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Divisional and Regenerative Morphogenesis in the Hypotrichous Ciliate, *Histriculus* sp.

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Synopsis. The morphogenetic processes in dividing and regenerating cells of the hypotrichous ciliate *Histriculus* sp. are described on the basis of light microscopic observations by using Protargol stain technic. The results presented herein confirm previous inclusion *Histriculus* among the genera of *Oxytrichidae* and reinforce the suggestion of a repressive capacity of the Adoral Zone of Membranelles on the stomatogenesis.

Morphogenetic processes in ciliates have been subjects of several extensive and interesting studies (Jerka-Dziadosz 1974) (Frankel 1973) (Aufderheide et al. 1980). The application of morphogenetic analyses for taxonomic and evolutive purposes has been proved to be of great value (Levine et al. 1980) as in *Hypotrichida*, in which their genera are separated into six families on the basis of their morphogenetical pattern (Borror 1979 a, b).

Some genera however, are only tentatively placed since available morphogenetical data are still fragmentary. In the case of the genus *Histriculus*, included among the *Oxytrichidae* on the basis of its morphology, the only available data are those concerning the ultrastructural and biochemical studies during the encystment differentiation (Matsusaka 1977) (Matsusaka 1979) (Matsusaka and Kimura 1981) (Sendo and Matsusaka 1982) (Calvo et al. 1983).

In the present paper, the morphogenetic pattern displayed by *Histriculus* sp. cells in both division and induced regeneration was studied by light microscopy on Protargol-impregnated specimens.

Materials and Methods

One stock of *Histriculus similis* and one stock of *H. muscorum* were used for this investigation. Both stocks were isolated from a sample of water collected in a small stream located at Puebla del Río (Sevilla).

Cultures were maintained in Petri dishes at 20 ± 1°C in Pringsheim's solution and fed daily with *Chlorogonium* sp., autotrophically grown as previously described (Gutierrez et al. 1983).

For light microscope observations, samples of growing or regenerating cells were fixed and stained with Protargol according to the technic described by Tuffrau (1967).

Regeneration was carried out by treating synchronized starved cells with an urea solution (3% (w/v) final concentration) for 30 s. Following this treatment, cells were transferred into fresh medium and fixed and stained in 0 min and subsequently at 15 min intervals to observe the different stages of the morphogenetic development.

All experiments were performed at room temperature (20 ± 1°C).

Results

Nondividing Cortical Structure. The ventral cortical anatomy of *H. similis* is diagrammatically represented in Fig. 1. Trophic cells show an ovoid body, no laterally flexible, of 140–180 μm long × 60–85 μm width.

The oral region is composed of the Adoral Zone of Membranelles (AZM) on the left and two Paroral Membranes (PMs) on the right. These two sets of structures converge posteriorly to meet at the cytostome (Pl. I 2). The AZM is composed of 35–44 membranelles, each of which has four kinetosomal rows arranged as can be seen in Pl. I 3. The remainder of the ventral ciliature is comprised of cirri divided into four groups: 8 frontals, 5 ventrals, 5 transverse and 52–58 marginals.

The dorsal ciliature is composed of six kineties, each with 20–25 small cilia, and three caudal cirri which are located in the posterior end of the body. Four of the kineties are longitudinal rows and the other two extend posteriorly from the anterior margin along 1/3 of the dorsal surface.

The nuclear structure consists of four macronuclear nodes and a variable number of micronuclei situated near them.

The above description is in agreement with Kahl's original description for *Histriculus similis* (Kahl 1932) and later confirmed by Borror (1972) and Corliss (1975, 1979).

Morphostatic cells of *H. muscorum* possess only two macronuclear nodes and their cortical anatomy is quite similar to *H. similis* cells.

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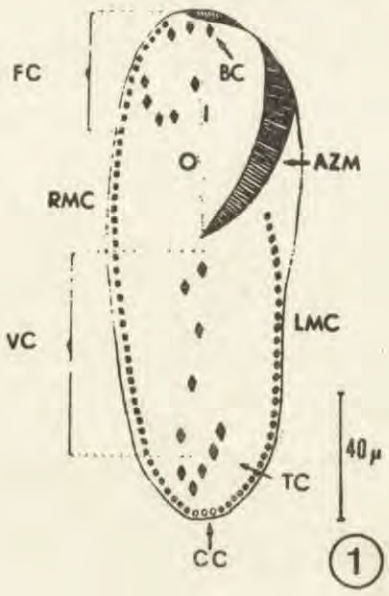


Fig. 1. Schematic diagram of ventral cortex illustrating the typical arrangement of the ciliature. FC — frontal cirri, BC — Buccal Cirrus, AZM — Adoral Zone of Membranelles, I — Inner Paroral Membrane, O — Outer Paroral Membrane, VC — Ventral Cirri, LMC and RMC — left and right marginal cirral rows, respectively, TC — Transverse cirri, CC — Caudal cirri

Prefission Cortical Morphogenesis. Since both species exhibit the same morphogenetic pattern, in the present paper the further descriptions will be only referred to *H. similis* cells.

The first event of cortical morphogenesis is seen to occur as the reorganization bands of the macronuclei have already pass about 1/2 of the length of each node. At this time, initiation of the Oral Primordium (OP) occurs by proliferation of a few kinetosomes which appear at the left margin of the first transverse cirrus (TC 1) (Pl. I 4). These kinetosomes immediately undergo a dramatic migration toward the cytostomal region loosing the continuity with TC 1. (Pl. I 5). The number of kinetosomes gradually increases and an uniform field in this region is formed (Pl. II 6) resulting a fully developed OP.

The next step is the formation of the Fronto-Ventro-Transverse Primordium (FVTP) and Paroral Primordium (PP) of the future opisthe. As two ciliary streaks extend from the right anterior margin of the OP, two ventral cirri disaggregate and the subsequent stringing out of their kinetosomes forms two primordial streaks each (Pl. II 7). From these six streaks, the closest cirral streak to the OP will form the PP and the remainder ones will give rise to the FVT system (Pl. II 9). Before the kinetosomes of old ventrals are incorporated into the posterior cirral streaks, other cirral streaks are initiated at the anterior region of the cell and develop into the new cirri of the future proter. Some parental frontal cirri disintegrate and rearrange their kinetosomes into the ci-

liary streaks in a similar form to that described for the incorporation of ventrals into the posterior field (Pl. II 7). At the same time, the anterior end of the paroral inner membrane begins to disaggregate giving rise to an anarchic field of kinetosomes.

Simultaneously, the rest of the OP differentiates into membranelles by alignement of kinetosomes from the right side of the OP to the left and posterior end (Pl. II 8). As the cirral streaks, both in proter and opisthe, grow in length and width, they break into lobes to form the new FVT cirri, transverse cirri being usually the last and largest ones (Pl. III 11).

The new Paroral Membranes in the future opisthe arise by arrangement of kinetosomes of the PP and the new Buccal Cirrus is originated from the anterior end of this primordium. On the other hand, the new one in the future proter derives from the right anterior portion of the "reorganizing" Paroral Membranes (Pl. III 12).

Shortly after cirral segregation from the FVTP, Marginal Cirral Primordia (MCP) appear asynchronously at four places, always formed closely at the right of the preexisting marginal rows (Pl. III 10, 11). These primordia develop into long ciliary streaks from kinetosomes originated from successively disaggregating parental cirri within each row. Subsequent fragmentation of each MCP gives rise to new marginal cirri. All old marginal cirri not incorporated into primordia are resorbed near the completion of the cortical morphogenesis.

The new cirri formed from each FVTP space out and migrate to their final cortical positions as the cell undergoes cytokinesis (Pl. III 13, IV 14).

The dorsal ciliature derives from old dorsal bristles rows in two sets. The first one originates by proliferation of kinetosomes of three parental dorsal rows (1–3) at two antero-posterior levels to form early dorsal primordia (Pl. IV 15). This process occurs while the FVT streaks are developing on the ventral surface of the organism. The dorsal primordia 3 then separate into two segments, each a primordium for a future row. The cell thus forms, for both the proter and the opisthe, four primordial streaks originating from three original rows (Pl. IV 16). Subsequently, the kinetosomes within the primordia proliferate and migrate both anteriorly and posteriorly. The posterior region of the rows 1, 2 and 4, later become organized in small basal plates which give rise to three new caudal cirri in each future tomites (Pl. IV 17).

A second set of two new kineties for the future proter and opisthe are later differentiating from the anterior ends of each new developing right MCPs and extends afterwards to the posterior region (Pl. III 13).

At the end of the cortical morphogenesis, all old ciliary organelles are resorbed after cytokinesis. During the cell constriction, the macro-

nuclear nodes fuse and fragmentate into eight lobes, four of them passing to each daughter cell (Pl. V 18).

Morphogenesis of Regeneration. The treatment with urea results in the removal of the anteromost membranelles of the AZM (with partial or total disorganization of the Paroral Membranes) and a variable number of frontal cirri on a morphostatic cell (Pl. V 19). The subsequent morphogenesis, which takes approximately 3.5 h, results in a complete set of ciliary structures.

The initial stage in these damaged cells can be seen at one hour after treatment and begins with the OP induction in association with the TC 1 (Pl. V 20). This kinetosomal field subsequently increases in size by kinetosomal proliferation and migrates toward the cytostomal region. However, an interesting difference with divisional morphogenesis can be appreciated, that is, the appearance of a new group of kinetosomes close at the left margin of ventral cirri located near the developing OP (Pl. V 20). Then, these cirri disaggregate into kinetosomes which will become incorporated into the early OP as it continues migration anteriorly (Pl. V 21).

Half an hour after initiation, the developing OP reaches peristomal vortex and the FVT primordium is formed by proliferation of kinetosomes from both right anterior region of the OP and two parental cirri which disintegrate and rearrange their kinetosomes into the cirral streaks (Pl. VI 22). This initially anarchic field becomes later arranged into five fine longitudinal rows from which new FVT cirri for the regenerating cell derive by fragmentation of cirral streaks into the individual cirri (Pl. VI 23).

The Paroral Primordium, formed in a similar fashion as in division, differentiates from its right anterior portion a new Buccal Cirrus. Simultaneously, the OP, which has organized into membranelles, fuses with the remaining parental membranelles following resorption of the cytostomal portion of the old AZM (Pl. VI 24). This Buccal Cirrus then migrates toward an anterofrontal cortical position and assumes a location within the frontal cirri group.

Marginal Cirral Primordia (MCP) appear simultaneously by disaggregation of existing marginal cirri which are in approximately the same latitudinal plane as the FVT primordia. These MCP occur about two hours after regeneration was induced and elongate afterwards to the right of parental marginal cirri as the primordia become fully developed. Subsequently, the new marginal cirri segregate from the primordia and migrate posteriorly and anteriorly along the length of the regenerating cell (Pl. VI 24).

During final stages of this regenerative morphogenesis, healing of

the AZM is completed and all new formed undergo cirri migration to their final positions as old cirri not incorporated into primordia are resorbed (Pl. VI 25).

The major future dorsal bristles develop from only three primordia which give rise later to four kineties by fragmentation of primordium of dorsal row 3 into two segments. As the newly formed dorsal rows migrate anteriorly and posteriorly along the dorsal surface, the posterior ends of the rows 1, 2 and 4 each develops a single caudal cirrus. The other two minor dorsal bristles primordia for rows 5 and 6 are derived from the anteromost portion of the right MCP of the cell.

Discussion

The morphogenesis of division displayed by *Histriculus* sp. resembles those patterns described for other genera of *Oxytrichidae* and thus reinforces its previous inclusion into this group. Thus, the sequence of prefission morphogenesis initiates with the appearance of the Oral Primordium adjacent to TC 1 followed by the PPs, the FVT primordia, MC primordia and dorsal bristle primordia respectively, as all morphogenetic features shared by oxytrichous ciliates (Martin 1982).

The most important difference between the morphogenetic pattern during division and regeneration processes is the participation of some ventral cirri in the developing OP in regenerating cells. This significant alteration of the typical morphogenetic pattern parallel closely the results described on regenerative morphogenesis in the *Oxytrichous* ciliates *Laurentiella acuminata* (Martin et al. 1983) and *Gastrostyla steinii* (Nieto et al. 1981), where similarly damaged cells shared an amplification of the area of appearance of the OP toward the anterior portion of the body.

The anterior amplification of the stomatogenic area in such regenerants, i.e., the participation of kinetosomes derived from several ventral cirri into the developing OP, can be explained with the model previously postulated by Martin, assuming a repressive effect of the AZM or related infraciliature on the stomatogenic area. This effect is lost as a consequence of the partial remove of the oral membranelles after the treatment with urea and resulting in the participation during the regeneration of some preexisting ventral cirri which are normally not included in the stomatogenic area. Such inhibitory action of the AZM has been also postulated by Tartar (1961) and Hyvert et al. (1972) in *Stentor* by using microsurgical technics.

REFERENCES

Aufderheide K. J., Frankel J. and Williams N. E. 1980: Formation and positioning of surface-related structures in *Protozoa*. Microbiol. Rev., 44, 252–302.

Borror A. C. 1972: Revision of the Order *Hypotrichida* (*Ciliophora, Protozoa*). J. Protozool., 26, 1–22.

Borror A. C. 1979 a: *Cladotricha* and phylogeny in the Suborder *Stichotrichina* (*Ciliophora, Hypotrichida*). J. Protozool., 26, 51–55.

Borror A. C. 1979 b: Redefinition of the *Urostylidae* (*Ciliophora, Hypotrichida*) on the basis of morphogenetic characters. J. Protozool., 26, 544–550.

Calvo P., Torres A., Navas P. and Pérez-Silva J. 1983: Complex carbohydrates in the cyst wall of *Histriculus similis*. J. Gen. Microbiol., 129, 829–832.

Corliss J. O. 1975: Taxonomic characterization of the superfamilial groups in a revision of recently proposed schemes of classification for the Phylum *Ciliophora*. Trans. Am. Microsc. Soc., 94, 224–267.

Corliss J. O. 1979: The Ciliated Protozoa: Characterization, Classification and Guide to the Literature. 2nd ed. Pergamon Press, London.

Frankel J. 1973: The positioning of ciliary organelles in hypotrich ciliates. J. Protozool., 20, 8–18.

Gutierrez J. C. and Pérez-Silva J. 1983: Ultrastructural aspects of the precyst and cyst cytoplasm of the hypotrichous ciliate, *Laurentiella acuminata*. Acta Protozool., 22, 203–210.

Hyvert N., Pelvat B. and de Haller G. 1978: Morphogenése expérimentale chez les ciliés: Sur le rôle de la zone de membranelles adorales dans la regeneration chez *Stentor coeruleus*. Rev. Suisse Zool., 79, 1060–1068.

Jerka-Dziadosz M. 1974: Cortical development in *Urostyla*. II. The role of positional information and preformed structures in formation of cortical pattern. Acta Protozool., 12, 239–274.

Kahl A. 1932: Urtiere order *Protozoa*. I. Wimpertiere order Ciliata. 3. *Spirotricha*. In: Die Tierwelt Deutschlands, (ed. Dahl F.) 25, 399–650.

Levine N. D., Corliss J. O., Cox F. E. C., Deroux G., Grain J., Honigberg B. H., Leedale G. F., Loeblich A. R., Lom J., Lynn D., Merinfeld E. G., Page F. C., Poljansky G., Sprague V., Vavra J. and Wallace F. G. 1980: A newly revised classification of the *Protozoa*, 27, 37–58.

Martin J. 1982: Evolution des patrons morphogénétiques et phylogénèse chez les *Sporadotrichina*. Protistologica, 18, 431–447.

Martin J., Fedriani C. and Pérez-Silva J. 1983: Morphogenetic pattern in *Laurentiella acuminata* (*Ciliophora, Hypotrichida*): its significance for the comprehension of ontogeny and phylogeny of hypotrichous ciliates. J. Protozool., 30, 519–529.

Matsusaka T. 1977: Induction of synchronous encystment in a hypotrichous ciliate, *Histriculus* sp. Exp. Cell Res., 110, 459–462.

Matsusaka M. 1979: Effect of cycloheximide on the encystment and ultrastructure of the ciliate, *Histriculus*. J. Protozool., 26, 619–625.

Matsusaka M. and Kimura S. 1981: Changes in macronuclear structures during encystment in a ciliate, *Histriculus muscorum*. Kunamoto J. Sci. Biol., 15, 49–58.

Nieto J. J., Calvo P., Torres A. and Pérez-Silva J. 1981: Régéneration chez *Gastrostyla steinii*. Acta Protozool., 20, 373–384.

Sendo Y. and Matsusaka T. 1982: Changes in two acid hydrolase levels during cyst differentiation of a ciliate, *Histriculus muscorum*. J. Protozool. 29, 125–129.

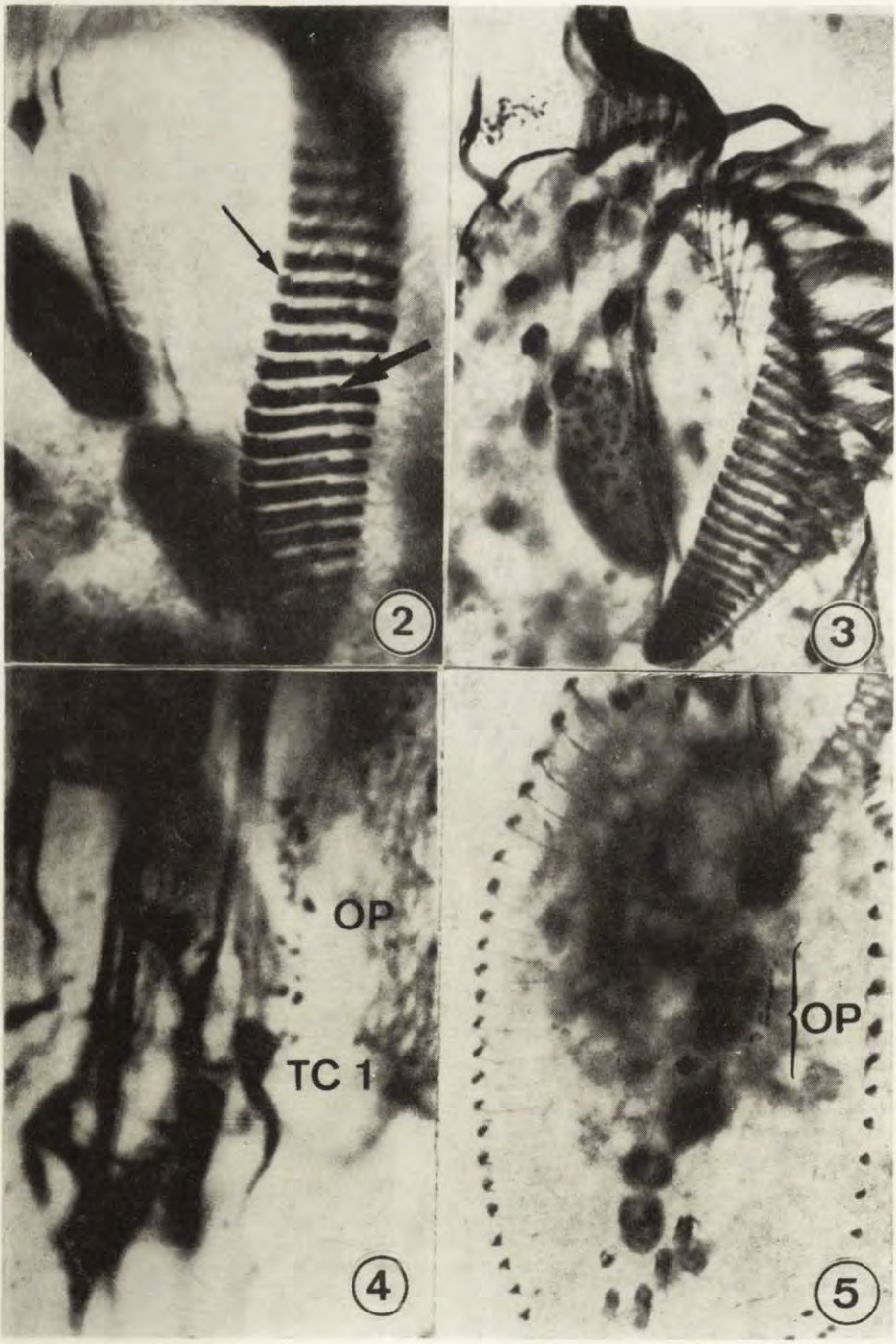
Tartar V. 1961: The Biology of *Stentor*. Pergamon Press, London.

Tuffrau M. 1967: Perfectionement et pratique de la technique d'impregnation au Protargol des infusories Ciliés. Protistologica, 3, 91–98.

EXPLANATIONS OF PLATES I–VI

All figures are Protargol stain photomicrographs of *Histriculus similis*. Except for Figs. 15–18, each figure is shown with the anterior end at the top in ventral view, with the viewer's left corresponding to the cell's right

2 to 3: Morphostatic cells
2: Partial view of the AZM. Each membranelle consists in four rows of kinetosomes. Two of these rows are long and extend for equal distances, the third is slightly shorter (large arrow) and the fourth is the shortest (small arrow). × 1400
3: Frontal cirri and Paroral Membranes. × 1100
4 to 16: Succesive division stages
4: The initiation of an Oral Primordium (OP), appearing at left margin of the first transverse cirrus (TC 1). × 2000
5: Anterior location of the OP in its early development. × 750
6: Initially unorganized appearance of the OP showing the proliferation of kinetosomes. × 520
7: Early stages of the formation of the fronto-ventro-transverse primordia (FVTP). Frontal and ventral cirri can be seen disaggregating (large arrows) as two ciliary streaks extend from the right anterior end of the OP (small arrows). × 880
8: The OP is organizating into membranelles (large arrow) as the FVTP are developing into streaks. × 950
9: Fully developed cirral streaks in a future opisthe showing the formation of the Paroral Primordium (PP). × 2200
10: Cell at the initiation of a Right Marginal Cirral Primordia (RMCP) in a future proter. Note the beginning of disaggregation at the anterior end of the parental Paroral Membranes (arrow). × 700
11: Early stages of segregation of new cirri from the cirral streaks. Marginal Cirral Primordia of both proter and opisthe are developing at right of parental marginal rows (arrows). × 720
12: Future proter of the cell shown in Fig. 11. Buccal Cirrus (BC) is seen differentiating from the anterior portion of the PP. × 1500
13: Cell after formation of all ciliature derived from the new FVTP. A new dorsal kineties of the future opisthe can be seen separating from the new marginal cirri (arrow). × 720
14–15: Migration of new cirri is in progress as the macronuclear nodes fuse and then fragmentate. × 500
14: Cell in cytokinesis. The arrows point to old caudal cirri. × 500
15–18: Succesive stages in the development of the dorsal kineties
15: Early dorsal primordia both in future proter and opisthe forming within dorsal rows 1–3. × 750
16: The primordia originally within row 3 have separated into two segments, each a primordium for a future row. × 750
17: Dorsal surface of a cell, prior to constriction. Note the formation of caudal cirri at the posterior end of three of the four new dorsal rows (arrows). × 600
18: Dorsal surface of a cell in cytokinesis. Note the resorption of the old caudal cirri (arrows). × 530
19–25: Regenerating cells
19: A cell stained immediately after removal of the anterior portion of the cell. The absence of several frontal cirri and the anteromost portion of the AZM can be seen. × 1000
20: Early stage in the development of the OP. Note the appearance of new kinetosomes close to a ventral cirrus (arrow). × 3500
21: Incorporation into the developing OP of kinetosomes from disaggregating ventral cirrus (arrow) as it migrates anteriorly. × 1500
22: An early stage of the FVTP development as it organizes into regular streaks (arrows). Note the breakdown of a ventral cirrus (large arrow). × 1500



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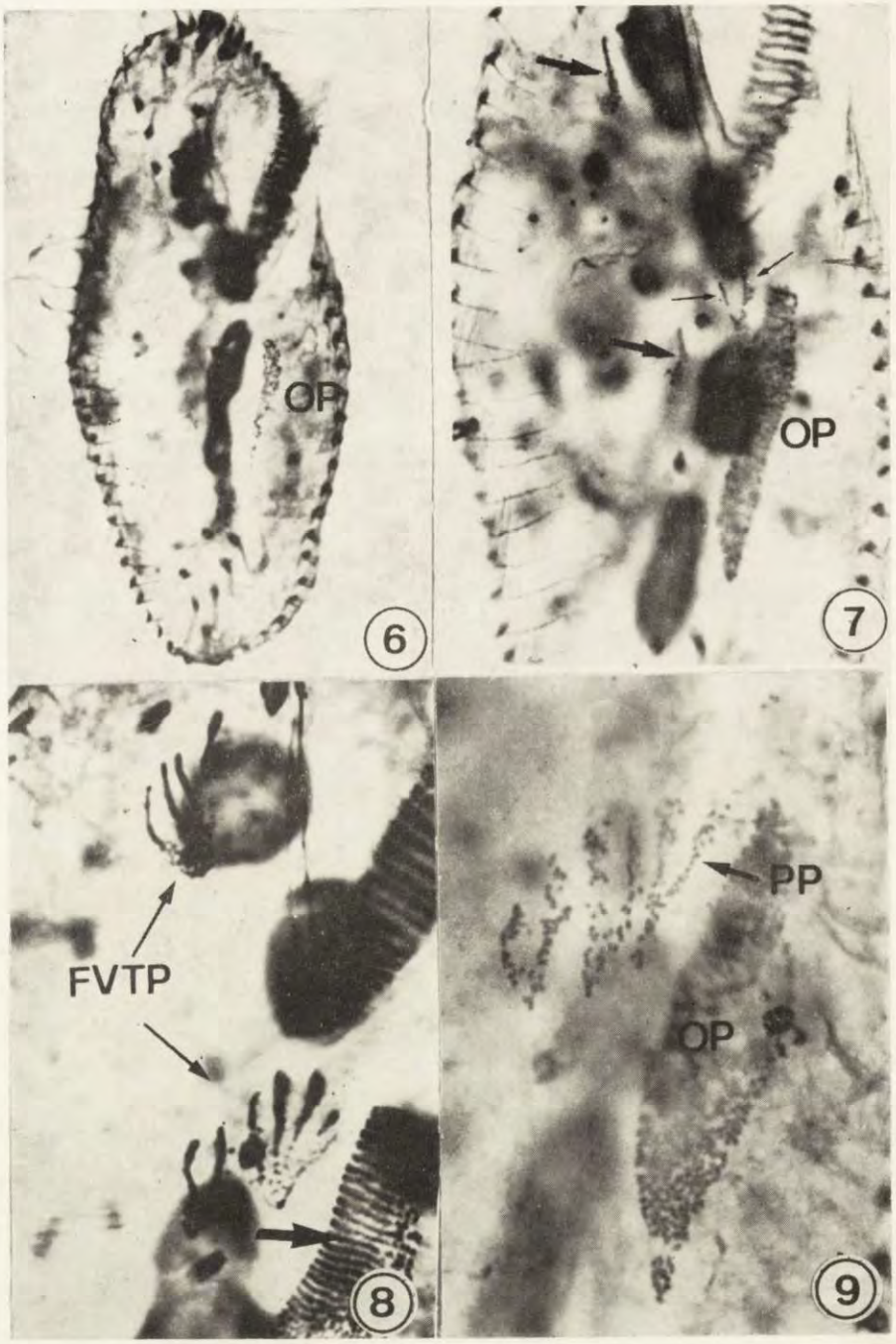

