ACTA PROTOZOOLOGICA

VOLUMEN XIII

Fasciculi 28–39

W A R S Z A W A 1 9 7 5

POLISH ACADEMY OF SCIENCE
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY
ACTA PROTOZOOLOGICA

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ACTA PROTOZOOLOGICA
VOL. XIII WARSZAWA, 31.X.1975 FASC. 28

Department of Cell Biology, M. Nencki Institute of Experimental Biology,
Polish Academy of Science, Pasteura 3, 00-973 Warszawa, Poland

Maria JERKA-DZIADOSZ and Irena JANUS

Discontinuity of Cortical Pattern During Total Conjugation of a Hypotrich Ciliate *Paraurostyla weissei*

Brak kontynuacji wzorca powierzchniowego w czasie koniugacji totalnej u *Paraurostyla weissei*

The process of conjugation in hypotrichs has been most extensively studied with respect to the changes in the nuclear apparatus. Many papers concern the so called "temporary conjugation" (Raikov 1972), where the fusion of conjugants is partial and temporary and two exconjugants result from separation of mates. The stages of nuclear reorganization, generally similar to the scheme given by Maupas (1889), were studied in such genera as *Pleurotricha* (Manwell 1928), *Oxytricha* (Gregory 1923, Kay 1946), *Stylonychia* (Alonso and Perez-Silva 1965, Tchang Tso-run et al. 1965, Moldenhauer 1965), *Euplotes* (Turner 1930, Hammond 1937, Katashima 1959, Diller 1966).

During conjugation, nuclear reorganization is followed by reorganization of the cortical organelles, which appear to be different in diverse genera. In the hypotriches that have been most studied in this respect, two cortical reorganizations take place during the interval from the beginning of conjugation to the first postconjugation division. Each one is different and the first is usually uncomplete when compared with normal divisional morphogenesis. This occurs in *Euplotes* (Hammond 1937, Hammond and Kofoid 1937, Diller 1966), *Stylonychia* (Tchang Tso-run et al. 1965, Diller 1965), *Oxytricha* and *Pleurotricha* (Manwell 1928).

In *Stylonychia* and *Oxytricha* both ventral and dorsal ciliature is replaced during conjugation whereas in *Euplotes* only the ventral ciliature is replaced while the dorsal cilia remain unchanged.

Since the cortical reorganizations occur during defined stages of the development of nuclear apparatus, these two processes were correlated, and most frequently the cortical changes were supposed to depend upon one component of the dual nuclear apparatus. Katashima (1959) suggested that the first cortical reorganization depend upon the old macronucleus, whereas Hammond (1937) suggested a correlation between cortical reorganizations with the haplo- and diploid generations of micronucleus. Recently Sapra

and Ammermann (1973, 1974) have shown, that the development of new nuclear apparatus and cortical reorganizations occurring during conjugation in *Stylonychia* both depend upon presynthesized mRNA. They showed that the disturbance of the cortical reorganization or arrest of nuclear divisions in conjugants and exconjugants of *Stylonychia* does not induce the cells to adjust the developmental program. They concluded that: "the nuclear and cortical changes proceed without any reference to each other in a defined course" (Sapra and Ammermann 1974).

Among hypotrichs a total conjugation very rarely occurs in which two specimens join completely, and after conjugation only one exconjugant is left, giving rise to a new clonal line. Total isogamontic conjugation was described in *Urostyla* by Heckmann (1965), Ilowaisky (1916) and Moldenhauer (1965).

The aim of our study was to follow the changes and the fate of cortical structures during total conjugation and formation of cortical primordia in the single exconjugant. We were interested in the following questions: (1) How do the two cortical patterns entering conjugation yield one pattern in the exconjugant, (2) How is the old ciliature resorbed and replaced by a new one, (3) How are the cortical features correlated with the changes in the nuclear apparatus.

In the Discussion we compare different types of cortical reorganizations which occur during conjugation with cortical reorganizations taking place in other morphogenetic situations described earlier (Jerka-Dziadosz and Frankel 1969, 1970) in *P. weissei* and other hypotrich ciliates.

Material and Methods

Cells used in this investigation were collected in several places in Poland. Conjugation was observed in the cultures derived from samples taken from ponds near Królkarnia and Sadyba in Warsaw in the years 1962 and 1972. The main observations on the total conjugation were performed on cells derived from samples isolated from the Pilica river in Warka (Poland) in August 1972. The studied species was designated as *Paraurostyla* (*Urostyla*) *weissei* Stein – the Polish strain. This strain differs insignificantly from the American strain in the number and disposition of cirri on the ventral surface (see Jerka-Dziadosz and Frankel 1969, Table 7, page 636).

The morphology and main stages of division of the Polish strain were given earlier (Jerka-Dziadosz 1965) and precise study on the cortical development of the American strain of *P. weissei*, and also the considerations on the cortical variability and species boundaries were given in the paper of Jerka-Dziadosz and Frankel (1969).

The culture methods were the same as in earlier studies already cited. The culture medium is Pringsheim solution, the food *Tetrahymena pyriformis* GL-C washed from 2% proteosepeptone.

Total conjugation in *P. weissei* occurs spontaneously in the cultures freshly established from isolations from ponds after maintenance for 2–3 weeks in the laboratory conditions. Conjugation was induced by two days starvation of the culture. The mating pairs were seen

in the culture dishes for about two weeks whereas the starvation of cultures in a later period caused a sporadic cases of autogamy.

The studies were performed on living and stained material. The mating pairs were isolated into depression slides and periodically observed. At different times after pairing the conjugants were fixed and stained with Protargol following the method described earlier (Jerka-Dziadosz and Frankel 1969). Preparations of the nuclear apparatus using the Feulgen method were also performed. Clones were derived from about 20 exconjugants and some of them were cultivated and occasionally checked with respect of the occurrence of intraclonal conjugation. It has been observed to happen spontaneously in one of the clones after half a year of cultivation. In two clones (79 and 99) the conjugation was induced by addition of 0.1% $MgSO_4$ to the culture medium in the proportion 1:1. The mating pairs appeared in the culture 24 h after addition the inducing solution.

Results

A general description of morphological features of *P. weissei* has been presented in earlier papers (Jerka-Dziadosz 1965, Heckmann 1965). Figure 1 represents a scheme of disposition of the locomotory and feeding organelles. This scheme was made on the basis of protargol stained specimens. The oral ciliature consists of an adoral zone of membranelles (AZM), which bounds the peristome on the left side (as left and right we refer to animals left and right, not the viewers) and on the anterior margin. The distal part of AZM passes to the right anterior side of the cell. The right side of the peristome is bounded by two undulating membranes (UMs). The peristome is equipped with fibers running through the oral groove inside the cell.

Eight, rarely seven, frontal cirri are situated posterior to the distal portion of the AZM in two transverse rows. The middle of the ventral surface is occupied by 4–5 (occasionally 6) longitudinal rows of ventral cirri. Posteriorly to these is located an oblique row, consisting of 7–9 transverse cirri. The most-right cirrus of this group is situated slightly anteribrly to the rest, as was noted in *U. hologamma* by Heckmann (1965). A single row of marginal cirri is situated at each of the lateral margins. On the posterior tip a small group of caudal cirri is seen. The dorsal side is covered by 5 meridionally oriented rows of short cilia. An opening of the contractile vacuole is located on the dorsal side, at the level of the left part of the AZM.

The conjugation of *P. weissei* appears always as a total isogamontic conjugation; during this process the left mate is always completely resorbed. Our observations of this process in living cells are in complete agreement with the description of the total conjugation in *U. hologamma* presented by Heckmann (1965). The whole process from the formation of mating pairs to the formation of one exconjugant lasts 190 h. The first part from the resorption of the left partner and resorption of the ciliature to the formation

of a zygocyst, lasts about 48 h. The development of the macronuclear anlagen in the nonciliated zygocyst takes about 4–6 days, and after this the single exconjugant forms its cortical structures two times before it finally divides.

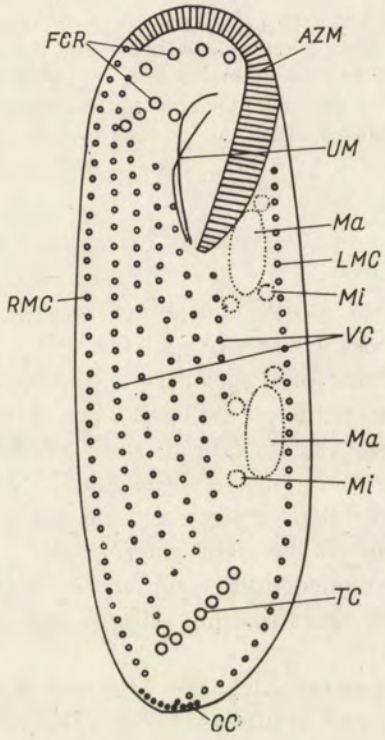


Fig. 1. Schematic representation of cortical anatomy of the ventral surface of *Paraurostyla weissei*, drawing made from a protargol stained specimen. Abbreviations: AZM – adoral zone of membranelles, CC – caudal cirri, FCR – frontal cirri, LMC – left marginal cirri, RMC – right marginal cirri, TC – transverse cirri, UM – undulating membranes VC – ventral cirral rows, Ma – macronuclei, Mi – micronuclei

Preconjugant cells do not differ from the other animals in the culture. They are 170–190 μm long. (All measurements given in this paper are made on the fixed and stained animals, therefore the actual measurements are probably larger).

The initial union of mates takes place by the joining of the anterior portions of AZM's in such a way that the membranelles of both zones coincide in position. Later the mates became situated parallel to one another with the ventral sides facing each other. After healing on the frontal area, the left partner twist around its long axis so that both ventral surfaces come to be on the same plane.

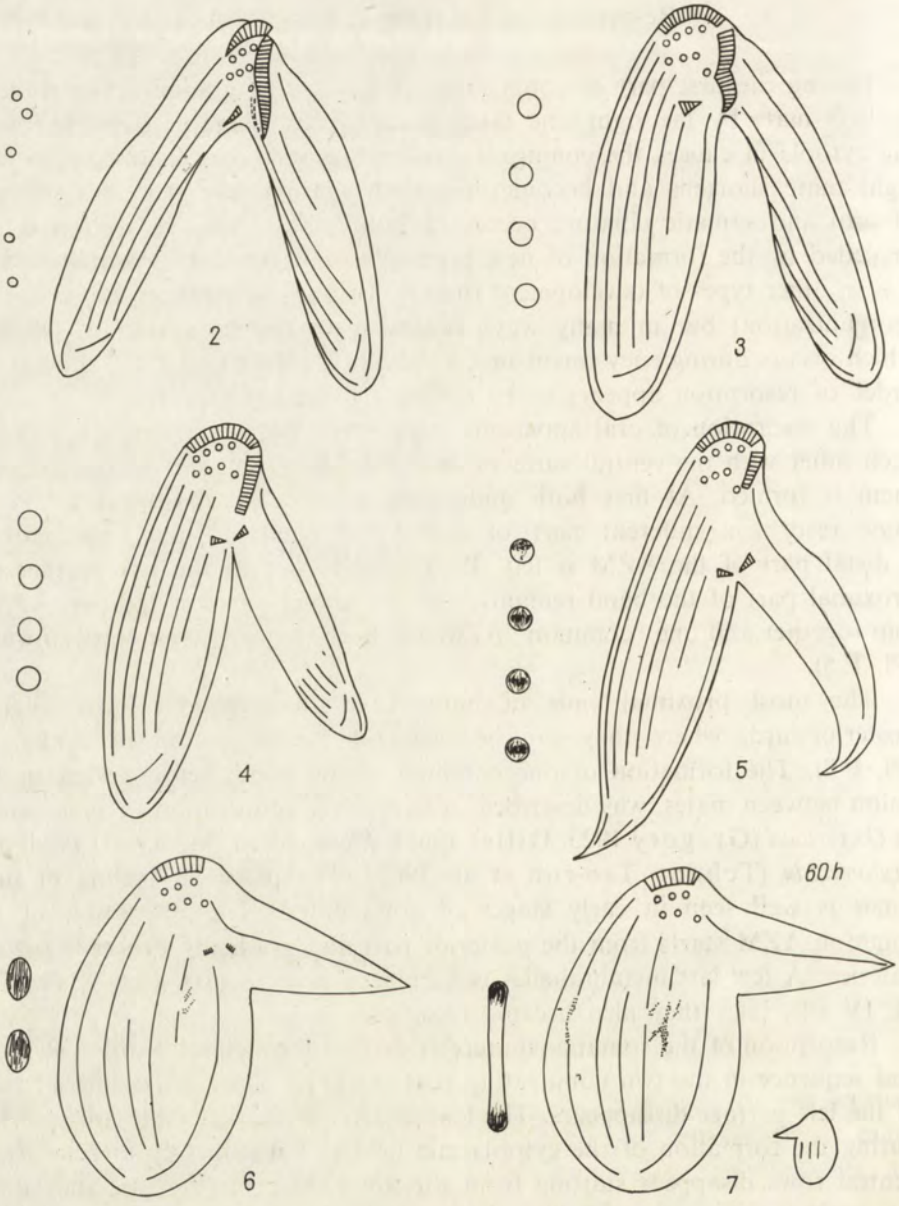
Resorption of Ciliature in Conjugants

During the first part of conjugation (Figs. 2–9) a gradual absorption of the left mate by the right one takes place. A considerable dehydration of the cytoplasm causes the conjugants to become very dark and opaque. The right mate shortens and becomes rounded. During this process resorption of oral and somatic ciliature occurs in both mates. This resorption is not preceded by the formation of new primordia and cortical reorganization as it is in other types of development (binary fission, regeneration, physiological reorganization) but in many ways is similar to the resorption of ciliature which occurs during encystment in *Oxytricha* (Hashimoto 1962) though the order of resorption appears to be different in many respects.

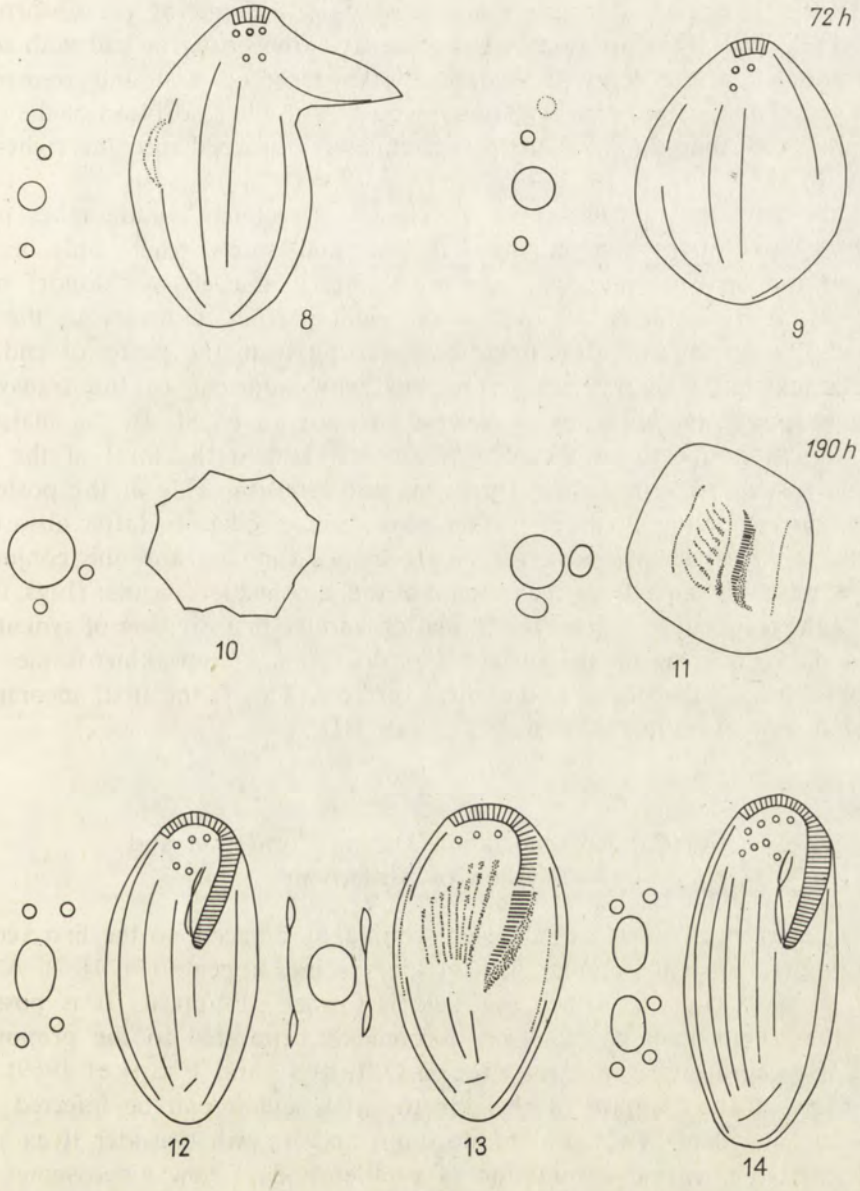
The resorption of oral apparatus starts when both conjugants are facing each other with the ventral surfaces and when a cytoplasmic bridge between them is formed. At first both undulating membranes disappear and each mate resorbs a different part of the AZM band. In the right partner a distal part of the AZM is left (Pl. I 1), whereas in the left partner the proximal part of the band remains. The remaining parts of the two AZM's join together and one "common" AZM for both mates is temporarily formed (Pl. I 5).

The most proximal ends of both AZM's detach and move slightly posteriorward, where they can be seen till the formation of synkaryon (Pl. I 2). The formation of one common adoral zone, being a sign of the union between mates, was described in temporary conjugation in such genera as *Oxytricha* (Gregory 1923, Diller 1965), *Pleurotricha* (Manwell 1928) and *Stylonychia* (Tchang Tso-run et al. 1965). The place of healing of both zones is well seen in early stages of conjugation. The resorption of the common AZM starts from the posterior part and gradually proceeds toward anterior. A few last membranelles can be seen in zygocysts (Fig. 9, Pl. II 6, Pl. IV 14), later they also became resorbed.

Resorption of the somatic ciliature is carried through with different speed and sequence in the two conjugating partners (Figs. 2–9). First, the ciliature of the left partner dissappears. The frontal cirri of the left mate are resorbed during the formation of the cytoplasmic bridge. Subsequently the cirri from ventral rows disappear starting from the anterior most (Fig. 4), and simultaneously the resorption of the right marginal cirri occurs also from anterior to posterior. The resorption of single cirri is usually performed in the same manner. At first the cilia, then the basal plate and finally the subpellicular fibers running from cirral bases disappear. After resorption of ventral rows, the transverse cirri are resorbed. This is accompanied by a rapid change in the shape of the body. Its posterior end, originally flat oval, becomes sharply tapered. At that time the caudal cirri are resorbed.



Figs. 2–14. Schematic drawing of ventral surface of conjugating pairs. The open circles represent the frontal cirri, the striped fields represent the AZM, the solid lines represent cirral rows. The primordial structures are indicated as fields of fine dots (6–8, 11 and 13). On the left side of each drawing the main features of the nuclear apparatus are represented. In Figs. 2–5 the drawing of the Mi applies to both mates, in Figs 6–9 only to the right conjugant. On the right side of some drawings the time (in hours) since mating is indicated. 2 – The formation of one common AZM, 3 – Beginning of resorption of the AZM, Mi are swollen 4 – The resorption of the ventral ciliature in the left mate. Mi in early prophase, 5 – Resorption of ventral and transverse cirri in the right mate. Tapering of both posterior



ends. Mi in parachute stage, 6 – Proliferation of kinetosomes on the ventral surface. Migration of pronuclei, 7 – The proliferation of kinetosomes of the dorsal primordia (small scheme at the right side). The division of synkaryon, 8 – The resorption of ventral primordia and old ventral ciliature. Second division of synkaryon, 9 – The formation of zygocyst. Resorption of the ventral ciliature. Differentiation of new nuclei, 10 – The zygocyst. The macronuclear anlagen and two Mi, 11 – The primordia of ventral ciliature in the zygocyst. The anlagen of Ma, Mi in prophase, 12 – Small exconjugant after the cortical reorganization. Note the incomplete cortical pattern. Single Ma and 4 Mi, 13 – The third cortical reorganization. Formation of normal pattern. Mi in telophase, Ma elongated, 14 – The exconjugant after third cortical reorganization. Normal pattern

The left marginal cirri are maintained until the end of the absorption of the left mate. They are resorbed starting from the posterior end with some cirri situated at the level of transverse cirri resorbed first and resorption than proceeding anteriorwards. Simultaneously with the resorption of the right marginal row the whole left mate is completely absorbed into the right one (Figs. 8, 9).

The resorption of ciliature in the right – "recipient" mate takes place a few hours later than in the left one and starts when only a few ventral and the right marginal cirri are visible in the left – "donor" mate (Fig. 4). It is characteristic that in the right partner, contrary to the left partner the ventral ciliature is resorbed starting from the posterior end. At first a few (2–3) ventral cirri from each row adjacent to the transverse cirri disappear and then the transverse cirri are resorbed. In the marginal rows on both sides a couple of cirri are resorbed at the level of the TC. This is associated with a slight furrowing and later, tapering of the posterior tip of the cell. Later, without further resorption of ciliature (after absorbing of the left partner) the posterior part becomes rounded and the conjugant swells, probably partially at the expense of the resorbed left partner (Figs. 6–8). Inside the recipient conjugant the formation and the first division of synkaryon takes place, whereas on the surface a proliferation of new kinetosomes can be observed on the dorsal and ventral surfaces. This is the first, incomplete cortical reorganization (Fig. 6, Pl. II and III).

Cortical Reorganization During Formation and Division of Synkaryon

On the ventral surface of the right conjugant, adjacent to the first ventral row of cirri, a small irregular field of kinetosomes appears (Pl. II 7). A few cirri, in the vicinity of which this field is formed, disappear. It is possible that the kinetosomes of these cirri become incorporated to the primordial field, as occurs during division (Jerka-Dziadosz and Frankel 1969). On the basis of the location of this kinetosomal field it can be inferred that it is an equivalent of an oral primordium and we will consider it as such, even though after the termination of proliferation of new kinetosomes this primordium does not differentiate neither into transverse membranelles of the AZM nor into the longitudinally oriented UM, but appears as a small group of ciliary aggregates (Pl. III 10, 12). It is nonetheless possible that this primordium became resorbed yet before it is fully formed and determined. Beside this incomplete primordium, no other ventral primordia are formed.

In the same time complete primordia of dorsal kineties are formed on the dorsal surface of the right conjugant (Fig. 7, Pl. II 8, 9 Pl. III 13).

They appear in the same mode as they have been described to form during physiological reorganization (Jerka-Dziadosz and Frankel 1969). In the three left rows of dorsal cilia, proliferation of new kinetosomes occurs in their equatorial part and short new kineties are formed (Pl. III 13). On the right margin of the conjuganting specimen a group of kinetosomes is formed with a visible participation of old marginal cirri (Pl. II 8). This later differentiates into abortive dorsal rows of the right side (Fig. 8, Pl. II 8, 9) Pl. III 10, 11). They move somewhat toward the dorsal side but appear rather randomly scattered, and do not form neatly ordered rows of bristles. Both left and right dorsal primordia differentiate; we however failed to follow the fate of the dorsal cilia from the previous generation.

Formation of the Zygocyst

After the second division of synkaryon, when the new Ma becomes determined further resorption of ciliature occurs on the surface of the recipient conjugant. The left mate is completely absorbed by the right one. The young zygocyst has an irregular bulgy shape and continues to resorb the remnants of the ventral and marginal cirri (starting in the posterior part and going anteriorwards) and also resorbs the rest of frontal cirri and the last several anterior-most membranelles of the AZM (Pl. IV 14). After the disappearance of the ventral ciliature the zygocyst becomes more or less spherical with small sharp protrusions. The cytoplasm is very dark and completely opaque. The cytoplasm is naked, with nothing resembling a cyst wall. In this form the zygocyst survives for 4–5 days. In protargol preparations of zygocysts fixed during 3–5 days after their formation, dark granules are seen on the surface, which correspond in size to the mucocysts of a normal cell (Pl. IV 17). The exact nature of these granules is difficult to ascertain without the use of the EM. Short cilia resembling the dorsal bristles are also seen on the surface. They appear to be randomly arranged – this impression can, however, be illusory because the surface of the zygocyst is wrinkled.

Cortical Reorganizations in Exconjugants

The zygocyst is formed during the third day of conjugation. During the subsequent 4 days it maintains itself in the form of a dark, opaque sphere with protrusions. During the fifth day a discrete changes can be noticed. The zygocyst slightly swells and the protrusions disappear.

Later the zygocyst became hemispherical and attaches with its flat surface to the substratum. Zygocysts fixed in this stage reveal a single set of

ciliary primordia (Fig. 11. Pl. IV 16, 18). The macronucleus is spherical and two micronuclei are in late prophase of mitosis. In many exconjugants the orientation of mitotic spindles does not correspond to the antero-posterior orientation of primordia.

The arrangement of the ventral primordia is represented on Fig. 11. The primordia of all categories of ciliature are seen: (1) The AZM primordium as a longitudinal field of kinetosomes differentiating into membranelles in its anterior portion, (2) The UM primordium as a thin band of kinetosomes situated parallel to the AZM primordium, (3) The primordia of the FVT complex as 5–8 meridionally oriented streaks of kinetosomes, (4) The marginal primordia as meridional streaks of kinetosomes situated on both sides of the oral-FVT primordium and parallel to it. Although the whole set of ventral primordia appears quite normal, an uncomplete ventral ciliature originates as a result of its differentiation. An extensive variability of the number of cortical elements exists in exconjugants after this reorganization. The cells possess 3–5 frontal cirri, 3–5 transverse cirri (very rarely more) and 3–4 rows of ventral cirri. The oral ciliature appears normal. The oral apparatus functions as can be inferred from the presence of food vacuoles containing *Tetrahymena* cells (Pl. IV 19). During the period when the primordia differentiate on the ventral surface, the primordia of new dorsal rows of cilia are also formed on the dorsal surface and the right margin.

About 12 h after the second reorganization (first exconjugant reorganization) a third cortical reorganization takes place. During this process the whole set of all categories of ciliature is formed in the same manner as during ordinary physiological reorganization. The AZM primordium originates to the left of the first ventral row of cirri, apparently without initial participation of old cirri (Pl. IV 19, 20). To the right of this primordium a complete primordium of the UMs appears, partially with the participation of the disaggregating old UMs. To the right of the UM primordium seven meridionally oriented streaks of kinetosomes that make up the primordium of the FVT complex originate (Pl. V 21). Primordia of new rows of cirri appear in both old marginal rows. As a result of differentiation of the primordia after completion of reorganization the exconjugants possess 7–8 frontal cirri, 7–9 transverse cirri and 4–5 rows of ventral cirri. New dorsal kineties appear as three rows of kinetosomes located in the three left rows of bristles and as two short rows of kinetosomes located at the right margin of the cell, close to the primordium of the left marginal cirri. The third cortical reorganization is invariably accompanied by mitosis of the micronuclei, and at the conclusion of this process the macronucleus elongates and divides. 12 h after this reorganization the cell divides for the first time. The formation of divisional primordia proceeds in the way characteristic for any binary fission (Jerka-Dziadosz and Frankel 1969).

Nuclear Reorganization

The nuclear apparatus of *P. weissei* consists of two ovoid macronuclei situated in the left part of the cell (Fig. 1), posteriorly to the lateral, ventral part of the AZM. The number of micronuclei in trophic cells varies from 3–9. Most frequently cells with 5 or 6 Mi were observed. The micronuclei form two groups each situated close to one of the Ma. Both groups often consist of a different number of Mi.

Table 1
Comparison between Number of Mi in Prophase of first Maturation Division and Total Number of Mi in Conjugants

Total Number of Mi	3	4	5	6	7	8	9	Total Number of Cells
Number of Mi in Prophase	Left Conjugants							
2	–	–	1	1	–	–	–	2
3	5	5	6	4	2	1	–	23
4	–	–	1	1	1	–	1	4
5	–	–	–	–	–	–	–	–
Total	5	5	8	6	3	1	1	29
	Right Conjugants							
2	–	–	–	1	–	–	–	1
3	2	2	3	6	1	–	–	14
4	–	–	4	7	2	–	–	13
5	–	–	–	1	–	–	–	1
Total	2	2	7	15	3	–	–	29

Stained specimens of *Paraurostyla* in an early stage of conjugation were analysed with respect of the number of Mi (Table 1). It was observed, that as in trophic cells the majority of conjugants possessed 5 or 6 micronuclei.

During the first few hours of conjugation, until the moment of formation of a cytoplasmic bridge, no changes in the nuclear apparatus are seen. After that, several micronuclei enter prophase of the first maturation division. The number of Mi entering this division is not constant and varies from 2–5, a number that is independent of the total number of Mi in the conjugant (Table 1). There is a significant difference observed between the number of Mi in prophase in the left and right conjugant, whose fates during conjugation differ, and there is more of the left conjugants with smaller number of Mi in

prophase of the first maturation division. This means that the right conjugant has a significant probability of activating four nuclei rather than three, compared to the left conjugant. All the data presented here are based on the observations made on protargol stained cells. This method was more useful than the traditional Feulgen method. Owing to very good staining of the achromatic structures, it was possible to observe the stages of the formation of synkaryon and the early anlagen of Ma. It was also important to make simultaneous observations of nuclear and cortical development.

The fragmentation of the old macronucleus begins after completion of the cytoplasmic bridge, when the mates are still facing each other by the ventral surfaces. Both Ma in each conjugant elongate considerably and later fragment into irregular, progressively smaller fragments. During the whole process of conjugation the fragments are seen as heavy bodies that are Feulgen positive and darkly stained with protargol. They disappear completely after the third reorganization, when the new Ma is fully developed.

At the same time that the fragmentation of the old Ma starts, changes in the micronuclei entering the first maturation division are visible in both partners. They undergo a slow swelling and appear as 3 or 4 clearly distinguishable nuclei occupying the same position in the cell as is taken by Ma in trophic cells. The micronuclei show a swollen nuclear envelope (protargol staining) and well stained chromatin (Feulgen preparations). In later stages of prophase the micronuclei become lighter and a darker granules – probably the chromosomes become visible inside. The final stage of prophase, the so called parachute stage, is clearly visible in *P. weissei* (Pl. VI 2, 6). In such nuclei the chromatin material is clumped on one of the nuclear poles and fibers run from it to the centrosome located in the center of the nucleus. The prophase of the first maturation division lasts about 10 h and during this stage all cirri except the left marginal are resorbed at the surface of the left partner, whereas in the right mate the transverse cirri and posterior-most ventral cirri are resorbed. The micronuclear prophase occurs synchronously in both mates.

The conjugants undergo the subsequent stages of the first maturation division relatively quickly (Pl. III 27, 28, 29). The nuclei enter the second maturation division while still in telophase of the first division. Two or three products of the first division undergo the second division, which proceeds rather quickly. The chromosomes are rod-shaped (Pl. VI 28, 29). In *P. weissei* telophase of the second maturation division is synchronous in all nuclei of both mates. We did not observed a third division. Probably, as was noticed by Moldenhauer 1965 in *U. polimicronucleata*, the third division is absent, and one of the products of the second maturation division in each mate differentiates into the pronucleus: migratory in the left ("donor") conjugant, and stationary ("recipient") in the right conjugant (Pl. VI 30).

The interior of the pronuclei is faintly stainable with protargol while the nuclear envelope is readily distinguishable. The pronuclei are surrounded by characteristic fibrous material forming a "halo" (Pl. VI 30). During the migration of pronuclei the fibrous halo disappears gradually whereas inside the pronuclei rod shaped chromosomes become visible (Pl. VII 31). Fusion of pronuclei occurs when chromosomes of both pronuclei are in metaphase plate (Pl. VII 32), as in *Stylonychia* (Ammermann 1971 b, Jareno et al. 1969).

