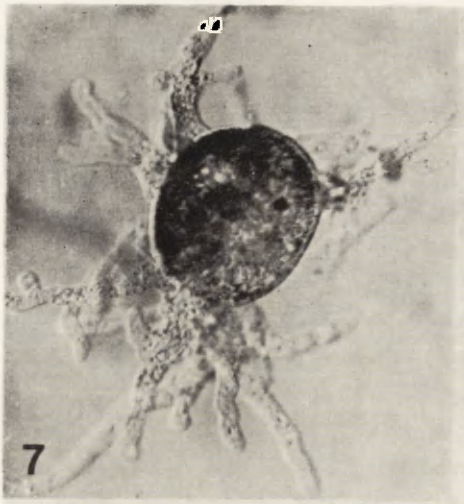

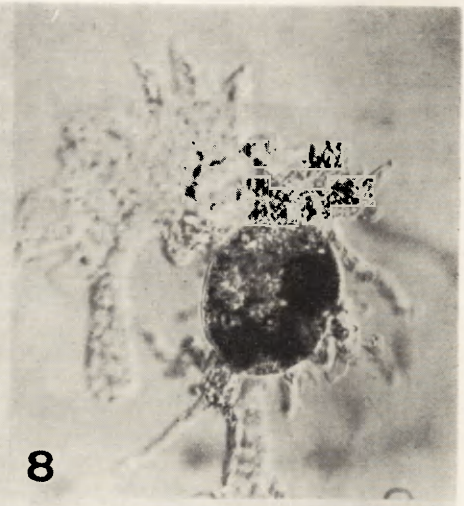

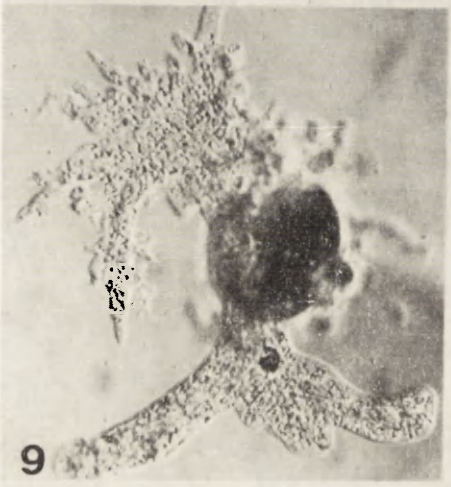

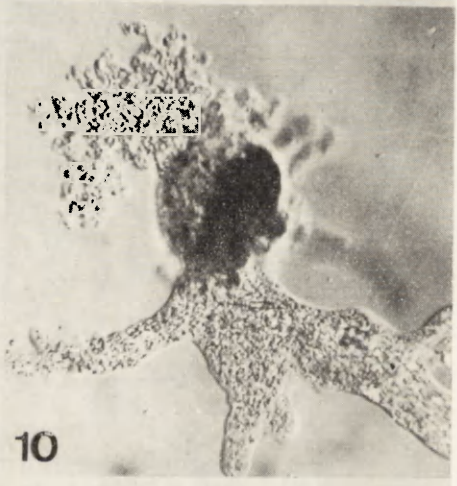

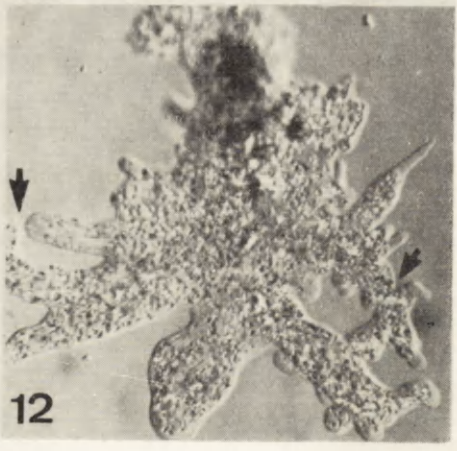