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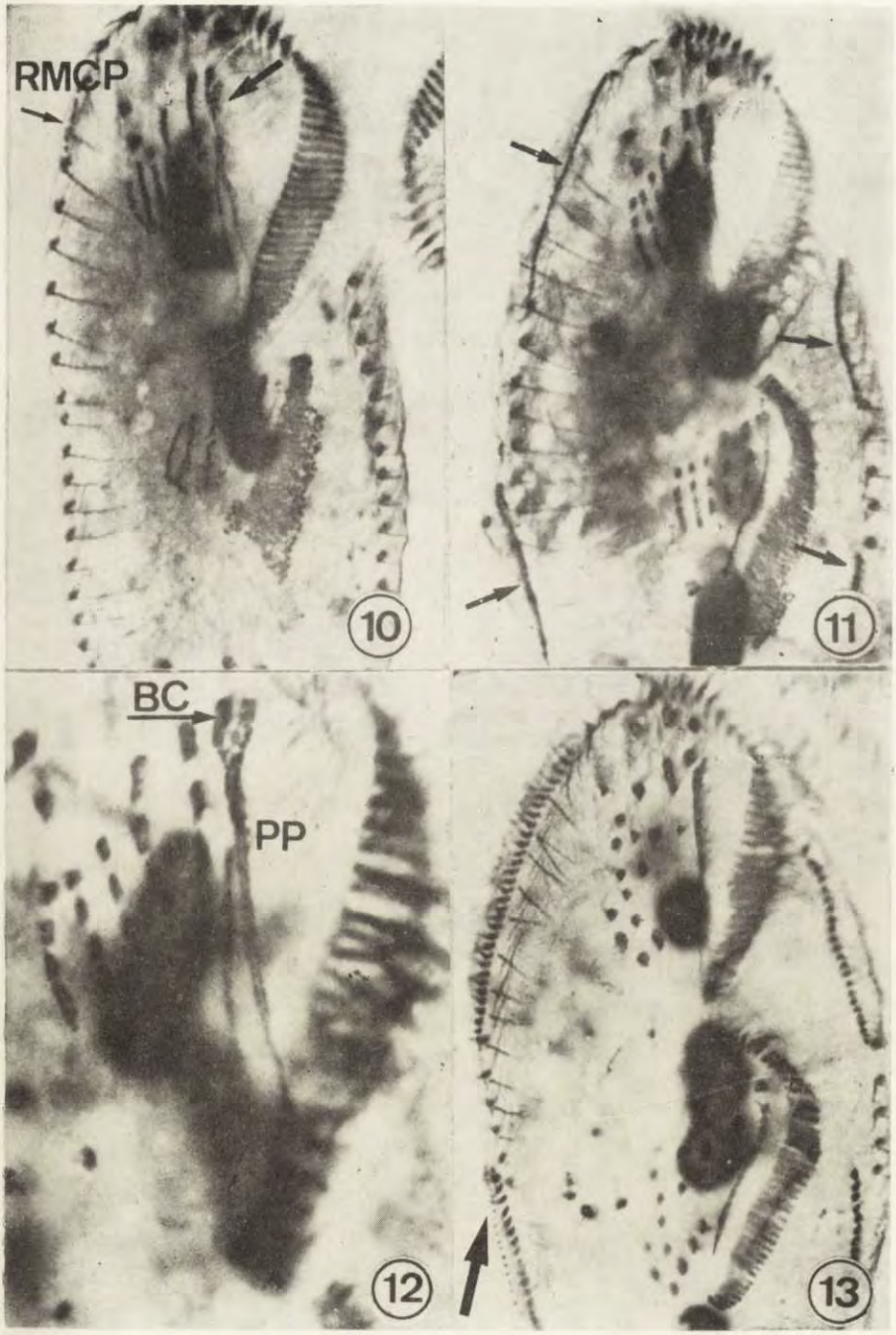

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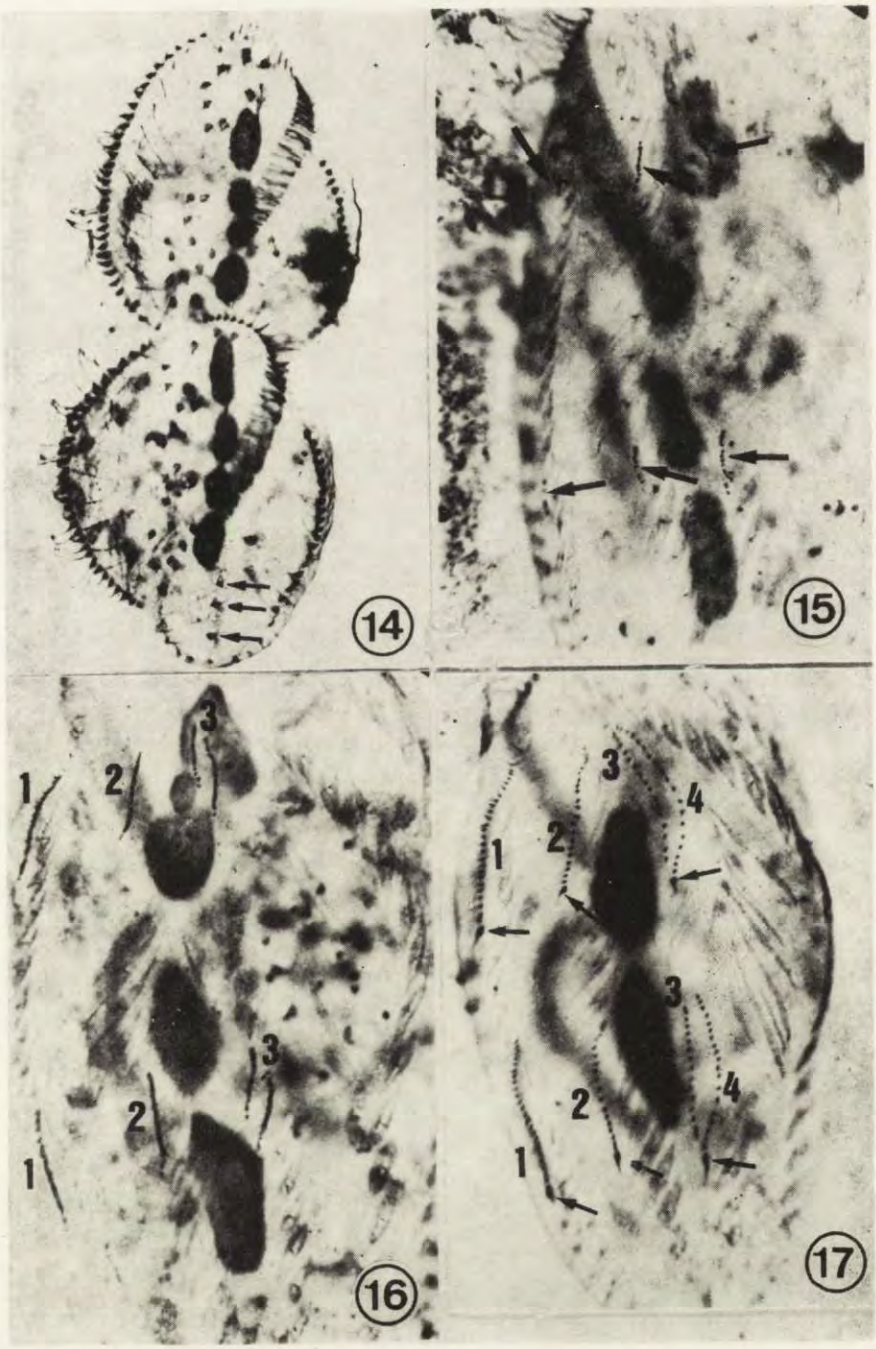

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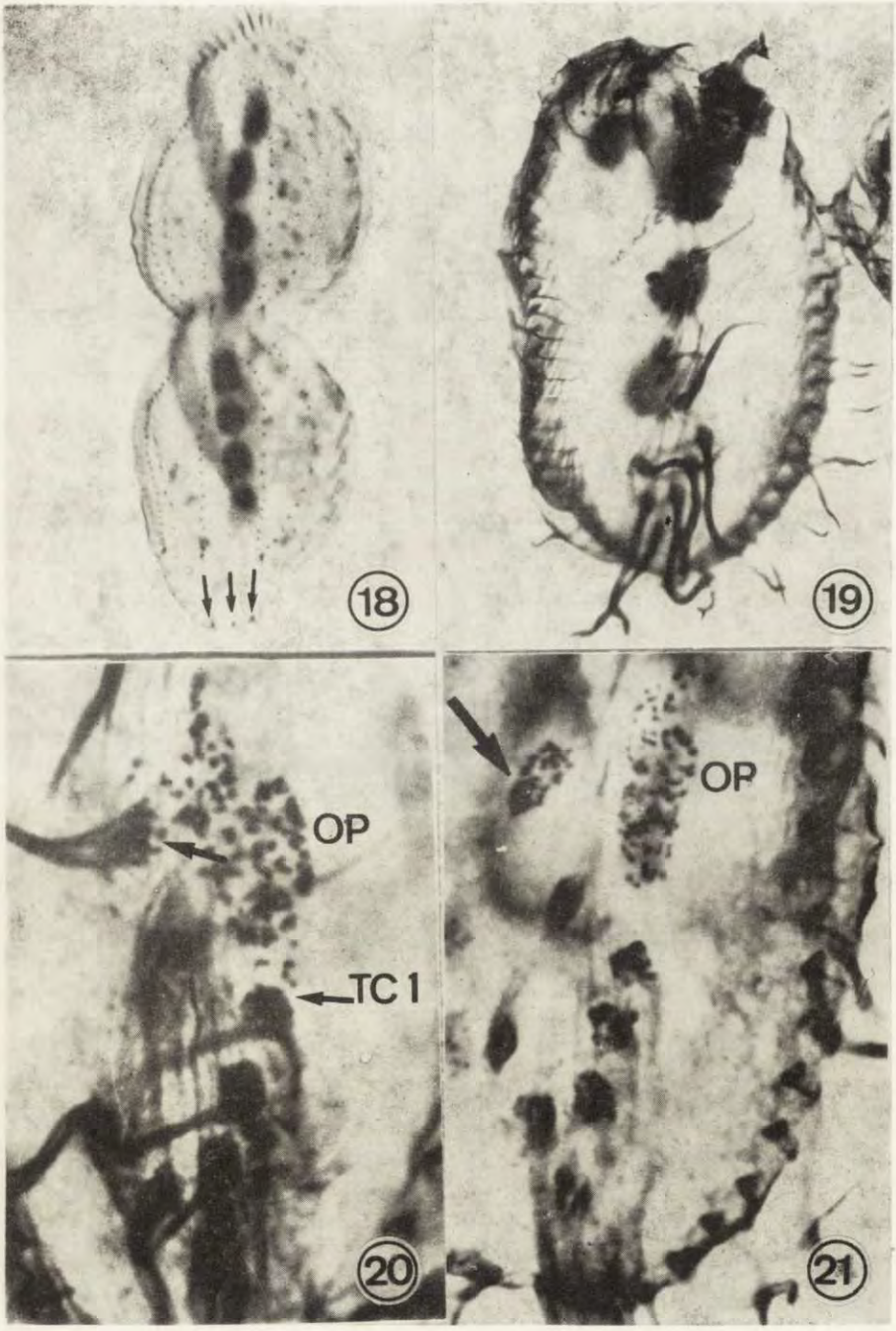

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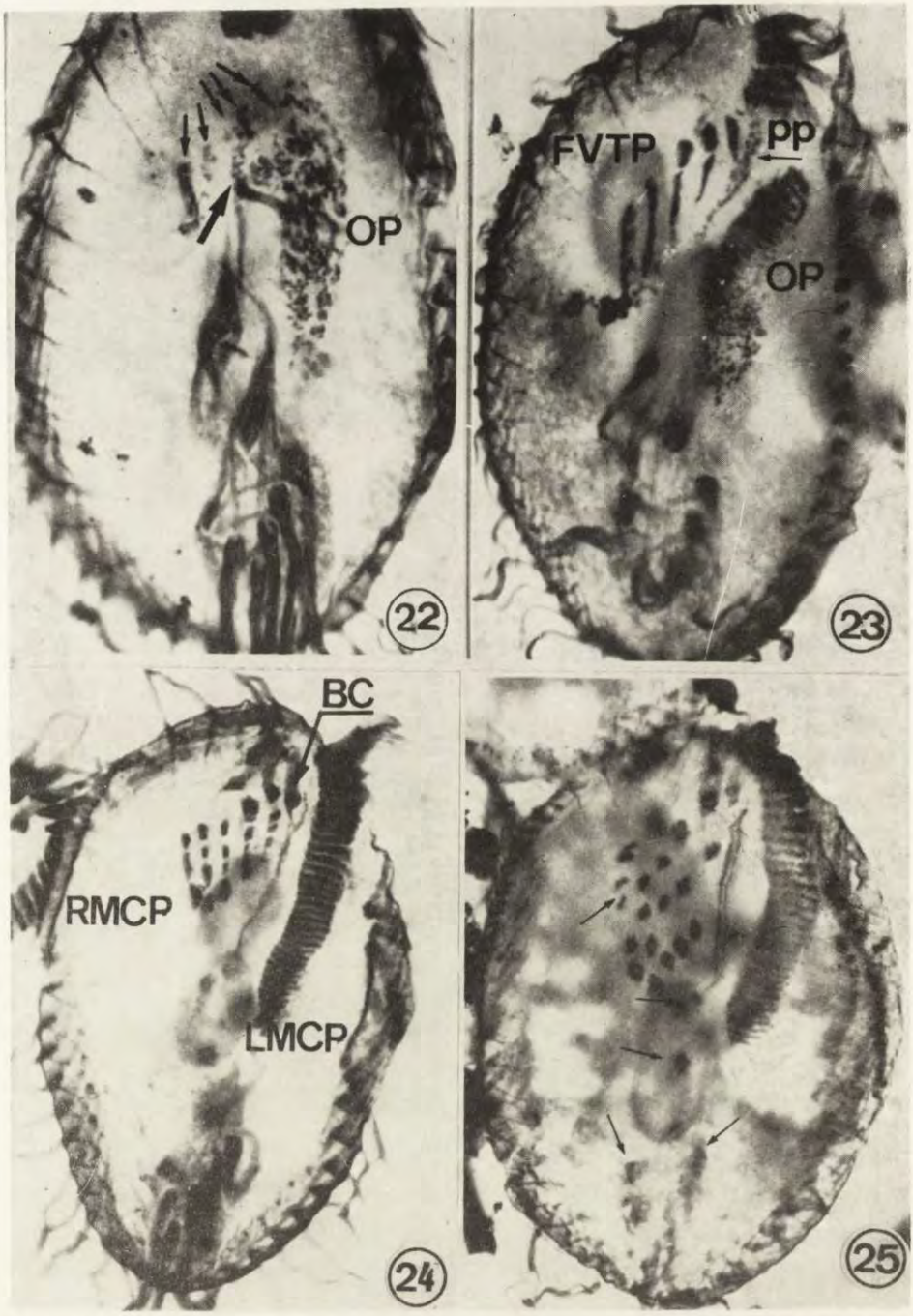

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23: The OP migrates toward the resorbing posterior end of the AZM as the FVTP is aligned into streaks and the PP is well developed. × 1300
24: Initiation of the RMCP and LMCP by disaggregation of existing marginal cirri. The cirral streaks have differentiated into individual crri as the OP has faused to the AZM to reestablish a complete AZM. Note the formation of Buccal Cirrus (BC) from the right anterior portion of the PP. × 1200
25: Cell near completion of regeneration. At this stage, the new structures are migrating to their final positions as old cirri are resorbed (arrows). × 1500

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Susceptibility of Food Chemoreceptors in Carnivorous *Protozoa*

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Synopsis. The effects of some factors (changes in temperature of cultivation, quality of applied food, then the influence of starvation, ethanol and Tween 40 solutions), which are capable to modify the physicochemical properties of cell surface membrane, on chemosensitivity in carnivorous ciliates, *Dileptus anser* and *Didinium nasutum* towards different phagocytosis inducers, were investigated *in vivo*. The quantity of specific chemical feeding inducers was found as unequal in diverse *Dileptus* clones. The agents evoking fluidization of the cell surface membrane are suggested as modulating the activity of feeding chemoreceptors. It is also supposed, on the base of own and other authors findings that alterations of feeding chemosensivity in these ciliates are concerned with adenylocyclase complex.

Carnivorous *Protozoa* possess a well developed chemoreceptor system responsible for discrimination and estimation of chemical properties of their prey (Seravin and Orlovskaja 1972, 1977). With the help of elaborated earlier method of chemical food models (CFM) it was possible to find several inducers of feeding responses engaged in the process of the capture and ingestion of food model objects by observed carnivorous species. The following compounds are, among others, able to induce the process of phagocytosis: adrenaline, some aminoacids, spermidine, phosphatidylcholine, phosphatidylethanolamine and some neutral detergents such as Tweens 40, 60 and 80 (Seravin and Orlovskaja 1977, Samovar and Orlovskaja 1979, Orlovskaja 1982). Particles of neutral adsorbents, carbon or starch, soaked in solutions of feeding inducers are actively eaten by predators. On the other hand, however, it was also shown that intensity of ingesting of one and the

same type of CFM by one species or clone may be not always alike (Orlovskaja and Seravin 1978).

It is known from Hevitt's (1978) work that transduction of cell sensibility is growing proportionally to the number of ligandly joined receptors. Accordingly to this fact the activity of phagocytic reaction should reflect the activity of protozoan chemoreceptor complexes.

Most of cytoreceptors are closely associated with plasma membrane of the cell surface. As it is known from several findings the properties of the large group of membrane-bound enzymes depend on physico-chemical state of the membrane (Cuatrecasas 1974, Pertseva 1981). There are such enzymes as: Na^+, Ka^+-ATP-ase, 5-nucleotidase, as well as sarcoplasmic reticulum enzymes: Ca^{+2}, Mg^{+2}-ATP-ase and others (see Rimon et al. 1980).

Lipid composition and microviscosity of the membranes seems to be their main physico-chemical characteristic. Recently, as a common model object used by "membranologists" in such kind of studies is the ciliate, *Tetrahymena*. The investigations of the last years show that the correlation between membrane lipids composition, the level of unsaturated fatty acids determining this composition and probably fluidity of *Tetrahymena* cell membrane can be easy modified *in vivo* (Fukushima et al. 1976, Shimonaka and Nozawa 1977, Thompson and Nozawa 1977). Starvation, changes in temperature of cultivation, addition to the culture medium of the exogenic fatty acid, or certain compounds influencing the cell membrane viscosity (ergesteron, hexadecyglicerol, ethanol) effect the alterations in fluidity of membrane lipids and, respectively, in activity of the enzymes (Thompson et al. 1972, Nozawa et al. 1974, Nozawa and Thompson 1979, Shimonaka and Nozawa 1977, Shimonaka et al. 1978, Thompson and Nozawa 1977, Nandini-Kishore et al. 1979, Kasai et al. 1981).

In presented work the effect of agents influencing *in vivo* on physico-chemical properties of the cell membrane in carnivorous *Protozoa* and alterating their chemosensitivity towards several inducers of phagocytosis has been studied. In experiments starvation, changes in temperature of the cultures and quality of food, then, the action of ethanol and Tween 40 solutions were applied.

Material and Methods

The experiments have been carried out on clonal cultures of *Dileptus anser* and *Didinium nasutum*. *Tetrahymena pyriformis* was used as a food for them. The method of chemical food models (CFM) was applied as a test of ciliates che-

mosensitivity (see Seravin and Orlovskaja 1977). The following compounds were used as inducers of phagocytosis: 10^{-3}M conc. of several aminoacids (Reachim), 0.2% conc. of phosphatidylcholine (Reachim), 0.2% conc. of phosphatidylethanolamine (FGB), 10^{-3} M conc. of spermidine (Serva), 0.5% conc. of Tween 40 (LTB) and water extract of hen liver.

Feeding response of the animals towards different CFM types was observed on 1 ml volume depression slides where 20–60 specimens together with 0.1 ml of culture medium were placed. Then, 0.01 ml of earlier prepared CFM was there added. The reaction of animals was estimated after 1 h of stay in the presence of feeding inducers. The intensity of reaction was characterized by the portion of specimens, eating CFM. Every variant of feeding reaction was repeated 6–9 times paralelly in experimental and control conditions. The mean number of phagocytizing specimens and its standard error were calculated for each variant of repeated series. To estimate the level of probability, Student's statistic method was used.

Observations on Influence of Starvation on Activity of Feeding Chemoreceptors in *Dileptus anser*

Three clones of *D. anser*, conventionally called: 10–34 "Bolšaja izora" and EK-1 were used in these experiments. The animals were cultivated as previously in Prescott solution, at *t* 20°C and pH 6.8 (Prescott and James 1955). After 1 h from the last feeding of *Dileptus* by living *Tetrahymena* the testing feeding with CFM took place, then repeated after each 24 h of starvation (each of these testing feedings continued during 1 h).

Observations on Influence of Various Temperature of Cultivation on Feeding Chemosensitivity in *Dileptus anser*

The experiments were carried out on two clones: of *D. anser* "P" and 24. Both of them were cultivated for 7 days in Prescott solution. The temperature in experiments was: 11, 17, 24 and 30°C, while control animals were kept always at 20°C. Before taking to the experiment all animals were starved for 48 h. Chemosensitivity towards five types of aminoacids: serine, methionine, cysteine, α-alanine and threonine, afterwards towards phosphatidylcholine and solution of Tween 40 was tested. Observations of food chemosensitivity were made at the temperature of cultivation.

Observations on Influence of Food Quality on Chemoreceptors Activity in *Dileptus anser* and *Didinium nasutum*

Clone 10–34 of *Dileptus anser* and clones 6 to 9 of Peterhof population of *Didinium nasutum* were used in these experiments.

During cultivation *Didinium* was fed by *Tetrahymena pyriformis*. To test the feeding response every from its four clones was divided to two parts (cultures).

The first one started to be fed by *Paramecium* (culture TP_1), the second, by *Tetrahymena* as previously (Culture TT_1) [1].

The proper experiment began after 30 days of applying such procedure. During long lasting experiments changes of food quality were made three times in following way: animals from culture TT_1 started to receive as a food *Paramecium* (culture TT_1P_2) and vice versa, these from TP_1 were transferred to the medium with *Tetrahymena* (culture TP_1T_2). Furthermore, with periodicity of 15–20 days analogical reciprocal changes of food objects in these newly received cultures were made. During every change of nutrition quality a part of *Didinium* culture was left as a control, fed in the same way as previously, 12–15 days after every change feeding response of *Didinium* towards "liver" CFM was tested. (Water extract of hen liver was used to prepare so called "liver" CFM described in the work of Seravin and Orlovskaja 1977).

In experiments with *Dileptus anser* changes of feeding response towards cysteine, phosphatidylcholine and "liver" CFM during cultivation the ciliate on normal, control ("C") *Tetrahymena pyriformis* and on phosphatidylcholine ("P") *Tetrahymena* were tested. Special "phosphatidylcholine" stain of *Tetrahymena pyriformis* was prepared after Irlina and Merkulova (1975), by addition to common aminopeptide nutrient medium phosphatidylcholine at 10^{-5}% conc. Strain incubated three days in these conditions was applied as food for *Dileptus*.

The Influence of Ethanol and Tween 40 Solutions on Feeding Chemosensitivity in *Dileptus anser*

The experiments were carried out on six *Dileptus* clones: P-1, 24, K-7, d-2, d-20, d-24. The boiled water from river Neva at temperature 20°C and ph 6.0–6.5 was used in this case as the culture medium for the ciliates. The animals were fed as always by *Tetrahymena pyriformis*. Observations of chemosensitivity were made on specimens incubated for 6 days with ethanol at concentrations: 5×10^{-2}% and 1.6%, with Tween 40 of 5×10^{-3}% conc. and on control animals growing without these compounds. Inducers of phagocytosis were: 24 aminoacids at 1×10^{-3}% conc., phosphatidylcholine at 0.2% conc., phosphatidylethanolamine at 0.2% conc. and Tween 40 at 0.5% conc.

Results and Discussion

The Influence of Starvation on Activity of Food Chemoreceptors in *Dileptus anser* (see also Orlovskaja and Seravin 1978)

Data received in experiments on the process of phagocytosis induced by different types of CFM applied in different stages of *Dileptus* starvation (clone "Bolšaja izora") are presented in Table 1. One hour starved animals were nearly not sensitive to anyone type of CFM (reaction

[1] To mark off the type of culture the letter system was used (T — *Tetrahymena* type culture, P — *Paramecium* type). The adequate cipher indicates the ordinal number of every change.

Table 1

The influence of starvation on chemosensitivity of *Dileptus anser*, clone "Bolšaja Izora"

Time of starvation (in hours)	The number of specimens (%) phagocytizing CFM of following types:		
	Cysteine	Phosphatidylethanolamine	Tween 40
1	8.2± 6.3	4.2± 1.4	0.7±1.8
24	52.3± 7.2	19.8± 7.3	36.1±6.7
48	54.5±13.3	45.5±12.7	62.1±9.9
72	39.8± 7.7	22.2± 8.7	42.0±7.9
96	21.9±10.6	18.0± 8.0	10.9±3.4
120	10.1± 3.7	6.0± 3.4	2.2±0.7
144	5.0± 7.4	0	0
168	8.3± 2.3	2.0± 1.3	0.5±0.3
192	5.6± 2.2	0	0

lower than 10%). Phagocytic sensitivity to all CFM types was restored after 24 h of starvation. Maximal level of feeding reactivity was reached after 48 h of standard feeding. Increasing period of starvation characterized in decreasing level of all types CFM phagocytosis. On the fifth day from the beginning of experiments sensitivity of *Dileptus* to food models was completely inhibited. Similar results were get with clones of *Dileptus* 10–34 and EK-1.

It is known that during starvation of *Tetrahymena pyriformis* utilization of membrane proteins and phospholipids is observed. In this situation appropriate amount of specific for this species neutral lipid, tetrahymanol, is growing. That causes the changes in fluidity of *Tetrahymena* cell membrane (Thompson and Nozawa 1977, Kasai et al. 1981). It is reasonably to suggest that similar events may take place also during the process of starvation in *Dileptus anser*. The alteration in sensitivity to the inducers of phagocytosis reflects, probably, the alterations in physico-chemical stage of membrane.

The Influence of Temperature on Feeding Chemosensitivity in *Dileptus anser*

The received results pointed out to specific dependence of *Dileptus* chemosensitivity on the temperature in the cultures. Results of these experiments are presented in Table 2. Phagocytosis of all types of CFM in clone "P" used here, kept at *t* 11°C, was completely inhibited (less than 5% of feeding response). When temperature in the culture was grown to 17°C phagocytic activity of animals also increased up to 15–20% of specimens towards all examining aminoacids. Reaction to

Table 2

The influence of temperature on phagocytosis of CFM in *Dileptus*, clone "P"

$t°$	The number of specimens (%) phagocytizing CFM of following types:						
	Serine	Methionine	Cysteine	α-Alanine	Threonine	Tween 40	Phosphatidylcholine
11	1.5±1.5	4.7±2.2	4.5±3.3	1.5±1.5	0	0	0
17	18.9±7.6	22.3±5.9	15.1±4.7	17.6±6.5	21.3±6.1	43.0±3.7	8.2±3.0
24	37.2±9.3	47.8±5.7	25.7±6.7	50.4±5.4	45.7±9.7	47.7±9.7	32.9±9.3
30	16.4±5.7	26.7±9.3	18.5±5.4	35.5±5.1	25.2±8.4	39.2±0.5	14.6±7.0

Tween 40 models was in this case near of its maximal level, i.e., 43.0 ± 3.7%. However, the difference between these two results, received at 11 and 17°C was statistically not significant. Phagocytosis of phosphatidylcholine models in *Dileptus* kept at *t* 17°C left on rather low level, 8.2 ±3.0%. When they were cultivated at *t* 24°C their sensitivity to all aminoacids and to phosphatidylcholine CFM increased to maximal values. Increasing of temperature to 30°C evoked decreasing of sensitivity to all types of CFM. Similar results were observed in experiments with *Dileptus* clone 24.