We did not find on our preparations a conjugant in the very moment of fusion of pronuclei. Probably this process lasts for a very short time. We did find, however, the next stage – the division of the synkaryon (telophase – Pl. VII 33). The nuclear envelope of a very elongated synkaryon as well seen, with a dumb-bell shaped appearance due to the presence of groups of chromosomes situated at both poles. This figure occupies almost 1/3 of the cell length and the spindle is oriented parallel to the long axis of the cell (as in previous divisions). The products of the first division of the synkaryon enter the second division very quickly, this division however is often asynchronous (Pl. VII 34). Rod-shaped metaphase chromosomes are well seen. One of the products of the first division divides into two micronuclei (Ist division of Mi) while the other one gives rise to the anlagen of the Ma and a pycnotic nucleus situated in the anterior part of the cell (Pl. VII 35, 36). All divisions of the synkaryon and its products take place in the region of cytoplasm which in trophic cells is occupied by the nuclear apparatus, whereas the fragments of old Ma are dispersed all over the cytoplasm.

The macronuclear anlagen enlarges considerably, and in an early phase of its development is Feulgen-negative (Pl. VIII 37). In the zygocyst it occupies a central position and grows further. In the two-day old zygocyst (4 days since the beginning of conjugation) the anlagen in living cells is seen in the center as a light sphere, surrounded by very dark cytoplasm. This probably corresponds to the polytenization stage of macronuclear development (Ammermann 1971 b). The protargol preparations of this stage revealed the existence of long tape-like polytenic chromosomes (Perez-Silva and Alonso 1966). Dark and light bands of unequal width are well seen (Pl. VIII 38).

Later the anlagen sharply decreases in size, and appears more compact. This corresponds in time to the preparation for the formation of cortical primordia in the exconjugant. During the second cortical reorganization the Ma anlagen remains spherical (Pl. VIII 39, 40) and elongates after the termination of this process (Pl. VIII 41). After elongation we observed the formation of nucleoli and replication bands in the still single Ma. In the cytoplasm of the exconjugants the remnants of old macronuclear fragments can be observed. They disappear completely during the third cortical

reorganization. The new macronucleus divides by the end of the third cortical reorganization (Pl. VIII 42), and few hours later the replication bands appear on distal ends of both nuclei. At the termination of the S period (Jerka-Dziadosz and Frankel 1969) the cell enters the first postconjugation division.

Divisions of micronuclei are invariably associated with each cortical reorganization. During the first uncomplete cortical reorganization of the right conjugant there is the first division of the new Mi. The second division of both Mi takes place during the differentiation of cortical primordia of the first reorganization of exconjugant (Pl. VIII 40). Very often the mitotic spindles of dividing Mi are oriented diagonally or even perpendicularly to the antero-posterior axis of the still rounded exconjugant.

The third division of new Mi occurs during the second reorganization of the exconjugant, at the stage of differentiation of new ciliature. The mitotic spindles are oriented longitudinally. After the division of Ma, the micronuclei are groupping around the two Ma.

Autogamy

A small number of autogamonts was found in slightly starved cultures after the termination of conjugation. Only limited observations were made, owing to the scarce material. The autogamonts possess an elongated shape and darker than trophic cells cytoplasm. In later stages of autogamy the anterior part of the cell curves to the left, and the posterior part of the cell is tappered, probably because of the resorption of the transverse cirri. Later it subsequently round up till it reach a spherical shape similar to the zygocyst of the conjugating pair. This stage of autogamy lasts about 70 h – that is the same amount of time as during conjugation. The zygocyst stage respectively lasts 5–6 days, and one exautogamont differentiates during the next 24 h.

The process of resorption of ciliature was not followed in details. We stained only few specimens in early stages of autogamy. On their ventral surfaces the UM's and lateral parts of AZM's were resorbed (Pl. V 23, 24). The distal part of AZM contained only about 5 membranelles, and small proximal part was retained near the previous peristomal region. The mode of resorption of the oral ciliature is similar to that of the right conjugant. We did not follow the subsequent stages of its resorption, nor was the resorption of the ventral ciliature observed.

The micronuclei of the fixed autogamonts showed (Pl. V 23, 24) the beginning of the first maturation division – that is the stage of large, swollen

darkly stained Mi. Here also, as in conjugation, not all Mi present in the cell entered the first maturation division.

Further observations on the nuclear apparatus were not performed.

Discussion

Resorption of Ciliature During Total Conjugation

The ciliature of hypotrich ciliates can be resorbed during the whole cell cycle, as well as during the clonal cycle. First, the primordia of ciliature can be resorbed before they fully differentiate. This occurs after an experimental intervention such as microsurgical operation or UV irradiation (Hashimoto 1961, Jerka-Dziadosz 1967, Wise 1965).

Secondly, the adult ciliature may be resorbed in two situations: during encystment, when the whole ventral ciliature is resorbed (Hashimoto 1962, Grimes 1973) or when new replacing material is prepared – that is when primordia of new ciliature are differentiating as in division, physiological reorganization and regeneration (Wallengren 1902, Dembowska 1925, 1938). In microsurgical studies (Jerka-Dziadosz 1968) it has been shown that the resorption of old ciliature can be prevented, when the primordia of developing ciliature are microsurgically removed. The presence of the nuclear apparatus was not necessary for the completion of resorption.

Total conjugation of *P. weissei* is a second process, besides encystment, in which the resorption of ciliature proceeds in a time prior to the formation of primordia of new ciliature. The resorption of the locomotory and feeding systems occurring during total conjugation in *P. weissei* is unique. The resorption of ciliature is a long lasting process and it proceeds faster in the left than in the right partner. First the UM's are in both partners resorbed, then the ventral parts of the AZM. Similarly during encystment of *Oxytricha* (Hashimoto 1962) and *Urostyla* (Jerka-Dziadosz, unpublished) the structures resorbed first are the UM and subsequently the AZM. In conjugating cells of *P. weissei*, similarly as in *Euplotes* (Hammond 1937, Diller 1966) the proximal parts of AZM detach from the rest and move posteriorwards. In *Euplotes* they are withdrawn to the cytoplasm, in our species they are resorbed superficially (Pl. II 6).

The sequence of resorption of ventral ciliature differs in both mates. In the left partner the following cirri are resorbed successively: frontal, ventral, right marginal, transverse, caudal – later left marginal and dorsal cilia together with the absorption of the whole pellicle of the left mate. The direction of resorption is from anterior to posterior and from right to left side. Only the left marginal row is resorbed starting from the posterior end.

It can be mentioned here that during encystment of *P. weissei* (authors unpublished observations) the marginal rows are resorbed in the opposite order: the right marginal row is resorbed from the posterior end and the left from the anterior end. Hashimoto (1962) noticed a similar pattern during encystment of *Oxytricha*.

In the right partner of conjugating *P. weissei* the following cirri are resorbed successively: first several cirri located in the posterior part of the animal adjacent to the transverse cirri, then the transverse cirri, then in a scattered mode cirri of all categories are resorbed. The dorsal cilia of the right mate are probably resorbed after the primordia of new dorsal bristles develop during the first reorganization of the right conjugant.

The following conclusions can be drawn from the review presented above of the pattern of the resorption of ciliature:

(1) The resorption of the entire ciliature may occur without the presence of developing primordia not only during a vegetative process such as the encystment, but also during sexual processes such as conjugation and autogamy.

(2) The sequence and timing of resorption is different in various developmental situations (such as division, encystment, conjugation). (3) The sequence and timing of resorption may differ in two cells which are permanently joined together even though the same nuclear processess (meiosis, formation of pronuclei) proceed simultaneously in those cells. This last conclusion is interesting when compared with observations of Tartar (1966) who observed a very strong tendency for synchronization of development of oral primordia in grafted cells of *Stentor*. It means that the resorption of ciliature need not to be synchronous. It is worth mentioning, that the sequence of the formation (in contrast to resorption) of ciliature – is the same in all developmental situations even though it may be incomplete.

Cortical Reorganizations During Total Conjugation

In the species studied three cortical reorganizations occur during conjugation, from the moment of formation of the cytoplasmic bridge between mates till the first division of exconjugant – that is, the proliferation of kinetosomes and their differentiation into composite ciliary structures takes place three times.

As has been mentioned in the introduction cortical reorganization accompanying conjugation has been described in several representatives of hypotrichs such as *Euplotes*, *Stylonychia*, *Oxytricha*, and *Pleurotricha*. In these species the conjugation is partial and temporary, therefore the reorganization occur in both mates and both exconjugants. The most detailed observations were presented by Diller (1966). He observed (confirming

earlier report of Hammond 1937) that there are two cortical reorganizations in *Euplotes*. The first one occurs in "gamonts" when the Mi is in the parachute stage. This reorganization is incomplete: a small AZM and primordia of ventral cirri originate, whereas the primordium of paroral membrane and frontal cirrus No. 1 are not formed. The new oral apparatus is nonfunctional. The second reorganization takes place during the development of new macronuclear anlagen, after the exconjugant separate, and is apparently complete. The dorsal rows of bristles in both mates remain intact during cortical reorganizations, the new primordia are formed during the first division of the exconjugants. Tchang Tso-run et al. (1965) studied the changes of cortical structures during conjugation in *Stylonychia* using silver impregnation. They noticed, that during the first cortical reorganization which occurs during fertilization, an incomplete set of ventral primordia (lacking the UM primordium) originates on ventral side of both mates, and the primordia of ciliary rows are formed on the dorsal side. From their pictures it follows, that there are two more cortical reorganizations, before the exconjugant divides.

The difference between the incomplete ventral reorganization and the complete dorsal in *Paraurostyla* and *Stylonychia* is striking, and is exactly opposite of what happens during conjugation and autogamy in *Euplotes*, when the ventral cilature reorganizes and dorsal not. This examples suggest that the two surfaces are independent in developmental control.

In *P. weissei* the first cortical reorganization starts a short time prior the formation of the synkaryon and proceeds only in the right component. The second reorganization occurs in the zygocyst and the third in the exconjugant. The first two reorganizations are incomplete, the third one produces a complete set of primordia and does not differ from, for instance, a regeneration of an anterior fragment with one Ma and two Mi.

The two incomplete reorganizations differ in the character of deficiency in developing structures. During the first reorganization primordia of whole categories of ciliature are lacking. The primordium of UM, FVT and marginal cirri are not formed. On the ventral surface only a small group of kinetosomes (Pl. II 7) originates at a place corresponding topographically to the AZM primordium. Unexpectedly, on the dorsal surface primordia of three left kineties are formed, within the old rows of cilia, and the remaining two new rows develop on the right margin. It is interesting that the primordia of the right dorsal kineties are apparently formed with participation of old marginal cirri in a manner very similar to that by which the primordia of the right marginal cirri originate (Jerka-Dziadosz and Frankel 1969). It should be recalled here, that during divisional morphogenesis the marginal primordia are formed first, involving a disaggregation of several marginal cirri from the preceeding generation, and later the right dorsal primordia are formed next to the marginal primordium. From the observations on reorga-

nization of conjugants it follows that apparently the old marginal cirri of *P. weissei* can disaggregate no matter what category of primordial structure is formed as a result. The same was observed during regeneration of lateral fragments of *P. cristata*, where the AZM primordium develops with participation of old marginal cirri (Jerka-Dziadosz 1973).

The development and differentiation of the new dorsal bristles in the right conjugant proceeds almost normally – giving rise to a set of new dorsal ciliature. On the ventral surface only a very small, rudimentary oral ciliature differentiates, which is quickly resorbed. This abortive development of ventral primordia is difficult to explain. It seems that the fact, that the ventral surface of a conjugant is engaged in resorption, and therefore cannot fully respond to the morphogenetic signal may play a certain role. Only the highest point of the morphogenetic potential (Jerka-Dziadosz 1974) could still be sufficiently active to produce a relatively small number of kinetosomes, which soon are resorbed.

Hashimoto (1962) in his study on regeneration of precystic animals of *Oxytricha fallax*, observed that fragments obtained from cells being on stage B of encystment, when the oral structures are already resorbed and the ventral ciliature is being resorbed, were not able to form primordia of new regenerating ciliature. Hashimoto concluded: "... hence it is indicated that the oral primordium site on posterior fragments and cirral ones in anterior and posterior fragments are depotentiated." Recently Sapra and Ammermann (1974) have shown, that fragments of conjugating *Stylonychia* were not able to form cortical primordia.

The second cortical reorganization in *P. weissei* takes place in the zygocyst after the polytenization of macronuclear anlage, most probably just after or during the shrinkage stage of Ma development. The following features characterize the second reorganization: (1) The primordia of all categories of ciliature are formed, (2) The primordia of oral and ventral ciliature originate without any visible participation of preexisting ciliature (because it has been completely resorbed), (3) The incompletness of the second reorganization involves the fact that a smaller than usual number of elements is formed in each category of ventral primordia. We got the impression that the number of kinetosomes formed is smaller than normally, which in turn causes the differentiation of a smaller number of cirri. The arrangement of primordia, and the spatial interrelations between different primordia appear similar to that in the normal development.

Exconjugants after the second reorganization show fewer frontal cirri, fewer ventral rows and most frequently only 2–4 transverse cirri.

What is the basis of this deficiency? Ammermann (1968) stated that during conjugation of *Stylonychia* there is no RNA synthesis in the macronuclear anlagen nor in the fragments of the old Ma. Sapra and Ammermann (1973, 1974) using Actinomycin D, showed that RNA synthesis important for

the completion of conjugation takes place between the fifth and sixth hour of conjugation. On the basis of limited cytological observations of the nuclear apparatus of *P. weissei*, we suppose that the first and second cortical reorganizations probably occur at the expense of the RNA synthetized before or during the first few hours of conjugation. The zygocyst almost certainly carries a large pool of microtubule protein, therefore even a relatively small amount of preformed RNA should be sufficient for the formation of the necessary catalytic or assembly proteins. It seems that the "exhaustion of materials" cannot explain the uncomplete second reorganization.

A second possibility seems more plausible. As has been shown in an earlier study (Jerka-Dziadosz and Frankel 1969) during cortical development in *P. weissei* (division, physiological reorganization, regeneration of transverse fragments) the primordia of all categories of ciliature are formed with considerable participation from the preexisting ciliature. Some old cirri disaggregate and lose their subpellicular fibrillar structures, while their kinetosomes most probably become incorporated into the kinetosomal fields and streaks. It was also observed that kinetosomes of all categories of ciliature (except AZM and probably caudal cirri) may be incorporated into the primordia. Therefore the proliferation of new kinetosomes, although very extensive, is in a sense an addendum to the number of kinetosomes derived from disaggregating old cirri.

In exconjugants, the primordia of ventral ciliature appear on a completely "naked" cortex lacking any preformed ciliature. Therefore the primordia contain only newly formed kinetosomes. It is tempting to suggest that the number of kinetosomes thus formed corresponds to the number of newly synthetized kinetosomes which are added to the "disaggregated kinetosomes" from the old set in situations where the primordia are formed with participation of the preexisting cilature[1]. Thus the deficiency of cortical structures after the postzygotic reorganization may be caused by a lack of contribution of the non-existing preformed structures.

This hypothesis has predictable consequences: (1) If the dorsal kineties of the right conjugant persist unchanged in the zygocyst then the number of dorsal elements differentiating during the first postzygotic reorganization should be normal, not deficient. Unfortunately we were not able to make the necessary counts of dorsal bristles in zygocysts and exconjugants.

[1] One additional qualification should be made. It has not been documented yet, that the kinetosomes from disaggregating cirri persist unchanged in primordial structures. Grimes (1972), in an EM study on the formation of kinetosomal streaks with the participation of old frontal cirrus, stated that the cirrus disaggregates, however, in later stages of primordial development it is impossible to discriminate the new kinetosomes from the preexisting ones. The old kinetosomes could still be resorbed. As has been suggested by Wiliams and Frankel (1973) and Wiliams and Nelsen (1973), the macronuclear subunits from disassembled ciliary structures can be immediately reutilized *in situ* during the formation of new structures. Therefore it seems that the contribution of preexisting pattern can be attained by supplying assembled kinetosomes and/or by supplying building blocks.

(2) If during cystment of *P. weissei* the ventral ciliature is completely resorbed (as in *Oxytricha*, Grimes 1973), then during the excystment the primordia of the new ventral ciliature should contain fewer elements, as during the postzygotic reorganization Frankel 1973 described a genetically based trait "basal body deficient" in *Euplotes minuta*. This trait is revealed in the first place by the incomplete proliferation of kinetosomes, which in turn causes a reduction of the number of dorsal bristles, the lack of right caudal cirri and abnormalities in the ventral ciliature. This dificiency of cortical pattern was perpetuated indefinitely during the clonal life.

In our case the incompletness of cortical pattern lasts only few hours and is replaced by new pattern not differring from that of normal cells. The third cortical reorganization is apparently supported by the newly developed Ma, for the nucleoli are already visible. The third reorganization involves replacement of all cortical organelles. The arrangement of primordia is similar to that in ordinary physiological reorganization.

In conclusion of this section it should be stressed that during total conjugation of *P. weissei* each of the three generations of the ventral structures are formed on a different cortical background. The first generation is formed on cortex which is in the state of resorption, the second on the cortex lacking any preformed ciliature and the third one on the cortex with an incomplete pattern on it. This indicates that the morphogenetic stimulus signaled from the inside of the cell acquire a response in the cortex, and this response is modified according to the state of the cortex. It should be stressed that this modifications have a strictly epigenetic character.

Correlation of Ciliary and Nuclear Development During Total Conjugation

During the total conjugation of *P. weissei* two divisions of micronuclei occur, one pronucleus differentiates in each partner – the stationary in the right and the migratory in the left mate. After fusion of the pronuclei one synkaryon is formed, which divides twice yielding a macronuclear anlagen, a pycnotic nucleus and two micronuclei. Our observations of the karyological features occurring during total conjugation confirm the observations made by Moldenhauer (1965) on *Urostyla polimicronucleata*. This type of conjugation corresponds to the IV type of total isogamontic conjugation according to the classification given by Raikov (1972).

In the study of the pattern of DNA synthesis in Ma and Mi during the cell cycle of *P. weissei* (Jerka-Dziadosz and Frankel 1970) it was stated that, as in *Stylonychia* (Ammermann 1968) the S period of Mi takes place at the end of the cell cycle and is immediately followed by micronuclear division. After division there is a long period of G1 (about 85% of cell cycle).

Since cells entering conjugation are slightly starved, it appears therefore that the micronuclei entering pregamic divisions are in G1 (similarly as in *Oxytricha* – Luporini et al. 1973).

As has been shown in the Results section the cortical reorganizations which occur during conjugation of *P. weissei* always initiate at the same time as certain characteristic changes in the nuclear apparatus. The first incomplete reorganization starts during the migration of pronuclei and each subsequent reorganization is always accompanied by division of the Mi. Similar correlation can be inferred from the study on temporary conjugation of *Stylonychia* where the first reorganization occurs during formation of synkaryon (Moldenhauer 1965) and the second in the exconjugant (Tchang Tso-run et al. 1965). In *Euplotes* where there is different pattern of DNA synthesis in the division cycle of Mi (G2-D-S) there are three pregamic divisions of Mi during conjugation – the first one being an ordinary mitosis. The first cortical reorganization during conjugation initiates earlier than in *Urostyla* and *Stylonychia*: during the parachute stage of meiosis, the second reorganization occurs in exconjugants (Diller 1966).

In the study of the pattern of DNA synthesis in *P. weissei* (Jerka-Dziadosz and Frankel 1970) it was observed that the mitosis of Mi, which invariably accompanies cortical development during division and regeneration, is always preceded by DNA synthesis in Mi even though DNA synthesis in Ma is arrested (as in regeneration). If during conjugation there is DNA synthesis in pronuclei just prior the formation of the synkaryon (during the first reorganization) as there is in *Stylonychia* (Ammermann 1971 a) – then it could be presumed that the initiation of DNA synthesis in micronuclei and initiation of cortical development are tightly coupled. The constancy of this relationship seems to indicate something more than a mere coincidence. This problem will be further studied.

Acknowledgements

The authores wish to express their sincere thanks to the following colleagues : Drs D. Ammermann, J. Frankel, K. Golińska, K. Heckmann, J. Kaczanowska and S. Radzikowski, who read and commented on this manuscript.

Summary

Changes of cortical structures in *Paraurostyla weissei* during total isogamontic conjugation and formation of single exconjugant were studied and corelated with the changes in nuclear apparatus. During this process the left conjugant is absorbed by the right one, an unciliated zygocyst without walls is

formed. The oral and somatic ciliature is gradually resorbed in both mates, only dorsal cilia of the right mate remain. The timing and sequence of resorption of ciliature differ in both conjugants. During conjugation three cortical reorganizations occur. The first two reorganizations are uncomplete, third produces normal pattern. During the first reorganization the whole categories of primordia are missing (UM, FVT, marginal). During the second reorganization an uncomplete elements of primordia appear. Each of three generations of ventral ciliature are formed in different bakground. The first generation is formed on cortex which is in state of resorption of ciliature, the second on the cortex lacking preformed ciliary structures and the third one on the cortex with uncomplete pattern on it. The basis of the differences in cortical reorganizations are discussed, it is suggested that the cause is situated in the "state" of cortex itself.

Each cortical reorganization initiates simultaneously with characteristic changes in the nuclear apparatus. The first occurs during the migration of pronuclei, the subsequent ones are accompanied by the division of Mi. It is suggested that the initiation of proliferation of kinetosomes and the initiation of DNA synthesis in micronuclei (or pronuclei) are controlled in common.

STRESZCZENIE

Badano przemiany w strukturach powierzchniowych zachodzące podczas totalnej koniugacji izogamontycznej u orzęska *Paraurostyla weissei* w powiązaniu ze zmianami w aparacie jądrowym odbywającymi się w tym czasie. Podczas koniugacji lewy partner jest całkowicie absorbowany przez prawego i tworzy się zygocysta pozbawiona otoczki. Orzęsienie oralne i somatyczne obu koniugantów jest stopniowo resorbowane tak, że w zygocyście pozostają tylko rzęski dorsalne prawego koniuganta. Czas trwania i kolejność resorbcji orzęsienia są różne w obu partnerach. Stwierdzono występowanie trzech reorganizacji powierzchniowych w koniugacji *P. weissei*. Pierwsze dwie są niekompletne, trzecia daje w wyniku normalne orzęsienie. W czasie pierwszej reorganizacji nie tworzą się całe kategorie zawiązków (UM, FVT, marginalne); podczas drugiej reorganizacji tworzą się wszystkie zawiązki ale o zmniejszonej liczbie elementów orzęsienia. Każde z trzech pokoleń orzęsienia brzusznego powstaje na innym "tle". Pierwsze powstaje na powierzchni brzusznej będącej w stanie resorpcji, drugie na powierzchni pozbawionej preformowanego orzęsienia, trzecie pokolenie na powierzchni posiadającej niekompletne orzęsienie. Dyskutuje się podstawy różnic między reorganizacjami powierzchniowymi, sugeruje się że ich przyczyna znajduje się w "stanie" samego korteksu.

Każda z reorganizacji powierzchniowych rozpoczyna się równocześnie z charakterystycznymi zmianami w aparacie jądrowym. Pierwsza z nich występuje w czasie wędrówki pronukleusów, a następnym towarzyszy zawsze podział Mi. Sugeruje się, że inicjowanie proliferacji kinetosomów i inicjowanie syntezy DNA w mikronukleusach (lub pronukleusach) są kontrolowane wspólnie.

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Received 11 January 1974

EXPLANATION OF PLATES I–VIII

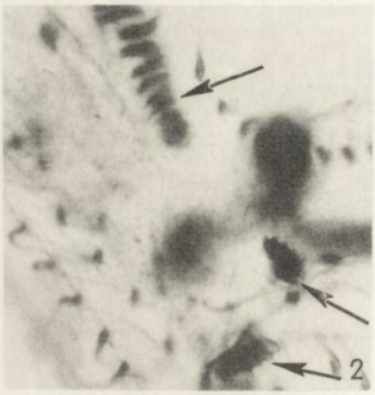
Paraurostyla weissei Stein. Protargol impregnated specimens engaged in total isogamontic conjugation. All photographs (except 30) are printed in the same magnification and so that the anterior end is up and the animals left correspond to the viewers right. The magnification (in parentheses) indicates that of the microscopic objective

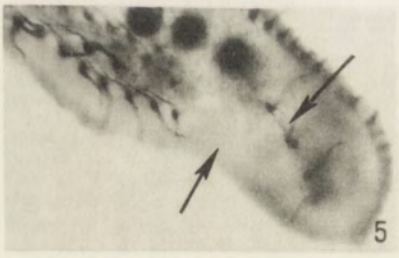
Plate I. Resorption of ciliature in early conjugants

1: The frontal areas during early conjugation. UMs of both partners are still present. The formation of one common AZM is seen (100×)
2: The resorption of the posterior part of AZM (upper arrow). The lower arrow point to the posterior parts of both AZMs which have detached from the rest and moved posteriorwards (100×)
3: The conjugating pair about 10 h after beginning of mating. Note one common AZM, the resorption of the ventral ciliature in the left partner. The beginning of fragmentation of Ma and swollen Mi are seen (40×)
4: Enlargement of the ventral surface of the left conjugant from phot. 5. The sequence of resorption of ventral ciliature can be observed. The lower arrow point to a basal plate with subpellicular fibers (cilia are missing), the upper arrow indicates the remnants of fibers (basal plate is missing) (100×)
5: The posterior part of the right conjugant at the beginning of resorption of transverse cirri (right arrow) and right marginal row (left arrow) (100×)

Plate II–III. Formation of primordial structures in the right conjugant

6: The conjugating pair fixed about 48 h after the beginning of mating. The ventral part of AZM is resorbed, as is the FVT and right marginal cirri in the left partner. In the right conjugant the transverse cirri are missing. The arrow indicates a very small group of kinetosomes (40×)
7: The kinetosomal field in the ventral surface of the right conjugant. Note the random arrangement of kinetosomes (100×)
8: The formation of the right dorsal primordia (left arrow). Note the remnants of subpellicular fibers of disaggregated marginal cirri (right arrow) (100×)
9: The beginning of differentiation of the right dorsal bristles (arrow) (100×)
10: Differentiation of the right dorsal bristles (left arrow). The right arrow points to the rudimentary oral primordium, partially resorbed (100×)
11: Movement of the dorsal cilia toward the dorsal side (100×)
12: The resorption of oral primordium (arrows) (100×)
13: The proliferation of kinetosomes in the left dorsal rows of cilia in the right conjugant (100×)





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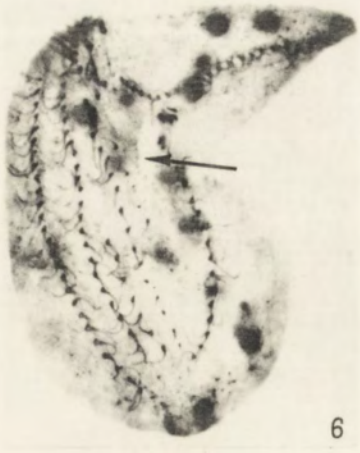

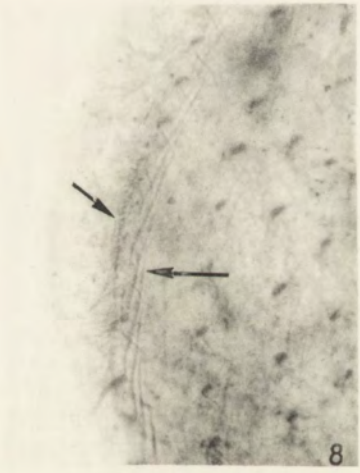

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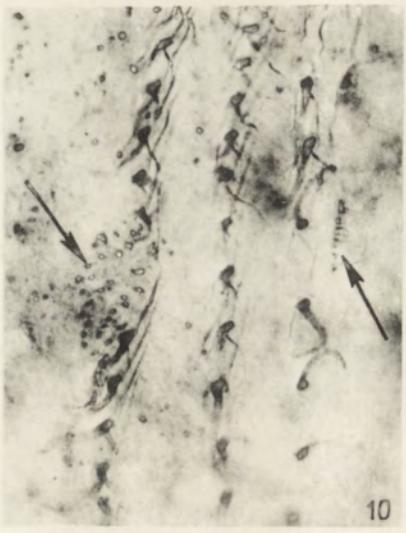

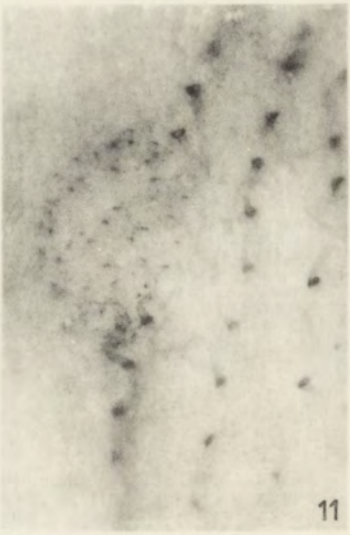

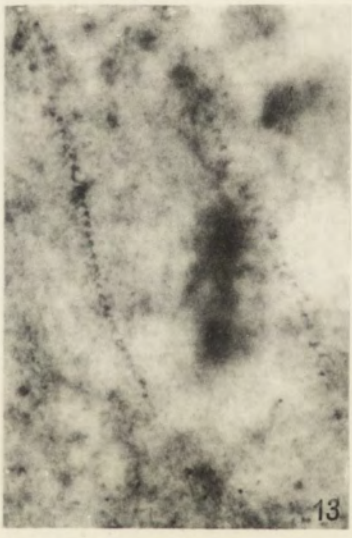

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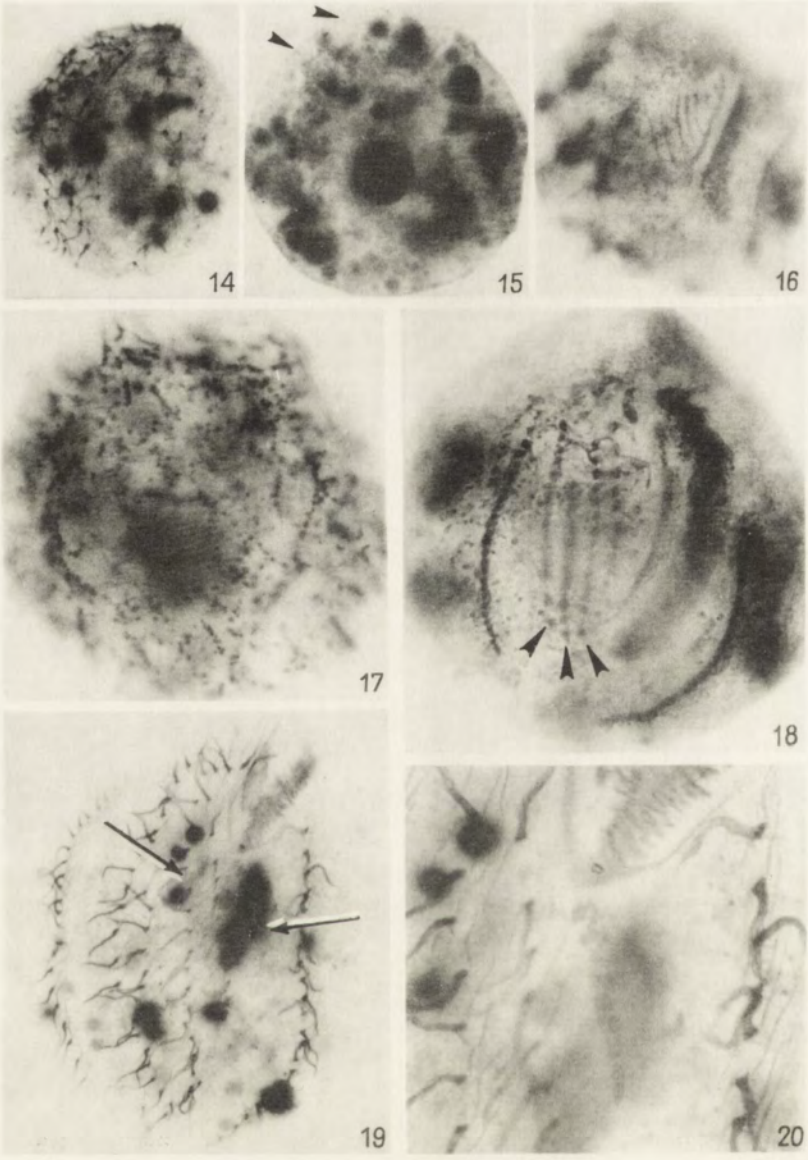