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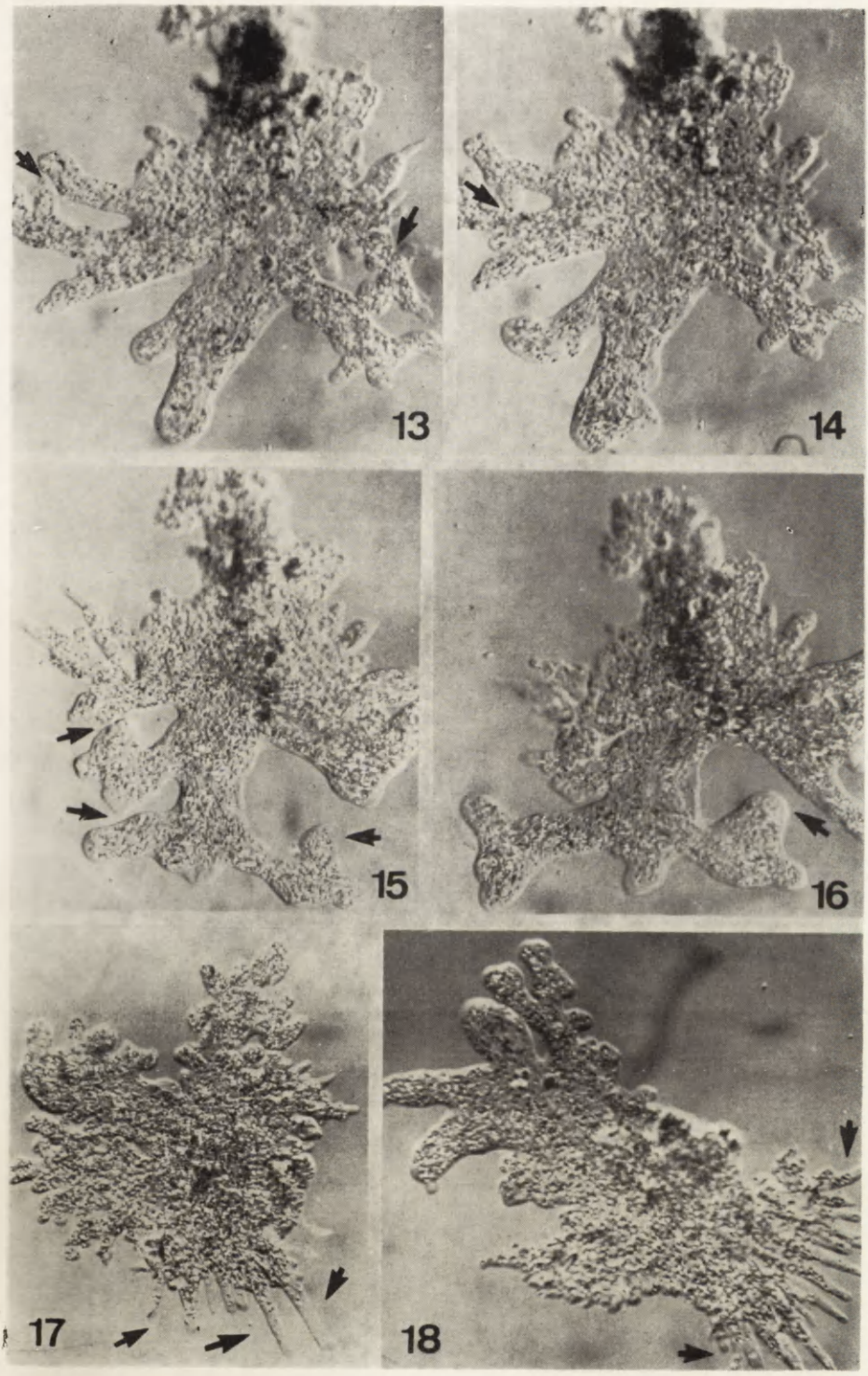

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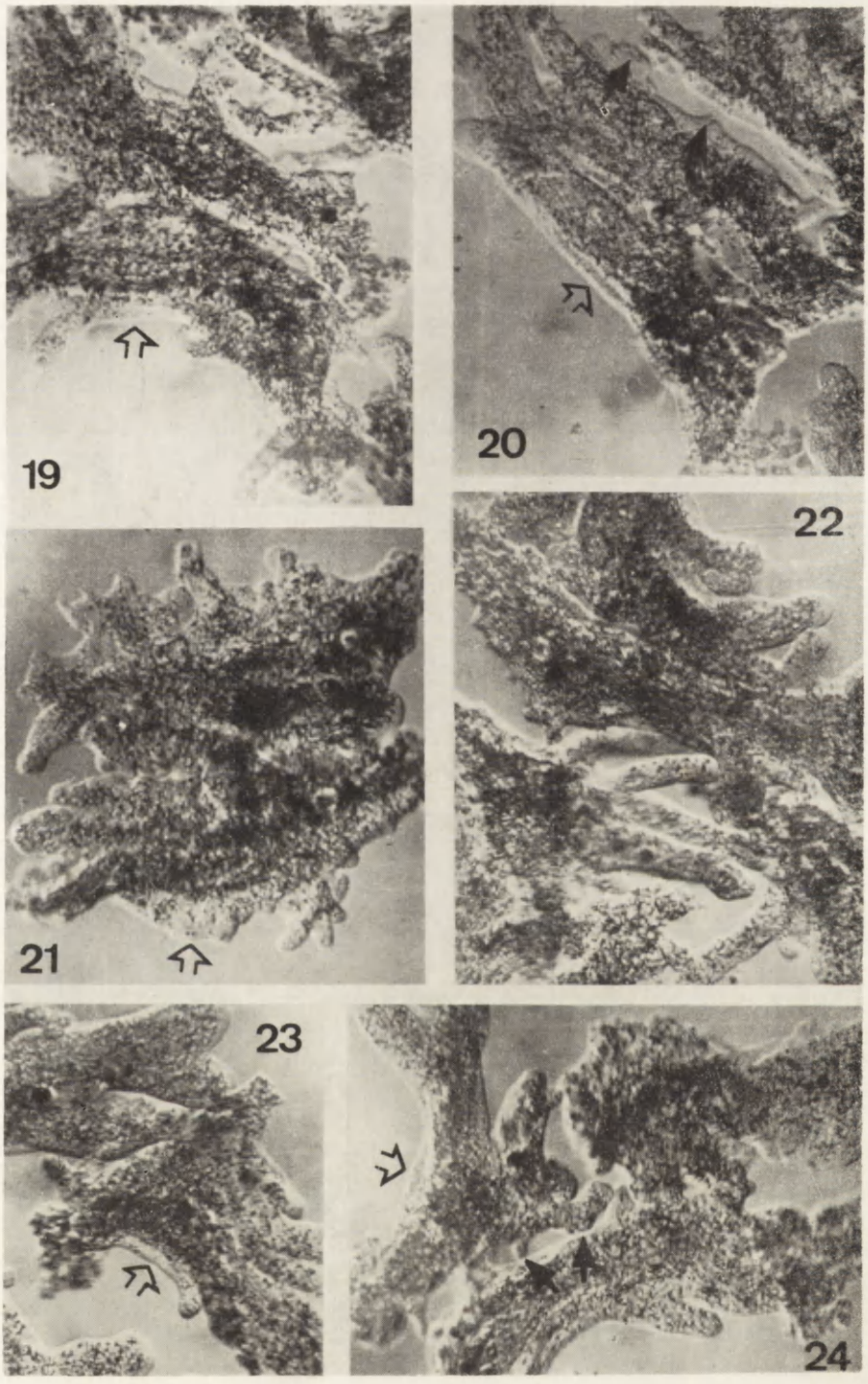