Data concerning changes of temperature during cultivation of *Tetrahymena pyriformis* show the influence of these changes on desaturase CoA activity, which affects alterations of saturated and unsaturated fatty acids, components of membrane phospholipids. That, of course, evokes alterations in the cell membrane fluidity (Fukushima et al. 1981, Nozawa 1981). Similar facts are also observed in Metazoan cells (Housley et al. 1976). May be that such events take place inside *Dileptus* cell membrane as well. Probably for effective action of chemical inducer of phagocytic processes an appropriate level of membrane fluidity is needed. Such optimal level, necessary for the work of receptor complex has been found when *Dileptus clone* "P" was cultivated at *t* 24°C.

The Influence of the Quality of Ingested Food on Chemoreceptors Activity in Carnivorous Ciliates, *Didinium nasutum* and *Dileptus anser*

Sensitivity of feeding response towards chemical inducers in predator species can be largely regulated dependently on properties of ingested food. Experiments performed on four clones of *Didinium nasutum* showed that "liver" CFM could be uptaken only in the case if animals were fed during cultivation by *Paramecium caudatum*. Specimens of the same clone growing in the cultures with *Tetrahymena py-*

riformis, were indifferent to "liver" CFM. Feeding sensitivity to the "liver" CFM in the ciliates cultivated in such conditions appeared in 20–30 days after change of food objects. This fact has been found in longlasting observations, which were carried out during four months. Results concerned two paralel lines of clone 8, TP_1 and TT_1 are presented in Fig. 1. It is possible to see there that specimens from TP_1 line uptake easy "liver" CFM. After removing to the medium with *Tetrahymena* (now already line TP_1T_2) they lost this sensibility. A part of

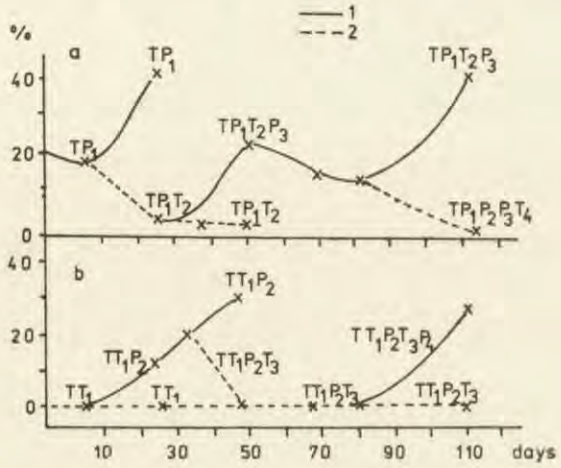

Fig. 1. Phagocytic reaction of *Didinium nasutum* during changes of food objects. a — Cultures fed at the beginning by *Paramecium*, b — Cultures fed at the beginning by *Tetrahymena*. *1* — Phagocytic reaction towards CFM in *Didinium nasutum* during cultivation on *Paramecium*, *2* — The same, during cultivation on *Tetrahymena*. *Ordinate*: percentage of specimens phagocytizing CFM. *Abscissa*: time in days

such culture was transferred again to the medium with *Paramecium* (line $TP_1T_2P_3$) and ability to uptake the "liver" CFM was restored. After the next changing of the food *Didinium* stopped ingesting of the "liver" models again (line $TP_1T_2\ P_3T_4$).

Similar results were received not only in experiments with paralel line TT_1 of clone 8, but also with all lines of clones 6, 7, 9.

In experiments with *Dileptus anser* changes in sensitivity towards "liver", phosphatidylcholine and cysteine CFM after 3–4 weeks cultivation of this predator in the medium with "normal" ("C") *Tetrahymena* and *Tetrahymena* reached by phosphatidylcholine ("P") were observed. Received results (Table 3) showed the significant increase in sensitivity towards all types of CFM in ciliates growing on "P" *Tetrahymena* line in comparison with these fed by "C" line.

It is known from the work of Thompson and Nozawa (1977)

Table 3

Feeding response of *Dileptus*, clone 10–30, cultivated on "P" and "C" *Tetrahymena* towards different types of CFM

Type of CFM	The number of specimens phagocytizing CFM, when *Dileptus* is growing on:	
	"C"-*Tetrahymena*	"P"-*Tetrahymena*
"Liver"	11.8±2.8	58.2± 8.1
Phosphatidylcholine	15.4±7.0	49.9±10.5
Cysteine	29.3±7.2	52.9± 7.7

that addition of exogenic unsaturated fatty acids to *Tetrahymena* culture medium effects in fluidity of its cell membrane. Phosphatidylcholine is one of very important phospholipid component of the cell membrane. It is characterized by accumulation of the greatest number of unsaturated bindings of fatty acids. The increase in unsaturated fatty acids in the membranes of ciliates modifies the fluidity of their membranes.

It is also suggested that greater chemosensitivity towards CFM in two investigated predators species simultaneous with changes of food quality, might be evoked by increase in the amount of required chemoreceptors or by activation the appropriate stages of transduction the inside stimulus to effectory structures. Inducable synthesis of chemoreceptors is well-known in other microorganisms (Keshland 1979). In *Tetrahymena* the changes in food substrate are known as regulating the processes of synthesis of specific enzymes during transcription (Roberts and Morse 1978). Probably in predator ciliates, the process of inducable chemoreceptor synthesis may take place after changing of the food quality. This way of chemosensitivity increasing with activate chemoreceptors of specific types, i.e., joined with qualitative changes of receptors activity. Similar alterations may appear also inside the cell membrane of *Didinium nasutum* when another than normal prey is experimentally applied.

The varied food properties evoke general increase in chemosensitivity of *Dileptus anser* and induces quantitative changes in its feeding reaction.

The modification of physico-chemical properties of membrane of the prey may be reflected in membrane composition of the carnivorous ciliated protozoan. It should be mentioned that in the earlier publication at this theme we gave another interpretation of the similar results (Orlovskaja et al. 1978).

The Influence of Ethanol and Tween 40 on Feeding Chemosensitivity in *Dileptus anser*

In these experiments feeding response of *Dileptus* towards various types of CFM in control and experimental groups was significantly different. In control specimens from six examined clones feeding response to cysteine CFM was practically absent (Table 4). After incubation at

Table 4

The influence of ethanol and Tween 40 on phagocytosis of cysteine CFM in *Dileptus*

Variant of experiment	The number (%) of phagocytizing specimens in clones[a]:					
	24	d-2	P-1	d-24	d-20	K-7
Ethanol 5×10^{-2}%	60.6±4.1	43.2±7.0	52.8±9.0	69.6±10.9	25.7±5.8	2.2±1.3
Tween 40 5×10^{-3}%	37.4±18.9	33.3±5.5	13.6±3.2	–	28.4±5.1	0
Control	1.5± 0.7	7.0±2.3	1.4±1.4	2.0± 1.0	1.1±0.7	0

[a] Without only clone K-7, the number of phagocytizing specimens in all clones in this experiment differs significantly from that in control ($P < 0.05$)

low concentration of ethanol (5×10^{-2} %) the process of phagocytosis of the cystein models was observed in 50–70% of specimens, while after stay at 5×10^{-3} conc. of Tween 40 in 15–40%. Clone K-7 seemed to be exceptional case. These ciliates were indifferent to the cystein inducer both in the control and in the experiment. It seems interesting that the ciliates of this clone tested periodically for 5 years showed the lack of sensitivity to cysteine CFM. Their reaction towards other CFM types was also enough low, both in control and experimental groups (Tables 5, 6, 7). They, however, fed easily "liver" CFM. Positive feeding response reached in such case was ca. 70% of control level.

Phagocytosis of phosphatidylethanolamine acid Tween CFM in *Dileptus* from another clones incubated at 5×10^{-2} % conc. of ethanol was 3–7 times more intensive than in control specimens (Table 5, 6). In clones P-1 and "24" feeding response to phosphatidylcholine CFM showed sufficiently significant increase (Table 7). In incubated at 5×10^{-2} % conc. of ethanol clones: d-2, d-20 and d-24 sensitivity to phosphatidylcholine was not observed. Since all "d" clones derived from one and the same population the above data might be explained as a result of their common origin.

Dileptus kept at 1.6% conc. of ethanol appeared full inhibition of

Table 5

The influence of ethanol and Tween 40 on phagocytosis of phosphatidylethanolamine CFM in *Dileptus*

Variant of experiment	The number (%) of phagocytizing specimens in clones:			
	24	d-20	d-24	K-7
Ethanol 5×10^{-2} %	69.6±2.6[a]	63.2±9.9[a]	98.9±1.0[a]	18.1±8.4
Tween 40 5×10^{-3} %	14.3±5.7	44.1±7.4	–	3.1±1.8
Control	11.0±4.6	25.8±8.9	1.9±1.9	3.7±2.1

[a] Differs significantly from control value ($P < 0.05$)

Table 6

The influence of ethanol on phagocytosis of Tween CFM in *Dileptus*

Variant of experiment	The number (%) of phagocytizing specimens in clones:			
	24	d-20	d-24	K-7
Ethanol 5×10^{-2} %	85.0±4.5[a]	34.1±8.3[a]	83.8±4.5[a]	5.0±1.6[a]
Control	20.8±6.8	11.3±2.5	31.6±16.0	3.0±1.4

[a] Differs significantly from control value ($P < 0.05$)

Table 7

The influence of ethanol and Tween 40 on phagocytosis of phosphatidylcholine CFM in *Dileptus*

Variant of experiment	The number (%) of phagocytizing specimens in clones:					
	24	d-2	P-1	d-24	d-20	K-7
Ethanol 5×10^{-2} %	93.2±2.6[a]	49.5± 8.1	78.8±5.0[a]	82.9±12.2	23.5±4.3	11.4±2.3
Tween 40 5×10^{-3} %	76.3±5.2	41.4±12.2	55.8±5.2	–	20.6±4.9	6.6±2.6
Control	62.7±7.0	62.1± 8.4	35.0±9.8	68.3±8.4	13.4±3.5	6.9±1.9

[a] Differs significantly from control values ($P < 0.05$)

phagocytosis towards all CFM types. However, these animals ingested intensively *Tetrahymena*, their normal food. This clearly shows that 1.6% conc. of ethanol does not damage wholly the mechanism of phagocytosis in *Dileptus*, but involves in deprivation of chemosensitivity towards certain inducers of feeding response.

Cultivation of any clones of *Dileptus anser* at 5×10^{-3} % conc. of Tween 40 expressed in increasing of phagocytic activity in relation to aminoacids, though feeding response to phospholipid FCM was not changed (Table 6, 7). Similar result were also received earlier (Orlovskaja and Brutkowska 1978).

Thus, due to the facts received in this work it is seen that sensitivity of *Dileptus* to cysteine and phosphatidylethanolamine CFM is stimulated by low concentration of ethanol, while Tween 40 effects only on aminoacid receptors. Chemoreceptors sensitivity observed in ethanol and Tween 40 solutions is probably of different character.

Ethanol effects specifically on animal membranes. When added in low concentrations to *Tetrahymena* culture medium it influences on changes in lipid composition and in this way affects fluidity of the membranes (Nandini-Kishore et al. 1979). Used at concentrations of 1% effects similarly on the synaptic plasma membranes from the brain of rats (Sun and Seaman 1980) and on bilaminar artificial membranes made from synaptosomal lipids of mice (Johnson et al. 1980). It is quite probably that also here ethanol acts as a compound influencing on the fluidity of cell membrane of *Dileptus* and evokes the increased activation of chemoreceptor complexes.

Tween 40 appears as inducer of phagocytosis in many protozoan species (Seravin and Orlovskaja 1977). In reaction with phospholipids it occupies competitively the sites of respective receptors and in such case no increase in chemosensitivity is observed. Reaction of Tween 40 with cysteine models shows cumulative effect summarizing activity of two receptors: Tween and cysteine.

All these results confirm the earlier findings concerning the heterogenic character of chemoreceptors system engaged in chemosensitivity in *Dileptus anser* (Orlovskaja and Seravin 1978, Orlovskaja and Brutkowska 1978).

Another type of experiments carried out on *Dileptus* incubated for 6 days in 5×10^{-2} conc. of ethanol and exposed afterwards to 24 different aminoacids, potential inducers of phagocytosis, showed that the action of ethanol gives an increase in the number of aminoacids — effective inducers in comparison with control (see Table 8).

From three clones used in these experiments specimens of only two: P-1 and 24 were sensitive to aminoacid CFM. Anyone of 24 aminoacids did evoke feeding response in animals from clone K-7, both in control and experimental groups. May be they are devoid of the aminoacid receptors.

In control ciliates of clone 24 only three aminoacids: serine, threonine and methionine gave positive feeding response. In control of clone

Table 8

The influence of ethanol on phagocytosis of aminoacid CFM in *Dileptus*

Substances induced in CFM	The number (%) of phagocytizing specimens			
	In control		After incubation in ethanol	
	clone P-1	clone 24	clone P-1	clone 24
DL-Serine	84.3± 6.5	54.2±13.6	82.3± 5.3	40.5±10.0
DL-Threonine	18.7± 2.2	12.7± 3.5	75.6± 6.4	14.3±44.7
DL-Methionine	39.5± 7.5	11.9± 3.8	58.2± 8.5	31.2± 7.1
L-Cysteine	9.6± 1.9	0	52.8± 9.0	60.6± 4.1
DL-Cysteine	0	0	17.0± 4.3	0
DL-Valine	27.8± 7.3	0	61.2± 5.6	47.7±15.0
DL-α-alanine	43.0±14.2	0	86.2± 4.1	24.4± 5.5
DL-β-alanine	0	0	34.7± 7.8	0
DL-β-phenyl-α-alanine	15.4± 3.6	0	59.8±10.4	13.1± 5.6
L-Histidine	0	0	16.6± 4.2	16.1± 4.5
L-Arginine	0	0	12.3± 6.4	0
Glicyne	0	0	32.3± 6.1	12.5± 4.0
L-Asparagine	0	0	34.3± 7.2	0
L-Glutamine	0	0	17.7± 5.6	0
DL-Leucyne	0	0	54.3±12.6	0
L-Isoleucyne	0	0	0	0
DL-Tryptophan	0	0	26.5± 3.3	0
DL-Tyrosine	0	0	21.7± 3.1	0
L-Proline	0	0	16.6± 5.9	0
DL-Ornitine	0	0	18.9± 7.5	0
DL-Asparginic acid	0	0	0	0
L-Glutaminic acid	0	0	0	0
DL-Lisine	0	0	0	0
Ninhydrine	0	0	0	0

P-1 — seven: serine, threonine, methionine, cysteine, valine, α-alanine and β-phenyl-α-alanine.

Dileptus of clone 24 incubated at 5×10^{-2} conc. of ethanol became sensitive towards nine aminoacids and of clone P-1 — towards ninteen. Aminoacids — inducers for specimens of clone 24 were comprised inside the list of these characteristic for clone P-1. Intensivity of "ethanol" *Dileptus* reaction towards aminoacid inducers significantly exceeded that, what was possible to see in control specimens. (All results are presented in Table 8). Ethanol undoubtedly effects strongly an increase of sensibility in all aminoacid receptors in *Dileptus*.

Increasing in the number of aminoacids — inducers of phagocytosis in these experimental conditions, may be explain or, as activation of new receptors, or, as alterations in properties of one and the same receptor existing earlier and functioning in control cells. The impression arises that during the action of ethanol changes in character of receptor

itself to recognition the inducer and decreases its ability to selection of aminoacids. Thus, in this case some common signs characteristic for aminoacids is sufficient to receptor activation.

It was already suggested earlier that in the cells of carnivorous *Protozoa* the cyclic nucleotides are involved in transduction of feeding stimuli from receptors to effectors. The intensity of such feeding response depends probably on activity of adenylcyclase complex and on the level of cyclic AMP inside the cell (Samovar and Orlovskaja 1979). Adenylcyclase activity is closely joined with fluidity of the cell membrane and with the composition of phospholipids. Alterations in viscosity of lipids surrounding adenylcyclase modify activity of this enzyme (Klein et al. 1978). Action of low concentrations of agent extending the membrane stimulate activity of adenylcyclase, whereas high concentrations of the same compound may inhibit its work (Dipple and Housley 1978). It is suggested that activation of adenylcyclase in the first case is evoked due to splitting of physical lipid "chain" surrounding enzyme and receptor. The high concentrations of the same agent effect translocations of phospholipids surrounding directly the enzyme and thus inhibit its action.

All referred above findings are in whole agreement with hypothesis concerning participation of adenylcyclase complex in mechanism of protozoan chemosensitivity and can be interpreted as following.

According to contemporary opinions (among them also Pertseva 1981), adenylcyclase complex represents at least four properties: receptive, catalizing, coordinating and regulating. Recepting and catalizing compounds are swimming freely in lipid matrix of the cell membrane. Their cooperation activates catalizatory functions of the whole adenylcyclase complex. Regulating component seems to be GTP. The role of joining factor may be concerned, after many authors, with phospholipids surrounding the whole complex. Agents able to decrease viscosity of the membrane like, for example, low concentrations of ethanol and others, make possible the quick joint action of receptory and catalizatory compounds. This fact might explain the observed in many cases increase of chemosensitivity in predators towards examined inducers of phagocytosis. When concentration of ethanol grows to 1.6%, maximal fluidity of *Tetrahymena* cell membrane is observed. After incubation of *Dileptus* in the same concentration of ethanol the full inhibition of chemosensitivity is observed. The loss of feeding response towards chemical inducers is seen after exposition to high temperatures in animals fed before one hour. It can be permitted that in these cases phospholipids, surrounding adenycyclase, migrate, and thus the activation of enzyme becomes impossible.

REFERENCES

Cuatrecasas P. 1974: Membrane receptors. Annu. Rev. Biochem., 43, 169–214.
Dipple I. and Housley M. D. 1978: The activity of glucagon stimulated adenylate cyclase from rat liver plasma membranes is modulated by the fluidity of its lipid environment. Biochem. J., 1974, 179–190.
Fukushima H., Umeki Sh. and Nozawa Y. 1981: Alterations in *Tetrahymena* microsomal desaturase activity and the level of electron transport components during temperature adaptation. In: Progress in Protozoology, abstr. VI Intern. Congr. Protozool., Warszawa, Poland, 107.
Fukushima H., Watanabe T. and Nozawa Y. 1976: *In vivo* manipulation of membrane lipids by 1.0-hexadecyl glycerol feeding in *Tetrahymena pyriformis*. Biochem. Biophys. Acta, 436, 249–259.
Hewitt I. A. 1978: Diffusion gradients, membrane receptor and the acquisition of orientational information by cells. J. Theor. Biol., 74, 297–306.
Housley M. D., Hesketh T. R., Smith G. A., Warren G. B. and Metcalfe J. 1976: The lipid environment of the glucagon receptor regulates adenylate cyclase activity. Biochem. Biophys. Acta, 436, 495–504.
Johnson D. A., Friedman H. J., Cooke R. and Lee N. M. 1980: Adaptation of brain lipid by layers to ethanol inducer fluidization. Species and strain generality. Biochem. Pharmacol., 29, 1673–1676.
Irlina I. S. and Merkulova N. A. 1975: The growing in volume and mass of *Tetrahymena pyriformis* for biochemical purposes and the synchronization of division. Citologija, 17, 1208–1212.
Kasai R., Watanabe T., Fukushima H., Iida H. and Nozawa Y. 1981: Adaptive modification of membrane lipids in *Tetrahymena pyriformis* during starvation. Alterations in phospholipid composition and postitional distribution of fatty acid chains. Biochim. Biophys. Acta, 666, 36–46.
Klein J., Moore L. and Pasten I. 1978: Effect of liposomes containing cholesterol on adenylate cyclase activity of cultured mammalian fibroblasts. Biochim. Biophys. Acta, 506, 42–53.
Koshland D. E. 1979: A model regulatory system: Bacterial chemotaxis. Physiol. Rev., 59, 811–862.
Nandini-Kishore S. G., Mattex S. M., Martin Ch. E. and Thompson G. A. 1979: Membrane changes during growth of *Tetrahymena* in the presence of ethanol. Biochim. Biophys. Acta, 551, 315–327.
Nozawa Y. 1981: Environmental adaptation of *Tetrahymena* membrane. Role of membrane lipids. In: Progress in Protozoology, abstr. VI Intern. Congr. Protozool., Warszawa, Poland, 272.
Nozawa Y., Iida H., Fukushima H., Ohki K. and Ohuishi S. 1974: *Tetrahymena* membranes: temperature-induced alterations in fatty acid composition of various membrane fractions in *Tetrahymena pyriformis* and its effect on membrane fluidity as inferred by spin label study. Biochim. Biophys. Acta, 357, 134–147.
Nozawa Y. and Thompson G. A. 1979: Lipids and membrane organization in *Tetrahymena*. In: Biochemistry and Physiology of *Protozoa* (Levandovsky M. and Hunter S. H., eds.), 275–338.
Orlovskaja E. E. 1982: Effect of ethanol on the activity of aminoacid receptors in the carnivorous ciliate *Dileptus anser*. Citologija, 24, 1075–1080.
Orlovskaja E. E. and Brutkovska M. 1978: Dejstvie detergentov na piščevuju reakciju kiščnoj infusorii *Dileptus anser*. In: Povedenie Vodnich Bespozvonočnich. Nauka, 28–29.
Orlovskaja E. E., Karvanen L. N. and Seravin L. N. 1978: Adaptivnij charakter izmenenija piščevogo povedenija kiščnich infuzorij pri smene piščevich objektov. Vestn. Leningr. Univ., Ser. Biol., 21, 24–27.
Orlovskaja E. E. and Seravin L. N. 1978: Mezklonalnie raslíčia piščevoj reakcii u kiščnoj infuzorii *Dileptus anser*. In: Fauna i Sistematika Odnokletočnyh Životnih. Leningrad, AN SSSR, 78, 51–58.
Pertseva M. N. 1981: On the role of membrane lipids in the function of hor-

mone-sensitive adenylate cyclase system. J. Ceoluz. Biochim. i Phisiol., 17, 281–286.
Prescott D. M. and James T. W. 1955: Culturing of *Amoeba proteus*. Exp. Cell. Res., 8, 256–258.
Roberts Ch. T. and Morse D. 1978: Genetic regulation of galactokinase in *Tetrahymena* by cyclic AMP, glucose and epinephrine. Proc. Natl. Acad. Sci. USA, 75, 1810–1814.
Rimon G., Hanski E. and Levitzki A. 1980. Temperature dependence of β-receptor, and sodium fluoride stimulated adenylate cyclase from turkey erythrocytes. Biochemistry, 19, 4451–4460.
Samovar A. G. and Orlovskaja E. E. 1979: Effect of biological active substances on the feeding response of the carnivorous ciliate *Dileptus anser*. Citologija, 21, 340–345.
Seravin L. N. and Orlovskaja E. E. 1972: Vybor pišči u prostejših. Vestn. Leningr. Univ., Ser. Biol., 15, 7–19.
Seravin L. N. and Orlovskaja E. E. 1977: Feeding behaviour of unicellular animals. I. The main role of chemoreception in the food choice of carnivorous *Protozoa*. Acta Protozool., 16, 309–332.
Shimonaka H., Fukushima H., Kawai K., Nagao S., Okano Y. and Nozawa Y. 1978: Altered microviscosity of *in vivo* lipid — manipulated membranes in *Tetrahymena pyrifomis*: A fluorescence study. Experientia, 34, 586–587.
Shimonaka H. and Nozawa Y. 1977: Subcellular distribution and thermally-induced transition of adenylate cyclase activity in thermotolerant *Tetrahymena* surface membranes. Cell Struct. Funct. 2, 81–91.
Sun A. Y. and Seaman R. N. 1980: Physicochemical approaches to the alcohol-membrane interaction in brain. Neurochem. Res., 5, 537–545.
Thompson G. A. and Nozawa Y. 1977: *Tetrahymena*: a system for studying dynamic membrane alteration within the eucaryotic cell. Biochim. Biophys. Acta, 472, 55–92.
Thompson G. A., Raymond J., Bambery R. J. and Nozawa Y. 1972: Environmentally produced alterations of the tetrahymanol: phospholipid ratio in *Tetrahymena pyriformis* membranes. Biochim. Biophys. Acta, 260, 630–638.

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The Adaptation of *Tetrahymena pyriformis* GL to the Continuous Presence of Colistin in the Medium as Observed in Selected Physiological Functions

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Synopsis. The effect of the polypeptide antibiotic, colistin on the adaptation of *Tetrahymena pyriformis* GL was investigated. The ciliates were found to adapt to the continuous presence of low, nonlethal concentrations of the drug in the medium, by means of physiological adaptation. Disturbances in the investigated physiological functions of the cell, i.e., the culture growth phases, the processes of cytokinesis and phagocytosis, and the recovery were related to the antibiotic level in the culture. It was also proved that the employed colistin concentrations did not produce the synchronization of cell division. Additionally, the antibiotic did not seem to affect the ciliary reversal in *Tetrahymena* due to the presence of potassium ions and thus did not reveal the property characteristic of detergents.

The effect of inhibitors of cell metabolism upon eucaryotic organisms, including ciliated protozoa, has been a subject of numerous studies and many disturbances in the cell cycle and other physiological processes have been reported. These are due to the specific character of the inhibitor, its concentration and the time of action on the one hand, and the general physiological status of the cells when exposed to the agent, on the other hand (Mitchison 1978).

The addition of inhibitors of cell metabolism to the *Tetrahymena* culture produces a temporary blockage of physiological functions of the cells related to the concentration of the drug with the subsequent reactivation while still in the presence of the inhibitor. This type of cell response has been referred to as "recovery" (Frankel 1965), "endogenous recovery" (Wunderlich and Peyk 1969 b) or "physiological, or phenotype adaptation" (Nyberg et al. 1978).

Does, however, the ciliate's response to the low concentrations in

the medium of agents affecting cell metabolism depend solely on the inhibitor employed? Or is it due to the development or triggering of some cellular mechanism neutralizing the inhibitory effect of the drug? Studies by Kiersnowska (1981) on the effect upon *Euplotes minuta* and *Chilodonella cucullulus* of cyclohexamide, a substance easily penetrating into the cell, suggest that the ciliate's response to the low toxin levels might in fact result from the development or triggering of such mechanism.

In the above studies there were employed inhibitors easily passing into the cell. However, there are not many papers discussing the ciliate response to the compounds which do not directly enter inside the cell (Németh and Csik 1961, Hayes 1977, Rebandel 1981, Rebandel and Karpińska 1981, Rebandel et al. 1981, Szablewski 1981, 1982).

Studies conducted with the use of inhibitors which do not penetrate into the cell are also interesting for other reasons. Our present knowledge of substances easily crossing the cell wall suggests that on entering the cell, the inhibitors induce or activate some still unknown system which either effects or increases the ciliate's resistance to the drug. The inactivation of the toxin through metabolism, e.g., the oxidation-reduction reaction does not seem likely. It has been suggested that the adaptation might be achieved by the production in the cell of some macromolecules responsible for the modifications in the passage of the inhibitor into and/or from the cell (Frankel 1970, Roberts and Orias 1974). Thus, it would be of interest to investigate whether a long-term exposure of ciliates to a substance which does not penetrate into the cell and additionally affects the permeability of the cell membrane might produce a physiological adaptation-like response.