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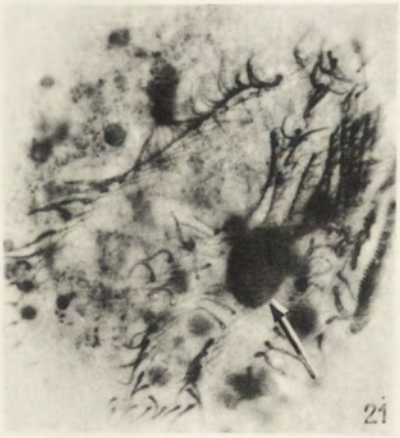

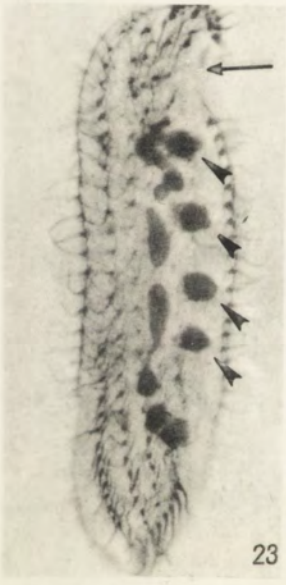

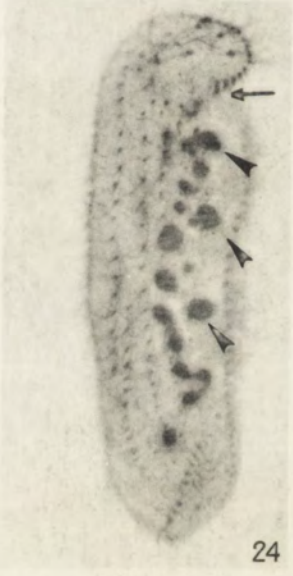

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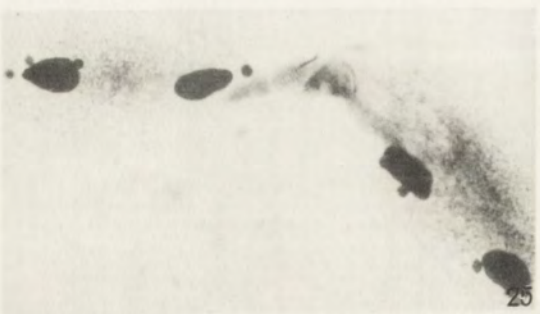

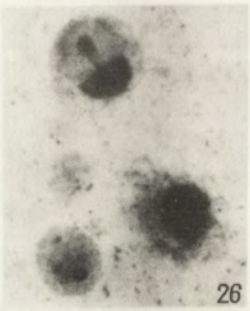

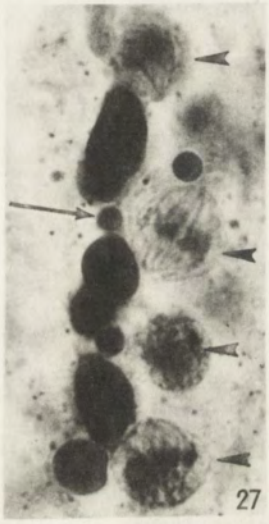

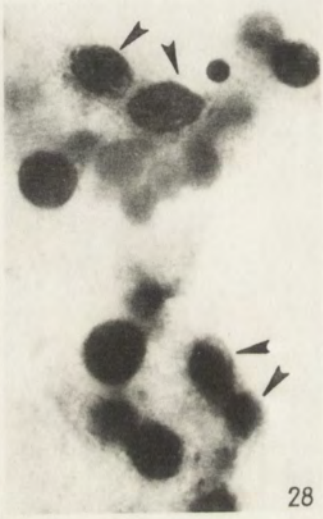

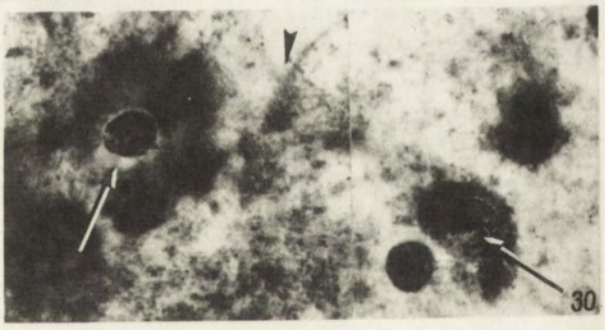

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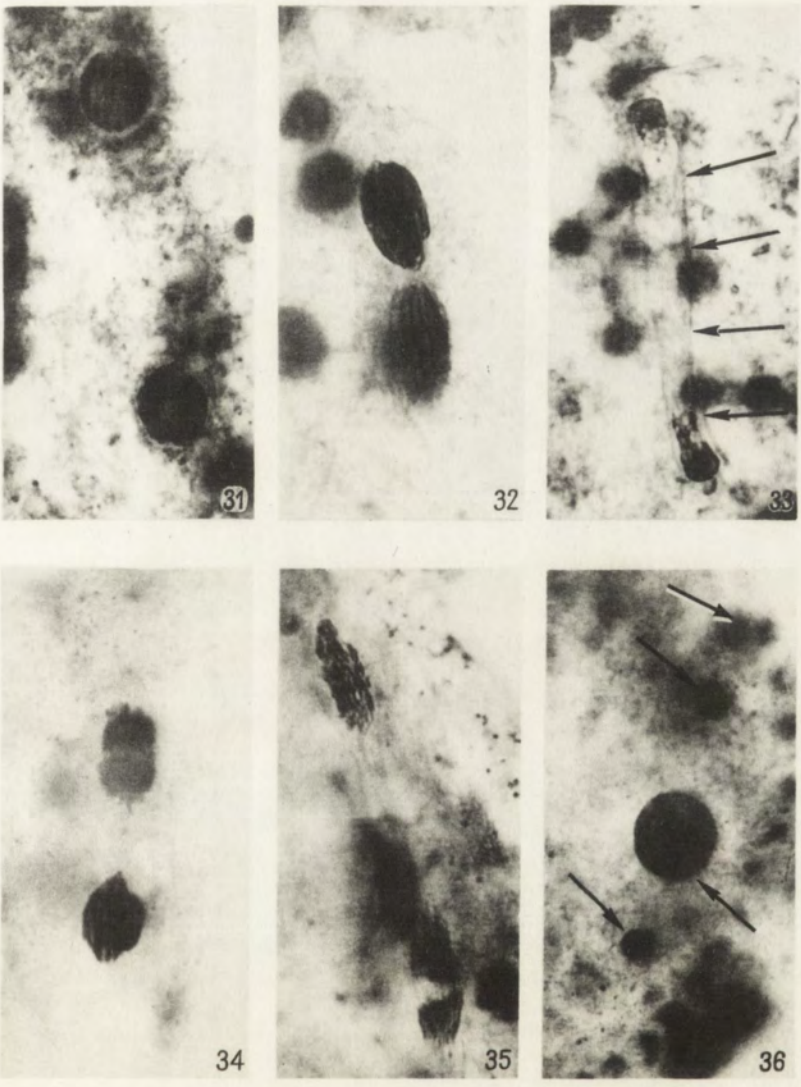

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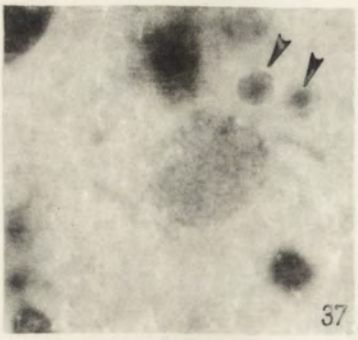

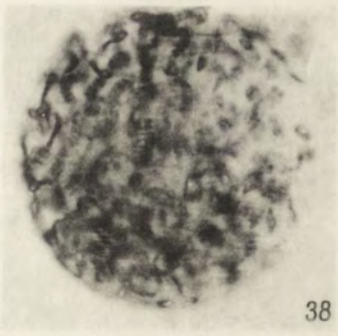

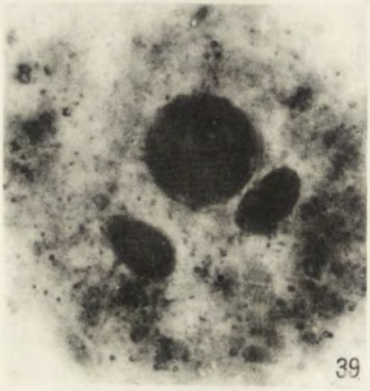

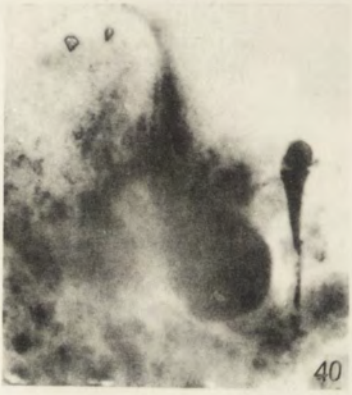

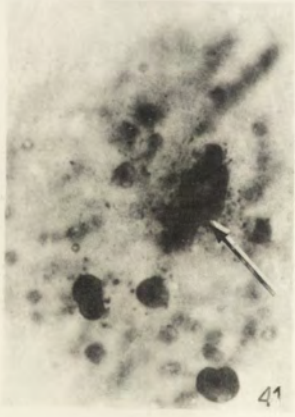

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Plate IV – The zygocyst and reorganization of exconjugant

14: The young zygocyst with the remnants of the ventral ciliature of the right partner (40×)
15: The three-day old zygocyst. Note the dorsal cilia (arrows) (40×)
16: The primordia of the ventral ciliature in a zygocyst fixed 10 days after beginning of conjugation (40×)
17: The dorsal side of zygocyst, shortly before the formation of primordia. The dark granules probably represent mucocysts (100×)
18: An exconjugant in a later stage of differentiation of the ventral primordia. Note that there are only three transverse cirri (arrows) (100×)
19: The exconjugant at the beginning of formation of the AZM primordium during the third cortical reorganization. The right arrow indicates a single Ma, the left arrow points to a food vacuole containing *Tetrahymena*. Note the three transverse cirri (40×)
20: An enlargement of part of the ventral surface of exconjugant from phot. 19. The arrow points to the kinetosomal field forming without participation of old cirri (100×)
21: The primordia of ventral ciliature in exconjugants, formed during the third reorganization. Note the single Ma in the center (arrow) (40×)
22: The normal ventral ciliature after the third reorganization (40×)
23 and 24: Autogamonts in an early stage of the first pregamic divisions of Mi. The UMs are resorbed. Note the curved anterior part of the cells (upper arrows). The macronuclei are fragmentating, the Mi are in prophase of the first maturation division (oblique arrows). The ventral ciliature is still complete (40×)

Plate VI–VIII. Nuclear reorganization during conjugation

25: A pair in an early stage of conjugation. The nuclear apparatus is normal. The Mi are grouped around the Ma (40×)
26: The micronuclei of the right mate in the parachute stage (100×)
27: The micronuclei in metaphase of the first pregamic division (right arrows). Note the difference in size compared to Mi not entering meiosis (left arrow) (100×)
28: The beginning of the second pregamic division. Upper arrows indicate the dividing nuclei in the left conjugant. The lower arrows indicate the dividing nuclei in the right partner (100×)
29: Telophase of the second maturation division in the right mate (100×)
30: The formation of pronuclei. A fibrous "halo" is seen around the pronuclei. This photograph is combined from two prints of the same focal level. The anterior end of the conjugating pair is on the viewer's left, the left side is up. The middle arrow indicates the border between the two mates.
31: The migration of pronuclei. The "halo" is still seen. The beginning of formation of rod shaped chromosomes can be observed. The ventral surface of this specimen is shown on phot. 7, 9 and 13.
32: The pronuclei just before fusion (Feulgen preparation) (100×)
33: Telophase of the first division of synkaryon. Note the tube-like nuclear envelope (arrows) (100×)
34: The beginning of the second division of synkaryon (note the rod-shaped chromosomes) (100×)
35: Telophase of the second division of synkaryon. Note the asynchronous division of both nuclei (100×)
36: The four products of division of synkaryon. Two Mi (left arrows), anlagen of Ma (right black arrow) the pycnotic nucleus (white arrow) (100×). The ventral surface of this cell is represented on phot. 11 and 12.
37: The macronuclear anlagen of young zygocyst (Feulgen preparation). Two Mi are seen close by (arrows) (100×)
38: An isolated slightly flattened macronuclear anlagen of Ma with the polytene chromosomes well visible (100×)
39: The nuclear apparatus of a zygocyst with the early primordia on the ventral surface (the same specimen as on phot. 16). The Ma is compact, Mi are in early division (100×)
40: The nuclear apparatus of a zygocyst at the end of the differentiation of ventral primordia. Note the spindle of Mi. Single round-shaped Ma is seen (100×)
41: The exconjugant entering the third cortical reorganization. The arrow points to the single, elongated Ma with RB (not visible). The dark granules in the cytoplasm represent the remnants of the old Ma (40×). The same specimen as on phot. 19 and 20
42: The exconjugant at the end of differentiation of the third generation of primordia. Note the dividing Ma (25×)

ACTA PROTOZOOLOGICA
VOL. XIII WARSZAWA, 31.X.1975 FASC. 29

Institute of Zoology and Comparative Anatomy, University of Pisa, A. Volta 4, Pisa, Italy

N. RICCI, R. BANCHETTI, R. NOBILI and F. ESPOSITO

Conjugation in *Oxytricha* sp. (*Hypotrichida, Ciliata*)[1] I. Morphocytological Aspects

La coniugazione in *Oxytricha* sp. (*Hypotrichida, Ciliata*) I. Aspetti morfocitologici

From the time of the first ultrastructural study of *Paramecium* by Jakus et al. (1942) until today, the ciliates have been the object of extensive research aimed at revealing both their fine structure in general, and the nature of morphogenetic processes. Another series of studies, within the latter line, have helped to clarify some of the problems posed by the mating phenomena. These studies dealt with *Tetrahymena pyriformis* Ehrb. (Elliot and Tremor 1958), *Paramecium caudatum* Ehrb. (Schneider 1960, 1963), *P. multimicronucleatum* Powers et Mitchell (Inaba et al. 1966), *Euplotes vannus* O. F. Müller (Nobili 1967), *Blepharisma intermedium* (Ototake 1969, Jenkins 1973). Previous observations of the mating process (Maupas 1889, Diller 1965 and Ricci et al. unpublished results) in *Oxytricha* prompted us to investigate the morphological and nuclear events that take place during conjugation phenomena. This study was carried out with both the fotic and the electron microscopes, since the available literature on the ultrastructure of *Oxytricha* was scarse and only concerned with other aspects of this hypotrich (Chakraborty 1967, Grimes 1972, 1973). On the other hand, this organism shows notable and interesting mating differences from those observed in *Euplotes vannus* (Nobili 1967), the only hypotrich studied from this aspect to date.

Materials and Methods

Stocks 13 and 19 from a species of *Oxytricha*, presumably *O. bifaria*, were used; these stocks belong to complementary mating types and were collected from a fresh-water canal near Pisa. The stocks were kept in 0.5 Erlenmeier flasks at 22 C, with a fission rate of one division per day in lettuce medium inoculated with *Aerobacter*. One day after feeding, cells were washed with and suspended in uninoculated lettuce medium. The following day these

[1] Study carried out with the help of the C. N. R.

cells were mixed together. The couples were isolated as soon as they formed in order to obtain a sure time sequence. Fixation was carried out at various time intervals from the onset of conjugation: (a) up to 10 min; (b) 20–30 min; (c) 1 h; (d) 8–10 h; (e) 17–19 h. Different fixatives were used to ascertain which was most suitable: (1) 1% OsO_4 in buffered phosphate 0.1 M, pH 7.4; (2) 1.5 glutaraldehyde (one part) and 2% OsO_4 (two parts) in buffered phosphate 0.05 M, pH 7.3; (3) 2% OsO_4 in buffered phosphate of Millonig 0.1 M pH 7.3. Fixation was 10 min at 4°C. The best fixation was obtained by using fixative (3), although the other gave satisfactory results. Single couples, transferred with micropipette through alcohols and acetone dehydration series, were embedded in the Araldite-Epon mixture. The sections obtained with the Porter-Blum microtome were stained with uranyl acetate for 15 min and Reynolds for 10 min. The microphotographs were made under the Siemens Elmiskop 101 microscope. Couples were fixed for the optic microscope at the same time intervals in a 1 : 1 mixture of OsO_4 2% in Millonig buffered phosphate 0.1 M, pH 7.3 and of phosphomolibdic acid 0.5%, washed in distilled water, stained with haematoxylin Delafield and transferred onto a gelatinated slide. Other live couples were studied in order to follow the mating process in each phase. Finally, in the aim of reconstructing three-dimensional models of couples, semifine serial sections of 1 μm in thickness and stained with 1% toluidin blue and 1% methylene blue were used.

With the "split-pair" method (Sonneborn 1950) the couples were separated in the first phases of conjugation reaction until tightly pairs appeared that were no longer separable. The split cells of each couple were singly isolated in the outer depressions of a three depression slide with uninoculated lettuce medium and examined in the next 24–48 h. This made it easy to recognize the animals in which the meiotic process had become irreversible, thus undergoing an autogamous process, with the formation of a new macronucleus visible in vivo. The non-autogamous partners divided as normal vegetative cells.

Results

In the conjugation process of *Oxytricha*, one distinguishes an initial "tête-à-tête" phase, during which the two cells have contact through their anterior extremities, which are partially raised from the substrate (Fig. 1, stage A).

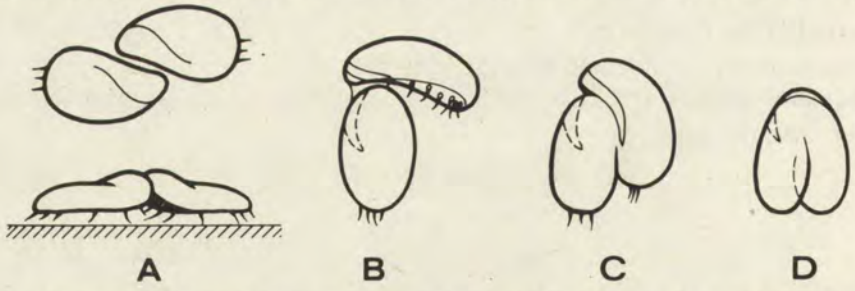

Fig. 1. Diagram illustrating the conjugation reaction of *Oxytricha*. A–D – subsequent stages of conjugation

In this phase, the partners, placed longitudinally on the same axis, may still separate by a push from other animals. In this instance, each of the two displaced cells explores repeatedly, using a rapid alternating forward and

backward movement while rotating clockwise, the entire area the center of which is the position occupied at the moment of separation (contact may or may not reoccur). The successive phase is characterized by a rapid clockwise movement of one of the two partners with respect to the other which acts as the fulcrum. In this stage, the cells are already united and can be clearly differentiated. In fact the one fixed to the substrate acts as an axis around which the other rotates; and, in addition, the cytoplasmic fusion involves the anterior right margin of the AZM of the fixed cell and the anterior left peristomal area of the rotating one. This second fact allows the partner that is turning to assume an oblique position with respect to the substrate, so that the ventral surface is almost perpendicular to the substrate (Fig. 1, stage B). Once the rotation is completed, the couple presents a typical morphology in which the right[2] partner forms a sort of hood for the other cell. In this stage the AZM of the left partner are normal except for the right frontal portion where cytoplasmic fusion has begun, and those of the right partner are complete (Fig. 1, stage C). Finally, in the couple that by now is typical ($\simeq$ 35 min from the beginning of the "tête-à-tête"), one observes a single cytostome (Fig. 1, stage D). At this point the couple moves around with the same agility of a single cell, in contrast with the preceding phases of almost complete immobility. The pair presents this aspect throughout most of the mating process: the now (2–3 h) very extensive cytoplasmic bridge extends in length over the entire anterior third of the cells thus permitting the exchange of the nuclei-gametes, that in the meantime have matured. Finally, near the end of conjugation, that is about 19 h from the beginning of conjugation, progressive enlarging and shortening of the two partners occur, and a small cytoplasmic bridge connects the two mates. Exconjugants appear as rounded cells, with a very noticeable macronuclear "anlage" in the center and with normal AZM, formed "de novo" in the last hours of conjugation by a midventral streak present in each partner.

Couples in the initial mating phases described above (Fig. 1, up to stage C) were isolated and pipetted several times, until the partners separated; the earlier the conjugation the easier the separation was: in fact, within the first 30 min of the reaction, it is easy to separate the conjugants, which show a quite low mortality rate after separation.

This experiment was performed in order to see up to what point the mating phenomenon is irreversible, as far as the nuclear processes are concerned. The results obtained are shown in Table 1. The fate of the partners taken from couples of different ages, left and right, was followed for two days. From the "tête-à-tête" until the beginning of rotation, both partners of 18 pairs (Table 1: columns "A" and "B") behaved similarly both dividing as

[2] For brevity, throughout this study the partner that remains still will be referred to as the left, and the rotating one as the right.

vegetative cells. The only exception observed may depend on an error made during the pair isolation procedure. As the rotation of the right partner proceeds, an ever greater number of animals appear in which the micronucleus is irreversibly engaged in meiosis. The critical point seems to be the stage in which the two partners appear at right angles (Table 1, stage and column C).

Table 1
Results of split-pair experiments

STAGE	A		B		C		D		E	
TIME	2'– 10'		15'		17'		19'		35'	
ASPECT										
ANGLE	180°		120°		90°		50°		30°	
PARTNER	L	R	L	R	L	R	L	R	L	R
V.	12	12	5	4	24	15	8	4	3	1
A.	–	–	1	2	9	21	9	12	15	18
TOTAL	12	12	6	6	33	36	17	16	18	19

Explanation: V – the cell has evolved in a vegetative direction. A – the cell has evolved in an autogamous direction.

In fact, during this phase, some of the ex-partners evolve into clearly autogamous organisms. It is also interesting to note that the right partner is always more advanced than the other in the meiotic process: 21 right ex-partners out of 36 against 9 out of 33 left ones became autogamous cells. The different behaviour of the left and right partners is significant with a $P < 0.02\%$. Moreover, the right cell only undergoes autogamy whenever there is a discrepancy between the two split-partners of a pair. During the successive "D" stage, one observes an analogous phenomenon, in as many as 12 out of 16 right ex-partners and 9 out of 17 left ones which evolve into autogamous cells. Finally, one may say that both partners are irreversibly engaged in the sexual process when the rotation is complete and the couple has assumed a typical aspect (stage "E").

An ultrastructural investigation of the conjugation phenomenon reveals that there is already an initial adhesion between the two partners immediately

after the "tête-à-tête". In cross sections of pairs in conjugation for no longer than 10–15 min, the cytoplasm of the two cells undergoes a certain vacuolization near the expected adhesion region. Thus, protuberances of scarse or only slightly dense cytoplasm are formed, surrounded by the pellicle, and extending towards the partner's cytoplasm. These formations in the right cell are evident at the UM level (Pl. I 1, 2). In frontal sections, taken in an immediately successive phase (only a very few minutes later) pellicle fusion of the two animals was observed. A vacuolized cytoplasmatic "sack" of the left partner extends to fill the gap between the two mates within the joined pellicles. Cytoplasmic vacuolization of the right cell is less extensive (Pl. II 3).

During the last stage of the bridge formation, a cellular membrane lysis occurs bringing about the disappearance of every discontinuity between the cells, a process that also includes the reabsorption of the right partner's AZM (Pl. II 4). At the level of the reabsorption zones myelin-like and lysosomal figures are present (Pl. III 5), accompanied by a relative mitochondrial clustering (Pl. I 1, 2) in the cytoplasm immediately adjacent the bridge area.

Couples fixed from 5 to 15 h revealed a very extensive cytoplasmatic bridge that extends over almost the entire dorsoventral height of the partners (Pl. III 6) and approximately the anterior third of their length. After 17–18 h, there is a progressive narrowing of the contact area between the conjugants, until it reaches a stage in which the partners remain united by a thin cytoplasmic bridge, with a finely and densely vesiculated structure (Pl. IV 7, 8). Finally, these residuous and weak connections are interrupted and the two partners separate.

Discussion

The mating process of *Oxytricha bifaria*, conforming to the *Oxytrichidae* pattern as described by Maupas since 1889, can be summarized as follows: (1) the contact zone of the partners is not symmetric, (2) the behaviour of the two partners is different: one remains still and the other rotates around it, (3) due to this behaviour, the two conjugants assume a parallel position with the ventral surfaces on the same side rather than symmetric, as in the case of other hypotrichs, (4) finally, the partner that rotates, having assumed the definitive position in the pair, loses the oral ciliature and the front ventral cirri. Maupas (1889) and more recently Diller (1965), in a short abstract, described the loss of the oral ciliature of one partner, after observing one oral apparatus per couple: it is now clear that it is always the same cell that reabsorbs its own ciliature. The marginal, transversal and caudal cirri are normal in the two partners. The reabsorption of the ciliature takes place in the first hour of the coupling. Under the electron microscope, it was

possible to observe cases of lysis of the basal bodies, of both AZM and cirri. The new oral apparatus that forms in the two partners as conjugation goes on originates from a ventral streak that, in the partner lacking oral apparatus, has no contact with preexisting structures. The "de novo" origin of this structure is consistent with Grimes' findings (1972, 1973) on the ciliary-pellicle reorganization of the excystate forms.

The different behaviour of the two partners at the time of conjugation is probably casual. It is difficult to imagine that in a species with a multiple mating type system this behaviour depends on a sexual differentiation pre-existing to the cell contact during mating interaction. Nevertheless, the result is noteworthy: (1) the animal that rotates loses its oral ciliature and the front-ventral locomotory apparatus, due to its positioning in the mating reaction and (2) seems to be "activated" at the nuclear level before the other.

Using the "split-pair" technique in various moments of the mating reaction, there are three developmental routes that the separated cells may follow: (a) both partners return to the vegetative state, (b) both undergo an autogamous process, c) one partner behaves like a vegetative cell, while the other starts an autogamous process. Every time (c) occurs it is always and uniquely the rotating partner that terminates the sexual process. This means that the irreversible activation of the micronuclear meiotic process takes place first in the right partner. Sonneborn (1950) observed with the same technique that in *Paramecium aurelia* usually one of the separated partners became autogamous whereas the other divided again; however, in *Paramecium* the cells are identical, and, as far as we know, behave alike in the conjugation reaction. Further investigation is necessary to clarify the mechanism responsible for this anticipation of the irreversibility of the nuclear phenomena of the right partner. The point of no return to a vegetative situation, nevertheless, is reached by the two partners within the space of a few minutes when the couple passes from a position in which the partners are at right angles to that in which they are parallel and already firmly united. This could signify that the micronucleus in both cells has already been activated. Dini et al. (in press) has demonstrated that the micronucleus of *Oxytricha* lacks the G_2 phase, and Luporini et al. (1973) have ascertained that the meiotic S phase is already in progress one hour after conjugation begins. However, it is not known if the meiotic replication of the micronuclear DNA has already started in the D, E phases of the mating reaction, although it seems probable that the beginning of micronuclear meiosis is the response to a more general activation of the cells triggered by membrane fusion during the mating phase.

The ultrastructural analysis showed that cytoplasmic modifications related to the future fusion area take place even before a true cytoplasmic bridge

forms between the partners[3]. In *Oxytricha* the membrane fusion appears to correspond to the moment in which the cell developmental fate becomes determined. It is at this stage, when no exchange of corpuscular material has yet occurred between the partners, that even the meiotic process and thus the future caryogamy, whether autogamous or heterogamous, are determined.

Summary

The mating processes of *Oxytricha* were studied in live and fixed individuals under the light and the electron microscopes. The results revealed a continuity between the pellicles of the two partners occurring a few minutes after the onset of conjugation. A cytoplasmic bridge then forms involving initially only the apical portion of one of the partners and the peristomal area of the other partner.

As conjugation proceeds, this bridge extends to include almost the entire anterior third of the united cells. During conjugation only one oral apparatus is present per couple. The induction of meiosis occurs earlier in the partner that has lost its oral apparatus.

RIASSUNTO

Le modalità della coniugazione in *Oxytricha* sp. sono state studiate al microscopio ottico su individui vivi e fissati; le modificazioni al livello della regione di fusione sono state osservate al microscopio elettronico. Dai risultati é emerso che una continuità tra le pellicole esterne si ha già dopo pochi minuti dall'inizio della coniugazione. Successivamente si costituisce un ponte citoplasmatico che interessa inizialmente la porzione apicale di un partner ed una fascia ventrale, limitata dalla concavità delle AZM, dell'altro.

In un secondo tempo tale ponte si estende giungendo ad interessare circa l'intero terzo anteriore dei coniuganti. Durante la coniugazione solo un apparato orale è presente, dacché quello di un partner viene riassorbito. L'induzione della meiosi è più precoce nel partner che perde il proprio apparato orale.

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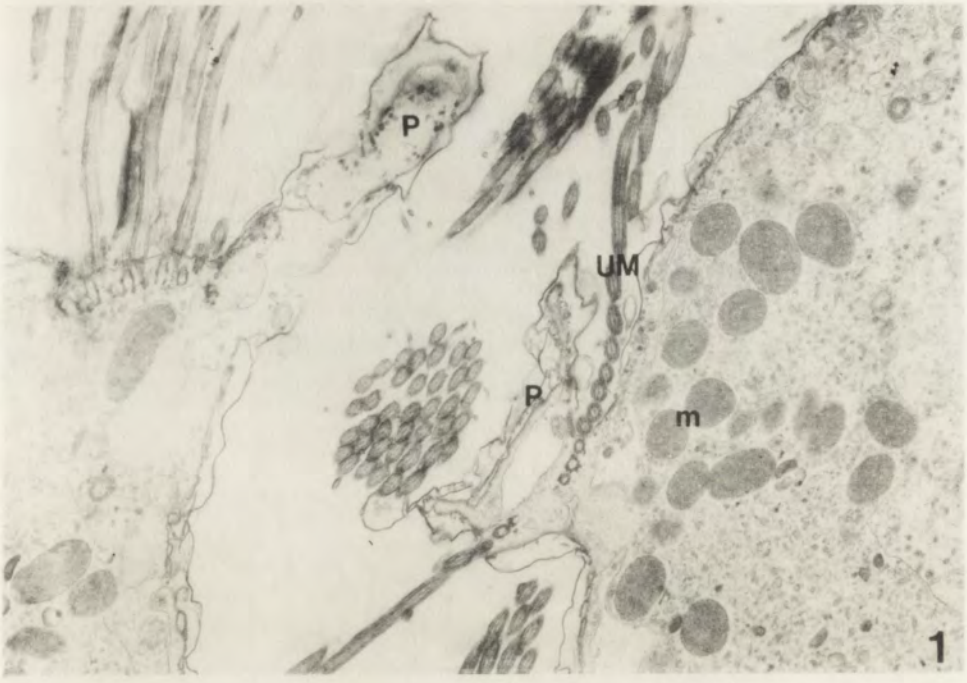
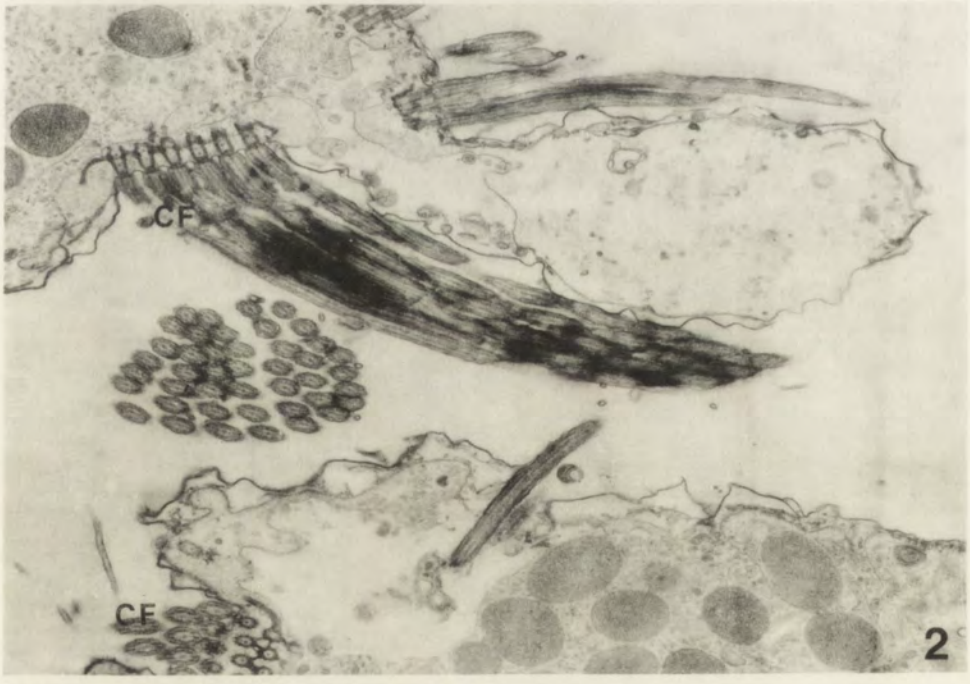
Received 6 March 1974

EXPLANATIONS OF PLATES I–IV

Conjugation in *Oxytricha* sp.