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The Radiation Changes of Activation Energy of Phosphate Ions Transport across the *Spirostomum ambiguum* Membrane and Accumulation of these Ions in Cells

Synopsis. The activation energy of passive phosphate ions transport across the *Spirostomum ambiguum* membrane was determined, and it was found that with increase of gamma radiation dose the activation energy decreases. The accumulation process of the phosphate ions in irradiated cells was investigated in the presence and in the absence of radioprotective substances in the medium.

The protozoa living in water medium contain about 90 per cent of water (Grobnicka and Wasilewska 1925). Radiation damage in biological systems is caused to a large extent by the action of ionizing radiation on cellular water. The highly reactive products of the water radiolysis process, such as $\dot{OH}$, $\dot{H}$, e_{eq} can diffuse and interact with biological macromolecules. The author (Balcerzak 1973) has shown that the mortality of *Spirostomum* is caused mainly by $\dot{OH}$ radicals.

The ionizing radiation also in the sub-lethal doses range causes the damage of the biological membranes and cellular structures. Michel (1976) reported, that the ionizing radiation provoked release of polysaccharide from cell wall of *Micrococcus radiodurans*. Rink (1975) observed damage to membrane bound sulphydryl groups and change of permeability in yeast cells. Sato and Kojima (1974) proved that due to the irradiation the cell membrane of yeast looses its negative charges. Myers and Johnson (1974) observed, that the ionizing radiation induced an outflow of K^+ ions from *E. colli* cells. According to the Alexander's et al. (1964) hypothesis, ionizing radiation causes changes in permeability of cellular and intercellular membranes, which lead ultimately to the death of the cells. The aim of this research was to investigate the effect of the gamma radiation on the phosphate ions

transport across the *Spirostomum ambiquum* membrane and on the accumulation process of these ions in the irradiated cells. Phosphates are commonly met in the composition of cellular structures, and play an important role in the cell's energy processes. Balcerzak (1978) has found that the transport of phosphate ions across the *Spirostomum ambiquum* membrane is passive. The activation energy of this process is an important parameter determining the peneration of the phosphate ions across the cellular membrane. Since the ionizing radiation causes structural changes of the *Spirostomum ambiquum* membrane (Balcerzak 1967, Balcerzak and Rostowska 1978) an the value of activation energy is dependent on the membrane structure, then there should appear a radiation induced change of the magnitude of the energy barrier for the phosphate ions transport. It is also known that the ionizing radiation inhibits the process of building — in of the phosphate ions into the cellular organic compounds, e.g., nucleic acids (Ord and Stocken 1956). Protoza show great ability to accumulate the phosphate ions from the medium and are useful in this type of research. In particular, the mechanism for accumulation of these ions in relation to the radiation dose was examined.

Material and Methods

The ciliate *Spirostomum ambiguum* was selected for carrying out the experiments. It was cultivated in a solution of the following composition: 11 mM NaCl + 0.05 mM Na_2HPO_4 + 0.05 mM NaH_2PO_4 + 0.05 mM KCl. The solution was prepared with distilled water and pH was adjusted to 7.0 by addition of a buffer NaH_2PO_4 and NaOH. Some boiled oat grains were introduced into the culture as a source of food for bacteria on which *Spirostomum ambiguum* was fed. About 20 000 animals were taken from the culture and transferred to a medium of the same chemical composition, pH and temperature but containing labelled phosphate ions. The specific activity of the medium was 5 μCi/ml. The culture was kept at a constant temperature of 20°C and pH was adjusted to 7.0. After two days of incubation in active medium, protozoa were separated and put into five test tubes in equal volumes. Each tube with protozoa was placed in thermostate in which the temperature values (14, 18, 20, 22, 25) °C were maintained with a tolerance of ±0.1°C. For every measurement of the permeability constant, 300 animals were taken from the active medium. The protozoa were washed three times with non-radioactive solution of the same chemical composition and pH as the sample taken from the culture. Measurements of activity drop of the constant number of animals transferred to the non-radioactive medium were carried out every 15 min using a β-scintillating counter.