Accordingly, colistin was employed in the present study. It is a polypeptide antibiotic acting on the cell membrane. The studies on *Procaryota* (Few and Schulmann 1953, Newton 1953, 1954 a, b, 1955, Sebek 1967) as well as on eucaryotic cells and phagocytes (McKay and Kay 1964, Axline et al. 1967, Jawetz 1970) demonstrated that the polymyxins, including colistin, do not enter the cell. Through incorporation in the bacterial cell membrane (Kuryłowicz 1979), colistin changes its structure, increasing simultaneously its permeability (Sebek 1967, Russel 1977) and affecting bacteria in much the same way as detergents (Korzybski et al. 1977). As a result such compounds as purines, monosaccharides, nucleotides and aminoacids are released from the cell (Russel 1977). The polymixins damage bacterial cell wall, enhancing the penetration of other antibiotics into the cell. The polymyxins have been found to be synergists of

erythromycins and sulphonamides, as they facilitate their penetration (Kawamata and Nakajima 1965).

In the present study the analysis of the findings focused on the question whether *Tetrahymena* were capable of phenotype adaptation in the presence of nonlethal colistin concentrations in the medium. The effect of such drug levels was assayed on the cell cycle the duration of cytokinesis, the frequency of cells at this stage, and the process of phagocytosis.

It was also considered worthwile to investigate whether the mechanism of colistin effect on *Tetrahymena* cell membrane would resemble the mechanism of action of detergents. In the latter case ciliary reversal occurs in the protozoa (Brutkowska et al. 1974, Dryl 1974). The detergent properties of the antibiotic could be established by adding to the colistin-containing medium potassium ions at concentrations usually leading to the reversal and checking if the cells behave accordingly.

To ensure the uniform pattern of experiments whereby all changes in cell function are due to the inhibitor added to the culture, the following factors should be considered in the case of ciliates:

(1) Change in the protozoan environment after the addition of antibiotic.

(a) possible change in pH of the medium after the addition of colistin,

(b) possible changes in the antibiotic activity in the course of the experiments.

(2) Changes in the behaviour of the protozoa due to:

(a) varying sensitivity of the ciliates to the inhibitor in the course of their cell cycle (Mitchison 1978),

(b) varying sensitivity of the cells to the inhibitor in the course of their sexual cycle, measured as the number of generations after the last sexual process, i.e., conjugation or autogamy (Smith-Sonneborn and Rodemal 1976, Williams 1980),

(c) varying sensitivity of *Tetrahymena* related to the cultural growth cycle the ciliates were undergoing when the antibiotic was added (Satir 1967).

In view of the above, experimental conditions were accordingly selected and additional control tests performed. In studies conducted with an appropriate cell density of the cultures, i.e., several thousand cells per ml, it may be assumed that fractions of the ciliates undergoing particular phases of the cell cycle are similar and comparable in all cultures used in the experiments (Frankel 1960). The use of the amicronucleate strain *Tetrahymena pyriformis* GL rules out sexual processes and accordingly the observed colistin action should not depend on the

age of the cells in their sexual cycle. Since the drug was added when the cells were undergoing the same culture growth phase, different sensitivity of the ciliates to colistin related to the growth phase of the culture was excluded.

Material and Methods

Material and Cultivation

The organism used in the study was an amicronucleate strain of the ciliate *Tetrahymena pyriformis* GL. The stock cultures were maintained in an axenic condition in 20 ml culture tubes, 16 mm in diameter, containing 5 ml of the growth medium: 1.5% proteose-peptone + 0.1% yeast extract (Difco). Water employed to prepare the medium was three times distilled and deionized. To ensure the optimum thermal conditions, the cultures were kept in an incubator at 28°C. 0.5 ml of cell suspension from the stock culture was inoculated into 5 ml of the medium every seven days. The experimental cultures, i.e., with colistin and the controls, i.e., antibiotic-free were maintained in 200 ml flasks containing 50 ml of the medium, both the medium and the temperature being the same as in the stock cultures. The exponential growth phase was achieved by transferring 0.5 ml of cell suspension from the stock culture into a culture tube containing 5 ml of the medium. The tube was kept slanted in the incubator to ensure a better oxygen supply. After 24 h the tube contents were poured into a flask containing 50 ml of the medium (second inoculation). The third inoculation was performed after another 24 h by transferring 5 ml of cell suspension from the second inoculation into a flask containing 45 ml of the medium (experimental culture). The experiments were started after 3 h from the time of inoculation. The ciliates employed in the study divided asynchronously.

The Antibiotic Employed

The antibiotic used in the study was produced by the Polfa Pharmaceutical Company at Tarchomin. It is a mixture of colistin A (polymyxin E_1) and colistin B (polymyxin E_2), cf. Fig. 1. Its molecular mass is 1400. Colistin employed in the study was the sulphate salt, since it is well soluble in water. As a salt, the antibiotic is fairly stable at a pH 2–6, while at a pH above 6 its activity decreases (Korzybski et al. 1977). Colistin was added directly to experimental cultures.

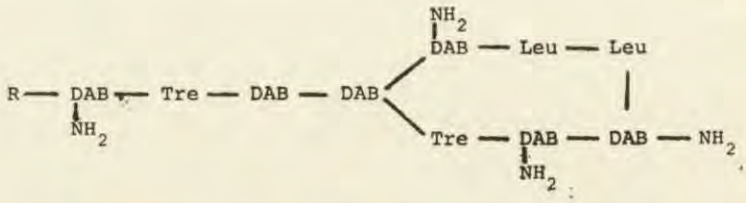

Fig. 1. Chemical structure of the molecule of colistin A and B: colistin A — R = isopelargonic acid, colistin B — R = isoheptacarboxylic acid, DAB = diaminobutyric acid

The antibiotic concentration in the culture was expressed as mM/l (milimole per liter). Following initial exploratory trials in the above conditions as well as earlier findings (Szablewski 1981), two concentrations of the drug were chosen: 0.05 mM/l = 0.07 g/l and 1 mM/l = 1.4 g/l, the former being the lowest dose which produced disturbances in the investigated physiological functions and the latter being the highest antibiotic level which did not cause any cell elimination in the exploratory trials.

The Control Trials to Demonstrate the Uniform Pattern of the Experiments

(a) the biological trial with naive cells to check the biological activity of colistin

Proper concentrations of colistin were added to two flasks containing the ciliates, the third one, antibiotic-free, serving as control. After 48 h the cultures were filtered and cells from the culture, which was inoculated after the lapse of 48 h, were added to that medium. Thus *Tetrahymena* from both cultures were in the same phase of culture growth at the beginning of the experiment. Subsequently, the course of phagocytosis was investigated according to the method described under E (Material and Methods), in 45 min and in 3 h following the cells' exposure to the antibiotic.

(b) the pH of the medium

The pH of the medium in the control and experimental cultures was measured at the beginning of the experiment and after 24 h with a Radelkis pH-meter, a combined glass-calomel electrode.

Statistical Methods

The results were compared using the "Two way ANOVA with replication" variance analysis method (Sokal and Rohlf 1969) with the confidence level of 95%. The method allows to establish the significance of differences between particular trials as well as the relationship to the duration of the experiment.

A Detailed Discussion of the Methods Employed

Due to the nature of the study, the experiments were mostly conducted in the exponential growth phase, particular growth phases of the cultures being determined according to the criteria developed by Monod (Satir 1967).

A. The studies on the Rate of *Tetrahymena pyriformis* GL Cultivation

Proper concentrations of the antibiotic were added to the experimental cultures 3 h after the inoculation. The time of the drug addition was referred to as 0 time point. The cell density, measured on an electronic cell counter was then ca. 2500 cells per ml. The increase in the cell count (dV) in particular cultures at a given time point was calculated according to the formula

$$dV = \frac{V_t}{V_0},$$

where V_t — density of the culture at a given time point, V_0 — density of the culture at 0 time point.

In the first four hours of the experiment, beginning at 0 time point, the samples were taken every half hour and in the remaining eight hours, every

two hours. Since longer observation of the rate of cell cultivation was required in the control culture and the one containing 1 mM/l colistin, they were sampled to calculate culture densities every three hours between the 12th and 24th hour of the experiment, and every four hours between the 24th and 36th hour of the experiment.

B. Studies on the Duration of Cell Generation

The experiments were conducted on cells isolated according to the method of Frankel (1965). A single dividing cell with cleavage well visible under a stereoscopic microscope was transferred using a micropipette onto a plate with three divisions containing 0.1 ml of growth medium each. After the division one of the daughter cells remained in the medium (control), while the other was transferred with a micropipette to the same amount of medium containing the antibiotic. To prevent evaporation, the medium was covered with paraffin oil (Frankel 1965). The observations using a stereoscopic microscope were carried out at 25°C. The study was conducted on 50 cells. The obtained results were used to calculate the so-called per cent prolongation according to Heyer and Frankel (1971), employing the following formula

$$\text{per cent prolongation} = \frac{GT_2 - GT_1}{GT_1} \times 100\%,$$

where: GT_1 — generation time in a control cell, GT_2 — generation time in a cell placed in atibiotic-containing growth medium.

C. Studies on the Duration of Cytokinesis

The experiment was complementary and it was performed on cells from the control cultures and those maintained for 12 h in 1 mM/l colistin concentration. Isolated ciliates were employed in the study. The cells were placed with a micropipette in cups containing 0.1 ml of growth medium without or with the antibiotic. To prevent evaporation, the growth medium was covered with paraffin oil. The time between the appearance of cleavage and the complete separation of cells was measured. The observations, with the use of a stereoscopic microscope, were carried out at room temperature. The experiment was performed on 20 cells from each sample.

D. The Study on the Cell Fraction Undergoing Cytokinesis

The time of the addition of the drug was the same as in Experiment A. The samples were taken at 0 h, 0.75 h (45 min), 2 h, 3 h, 4.5 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h and 24 h, the time of sampling being a partial modification of the system developed by Frankel (1969 a). The specimens for *Tetrahymena* observation were prepared according to the method of Chatton-Lwoff, modified by Frankel and Heckmann (1968). Cells with pronounced cleavage, in the sixth stage of stomatogenesis were assumed to undergo cytokinesis. The sample used to investigate this phase of cell cycle comprised 200 ciliates. And optic microscope with immersion was used and the total magnification was 1600 ×.

E. The Study on the Course of Phagocytosis

In this part of the experiment the number of food vacuoles appearing in the cell in the course of absorbing ink suspension (Rotring) diluted with distilled

water in the ratio 1 : 100 was counted, the ink being a frequently employed inducer of phagocytosis (Elliott and Clemons 1966, Orias and Pollock 1975, Rasmussen et al. 1975, Rasmussen 1976). The duration of feeding was established experimentally as 15 min. Times of addition of the antibiotic and of sampling were the same as in the previous experiment. 0.1 ml of ink suspension was added to cups placed in an incubator and containing 1 ml of growth medium with proper concentrations of the drug and in case of the control, without the antibiotic. Eventually the cells were fixed with 0.1 ml of 10% formalin solution and food vacuoles were counted in 100 cells, employing the magnification of 320 ×.

F. The Study on the Detergent Effect of Colistin on the Cell

The study concerned the determination of the threshold KCl concentration, which produced in *Tetrahymena* the phenomenon referred to as continuous ciliary reversal. Twenty four hours after the second inoculation the cells were washed out from growth medium by centrifugation. The ciliates were centrifuged three times in an electrical centrifuge at ca. 200 rotations per minute, for 5 min. The observations started 2 h after the washing. The study was performed in dishes with three depressions.

The control sample was prepared and investigated as follows: 0.09 ml of cell suspension was transferred into the depression and 0.01 ml of KCl solution at the proper concentration was added. Concentrations of stock solutions of KCl were 10 times higher than the effective concentration of KCl obtained after blending with *Tetrahymena* suspension. In trials with the antibiotic 0.08 ml of cell suspension + 0.01 ml of stock KCl solution at the proper concentration + 0.01 colistin at the initial concentrations of 0.5 mM/l or 10 mM/l were transferred into a depression in the dish. In this way the drug concentration in the samples was 10 times lower. Thus it was possible to establish what KCl concentration induced a continuous ciliary reversal in at least 50% of cells. At each session approximately 15 cells were studied with the aid of a stereoscopic microscope.

Results

The Initial Exploratory Tests

(a) the biological test on naive cells to demonstrate biological activity of colistin at 48 h of the experiment

In the control sample 45 min (0.75 h) after inoculation 30% of the cells did not take up ink suspension, while the average food vacuole count in a cell was 4.52. Thirty nine per cent of the *Tetrahymena* removed from the medium obtained by filtration of the culture with 0.5 mM/l colistin did not absorb ink suspension, while the average food vacuole count in a cell was 4.13. On the other hand, 100% of the ciliates inoculated into the growth medium with the antibiotic at a concentration of 1 mM/l added 48 h earlier and then filtered, did not take up ink suspension after 45 min. Similar studies performed 3 h after

Table 1

Biological test with naive cells. The change in the rate of occurrence of cells with a definite number of food vacuoles according to colistin concentration and the duration of its action. Total number of investigated cells — 100

Time		Number of food vacuoles in cell										
		0	1	2	3	4	5	6	7	8	9	10
45 min	Control	30	4	0	4	4	12	10	8	12	8	8
	Colistin 0.05 mM/l	39	0	4	0	4	4	10	15	16	4	4
	Colistin 1 mM/l	100	0	0	0	0	0	0	0	0	0	0
3 h	Control	26	0	1	3	15	13	10	14	10	4	4
	Colistin 0.05 mM/l	24	1	1	9	10	13	15	16	6	5	0
	Colistin 1 mM/l	90	1	5	1	1	2	0	0	0	0	0

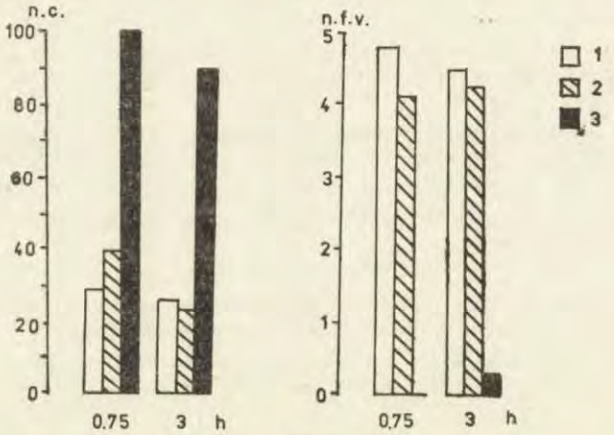

Fig. 2. Biological test with naive *Tetrahymena* cells. The change in the number of cells (n. c.) which did not absorb ink suspension (A) and the mean number of food vacuoles (n. f. v.) in a cell (B) according to colistin concentration in the medium. *1* — control, *2* — colistin 0.05 mM/l, *3* — colistin 1 mM/l. Total number of investigated cells — 100

0 time point demonstrated that 26% of the cells from the control sample did not develop food vacuoles, while the average food vacuole count in a cell was 4.47. In the *Tetrahymena* cultured with colistin at 0.05 mM/l the values were 24% and 4.30 respectively and in those cultured with the drug at 1 mM/l 90% and 0.28 (Table 1, Fig. 2).

(b) the effect of colistin and of the duration of the experiment on the pH of the growth medium

The pH of the growth medium with the drug did not differ significantly from the pH of the colistin-free medium, whereas the pH of

the medium changed only slightly after 24 h. Accordingly, it may be said that the differences of ±0.05 in the pH of the medium do not affect the investigated physiological parameters (Szablewski 1981).

Basic Results

A. The Effect of Colistin on the Rate of *Tetrahymena* cultivation

The investigations performed demonstrated that the presence of colistin in the growth medium effected changes in the rate of *Tetrahymena* cultivation. In the control sample culture growth phases were as follows: (1) the lag phase (0–1.5 h), (2) the exponential growth phase (1.5–18 h), (3) the decelerated growth phase (18–24 h), (4) the stationary phase (24 h to the end of the experiment). (See Table 2, Fig. 3).

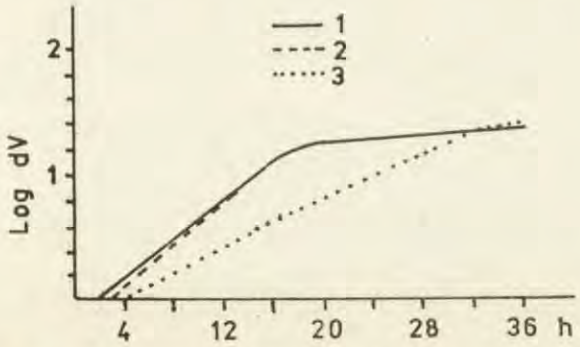

Fig. 3. The rate of *Tetrahymena pyriformis* GL cultivation according to colistin concentration in the medium. For the culture incubated with 0.05 mM/l colistin the observation was carried out for 12 h. dV — coefficient of cell increase with time. Denotations of control and colistin concentration — see Fig. 2

With the addition of 0.05 mM/l colistin to the culture only the lag phase was prolonged by ca. 20 min as compared to the control, the rate of other phases of *Tetrahymena* growth being similar to the control culture. A variance analysis showed that the differences in the dynamics of cell cultivation observed during the 12 h of the experiment in both samples were not statistically significant. Since a further increase in the cell count was assumed to be the same as in the control culture, the observations of growth phases in the culture containing colistin at 0.05 mM/l were stopped at that time (Table 2, Fig. 3). Additionally, a relationship between the alterations in cell density and the duration of the experiment was established, the increase in cell count being pro-

Table 2

The coefficient of cell count increase with time (dV) and the standard deviation (SD). The density of each culture at 0 time point appeared in calculations as 1. For the culture incubated with 0.05 mM/l colistin the observation was carried out for 12 h. Time given in hours starting from the antibiotic addition

	Time in h													
	0	2	4	6	8	10	12	15	18	21	24	28	32	36
Control	1.0	1.02	1.34	2.12	2.97	4.32	7.11	10.90	16.25	19.36	21.00	22.43	22.84	25.08
SD	0.0	0.03	0.15	0.27	0.24	0.49	0.90	0.20	1.66	1.34	1.37	1.45	1.50	1.66
Colistin 0.05 mM/l	1.0	1.02	1.36	1.87	2.90	4.20	6.29							
SD	0.0	0.05	0.08	0.27	0.31	0.57	0.82							
Colistin 1 mM/l	1.0	1.00	1.05	1.28	1.57	1.74	2.37	4.10	4.98	7.31	10.76	13.69	22.96	28.52
SD	0.0	0.00	0.04	0.07	0.24	0.24	0.32	1.11	1.20	1.18	1.88	1.68	1.70	1.45

portional to the duration of their immersion in colistin. The correlation was statistically significant.

The addition of 1 mM/l colistin to the culture produced more pronounced changes in the culture growth phases. The lag phase lasted for the first four hours of the experiment, the exponential growth phase from the 4th to the 32nd h, the decelerated growth phase from the 32nd to 36th h while the stationary phase began at 36 h (Table 2, Fig. 3). A variance analysis demonstrated that the addition of 1 mM/l colistin to the culture effected statistically significant changes in the rate of *Tetrahymena* cultivation as compared to the other two samples. Also in this culture the increase in cell count with time was statistically significant.

The findings concerning the rate of ciliate growth demonstrate that after the addition of 0.05 mM/l of the antibiotic to the population, the stationary phase was achieved at the same time and with the same number of cells as in the control sample. Prolongation of the lag phase by 20 min did not seem to significantly affect the achievement of the stationary phase by the culture. At 1 mM/l of colistin, however, the stationary phase began with a similar number of cells as in the control, but a certain shift in time was observed.

B. The Effect of Colistin on the Time of Generation in *Tetrahymena pyriformis*

In long-term experiments a direct observation of the duration of many consecutive cell cycles is not feasible. Accordingly, in the present study the observation was limited to the two consecutive cell cycles. The time of generation in cells cultured with the antibiotic was found to be proportional to its concentration in the medium.

In *Tetrahymena* from the control culture the average generation time was 3.5 h, the minimum time being ca. 3 h and the maximum time ca. 4 h. With the concentration of colistin equal to 0.05 mM/l the period between cell divisions was lengthened by ca. 30 min as compared to controls. The per cent prolongation was 14.5%. The prolongation of cell generation time by ca. 30 min constituted a statistically significant difference as demonstrated by variance analysis.

When the drug at a concentration of 1 mM/l was added to the culture, the time of *Tetrahymena* generation was much more markedly prolonged. It was 8.5 h on the average, the minimum time being ca. 7 h and the maximum period ca. 10 h. The per cent prolongation was then ca. 143%.

It was also established that the prolongation of the generation time

in cells exposed to the two investigated concentrations of colistin concerned only the first cell cycle, subsequent cell divisions occurring at the rate similar to the one observed in the control culture.

C. The Effect of Colistin at 1 mM/l on the Duration of Cytokinesis

The duration of cytokinesis in control cells and in those adapted to colistin after 12 h of *Tetrahymena* exposure to the antibiotic was investigated in the experiment. In control cells the duration of cytokinesis averaged 18 min, while in the antibiotic sample it was 19 min. Prolongation of cytokinesis by one minute, i.e., 5.5% was statistically insignificant and within limits of error. Accordingly, it may be assumed that the colistin concentration employed in the experiment did not prolong the duration of cytokinesis after 12 h of *Tetrahymena* exposure to the drug.

D. The Effect of Colistin on the Frequency of Cells in the Stage of Cytokinesis

The studies on the effect of colistin upon *Tetrahymena pyriformis* GL demonstrated that the antibiotic produced alterations in the frequency of cells in the stage of cytokinesis, dependent on the drug concentration in the growth medium and the culture growth stage.

In the control medium the lowest fraction of cells undergoing cytokinesis was observed in the lag phase (3–4%). The number of ciliates in this stage of cell cycle increased with the completion of the phase. The maximum fraction of cells undergoing cytokinesis was observed in the exponential growth phase (5–6%) and it remained at that level until the end of the experiment (Fig. 4).

At the 0.05 mM/l colistin produced a total inhibition of cytokinesis after 45 of *Tetrahymena* exposure to the antibiotic. After 2 h a few ciliates (0.5%) undergoing cytokinesis were observed. After 3 h, however, the fraction of such cells achieved the control level, at which it remained until the end of experiment. The differences in percentage between the two samples observed in the period were not statistically significant (Fig. 4).

The addition of 1 mM/l colistin to the population led to a complete arrest of cytokinesis for three hours from the zero time point. Ciliates with cleavage (1%) appeared after 4.5 h. Subsequently the fraction increased to reach the control level, observed after 12 h of *Tetrahymena* exposure to the antibiotic. From that moment on, until the end of experiment the number of dividing cells in the investigated sample remained at the control level (Fig. 4).

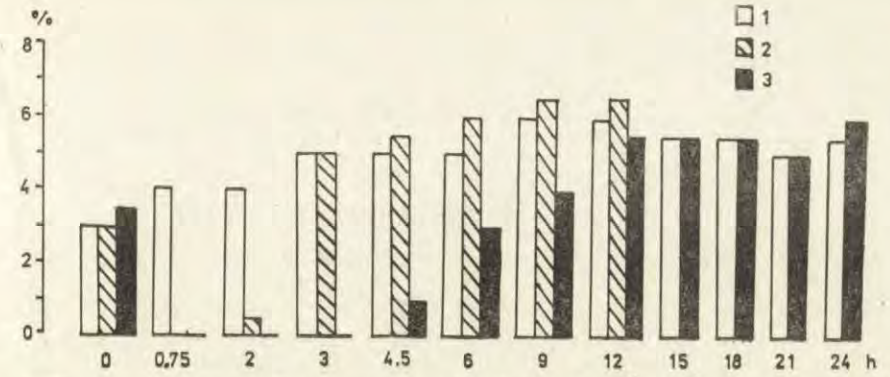

Fig. 4. The percentage of *Tetrahymena* cells (%) undergoing cytokinesis according to colistin concentration and the experiment duration. With the drug at 0.05 mM/l in the medium the observation was carried out for 12 h. Denotations of control and colistin concentrations — see Fig. 2. Total number of investigated cells — 200

E. The Effect of Colistin upon the Course of Phagocytosis

Studies on the uptake of ink suspension by *Tetrahymena* and the formation of food vacuoles revealed the relationship between the process and the antibiotic concentration in the culture as well as the culture growth phase.

In the control population the number of cells which did not absorb ink suspension was highest (ca. 30%) in the lag phase, the mean number of food vacuoles and their maximum number in a single cell being 3.76 und 9 respectively. At the beginning of the exponential growth phase a gradual decrease down to ca. 26% in the number of ciliates which did not take up ink suspension was observed. The mean number

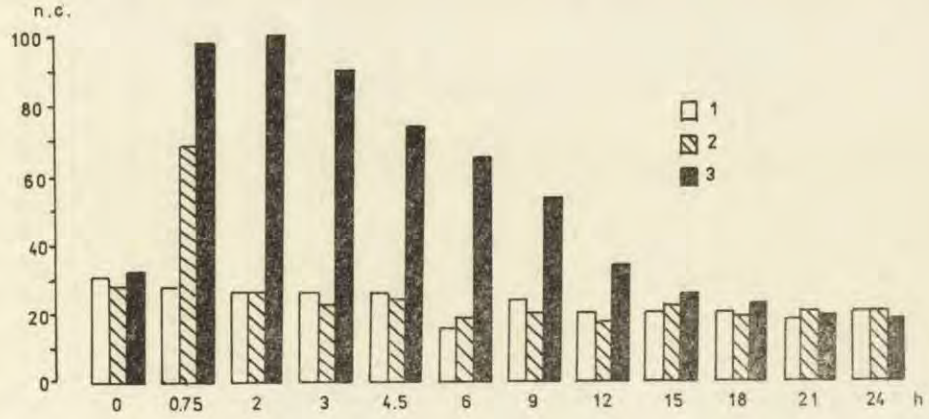

Fig. 5. The number of *Tetrahymena* cells (n. c.) which did not absorb ink suspension according to colistin concentration in the medium and the experiment duration. Denotations of control and colistin concentration — see Fig. 2. Total number of investigated cells — 100

Table 3

The change in the rate of occurrence of cells with a definite number of food vacuoles according to colistin concentration and the experiment duration. Total number of investigated cells — 100

	Time in hours	Number of food vacuoles in cell										
		0	1	2	3	4	5	6	7	8	9	10
Control	0	30	3	4	7	8	12	12	14	8	2	0
	0.75	27	4	5	4	10	14	11	13	8	4	0
	2	26	4	2	6	12	10	16	10	12	4	0
	3	26	2	0	2	16	14	12	12	12	3	1
	4.5	26	1	3	5	10	9	15	10	12	5	4
	6	16	0	6	4	18	12	16	12	6	4	6
	9	19	5	2	8	14	10	16	10	8	6	2
	12	20	2	0	6	18	14	10	12	6	8	4
	15	21	3	3	5	11	12	6	12	12	6	9
	18	20	2	2	8	8	14	16	14	6	4	6
	21	20	3	5	7	9	6	8	14	16	6	6
	24	20	6	3	5	9	10	12	14	7	9	5
Colistin 0.05 mM/l	0	29	5	6	6	9	12	12	14	4	3	0
	0.75	68	4	5	10	10	0	3	0	0	0	0
	2	26	0	10	12	20	12	6	8	2	4	0
	3	22	0	0	10	9	15	14	16	7	4	3
	4.5	24	4	6	6	3	10	11	19	10	4	3
	6	19	0	3	2	4	22	14	18	16	4	0
	9	16	2	4	4	14	16	16	14	10	4	0
	12	18	0	6	6	12	12	14	12	12	6	2
	15	21	2	5	6	8	7	8	14	14	6	9
	18	19	3	3	7	7	13	14	16	8	6	4
	21	21	4	6	3	8	10	7	10	16	10	5
	24	20	1	4	4	10	12	16	18	4	8	3
Colistin 1 mM/l	0	32	4	5	6	9	11	11	14	6	2	0
	0.75	98	2	0	0	0	0	0	0	0	0	0
	2	100	0	0	0	0	0	0	0	0	0	0
	3	90	2	4	0	2	2	0	0	0	0	0
	4.5	74	6	4	4	4	2	2	4	0	0	0
	6	66	8	6	0	8	6	6	0	0	0	0
	9	54	18	6	8	6	4	2	2	0	0	0
	12	34	12	2	18	10	6	12	4	2	0	0
	15	25	7	12	16	16	4	8	2	5	5	0
	18	23	4	10	8	8	18	8	6	5	5	5
	21	20	4	0	12	8	12	12	12	8	6	6
	24	18	3	5	8	11	10	13	12	12	6	2

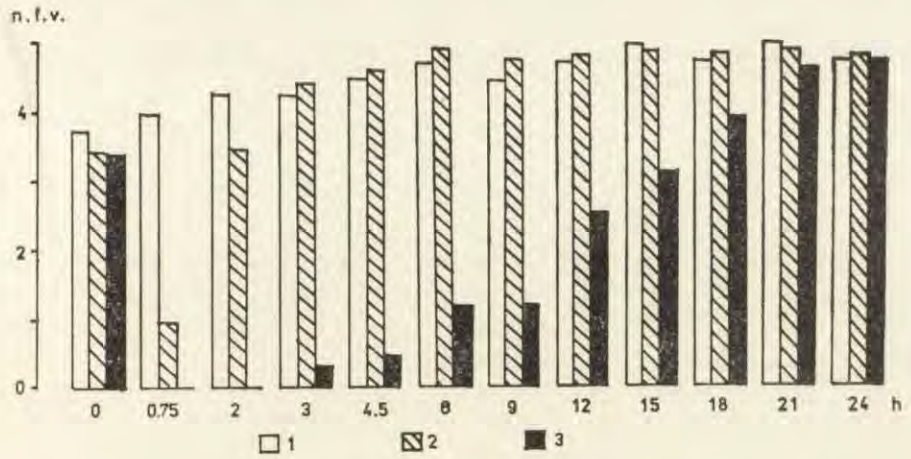

Fig. 6. The average number of food vacuoles in the *Tetrahymena* cell (n.f.v.) according to colistin concentration in the medium and the experiment duration. Denotations of control and colistin concentrations — see Fig. 2. Total number of investigated cells — 100

of food vacuoles in a cell was then 4.22, while their maximum number remained 9. The highest phagocytosis rate could be observed during the subsequent culture growth phases. 24.4% of the cells on the average did not take up then ink suspension, while the mean number of food vacuoles in a cell was 4.56, their maximum number being 10 (Table 3, Fig. 5 and 6).