1: Transversal section of two animals 10 min from the beginning of the "tête-à-tête"; the cytoplasmic protuberances (P), a relative clustering of mitochondria in the adjacent region (m), and the undulating membrane of the right partner (UM) are shown (5000 × 1.7)
2: Successive transversal section of the same couple that is more caudal with regard to 1. Frontal cirri of the partners (CF) and a more extensive cytoplasmic protuberance of the left partner are evident (6000 × 2)
3: Frontal section of a couple approximately 15–17 min from the beginning of the "tête-à-tête": the pellicular membranes have now fused to form a cytoplasmic bridge, contained between the AZM of the left partner (AZM_L) and those of the right (AZM_R), at this level there is evidence of a relative mitochondrial clustering (4000 × 1.8)
4: Frontal section of a couple approximately 60 min from the onset of conjugation. The cytoplasmic bridge, already quite extensive, expands to involve most of the right partner's AZM that are then reabsorbed (Arrow "←") (6000 × 1.6)
5: Frontal section of a couple of approximately 60 min: series of myelin-like structures observed at the level of the fusion zone (12000 × 1.6)
6: Transversal section of a couple of about 7 h: one can see the large extension of the cytoplasmic bridge; (M) macronucleus; (m) micronucleus (2400 × 2.3)
7: Transversal section of a couple at 17 h. The cytoplasmic bridge is now fragmented into numerous lobes, whereas the pellicle connections are still present (2000 × 2.6)
8: High magnification of the same couple of 7 showing the residues of the cytoplasmic bridge (10000 × 2.1)





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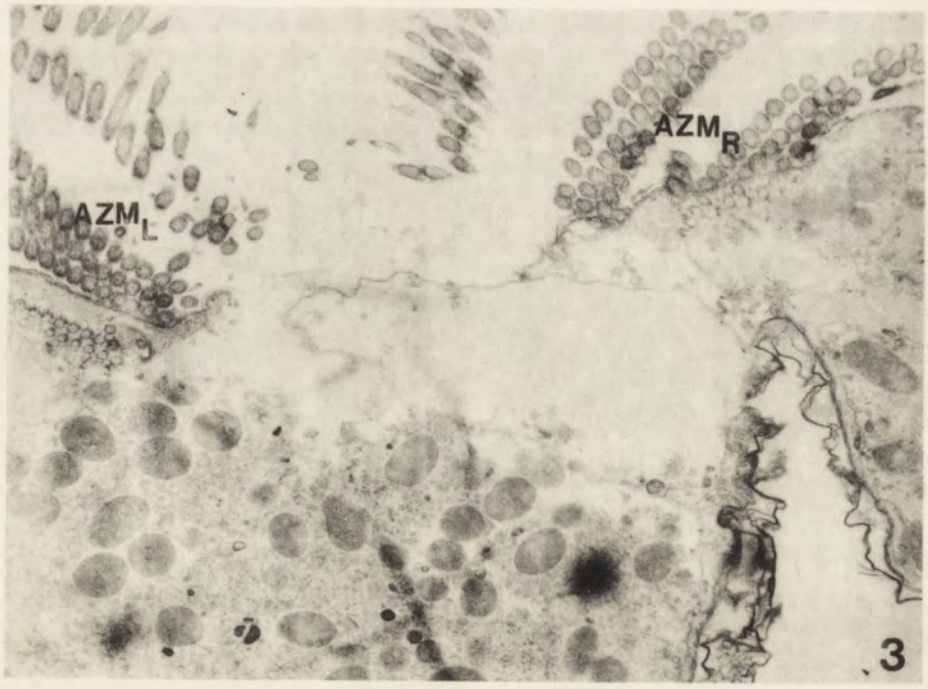

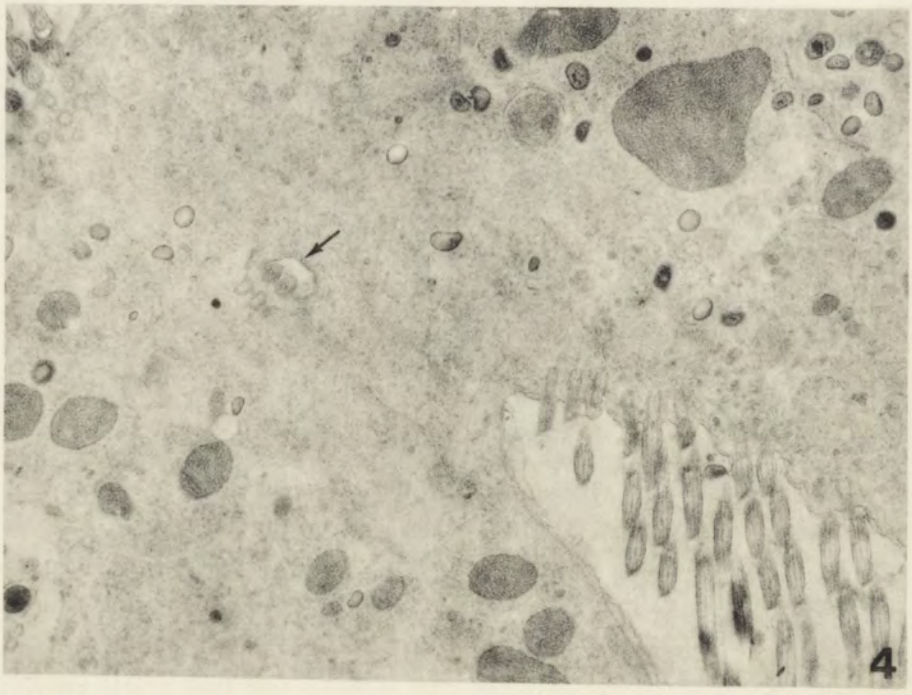

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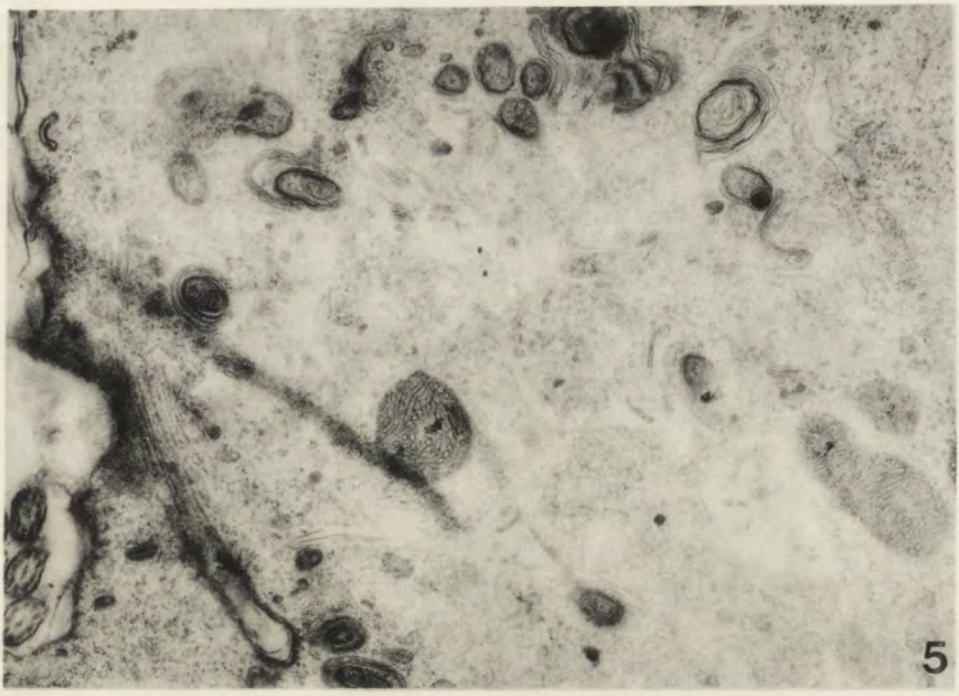

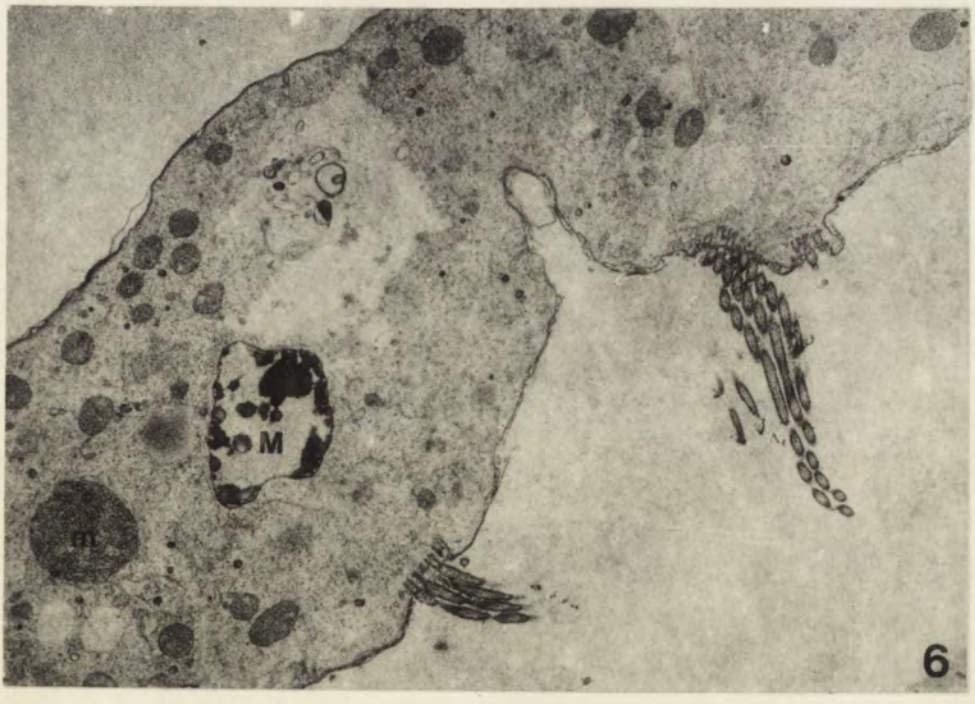

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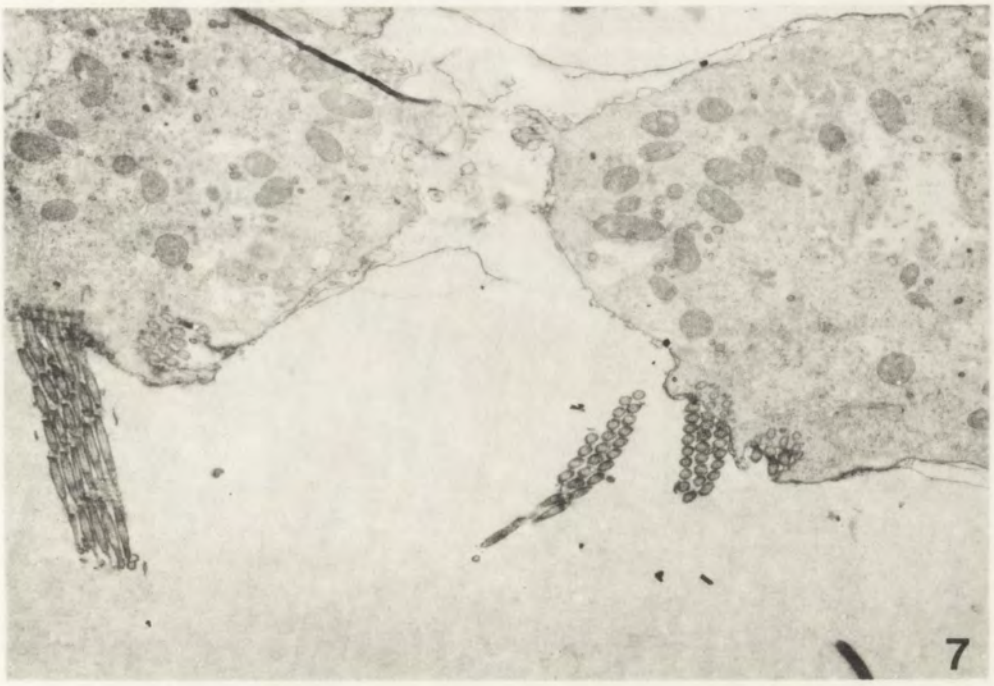

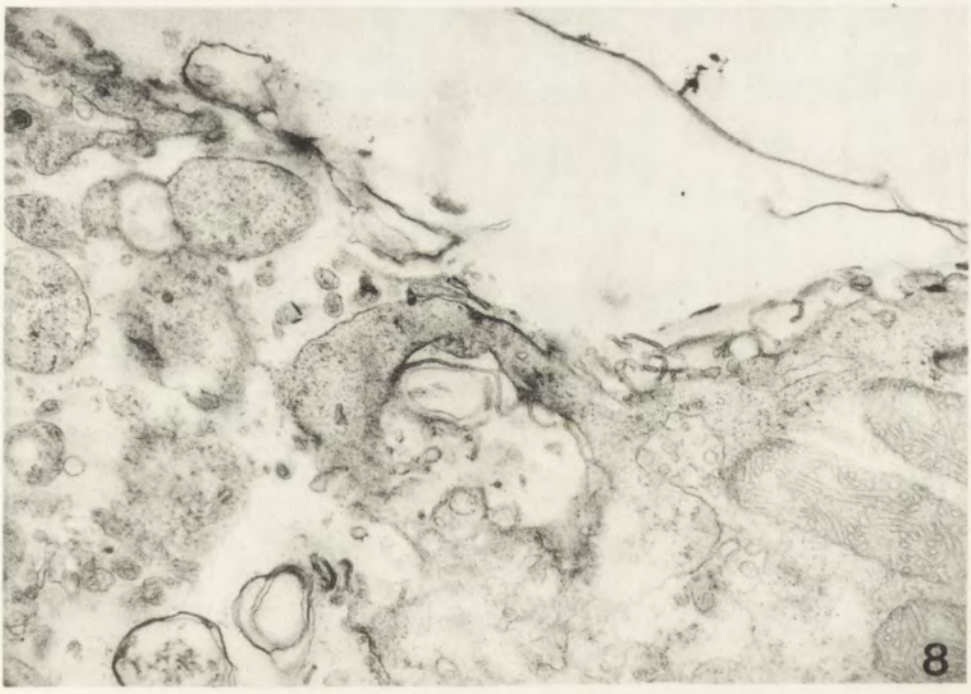

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ACTA PROTOZOOLOGICA

VOVOL. XIII WARSZAWA 31.X.1975 FASC. 30

Institute of Zoology, Warsaw University, Warsaw, Krakowskie Przedmieście, Poland

Janina KACZANOWSKA

Shape and Pattern Regulation in Regenerants of *Chilodonella cucullulus* (O. F. M.)

Regulacja kształtu i wzoru powierzchniowego u regenerantów *Chilodonella cucullulus* (O. F. M.)

It has been previously proposed (Kaczanowska 1974) that in early didividers of *Chilodonella cucullulus* there are spatial coordinates used in spspecification of the map of sites of organellogenesis. It was found that the sitsite of the oral apparatus (or the oral apparatus itself) and the boundadaries of the ventral surface of *Chilodonella* form reference points for spatial dedetermination of sites of organellogenesis of daughter cells. It was anticicipated that these processes would display a definite sequence of positioning, wlwhich would be at least partly under genic control. It was expected that gegenetic factors in some way would determine longitudinal sectors of compepetence for organellogenesis rather than the exact pattern of disposition ofof organelles. Therefore some randomness of pattern within the limits set byby a particular genome would be manifested through observed intraclonal pcpolymorphism.

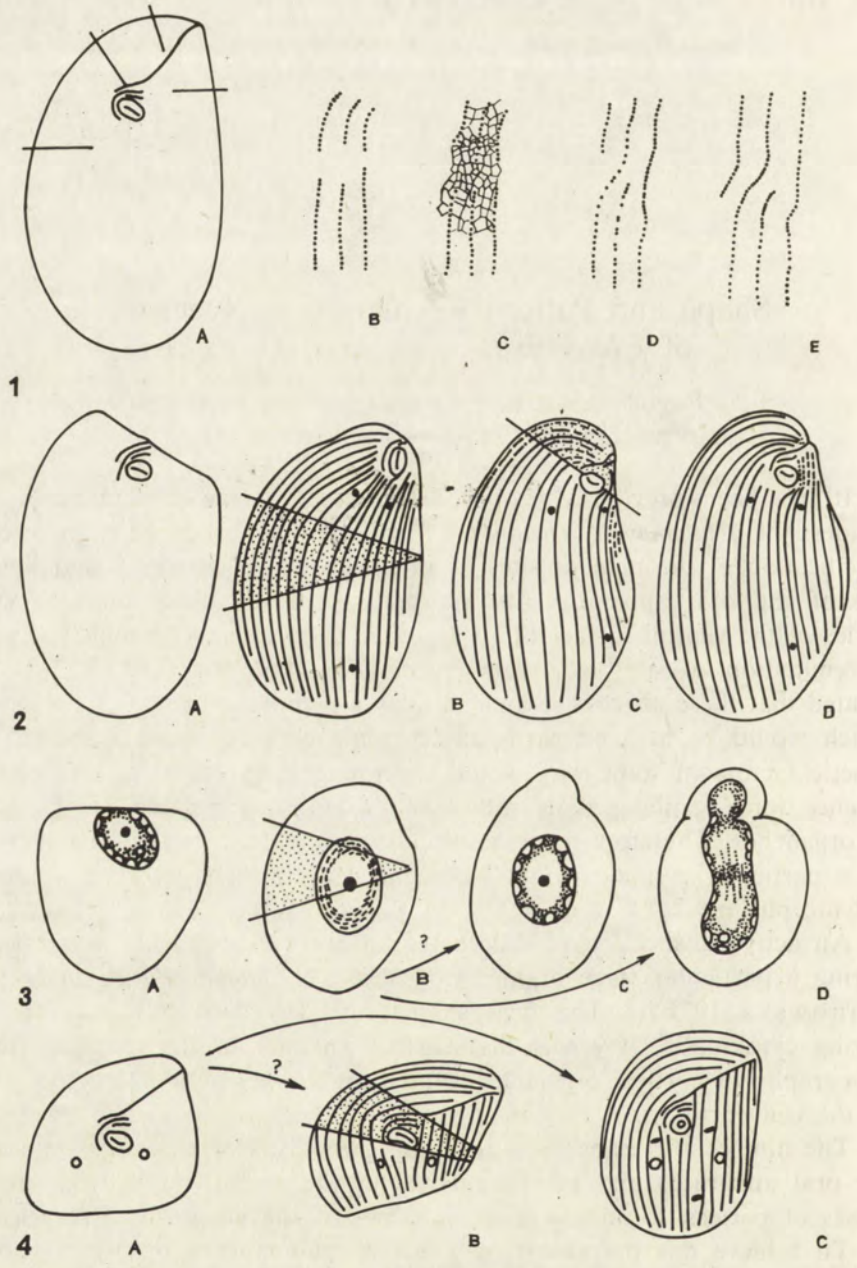
An active dislocation of differentiating cortical organelles was observed diduring a particular stage of morphogenesis of *Chilodonella cucullulus* (Kaczczanowska 1971 b). The final shaping of descedant cells was achieved diduring cytokinesis. It seems that further changes in the shape or in the totopography of cortical organelles result from isometric or allometric growth ofof the cell cortex.

The aim of this paper was to study the effects of traumatic injuries of ththe oral apparatus and the boundaries of the ventral surface on the procecesses of pattern formation and moulding of the shape in disturbed cells.

To achieve this purpose, four kinds of traumatic injury were followed:

(1) Slight local incisions were made in various parts of the ventral surface leleading to local interruptions of a few kineties and the cortex without disturbibing the overall proportions of the cell body (Fig. 1 A).

(2) The preoral part of the cell was removed, including the preoral part ofof ciliature, but without injury to the oral apparatus (Fig. 2 A).



Figs 1–4. Diagram of types of operations performed on *Chilodonella cucullulus* and following regeneration. 1 A, 2 A, 3 A, 4 A – Types of operations performed. 1 B, 1 C, 1 D, 1 E – Tentative model of reconstruction of the kinetome after incisions. 2 B, 2 C – Tentative model of zone of allometric growth of the cortex marked by the stippled area in the drawings. 3 B, 3 C, 3 D – Macronuclear behavior in operated cells. 4 B, 4 C – Stomatogenesis in situ in promers

(3) The anterior part of the ciliate including the oral apparatus was amputated. Small posterior fragments (opimers) lacking the anterior 1/3 to 1/2 of the cell body were observed (Fig. 3 A).

(4) The posterior 1/3 to 1/2 of the cell body including the cytoproct and the posterior contractile vacuole pores was amputated. Small anterior fragments (promers) were followed (Fig. 4 A).

Studies concern only transversal sections of specimens. Therefore all generalizations concerning the shaping and the pattern formation after injury are strictly restricted to this kind of regenerants.

The results suggest that the amputation of part of the ventral surface does not per se elicit a total morphogenesis if the oral apparatus is left intact. However, changing of the boundaries of the ventral surface can shift the zone of the maximum growth of the cortex. Reorganizational morphogenesis with in situ oral replacement was observed instead of division in small promers. In such cases the site of the oral apparatus and the existing boundaries serve as reference points for specification of sites of contractile vacuole pores (CVPs).

The amputation of the oral apparatus brings about total regeneration. The newly arising map of disposition of cortical organelles is adjusted to the existing size of the fragment, but stomatogenesis is always performed along the stomatogenic kineties present in the fragment. Regulatory monitoring of the pattern of CVPs with respect to the existing boundaries of the fragment is observed as in the case of promers.

Both in promers and opimers a series of events in the macronucleus parallel to those observed during divisional morphogenesis (Radzikowski 1965), (but not necessarily followed by macronuclear division) accompanied stomatogenesis and formation of new set of CVPs and cytoproct. The old organelles (if present in the fragments) were resorbed during this processes. All experiments concern the fate of the viable nucleated fragments.

Some theoretical implications of these results are discussed.

Material and Methods

The morphology of *Chilodonella cucullulus* of stocks B_1 and X described elswehere (Kaczanowska and Kowalska 1969, Kaczanowska 1974). The study of regeneration was performed on cells injured by micro-needle operations. Since the cells were not selected before operations, the ontogenic stage of the cells during traumatization was unknown in the vast majority of the cases. The operated cells were fixed at various times after injury. They were silvered by Chatton-Lwoff method (after Corliss 1953). Most of the material was fixed 5–6 h after operations.

The comparison of the topography of cortical organelles in dividers and regenerants was based on drawings of silvered specimens. The same populations of ciliates were used in a study of divisional and regenerative morphogenesis (Kaczanowska 1974). All drawings

were made at the same magnification with the aid of a camera lucida. The photomicrographs of total individuals were taken by Exa-125 with 4× eyespiece.

Details of the argyrome were investigated on a series of specimens silvered with a dry method of Klein (1958).

All measurements of the distances between the marker points were made on the drawings, and they were used for calculations in a millimeter scale. The error of estimations on the drawings was limited to ± 1 mm.

The Cortical Pattern of Uninjured *Chilodonella cucullulus*

The general morphology and differences between stocks B_1 and X are analysed elsewhere (Kaczanowska 1974). In this paper those features necessary for this study are brieflly reviewed.

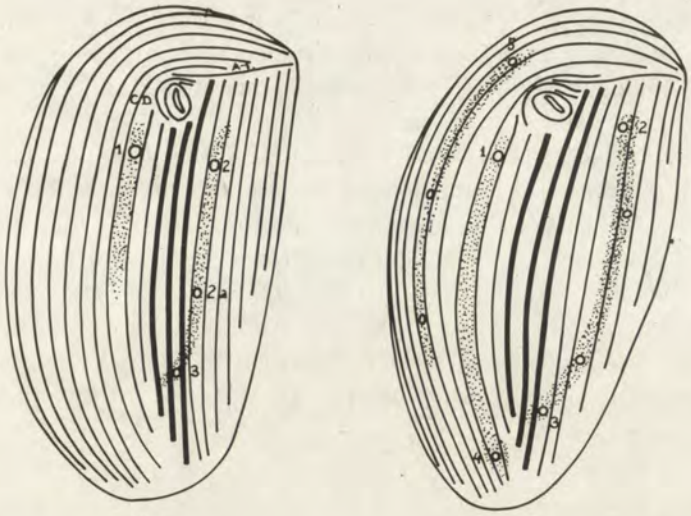
Chilodonella cucullulus is a flat, asymmetric holotrichous ciliate. The flat ventral surface is covered with kineties, while the dorsal surface is not ciliated but possesses one oblique kinety X (after Radzikowski 1966) in the anterior part of the cell body. This kinety X originated from the ventral surface kinetome (Chatton et al. 1931) and is displaced dorsally to the opisthe during cytokinesis. In the anterior derivate of division (proter) this kinety is transmitted with no change from the parental specimen. Therefore in successive generations the same preoral part of the ciliature with the same dorsal kinety X is always transmitted to the successive proter cells.

The longitudinal somatic kineties of the ventral surface 16–21 in number in stock B_1 and 17–19 in stock X, are arranged parallel to the long axis of cell. The right kineties are longer than the others and they are curved in the area anterior to the oral apparatus.

The oral apparatus is positioned centrally about one third of the distance from the anterior to the posterior end along the long axis of the cell body. Some postoral kineties, 2–4 in number are positioned posterior to the oral apparatus. The left kineties run parallel to these and they meet the anterior curved ends of the right system of ciliature. A suture between the left and right systems of kineties in the anterior left part of the ventral surface is occupied by the preoral kinety A-1 extending from the oral apparatus up to the apex on the anterior left margin of the ventral surface. Two additional circumoral kineties (B_1 and CD_1 after Kaczanowska 1971 b) and preoral kineties A-1 form the oral ciliature of *Chilodonella* (Fig. 5). The oral apparatus consists of an oral basket, a cytopharyngeal tube and the oral lips (Sołtyńska 1971).

A cytoproct is localized at the dorsal surface parallel to the right margin of the ventral surface at its posterior part in specimens of stock B_1. In stock X it lies at the posterior end of the cell (Fig. 6).

The contractile vacuole pores (CVPs) are permanent structures in the morphostatic period but vary in number and disposition. CVPs are resorbed



Figs 5–6. The general outlines of *Chilodonella cucullulus*. 5 – The ventral surface of the opisthe cell of stock B_1 6 – The ventral surface of the proter cell of stock X, heavy lines-stomatogenic kineties, stippled areas – the sectors of ability for CVPs formation, 1, 2, 3, 4, 5 – obligatory set of CVPs, 2 a and with no denominations – facultative CVPs

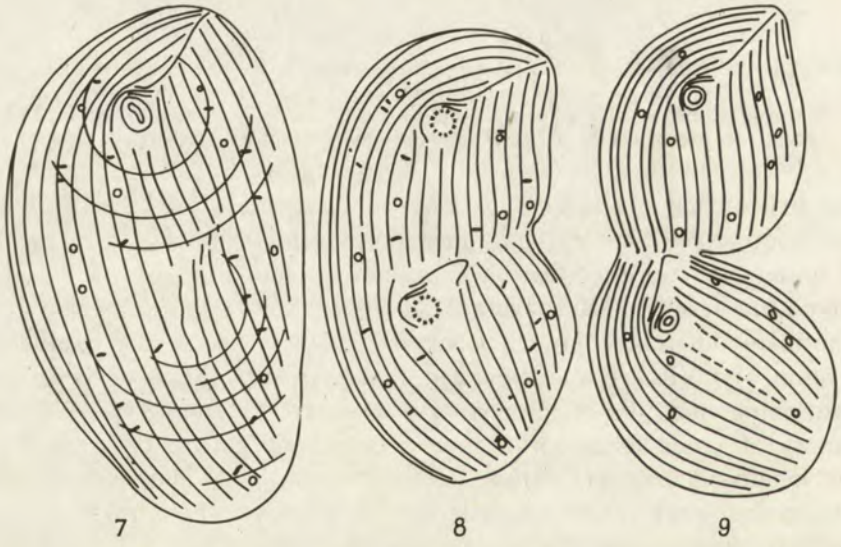
during division but not in conjugational morphogenesis (Janus 1972). New sets of contractile vacuoles with their pores arise simultaneously for incipient proter and opisthe. During cytokinesis some of them are stabilized, but some are resorbed (Kaczanowska 1971 b).

The main difference between stock B_1 and X concerns the number and disposition of CVPs. It was suggested that there are the same coordinates of positioning of CVPs in both stocks, but there are numerical differences in sectors of competence for organellogenesis. CVPs are differentiated in certain longitudinal sectors parallel to the boundaries of the ventral surface, and there are some privileged distances (radii) from the center of stomatogenesis to these sectors which determine the exact sites of CVP primordia. This positioning is thought to take place during an early stage of divisional morphogenesis (Kaczanowska 1974). Not all, but only some of the points competent to differentiate CVPs may be manifested in a given cell. Some randomness in expression of the pattern of competent sites is suggested, resulting in polymorphism within each of these stocks.

In stock B_1 there are only two longitudinal sectors (Fig. 5): in the right only one anterior CVPs is formed, while in left sector there can be 2–4 CVPs. An anterior CVP-2 and a posterior CVP-3 are always present. Additional CVP-2a, CVP-2b and CVP-2c may be formed in this left sector. These additional CVPs are represented only in the opisthe derivate of divisional morphogenesis (Kaczanowska and Kowalska 1969). In proters

there is a sector distance of five kineties separating CVP-1 and CVP-2, while in the majority of opisthes this distance is enlarged to six kineties. Therefore intraclonal dimorphism is always manifested due to differences in two sister cells.

In stock X (Fig. 6) there are at least five CVPs (on the average 8–9) diposed in three sectors of competence. These are: extreme right sector with at least one preoral CVP-5 and with additional CVPs, the right sector represented by anterior CVP-1 and posterior CVP-4, and the left sector with a variable number of CVPs. In the left sector at least the anterior CVP-2 and the posterior CVP-3 are observed in each cell. The distance separating left and right sectors (i.e., distance between CVP-1 and CVP-2) is seven-kineties in the majority of the proters and in opisthes as well (Fig. 6).



Figs 7–9. Schema of cortical morphogenesis of *Chilodonella cucullulus* during division of specimen of stock X. 7 – Early divider. Some of presumed radii are presented, round circles-formed CVPs, slits-CVP primordia, 8 – Morphogenetic movement of the oral segments in the forming opisthe cell. Differentiation of the oral apparatusses in the forming proter and opisthe descendants, 9 – Cytokinesis. The two daughter cells manifest a different number and distribution of CVPs

The sequence of nuclear events and changes in the kinetome during the cell-cycle seems to be identical in both stocks (Kaczanowska 1974). The main stages of cortical morphogenesis during division are represented by the drawings of dividers of stock X (Fig. 7, 8, and 9).

Results

(1) Slight local incisions without disturbing the overal boundaries and proportions of the ventral surface (Fig. 1 A, B, C, D, E, Pl. I 1, 2, 3, 4).