Before each measurement the liquid from the protozoan sample was removed. In order to determine the permeability constant across the membrane of cells under investigation, the dependency given by Carter (1957) was used:

$$\ln\left[\frac{C(t)}{C(t=0)}\right]=-kt \tag{1}$$

where C_t is the concentration of labelled phosphate ions inside the cell, k is the permeability constant, t — the time of keeping the cells in non-radioactive medium. The process of phosphate transport across the protozoa membrane is an activation process and the permeability constant satisfies Arrhenius's relationship:

$$k=k_0\exp\left(-\frac{E}{RT}\right) \tag{2}$$

where k_o is the constant determined experimentally, T is the temperature in Kelvin's scale, R is the gas constant, and E is activation energy. Activation energy was determined from the linear dependency between the logarithm of permeability constant and reciprocal of temperature in Kelvin's scale.

The animals were irradiated with gamma rays emitted by Co-60. The dose rate was 10^5 rads/h. In research on the accumulation of phosphate ions in the cells of protozoa, the animals taken from the culture were distributed among four test tubes. Three of the tubes were irradiated with dose of 5×10^4 rads, 7×10^4 rads and 11×10^4 rads, while the ciliates in the fourth tube were not irradiated. Afterwards P-32 was introduced into each test-tube in the form Na_2HPO_4. The specific activity of the medium equalled 5 μCi/ml. The culture was kept at a temperature of 20°C. At several hours intervals, 300 animals were taken from each tube, washed and its radioactivity was determined.

Results

The activation energy of the phosphate ions transport process across the *Spirostomum ambiquum* membrane was determined for pH 7.0 with the ionic strength of the medium equalling 11 mM. Figure 1 shows the dependency between the logarithm of permeability constant and the reciprocal of temperature in Kelvin's scale. Determined by using the method of least squares, the activation energy of the phosphate ions transport process across the protozoa membrane under investigation equals:

$$E = (25.9 \pm 0.7)\ \text{kcal/mole}$$

Figure 2 show the dependency of the logarithm of permeability constant as a function of the reciprocal of temperature in Kelvin's scale for protozoa irradiated with 7×10^4 and 10^5 rads. The pH of the medium was 7.0 and ionic strength was 11 mM.

In these conditions, the activation energy equals (17.2 ± 0.8) kcal/mole and (11.9 ± 0.7) kcal/mole respectively. The obtained result show that gamma radiation, with an increase of the dose markedly reduces the activation energy of the phosphate ions transport process across the ciliate membrane. Since the phosphate ions transport process across the

exposed to gamma radiation. It appears from the obtained experimental data, that with the dose of 10^5 rads, thirty hours after the irradiation the protective substances in concentration 10^{-4} M used separately in the experiments (potassium bromide, cysteamine, glutathione) have no evident effect on the radiation inhibited synthesis of phosphates in the cell.

Discussion

The value of the activation energy of phosphate ions transport across the *Spirostomum ambiguum* membrane, equalling (25.9 ± 0.7) kcal/mole, which I have obtained in my experiments, corresponds to the values obtained for these ions by Przestalski (1962), Deuticke (1970), Passow (1969) for the erythrocytes membranes.

The radiation induced reduction of activation energy in the passive phosphate ions transport process across the membrane of protozoa under investigation points to the destruction of the cellular membrane.

Following the lowering of the energy barrier, the penetration of the phosphate ions across the irradiated membrane is facilitated. The ionizing radiation substantially influences on the passive transport of phosphate ions across the cellular membrane. Since the gama radiation energy in the cell is absorbed in about 90 per cent by water, then — due to the interaction of water radiolysis products — the intercellular membranes are probably also destroyed.

The obtained results confirm the hypothesis of Bacq et al. (1964) about the radiation induced changes of permeability of cellular membranes. Using the radioprotective substances, which are good scavengers of the OH radicals, the biological membranes can be protected from the radiotional reduction of activation energy.

During the accumulation process of the phosphate ions in the *Spirostomum* cells, the two coupled processes occur. One is the transport of the phosphate ions from the medium across the cellular membrane into the cell, and the other is the process of a quick building-in of those phosphate ions, which penetrated inside the cell, into intracellular organic compounds (Balcerzak 1967, Bunow and Caplan (1977)). In the irradiated cells the process of building-in of the phosphate ions into the cellular organic compounds is inhibited, while the irradiation of the cells accelerates the passive transport of the ions from the medium to the inside of the cell. This is why during irradiation the concentration gradient of the ions occurring on both sides of the membrane disappears relatively quickly, while the passive flow of the ions from the medium to the inside of the cell is stopped.