With the addition of colistin at 0.05 mM/l the percentage of cells which did not develop food vacuoles increased to 68% after 45 min of exposure, the mean number of food vacuoles decreased to 1.02, while their maximum number in a single cell was 6. However, after 2 h of exposure to the drug the cells returned to the control level (Table 3, Fig. 5 and 6). After 45 min of antibiotic treatment there were no statistically significant differences in phagocytosis rate between experimentals and controls, apart from the rate of food vacuole formation.

The addition of 1 mM/l colistin to the culture produced a significant inhibition of phagocytosis after 45 min. Ninety eight per cent of the cells did not take up ink suspension and the mean number of food vacuoles in a cell dropped to 0.02 with the maximum number being 1. After 2 h of exposure to the antibiotic at such concentration phagocytosis was completely arrested. Later, the number of ciliates which failed to form food vacuoles gradually decreased, to return to normal limits after 21 h (Table 3, Fig. 5 and 6).

A correlation was also established between the number of food vacuoles formed in a cell and the concentration of the antibiotic added

to the culture (Fig. 7): the higher the colistin concentration, the smaller the number of food vacuoles formed. It is necessary, however, to consider the discrepancies between the above findings and the observations presented in Results (a). However, in the case of the biological test on naive cells, the ciliates were exposed to the antibiotic immediately after inoculation, while in the other experiment 3 h elapsed between the inoculation and the addition of colistin to the culture. Accordingly, in the two experiments, cells were undergoing different growth phases when exposed to the drug. Such a conclusion may be confirmed by the relationship between phagocytosis in *Tetrahymena* and the culture growth phase, demonstrated in part E of the paper.

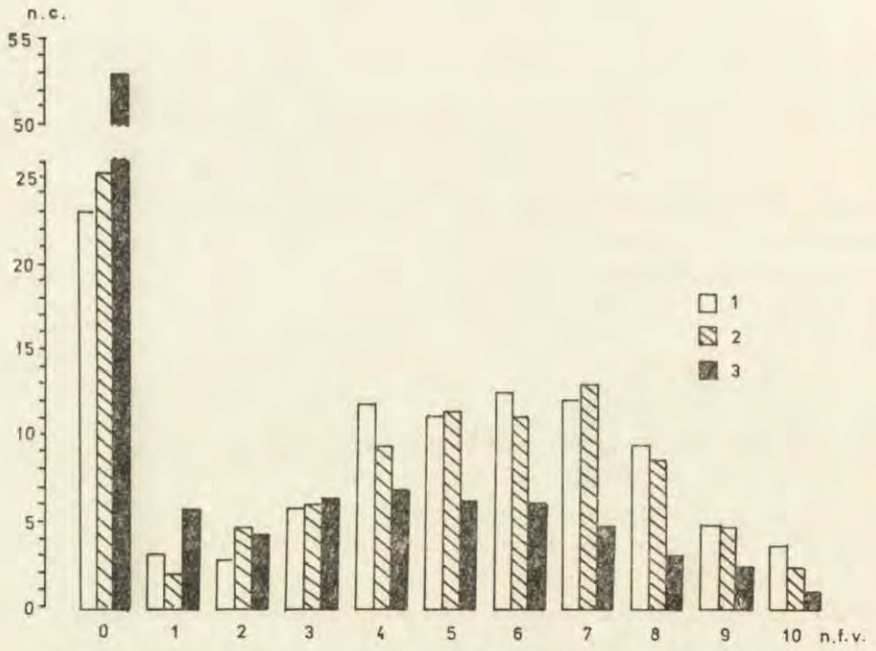

Fig. 7. The average number of *Tetrahymena* cells (n. c.) with a definite number of food vacuoles (n.f.v.) according to colistin concentration in the medium. Denotations of control and colistin concentrations — see Fig. 2

F. The Study on the Detergent Properties of Colistin

The antibiotic at both concentrations used in the study did not seem to produce changes in the threshold KCl concentration leading to continuous ciliary reversal in *Tetrahymena* (Table 4), since the differences in the number of controls and experimentals undergoing ciliary reversal were not statistically significant.

Table 4

The percentage of cells with continuous ciliary reversal according to KCl and colistin concentrations in the medium

Concentrations of KCl in mM/l	Per cent of cells in continuous ciliary reversal		
	Control	colistin 0.05 mM	colistin 1 mM
1	0	0	0
2	0	0	0
3	2.77	4.44	0
4	23.33	23.33	21.11
5	67.77	68.88	56.66
6	82.22	83.33	71.11

Discussion

The results of the studies on the action of colistin on *Tetrahymena pyriformis* GL presented above demonstrated the ability of the ciliates to survive in the presence of certain antibiotic concentrations for longer periods of time. The phenomenon seems to be due to the protozoan capability to adapt to the conditions markedly different from these occurring in nature. The analysis of findings reveals that *Tetrahymena* adapt to the changes in the medium through physiological adaptation A similar response of such ciliates has been observed also with other inhibitors of cell metabolism (Frankel 1965, 1969 b, 1970, Gavin and Frankel 1966, Mazia and Zeuthen 1966, Rasmussen and Zeuthen 1966, Rosenbaum and Carlson 1969, Wunderlich and Peyk 1969 b, Roberts and Orias 1974, Wang and Hooper 1978).

The adaptation of *Tetrahymena* to the continuous presence of colistin in the medium by means of physiological adaptation is confirmed by, among other facts, the results of studies on changes in the rate of their growth following the addition of colistin to the culture medium. At 0.05 mM/l the process of adaptation progressed fairly quickly, while at 1 mM/l the change in the rate of growth in the investigated sample as well as the return to the control level took much more time. It should be remembered, however, that the cells used in the experiments divided asynchronously. Accordingly, some of them, being at the stabilization point in the cell cycle, did not respond to the presence of colistin in the medium and completed the stage of cytokinesis revealing their response to the drug not earlier than the next cell cycle. After the complete adaptation of *Tetrahymena* to colistin through physiological adaptation, the growth rates in experimentals and controls were the same. It must

be also kept in mind that the prolongation of the first generation time effected by the inhibitor addition to the culture medium is related to its concentration.

The frequency of cells undergoing cytokinesis decreases following the addition of colistin to the culture medium. Subsequently, however, the number of organisms in this stage of cell cycle gradually increases, to reach eventually the control level. The phenomenon might suggest that:

(1) If the physiological adaptation is a two-stage event, the phases of stomatogenesis and cytokinesis begin in the first stage and they occur at a slower rate than in cells from inhibitor-free cultures. In the second stage the investigated physiological functions would achieve the same rate as in the control cultures.

(2) If, on the other hand, the physiological adaptation is a one-stage event developing according to the principle of all-or-none, then a gradual increase in the number of cells undergoing cytokinesis might be due to the asynchronous divisions of the ciliates and shifts in the culture growth phases as compared to the controls.

The first concept is negated by the findings concerning the duration of cytokinesis, which was not affected by colistin at concentrations employed. Also alterations in the time of *Tetrahymena* generation produced by colistin took place only in the first generation.

Since *Tetrahymena* cease to form food vacuoles approximately 20 min prior to division (Chapman-Andresen and Nilsson 1968), and since cell divisions are asynchronous, a gradual increase in the number of food vacuoles in the cell might be explained in the following way. The ciliates from the antibiotic-containing growth medium were either in the first cell cycle if exposed to colistin before the stabilization point, or in the second cell cycle if the exposure occurred after this point. However, the phagocytosis rate characteristic of the control population was achieved after all the cells had adapted to the presence of colistin in the medium. A shift in the growth phases of the antibiotic-containing culture as compared to the control also plays a significant role in this case.

The findings concerning ink suspension uptake after 2 h of *Tetrahymena* exposure to 1 mM/l colistin might suggest the damage of the ciliates' oral apparatus by the antibiotic. This in turn could produce a complete arrest of phagocytosis, similar to the one observed by Orias and Pollock (1975). Were this assumption correct the uptake of ink particles by the cells would occur much later, possibly during the following cell cycles. However, the results obtained in the present study seem to indicate that the disturbed course of phagocytosis in *Tetrahymena* produced by colistin at the concentration of 1 mM/l might be

related merely to the functional changes in the oral apparatus. Also, colistin does not seem to produce physical and chemical changes in the medium capable of arresting phagocytosis, as the absence of macromolecules in the culture medium was earlier proved (Rasmussen 1973) to inhibit the process. In such case food vacuoles would not appear following the addition of the antibiotic to the culture during the entire experiment. On the other hand, the present study appears to confirm the observations by other authors (Rasmussen and Kludt 1970, Rasmussen and Modeweg-Hansen 1973), who claim that in *Tetrahymena* cytokinesis is a process related to phagocytosis. Only in specific conditions the cell growth and division can occur without phagocytosis (Rasmussen and Orias 1975).

Although the actual nature of adaptation remains unknown, the suggested explanations focus mostly on the development or triggering of some mechanism diminishing the effect of the particular inhibitor. Thus, *Tetrahymena* adaptation may be due to an increase in the physiological activity of a particular system susceptible to the given inhibitor. In the case of adaptation to cycloheximide, the theory seems to be confirmed by the following facts:

(1) The presence of numerous copies of a given gene or genes in the macronucleus; the DNA amount in the *Tetrahymena* macronucleus is 45 times as great as the DNA content of the haploid set of five chromosomes of the micronucleus (Allen and Nanney 1958).

(2) An increase by ca. 20% in the average content of macronuclear DNA in the *Tetrahymena* cell observed prior to the start of division, with cycloheximide continuously present in the medium (Wang and Hooper 1978).

(3) The occurrence of the additional S phase in the *Tetrahymena* macronucleus in the conditions inhibiting and delaying cell division such as actinomycin D shock (Cleffmann 1968) and high temperature shock (Scherbaum et al. 1959, Hjelm and Zeuthen 1967).

In the case of a long-term action of low concentrations of cell metabolism inhibitors upon *Tetrahymena*, there may appear a cell or cells which are either more resistant or totally unsusceptible to the substance employed in the experiment. Due to such a genetic change or mutation, the cells will multiply more rapidly and thus their "offspring" will dominate over other cells, producing in effect a differentiation of the population according to the cells' susceptibility to a given inhibitor. As far as the physiological adaptation is concerned "domination" of the culture by the resistant cells cannot be considered, since:

(1) the arrest of cell division may last shorter than the mean generation time in cells not treated with the inhibitor;

(2) the time necessary to duplicate the culture after the completion

of the lag phase may be almost the same as before the addition of the inhibitor.

On the other hand, due to a specific structure of the macronucleus in ciliates (Nanney 1980), the long-term effect of the inhibitor might reveal the presence of resistant cells resulting from the increased number of gene copies in the macronucleus. However, with long-term action of low, non-lethal doses of the inhibitor the probability of a positive selection of a resistant macronuclear (somatic) variant seems slight (Kiersnowska 1981).

Studies employing an agent such as colistin, which changes the permeability of cell membrane, do not supply sufficient evidence to elucidate the mechanism responsible for the adaptation of the protozoan cell to the continuous presence of the drug in the medium. The findings demonstrate, however, that *Tetrahymena* are capable of the same physiological adaptation when exposed both to the agents easily penetrating into the cell and to the ones unable to cross the cell membrane, although with some inhibitors, e.g., puromycin (Frankel 1967) either adaptation does not occur or the process is incomplete, as when actinomycin C is employed (Jauker 1970). This results from the stable binding of particular chemical molecules in the cell by the inhibitors. Still, the presented examples are an exception rather than a rule in the process of physiological adaptation. Kiersnowska's suggestion (1981) that the ciliates' response to the continuous presence of cell metabolism inhibitors in the medium is their specific feature, depending on the occurrence in the cell of certain mechanisms neutralizing the inhibitory effect of the drug seems highly probable. Such type of adaptation may develop in all cells and the mechanism responsible for the resistance to a given inhibitor appears to be triggered in all cells simultaneously, irrespective of the cell cycle phase a given organism is undergoing.

Synchronized phases of cell cycle may appear in the presence of the inhibitor only when the developmental processes run a nearly normal course in all stages of the cycle, except one, the most susceptible critical phase. Two critical stages may be distinguished in the cell cycle, the transition from the G_1 phase to S_{DNA} and the moment immediately prior to cell division (Nachtwey and Giese 1968, Mitchison 1977, 1978). The continuous presence of colchicine or colcemid in the medium leads to the inhibition of living processes in *Tetrahymena* undergoing the most sensitive stage of the cycle, while cytokinesis remains unaffected by the substances (Wunderlich and Peyk 1969 a, b) and the process of physiological adaptation allows their synchronous emergence from the phase. Nelsen (1970), on the other hand, proved that colchicine "pushes" *Tetrahymena* into the phase of resorption of differentiating oral kineties for the posterior daughter cell when they

are most susceptible to the inhibitor effect. Thus, the course of the cortical division morphogenesis is temporarily arrested. Subsequently, the cells resume the process of morphogenesis, but the divisions are continued without the cells "entering" the stage of resorption. If colistin had actually effected the synchronized cell divisions in *Tetrahymena*, the number of cells undergoing cytokinesis would have been significantly greater than in control cultures. No accumulation of cells undergoing any particular stage of cell cycle was, however, found in any cultural growth phase following the antibiotic addition, especially when the lag phase had been completed. Accordingly, it may be concluded that the employed colistin concentrations did not effect synchronization of cell division. The conclusion is confirmed by the time of ciliates' generation. The differences in the duration of generation in ciliates from the same culture are due to the different durations of cell cycles in particular asynchronously dividing cells (Smith and Martin 1974).

Besides, colistin does not seem to exert the effect typical of many detergents, i.e., it does not modify the duration of ciliary reversal effected by potassium ions, as no difference was established in the threshold KCl concentration producing continuous ciliary reversal between controls and cells exposed to the antibiotic.

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REFERENCES

Allen S. L. and Nanney D. L. 1958: An analysis of nuclear differentiation in the selfers of *Tetrahymena*. Am. Nat., 92, 139–160.

Axline S. G., Yaffe S. J. and Simon H. J. 1967: Clinical pharmacology of antimicrobials in premature infants. II. Ampicillin, methicillin, oxacillin, neomycin and colistin. Pediatrics, 39.

Brutkowska M., Dryl S. and Mehr K. 1974: Action of detergents on motility of *Tetrahymena pyrifomis* GL. J. Protozool., 21, 440–441.

Chapman-Andresen C. and Nilsson D. M. 1968: On vacuole formation in *Tetrahymena pyriformis* GL. C. R. Trav. Lab. Carlsberg, 36, 405–432.

Cleffmann G. 1968: Regulierung der DNS-menge in Macronucleus von *Tetrahymena*. Exp. Cell Res., 50, 193–207.

Dryl S. 1974: Behaviour and motor response of *Paramecium*. In: *Paramecium*, a Current Survey (ed. W. J. Van Wagtendonk), Elsevier Scientific Publishers Company, Amsterdam–London–New York, pp. 165–218.

Elliott A. M. and Clemmons G. L. 1966: An ultrastructural study of ingestion and digestion in *Tetrahymena pyriformis*. J. Protozool., 13, 311–323.

Few A. V. and Schulman J. H. 1953: The absorption of polymyxin E by bacteria and bacterial cell walls and its bactericidal action. J. Gen. Microbiol., 9, 454.

Frankel J. 1960: Morphogenesis in *Claucoma chattoni*. J. Protozool., 7, 362–376.

Frankel J. 1965: The effect of nucleic acid antagonists on cell division and oral organelle development in *Tetrahymena pyriformis*. J. Exp. Zool., 159, 113–148.

Frankel J. 1967: Studies on the maintenance of oral development in *Tetrahymena pyriformis* GL–C. II. The relationship of protein synthesis to cell division and organelle development. J. Cell Biol., 34, 841–858.
Frankel J. 1969 a: Participation of the undulating membrane in the formation of oral replacement primordia in *Tetrahymena pyriformis*. J. Protozool., 16, 26–35.
Frankel J. 1969 b: The relationship of protein synthesis to cell division and oral organelle development in synchronized *Tetrahymena pyriformis* GL–C: An analysis employing cycloheximide. J. Cell. Physiol., 74, 135–148.
Frankel J. 1970: An analysis of the recovery of *Tetrahymena* from effects of cycloheximide. J. Cell. Physiol., 76, 55–64.
Frankel J. and Heckmann K. 1968: A simplified Chatton-Lwoff silver impregnation procedure for use in experimental studies with ciliates. Trans. Am. Microsc. Soc., 87, 317–321.
Gavin R. H. and Frankel J. 1966: The effects of mercaptoethanol on cellular development in *Tetrahymena pyriformis*. J. Exp. Zool., 161, 63–82.
Hayes A. W. 1977: Effect of the mycotoxin, penicillin acid, on *Tetrahymena pyriformis*. Toxicon, 15, 497–504.
Heyer C. B. and Frankel J. 1971: The kinetics of resensitilization of *Tetrahymena* following recovery from effects of cycloheximide. J. Cell. Physiol., 78, 411–418.
Hjelm K. K. and Zeuthen E. 1967: Synchronous DNA synthesis following heat-synchronized cell division in *Tetrahymena*. Exp. Cell Res., 48, 231–232.
Jauker F. 1970: Anpassungerscheinungen von *Tetrahymena pyriformis* on langdauernde Actinomycin-behandlung. Cytobiologie, 1, 208–227.
Jawetz E. 1970: Polymyxins, colistin, bacitracin, ristocetin and vancomycin. In: Antimicrobial Therapy (ed. B. M. Kagan), W. B. Saunders Co., Philadelphia–London–Toronto, p. 91.
Kawamata J. and Nakajima K. 1965: Mode of action of colistin. Antimicrob. Agents Chemother., 403.
Kiersnowska M. 1981: Wpływ cykloheksymidu na rozwój *Euplotes minuta* i *Chilodonella cucullulus*. Doctoral Diss.
Korzybski T., Kowszyk-Gindifer Z. and Kuryłowicz W. 1977: Antybiotyki, pochodzenie, rodzaje i właściwości. T. II. PZWL, Warszawa, 1771 p.
Kuryłowicz W. 1979: Antybiotyki, aktualny stan wiedzy. PZWL, Warszawa, 224 p.
Mazia D. and Zeuthen E. 1966. Blockage and delay of cell division in synchronous populations of *Tetrahymena* by mercaptoethanol. C. R. Trav. Lab. Carlsberg, 35, 341–361.
McKay D. N. and Kay D. 1964: Serum concentrations of colistin in patients with normal and impaired renal function. New Engl. J. Med., 270.
Mitchison J. M. 1977: Cell cycle control models. In: Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells. (eds. B. Drewienko and R. M. Humprey), The Williams and Wilkins Company, p. 23–33.
Mitchison J. M. 1978: Biologia cyklu komórkowego. PWRiL, Warszawa 339 p.
Nachtwey D. S. and Giese A. C. 1968: Effects of ultraviolet light irradiation and heat-shocks on cell division in synchronized *Tetrahymena*. Exp. Cell Res., 50, 167–176.
Nanney D. L. 1980: Experimental Ciliatology. (ed. J. Wiley and Sons).
Nelsen E. N. 1970: Division delays and abnormal oral development produced by colchicine in *Tetrahymena*. J. Exp. Zool., 175, 69–83.
Németh G. and Csik L. 1961: Effect of penicillin on *Tetrahymena pyriformis* GL. I. Multiplication and change of form. Acta Biol. Acad. Sci. Hung., 11, 405–410.
Newton B. A. 1953: The release of soluble constituents from washed cells of *Pseudomonas aeruginosa* by the action of polymyxin. J. Gen. Microbiol., 9, 54.
Newton B. A. 1954 a: Site of action of polymyxin on *Pseudomonas aeruginosa*: antagonism by cations. J. Gen. Microbiol., 10, 491.
Newton B. A. 1954 b: The absorption of polymyxin by cell wall preparations from *Pseudomonas aeruginosa*. J. Gen. Microbiol., 10.
Newton B. A. 1955: A fluorescent derivative of polymyxin: its preparation and

use in studying the site of action of the antibiotic. J. Gen. Microbiol., 12, 226–236.
Nyberg D., Et-Mallakah R. S. and Szeto G. 1978: Acclimation of *Paramecium* to cooper. J. Hered., 69, 423–426.
Orias E. and Pollock N. A. 1975: Heat-sensitive development of the phagocytic organelle in a *Tetrahymena* mutant. Exp. Cell Res., 90, 345–357.
Rasmussen L. 1973: On the role of food vacuole formation in the uptake of dissolved nutrients by *Tetrahymena*. Exp. Cell Res., 82, 192–196.
Rasmussen L. 1976: Nutrient uptake in *Tetrahymena pyriformis*. Carlsberg Res. Commun., 41, 143–167.
Rasmussen L., Buhse H. E. Jr. and Groh K. 1975: Efficiency of filter feeding in two species of *Tetrahymena*. J. Protozool., 22, 110–111.
Rasmussen L. and Kludt T. A. 1970: Particulate material as a prerequisite for rapid cell multiplication in *Tetrahymena* cultures. Exp. Cell Res., 59, 457–463.
Rasmussen L. and Modeweg-Hansen L. 1973: Cell multiplication in *Tetrahymena* cultures after addition of particulate material. J. Cell Sci., 12, 275–283.
Rasmussen L. and Orias E. 1975: *Tetrahymena*: Growth without phagocytosis. Science, 190, 464–465.
Rasmussen L. and Zeuthen E. 1966: Cell division in *Tetrahymena* adapting to p-DL-fluorophenylalanine. Exp. Cell Res., 41, 462–465.
Rebandel H., Gierczak A. and Karpińska A. 1981: Toxic action of Colistin and Penicillin V and G on *Tetrahymena*. I. Lethal effect and influence on multiplication. Acta Protozool., 20, 177–184.
Rebandel H. and Karpińska A. 1981: Toxic action of Colistin and Penicillin V and G on *Tetrahymena*. II. Inhibition of phagocytic activity. Acta Protozool., 20, 291–298.
Rebandel H. 1981: Toxic action of Colistin and Penicillin V and G on *Tetrahymena*. III. Effects on pairing for conjugation. Acta Protozool., 20, 299–308.
Roberts Ch. T. and Orias E. 1974: On the mechanism of adaptation to protein synthesis inhibitors by *Tetrahymena*. J. Cell Biol., 62, 707.
Rosenbaum J. L. and Carlson K. 1969: Cilia regeneration in *Tetrahymena* and its inhibition by colchicine. J. Cell Biol., 40, 415–425.
Russel S. 1977: Antybiotyki, PWN, Warszawa, 323 p.
Satir B. 1967: Effect of actinomycin D on cultural growth phases and on the pattern of total RNA synthesis in *Tetrahymena*. Exp. Cell Res., 48, 253–262.
Scherbaum O. H., Louderback A. L. and Jahn T. L. 1959: DNA synthesis, phosphate content and growth in mass and volume in synchronously dividing cells. Exp. Cell Res., 18, 150–166.
Sebek O. K. 1967: Polymyxins and circulin. In: Antibiotics, Vol. I: Mechanism of Action. (eds D. Gottlieb and P. D. Shaw), Springer Verlag.
Smith J. A. and Martin L. 1974: Cell Cycle Control. (eds. G. Padilla, Cameron J. L. and A. Zimmermann), Academic Press, New York.
Smith-Sonneborn J. and Rodermal R. S. 1976: Loss of endocytic capacity in aging *Paramecium*. The importance of cytoplasmic organelles. J. Cell Biol., 71, 575–588.
Sokal R. R. and Rohlf F. J. 1969: Biometry: W. H. Freeman and Co., San Francisco, 776 p.
Szablewski L. 1981: The effect of selected antibiotics on *Tetrahymena pyriforms* GL. Acta Protozool., 20, 309–322.
Szablewski L. 1982: The effect of penicillin G (sodium salt) on *Tetrahymena pyriformis*. Acta Protozool., 21, 163–171.
Wang T. Ch. and Hooper A. B. 1978: Adaptation to cycloheximide of macronuclear synthesis in *Tetrahymena*. J. Cell. Physiol., 95, 1–12.
Williams D. B. 1980: Clonal aging in two species of *Spathidium* (*Ciliophora*: *Gymnostomatida*). J. Protozool., 27, 212–215.
Wunderlich F. and Peyk D. 1969: a: Antimitotic agents and macronuclear division of ciliates. I. Colchicine and colcemid in exponentially growing cultures of *Tetrahymena pyriformis* GL. Naturwissenschaften, 56, 285–286.
Wunderlich F. and Peyk D. 1969 b: Endogenous recovery from colchicine and colcemid — a new method of synchronization in *Tetrahymena pyriformis* GL. Exp. Cell Res., 57, 142–144.