The whole process was observed in slides silvered by the dry method of Klein. In no case this operation did bring about the total morphogenesis of the oral apparatus and other cortical organelles. It is not excluded that it can cause delay in entering the following divisional morphogenesis. The timing of wound healing showed wide variability. Reconstruction of normal cortical pattern can be achieved even in the next generation. Furthermore, reconstruction of a normal kinety can be attained in one kinety, while an adjacent one has not yet completed it (Pl. I 2, 3). After the closure of the cut edges of the incised region there is a process of local disappearance of the silvernet. The normal mesh of the argyrome reappears earlier than the reconstruction of the pattern of the kinety (Pl. I 1, 2, 3). If there were some local changes in the contour of the ciliate (Pl. I 4), there them occurs a re-shaping of the cortex which restores the normal phenotype. In the vicinity of the "naked" argyrome there are changes in the adjacent interrupted ends of kineties. The space between kinetosomes becomes greater than in a normal kinety. There is an impression that the kinetosomes are more widely separated from one another than usually (Pl. I 1, 2). In the next stage the free ends of the same disrupted kinety meet and there is a process of crowding of kinetosomes in the region of healing (Pl. I 2, dark arrow). It can be presumed that rarefaction on kinetosomes precedes their local proliferation, since at the moment of rejoining of the tips of an interrupted kinety, a large number of kinetosomes can be observed in the region of healing. This rejoining of the ends of a disrupted kinety restores its integrity. Using the phase contrast microscope the reconstruction of the fibrillar system of the kinety was confirmed.

As was stated before, the course of this process is out of the phase even between adjacent kineties. This statement is based on the observation of improper reconstruction of a kinety due to joining of segments of adjacent incised kineties. In such cases the reconstruction of one complete kinetal unit stops its further development, while the unpaired deviated segments pass by and are not inhibited from further growth (Pl. I 2, 3). The same course of events was observed in the preoral region also (Pl. I 3, 4). The preoral part of the ciliate lies out of the zone of the growth of the cell cortex (Kaczanowska 1971 a) and it is transmitted to the proter of the next generation without no major changes. However, after injury it can perform all stages of healing.

It was not determined whether or not both the anterior and posterior segments of an interrupted kinety contribute to healing to the same degree.

It can be presumed that local injury influences the adjacent region in such a way that adjacent kinetosomes are displaced, and in the next stage the gaps are filled by new kinetosomes. It seems that local regeneration is not limited to step by step terminal growth of the cut ends of a kinety but covers a considerable region. All kinetosomes (at least in the stage which is visualized in silvered specimens) are always positioned according to the polarity of the cortex and there is no trace of an anarchic field of kinetosomes. The cases of passing by of the free ends of an adjacent kinety next to an improperly rejoined kinety are consistent with this statement because these are cases of segments of kineties displaced during injury.

(2) Amputation of the preoral part of the cell, without injury to the oral apparatus (Fig. 2 A, B, C, Pl. II 5, 6).

If the preoral part is amputated but the preoral kinety A-1 and apex is left intact, local growth of the cell cortex and kinetome is observed. However, there are different results if the preoral kinety A-1 and apex are removed (Pl. II 5, 6). This kind of amputation does not bring about total morphogenesis. There is a local growth of the edge of the wound as in the previous case, but there is also a change in growth of the remaining cortex. This kind of operation elicits very distinct allometric growth of the right portion of the ventral surface. This growth is visualized because the oral apparatus is very distinctly displaced to the left and the oral slit previously obliquely oriented takes on a longitudinal orientation (Pl. II 5, 6) that is as normally observed only in predividers (Kaczanowska and Kowalska 1969). Local growth of the wounded region and postoral allometric growth of the right part of the ventral surface taken together result in restoration of the shape of the ventral surface.

If the same injury was inflicted on predividers they were able to complete normal divisional morphogenesis regardless of their deficiency and the above phenomena were observed subsequently in the defective proter products (Pl. II 7).

(3) Amputation of the anterior part of the cell body including the oral apparatus (Fig. 3 A, B, C, D; Pl. II 8, Pl. III 9–14).

This kind of operation was performed on specimens of stock X and on specimens of stock B_1 at an early age in the cell cycle (2–4 h after the last cell division). In all cases this kind of injury stimulates a regenerative total morphogenesis during the following 7–8 h. In some cases, the operation involves injury to the nuclear apparatus. It was observed that amacronuclear fragments can survive about 8 h (Pl. IV 16). They are able to maintain coordinated cell movement and to digest swallowed food vacuoles. They are, however, incapable of performing total morphogenesis. Finally all of them died out.

Advanced dividers with part of the ciliature amputated and the macronucleus removed are still able to complete cytokinesis and to maintain final

differentiation of new cortical organelles and resorption of the old ones (three cases recorded). Even a small portion of the macronucleus is able to reconstruct its normal shape and to assume a central position in the fragment after about 2 h. The fate of micronucleus was not investigated.

During this first two hours an allometric growth at the right portion of the cell body was observed. About four hours after injury a large number of nucleoli were observed in the macronucleus as in early predividers (Radzikowski 1965). About 5–6 h after operation, the nucleoli disappeared and a rosette like distribution of chromatin strands was formed as in more advanced predividers (Radzikowski 1965, Fig. 3 B).

At the rosette-like stage of chromatin in macronucleus there are the first signs of appearance of new oral kineties (Pl. III 9–11) and CVP primordia. In all cases the new oral kineties of regenerants are differentiated within the previous stomatogenic kineties and subapically with respect to the anterior boundary made during operation of the fragment. The continuity of stomatogenesis in the same stomatogenic kineties is clearly deduced because there are three kineties to the right of the posterior old CVP-3 in stock B_1 which are involved in stomatogenesis both in dividers and regenerants (Pl. III 9, 10).

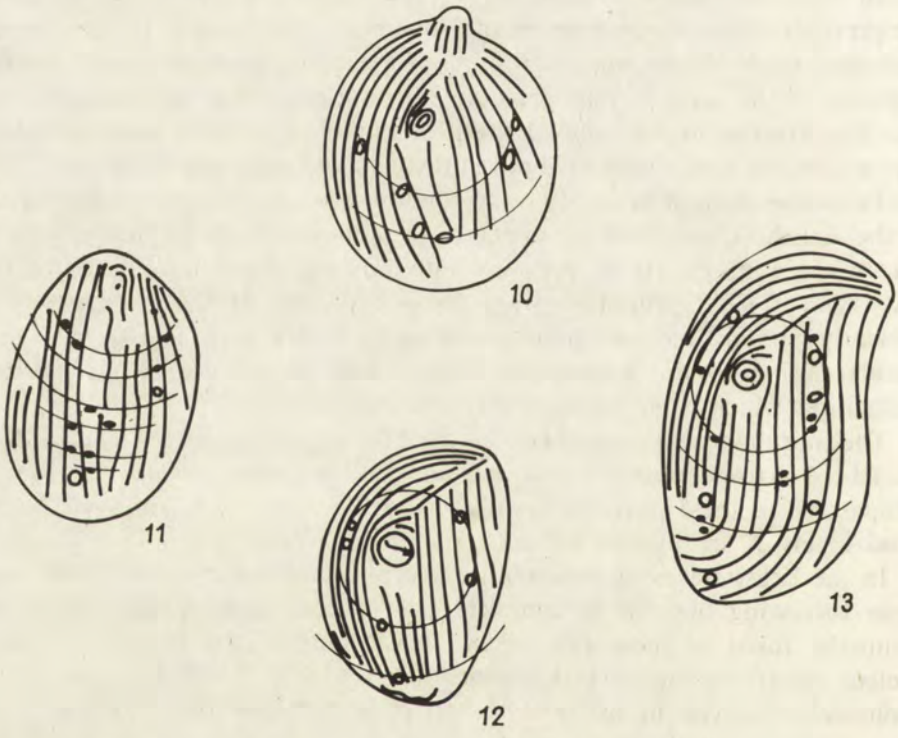
This disposition of new CVP primordia corresponds to the pattern observed in the opisthe component of dividers. In some cases the additional CVPs (eg., CVP-2a in Pl. III 9, 10) were not induced. An independence of the new sites of CVP primordia from the topography of the parental CVPs is clearly demonstrated in specimens of stock B_1 (Pl. II 8, III 10). The new double CVP-3 in this regenerant is positioned posteriorly to old CVP-3, a situation which is never observed in dividers.

The morphogenetic movements of the kinetome (e. g., of segment of A-1, Pl. III 14 arrow), resorption of old and differentiation of new CVPs are comparable to these phenomena in opisthes. In some cases, however, there is no kinety X in regenerated cells.

In the latest stages of regenerative morphogenesis the macronucleus can begin furrowing but fail to complete this process, and the two parts are eventually fused or there can be no furrowing and the collapsed macronucleus reverts to the normal interfission stage (Fig. 3 C). The latter situation was observed in living cells and it is not sure that the furrowing was not suppressed by the pressure of the cover slip. During the moulding of the preoral part of regenerants a very small portion of the cortex is sometimes out off from the anterior apical tip of the cell. This process simulates the moulding observed during cytokinesis (Pl. III 14, Fig. 10). A small part of the macronucleus can be pinched off in this portion too (Fig. 3 D). It is interesting to note that the same various fates of the macronucleus that were observed in regenerants (Figs. 3 A, B, C, D) were also observed in predividers deprived of the parental oral apparatus. Delayed divisional morphogenesis lasted about the same time as regeneration (i.e.,

about 6–8 h) leading to a normal opisthe and defective proter (Pl. II 8). The cycle of the micronucleus was investigated neither in regenerants nor in injured dividers. It is evident from the above that in the present studies there is no meaningful criterion of difference between delayed division of an injured pre-dividing cell and total regeneration of a non-dividing cell.

The case of stock X offers a possibility for testing whether the distribution of CVP primordia in opimers is consistent with the model proposed for dividers (Kaczanowska 1974). Seventeen opimers (or delayed dividers but with a large anterior portion removed fixed about 5 h after the operation in the stage of formation of CVP primordia (Pl. III 11, 12) were analyzed. It was tentatively found that the center based on topography of



Figs 10–13. Regenerants of *Chilodonella cucullulus* of stock X 10 – A regenerant of stock X seen 6 h after removal of the oral apparatus. The anterior portion of the cortex had been cut off. Only two radii cover all CVPs. 11 – An early regenerant of stock X deprived of its oral apparatus and the extreme left and right somatic kineties. This drawing corresponds to phot. 11 (Plate II). The stomatogenic kineties retains their function, but some abnormalities in topography of CVP primordia are observed, because of the narrowing of the ventral surface. 12 – The promer of stock X after in situ oral replacement. Two radii cover the set of CVPs. The cytoproct is differentiated at the posterior end of the cell body. 13 – The promer of stock X with curved ends of postoral kineties. The regulation of the pattern of CVP primordia (slits) is in respect to the new boundaries of the ventral surface

CVP-1, CVP-2 and CVP-5 can serve as the reference point for the other CVPs. In the extreme case only two radii from the center described the topography of all CVPs (Fig. 10).

All observed opimers have a very short postoral axis (measured from the center to the posterior end of the ventral surface) which varied from 31–51 μm in comparison to 40–58 μm in dividers. Surprisingly there is no significant correlation of the total number of CVP primordia and the postoral length of the fragment ($k = 0.15$), though the total number of primordia varied from 5–13 with an average number being 8.5 ± 1.4 in comparison to 10.6 ± 2.1 primordia in opisthes of dividers. The existence of CVP-4 and the cytoproct at the posterior end proved that the full pattern was realized in all seventeen specimens (Pl. IV 15). The total number of radii was reduced in regenerants and varies from 2–6 with an average number of 4.3 ± 0.82 in comparison to 5.97 ± 0.89 radii in opisthes. However, there is no correlation between the total number of radii and the postoral length of the regenerants ($k = 0.21$).

In some specimens the first radius (i. e., a distance from center to CVP-1, CVP-2 and CVP-5) is particularly short in regenerants. There is a minimal value of about 10 μm vs. 12 μm of minimal value of R_1 for dividers. Such low values were observed in some small opimers (3 cases). In these cases the second radius was within the limits of length of the first radius of dividers. The average value calculated for the first radius was 12.6 ± 0.9 (Table 1).

Table 1
The average value and standard deviation of the length of particular radius of *Chilodonella cucullulus* in 17 opimers of stock X and in the opisthes (after Kaczanowska 1974)

R	No. of cases	Mean value of R	sd.	No. of cases	Mean value of R	sd.
R_1	17	12.63	0.9	100	13.7	0.8
R_2	13	17.00	1.0	72	18.1	1.1
R_3	14	21.46	1.2	86	22.6	1.4
R_4	10	27.14	0.89	78	27.2	1.2
R_5	11	31.63	1.5	75	31.7	1.7
R_6	2	37.50	0.5	28	35.6	1.1

There is significant ($\alpha = 0.01$) correlation between the length of the first and last radii on the one hand and the length of the postoral axis in opimers on the other (respectively $k = 0.51$ and $k = 0.59$). The null hypothesis (H_0) of regular periodicity of R_1, R_2, R_3, R_4, R_5 and R_6 can be accepted at the 0.01 level of significance.

From the above, one can conclude that in opimers the same kind of topographical relationships hold as in dividers. The study of opimers proved that astomatous cells are able to specify a new topography. These events are phased with the nuclear events which occurred in a regular sequence from the moment of traumatization.

It seems that the boundaries of the ventral surface and stomatogenic sector serve as references for new positioning. The analysis of photomicrographs 11 and Fig. 11 is particularly instructive in this respect. This is the case of a specimen from stock X deprived of the anterior part including the oral apparatus and four extreme right and three extreme left somatic kineties. While the continuity of stomatogenic kineties is left intact during morphogenesis, the narrowed width of the ventral surface is reflected through the proportionally more dense and irregular disposition of sectors of CVP primordia.

(4) The removal of the posterior part of the cell. Regeneration of the promers (Fig. 4 A, B, C, D, Pl. IV 17, 18).

In specimens of stock B_1 and X the posterior part including a cytoproct and posterior CVPs was amputated, while the anterior portion of the cell including the oral apparatus was kept. In some promers fixed about 5–6 h after injury characteristic changes in the macronucleus were observed, similar to observed in opimers. These events were followed by the resorption of the oral apparatus and in situ oral replacement (Figs. 12, 13). In such cases a new pattern of CVP primordia and new cytoproct were observed. Stomatogenesis in situ and the pattern of disposition of CVPs corresponds to the morphogenesis of proter derivate in dividers.

However, some promers with a morphostatic macronucleus were found in silvered slides even 15–17 h after operation. These cells had been of unknown age during the operation and they could represent the defective opisthe product of specimens injured in a predivisional stage. Such abnormal promers were observed from 7 h to 17 h (or even longer) after the operation and they displayed an abnormal shape of the ventral surface. This kind of deformation of the shape (Pl. IV 17, 18, Fig. 4 B) can be intepreted as the result of allometric growth of the right portion of the ventral surface in its equatorial zone. In the case of a very short promers this equatorial zone covers the level of the oral apparatus or even the preoral portion of the ventral surface. In this way very wide and short specimens can be formed. In longer promers the equatorial zone of the allometric growth can lie posteriorly to the oral apparatus. In such cells the anterior CVP-1 and CVP-2 are displaced far from the oral region. In both cases oral replacement and formation of new CVPs and a cytoproct was occasionally observed (Pl. IV 17). These processes were always phased with macronuclear rosette – like stage.

Discussion

Mechanical injuries to the cortex of the ventral surface are healed in situ without regard to the place of injury. The process of healing of the cortex and restoration of the kinetome seems to be out of phase with the nuclear cycle. These data are consistent with evidence on the mutual independence of DNA synthesis and kinetosome proliferation in *Stentor* (Younger et al. 1971).

A sequence of restoration of the interrupted kinety was observed. However, there is no synchrony of these events in different kinetal units. It seems that the kineties at the edge of the wound are restored faster than those which are interrupted at the center of wounded region. There is no mediolateral gradient of the velocity of healing. Examples – the most central kinety of the wounded region (Pl. I 1), and conversely the most external kinety (Pl. I 2 black arrow) attained the stage of proliferation of kinetosomes first while others were more delayed. An appearance of an additional kinety through the improper joining of ends of kineties seems to be a random process with respect to the localization of the wound on the ventral surface of *Chilodonella*.

It is concluded that each part of the ventral surface and kinetome is able to grow and to form the structural elements of the kinetome according to polarity and cytoplasmic guidance (Frankel 1973) factors and short-range interactions of kinetosomes (Sonneborn 1974). Then the suppression of the growth of the preoral part of the ventral surface of the uninjured cell is rather a function of its position than of the intrinsic inability of this part to grow.

Drastic surgical alteration of the general size, shape and proportion of the ventral surface does not immediately trigger either subsequent total reprogramming of the pattern or regeneration of the deprived organelles if the oral apparatus is kept intact. It is only during morphogenesis with concomitant stomatogenesis when the whole new set of organelles is restored. It is not excluded that amputation of the portion of the ventral surface including the cytoproct and/or CVPs or the preoral part of ciliature may delay the start of oncoming morphogenesis.

Opimers deprived of the preoral part of the ventral surface and promers without the posterior part represent the classes of specimens with a shortened antero-posterior axis of the cell body. In such specimens regeneration in situ of the kinetome was followed by some shifting of the zone of the maximum growth of the cortex into the equatorial zone of the fragment. This long-range effect is not consistent with the stability of this zone observed in traumatized *Paramecium* (Chen-Shan 1969). There is right-left gradient of the degree of growth in the equatorial zone of regenerants with the

most growth at the right edge. It is not known if the area of growth is wider in the right part of the ventral surface, or if right part merely grows more intensely. It is necessary to point out that data on the shift of the zone of maximum growth of the cortex do not refute the possibility of membrane and fibrillar synthesis all over the cell surface. This conclusion is interpreted strictly in terms of maximum growth zone between cortical markers (e.g., between the oral apparatus and CVPs in Fig. 4 C). In very short promers this equatorial zone of growth of cortex can include even the preoral part of the ventral surface (Fig. 4 B). Abnormal shaping of the flat ventral surface can be observed (Fig. 4 B, Pl. IV 17, 18) as an effect of this growth.

The observed restoration of the kinetome and equatorial growth of the ventral surface (even leading to abnormal shaping) can be generalized as the expression of some regulative growth after traumatization. The cytoplasmic guidance factors operate within the zone of this growth (after Frankel 1973) but the control of the position of the zone depends on the existing boundaries of the ventral surface.

Specimens deprived of a portion on the ventral surface including the cytoproct and/or some CVPs can enter asynchronously into subsequent morphogenesis. New stomatogenesis is phased with a series of nuclear events and with a new positioning of CVPs and the cytoproct. Reorganizational morphogenesis with in situ oral replacement is observed instead of division in short promers. This morphogenesis corresponds to that observed in the proter component of dividers. This fact is consistent with results of Golińska and Jerka-Dziadosz 1973 concerning the existence of minimal cell size indispensable for division of *Urostyla* and *Dileptus*. In the promer shown in Fig. 13 some kineties are longer and twisted. The new pattern of CVPs is proportionated to dimension of the ventral surface and not to the length of kineties.

An amputation of the oral apparatus triggers the series of nuclear and cortical events leading to restoration of a normal individual. The sequence of these events corresponds to morphogenesis of the opisthe component of a divider. In all cases which are thought to be regeneration of the transversal fragments of the interphasic cells but not delayed division of an injured divider, the oral kineties are formed subapically within the stomatogenic kineties. The conclusion that the new boundaries play a role in positioning of the site of stomatogenesis is deduced since all regenerants restored the normal proportions of the ventral surface. However, even in very small fragments (of about half of the length of the normal postdivider) and amputated at early interfission period a portion of the cortex can be pinched off by the cell during final shaping of the ventral surface (Fig. 10). Hence it seems that this is not a remnant of cytokinetal zone of the fragment. It is rather true, that the moulding of the new ventral surface involves some contractile processes similar to cytokinesis.

All available data on regenerants of *Chilodonella* corresponds to those in *Stentor* reviewed by Tartar (1961, pp. 116, 123, 153, 204, 222, 229). In *Chilodonella* a new site of stomatogenesis and the boundaries of the ventral surface are used in determination of the topography of CVP primordia in regenerants. The coordinates and reference points used in description of the spatial pattern of organellogenesis in dividers (Kaczanowska 1974) hold in the case of regenerants too (Figs. 7, 10, 13 and Table 1).

Acknowledgements

I am much indebted to Professor Joseph Frankel for penetrating discussions throughout the course of this work. Thanks are due to Professor Earl D. Hanson, Professor Joseph Frankel and Doctor Sc. Maria Jerka-Dziadosz for helpful corrections and critical reading of this manuscript.

Summary

The effects of surgical injuries of the oral apparatus and of the boundaries of the ventral surface of *Chilodonella cucullulus* were studied during regeneration of the viable, nucleated fragments.

Studies were performed on two stocks of *Chilodonella cucullulus* namely: B_1 and X which differs in a number and a disposition of contractile vacuole pores. Analysis concern only transversal sections of specimens. Therefore all generalization concerning the shape and pattern regulation after injury are restricted to the transversal regenerants.

The attention was focussed on the process of restoration of kinetome after incisions made in various part of the cell body and on the process of growth and pattern formation after removal of some portion of the cell body.

The results suggest that the amputation of a part of the ventral surface including the organelles as the cytoproct and the contractile vacuoles does not per se elicit a total morphogenesis if the oral apparatus is left intact. However, changing of the boundaries of the ventral surface can shift the zone of the maximum growth of the cell cortex.

The amputation of the oral apparatus brings about total regeneration. It is only during morphogenesis with concomitant stomatogenesis when the whole set of organelles is restored. This process is accompanied with a sequence of changes in macronucleus.

In these kinds of fragments the stomatogenesis is always performed along the stomatogenic kineties present in a fragment. A new site of stomatogenesis and the boundaries of the ventral surface of the fragment are used in determination of a set of new CVP primordia in regenerants deprived of the oral apparatus.

It was found that the coordinates and reference points used in description of the spatial pattern of organellogenesis in dividers (Kaczanowska 1974) hold in the case of regenerants too.

STRESZCZENIE

Przeprowadzono badania nad regeneracją różnego typu jądrowych fragmentów dwóch szczepów B_1 i X orzęska *Chilodonella cucullulus*. Analiza dotyczyła jedynie fragmentów poprzecznych.

Stwierdzono, że uszkodzone kinety regenerowały *in situ*. Opisano przebieg tego procesu i stwierdzono, że nie jest on zsynchronizowany z określonymi przemianami w makronukleusie.

Amputacja takich organelli jak cytoproct i otwory wodniczek tętniących nie wywołała ogólnej reorganizacji fragmentu o ile tylko nie uszkodzony był aparat gębowy. Zmiany kształtu fragmentu, oraz zmiany kształtu powierzchni brzusznej po amputacji części komórki mogą powodować zmianę położenia strefy najbardziej intensywnego przyrostu powierzchni w okresie międzypodziałowym.

Uszkodzenie lub usunięcie aparatu gębowego *Ch. cucullulus* powoduje zupełną reorganizację. Tej całkowitej regeneracji towarzyszą określone przemiany w makronukleusie, resorpcja starego kompletu i powstanie nowego kompletu organelli komórkowych. Miejsce wytworzenia nowej gęby jest wyznaczone przez istniejący segment stomatogenny, oraz przez granicę powierzchni brzusznej fragmentu. W wyniku takiego pozycjonowania regenerant ma proporcjonalne ułożenie wszystkich zawiązków organelli.

Te same koordynaty i punkty odniesienia użyte w modelu pozycjonowania zawiązków organelli w podziałowcach, stosuje się również do opisu pozycjonowania zawiązków w regenerantach.

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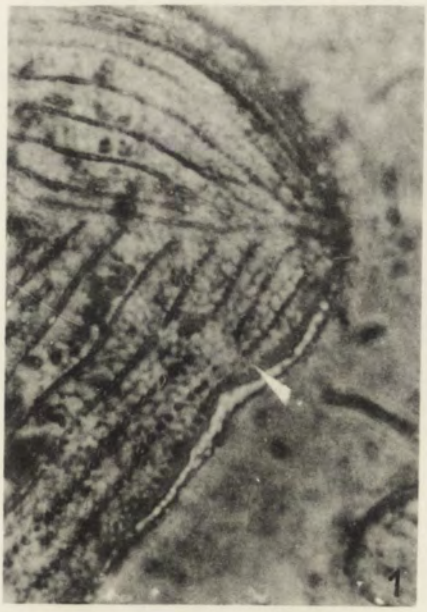
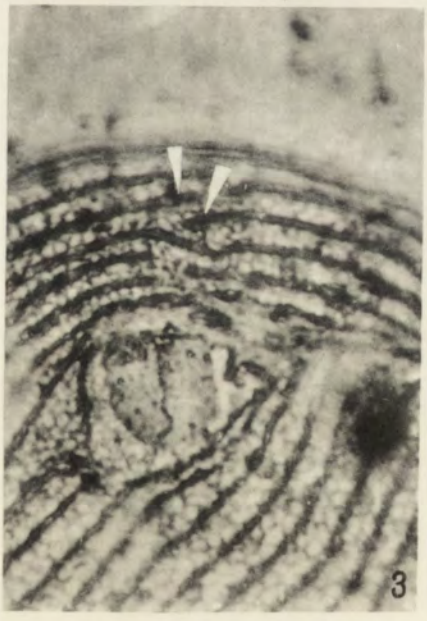
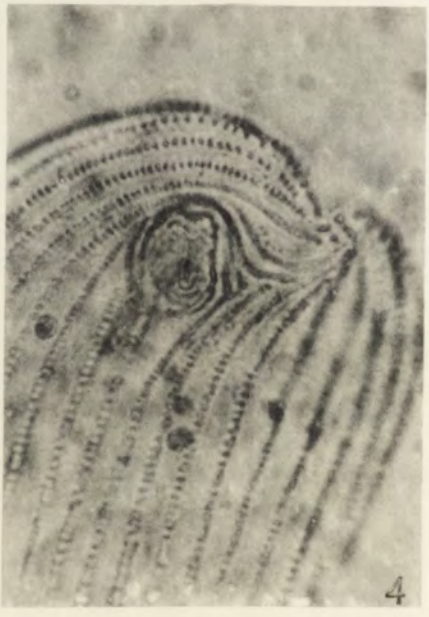
EXPLANATION OF PLATES I–IV

Specimens of *Chilodonella cucullulus* presented on photomicrographs 1, 2, 3 were stained with Klein's dry silver method, the remaining ones with the Chatton-Lwoff silver method. Photomicrographs 1–3 × 1950, phot. 3 × 1300 others × 650

1: Specimen of stock B_1 fixed 6 h after an incision (flesh). Rarefaction of kinetosomes in wounded region. Differentiated argentophilic network is visible
2: Specimen of stock B_1 fixed 12 h after transverse incision. Proliferation of kinetosomes in the region of meeting of the ends of the interrupted kinety (black arrow). Ends of broken kineties join at random (white arrow)
3: Specimen of stock B_1 fixed 18 h after the operation. The incision was made in preoral part of the ventral surface. Ends of one interrupted kinety (fleshes) fail to join, while the others join succesfully
4: Specimen of stock B_1 in which the preoral region of the ventral surface had been removed fixed 5 h after the operation. Regulation of the outline of the ventral surface and in situ growth of the cell cortex are marked by the rarefaction of kinetosomes
5: Specimen of stock X, deprived of the preoral part of the body, fixed 6 h after the operation. Abnormal direction of the oral slit indicates the allometry of the growth in postoral region
6: Specimen of stock B_1 deprived of the preoral part, including oral kineties, fixed 5 and one half hours after the operation. There is a strong allometric growth of the right portion of the ventral surface. No signs of morphogenesis are visible
7: Predivider of stock X, with the preoral part of the ventral surface amputated, fixed 5 h after the operation. Divisional morphogenesis is in progress despite the wound
8: Predivider (?) of stock B_1 deprived of oral apparatus and fixed 5 and one half hours after the operation. The posterior old CVP-3 (black arrow) is visible in front of the new primordium of CVP-3 (white arrow). This kind of topography was never observed in normal dividers
9: An early stage of morphogenesis in a regenerant of stock B_1 with the oral opparatus amputated. The specimen was fixed 5 and one half hours after the operation
10: An early stage of morphogenetic movements in a regenerant of stock B_1, fixed 6 h after the removal of the oral apparatus. Some of the anterior segments of the stomatogenic kineties do not belong to the newly formed ventral surface pattern of regenerant (flesh). It is supposed that they will be cut off during final shaping of the cell
11: Early morphogenesis in a regenerant of stock X deprived of the oral apparatus and some lateral kineties. The specimen was fixed 6 h after the operation. Stomatogenic kineties are the same as in the unoperated cell, but abnormalities in pattern of CVP primordia are clearly seen as due to the narrowing of the ventral surface
12: An early stage of morphogenesis in a regenerant of stock X deprived of the oral apparatus, fixed 5 and one half hours after the operation. This stage corresponds to phot. 9 of the B_1 stock cell
13: Early morphogenetic movement in the regenerant of stock X deprived of its oral apparatus and fixed 6 h after the operation. This stage corresponds to that presented in phot. 10 for the B_1 stock cell
14: A final shaping of the regenerant of stock X fixed 6 h after the removal of the oral apparatus. A new set of CVPs and the oral apparatus are differentiated. Preoral A-1 kinety has a snaky shape (arrow). The anterior portion of the cell had been pinched off. However, details of the anterior end are invisible on the picture since they are out of the focus
15: The regenerant of stock X after completion of morphogenesis fixed 6 h after the operation. The shape and pattern of organelles are normal, however, the preoral kinety still remains snaky
16: The amacronuclear fragment of stock B_1, fixed 6 h after the amputation of the oral apparatus. The old CVP-3 is clearly seen
17 and 18: present the ventral and dorsal surface of the same specimen of stock B_1. This is a short promer during early morphogenesis. The shape of the ventral surface is highly abnormal. The new set of CVP primordia (slits) corresponds to the pattern of a proter. The primordium of new cytoproct is visible on phot. 18 (arrow)







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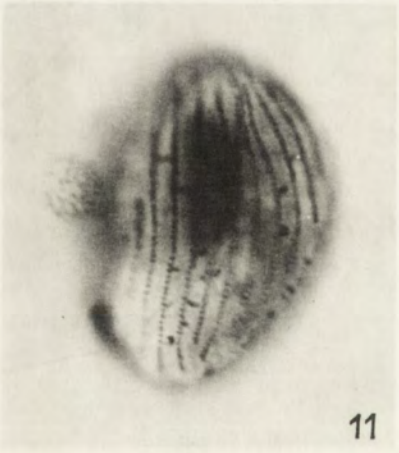

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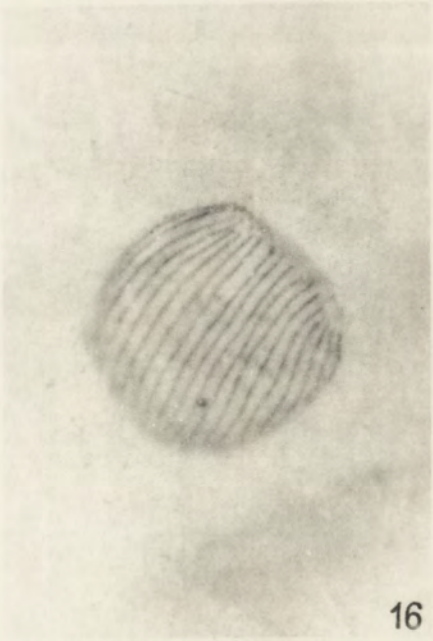

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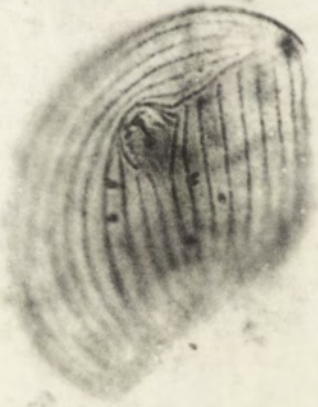

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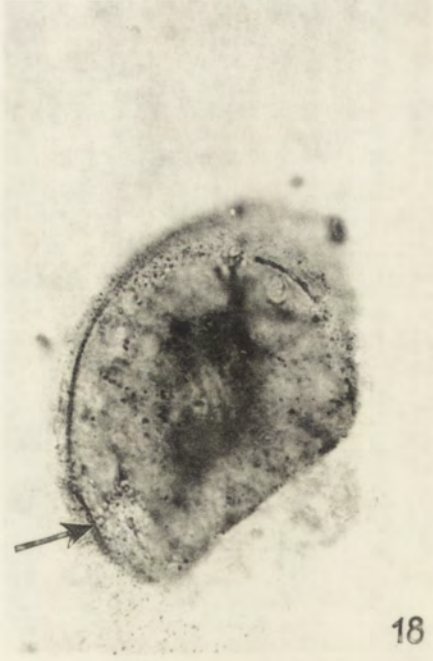

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VOL. X. XXIII WARSZAWA, 31.X.1975 FASC. 31

Institut Zoologique de l'Université Jagiellone, 30-063 Kraków, Oleandry 2, Pologne

Anna CZAPIK

Les observations sur *Trimyema compressum* Lackey (*Ciliata, Trichostomata*)

Obobserwacje nad *Trimyema compressum* Lackey (*Ciliata, Trichostomata*)

Le genre *Trimyema* a été créé par Lackey (1925) qui a nommé *Trimyema compnpressum* un petit cilié trouvé dans les bassins d'épuration d'eaux résiduelles. Presqsque en même temps (1926) Kahl a trouvé des ciliés pareils dans les eaux x douces ainsi que salées, riches en matière organique et les a décrits sous ls le nom *Sciadostoma*, en distinguant deux espèces: *S. difficile* et *S. marinum*. Plus s ttard il a réalisé que *Sciadostoma difficile* est identique avec *Trimyema compnpressum* Lackey et dans le supplément de sa grande monographie des ciliés és il a restitué au cilié son nom original. Tous les deux auteurs ont décritrit la morphologie des espèces citées en s'appuyant sur les observations in vivivo. C'était Klein (1930) qui pour la première fois dans son étude sur le sysystème argentophil des ciliés a décrit *Trimyema compressum* en tenant compmpte des préparations imprégnées à l'argent d'après sa propre méthode. Le c côté faible de cette méthode appellée "sèche" est qu'elle entraine les déforormations de la forme du corps.