On the graph showing the accumulation of the phosphate ions in cells one may observe a characteristic plateau remaining there up to more than thirty hours after the irradiation. After this time the renewal of radiation damaged mechanisms of the phosphate synthesis takes place and free phosphate ions are bound in the cell. This process causes the recovery of the concentration gradient on both sides of the membrane, and is a stimulus for the continuing flow of ions from the medium to the cell's inside, which in effect causes an increase of accumulation. The application of radio-protective substances which are good scavengers of OH radicals (Adams et al. 1967) did not have any evident influence on the inhibition of the radiation process of building-in of the phosphate ions into organic compounds. Still, in protozoa irradiated in presence of the radioprotective substances, there was a quicker process of renewal and the greater rise of accumulation than in those protozoa during the irradiation of which the radioprotective substances were not used. The cells of ciliata *Spirostomum ambiguum* proved to be an excellent material for this type of research because they contain a large amount of water, have relatively large size and it is easy to obtain a large number of them in the culture.

RÉSUMÉ

On a determiné l'énergie d'activation du transport passif de ions phosphatiques par la membrane de ciliate *Spirostomum ambiguum*. On a constaté, qu'au fur et mesure croissment dose de gamma radiation, l'énergie d'activation diminue. On a examiné de procès d'accumulation de ions phosphatiques dans les cellules d'infuisoire avant et après la radiation en présence de substances radioprotectives.

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The Bicarbonate Ion as a Factor Influencing Cell Growth in *Polytomella agilis*

Synopsis. The presence of 5.0 mM or more of Na on K bicarbonate in the medium reduced the rate of logarithmic growth and peak cell numbers of the colorless *Polytomella agilis* (Doflein, 1916). Although bicarbonates increased extracellular pH, these effects were not produced by adjusting pH with Na or K hydroxides. Extension of the lag phase of growth was observed as pH increased with addition of the hydroxides but not with bicarbonates. The conclusion that bicarbonate anions *per se* act specifically on the cell and in particular the surface membrane is discussed.

Bicarbonate salts have been demonstrated to influence the growth of viruses (Hsiung and Melnick 1958, Vonka and Benyesh-Melnick 1966) bacteria Hardy and Munro 1966, Surgalla et al. 1964, Wegner et al. 1968), protozoa (Hoover and Gittleson 1968, Levedahl and Tremmel 1966, Nelson and Jones 1964), chick heart fibroblasts (Harris 1952, Harris 1954) and rabbit ova (Kane 1975). The processes of encystment (Nelson and Jones 1961, Nelson and Jones 1964), respiration (Hamner and Williams 1964), differentiation (Cantino and Lovett 1964, Haywood and Roote 1932), photosynthesis (Cramer and Myers 1952, Myers 1946) and photophosphorylation (Batra and Jagendorf 1965) are known to be affected by the presence of bicarbonates.

At this time controversy still exists concerning how bicarbonate salts bring about such changes in cellular activity. Some workers (McElroy et al. 1958, Nelson and Jones 1961, Vonka and Benyesh-Melnick 1966) believe that a change in extracellular pH affected by

[1] This study was completed while the authors were at the University of Kentucky, Lexington, Kentucky.

bicarbonates is the primary cause whereas others (Batra and Jagendorf 1965, Creese 1949, Harris 1954) consider failure to maintain intracellular pH as the direct effect of bicarbonate. Sears and Eisenberg (1961) show that bicarbonate salts caused marked decreases in interfacial tension between water and oil phases with concomitant increase in hydration of the interface indicating that the effect may be at the level of the surface membrane. It has also been suggested that the bicarbonate anion might even change the conformation of an enzymatic protein because it could combine with subsites on the active portion of the enzyme (Lysiak et al. 1975).

We present data here to show that bicarbonate anions *per se* exert an influence on cellular growth and that this effect cannot be attributed to extracellular pH, osmotic pressure, cations or other anions.

Materials and Methods

Experiments were carried out on *Polytomella agilis* (Doflein 1916) obtained from the Indiana Algal Culture Collection, Indiana University, Bloomington, Indiana (now at the University of Texas). *P. agilis* were grown axenically in medium consisting of 0.1% sodium acetate (Mallinckrodt), 0.1% bacto-tryptone (Difco) and 0.2% bacto-yeast extract (Difco). The medium was autoclaved for 20 min at 121°C. Stock cultures were maintained in screw top test tubes and were transferred every three or four days.

The medium was autoclaved in 13 × 100 mm Bausch and Lomb Spectronic-20 test tubes stoppered with sterile cotton and covered with aluminium foil. Bicarbonate solutions, 2.5, 25.0, and 250.0 mM were sterilized by means of Millipore filtration, pore size 0.45 μm. These solutions were then added aseptically to the autoclaved medium in the Spectronic-20 test tubes to reach the desired concentration. Inocula of cells were taken from 24–30 h mid-log phase cultures growing in 100 ml batches in 250 ml Erlenmeyer flasks. All experiments were carried out in quadruplicate at 20°C in the dark.