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Parastasia caudata sp. n. (*Euglenida*) — a Parasite of Copepods

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Synopsis. Parastasia caudata sp. n. (*Euglenida*), inhabiting gut of copepods from small water bodies from the environs of Warszawa and Leningrad, is described. The life cycle of this parasite has been investigated, the trophozoites, palintomic division and flagellate forms are described and compared with other representatives of the genus *Parastasia*. Presence of the stigma in both parasitic and free living stages is found to be a characteristic feature of the new species as well as formation of the flagellum by the parasites just after leaving host gut, casting it off before palintomic division and reconstituting after the division. Before formation of the flagellum the parasites move in water in the same manner as in the host gut (metabolic motion). Flagellate forms, swimming in water, elongate and form a characteristic tail. Post-divisional flagellate specimens swim quickly in a characteristic manner due to a very long flagellum, three times longer than the body.

Investigations on parasitic flagellates inhabiting copepode gut are still furnishing new data. Despite many papers dealing with this subject (Michajłow 1964 a, b, 1965, 1966, 1972, Palienko 1982 a, b, Wita 1978 a, b, 1984, Wita and Sukhanova 1983) new species are still described and new properties of their biology are recognized.

In the present paper the new species, *Parastasia caudata* sp. n. is described as well as its original life cycle [1].

[1] The paper was partly done in Laboratory of Unicellular Organisms of Institute of Cytology of the USSR Academy of Sciences, Leningrad.

Material and Methods

The new species, *P. caudata* was found in plankton collected from a small pond near Warsaw in spring and summer 1978 and in summer 1979. The same species was found also in summer 1980 and 1983 in copepods from a pond near Leningrad. The copepods were surveyed alive. The parasites, taken out of the gut or emerging spontaneously from the gut, were cultured individually in a drop of water in a humid chamber on the objective slide in room temperature. Individual cultures allow to survey a sequence of changes in these protozoans during their life cycle.

In the environs of Warsaw the following copepods were found to harbour *P. caudata* sp. n.: *Eucyclops serrulatus* (Fischer), *Acanthocyclops viridis* (Jurine) and *Mesocyclops leuckarti* (Claus) — the main host of this parasite. In the environs of Leningrad *P. caudata* was found in *Acanthocyclops viridis* (Jurine), *A. bisetosus* (Rehb.), *A. vernalis* (Fischer), *Macrocyclops fuscus* (Jurine), *M. albidus* (Jurine), *Mesocyclops leuckarti* (Claus) and *Eucyclops serrulatus* (Fischer).

In Poland the prevalence of *P. caudata* was low — only a few per cents of copepods were infected, and the intensity of infection reached from 1 to 13 specimens per host. In USSR the prevalence and the intensity of infection were very low (only 1–3 protozoans per host), frequently single specimens of *P. caudata* were found in the gut, sometimes together with *P. fennica*. In both territories the parasite was more frequent in female cyclops. The parasites inhabited the whole gut. Usually in one copepode the parasites of different size were observed.

Description of the species is based mainly on living protozoans. Cytochemical investigation on this species will be a subject of another publication. Living protozoans were measured in the moment of maximum elongation of the body (relaxation) during their forward movement (metabolic motion during creeping on the substrate or swimming with the aid of the flagellum).

Results

Description of the Species. Life Cycle

Trophic and Pre-Divisional Phase

Trophic specimens of *P. caudata* sp. n. (Fig. 1) are spindle shaped, with rounded anterior and tapered posterior end prolongating into a "tail". In transverse section the body is rounded. Young trophozoites as well as mature ones living in the gut do not have a flagellum and move due to metabolic movements opposite to peristaltic movements of the copepode gut.

The smallest specimens found in the gut, probably shortly after being swallen by the host, were 30 μm long and about 10 μm wide, the longest were about 120 μm long and 20–25 μm wide. Most frequently specimens measuring 60–80 μm were observed.

Development of the parasite in the host gut lasts 8–10 days. During

this time the protozoan does not divide but intensively feeds and stores paramylon used later during free life in water.

After leaving host intestine an aflagellate specimen of *P. caudata* moves further performing metabolic movements characteristic of the *Parastasia* species. When creeping on the substrate the protozoan elongates and the contraction runs down along the longitudinal body axis, causing the forward movement of the protozoan. During the contraction 1 or 2, rarely 3, swellings appear simultaneously on the body.

Fig. 1. Trophozoite of *P. caudata* sp. n. just after leaving host gut

One to two hours after leaving host gut in the anterior body part a flagellum about 60 μm long (equal to the body length) (Fig. 2 a) is formed. The flagellum is directed posterior forming an arch, sometimes a loop formed by it may be seen. Even quite small protozoans may produce the flagellum after emerging from the host gut. Just after the flagellum being formed the protozoan ceases creeping on the substrate and begins to swim in a characteristic manner (Fig. 2), only rarely producing metabolic swellings. The flagellate swims along a broken line rotating simultaneously around the body axis; the anterior, more mobile body end performs oscillatory movements and the tail acts as a rudder. Formation of the tail by swimming specimens is a characteristic feature of the described species. The tail is usually blunt but sometimes may

be tapered. It is 20–30 μm long but the length may change during metabolic movements. It is transparent due to lack of paramylon grains inside. Sometimes, the flagellate stands upright with the posterior end based on the ground; the tail takes part in fastening of the protozoan to the substrate. Generally however, the flagellate form does not attach to the substrate. Smaller specimens, measuring about 60 μm, swim more quickly than larger ones measuring about 100 μm. The latter may elongate even up to 150 μm.

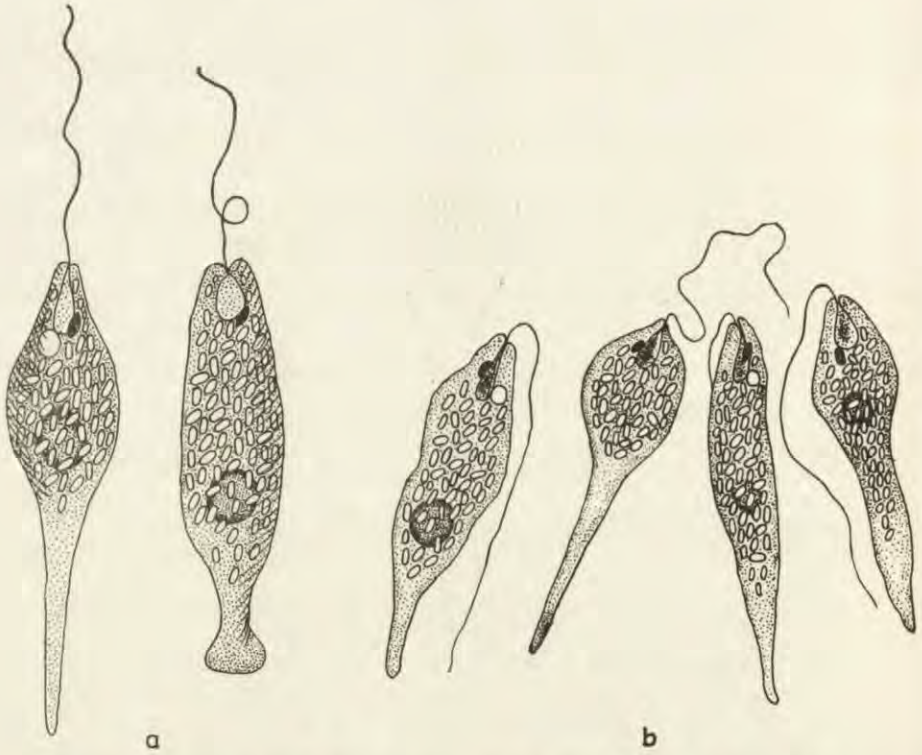

Fig. 2. *P. caudata* sp. n., a specimen 2–3 h after leaving host gut

The pellicle of the flagellate bears subtile spiral stripes difficult to observe in light microscope. It is elastic, enabling great changes of the body shape.

The cytoplasm of swimming specimens circulates and grains of paramylon gather mainly in the anterior portion of the body (Fig. 1).

In the anterior body part there is an opening leading to a canal dilating into an oval reservoir about 7 μm in the diameter, especially well visible during metabolic movements when the anterior body part is elongated. The reservoir is accompanied by the contractile vacuole

exhausting the content to it in every few seconds. Near the reservoir, but opposite to the vacuole, a pale pink bacilliform stigma composed of small granules is situated. It measures 1.5–2.0 μm in length and 1–1.2 μm in width. After swimming some time in water the stigma becomes more intensively pigmented. No chromatophores and pirenoids have been observed.

As the size and shape of grains are concerned the paramylonium is differentiated. The largest oval grains are 3–3.5 μm long and 2 μm wide. Other ones are 2–3 μm long and 1.5 μm wide. Sometimes they are almost sphaerical. In elongating specimens the grains are grouped mainly in the anterior part of the body.

Sphaerical nucleus, about 6 μm in the diameter, with central nucleolus visible in light microscope, is situated in the posterior half of the body. It may be slightly shifted onwards or backwards during metabolic movements.

Reproductive Phase

About 24 h after leaving the host gut mature specimens cast off the flagellum, attain sphaerical shape and begin to divide by palintomic division, the whole time performing metabolic movements. Premature specimens, get off from the gut, produce flagellae and swim energicall during 2–3 days. After that time they cast off the flagellum and begin to divide but frequently the division is not complete and post-divisional flagellate forms are not produced. After some time these protozoans become dead.

Palintomic division lasts usually 24 h, the successive divisions occur in 5–6 h intervals. They may be unequal, giving the offspring of different size, provided with the stigma but without the flagellum. The offspring form fairly loose aggregations. Sometimes the descendants of the first division separate completely while those of the successive divisions rest loosely aggregated. The fission rate is not the same in all specimens, it seems to depend on the stage of maturity of the protozoan emerging from the host gut. Three, sometimes four, palintomic divisions result in production of 8 or 16 descendant individuals (Fig. 3 a, b, c, d) which creep out and transform into flagellate forms called post-divisional flagellates, different from those formed by trophozoites.

The post-divisional flagellate forms measure usually 15–20 μm by 4–5 μm (Fig. 4). Some specimens measuring 20–28 × 10 μm were also observed. The size of the post-divisional forms depends on the size of the parental cell and mainly on the number of divisions undergone by parental individual. A thin flagellum grows out on the anterior body end; it measures about 60 μm and is about three times longer than the body. Flagellate specimens are provided with the stigma. Sometimes,

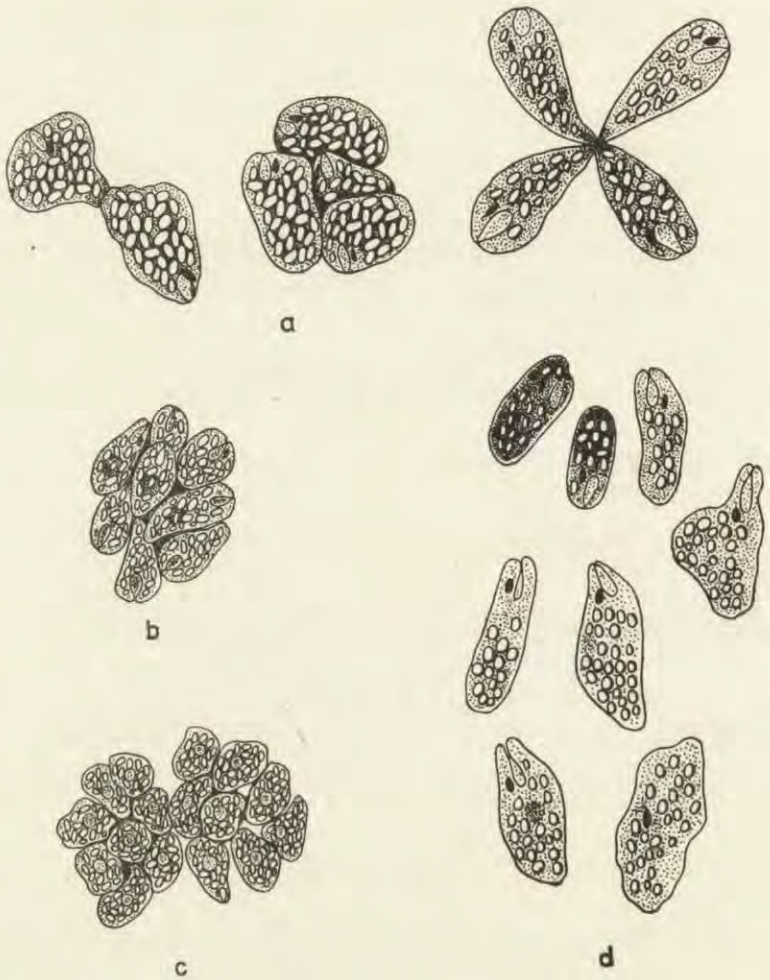

Fig. 3. Palintomic division of *P. caudata* sp. n.; a–d successive stages of palintomy

in specimens resulting of the Ist or the IInd division, some stigmae may be seen in the cytoplasm, not only in the anterior body end. The cytoplasm contains some or several small paramylon grains of different size. Sphaerical nucleus measures 5–6 μm in the diameter. The post-divisional flagellate specimens swim quickly in a characteristic manner due to proportionally very long flagellum. During swimming its body is flattened and twisted and bears also a swelling similar to that produced by metabolic movement. Sometimes a metabolic wave runs along the body, especially when the flagellate stops. These flagellates do not attach to the substrate. In unfavourable conditions (e.g., drying) they lose the flagellae and begin to creep on the substrate performing meta-

bolic movements. They are very delicate and quickly die. Post-divisional flagellates live 2–3 days, sometimes only 1–2 days, and die if not swallowed by cyclops. They fell down to the bottom, attain sphaerical shape, loss the flagellum and undergo vacuolization. They die after 2 days.

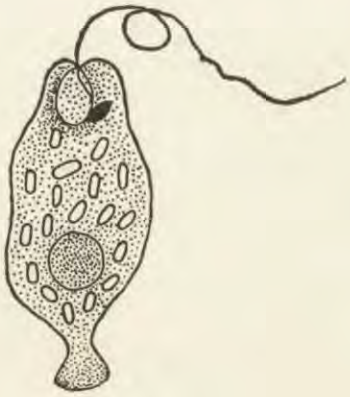

Fig. 4. Post-divisional flagellate form of *P. caudata* sp. n.

In experimental conditions the life span of *P. caudata* sp. n., from the moment of emerging from the host gut by the trophic stage to the death of the flagellate form, lasts 5–6 days (1–3 days for divisions, 2–3 days of life of the flagellate form). The whole life cycle of the described species lasts 13–16 days (8–10 days of the trophic stage, 5–6 days of the reproductive stage).

Discussion

Morphological features of the trophic and flagellate forms of the described species, as well as the course of the life cycle, allow to allocate it within the genus *Parastasia* Michajłow, 1972. However, this species differs clearly from all up to date described *Parastasia* species (Michajłow 1972, Palienko 1982 a, b, Wita 1978 a, b, 1984) so it is described as a new one, *P. caudata* sp. n.

The characteristic feature of *P. caudata* sp. n., beside the presence of the tail, is formation of the flagellum two times in the life cycle — by specimens emerging from the host gut and after palintomic division. The most similar in this respect is *P. macrogranulata* Wita, 1984 which also forms the flagellum just after leaving host gut but retains it during the whole free life, including the period of palintomy. Other species produce the flagellum after palintomic division. Formation of the flagellum just after leaving host gut is usually regarded as abnormal, being

the most frequently observed in immature trophic specimens taken out of the host gut.

P. caudata sp. n. is characteristic by the presence of stigma during the whole life cycle, in trophic as well as in flagellate form. Until now the stigma has been described in trophozoites of *P. hanoiensis* (Mich.) (Michajłow 1964, 1972), *P. sophiensis* (Mich.) (Michajłow 1965, 1972) and in trophozoites and flagellate specimens of *P. ucrainica* Wita (Wita 1978) and *P. macrogranulata* Wita (Wita 1984).

Out of all described species of the genus *Parastasia* *P. caudata* sp. n. is the most similar to *P. macrogranulata* (Wita 1984) and *P. hanoiensis* (Michajłow 1964).

In comparison with *P. macrogranulata* the trophic form of the new species has larger body dimensions while the flagellate forms are similar. This may be the result of greater number of palintomic divisions undergone by *P. caudata*. In flagellate and post-divisional flagellate forms of *P. caudata* the flagellum is much longer than in *P. macrogranulata*. The paramylon grains are more numerous but half as large as in *P. macrogranulata* and even in specimens spontaneously leaving host gut they do not fill tightly the cytoplasm as in the case of *P. macrogranulata*. Moreover, the paramylon grains in *P. caudata* are most frequently oval while larger grains of *P. macrogranulata* have various shapes (sphaerical, oval and other). Smaller number of paramylon grains in *P. caudata* than in other species causes greater transparency of its body in comparison with *P. macrogranulata* and *P. fennica*. Both species, *P. macrogranulata* and *P. caudata* differ greatly by the structure of the trophic and the flagellate forms as well as by the course of the life cycle, longer in *P. caudata*. Moreover, some differences may be observed in the character of motion of the flagellate forms.

P. caudata differs from *P. hanoiensis* (Mich.) (Michajłow 1964, 1972) by greater body dimensions of mature parasitic stage and less numerous paramylon grains, although similar in shape and size. The nucleus of *P. caudata* is about 2.5 times smaller than the nucleus of *P. hanoiensis*. The latter species may also form a tail but smaller than in *P. caudata*. A common feature of both compared species is occurrence of the stigma of similar dimensions in the parasitic stage. However, in post-divisional flagellate form of *P. hanoiensis* the stigma was not observed.

The flagellate forms of *P. caudata* are almost twice as large as the corresponding *P. hanoiensis* stages and have twice as long flagellum. They are, however, very similar in shape being flattened and twisted. Also the nucleus of the post-divisional flagellate of *P. caudata* is twice as large as the nucleus of *P. hanoiensis*. The common feature of both

species is that flagellate specimens swim in water after leaving the parental aggregation and do not attach to the substrate. The life cycle of *P. caudata* lasts two times longer than in *P. hanoiensis* although *P. caudata* undergoes only 3, sometimes 4 palintomic divisions while *P. hanoiensis* bears 5 divisions resulting in production of 32 offsprings. The post-divisional specimens of *P. caudata* form usually a loose aggregation while *P. hanoiensis* form a kind of a "colony".

As it has been mentioned above the trophozoites of *P. caudata* form the flagellum after leaving host gut and cast it off before palintomic division. In *P. hanoiensis* the specimens taken out of the host gut formed also the flagellum, their body elongated attaining spindle shape and formed a transparent tail. These specimens, similarly as those of *P. caudata*, stopped metabolic motion and performed progressive and rotatory movements with the aid of the flagellum. But in contrast to *P. caudata* they did not divide.

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REFERENCES

Fize A. and Michel R. 1972: Ultrastructure d'*Astasia fennica* Michajlow (*Euglenida*) parasite du tube digestif de Copépodes *Cyclopidae*. Protistologica 8, 143–153.

Michajłow W. 1964 a: *Astasia hanoiensis* n. sp. (*Flagellata*) from the intestine of *Copepoda* and its life cycle. Bull. Acad. Pol. Sci., Cl. II, XII, 7, 313–317.

Michajłow W. 1964 b: *Astasia norvegica* sp. n. (*Flagellata*), parasite of some species of *Cyclopoida* (*Copepoda*) in Norway. Acta parasit. pol., 12, 339–355.

Michajłow W. 1965: *Astasia bulgarica* sp. n. and *Astasia sophiensis* sp. n. *Euglenoidina parasitica* found in Bulgaria. Acta parasit. pol., 13, 7–17.

Michajłow W. 1966: *Astasia fennica* sp. n. (*Euglenoidina*) a parasite of some copepod species from the environs of Helsinki (Finland). Bull. Acad. Pol. Sci., Cl. II, XIV, 6, 439–442.

Michajłow W. 1972: Euglenoidina Parasitic in *Copepoda*. An Outline Monograph, PWN.

Palienko L. P. 1982 a: *Parastasia oligomera* (*Protozoa, Euglenoididae*) — novyj vid evglenoidid iz kišecnika ciklopov. Vest. Zool., Kiev, No. 1, 77–79.

Palienko L. P. 1982 b: *Parastasia breviflagellata* sp. n. — novyj vid evglenoidid (*Protozoa, Euglenoididae*) iz kišecnika ciklopa *Eucyclops serrulatus*. Vest. Zool., Kiev, No. 3, 78–80.

Wita I. 1978 a: *Parastasia ukrainica* sp. n. (*Euglenoidina*), a parasite of *Copepoda* in water bodies of the environs of Kiev. Bull. Acad. Pol. Sci., Cl. II, XXVI, 7, 489–494.

Wita I. 1978 b: *Parastasia kieviensis* sp. n. (*Euglenoidina*) parasite of some *Copepoda* from the Ukraine. Bull. Acad. Pol. Sci., Cl. II, XXVI, 8, 555–560.
Wita I. 1984: A new flagellate species *Parastasia macrogranulata* sp. n. (*Euglenida*) parasitizing *Copepoda* in the environs of Leningrad. Acta parasit. pol., 29.
Wita I. and Sukhanova K. M. 1983: Studies on the biology and cytology of *Parastasia fennica* (Michajłow) (*Flagellata, Euglenida*), a parasite of the intestine of cyclopidae (*Copepoda*). Acta Protozool., 22, 55–70.

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Etude sur les Thécamoebiens du mésopsammon

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Synopsis. Dans ce travail, nous étudions l'influence du milieu sur la morphologie des populations thécamoebiennes du psammon supralittoral et nous envisageons l'hypothèse de schémas phylogénétiques partiels.

Au cours de nos études sur le mésopsammon des plages de la mer du Nord en Belgique, nous avons constaté, que de nombreuses espèces formant les populations thécamoebiennes de ce type de biotope, avaient des mensurations sensiblement réduites par rapport aux mêmes espèces décrites d'autres plages et que les très petites espèces sont toujours plus abondantes.

Il est certain que le fasciès granulométrique du substrat joue un rôle dans la prolifération des espèces très petites.

Dans cette étude, nous avons recensé la population thécamoebienne du mésopsammon d'une plage, où la diversité spécifique est assez grande pour donner une idée de l'importance du substrat sur l'association des espèces et sur leurs dimensions.

Matériel et méthode

Plusieurs échantillons ont été prélevés dans un rayon de 3 m, sur une plage à Oostende; à 2 m des vagues a marée haute et 30 cm de profondeur. Chaque échantillon consiste en 100 cc d'eau contenant environ 30 gr de sable sec.

Au laboratoire, les échantillons ont été placés en éprouvettes et fixés par quelques gouttes de picro-formol de Boin.

Sur la côte belge, la salinité de l'eau baignant le mésopsammon est proche de celle de la mer: environ 31,666 NaCl/l, pH moyen 7,34; l'écart du pH entre le haut et le bas des plages est minime, l'expérience nous a montré que les fluctuations saisonières n'ont pas grande influence sur les populations de Thécamoebiens.

Considérations sur l'adaptation au biotope

Les plages sableuses de la côte belge ont une faible déclivité et une zone de balancement des marées très grande, elles sont constituées de sable fin et plus particulièrement celui présent dans la région mésopsammique, où les grains les plus fins s'accumulent et réduisent l'espace vital disponible. Cette zone en raison de ses propriétés absorbantes favorise la fixation des oligo-éléments principaux.

Les conditions physiques, température, pH, salinité, oxygénation et éclairement ont naturellement une influence sur la distribution biotique des Protozoaires d'une façon générale, mais beaucoup moins grande que la nature et la quantité de nourriture disponible pour espèces peu mobiles adaptées à ce type de biotope, véritables microcavernes, où l'eau circule lentement, sous l'effet du flux, du reflux et des mouvements ascensionnels.

La structure particulière des régions pseudostomiennes; col large et évasé, leur permettant une fixation parfaite aux grains de sable, réduit les déplacements de ces Thécamoebiens au strict minimum. L'acquisition de ces particularités morphologiques, évolution du type Acrostome vers le type Cotylostome: évasement du pseudostome en une collerette mince et souple dans la lignée *Ogdeniella* et l'inclinaison de la panse de la thèque jusqu'à devenir parallèle au substrat dans la lignée des *Psammonobiotus*, sont des caractères d'adaptation au biotope.

Que la pénétration des Thécamoebiens dans le psammon vienne de la mer vers le continent, ou des biotopes continentaux vers la mer, il y a dans ce milieu sinon formation d'une lignée phylogénétique commune, au moins une possibilité d'envisager des schémas partiels et de dégager les tendances qui se manifestent dans les lignées, dont la morphologie générale est semblable ou ressemblante.

Parmi ces caractères évolutifs, il faut considérer la réduction des dimensions permettant à certaines espèces de s'adapter à des espaces vitaux extrêmement réduits, ce qui tend à expliquer que nous n'avons jamais rencontré d'espèces psammophiles de grande taille dans ces plages. D'autre part, il est connu que la quantité d'eau contenue dans l'espace intergranulaire d'un sable brut est faible de l'ordre de 20% du volume considéré (Fauré-Frémiet 1951), seules de très petites espèces peuvent vivre dans les espaces les plus larges, délimités par les plus gros grains toujours comblés par les grains les plus fins.

Considérations phylogénétiques

Si comme Golemansky (1982) on émet l'hypothèse que la pénétration des Thécamoebiens dans le psammon supralittoral s'est faite du continent vers la mer, force nous est de constater que de nombreuses

espèces dulcaquicoles ou terricoles peuvent être à l'origine de quelques lignées; comme par exemple: les espèces Acrostomes des genres *Pseudodifflugia*, *Difflugiella*, *Nadinella* et *Cryptodifflugia* dont certaines ont tendance a former un col, ou encore, que des espèces des types Plagiostome simple ou Acrostome arcé suivant la classification des types morphologiques de Bonnet (1975) pourraient être à l'orgine des lignées *Centropyxiella* ou *Micropsammella*, il est possible que l'implantation et l'évolution de cette faunule caractéristique s'est faite par les eaux souterraines continentales.

En sens inverse, venu de la mer, on trouve actuellement uniquement deux genres: *Lagenidiopsis* et *Volutella*, mais qui ne peuvent pas dans l'etat actuel des connaissances être classés parmi les Thécamoebiens, ils seraient plustôt à rapprocher des Foraminifères, ou dans un groupe intermédiaire.

Cas des *Centropyxiella*

Sur les neuf espèces de *Centropyxiella* décrites, nous en avons retrouvé six dans cette biocénose. Dans ce groupe également, nous constatons une réduction sensible de la taille par rapport aux types décrits.

Si dans la lignée des *Centropyxiella*, la morphologie générale est proche des *Psammonobiotus*, par contre la structure de la thèque est différente, moins hyaline et beaucoup plus chargée de particules minérales volumineuses, ce qui leur donne un caractère plus pierreux.

Le caractère les différenciant le mieux des *Centropyxis* est la conformation de la région du pseudostome, dont les bords formant collerette s'évasent vers l'extérieur (Cotylostomie), tandis que chez les *Centropyxis*, ils s'invaginent vers l'intérieur (Plagiostomie). Si comme pour les genres précédents, nous émettons une hypothèse phylogénétique, le genre qui forme le chaînon le plus probable entre ces deux types morphologique est *Centropyxis* et plus particulièrement *C. constricta* (Ehrenberg) Deflandre, espèce banale d'eau douce de biotopes muscicoles et terricoles, dont de nombreux spécimens ont déjà été signalés dans les milieux salins Valkanov (1936). Boltovskoy E. et Boltovskoy A. (1968) et Golemansky (1970), nous l'avons recontré sur plusieurs plages de la mer du Nord.

Partant de cette espèce, dont le pseudostome est très légèrement rentrant, on aboutit facilement à *Centropyxiella oopyxiformis* Chardez, dont le pseudostome est simplement tronqué dans le prolongement de la face ventrale plane, puis, poursuivant le schéma, on constate progressivement se former un évasement des bords pour former cette vaste collerette définition même du type Cotylostome.