F Fauré-Fremiet (1962) a trouvé une abondante population de *Trimyema marirrinum* aux environs de Concarneau dans une cuvette rocheuse remplie d'eauau marine sursalée et riche en matière organique pourissante; il a redécrit cette te espèce en s'appuyant sur les préparations faites d'après la méthode de C Chatton.

J Jankowski (1964) cite l'espèce *Trimyema compressum* parmi les autres ciliés és saprobiotiques et décrit sa morphologie en s'appuyant sur les préparatioions faites d'après la méthode sèche.

Materiel et méthodes

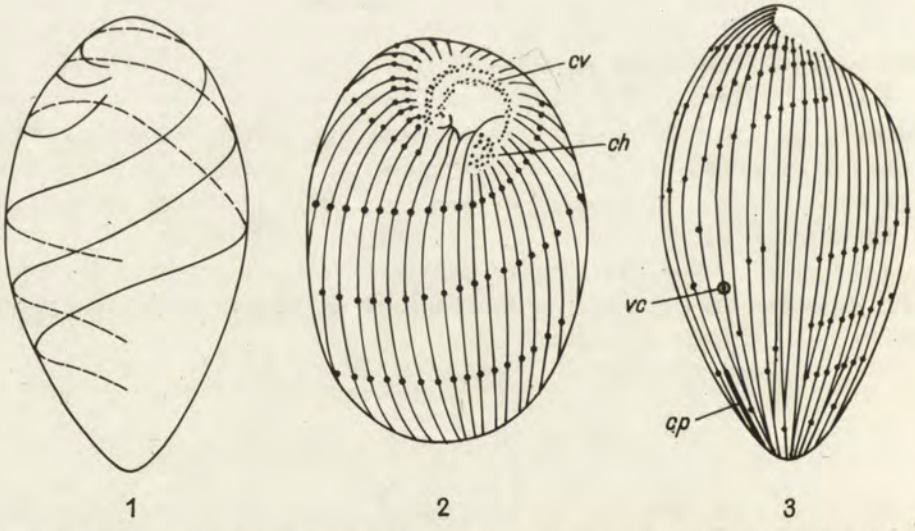
J' J'ai trouve espèce *Trimyema compressum* Lackey en examinant les prélevements d'une petite te rivière Prądnik (Parc National d'Ojców prés de Cracow). Ces ciliés vivaient aux endroroits à courant lent où il y avait une couche de vase riche en débris organiques. Ils étaientent commun mais pas nombreux. Pour obtenis un nombre plus grand d'individus j'ai mis

un peu de la vase dans un petit aquarium et j'ai y jeté quelques grains du riz, que je remplaçais au fur et à mesure qu'ils se décomposaient. Dans cette culture les ciliés se sont multipliés très vite; la culture existe depuis un an et elle prospère toujours. Les prèparations faites d'après la méthode de Chatton modifiée par Corliss donnent un image très clair de la morphologie de cette espèce.

Morphologie et biologie

La forme du corps de *Trimyema compressum* ressemble en général à celle de *T. marinum* avec cette différence que le pôle posterieur est ici conique, non arrondi comme chez l'autre espèce. Quand on observe ce petit cilié (40 μm en moyenne), pendant qu'il nage librement, son corps semble être rigide, mais quand écrasé par la lamelle il rampe parmi les algues on voit qu'il est doué d'une certaine métabolie. Il ne forme aucune sorte de kystes; les individus affamés meurent sans s'enkyster. La bipartition se passe aussi à l'état actif.

Sur la partie apicale il y a le vestibule au fond duquel se trouve le cytostome. La ciliature est fort reduite et limitée à deux cinéties entourant la partie apicale et trois ceintures parallèles des cils qui commençant à côté droit de la bouche contournent le corps, en suivant une ligne helicoïde (Fig. 1, Pl. I 1). Les cinéties apicales, composées de cinétosomes rangés d'une façon irregulière après avoir contourné le sommet du corps entrent dans le vestibule où ils forment un champ ciliare (Fig. 2, Pl. I 3). Les cinéto-



Figs 1–3. *Trimyema compressum* Lackey. 1 – La scnema representant la disposition des ceintures des cils, 2 – La region ventro-apicale, cv – cinéties vestibulaires, ch – champ ciliare, 3 – Le côté droit de l'animal, vc – porus de la vacuole contractile, cp – cytopyge

somes de trois ceintures des ciliés sont rangés sur 52 fines fibrilles meridiennes qui parcourent le long du corps et tout près du pôle postérieur deviennent irrégulières en formant un réseau. Au-dessous de la dernière ceinture des cils quelques cinétosomes isolés, situés irrégulièrement sur des fibrilles meridiennes sont dispersés. Le cytopyge situé sur le côté latero-dorsal droit du corps est visible comme une ligne droite, assez épaisse; le porus de la vacuole contractile se trouve à côté de lui (Fig. 3). Sur le pôle postérieur il y a un long cil caudal.

Discussion

A cause de la ciliature assez spéciale de *Trimyema* Kahl (1926) a créé pour ce genre une famille à part dans l'ordre *Trichostomata*. Fauré-Fremiet (1962) accentue la différenciation de deux cinéties vestibulaires qui manquent chez les autres *Trichostomes*. D'après Jankowski (1964) la partie apicale serait entourée par 4 cinéties. Malheureusement l'auteur ne donne aucune photo confirmant cette constatation, seulement un dessin. Fauré-Fremiet remarque, que la famille *Trimyemidae* présente une des nombreuses possibilités existantes dans cette ordre, d'utiliser les extrémités antérieures des cinéties somatiques pour constituer la ciliature vestibulaire. Corliss (1956) a remarqué aussi cette diversité et en a conclu l'origine polyphyletique des *Trichostomes*.

Tous les chercheurs cités qui ont étudié les représentants du genre *Trimyema* les trouvaient dans les eaux riches en matière organique en décomposition et pleines de bactéries dont ces ciliés se nourissent. Liebmann (1951) inclut *Trimyema compressum* à son système des saprobiontes comme un organisme typique de la zone polysaprobe. Il le considère comme anaërobe facultatif, qui peut vivre en présence de H_2S (bien qu'il ne soit pas lié à ce gaz) accompagné par des espèces caracteristiques du sapropel (par exemple *Metopus* et *Caenomorpha*). Mes propres observations s'accordent avec ces donnés: j'ai trouvé *Trimyema compressum* dans la vase riche en matière organique mais sans H_2S. Dans la culture sur les grains du riz pourissant ce gaz a apparu bientôt c'est qui était mis en évidence non seulement par l'odeur mais par la présence de tels organismes que les bactéries sulfuriques du groupe *Beggiatoa* ainsi que les ciliès du genre *Metopus*. Dans ce milieu changé *Trimyema compressum* continuait à vivre et à se multiplier.

Resumé

Le genre *Trimyema* se distingue parmi les autres ciliés trichostomes par sa ciliature reduite, limitée à trois ceintures des cils et par la présence de deux cinéties vestibulaires décrites pour la première fois par Fauré-Fremiet

chez *Trimyema marinum*. L'espèce *Trimyema compressum* vit dans les eaux riches en matière organique et contenant beaucoup de bactéries. Cette espèce ne forme aucune sorte de kystes. La bipartition se passe aussi à l'état actif.

STRESZCZENIE

Rodzaj *Trimyema* wyróżnia się wśród rzędu *Trichostomata* zredukowanym orzęsieniem, ograniczającym się do trzech pasów rzęsek oraz obecnością dwóch kinet przedsionkowych opisanych po raz pierwszy przez Fauré-Fremiet u gatunku *T. marinum*. Gatunek *Trimyema compressum* żyje w wodach bogatych w materię organiczną i zawierających dużo bakterii. Gatunek ten nie tworzy żadnych cyst, podział odbywa się w stanie aktywnym.

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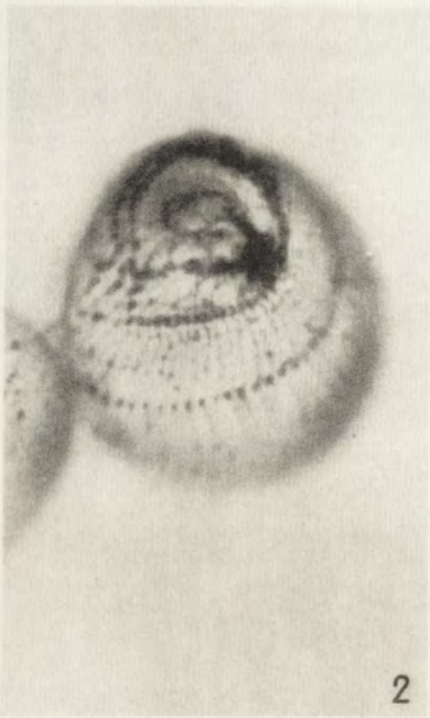
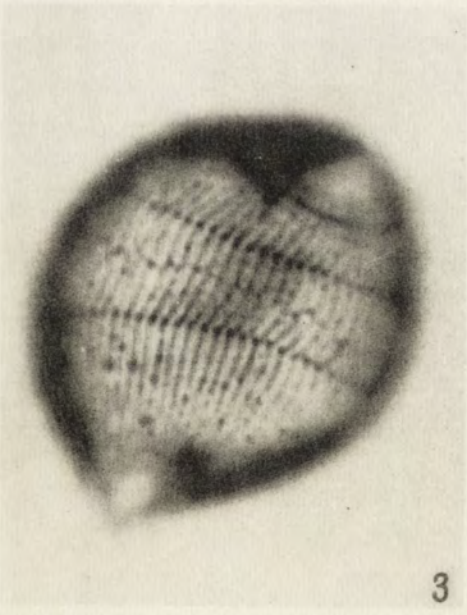
Received 2 February 1974

EXPLICATION DE PLANCHE I

Trimyema compressum Lackey

1: la vue général du corps montrant la disposition des ceintures des cils (préparation imprégnée à l'argent)
2: la vue de la region apicale montrant deux cinéties vestibulaires
3: le côte gauche de l'animal
4: le côté droit de l'animal montrant le cytopyge, le porus de la vacuole contractile ainsi que les bouts des ceintures des cils





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ACTA PROTOZOOLOGICA
VOL. XIII WARSZAWA, 31.X.1975 FASC. 32

Institute of Cytology of the Academy of Sciences of the USSR, Leningrad F-121 Prospekt Maklina 32, USSR

I. G. BEREZINA

An Electron Microscope Study of Endosymbionts in *Blepharisma japonicum*

Электронно-микроскопическое исследование эндосимбионтов *Blepharisma japonicum*

A great number of various symbionts have been described in *Ciliata* (Fauré-Fremiet et al. 1964, Ball 1969). They are found to infect the macronucleus, micronucleus, or cytoplasm. In many cases the nature of the host-symbiotic interaction has not been definitely settled. It is usually supposed that the possibility of the existence of one or another symbiont in an eukaryote cell cell is mainly dependent on the host cell genotype (Jenkins 1970). The host-symbiotic interaction has been studied in detail in the genus *Paramecium* (Preer and Jurand 1968, Weis 1968, Beale et al. 1969, Jenkins 1970, Stevenson 1970, 1972, Ossipov and Ivakhnyuk 1972, Preer et al. 1972).

This paper contains the results of an electron microscopic study on endosymbionts discovered in *Blepharisma japonicum* (Suzuki). One of the most interesting characters in ciliates of this genus is its pigmentation. When exposed to light, the pigment subjects the cell to a lethal photooxidation. A number of organisms (for example, *Stentor coeruleus*, some species of bacteria) contain pigments that respond to light. But in all these cases the cells are not injured during this process (Giese 1973).

Materials and Methods

Blepharisma japonicum has been received from the Zoological laboratory of the University of Clermond-Ferrand (France). The cells were cultivated in total darkness at 17° C in the lettuce medium; *Aerobacter aerogenes* was used as food (Berezina 1969). Parts of the ciliates were exposed to the artificial light of a luminescent lamp (40 w). After 36 h of the exposure a considerable loss of the blepharisma pigment was observed. Six days after the onset of illumination colorless cells were killed.

Organisms prepared for electron microscopy were fixed with (1) 1% osmium tetrooxide buffered at pH 7.4 with 0.1 M cacodilate buffer for 40 min at 23° C, or (2) a mixture of glutaraldehyde and formaldehyde in 0.1 M cacodilate buffer at pH 7.4 (Karnovsky 1965) for 40 min at 20° C followed by 1% osmium tetrooxide in the same buffer for 30 min at 20° C. The material was dehydrated in an ethanol/water series, placed in propylene oxide and

embedded in Epon 812. Sections were cut on an LKB ultramicrotome with glass knives, stained in a saturated aques solution of uranyl acetate for 4 h, post-stained in lead citrate and examined with a JEM – 5 g electron microscope.

Results and Discussion

Electron microscopic examination of dark red *Blepharisma japonicum* has revealed the presence of symbionts within the cytoplasm, but none in the macronucleus or in the micronucleus. All symbionts had a rod-shaped form and were 0.7–1.4 μm in length (Pl. I 1). The symbionts were surrounded by two membranes resembling their outward appearance a gram-negative bacteria. The central area of the symbiont cell was as a rule occupied by a clearly delimited nucleoid, such as is commonly found in free-living bacteria. The symbiont's cytoplasm was crowded with ribosomes (Pl. I 1). In the red blepharisma cytoplasm the symbionts occurred very seldom. The symbiotic division forms were never found.

If a deeply pigmented blepharisma is bleached by the light, two morphologically different symbiotic forms constantly appear in its cytoplasm (Pl. I 2). Most of symbionts of the bleached ciliates are rod-shaped and are surrounded by two membranes. Their ultrastructural features are very similar to that formerly described for bacteria from red ciliates. The rod-shaped bacteria of the light bleached blepharisma have also clearly delimited nucleoid and ribosomes (Pl. I 2, II 7, 8). The bleached ciliates, however, contain much more symbionts that the red ones, many of the symbionts are found during the binary fission (Pl. I 4, II 7).

The second symbiotic form which has been revealed in the bleached blepharisma is oval bacteria averaging 0.9–1.6 μm in diameter (Pl. II 5, 6). They are less frequent at the section than the rod-shaped bacteria. The oval symbiont like a rod-shaped ones is bounded by two membranes and contains a nucleus and ribosomes (Pl. II, 5, 8). The main characteristic of the oval forms is the presence of numerous spherical virus-like particles approximately 30–50 nm in diameter in their cytoplasm (Pl. I 3, II 5, 6). These particles are localized under the cytoplasmic membrane of the oval bacterium. As a rule the virus-like elements are closely arranged forming a paracrystal cluster (Pl. II 6). The quantity of "viruses" in a given plane of the microscopic section varies between several particles and two hundred. The oval symbionts bearing virus-like particles are also capable of dividing like the rod-shaped bacteria (Pl. II 8).

Various morphological transitions exist between two main morphological forms of the symbionts above described in the bleached blepharismas. Not unfrequently slightly rounded rod-shaped bacteria occur in the bleached blepharisma cytoplasm (Pl. I 4). They are also bounded by two membranes,

contain a clearly delimited nucleoid and several virus-like particles. The size of this symbionts ranges from 0.9 μm to 1.5 μm in length. In view of the pronounced resemblance between different morphological symbiotic form of the same cell (óval rod-shaped and transitional forms) in their size and ultrastructure it can be concluded that we are dealing with a species of the intracellular bacteria. The appearance of the oval symbionts bearing virus-like elements in the light bleached ciliates probably may explain the fact that the rod-shaped symbionts are lysogenic bacteria, capable of forming viruses at the photodynamic action on *B. japonicum*.

The formation of phage-like particles in the ciliate symbiotic bacteria have been studied in most detail on kappa – a bacterial endosymbiont of *Paramecium aurelia* (Preer et al. 1971, 1972). In kappas the appearance of phage-like elements is connected as a rule with the formation of R-body and with the loss of reproducibility. In contrast to the kappas the oval symbionts of *B. japonicum*, containing virus-like elements have a clearly delimited nucleoid and are capable of divisions at least in the beginning of the phage formation. The subsequent development of the oval bacteria and containing in them virus material is not known. Whether the blepharisma symbionts and their virus-like particles are infective or not also is not clear.

Within the red and light bleached blepharisma cytoplasm the symbionts always lie free and are never enclosed in membrane-bounded vacuoles (Pl. I 1, 2, II 7). The buck symbionts have been found within the endoplasm (Pl. I 1). Sometimes they lie next to the macronucleus (Pl. I 3). As the exposure of cells to a light proceeds (from 2 to 6 days) the number of symbionts on the cell section sharply is increasing and groups of bacteria at different developmental stages come into view in the section (Pl. I 4, II 8). Many bleached blepharisma symbionts were found during binary fission (Pl. I 4, II 7, 8). No distinctive changes in the ultrastructure of the macronucleus, micronucleus, cytoplasm and mitochondria indicating pathological alterations in the light-bleached blepharisma have been discovered. Only partial shedding of pigment from the subpellicular granules have been observed.

On the basis of the above data it is difficult to explain the reason why an exposure to light evokes both the increase of the symbiotic number and formation of virus-like elements in symbiotic bacteria. It may be only suggested that the observed alterations in the number of the symbionts and in their ultra-structure are induced by the photodynamic effect in the host eukaryotic cell as a result of injury of some cell protective devices, which had repressed early the bacterial reproduction. On the other hand it is not unlikely that the prolonged exposure of blepharisma to a light immediately affects the rod-shaped symbionts, influences bacterial division rates and evokes formation of virus material in bacteria. The existence of close correlation between infusorian cultivating conditions and appearance of virus-

like structures in the bacterial symbionts (kappas) have been reported for *Paramecium aurelia* (Stevenson 1972).

In *Blepharisma* the light bleached effect consists in extraction of red pigment from subpellicular granules (Inaba et al. 1958, Kennedy 1965, 1966). According to Giese (1973) the pigment might well screen a cell a certain against UV radiation of sunlight. In view of all this it may be supposed that the above alterations in the symbiotic ultrastructural form and number caused by exposure of a cell to a light are associated with pigment shedding which is one of the most important protective devices of *Blepharisma* during its exposure to light.

Summary

Rod-shaped bacterial symbionts have been revealed electron microscopically within the cytoplasm of dark red *Blepharisma japonicum*. These symbionts are bounded by two membranes, contain a clearly delimited nucleoid and ribosomes.

In blepharisma exposed to a light for several days two different morphological forms of bacterial symbionts (rod-shaped and oval) have been observed. The ultrastructure of rod-shaped bacteria from bleached ciliates resembles in its general appearance that of rod-shaped bacteria from red blepharisma. An oval bacterial symbiont from a ciliate which has been exposed to light is surrounded by two membranes, and contains a nucleoid with virus-like particles. Various morphologically transitional forms between rod-shaped and oval bacteria are seen within the bleached blepharisma cytoplasm.

It is supposed that the described symbiotic forms of *B. japonicum* represent different developmental stages of one and the same species of Bacteria. The appearance of the oval bacterial symbionts bearing virus-like particles seems to be connected with the photodynamic action on the blepharisma cell.

РЕЗЮМЕ

В цитоплазме красной инфузории *Blepharisma japonicum* с помощью электронного микроскопа были обнаружены палочковидные симбиотические бактерии. Обнаруженные эндосимбионты ограничены двумя мембранами, содержат четко различаемый нуклеоид и рибосомы.

При обесцвечивании блефаризм светом в течение нескольких дней в их цитоплазме, наряду с палочковидными симбионтами, появляются овальные симбиотические бактерии Характерной особенностью овальных бактерий является присутствие в их цитоплазме

большого количества вирусоподобных грнаул. Одновременно с овальными и палочковидными симбионтами на срезах обесцвеченных инфузорий изредка встречаются слегка округливщиеся палочковидные бактерии, содержащие несколько вирусоподобных частиц.

Предполагается, что обнаруженные в цитоплазме обесцвеченных инфузорий различные морфологические формы симбионтов являются разными стадиями развития одного вида бактерии. Появление овальных симбионтов, содержащих вирусоподобные гранулы, по-видимому, связано с длительным воздействием света на клетку блефаризмы.

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Received 2 January 1974

EXPLANATION OF PLATES I–II

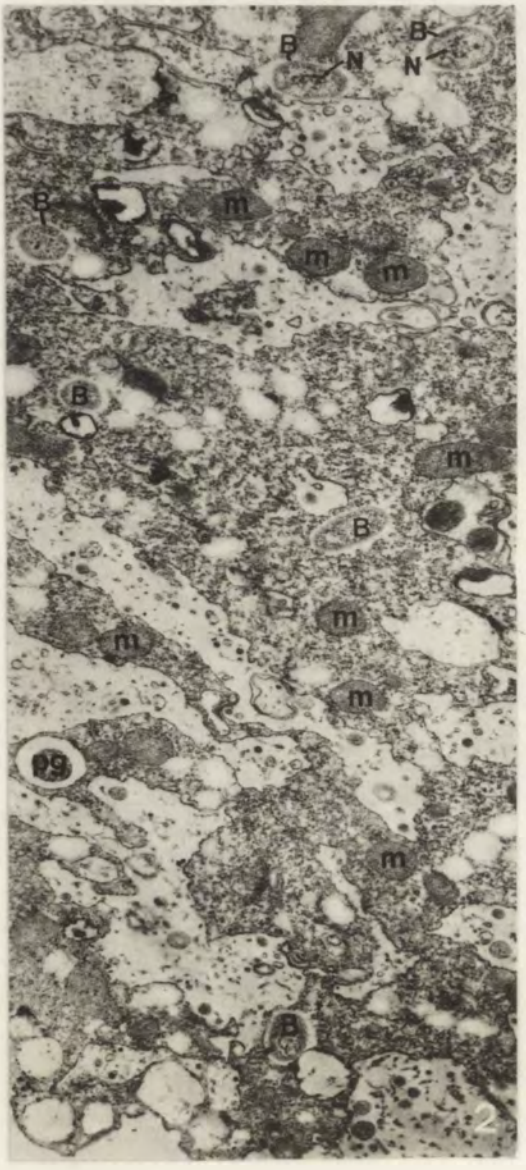
Electron micrograph of section of red *Blepharisma japonicum*

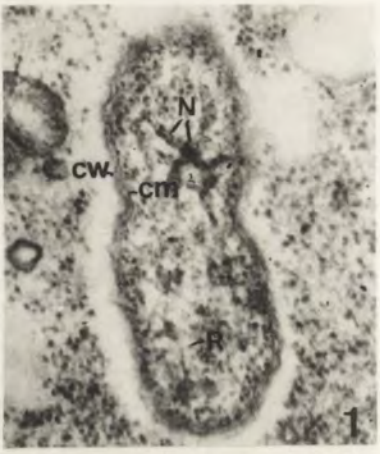
1: The rod-shaped symbiont showing a sectioned nucleoid and ribosomes (41000 ×)

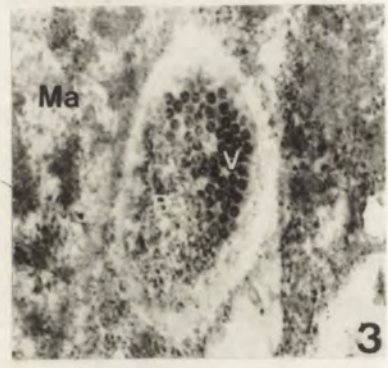
Electron micrographs of sections of light bleached *Blepharisma japonicum*

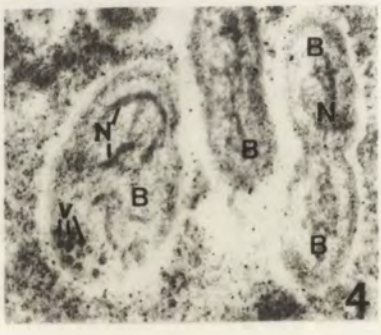
2: A longitudinal section of a bleached blepharisma showing rod-shaped bacteria, within a cytoplasm (3600 ×)
3: A section through the oval bacterium, bearing virus-like particles. The bacterial symbiont lies next to the macronucleus (36,000 ×)
4: A section through two rod-shaped bacteria and through a morphologically transitional bacterial form, containing several virus-like particles (37,000 ×)
5: An oval bacterium showing a sectioned nucleoid, virus-like material and ribosomes (40,000 ×)
6: A section through the oval bacterium containing virus-like elements (35,000 ×)
7: A section through the dividing rod-shaped bacterium (40,000 ×)
8: A dividing oval bacterium showing a sectioned nucleoid, virus-like particles and ribosomes. Several rod-shaped bacteria lie next to the oval symbiont (34,000 ×)

Abbreviations used: B – bacterium, cm – cytoplasmic membrane, cw – cytoplasmic wall, Ma – macronucleus, m – mitochondrium, N – nucleoid, pg – pigment granule, R – ribosome, v – virus-like particles.









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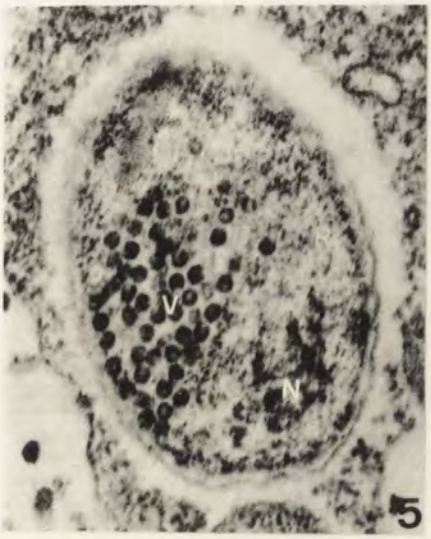

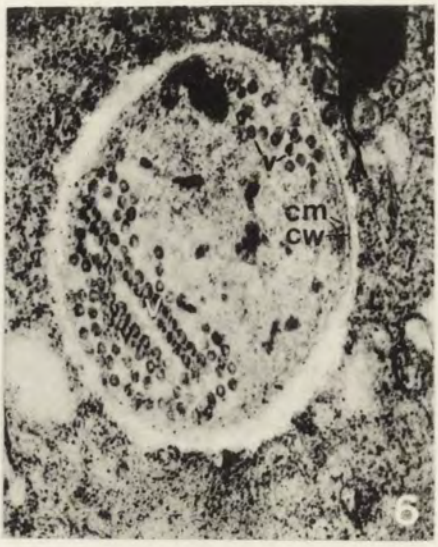

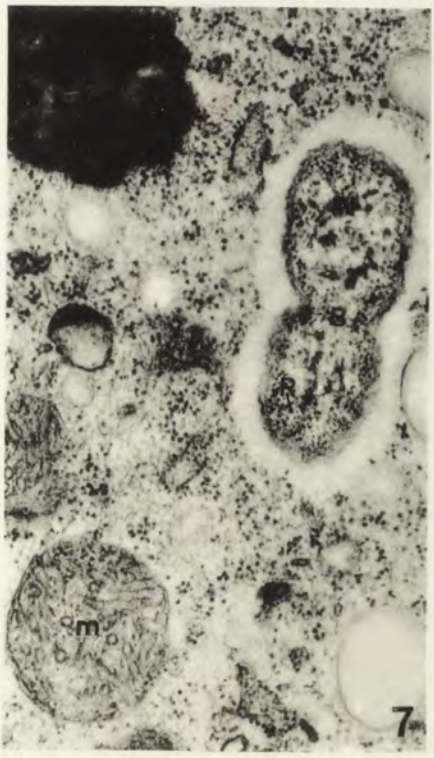

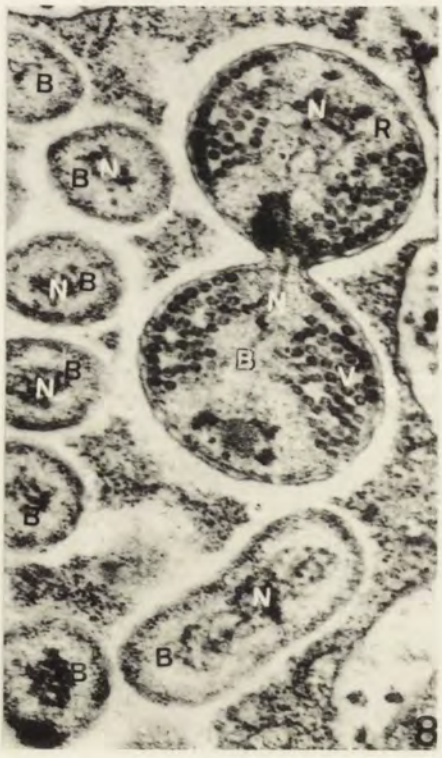

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ACTA PROTOZOOLOGICA

VOL. XIII WARSZAWA, 31. X. 1975 FASC. 33

Всесоюзный научно-исследовательский институт по болезням птиц. Ленинград 196006, Московский проспект, 99
All-Union Institut of Poultry Diseases, Leningrad 196006, Moscow Prospekt 99, USSR

Т. А. ШИБАЛОВА, Т. И. МОРОЗОВА и В. А. КРАВЦОВ
T. A. SHIBALOVA, T. I. MOROZOVA and V. A. KRAVTSOV

О соотношении роптрий и микронем у *Eimeria tenella* *(Sporozoa, Coccidia)*

Eimeria tenella Microneme-Rhoptry Relationship

Для спорозоита и мерозоита различных споровиков характерно наличие комплекса специфических органелл — роптрий и микронем, которые заполняют всю переднюю половину тела паразита этих стадий, частично простираясь до заднего конца. Изучению этих структур посвящено много работ. Однако до сих пор ещё остается неясным гомологичны ли роптрии и микронемы, едина ли их функция и какова эта функция, стабильна ли ультратонкая организация этих структур на разных фазах жизненного цикла паразита и при разных условиях его обитания, в частности, при развитии *in vivo* и *in vitro*, могут ли эти структуры быть использованы в качестве критерия для внутривидовой классификации.

Некоторые авторы (Aikawa 1966, Scholtyseck and Mehlhorn 1970), основываясь на том факте, что эти структуры исчезают у внедрившегося в клетку паразита, высказали предположение об их роли в процессе проникновения. Это предположение тем более вероятно, что в роптриях *Toxoplasma gondii* (Norrby and Lycke 1967, Lycke 1973), и *Selenidium* (Schrevel 1968) были найдены протеолитические ферменты, которые, возможно, растворяют мембрану клетки хозяина при внедрении паразита.

Большинство авторов указывают на очень тесную связь микронем и роптрий, при этом, однако, считают их разными структурами, называя мелкие из них микронемами, а крупные — роптриями. У разных видов *Sporozoa* подсчитано определенное количество роптрий и микронем.

Целью настоящей работы было изучение соотношения роптрий и микронем на разных фазах развития спорозоита и мерозоита *E. tenella in vitro* и в культуре ткани.

Материал и методы

В нашем материале комплекс роптрии-микронемы был исследован на некоторых фазах развития спорозоита и мерозоита *Eimeria tenella*: спорозоит в спороцисте, эксцистированный (свободный) спорозоит, спорозоит после внедрения в клетку хозяина, мерозоит, формирующийся в шизонте, внеклеточный (свободный) мерозоит и внутриклеточный мерозоит.