Growth was measured as optical density with a Bausch and Lomb Spectronic-20 colorimeter/spectrophotometer at 500 μm. This technique allowed growth measurements to be made without disturbing the cultures because samples did not have to be withdrawn from the tubes to obtain cell counts. O. D. changes with growth were calibrated against cell number/ml using the Sedgewick-Rafter direct cell counting method described by Scherbaum (1957), in order to obtain a standard curve (Fig. 1).

Microscopic examinations were made with hanging drop preparations to determine survival, motility or any morphological changes, at higher concentrations in which no growth occurred.

NaCl, KCl and $KHCO_3$ solutions were prepared in the same manner as the $NaHCO_3$ and added to the medium to give the same molar concentrations to test the influence of osmotic changes as well as that of the cation. NaOH and KOH were added to the medium to obtain pH's similar to those with the $NaHCO_3$.

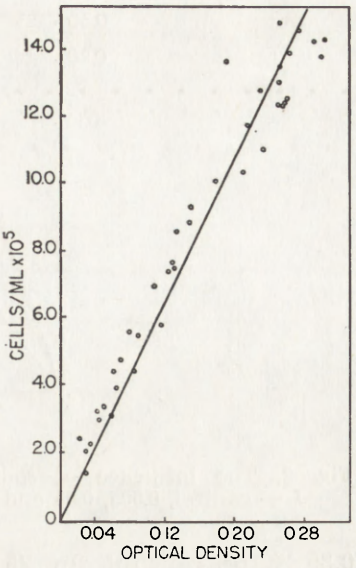

Fig. 1. The relationship between optical density and cell number of *Polytomella agilis*

Continuous pH measurements were made during growth on parallel cultures. The conditions described previously were employed and pH monitored with a Heath model EUW-301 pH recording electrometer using a Sargent combination electrode. The electrode was sterilized under an ultraviolet light and placed in the cultures which were at 20°C in a Forma model 2095-2 refrigerated and heated bath and circulator. Light was excluded by placing aluminium foil over the water bath. Measurements were made in duplicate for each bicarbonate concentration. Experiments showed the presence of the electrode in the culture had no effect on growth and since neither the pH nor cell growth measurements disturbed the cultures, we assumed them to be identical to the experimental situation.

A CO_2 electrometric sensor GL-8 (Chemtronics Inc.) was used to measure CO_2 partial pressure in cultures.

Results

Figure 2 shows the effects of $NaHCO_3$ on the growth of *P. agilis*. The control containing no added bicarbonate and the 0.005 0.05 and 0.5 mM solutions were identical in their effect on growth. The generation time was about 10 h. The cells reached a peak of 1.4×10^6 cells/ml. In the 5.0 and 15.0 mM cultures, peak populations decreased and generation times increased to averages of 13 and 28.5 h, respectively. Populations in the 5.0 mM medium peaked at 1.1×10^6 cells/ml and in the 15.0 mM at

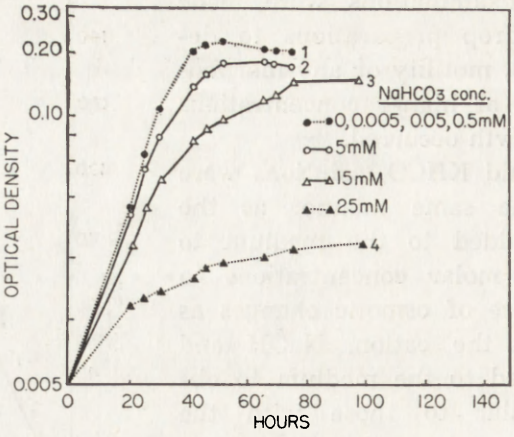

Fig. 2. The influence of sodium bicarbonate on growth of *Polytomella agilis*. *1* — control, 0.005, 0.05 and 0.5 mM, *2* — 5 mM, *3* — 15 mM, and *4* — 25 mM

0.85×10^6 cells/ml. In 25.0 mM bicarbonate the cultures grew more slowly but the cells continued to survive after 100 h. During this period the cultures only reached 0.25×10^6 cells/ml. All cells in 150 mM bicarbonate became immotile after 1 h, swelled and finally lysed. The same results were obtained when *P. agilis* were grown in $KHCO_3$.