Note sur la systématique

Tous les Thécamoebiens psammobiontes stricts appartiennent à la sous-classe des *Filosia*, les espèces à structure agglutinant éventuellement de fines particules minérales sont regroupées dans la famille des *Psammonobiotidae* Golemansky qui comporte les genres: *Psammonobiotus, Alepiella, Chardezia, Micropsammella, Rhumbleriella* et *Ogdeniella.*

La sous-famille des *Corythionellinae* Sudzuki, rassemble des espèces dont la thèque est constituée d'écailles circulaires endogènes; elle comporte les genres: *Pseudocorythion, Messemvriella, Corthionelloides et Micropsammelloides.*

La sous-famille des *Corythionellinae* Sudzuki, rassemble des espèces dont la thèque est faite de petites écailles endogènes, ovales, naviculaires ou vermiformes, on y trouve les genres suivants: *Corythionella, Pseudowailesella et Lesurella.*

Parmi tous les Thécamoebiens décrits du psammon, quelques genres et espèces sont actuellement classés dans d'autres familles, c'est le cas du genre *Centropyxiella* provisoirement placé dans la famille des *Centropyxidae.* Enfin, nous citerons les espèces: *Difflugia subterranea* et sa variété *inflata, Ellipsopyxis marinus, Pomoriella valkanovi, Difflugiella psammophila, Difflugiella lamingerae, Phryganella marinus* et *Pseudodifflugia andrevi,* toutes ces espèces sont réparties dans leur famille respective, il n'est pas encore démontré que ces espèces soient des psammobiontes stricts.

Les schémas 1 et 2 figurant des relations phylogénétiques possibles, que nous dressons ici, n'intéressent que les genres observés sur cette plage, qui peut être considérée comme une biocénose délimitée dans l'espace, où les conditions écologiques sont semblables. Les espèces dont les no. sont entourés d'un cercle ont été observées dans nos pérlèvements.

Les dimensions moyennes (longueur totale) relevées dans ces prélèvements se répartissent comme suit (en microns): *Ogdeniella elegans* 39, *O. taschevi* 27, *O. lucida* 19, *O. carinata* 16, *O. pussilla* 8, *Psammonobiotus golemanskyi* 88, *Ps. communis* 39, *Ps. minutus* 22, *Ps. septentrionalis* 11, *Micramphora pontica* 16, *Micropsammella retorta* 30, *Centropyxiella arenaria* 45, *C. gibbula* 36, *C. gibbulina* 22, *C. golemanskyi* 22, *C. oopyxiformis* 48 et *C. lucida minima* 40.

Conclusion

La diversité des formes et des dimensions que l'on constate chez tous les Thécamoebiens, est une conséquence directe du milieu et démontre

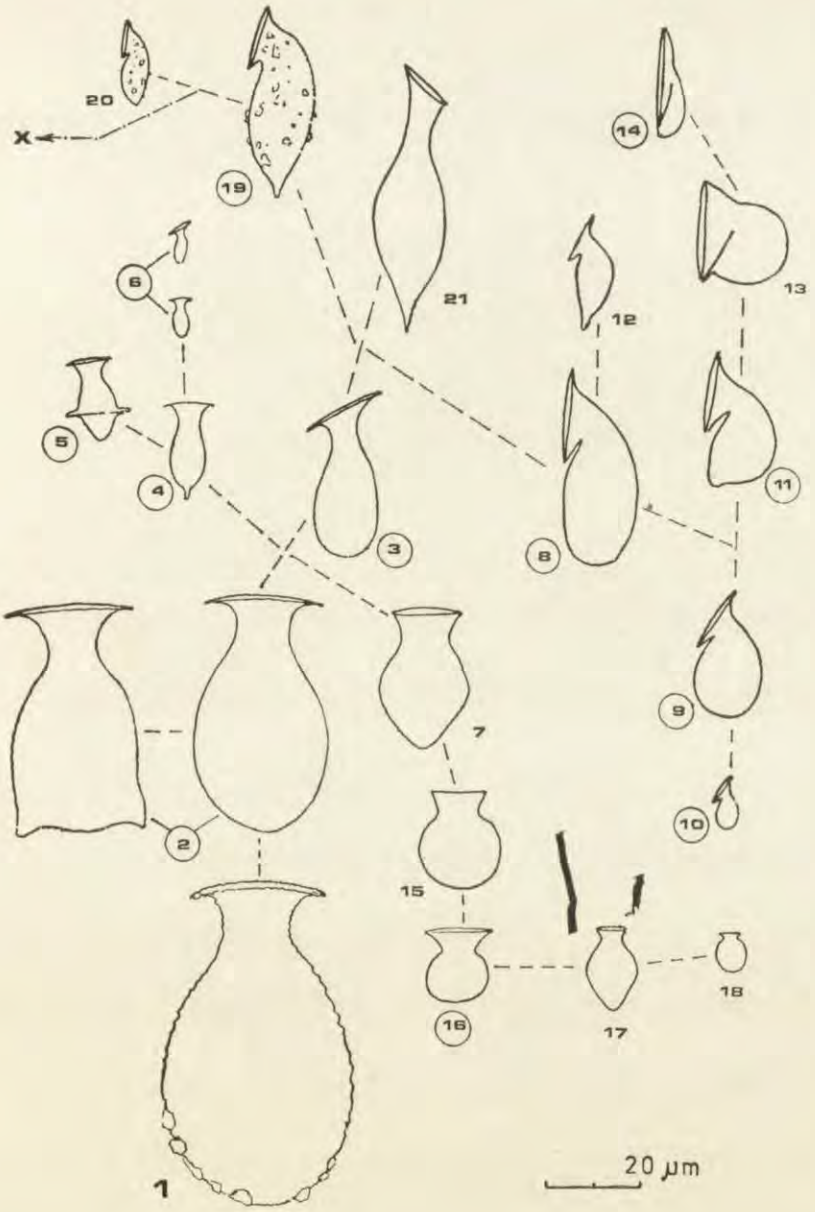

Fig. 1. *1* — *Ogdeniella maxima* Golemansky, *2* — *Ogdeniella elegans* Golemansky, *3* — *Ogdeniella taschevi* Golemansky, *4* — *Ogdeniella lucida* Golemansky, *5* — *Ogdeniella carinata* (Chardez) Golemansky, *6* — *Ogdeniella pusilla* (Chardez) Golemansky, *7* — *Ogdeniella conica* Golemansky, *8* — *Psammonobiotus communis* Golemansky, *9* — *Psammonobiotus minutus* Golemansky, *10* — *Psammonobiotus septentrionalis* Chardez, *11* — *Psammonobiotus golemanskyi* Chardez, *12* — *Psammonobiotus linearis* Golemansky, *13* — *Psammonobiotus balticus* Golemansky, *14* — *Psammonobiotus plana* Chardez, *15–16* — *Micramphora pontica* Valkonov, *17* — *Micramphora amphoriformis* Chardez et Thomas, *18* — *Micramphora atlantica* Chardez et Thomas, *19* — *Micropsammella retorta* Golemansky, *20* — *Miropsammella minima* Chardez et Thomas, *21* — *Chardezia caudata* Golemansky

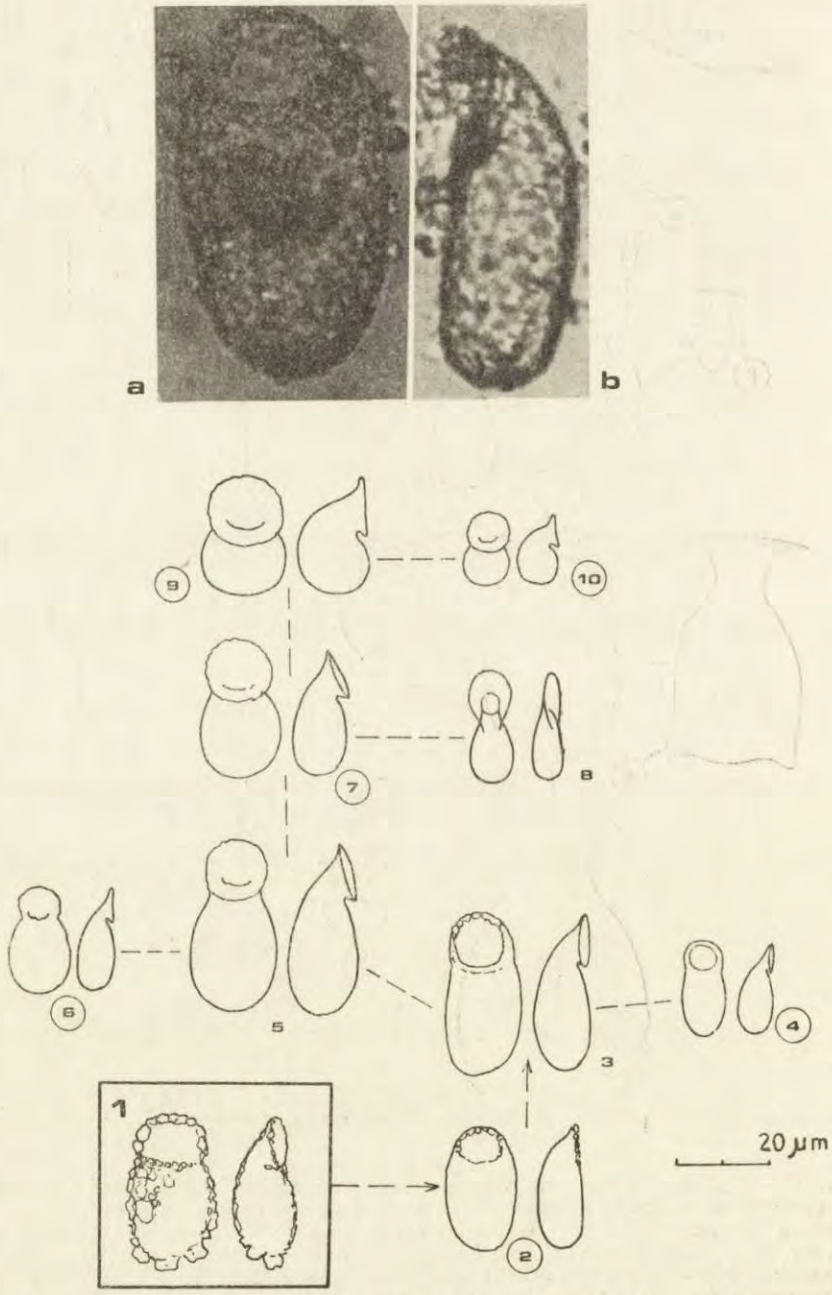

Fig. 2 *a* — *Centropyxiella oopyxiformis* Chardez (× 600), *b* — *C. oopyxiformis* (profil, × 600), *1* — *Centropyxis cassis* (Ehrenberg) Deflandre, *2* — *Centropyxiella oopyxiformis* Chardez, *3* — *Centropyxiella lucida* Golemansky, *4* — *Centropyxiella lucida* v. *minima* Chardez, *5* — *Centropyxiella elegans* Valkanov, *6* — *Centropyxiella golemanskyi* Chardez, *7* — *Centropyxiella arenaria* Valkanov, *8* — *Centropyxiella platystoma* Golemansky, *9* — *Centropyxiella gibbula* Valkanov, *10* — *Centropyxiella gibbulina* Chardez et Thomas

leur grand pouvoir d'adaptation à de nombreux biotopes. La structure même de l'habitat est un facteur déterminant qui joue un rôle important dans l'évolution des formes, permettant la vie de ces Protozoaires, présents dans des milieux aussi nombreux que variés.

SUMMARY

In this work, we are studying the influence of the surroundings on the morphology of the Testate Amoebae from the supralittoral psammal and we are considering the hypothesis of partial phylogenetic diagrams.

BIBLIOGRAPHIE

Boltovskoy E. et Boltovskoy A. 1968: Foraminiferas y Thecamoebas de la parte inferiore del Rio Quequen Grande. Mus. Argentino. Sc. Nat. "Bernardo Rivadavia" Hidrob., 2, 127–164.

Bonnet L. 1975: Types morphologiques et évolution de la thèque chez les Thécamoebiens. Protistologica, 2, 363–378.

Chardez D. 1977: Thécamoebiens du mesopsammon des plages de la mer du Nord. Rev. Verviétoise Hist. Nat., 4, 3–19.

Fauré-Frémiet E. 1951: Ecologie des Protistes littoraux. Ann. Biol., 27, 205–215.

Golemansky V. 1970: Contribution à la connaissance des Thécamoebiens des eaux souterraines du Golf de Gdańsk (Pologne). Bull. Inst. Zool. Mus., 31, 77–87.

Golemansky V. 1974: *Psammonobiotidae* fam. nov. une nouvelle famille de Thécamoebiens (*Rhizopoda testacea*) du psammal supralittoral des mers. Acta Protozool., 13, 137–142.

Golemansky V. 1982: Révision du genre *Ogdeniella* n. n. (= *Amphorellopsis* Gol. 1970), (*Rhizopoda Gromida*) avec considération sur son origine et évolution dans le milieu interstitiel. Acta Zool. Bulg., 19, 3–12.

Valkanov A. L. 1936: Notizen über die Brackwässer Bulgariens II. Ann. Univ. Sofia, III Fac. Phys. Math., 3 (Sci. Nat.), 32, 209–341.

Valkanov A. L. 1970: Beitrag zur Kenntnis der Protozoen des Schwarzen Meeres. Zool. Anz., 184, 241–290.

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Lepismatophila cruszi sp. n. New Cephaline Gregarine from Silver-fish *Acrotelsa collaris* (Fabricius) of West Bengal, India

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Synopsis. The paper contains the description of morphology and life history of a new species of cephaline gregarine (*Apicomplexa* : *Sporozoea*) of the genus *Lepismatophila* Adams and Travis, 1935 from the Silver-fish, *Acrotelsa collaris* (Fabricius). The gregarine has been named *Lepismatophila cruszi* sp. n. and has the following ratios: LP : TL = 1 : 2.75–19.0; WP : WD = 1 : 0.78–1.33. Information regarding the percentage of infection, seasonal intensity and type material have also been incorporated in the paper.

Cornwall (1915) first described a cephaline gregarine from a Silver-fish but did not assign it to any particular genus. Adams and Travis (1935) established a new genus *Lepismatophila* and described *Lepismatophila thermobiae* as the type species of the genus obtained from the firebrat, *Thermobia domestica* (Pack.). The characteristic features of the genus as proposed by them are as follows: (1) Sporadins solitary, (2) Epimerite a simple symmetrical knob, (3) Protomerite present throughout the trophozoite stage, (4) Cyst dehiscing by simple rupture and (5) Spores in uncoiling chains, ellipsoidal, devoid of processes. Since then a number of workers have described different species of *Lepismatophila* from various parts of the world (Table 1).

In our studies on the cephaline gregarines from insects we have also obtained a parasite from the common Silver-fish of the gangetic plain,

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Table 1

A chronological list of *Lepismatophila* species recorded so far from different parts of the world

Lepismatophila sp.	Hosts	Country	References
Lepismatophila thermobiae	*Thermobia domestica*	USA, England	Adams and Travis (1935)
	Lepisma saccharinum	England	
	Acrotelsa collaris	Ceylon	
L. cornwalli	*Ctenolepisma* or *Acrotelsa* sp.?	India	Bhatia (1938)
L. ctenolepismae	*Ctenolepisma longicaudatum*	Australia, Tasmania, Argentina	Lindsay (1939)
L. parva	*Ctenolepisma lineatum*	France	Tuzet et al. (1952)
L. campodeae	*Campodea augens*	France	,,
L. orientalis	*Peliolepisma calvum*	Ceylon	Crusz (1960)
	Ctenolepisma nigrum	,,	
L. pluciocampae	*Pluciocampa bureschi*	Bulgaria	Taschev and Golemansky (1973)
L. karnataki	*Ctenolepisma* sp.	India	Nimbargi and Rodgi (1974)
L. rhombocephala	*Ctenolepisma nigra*	,,	Haldar and Chakraborty (1977)
L. cruszi	*Acrotelsa collaris*	,,	Present study

Acrotelsa collaris (Fabricius) and as it differs from the known species of *Lepismatophila*, it is described here as a new species, and is named *L. cruszi* sp. n.

Material and Methods

The cephaline gregarine, *Lepismatophila cruszi* was obtained from the midgut and ventricular caeca of the Silver-fish, *Acrotelsa collaris* (Fabricius), collected from the old wooden furniture at Ranaghat, West Bengal. Smears of host gut content, fixed in Schaudinn's fluid and 5.0 μm thick sections of the infected host gut fixed in Bouin's fluid were stained in iron alum-haematoxylin. Gametocysts were collected from the hindgut of the host and were placed in moist chambers (Sprague 1941) for sporulation in living condition. Figures were drawn with a mirror type camera lucida. The ratios used in this paper are those of length of protomerite to total length (LP : TL) and width of protomerite to width of deutomerite (WP : WD). The following abbreviations are used in this paper: LE — length of epimerite, LP — length of protomerite, LD — length of deutomerite, LN — length of the nucleus, TL — total length, WE — width of epimerite, WP — width of protomerite, WD — width of deutomerite, WN — width of the nucleus.

Observations

Lepismatophila cruszi sp. n.

Development

The early developmental stages of the parasite within the epithelial cells are lacking, as revealed from a large number of serial sections studied by us so far. So, in all probability the mode of development is extracellular.

Trophozoite

Two distinct forms of the trophozoite are observed: (1) Free trophozoite (Fig. 1 *1–3*) and (2) Attached trophozoite (Fig. 1 *4–5*). These are separately described below.

Free Trophozoite

This is found to live free within the lumen of the ventricular caeca. The body has the usual three segments: the epimerite, the protomerite and the deutomerite. The epimerite of the trophozoite is sub-globular to almost globular in outline and its cytoplasm is hyaline. The protomerite is elliptical or conical in shape and is broadest at the base. The deutomerite is separated from the protomerite by a transverse ectoplasmic septum which is elevated at the middle in some cases. The deutomerite is broadest just behind the septum and then gradually narrows to a rounded posterior extremity. The cytoplasm in both protomerite and deutomerite is granular. The pellicle is moderately thick. The nucleus is circular in outline and has a distinct nuclear membrane. In some cases, there is a spherical endosome in the middle. The nucleus measures 12.7 μm to 21.2 μm in length (average 19.3 μm) and 10.6 μm to 21.2 μm in width (average 16.6 μm).

Attached Trophozoite

Occasionally a fully grown trophozoite remains attached with its epimerite to the intestinal epithelium of the host. The detailed structures of such a trophozoite are distinctly revealed in serial sections. The body as usual bears epimerite, protomerite and deutomerite. The epimerite is a petaloid or two-horned structure that pushes through the host gut epithelium towards the basement membrane. As a result a space is formed into which the epimerite is firmly lodged. The epimerite of the attached trophozoite measures 13.7 μm to 27.6 μm in length (average 20.6 μm) and 20.0 μm to 21.2 μm in width (average 20.6 μm). The protomerite is subconical or hemispherical in shape and measures 17.0 μm to

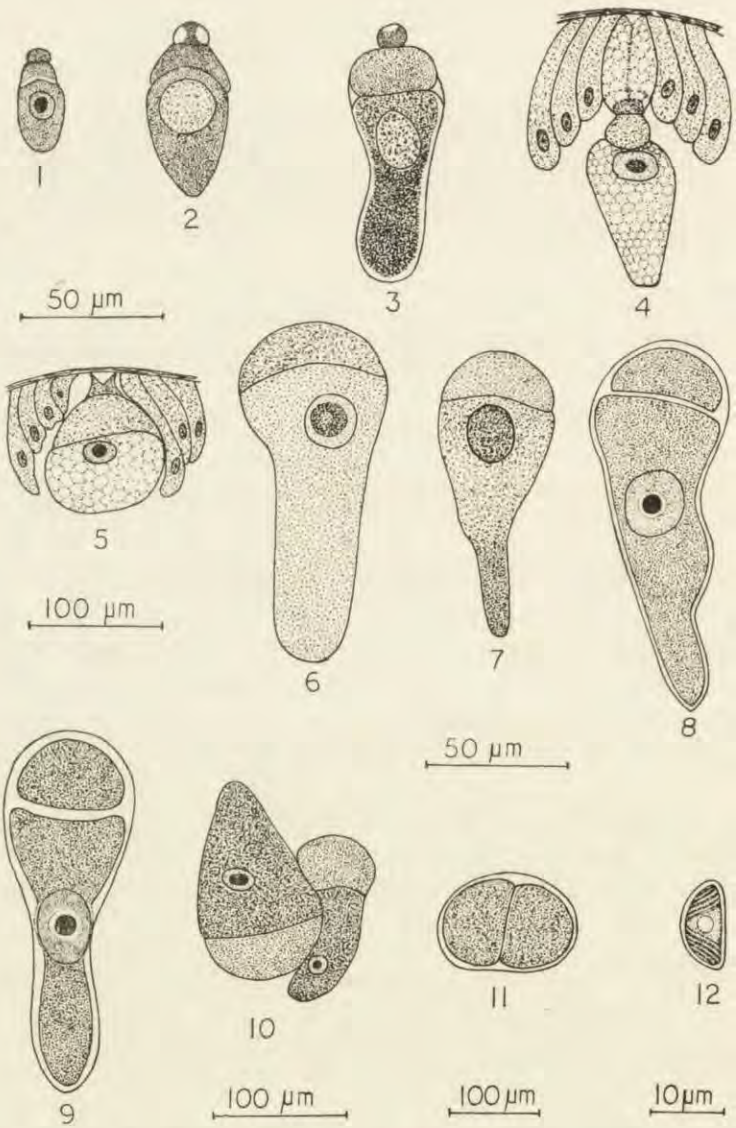

Fig. 1. *1–12* — Camera lucida drawings of the different stages in the life history of *Lepismatophila cruszi* sp. n., *1–3* — Free trophozoites of different shapes and sizes, all from smears (iron alum-haematoxylin), *4–5* — Trophozoites attached with the host gut epithelium by their epimerites, from sections (iron alum-haematoxylin), *6–9* — Sporadins showing variable structures of protomerite, deutomerite and nucleus (iron alum-haematoxylin), *10* — Sporadins in association (iron alum-haematoxylin), *11* — A freshly collected gametocyst, *12* — A spore showing eight slender sporozoites and residual cytoplasm (Lugol's iodine)

33.7 μm in length (average 25.3 μm) and 25.5 μm to 60.0 μm in width (average 42.7 μm). This is followed by the deutomerite and measuring 46.7 μm to 52.5 μm in length (average 49.6 μm) and 29.7 μm to 85.0 μm in width (average 57.3 μm). It is highly granulated and is separated from the protomerite by a clear septum. The nucleus is lodged immediately behind the septum, possesses a distinct nuclear membrane and encloses a big endosome and a few chromatin granules in it. The nucleus measures 18.5×12.0 μm on the average.

Sporadin

The sporadins (Fig. 1 *6–9*) are characteristically solitary and are found to live within the midgut lumen. In living specimens the cytoplasm appears milky-white under the microscope. The characters of protomerite and deutomerite and the structure of the nucleus are more or less the same as in the trophozoites. The only difference is that the pellicle in the sporadins is very thick.

Association

Typical caudofrontal or frontal associations (syzygy) are not observed. Instead, the two sporadins associate side-wise (Fig. 1 *10*) before enclosing themselves within the common cyst wall.

Gametocyst and Spore

A freshly collected gametocyst from the hindgut of the host is yellowish white in colour and is bean-shaped with one side convex and the other side slightly concave (Fig. 1 *11*). The cyst measures 138.8 × 99.9 μm. The enclosed gametocytes may be equal or unequal in size. With the gradual development inside the moist chamber, the cyst becomes deep black in colour. At about 96 h of development the cyst wall ruptures and the spores come out in uncoiling chains.

The spores are brownish in colour and measure 11.6 × 5.8 μm on the average. Each spore is boat-shaped with one side convex and the other side slightly concave and is provided with a thick spore wall (Fig. 1 *12*). At 110 h of development eight slender spindle-shaped sporozoites are formed within the spore. Some amount of residual cytoplasm is observed inside the spore after the formation of the sporozoites.

Measurements (in μm)

The summary of measurements of 25 specimens of trophozoites and sporadins with the mean within parenthesis is given below:

Trophozoite

TL = 36.1–150.0 (84.8)
LE = 5.3– 25.0 (11.1), WE = 8.5–30.0 (14.0)
LP = 7.4– 25.0 (14.1), WP = 12.7–50.0 (29.1)
LD = 23.3–100.0 (59.5), WD = 17.0–55.0 (32.2)
LN = 12.7– 21.2 (19.3), WN = 10.6–21.2 (16.6)

Sporadin

TL = 95.0–290.0 (185.6)
LP = 5.0– 45.0 (33.0), WP = 32.5–105.0 (72.5)
LD = 70.0–255.0 (152.6), WD = 35.0–110.0 (73.2)
LN = 20.0– 40.0 (30.6), WN = 15.0– 40.0 (28.6)
LP : TL = 1 : 2.75–19.0 (1 : 6.26), WP : WD = 1 : 0.78–1.33 (1 : 1.03)

Seasonal Intensity and Site of Infection

On an average, 54.5% of the insects are infected with this gregarine. The intensity of infection rises to the peak during the months of May to September. Young trophozoites are found within the ventricular caeca attaching to the epithelial cells by the epimerite. The fully grown trophozoites and sporadins are found to live free within the lumen of the midgut.

Material

Holotype, trophozoite on slide No. L/T-1, prepared from contents of ventricular caeca of the Silver-fish, *Acrotelsa collaris* (Fabricius) collected by T. K. Kundu at Kanaghat, West Bengal, India on 2 May, 1979. Paratypes, many other particulars are the same as for the holotype material.

The slides containing the holotype and paratype materials have presently been deposited at the Department of Zoology, University of Kalyani, and will finally be submitted to the National Collection of Zoological Survey of India, Calcutta.

Affinities

In having solitary sporadins, epimerite a simple knob and ellipsoidal spores, the gregarine is accomodated in the family *Lepismatophilidae* Kudo. The following characters justify the inclusion of the parasite in the genus *Lepismatophila* Adams and Travis: (1) Epimerite a simple knob, (2) Solitary nature of sporadins, (3) Cyst without ducts, and (4) Smooth, boat-shaped spores extruded in chains, without any filamentous process. It resembles *Lepismatophila karnataki* Nimbargi and Rodgi,

1974 in the general shape of the protomerite and spore, but differs from it in the characters of epimerite, structure of the nucleus, shape of the cyst and in the measurements of the different parts of the body. The LP : TL and WP : WD values also differ markedly in the two species. It also differs from *Lepismatophila orientalis* Crusz, 1960 in the general shape of the body of young as well as adult stages, absence of posterior processes and in the measurements of different body parts. Although the parasite resembles in the shape of the sporadin and spores that of *Lepismatophila rhombocephala* Haldar and Chakraborty, 1977, it differs from the latter in all other features. In having an epimerite which is spherical to hemispherical in the free trophozoites and a petaloid or two-horned structure in the attached trophozoites, unusually thick epicyte and spores with a residual cytoplasm, it can be separated from the previously described species under the genus. The parasite is, therefore, given a separate specific status and is named *Lepismatophila cruszi* new species. The specific name has been given after Prof. Hilary Crusz of Sri Lanka, who has contributed much in the understanding of the genus *Lepismatophila*.

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REFERENCES

Adams J. A. and Travis B. V. 1935: Two new species of gregarine protozoa from the firebrat, *Thermobia domestica* (Pack.) (*Thysanura*). J. Parasitol., 21, 56–59.

Bhatia B. L. 1938: Fauna of British India Sporozoa. London.