В опытах использовали местный чистый штамм № 1 *E. tenella* 14-го пассажа.

Техника эксцистирования спорозоитов *in vitro* и культивирования *E. tenella* в культуре ткани подробно описаны в предыдущих сообщениях (Шибалова 1968, 1969, 1973).

При изготовлении блоков для электронной микроскопии суспензию, содержащую эксцистированные спорозоиты с примесью спороцист и ооцист, центрифугировали при 600–1000 об/мин для получения осадка. Также готовили осадок внеклеточных мерозоитов из культуральной жидкости, взятой на 4–5 день после заражения спорозоитами культуры куриных эмбриональных клеток. Для изучения внутриклеточных спорозоитов, мерозоитов и шизонтов препараты для электронной микроскопии готовили из зараженных спорозоитами культур клеток куриного эмбриона через 1–24 ч после инокуляции их.

Клетки фиксировали 3–5% глутаральдегидом на какодилатном буфере с последующей обработкой в 1–2% растворе тетраокиси осмия в том же буфере, pH 7.2–7.4. Обезвоживали в спиртах возрастающей крепости и абсолютном ацетоне и заливали в эпон 812. Перед обезвоживанием клетки контрастировали водным раствором уранилацетата. Срезы готовили на ультрамикротоме УМТП-2, затем производили дополнительное контрастирование цитратом свинца. Просматривали срезы на электронном микроскопе УЭМВ-100 К производства СССР.

Результаты исследований

Спорозоит в спороцисте (Табл. 1) и свободный (эксцистированный) *in vitro* спорозоит (Табл. I 2–5, II 6–10)

На ультратонких срезах спорозоитов, находящихся в спороцисте, и свободных спорозоитов роптрии и микронемы выявляются в виде многочисленных отрезков разного сечения: округлых, овальных, эллипсовидных. Даже на продольных срезах спорозоитов редко удается получить срезанные по длине фрагменты роптрий и микронем. По-видимому, они очень извилисты. Все они покрыты элементарной мембраной (Табл. I 2–5). Создается впечатление, что все элементы этого комплекса содержат одинаковое вещество, которое морфологически выявляется как гомогенное или мелкозернистое, реже альвеолярное, имеющее высокую электронную плотность. Иногда внутри роптрий видны участки (Табл. I 2) или пузырьки (Табл. II 7) более низкой электронной плотности.

На поперечных срезах через апикальный конец спорозоитов внутри коноида выявляется небольшое число срезанных элементов комплекса роптрии-микронемы. На серийных срезах (Табл. I 3–5) можно видеть, что к вершине коноида их количество уменьшается и в самой верхней части его иногда выявляется выход лишь одной ветви (Табл. II 6). Это свидетельствует о том,

что при прохождении через коноид элементы комплекса роптрии-микронемы сливаются. На некоторых продольных срезах через коноид такое слияние различимо (Табл. II 7). Часть элементов, выявляемых в коноиде, содержат гомогенное электроннoплотное вещество, другие пусты (электроннопрозрачны) (Табл. I 3–5). Их диаметр в этом участке не одинаков — от 30 до 60 nm.

Ниже коноида количество срезов микронем и роптрий сильно увеличивается. На поперечных срезах таких участков (Табл. I 1, II 8, 9) видны многочисленные округлые или овальные элементы разного диаметра (от 50 до 200 nm). На продольных срезах (Табл. II 7–10) видно, что такие вариации диаметра обусловлены прежде всего тем, что некоторые ветви сужены в верхней части (60–90 nm) и расширены в нижней (100–250 nm).

Учитывая, что на поперечных срезах наряду с самыми мелкими элементами — микронемами (50–60 nm) и самыми крупными — роптриями (150–250 nm) выявляются элементы среднего диаметра, становится трудным производить дифференциацию и подсчет микронем или роптрий. С другой стороны, на продольных срезах расширенные колбовидные или ампуловидные ветви можно считать роптриями, но на основании продольных срезов невозможно подсчитать количество их у одного паразита.

При анализе большого количества срезов спорозоитов в разных плоскостях не удалось проследить какую-либо закономерность в количественном соотношении мелких (микронем) и крупных (роптрий) элементов этого комплекса. Так, (Табл. II 7) на продольном срезе свободного спорозоита различимы четыре расширенные ветви, на поперечном срезе — восемь (Табл. II 9). В других срезах можно было видеть одну, две, три более широкие ветви. У спорозоитов в спороцисте выявлялись 3–5 более расширечных элементов (Табл. I 1).

Внутриклеточный спорозоит (Табл. II 11–12)

У внедрившихся в клетки культуры ткани спорозоитов было отмечено увеличение количества расширенных элементов комплекса роптрий-микронем. На Табл. II 11 у внутриклеточного спорозоита отчетливо различимы девять продольно срезанных расширенных ветвей, морфологически сходных с тем, что описывают как роптрии. Они имеют ампуловидную форму: более широкие в нижней части и сужены по направлению к коноиду спорозоита. На поперечном срезе внутриклеточного спорозоита (Табл. II 12) обращают на себя внимание вариации диаметра элементов комплекса роптрии-микронемы — от 50 до 200 nm. Многочисленны расширенные ветви.

В процессе дальнейшего развития внутриклеточного спорозоита, как известно, происходит дезинтеграция структур комплекса роптрии-микронемы. Морфологически удавалось установить уменьшение количества этих структур и полное их исчезновение.

Мерозоиты, формирующиеся в шизонте (Табл. II 12)

На срезах мерозоитов в ранней стадии формирования их в шизонте вблизи коноида выявляется округлое осмиофильное тело 700 nm в диаметре, окруженное элементарной мембраной (Табл. III 13). Это зачаток будущего комплекса роптрий и микронем.

В процессе формирования мерозоитов происходит последовательное расщепление этой структуры на отдельные ветви, как это было показано у *E. stiedae* (Heller and Scholtyseck 1971, Heller 1972). Этот процесс протекает очень быстро: к моменту отделения мерозоита от остаточного тела шизонта роптрии и микронемы видны уже в виде многочисленных ветвей. При этом расширенные элементы обнаруживаются в небольшом количестве (один-два элемента).

Внеклеточный (свободный) мерозоит (Табл. III 14–15)

Мелкие элементы очень многочисленны, извилисты. На срезах они выявляются в виде округлых или эллипсовидных отрезков приблизительно одинакового диаметра (около 60 nm Табл. III 14–15). Среди них обнаруживались один-два-три расширенных элемента. На Табл. III 15 показан срез свободного мерозоита непосредственно под коноидом. Об этом свидетельствует диаметр мерозоита и радиальное расположение микротрубочек. Среди поперечно и касательно срезанных одинаковых по ширине элементов (диаметр 60 nm) виден один расширенный, имеющий диаметр 240 nm. Можно было бы предположить, что элемент с большим диаметром это роптрия, а все остальные — микронемы. Однако, учитывая сужение роптрии по направлению к коноиду (сравните с Табл. II 7) становится очевидным, что в этом случае мы не можем мелкие элементы дифференцировать как микронемы. Возможно, что это срезы суженных участков роптрий.

Привлекает внимание скопление рибосом у поверхности элементов комплекса роптрий и микронем.

Внутриклеточный мерозоит (Табл. III 16)

У внедрившихся в клетки культуры ткани мерозоитов так же, как и у внутриклеточных спорозоитов, наблюдается увеличение количества расширенных элементов комплекса роптрии-микронемы (Табл. III 16). Максимальный диаметр расширенных элементов достигал 300 nm.

Обсуждение

Роптрии и микронемы были описаны уже в первых исследованиях по ультраструктуре споровиков (Gustafson et al. 1954, Garnham et al. 1960). С накоплением новых данных и углублением исследований по этим струк-

турам у разных видов *Sporozoa* постепенно изменялись представления об их структурной организации и возможной функции. Изменились и их названия: токсонемы стали называть микронемами, парные органеллы — роптриями.

Шолтизек и Мельхорн (Scholtyseck and Mehlhorn 1970) на основании изучения разных видов *Eimeria* создали структурную модель взаимоотношения роптрий и микронем, полагая, что микронемы секретируют ферменты, которые выводятся наружу роптриями. Однако Хеллер (Heller 1972), последовательно проследив процесс формирования роптрий и микронем у мерозоитов *E. stiedae* предложил новую модель структур этого комплекса, значительно отличающуюся от предыдущей. По данным Хеллера роптрии и микронемы — это части одной очень разветвленной органеллы. При этом среди множества микронем у мерозоитов *E. stiedae* им было найдено лишь две роптрии.

Большинство авторов указывают на наличие определенного количества роптрий у разных представителей класса *Sporozoa* (см. обзор Scholtyseck and Mehlhorn 1970, Scholtyseck et al. 1973) придавая этому количеству значение систематического признака.

Наши сравнительные исследования роптрий и микронем на разных стадиях развития спорозоита и мерозоита у *E. tenella* показали, что план построения этих структур сходен с таковым у *E. stiedae* (Heller 1972) Это — одна сильно разветвленная органелла, ветви которой имеют разный диаметр. Однако мы не разделяем мнение что на основании морфологии роптрии и микронемы можно считать различными структурами.

При использовании метода ультратонких срезов не удается с уверенностью дифференцировать роптрии от микронем. Все ветви этой органеллы на срезах имеют сходную структуру, содержат морфологически одинаковое вещество. Разграничение микронем и роптрий только на основании разных диаметров затруднено. Не исключено, что каждая ветвь этой органеллы имеет или при определенном состоянии может иметь расширение, подобное роптриям. К такому заключению мы пришли потому, что внаших исследованиях у *E. tenella* количественное соотношение мелких и более крупных ветвей не было стабильным как у разных клеток в одном блоке, так и на разных фазах их развития. Отмечено незначительное увеличение расширенных ветвей у эксцистированного (свободного) спорозоита изначительное — у внедривши хся в клетки спорозоитов и мерозоитов.

Можно полагать, что термином роптрии и микронемы обозначается разное состояни ветвей одной органеллы.

Обращеет на себя внимание, что разные авторы указывают на различное количество роптрий у *E. tenella*: Мак Ларен и Паджет (McLaren and Paget 1968), Селиверстова (1970) сообщают о наличии у мерозоитов двух роптрий, Райли (Ryley 1969) — у спорозоитов отмечает четыре роптрии. Мельхорн и Сенод (Mehlhorn and Senaud 1973) недавно сообщили, что количество

роптрий не одинаково у разных генераций мерозоитов *E. falciformis*. Мерозоиты первой генерации имеют 2 роптрии, в то время как четвертой более двух.

На основании приведенных фактов становится очевидным, что число расширенных структур (роптрий) у спорозоита и мерозоита — признак непостоянный, зависящий от стадии развития паразитической клетки, от разных генераций (мерозоитов) и, по-видимому, от физиологического состояния органеллы, а поэтому вряд ли может служить целям систематики.

Наблюдение увеличения количества расширенных ветвей комплекса роптрии-микронемы у внедрившихся в клетки спорозоитов и мерозоитов позволяет согласиться с предположением Шолтизека и Мельхорна (Scholtyseck and Mehlhorn 1970) о том, что функция этой органеллы не ограничена процессом внедрения, а, по-видимому, распространяется на последующую фазу развития паразита.

Резюме

Комплекс специфических структур — роптрий и микронем был исследован на разных стадиях развития спорозоита и мерозоита *E. tenella* в культуре ткани. Показано, что роптрии и микронемы — это ветви одной органеллы, которые в передней части спорозоита и мерозоита суживаются, сливаются и выходят через коноид в виде протоков. Все ветви имеют сходное строение и различаются только по диаметру. Количественное соотношение мелких и более крупных ветвей не было стабильным как у разных клеток в одном блоке, так и на разных фазах развития спорозоита и мерозоита. Отмечено незначительное увеличение количества расширенных ветвей у эксцистированных (свободных) спорозоитов и значительное — у внедрившихся в клетки спорозоитов и мерозоитов. Сделан вывод о том, что число роптрий не может служить систематическим признаком.

SUMMARY

The complex of specific structures (rhoptries and micronemes) in different stages of development of *E. tenella* sporozoites and merozoites in tissue culture was studied.

It was shown that the rhoptries and the micronemes are branches of a single organellum. In the anterior region of the sporozoite and the merozoite they become narrower, confluent and spread in the conoid area in form of ductules. All the branches have similar structure and differ only in the diameter. The ratio of the smaller branches number to the larger ones was not stable as well in various individuals of the same block as in the different stages of development of the sporozoite and the merozoite. Only a slight increase in the number of enlarged branches was observed in excysted free sporozoites and significant increase in their number in intracellular phases of the sporozoite and the merozoite.

The authors conclude that the number of rhoptries cannot serve for taxonomical purposes.

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ПОДПИСИ К ТАБЛИЦАМ I–III

Ультраструктуры апикального комплекса *Eimeria tenella*

1: Поперечный срез спорозоита в спороцисте. Увеличение 14,000×

2: Свободный (эксцистированный *in vitro*) спорозоит. Отдельные элементы комплекса роптрии-микронемы покрыты элементарной мембраной. Мелкозернистое содержимое их имеет разную электронную плотность. Увеличение 56,000×

3–5: Срезы свободных спорозоитов на разных уровнях коноида. Обратите внимание на то, что в верхней части коноида (рис. 3) число элементов комплекса роптрии-микронемы меньше, чем в нижней (рис. 5). Некоторые из них электроннопрозрачные, другие электронноплотные. Увеличение: 3 — 85,000×, 4 — 100,000×, 5 — 75,000×

6: Срез через апикальный конец свободного спорозоита. Стрелкой отмечен один проток роптрий в центре коноида. Увеличение 42,000×

7: Продольно срезанные расширенные элементы комплеска роптрии-микронемы у свободного спорозоита. Увеличение 30,000×

8: Поперечный срез через апикальный конец спорозоита. Многочисленны мелкие ветви комплекса роптрии-микронемы. Увеличение 23,000×

9: Участок свободного спорозоита. Многочисленны расширенные элементы (роптрии на поперечном срезе спорозоита. Увеличение 23,000×

10: Свободный спорозоит. Поперечносрезанные мелкие элементы комплекса роптрии--микронемы имеют одинаковый диаметр (60 nm) с узкой частью роптрий. Наибольший диаметр роптрий приблизительно 270 nm. Увеличение 30,000×

11: Тангенциальный срез внутриклеточного спорозоита. Видны девять расширенных ветвей (роптрий). Увеличение 37,000×

12: Поперечный срез внутриклеточного спорозоита. Многочисленны расширенные элементы комплекса роптрии-микронемы. Увеличение 40,000×

13: Формирующийся мерозоит. Зачаток роптрий и микронем в виде округлого осмиофильного тела. Увеличение 15,000×

14: Участок внеклеточного (свободного) мерозоита. Многочисленны срезы мелких ветвей, одинаковые по диаметру. Увеличение 24,000×.

15: Внеклеточный мерозоит. Одна роптрия среди одинаковых по ширине микронем. Увеличение 40,000×

16: Мерозоит, внедрившийся в клетки культуры ткани. Обратите внимание на вариации диаметра элементов комплекса роптрии-микронемы. Увеличение 50,000×

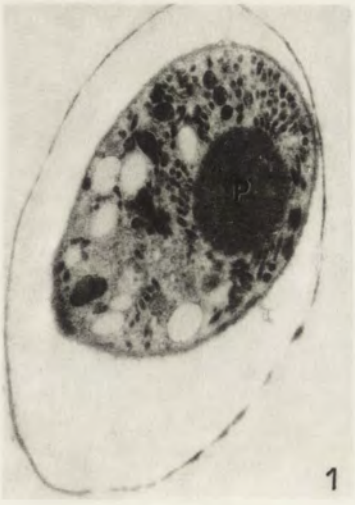
Обозначения на рисунках C — коноид, D — элементы комплекса роптрии-микронемы, проходящие внутри коноида, MN — элементы комплекса роптрии-микронемы с меньшим диаметром, MT — субпелликулярные микротрубочки, N — ядро паразитической клетки, P — парануклеарное тело, RH — роптрия, RA — зачаток структур комплекса роптрии и микронемы, V — везикула внутри роптрии.

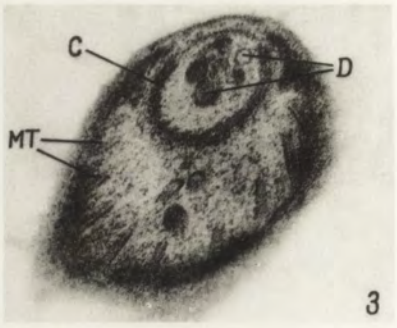
EXPLANATIONS OF PLATES I–III

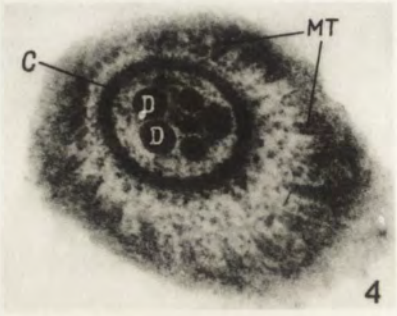
Ultrastructure of apical complex of *Eimeria tenella*

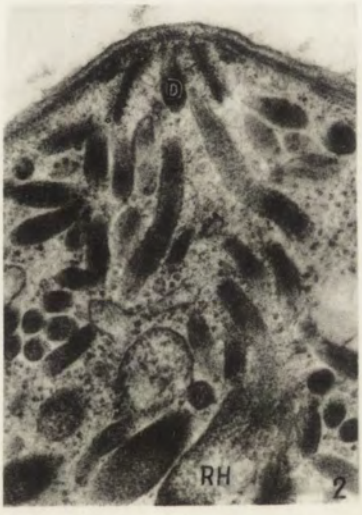
1: Transverse section through a sporozoite within a sporocyst. magn. 14,000 ×
2: Free sporozoite (excysted *in vitro*). Particular elements of the rhoptries-micronemes complex covered with the unit membrane. Fine granular content shows different electron density. magn. 56,000 ×
3 – 5: Sections through free sporozoites at various levels of the conoid. The number of elements of the micronemes-rhoptries complex in the apical part of the conoid (phot. 3) is greater than in its basal part (phot. 5). Some of these elements are transparent, the other ones are dense. magn. phot. 3 – 85,000 × 4 – 100,000 ×, 5 – 75,000 ×
6: Section through the apical end of a free sporozoite. The arrow indicates a tubule of the rhoptry in the centre of the conoid. magn. 42,000 ×
7: Enlarged elements of the rhoptries-micronemes complex of a free sporozoite in longitudinal section. magn. 30,000 ×
8: Transverse section through the apical end of the sporozoite. Numerous small branches of the rhoptries-micronemes complex are seen. magn. 23,000 ×
9: A part of a free sporozoite. Numerous enlarged elements (rhoptries) are seen in transversal section. magn. 23,000 ×
10: Free sporozoite. Small elements of the rhoptries-micronemes complex have the same diameter (60 nm) in transversal section as a narrow part of the rhoptry. The largest diameter of the rhoptry is about 270 nm. magn. 30,000 ×
11: Tangential section through an intracellular sporozoite. Nine enlarged branches of the rhoptries are seen. magn. 37,000 ×
12: Intracellular sporozoite in transversal section. Numerous enlarged elements of the rhoptries-micronemes complex are present. magn. 40,000 ×
13: Developing merozoite. An anlage of rhoptries and micronemes occurs in form of a round osmiophilic body. magn. 15,000 ×
14: A part of a free merozoite. In section plane numerous small branches of the same diameter are present. magn. 24,000 ×
15: Free merozoite. One rhoptry among micronemes of equal diameter, magn. 40,000 ×
16: A merozoite inside the cell of the tissue culture. Elements of the rhoptries-micronemes complex are of different diameters. magn. 50,000 ×

Abbreviations: C – conoid, D – element of the rhoptries-micronemes complex, in the conoid, MN – small diameter elements of the rhoptries-micronemes complex, MT – subpellicular microtubules, N – nucleus of a parasite, P – paranuclear body, RH – rhoptry, RA – anlage of the rhoptries-micronemes complex, V – vacuole inside the rhoptries.









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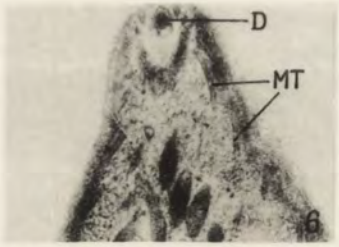

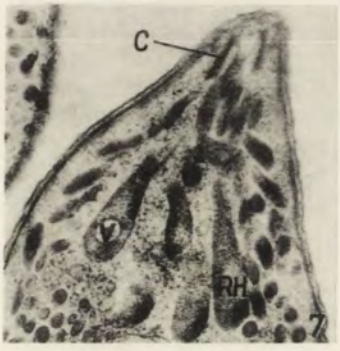

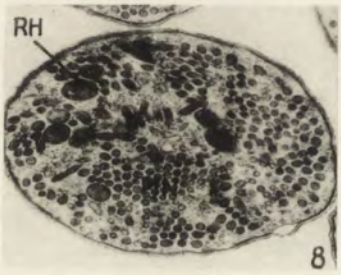

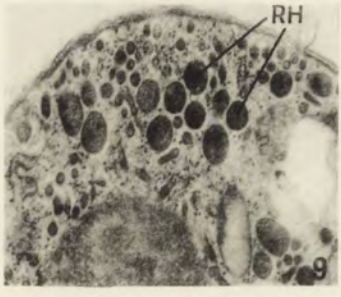

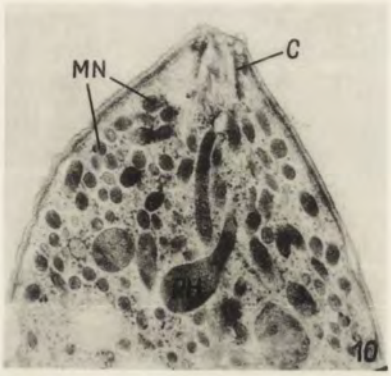

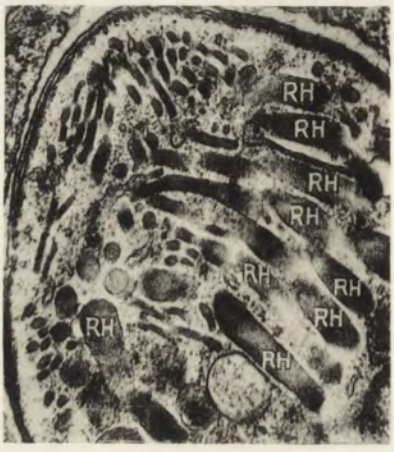

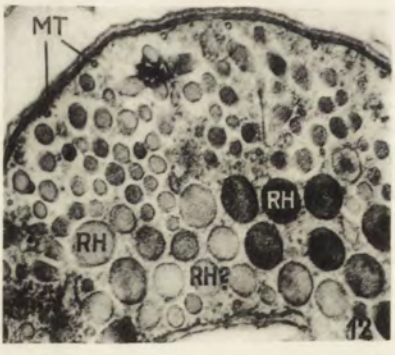

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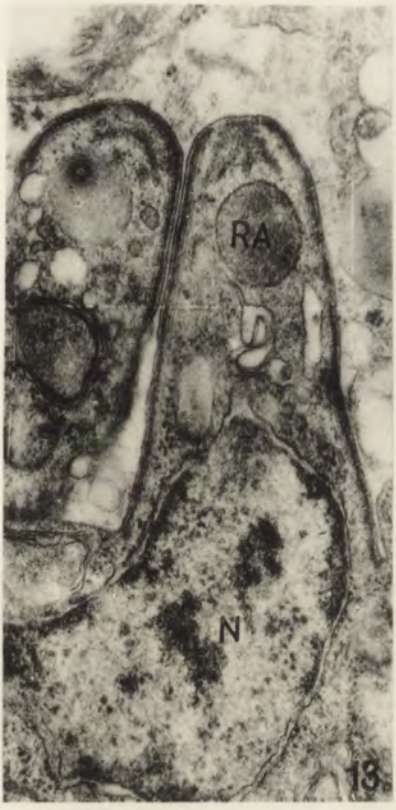

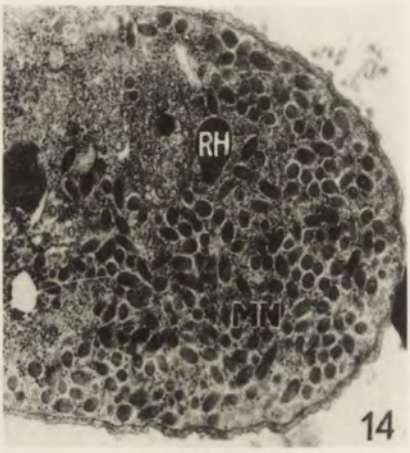

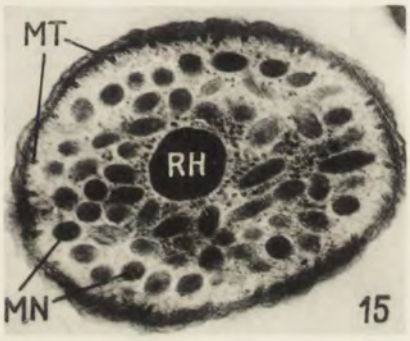

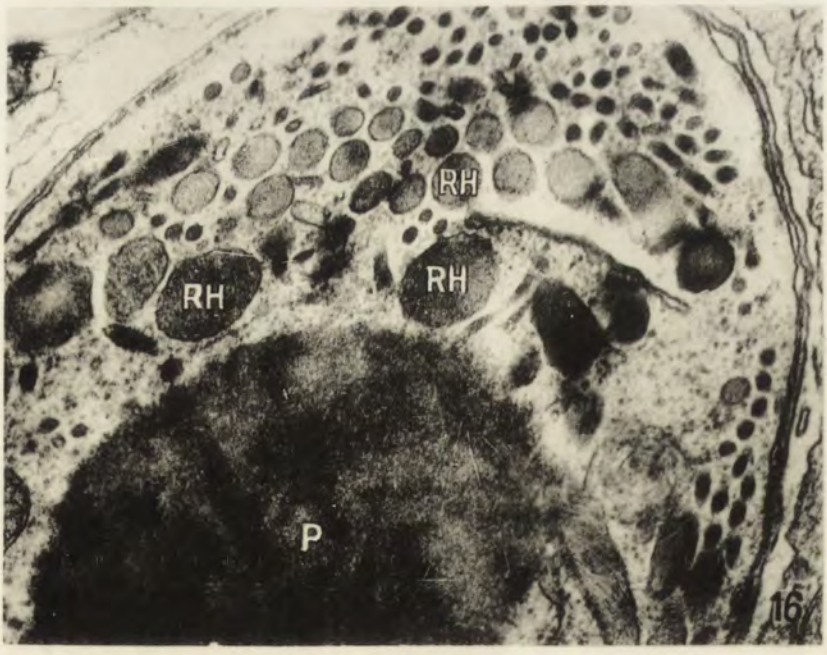

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ACTA PROTOZOOLOGICA
VOL. XIII WARSZAWA, 31.X.1975 FASC. 34

Department of Biology, University of California, Los Angeles, California 90024, USA

Gregory B. WILSON, Theodore L. JAHN and James R. FONSECA

Helical Nature of the Ciliary Beat of *Colpidium striatum*[1]

Caractère helicoidal du battement Ciliaire chez le *Colpidium striatum*

Our knowledge of the normal movement of individual body cilia of free-swimming protozoan ciliates has been derived from studies that have utilized six basic methods.

(1) Stroboscopic analysis of peripheral cilia (Gray 1922, 1928, 1930, 1953).
(2) Rapid fixation techniques as developed by Párducz (1967 and earlier), and modified by Grębecki (1964).
(3) High speed cinemicrographs of cilia on the periphery of the organism (Sleigh 1962, 1966, 1968).
(4) Single flash pictures of ciliary beating on living organisms (Machemer 1972, 1973, Aiello and Sleigh 1972).
(5) Scanning electron micrographs of fixed specimens (Horridge and Tamm 1969, Tamm 1972).
(6) High speed cinemicrographs of swimming organisms where ciliary movements are observed in two planes of reference – on the cell surface and on the periphery of the cell (Kuźnicki et al. 1968 a, 1968 b, 1970).

The development of these various methods was prompted by the fundamental problem that when functioning normally, cilia cannot be directly observed under the microscope due to their small size, rapid movement, optical properties which are almost identical to the cytoplasm of the cell and the dense number of cilia normally found covering many ciliates (Párducz 1967, Preston 1972, Kuźnicki 1970, Kuźnicki et al. 1970).

The methods of Párducz (1967) and Tamm (1972) attempted to overcome the above-mentioned problems by analyzing fixed specimens. When analyzing fixed specimens there is no guarantee that the actual form of the beat observed in the living specimen is preserved in the processes of fixation, dehydration or embedding. As pointed out by Kuźnicki (1970), a technique which involves fixation may lead to the production of artifacts. Methods 1, 3, and 4 are concerned primarily with analyses of peripheral cilia. When only peripheral cilia are analyzed, the optical illusion created

[1] Supported by NSF Grant GB 24840 and USPHS AM 14388

when the cilia bend out of the plane of focus may lead to erroneous interpretations (Kuźnicki et al. 1970).

Method (6), which analyzes the ciliary beat of freely-swimming organisms in two planes of reference does not appear to be plagued with the shortcomings mentioned for the other five methods. It would appear, therefore, to be the method of choice for the analysis of ciliary beating in free-swimming protozoans.

The advantages of using method six, as compared to the other methods mentioned above, have been exhaustively discussed previously by Kuźnicki (1970) and Kuźnicki et al. (1970) (and references contained therein). These papers contain a more detailed account of the advantages and methodology involved in the analysis of ciliary beating using this method.

Párducz (1967), Sleigh (1962, 1966, 1968), Machemer (1972, 1973) Tamm (1972) and other investigators, who have utilized methods 1–5 mentioned above, generally agreed that the ciliary beats of *Tetrahymena pyriformis*, *Opalina ranarum*, *Paramecium multimicronucleatum*, *Balantidium coli* and most other ciliates that they have studied, conform to either a "classical" planar discontinuous effective and recovery stroke as described by Gray (1922, 1928, 1930, 1953) or a three-dimensional effective and recovery stroke as described by Párducz (1967).

Recently evidence has been accumulating that not all ciliary organelles beat with the classical effective and recovery phases. Sleigh (1968) has shown that the caudal cirri of *Stylonychia mytilus* may show a helical beat and Párducz (1967) and Kuźnicki et al. (1970), working with the body cilia of *Paramecium* or *Colpidium*, have shown that in stationary organisms the cilia may show a conicoidal beat.

The best evidence to date showing that not all cilia beat with a classical discontinuous stroke has been obtained by Kuźnicki, Jahn and co-workers. Analyzing some of the same species of ciliates as were used by Párducz, Sleigh and other investigators, they have shown that the body cilia of *Paramecium multimicronucleatum* (Kuźnicki et al. 1970, Kuźnicki 1970), *Tetrahymena pyriformis* (Preston et al. 1970, Preston 1972), *Opalina ranarum* (Cheung et al. 1973, Cheung 1973) and *Colpidium striatum* (Wilson et al. 1974), beat with a helical wave traveling from base-to-tip. Unlike the work of other authors, high speed cinemicrographic techniques were always employed to make observations of ciliary beating of living organisms utilizing method six.

In the present paper the ciliary beat of free-swimming and stationary *Colpidium striatum*, and the process of ciliary reversal were analyzed using the methods of Kuźnicki, Jahn and co-workers. The results of the analyses are presented in detail and the normal motor responses and pattern of ciliary metachrony shown by *Colpidium* are described.

Materials and Methods

Cultures of *Colpidium striatum* were grown agnotobiotically in Pringsheim's wheat grain medium[2] or in Cerophyl sodium phosphate medium[3] to concentrations of 10^5 or 10^4 per milliliter, respectively. The organisms were then transferred in their media to a slide. All of the preparations examined were prepared as vaseline mounts to prevent evaporation, and to insure a sufficient distance between the slide and coverslip (about 720 μm) to allow full freedom in swimming.

A Red Lake Locam high speed movie camera, model 162-4DC, was used to film all the sequences. Eastman Ektachrome Commercial No. 7255 (ECO) film was used for color photography while black and white filming was done with Eastman 4×negative No. 7224 (4×N). A 100W tungsten-halogen light source was used. Framing rates of 200 to 400 fps were used with 4×N film and 16 fps with ECO. The framing rate was determined for each film by an internal timing light which marked the edge of the film.