Figure 3 gives the results of adding NaCl to the medium of *P. agilis*. The control 0.005 0.05, 0.5 and 5.0 mM solutions produced similar results. The cultures have peak numbers of 1.4×10^6 cells/ml and generation times of about 10 h. The 15.0 and 25.0 mM cultures declined slightly in peak cell numbers as compared with the control. Peak populations were

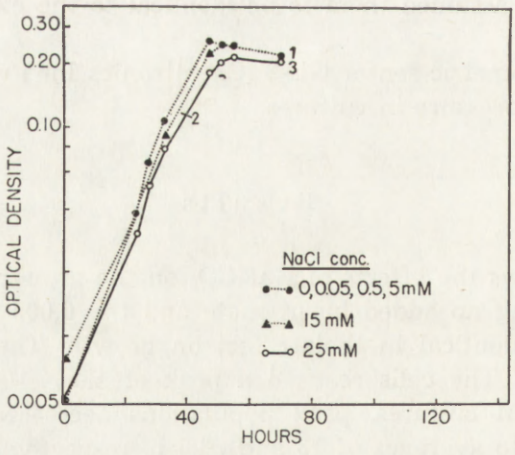

Fig. 3. The effect of sodium chloride on growth of *Polytomella agilis*. *1* — control, 0.05, 0.5 and 5.0 mM; *2* — 15 mM; and *3* — 25 mM

1.2×10^6 and 0.75×10^6 cells/ml and generation times were 14 and 24 h for the 15.0 and 25.0 mM concentrations, repectively. The same results were obtained with KCl.

The pH of cultures with added bicarbonate ranged initially between 6.8 in the control to 8.3 in the 25.0 mM solutions and increased with growth of the cultures in all concentrations (Fig. 4). Net increase in pH

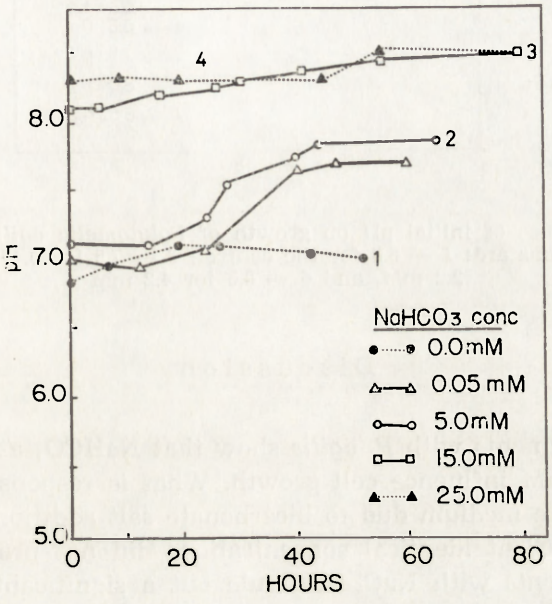

Fig. 4. Continuous pH measurements of *Polytomella agilis* cultures growing in sodium bicarbonate. *1* — control, *2* — 0.05 mM; *3* — 5.0 mM; *4* — 15.0 mM; and *5* — 25.0 mM

was inversely related to bicarbonate concentration, i.e., the higher the bicarbonate concentration the less the increase in pH during growth. The final pH was related directly to bicarbonate concentration. A maximum pH of 8.5 was reached in the 25.0 mM culture.

Figure 5 shows the results of adjusting initial pH to 7.5, 8.0 and 8.5 with the addition of NaOH to cultures without bicarbonate. The cultures attained peak populations very similar to the control at a pH of 6.8. Peak populations were 1.4×10^6 cells/ml. The only effect observed with change in pH was an increase in the lag phase with increased pH. The same results were obtained with KOH.

In all cultures, there was no change in partial pressure of CO_2 during growth in bicarbonate or in controls with no added HCO_3^- over that in media equilibrated with air.

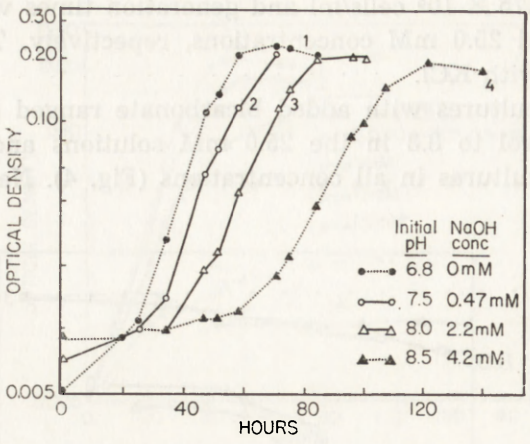

Fig. 5. The influence of initial pH on growth of *Polytomella agilis*. Initial pH and NaOH concentrations are: *1* — 6.8 for the control; *2* — 7.5 for 0.47 mM, *3* — 8.0 for 2.2 mM, and *4* — 8.5 for 4.2 mM

Discussion

These experiments with *P. agilis* show that $NaHCO_3$ at concentrations as low as 5.0 mM influence cell growth. What is responsible? Increased osmolarity of the medium due to bicarbonate salt addition may be ruled out because NaCl at identical concentrations did not produce the same effect. Experiments with NaCl also rule out a significant effect due to cations, at least in the absence of bicarbonate, because similar results would have been produced by the chloride, hydroxide and bicarbonate salts had the cation alone been responsible.