Cornwall J. W. 1915: *Lepisma saccharina* (?): its life history and anatomy and its gregarine parasite. Indian J. Med. Res., 3, 116–131.

Crusz H. 1960: New gregarines parasitic in Lepismatids from Ceylon. Proc. Zool. Soc. Lond., 135, 525–536.

Haldar D. P. and Chakraborty N. 1977: Observations on the cephaline gregarine, *Lepismatophila rhombocephala* n. sp. (*Protozoa* : *Sporozoa*) from silver fish. Arch. Protistenkd., 119, 361–366.

Lindsay E. 1939: Two gregarines from *Ctenolepisma longicaudata*, with notes on forms in other silver fish. Proc. R. Soc. Vict., 51, 99–111.

Nimbargi P. M. and Rodgi S. S. 1974: A new species of gregarine, *Lepismatophila karnataki*, sp. nov., from the silver fish, *Ctenolepisma* sp. J. Karnatak Univ. J. Sci., 19, 42–48.

Sprague V. 1941: Studies on *Gregarine blattarum* with particular reference to the chromosome cycle. Ill. Biol. Monogr., 18, 5–57.

Taschev D. and Golemansky V. 1973: *Lepismatophila pluciocampae* n. sp. (*Gregarinida, Stylocephalidae*) — une nouvelle gregarine de troglobie *Pluciocampa bureschi* Silv. (*Diplura, Campodeidae*). Zool. Anz., 190, 305–311.

Tuzet O., Manier J. F. et Ormieres R. 1952: Deux nouvelles especes de Gregarines du genre *Lepismatophila*, parasites de l'intestin de *Ctenolepisma lineata* Esch. et de *Campodea augens* Silvertri. Annls. Sci. Nat. Zool., 14 IIe, 87–93.

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Pyxinia reneae sp. n. and *Gregarina chaetocnemae* sp. n., New Cephaline Gregarines from the Coleopteran Insects of West Bengal, India

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Synopsis. The life history of two new species of cephaline gregarines (*Apicomplexa* : *Eugregarinida*), *Pyxinia reneae* sp. n. and *Gregarina chaetocnemae* sp. n. are described from the midguts of *Evorinea iota* (Arrow) and *Chaetocnema concinnipennis* Baly (*Coleoptera*) respectively from West Bengal, India. The characteristics of the gregarines are as follows: *Pyxinia reneae* sp. n. — its epimerite is a long ribbon set upon a smooth hemispherical projection on the protomerite, sporadins solitary, gametocysts oval to ellipsoidal, release of sporocysts by simple rupture, sporocysts ellipsoidal, 8.0 μm × 3.5 μm. *Gregarina chaetocnemae* sp. n. — its epimerite is a spherule, sporadins show caudofrontal association, relative size of primite and satellite variable, gametocysts ellipsoidal, extrussion of sporocysts through short sporoducts, in chain, sporocysts cylindrical and truncate, 8.0 μm × 3.2 μm.

Although numerous species of cephaline gregarines have been described from the coleopteran insects of the world (Schneider 1887, Léger 1892, Labbé 1899, Crawley 1903, Ellis 1913, Watson 1916, Foerster 1938, Grasse 1953, Obata 1953, Hoshide 1952, 1959, Geus 1969, Hoshide 1979, 1980), only 20 species of cephaline gregarines have so far been reported from Indian coleoptera by Misra (1941, 1942), Bhushanarao (1962), Amoji and Rodgi (1975), Chakraborty and Haldar (1975), Haldar and Chakraborty (1976, 1978, 1979 a, b), Devdhar and Amoji (1977), Patil and Amoji (1979 a, b), Kundu and Haldar (1981 a, b, c) and Sarkar and Mazumder (1983).

This communication describes the life history of two new cephaline gregarines under the genera *Pyxinia* Hammerschmidt, 1838 and *Gregarina* Dufour, 1828 from the coleopteran insects of West Bengal, India.

Material and Methods

The insects were collected from the various Jackfruit trees of Murshidabad, West Bengal, adjoining the border with Bangladesh during 1982 and 1983 and their guts were examined under a microscope for the protozoan parasite. The smears of the parasitized midguts were fixed in Schaudinn's and Bouin's fluids and both were subsequently stained with iron alum haematoxylin. The gametocysts, obtained from the host's gut or faeces, were kept in a moist chamber for their later phase of development. The sporocysts, after treatment with Lugol's iodine solution, were studied under the oil immersion lens of the Olympus research microscope. The figures were drawn with the aid of a camera lucida. The measurements were taken in micrometer (μm). The following abbreviations are used in this paper: LE — length of epimerite, LD — length of deutomerite, LN — length of nucleus, LP — length of protomerite, TL — total length, WE — width of epimerite, WD — width of deutomerite, WN — width of nucleus, WP — width of protomerite, LP : TL — ratio of the length of protomerite to total length and WP : WD — ratio of the width of protomerite to the width of deutomerite.

Observations

Pyxinia reneae sp. n.

Of the 29 insects examined, 6 have been found to be parasitized by this gregarine.

Trophozoite

These were almost fusiform with anterior complex epimerite, a conical protomerit and a cylindro-conical deutomerite with gradually tapering round posterior and (Fig. 1 *1*). The nucleus was, in the deutomerite, round with a large spherical karyosome in it. The epimerite was differentiable into a proximal small, smooth hemisphere and on the summit of it was a distal long ribbon. The largest trophozoite was 133.65 μm in dimension.

Measurements (the measurements of seven trophozoites are given in range with mean within parenthesis):

TL = 49.95–133.65 (96.75),
LE = 24.3 – 45.9 (33.5), WE = 3.24– 5.4 (3.7),
LP = 6.75– 16.2 (11.9), WP = 8.1 –18.9 (15.42),
LD = 18.9 – 78.3 (51.3), WD = 10.8 –29.7 (22.57),
LN = 4.05– 8.64 (6.75), WN = 4.05– 8.64 (7.32)

Sporadin

These were made up of a rhomboidal to spherical protomerite and a cylindro-conical deutomerite, the later being widest just below the

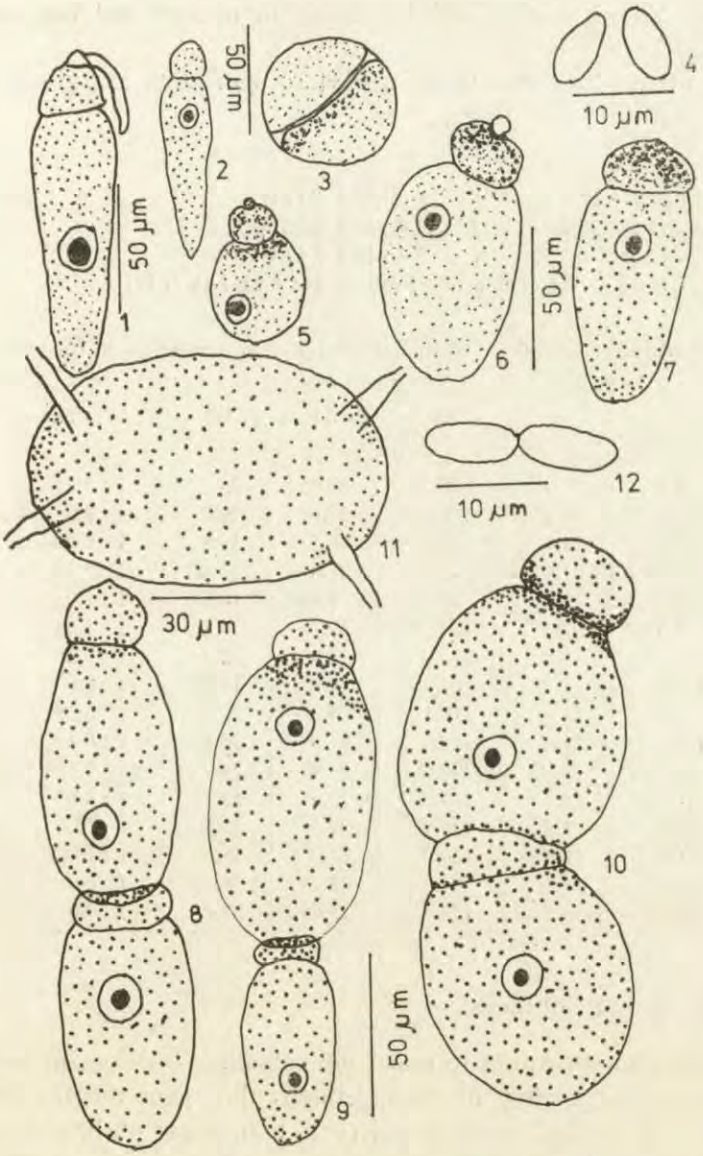

Fig. 1. *1–4* — The life history stages of *Pyxinia reneae* sp. n., *1* — A trophozoite with complex epimerite, *2* — A solitary sporadin, *3* — An early gametocyst with gametocytes, *4* — The ellipsoidal sporocysts, *5–12* — The life history stages of *Gregarina chaetocnemae* sp. n., *5–6* — The early and the fully-grown trophozoite respectively, *7* — A mature and solitary sporadin, *8–10* — Caudofrontal associations or syzygy stages showing relative variations in dimensions, *1* — Ellipsoidal gametocyst with four short sporoducts, *12* — Cylindrical sporocysts with truncate ends

septum (Fig. 1 2). A round nucleus with a karyosome was found in the deutomerite. The sporadins were solitary in nature and the largest dimension was 194.4 μm.

Measurements (the measurements of 16 sporadins are given in range with mean within parenthesis):

TL = 40.5–194.4 (138.73),
LP = 8.1– 27.0 (17.7), WP = 9.45–40.5 (27.84),
LD = 32.4–175.5 (120.66), WD = 10.8 –37.8 (30.75),
LN = 5.4– 13.5 (9.37), WN = 5.4 –16.2 (9.94),
LP : TL = 1 : 5.0–10.4 (7.80), WP : WD = 1 : 0.93–1.44 (1.17)

Detail measurements of 16 sporadins of *Pyxinia reneae* sp. n. (in μm)

Sl. No.	TL	LP	LD	WP	WD	LP : TL	WP : WD
(1)	194.4	18.9	175.5	31.05	35.1	1 : 10.3	1 : 1.13
(2)	145.8	16.2	129.6	27.0	29.7	1 : 9.0	1 : 1.1
(3)	194.4	24.3	170.1	40.5	37.8	1 : 8.0	1 : 0.93
(4)	164.7	21.6	143.1	31.05	32.4	1 : 5.35	1 : 1.14
(5)	40.5	8.1	32.4	9.45	10.8	1 : 5.0	1 : 1.04
(6)	144.45	27.0	117.45	29.7	29.7	1 : 5.35	1 : 1.0
(7)	137.7	18.9	118.8	24.3	32.4	1 : 7.29	1 : 1.33
(8)	118.8	14.85	103.95	18.9	18.9	1 : 8.0	1 : 1.0
(9)	137.7	21.6	116.1	24.3	27.0	1 : 6.38	1 : 1.1
(10)	135.0	21.6	113.4	27.0	35.1	1 : 6.25	1 : 1.3
(11)	125.55	17.55	108.0	24.3	29.7	1 : 7.15	1 : 1.22
(12)	140.4	16.2	124.2	27.0	33.75	1 : 8.67	1 : 1.25
(13)	140.4	13.5	126.9	24.3	35.1	1 : 10.4	1 : 1.44
(14)	140.4	13.5	126.9	27.0	35.1	1 : 10.4	1 : 1.3
(15)	113.4	18.9	94.5	29.7	36.45	1 : 6.0	1 : 1.23
(16)	145.8	16.2	129.6	25.65	32.4	1 : 9.0	1 : 1.26

Gametocyst and Sporocyst

These were small, round to ovoid white bodies — 90.0 μm to 110.0 μm in dimension, comprising of two semicircular gemetocytes being enclosed by a transparent, smooth cyst (Fig. 1 3). After 48 h, the sporocysts were released singly and also in cluster by the simple rupture of the gametocyst's wall. Each sporocyst was smooth, ellipsoidal and 8.0 μm × 3.5 μm in dimension (Fig. 1 4).

Infection locus: Midgut
Incidence: 6 infected out of 29 examined
Host: *Evorinea iota* (Arrow) (*Dermestidae*)
Locality: Murshidabad, West Bengal, India

Remark

At present 12 *Pyxinia* spp. are known (Levine 1979). Among them, *P. moebuszi* Léger and Duboscq, 1900 and *P. anobii*, Vincent, 1922 show similarity with the present parasite in the morphological features of the trophozoite and sporadin. However, the proximal hemispherical projection of its epimerite is distinct from the epimerites of the above mentioned gregarines. Moreover, the ellipsoidal sporocyst of the reported gregarine is different from the barrel-shaped and cylindrical sporocysts of *P. moebuszi* and *P. anobii* respectively. The present species also resembles *P. major* Hoshide, 1959 in the shape of its epimerite and sporadin. But it differs markedly from *P. major* by its smooth hemispherical projection of the epimerite (many firm cirri on the projection of *P. major*) and ellipsoidal sporocyst (spindle-shaped in *P. major*). Moreover, the gregarine is described from a different host. In having such distinctive features, the present gregarine has been considered to be a new species for which the name *Pyxinia reneae* sp. n. is proposed after Late Dr. Rene Ormieres, an illustrious Protozoologist of France.

Gregarina chaetocnemae sp. n.

Of the 20 insects examined, the midguts of 5 have been infected by the parasite.

Trophozoite

These were found in the lumen of the host's midgut. Each trophozoite was made up of very small, spherule-like sessile epimerite, round to oval protomerite and oval to cylindro-conical deutomerite (Fig. 1 *5–6*). There was a spherical nucleus with a small, round karyosome found in the deutomerite. The septum between the protomerite and the deutomerite was deep and distinct. The largest trophozoite found was 77.0 μm in length.

Measurements (the measurements of six trophozoites are given in range with mean within parenthesis):

TL = 39.8 –77.0 (50.2),
LE = 4.67– 8.41 (6.86), WE = 5.6– 8.4 (7.32),
LP = 9.3 –14.0 (10.74), WP = 11.7–18.7 (15.7),
LD = 13.3 –56.0 (33.3), WD = 21.0–28.0 (24.5),
LN = 9.3 –10.3 (9.7), WN = 9.3–10.3 (9.5)

Sporadin

These were also found in the midgut lumen of the host. Each sporadin was made up of hemispherical to slightly conical protomerite and

cylindrical deutomerite with round posterior end (Fig. 1 7). A round nucleus containing a small round karyosome was found in the deutomerite. The largest solitary sporadin measured 144.8 μm in length. Beside solitary forms, the sporadins also occurred in caudo-frontal association. The relative sizes of the primite and the satellite were highly variable (Fig. 1 *8–10*).

Measurements (the measurements of 20 solitary sporadins are given in range with mean within parenthesis):

TL = 32.7–144.8 (87.0),
LP = 9.3– 28.0 (16.8), WP = 16.3 –44.4 (27.5),
LD = 11.7–109.7 (70.2), WD = 18.7 –79.4 (65.5),
LN = 9.3– 19.6 (13.08), WN = 18.68–23.35 (20.22),
LP : TL = 1 : 4.0–8.0 (5.35), WP : WD = 1 : 1.25–2.3 (1.52)

Detail measurements of 20 sporadins of *Gregarina chaetocnemae* sp. n. (in μm)

Sl. No.	TL	LP	LD	WP	WD	LP:TL	WP: WD
(1)	112.0	18.6	93.4	32.6	46.7	1 : 6.0	1 : 1.4
(2)	56.0	14.0	42.0	23.3	37.3	1 : 4.0	1 : 1.6
(3)	58.4	14.0	44.4	23.3	37.3	1 : 4.2	1 : 1.6
(4)	98.0	18.6	79.4	35.0	49.0	1 : 5.2	1 : 1.4
(5)	32.6	9.3	23.3	16.3	18.3	1 : 3.5	1 : 1.4
(6)	53.7	9.3	44.4	18.6	25.6	1 : 5.7	1 : 1.4
(7)	93.4	11.7	81.7	28.0	35.0	1 : 8.0	1 : 1.2
(8)	107.4	18.7	88.7	32.6	37.3	1 : 5.7	1 : 1.1
(9)	144.7	28.0	116.7	44.3	77.3	1 : 5.2	1 : 1.8
(10)	98.0	18.6	79.4	28.0	51.3	1 : 5.2	1 : 1.8
(11)	95.7	16.3	79.4	32.6	39.6	1 : 5.8	1 : 1.2
(12)	88.7	18.7	70.0	28.0	46.7	1 : 4.7	1 : 1.7
(13)	93.4	14.0	79.4	28.0	32.6	1 : 6.7	1 : 1.2
(14)	116.7	28.0	88.7	29.4	46.7	1 : 4.2	1 : 1.6
(15)	70.0	14.0	56.0	28.0	46.7	1 : 5.0	1 : 1.7
(16)	137.7	28.0	109.7	32.6	74.7	1 : 4.9	1 : 2.3
(17)	77.0	14.0	63.0	23.3	46.6	1 : 5.5	1 : 2.0
(18)	60.7	11.7	49.0	21.0	32.6	1 : 5.2	1 : 1.5
(19)	51.4	11.7	39.7	18.6	23.3	1 : 4.4	1 : 1.2
(20)	93.4	18.6	74.8	32.6	49.0	1 : 5.0	1 : 1.5

Measurements of five associative or syzygy forms are given below:

Total Length	Length of Primite	Length of Satellite
231.17	128.43	102.74
225.56	132.16	93.40
197.55	123.76	73.79
135.42	91.07	44.37
149.44	70.05	79.39

Gametocyst and Sporocyst

The gametocyst was a small ellipsoidal white body measuring 95.5 μm × 62.5 μm. At maturation after 24 h, two pairs of symmetrically arranged, 28.0 μm long sporoducts were developed (Fig. 1 *11*). The sporocysts were released in long chain. Each sporocyst was cylindrical with truncate ends and was 8.0 μm × 3.2 μm in dimension (Fig. 1 *12*).

Infection locus: Midgut
Incidence: 5 infected out of 20 insects examined
Host: *Chaetocnema concinnipenis* Baly (*Chrysomelidae*)
Locality: Murshidabad, West Bengal, India

Remark

The gregarine in study resembles *G. spraguei* Haldar and Chakraborty, 1978 in the shape of its epimerite and gametocyst and also in the number and nature of the sporoducts. However, it differs from the latter by the smaller dimension of its epimerite (epimerite is 11.5 μm in *G. spraguei*), variable relative size of primite and satellite (primite is smaller than satellite in *G. spraguei*) and cylindrical sporocyst (ovoidal sporocyst in *G. spraguei*). The present gregarine also shows similarity with *G. bilobosa* Kundu and Haldar, 1981 in having ellipsoidal gametocyst and cylindrical sporocyst and with *G. ampullaria* Hoshide and Hoshide, 1969 in the shape of the epimerite and the gametocyst and also in the nature of the sporoduct. However, the present species differs from the former by its small spherule-like epimerite (bilobose epimerite in *G. bilobosa*) and 8.0 μm × 3.2 μm sporocyst (6.0 μm × 3.0 μm in *G. bilobosa*). It is also distinct from the later gregarine by its variable ratio of the primite-satellite length (primite is larger than satellite in *G. ampullaria*), greater ratio of LP : TL (LP : TL = 1 : 3–4 in *G. ampullaria*). The reported gregarine has, therefore, been considered to be a new species and described as *Gregarina chaetocnemae* sp. n. after the name of its host.

Material

Holotype and paratypes of *Pyxinia reneae* sp. n. on slide No. Pc-12 and of *Gregarina chaetocnemae* sp. n. on slide No. Gc-8 are kept in the Department of Zoology, R.B.C. College and soon will be deposited to the National Collection of the Zoological Survey of India, Calcutta.

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REFERENCES

Amoji S. D. and Rodgi S. S. 1975: *Pileocephalus rhytinotus*, a new gregarine parasite of the beetle, *Rhytinota impolita* Fairm. J. Karnatak Univ. Sci., 20, 230–239.

Bhushanarao K. S. P. 1962: On a new cephaline gregarine (*Protozoa*) from the gut of the beetle *Gonocephalum brachyelytra*. Proc. Ind. Congr. Zool., Part II, 265–266.

Chakraborty N. and Haldar D. P. 1975: Observation on a species of *Stylocephalus* (*Protozoa* : *Sporozoa*) from a beetle. Proc. 62nd Ind. Sci. Congr., Part III, 214.

Crawley H. 1903: List of polycystid gregarines of the United States. Proc. Acad. Nat. Sci. Phila., 55, 41–58.

Devdhar M. J. and Amoji S. D. 1977: *Stylocephalus* Gregarines found in Tenebrionid Beetle, *Gonocephalum hoffmannseggi* Stev. Arch. Protistenkd., 119, 75–85.

Dufour L. 1828: Note sur le gregarine nouveau genre de ver qui vit en tropeau dans les intestins de divers insectes. Annls. Sci. Nat., 13, 366–368.

Ellis M. M. 1913: A descriptive list of the cephaline gregarines of the new world. Trans. Am. Microsc. Soc., 32, 259–266.

Foerster H. 1938: Gregarinen in Schlesischen Insekten. Z. Parasitenkd., 10, 157–210.

Geus A. 1969: Die Gregarinida, Die Tierwelt Deutschlands. Teil 57, 1–608.

Grasse P. P. 1953: Traite de Zoologie, Paris, Masson et Cie, I(2), 545–797.

Haldar D. P. and Chakraborty N. 1976: *Retractocephalus* — A new genus of Cephaline Gregarines (*Protozoa* : *Sporozoa*) from insects. Curr. Sci., 45, 668–669.

Haldar D. P. and Chakraborty N. 1978: Observations on three new species of Cephaline Gregarines (*Protozoa* : *Sporozoa*) from insects. Acta Protozool., 17, 233–244.

Haldar D. P. and Chakraborty N. 1979 a: Observations on three new species of Cephaline Gregarines (*Protozoa*: *Sporozoa*) of the genus *Hirmocystis* Labbé from Coleopteran Insects. Z. Parasitenkd., 59, 121–130.

Haldar D. P. and Chakraborty N. 1979 b: Observations on a new Cephaline Gregarine *Stylocephalus apapillatus* n. sp. (*Protozoa* : *Sporozoa*) from a Beetle, *Gonocephalum* sp. Arch. Protistenkd., 122, 154–163.

Hammerschmidt K. E. 1838: Helminthologische Beytraege, Isis, 5, 531–558.

Hoshide H. 1952: Studies on Gregarines parasitic in Japanese insects III. Zool. Mag. (Tokyo), 61, 269–274.

Hoshide H. 1959: Studies on the Cephaline Gregarines of Japan III. Description of the members belonging to the families *Didymophydae*, *Actinocephalidae*, *Acanthosporidae*, *Stylocephalidae*, *Dactylophoridae*. Bull. Fac. Educ., 8, 35–101.

Hoshide H. and Hoshide K. 1969: *Gregarina ampullaria* n. sp. from *Altica caerulensis* Baly and two other already known gregarines from *Chrysomelidae*. Bull. Fac. Educ. Yamaguchi Univ., 18, 35–43.

Hoshide K. 1979: Notes on the Gregarines in Japan 10. Three new and seventeen already known species of gregarines from Japanese *Tenebrionidae*. Bull. Fac. Educ. Yamaguchi Univ., 29, 31–75.

Hoshide K. 1980: Notes on Gregarines in Japan 11. A new and two already known species of gregarines from Japanese *Coccinellidae*. Bull. Fac. Educ. Yamaguchi Univ., 30, 49–59.

Kundu T. K. and Haldar D. P. 1981 a: Observations on two new species of cephaline gregarines (*Protozoa* : *Sporozoa*) of the genus *Gregarina* Dufour, 1828 from insects. Z. Parasitenkd., 64, 233–241.

Kundu T. K. and Haldar D. P. 1981 b: Observations on a new cephaline gregarine (*Protozoa* : *Sporozoa*), *Brustispora indicola* gen. nov., sp. nov. from a Coccinellid Beetle, *Stethorus* sp. Arch. Protistenkd., 124, 471–480.

Kundu T. K. and Haldar D. P. 1981 c: Observations on two new species of cephaline gregarines (*Protozoa* : *Sporozoa*) of the genus *Hirmocystis* Labbé from Insects. Acta Protozool., 20, 323–335.

Labbé A. 1899: *Sporozoa*. In: Das Tierreich. Lief. 5 R. Friedlander und Sohn, Berlin.

Léger L. 1892: Recherches sur les Gregarines. Tabl. Zool., 3, 1–183.
Léger L. and Duboscq O. 1900: Les Gregarines et l'epithelium intestinal C. R. Acad. Sci. Paris, 130, 1566–1568.
Levine N. D. 1979: New genera and higher taxa of septate gregarines (*Protozoa, Apicomplexa*). J. Protozool., 26, 532–536.
Misra P. L. 1941: Observations on a new gregarine *Stylocephalus bahli* sp. nov. from alimentary canal of an Indian beetle *Gonocephalum helopioides*. Rec. Indian Mus. (Calcutta), 43, 43–72.
Misra P. L. 1942: A new cephaline gregarine, *Stylocephalus indicus* sp. nov. from a beetle. Rec. Indian Mus. (Calcutta), 44, 339–340.
Obata K. 1953: Reports on some gregarines from Japanese insects. J. Sci. Hiroshima Univ., Ser. B, Div. Zool., 14, 1–34.
Patil C. C. and Amoji S. D. 1979 a: Eugregarine parasites of *Coleoptera* from North-East region of Karnataka. II. *Leidyana gnyanagangai* n. sp. found in the gut of *Rhytinota impolita* Fairm. Arch. Protistenkd., 122, 333–339.
Patil C. C. and Amoji S. D. 1979 b: Eugregarine parasites of *Coleoptera* from North-East region of Karnataka. II. *Leidyana gnyanagangai* n. sp. found in the gut of *Rhytinota impolita* Fairm. Curr. Sci., 48, 736–737.
Sarkar N. K. and Mazumder S. K. 1983: Observations on a new species of cephaline gregarine *Stylocystis chowdhurya* n. sp. (*Apicomplexa* : *Eugregarinida*) from *Cryptophagus* sp. (*Insecta* : *Coleoptera*). Arch. Protistenkd., 127, 189–192.
Schneider A. 1887: Gregarines nouvelles ou peu connues. Tabl. Zool., 2, 67–85.
Vincent M. 1922: On the life history of a new gregarine: *Pyxinia anobii* n. sp., intestinal parasite of *Anobium paniceum* L. (*Coleoptera*). Parasitology, 14, 299–306.
Watson M. E. 1916: Studies on gregarines including description of twenty one new species and a synopsis of the eugregarines records from the *Myriapoda*, *Coleoptera* and *Orthoptera* of the world. Ill. Biol. Monogr., 2, 211–468.

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Prenumeratę ze zleceniem wysyłki **za granicę** przyjmuje RSW „Prasa-Książka-Ruch", Centrala Kolportażu Prasy i Wydawnictw, ul. Towarowa 28, 00-958 Warszawa, konto NBP XV Oddział w Warszawie Nr 1153-201045-119-11, w terminach podanych dla prenumeraty krajowej.

Prenumerata ze zleceniem wysyłki za granicę pocztą zwykłą jest droższa od prenumeraty krajowej o 50% dla zleceniodawców indywidualnych i o 100% dla zlecających instytucji i zakładów pracy.

Bieżące i archiwalne numery można nabyć lub zamówić we Wzorcowni Ośrodka rozpowszechniania Wydawnictw Naukowych PAN, Pałac Kultury i Nauki, 00-901 Warszawa, oraz w księgarniach naukowych „Domu Książki".

Cena zł 100.—

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