Changes in extracellular pH is a popular explanation for the effect of bicarbonates since addition of bicarbonate raises the pH of the medium (Fig. 4) by (1) dissociation of the bicarbonate salt: $NaHCO_3 \leftrightharpoons Na^+ + HCO_3^-$ and (2) hydrolysis of the bicarbonate anion: $HCO_3^- + H_2O \leftrightharpoons H_2CO_3 + OH^-$. Experiments with NaOH matching the initial pH produced by bicarbonates in solution show that adjustment of extracellular pH does not simulate the bicarbonate effect. Rate of logarithmic growth and peak cell number attained at a pH of 8.5 is no different than the control (pH 6.8); whereas, at the lower pH of 8.3 produced by the presence of $NaHCO_3$, the growth rate is inhibited and the peak cell number reduced. In contrast to this bicarbonate effect, increased pH produced by the hydroxides extends the lag phase of growth (Fig. 5). Apparently, the HCO_3^- suppresses this pH effect because no significant change in lag phase is observed with addition of bicarbonates (Fig. 2).

Increased pH in the medium brings about dissociation of acetate which is essentially complete at pH 7.9 (Danforth and Wilson 1957). One might expect, then, an extracellular pH change to influence growth, particularly since acetate is one of the major components of the medium. However, this must be of little consequence because the addition of NaOH to raise the pH even above 7.9 did not produce the bicarbonate effect.

Evidence does exist which indicates that bicarbonate anions may act directly on the cell membrane. This is predicted by the membrane model of Sears and Eisenberg (1961) who found that sodium and potassium bicarbonates at about 5.0 mM affected interfacial tension at an oil/water interface. Specifically, Sears and Eisenberg indicate that H^+ is removed from the cell membrane because of the stronger affinity of HCO_3^- for H^+ than for Na^+ and H_2CO_3 is formed as in equation 2 above. Na^+ and K^+ have a thicker water shell than H^+, the membrane becomes hydrated and swelling occurs. This has been observed in 100.0 mM and higher concentrations of $NaHCO_3$ for *P. agilis* and *Paramecium multimicronucleatum* (Gittleson 1967, Gittleson and Sears 1964). Further support for a membrane effect comes from measurements of electrophoretic mobilities which show that the surface charge of *P. agilis* is altered in the bicarbonate media that influence growth (Gittleson et al. 1970).

Bicarbonate is used extensively in tissue and cell cultures in concentrations equal to and above those used in the experiments reported here. For example, there is 154.0 mM $NaHCO_3$ in Krebs' balanced saline solution, 4.3 mM in Eagle's medium 33.5 mM in Trovell's T8 medium, and 6.5 mM in White's medium. Although bicarbonate is necessary for maintaining or enhancing growth of some organisms such as *Treponema microdentium* (Hardy and Munro 1966), *Escherichia coli* (Wegner et al. 1968) and chick heart fibroblasts (Harris 1952), growth of other cells such as *Polytomella agilis*, *Euglena gracilis* (Levedahl and Tremmel 1966) and an attenuated poliovirus (Hsiung and Melnick 1958) is inhibited by the presence of bicarbonate anions. Therefore, in selecting a medium it is important to know how bicarbonate affects growth of the organism — certainly a critical matter in bicarbonate buffer systems containing up to 50.0 mM of bicarbonate (Paul 1960).

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RÉSUMÉ

La présence de 5.0 mM ou d'avantage du bicarbonate de pottasse ou du bicarbonate de soude dans le bouillon de culture amoindrissait la vitesse de l'accroissement lagarithmique, et aussi les nombres maximums du *Polytomella agilis incolore* (Doflein, 1916). Les bicarbonates ont aussi augmenté le pH extracellulaire, mais la régulation du pH avec l'hydroxide de pottasse ou l'hydroxide de soude n'a pas producit tous ces resultats ci-dessus. L'extension de la phase lente de l'accroissement était observée au même temps que le pH augmentait à cause de l'addition des hydroxides, mais pas avec les bicarbonates. La conclusion que les anions de bicarbonate *per se* agissent spécifiquement sur la cellule, et sur la membrane cellulaire en particulier, est discutée.

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— do dnia 25 listopada na styczeń, I kwartał, I półrocze i cały rok następny,
— do dnia 10 każdego miesiąca (z wyjątkiem grudnia) poprzedzającego okres prenumeraty.

Jednostki gospodarki uspołecznionej, instytucje i organizacje społeczno-polityczne oraz wszelkiego rodzaju inne zakłady pracy składają zamówienia w miejscowych Oddziałach RSW „Prasa-Książka-Ruch".

Zakłady pracy w miejscowościach, w których nie ma Oddziałów RSW, oraz prenumeratorzy indywidualni zamawiają prenumeratę w urzędach pocztowych lub u doręczycieli.

Prenumeratę ze zleceniem wysyłki za granicę, która jest o 50% droższa od prenumeraty krajowej, przyjmuje Biuro Kolportażu Wydawnictw Zagranicznych RSW „Prasa-Książka-Ruch", ul. Wronia 23, 00-958 Warszawa.

Bieżące i archiwalne numery można nabyć lub zamówić we Wzorcowni Wydawnictw Naukowych PAN-Ossolineum-PWN, Pałac Kultury i Nauki (wysoki parter), 00-901 Warszawa, oraz w księgarniach naukowych „Domu Książki".

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