The movie camera was attached to a Zeiss Universal Research Microscope, equipped with phase and Nomarski differential interference contrast optical systems. In this study the best cinemicrographs were obtained using oil immersion phase contrast optics (650× magnification) at a film rate of 200 fps, with the organisms freely swimming in a medium of normal viscosity.

Analysis of the high speed cinemicrographs was accomplished using a flickerless L. W. photo-optical analyzer employing projection rates between 1 and 16 fps. Selected sequences were traced or printed to confirm conclusions gained from repeated low speed analysis of the cinemicrographs.

Observations of cilia on the cell surface in addition to those of peripheral cilia of freely swimming and stationary *Colpidium* were made, using procedures similar to the pioneer high speed cinemicrographic studies of Kuźnicki et al. (1968 a, 1969 a, 1970) on the ciliary beat of *Paramecium multimicronucleatum*, which were done in this laboratory.

Results

General Description of *Colpidium striatum*

Colpidium striatum is a holotrich ciliate classified in the order *Hymenostomatida* (Corliss 1961, 1967). It is related to both *Paramecium* and *Tetrahymena*.

The organism is generally 60 to 90 μm long and 20 to 30 μm wide. The cilia are arranged in rows which run the length of the body (anterior to posterior). There are 27 to 30 rows on the organism. The rows are spaced at intervals of between 0.8 and 1.8 μm, while the cilia in each row are spaced at intervals between 2.4 to 3.0 μm. The cilia are more closely

[2] Pringsheim's medium: 1 part 0.2 g K_2HPO_4 and 0.2 g NaCl in 500 ml H_2O; 1 part $Ca(NO_3)_2$, 2.0 g; $MgSO_4$, 2.0 g; and $FeSO_4 \cdot 7\ H_2O$, 2.0 g in 500 ml H_2O; and 20 parts H_2O. To 125 ml of this solution 40 boiled wheat grains were added.

[3] Cerophyl sodium phosphate medium: 2.5 g Cerophyl (Cerophyl Laboratories, Inc. Missouri) per 1000 ml H_2O. Boil, filter and autoclave, then add 1.0 g $Na_2HPO_4 \cdot 12\ H_2O$ per 1000 ml.

packed at the anterior end than the posterior end, for the distance between rows increases slightly as they progress posteriorly. The cilia are approximately 10 μm long and 0.27 μm in diameter.

General Patterns of Movement and Metachrony

When freely swimming *Colpidium* is observed to exhibit motor responses similar to those of *Paramecium*, as described by Dryl and Grębecki (1966) and Kuźnicki (1970) (forward movement with left or right spiralling (FLS or FRS), continuous ciliary reversal (CCR), periodic ciliary reversal (PCR), and partial ciliary reversal (PaCR)). Among the five basic motor responses noted above, LFS and PaCR are the patterns most commonly observed for *Colpidium*.

When *Colpidium* is freely swimming and the cilia are working in a coordinated manner, the somatic ciliature of *Colpidium* shows a "dexioplectic metachronism". The Knight-Jones (1954) classification of metachronal coordination, defines dexioplectic metachrony as metachronal coordination in which the effective stroke of the cilia is directed rightward from the propagation of the metachronal waves. The cilia of *Colpidium*, as will be described shortly, do not have a classical planar discontinuous beat as described by Gray (1922, 1928, 1930, 1953) or a three-dimensional effective and recovery stroke as described for the cilia of some protozoa by Párducz (1967), Sleigh (1968), and Machemer (1972). Rather, the cilia of *Colpidium* beat with the form of a three-dimensional traveling helical wave propagated from base to tip, which is similar to the form of beat described for *Paramecium multicronucleatum* Kuźnicki 1970, and Kuźnicki et al. 1970) and *Tetrahymena pyriformis* (Preston et al. 1970, Preston 1972). Instead of saying that the effective stroke of the cilium is directed rightward in relation to the propagation of the metachronal wave, we must say that the traveling helical wave of each cilium is directed toward the right from the longitudinal axis of the organism and that the metachronal wave moves anterior to posterior following a course down the longitudinal axis of the organism. As shown in plate I, the cilia beat at an angle of about 30° to the right of the propagation of the metachronal waves when the organism is moving forward with left spiraling (FLS).

Form of the Ciliary Beat in Swimming *Colpidium*

Repeated low speed (2–16 FPS) and frame by frame analysis of selected sequences from high speed movies of freely-swimming *Colpidium*, revealed that the cilia of *Colpidium* beat with a continuous traveling helical wave propagated from base to tip.

Plates II and III present a typical picture of the beat of the body cilia of *Colpidium*, as observed on the cell surface. Eighteen successive frames are shown. The frame rate was 200 per second and the original magnification was 650×. The cilia are beating with a frequency of about 25/sec. As is shown, the cilia beat with a continuous undulatory wave which is propagated from base to tip. In the eighteen frames presented, the cilia pass through about $2^1/_2$ cycles, but notice that a return stroke is never observed.

Plate IV presents the ciliary beat of *Colpidium* as observed in lateral view on the cell periphery. As in Plates II and III, the cilia beat with a continuous traveling undulatory wave, and no return stroke is ever observed. The cilia in Plate IV were filmed at 200 FPS and the ciliary beat is about 25/sec (as in Plates II and III).

Since the beat is seen as an undulatory wave in both surface and profile views (Plates II–IV), the wave can actually be described as a 3-dimensional traveling wave and approximates a traveling helical wave (Kuźnicki et al. 1970). Figure 1 shows a diagram of the traveling helical wave

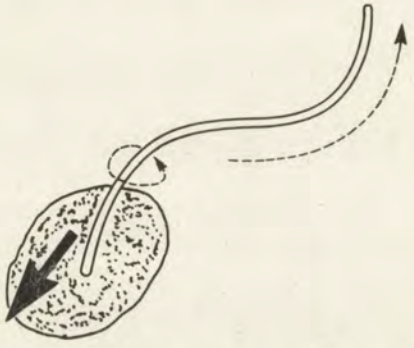

Fig. 1. Diagramatic illustration of the traveling helical ciliary beat as observed in freely swimming *Colpidium striatum* (Plates II–IV). The large arrow indicates the direction of movement of the organism. The dotted arrow indicates the movement of the continuous traveling helical wave (base-to-tip). The cilium appears to gyrate about its axis (as indicated by the dotted arrow near the base of the cilium) as the helical wave propagates from base to tip, however, this gyration is more apparent than real. Based on the drawing by Kuźnicki (1970) of the traveling helical wave in an individual body cilium of swimming *Paramecium*

as it appears in an individual cilium of *Colpidium* when it is beating at a frequency of about 15–25/sec. The wave is composed of 2 undulatory traveling waves at right angles to each other and 90° out-of-phase. If the undulatory waves are sine waves the combination is a perfect helix. The cilium does not rotate on its axis but merely undulates in 2 planes simultaneously, 90° out-of-phase (Jahn and Bovee 1968, Kuźnicki et al. 1970). The simultaneously undulation of the two waves causes the cilium to appear to rotate or gyrate about its axis.

When the cilia of *Colpidium* are beating quickly (15–25 beats per sec) there are about 1 to $1^1/_4$ wave lengths present along the length of the cilium at any one given time. The ratio of amplitude to wave length is about 1 : 4. The ciliary wave of *Colpidium* is not a true sine wave but it does have a symmetrical form and as seen in the motion picture (Wilson et al. 1974) and Plates II–IV, the waves do travel from base to tip.

The Ciliary Beat in Slow-Moving *Colpidium*

When the ciliary beat of *Colpidium* drops below 10 per second, as in slow-moving organisms, the basic form of the helical beat changes. The amplitude greatly increases and the ratio of amplitude to wave length changes from about 1 : 4 to about 1 : 2. Because it is moving slowly the illusion that the cilium is rotating is even more apparent. At times it may even appear to look like a spiral.

The Conical Beat of Cilia in Stationary *Colpidium*

In stationary *Colpidium* the cilia show a conicoidal or rotational form of movement. Plate V and Fig. 2 show what this form of beat looks like

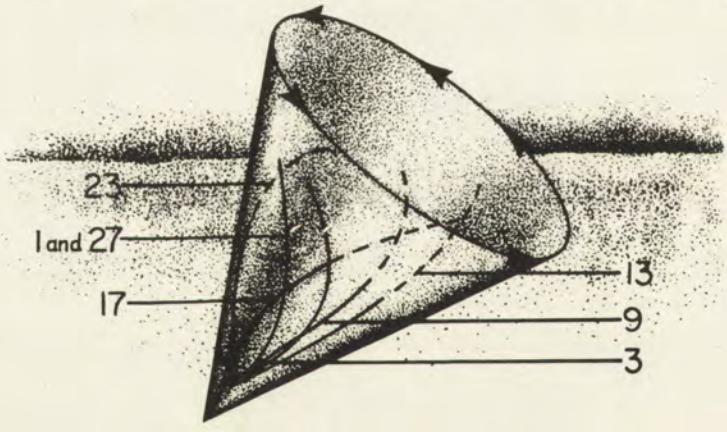

Fig. 2. Diagram showing the conicoidal ciliary beat in stationary *Colpidium striatum*. The cilium shown is indicated by the arrow in plate V. The cilium is rotating counter clockwise at 7 rotations per second. The solid parts of the cilium indicate the sections of the cilium which are in focus in the frame indicated. The dotted parts indicate those parts of the cilium which are out of focus. The cilium sequentially rotates out of the focal plane and then in again (Frame 1–27, Plate V)

when a cilium is viewed in profile near the cell periphery. As shown, the cilium rotates slowly in a counter-clockwise fashion (about 7 rotations per second), and never acquires a straight line appearance in any plane. Coni-

coidal movement or "rotational" movement is not simply a pre-mortal symptom or pathological symptom as originally felt by Párducz (1954, 1967) when he observed it for cilia on hyaline blebs of stationary *Paramecium* or on intact *Paramecium*, *Colpidium*, *Didinium* and *Opalina*, when the organisms were treated with a harmful substance (narcotics, inorganic salts, etc.). Rather, conicoidal ciliary movements are normal phenomena observed in stationary protozoan ciliates. This has been well documented for *Paramecium multimicronucleatum* by Kuźnicki et al. (1970) and Kuźnicki (1970). In this study, *Colpidium*, which when stationary showed cilia performing a conicoidal beat (Plate V, Fig. 2), subsequently many of these organisms began to move in a normal fashion. This indicates that conicoidal ciliary movement in *Colpidium* is merely a transitory state accompanying a condition in which the organism is stationary. When the organism begins to move a normal continuous traveling helical ciliary form of beat is observed.

Ciliary Reversal in *Colpidium*

During turning movements and complete ciliary reversal in *Colpidium*, each cilium rotates about its point of attachment to the cell. The reversal or turning movement may occur in either direction dependent upon the new direction of movement. In ciliary reversal the cilia are observed to travel 180° from their previous positions, thus causing the organism to move in the opposite direction.

The process of ciliary reversal in *Colpidium* is shown in Plate VI. Plate VI presents every sixth frame in a series of 179 frames taken from a high speed movie shot at 200 FPS. The cilia are shown on the surface as viewed from above the organism (650× magnification). Prior to frame 1 and after frame 179, the cilia were observed to beat with a continuous helical wave. As shown, successive rows rotate 180° until all the cilia have changed direction.

Figure 3 presents a diagramatic representation of the successive stages of a cilium during reversal. As shown in Plate VI and Fig. 3, the cilia change position following a course which is quite analogous to the recovery phase of a planar discontinuous ciliary beat (Gray 1928). As can be seen in Plate VI, the entire cilium is always in the same plane of focus, which indicates the relative planar nature of the reversal process.

As shown in Fig. 3, the process of reversal occurs with an active bending and rotation starting at the basal part of the cilium (position 2). The basal part sequentially rotates and the bend progressively encompasses the entire cilium as the distal part of the cilium is "dragged" by the basal part (positions 2–5). The cilium then straightens out and the reversal sequence is completed (positions 6 and 7).

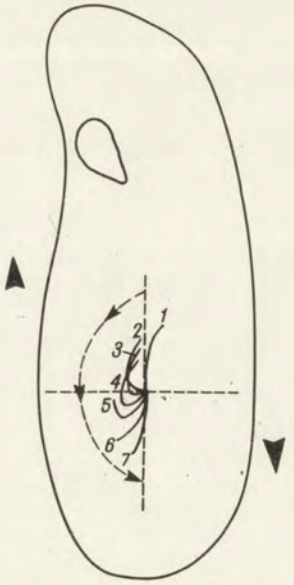

Fig. 3. Diagram showing ciliary reversal in *Colpidium striatum* as seen on the cell surface. The large arrows indicate the direction of movement of the cilium prior to and after reversal has occurred. Seven stages of the reversal process are shown. The direction of reversal is indicated by the dotted arrow. The entire cilium is always in the same focal plane, indicating the relatively planar nature of the reversal process

The high speed movies of the reversal progress in *Colpidium* were compared to the original movies obtained by Kuźnicki et al. (1969 a, b) on *Paramecium*. Comparison of the reversal process as shown by these two ciliates demonstrated that they are nearly identical. Refer to Kuźnicki (1970) for some cinemicrographs showing reversal in *Paramecium*. It should be noted that in *Colpidium* regardless of the speed with which the cilia undergoes reversal, the basic stages of ciliary reversal as shown in Fig. 3 remain the same.

Discussion

Observations of the ciliary beat in free-swimming *Colpidium striatum* have never before been attempted. The only descriptions of the ciliary beat of *Colpidium* are those of Párducz (1967). Párducz observed specimens of *Colpidium* which had been fixed with osmium tetroxide and subsequently stained and at times even dehydrated and mounted in balsam. Using fixed specimens obtained in this manner he postulated and described a three-dimensional discontinuous beat for the cilia of *Colpidium*, *Opalina*, and *Paramecium*. Very little analysis was actually done on specimens of *Colpidium*, instead Párducz simply drew analogies to the form of beat he had worked out for *Paramecium*.

In this study we have used the methods of Kuźnicki, Jahn and co-workers (Kuźnicki et al. 1968 a, b, 1970; Kuźnicki 1970; Preston et al. 1970; Cheung et al. 1973; Boggs et al. 1970) to study the ciliary beat of *Colpidium striatum*. Using these methods we have determined that:

(1) The ciliary beat of *Colpidium* is a traveling helical wave similar to that observed for *Tetrahymena pyriformis* (Preston et al. 1970, Preston 1972) and *Paramecium multimicronucleatum* (Kuźnicki 1970, Kuźnicki et al. 1970). The wave is distally directed and thus exerts a locomotory force from base to tip which pushes the organism in a direction opposite to the movement of the wave (Plates II–IV, Fig. 1).

(2) In slow-moving organisms the ciliary beat may appear to take the form of a spiral because of an increased amplitude and apparent gyration about the base of the cilia as the wave is propagated toward the tip. This gyration is more apparent than real.

(3) In stationary *Colpidium* the ciliary beat is conicoidal (Plate V, Fig. 2).

(4) *Colpidium* shows motor responses similar to those observed in freely swimming *Paramecium* (Kuźnicki 1970).

(5) *Colpidium* when swimming normally shows a dexioplectic form of metachrony.

Dexiopletic metachrony as used in reference to the ciliary beat of *Colpidium* is defined in this paper as a form of metachronal coordination in which the traveling helical wave of each cilium is directed toward the right from the longitudinal axis of the organism while the metachronal wave moves anterior to posterior following a course down the longitudinal axis of the organism (see Results, Plate I). That a coordinated type of ciliary movement can occur and result in metachrony when cilia (or flagella) are beating with traveling undulatory waves (helical waves) is supported by the work of Cleveland and Grimstone (1964) on *Mixotricha paradoxa*, by the papers of Jahn and Bovee (1968) and Jahn and Landman (1965) and by the theoretical papers of Machin (1958, 1963).

The locomotion of *Mixotricha* is primarily caused by symbiotic spirochetes. Each spirochete swims by means of a traveling helical wave as described for *S. cristispira* and *S. plicatilis* by Jahn and Landman (1965), and which is similar to the ciliary beat of *Colpidium* and *Paramecium* (Kuźnicki et al. 1970). As shown by Cleveland and Grimstone (1964), the thousands of spirochetes attached to *Mixotricha* can beat with abolutely perfect metachronal waves. The papers of Machin (1958, 1963) describe a plausible theory on how synchronization or coordinated beating (metachrony) of flagellar or ciliary organelles occurs.

The results obtained in this present investigation and the work of Kuźnicki et al. (1970), and Kuźnicki (1970) on *Paramecium*, Preston et al. (1970, 1972) on *Tetrahymena* and Cheung et al. (1973) on *Opalina*, demonstrate that the ciliary beat in some ciliates is helical in form. Not all

cilia show a planar effective and recovery stroke as originally postulated by Gray (1922, 1928, 1930, 1953), or a three-dimensional effective and recovery stroke as described by Párducz (1967), Tamm (1972), Sleigh (1962, 1966, 1968) and Machemer (1972, 1973). It has been demonstrated by Boggs et al. (1970), using the high speed cinemicrographic methods of Kuźnicki, Jahn and co-workers, that the somatic cilia of *Spirostomum* do beat with an effective and recovery stroke. This finding indicates strongly that the methods of Kuźnicki, Jahn and co-workers are quite valid, and that the helical form of ciliary beat is authentic. If the helical form of ciliary beat were merely an artifact resulting from abnormal ciliary behavior caused by the methods employed or from erronous interpretations of the results; one would expect all ciliates analyzed to show this type of beat when treated in an identical manner. The study of Boggs, Jahn and Fonseca (1970) shows that the helical form of ciliary beating is not observed for all ciliates when the methods of Kuźnicki, Jahn and co-workers are used, but only in those protozoans which actually do swim by means of a continuous traveling helical ciliary beat.

It has been shown that the flagella of different protozoan flagellates may beat differently, although they are structurally identical (Jahn and Bovee 1967). Some flagella beat with planar undulatory waves (*Ceratium* and *Prymnesium*), some with traveling helical waves (*Trachelomonas*) and some with a spiral wave (*Peranema*). Some flagella even beat with a latero-posterior beat (*Trichomonas* and *Entosiphon*) (Jahn and Bovee 1967, Jahn and Votta 1972) which is somewhat similar to the form of beat postulated by Gray (1922, 1928, 1930, 1953) and others (Párducz 1967, Sleigh 1968, Tamm 1972, Machemer 1972) for most cilia.

It has also been rather well documented that flagella and cilia are nearly identical biochemically, structurally and metabolically (Afzelius 1959, 1969; Allen 1967, 1968; Grimstone 1966, Pitelka 1963, Pitelka and Child 1964). Therefore it should not be too difficult to conceive that they may function in similar ways. The existence of the traveling helical form of ciliary beat in some protozoa and the variations of the effective and recovery type of ciliary beat shown by other protozoans, suggest that ciliary orgenelles may function identically to flagella.

Summary

The ciliary beat of free-swimming and stationary *Colpidium striatum* and the process of ciliary reversal were analyzed using the high speed cinemicrographic methods of Kuźnicki, Jahn and co-workers with *Colpidium* in medium of normal viscosity. In addition the normal motor responses and pattern of ciliary metachrony shown by *Colpidium* were ascertained.

Using the above methods the following was determined:

(1) The ciliary beat of freely swimming *Colpidium* is a continuous traveling helical wave propagated from base to tip.

(2) In stationary *Colpidium* the ciliary beat is conicoidal.

(3) *Colpidium* shows motor responses similar to those observed in freely swimming *Paramecium* (Kuźnicki 1970).

(4) *Colpidium* when swimming freely shows a dexioplectic form of metachrony. "Dexioplectic metachrony" is redefined to apply to helical forms of ciliary beating.

RÉSUMÉ

On a analysé le battement ciliaire chez le *Colpidium striatum* en nage libre est en arrêt, en conditions de la viscosité extérieure normale, par la méthode de la cinématographie à cadence accélérée de Kuźnicki, Jahn et colaborateurs. En plus, on a contrôlé les réactions motrices normales et le pattern de la métachronie du travail des cils du *Colpidium*.

Les técniques employées ont permis d'établir que:

(1) Le battement d'un cil d'un *Colpidium* en nage libre représente une onde helicoïdale continue se déplaceant de la base du cil à son extrémité.

(2) Chez un *Colpidium* en arrêt le battement ciliaire est conicoïdal.

(3) Le *Colpidium* présente les réactions motrices pareille à celles du *Paramecium* en nage libre (Kuźnicki 1970).

(4) Le *Colpidium* en nage libre montre une métachronie déxiopléctique. On présente une nouvelle définition de le "métachronie déxiopléctique" applicable au battement ciliaire de forme hélicoïdale.

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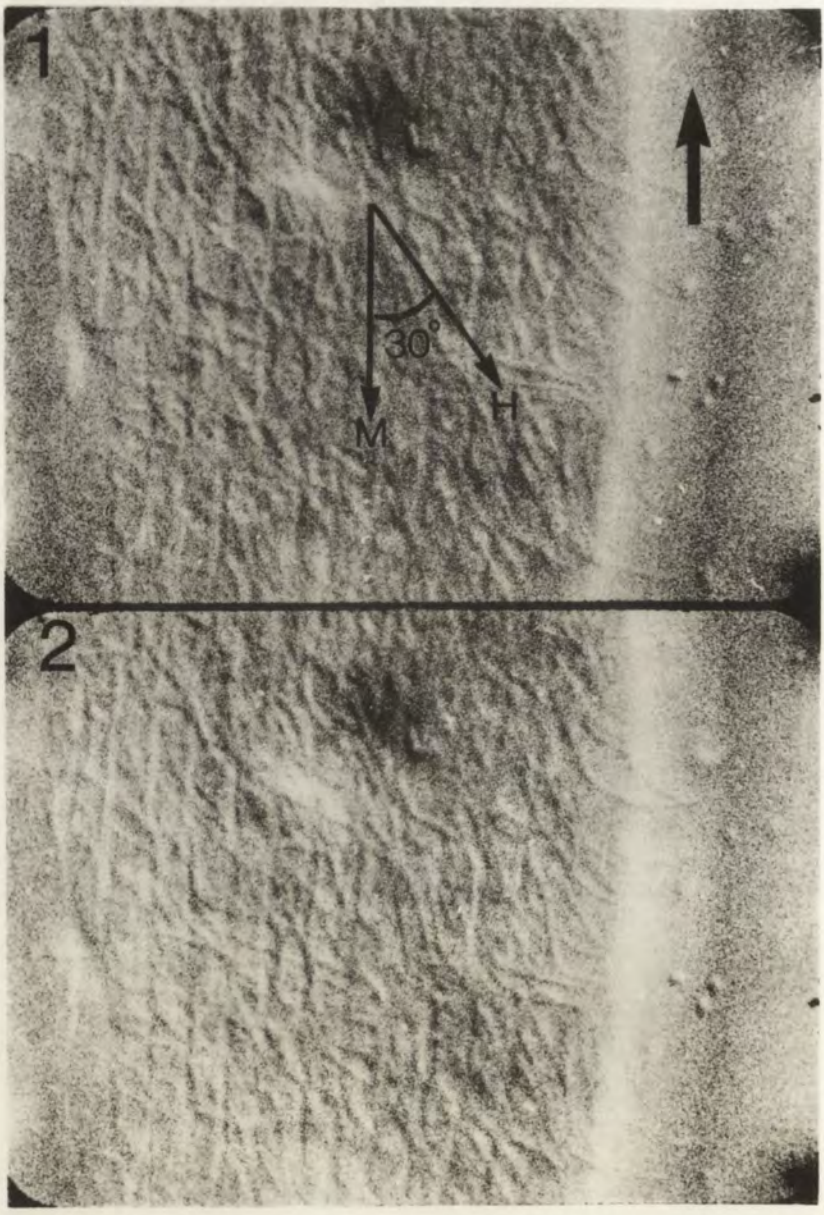

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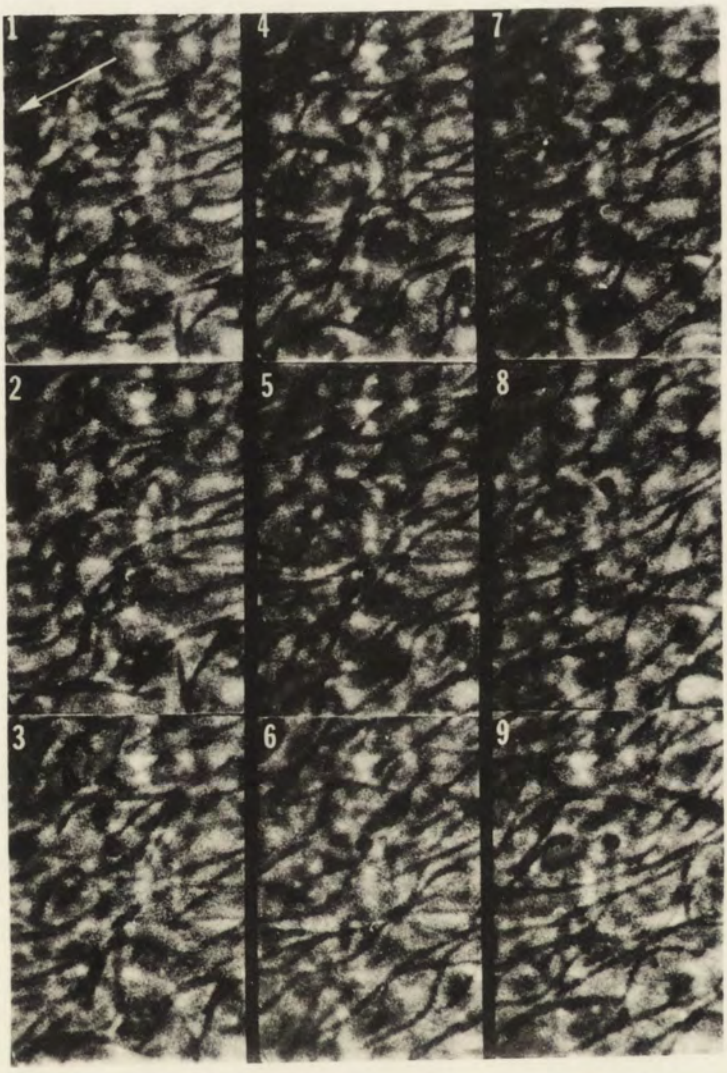

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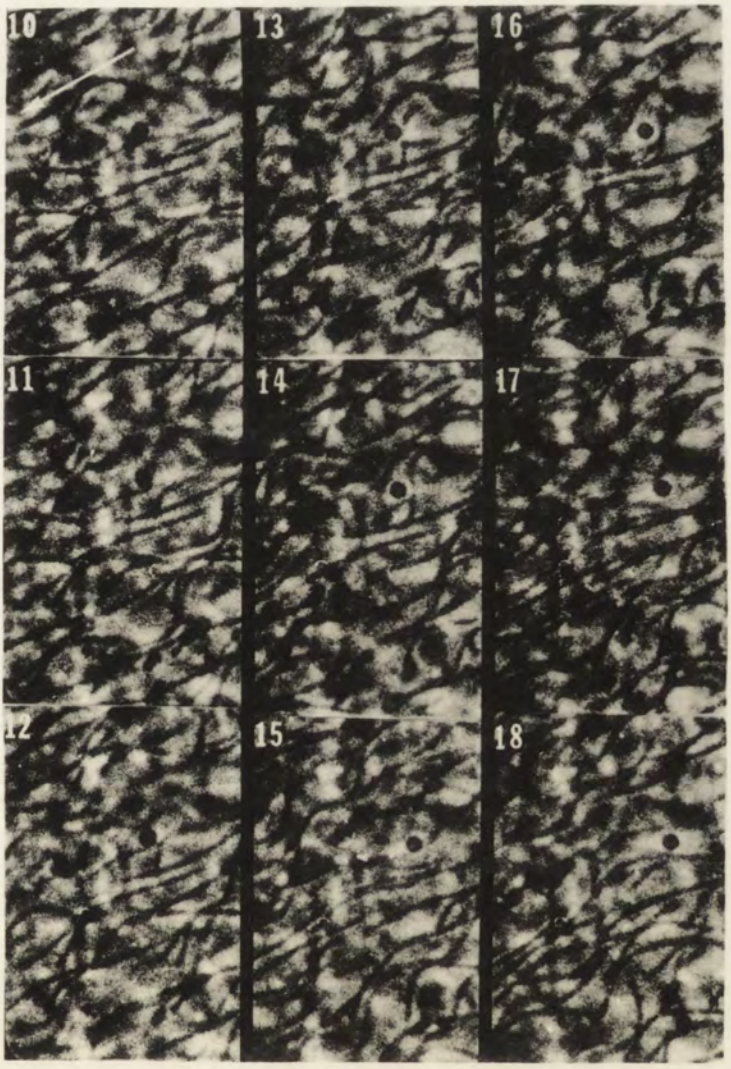

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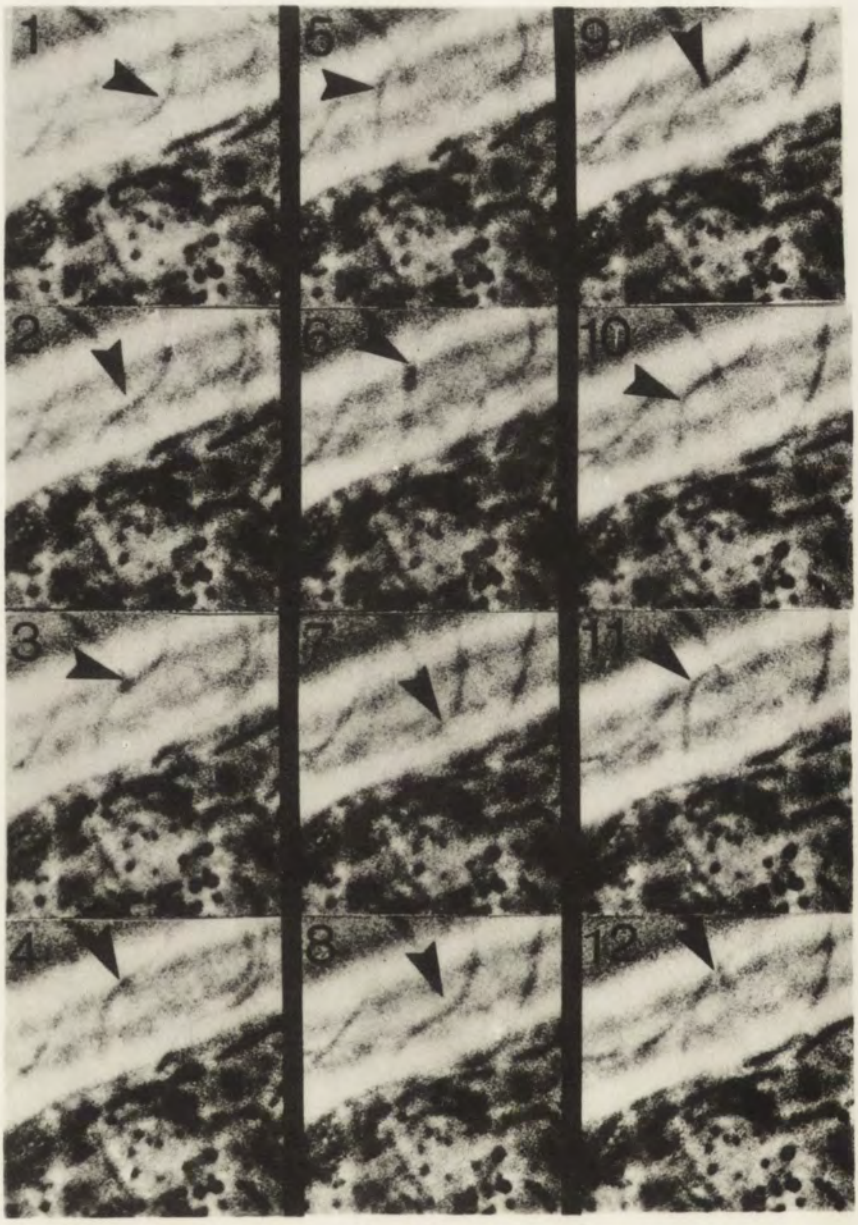

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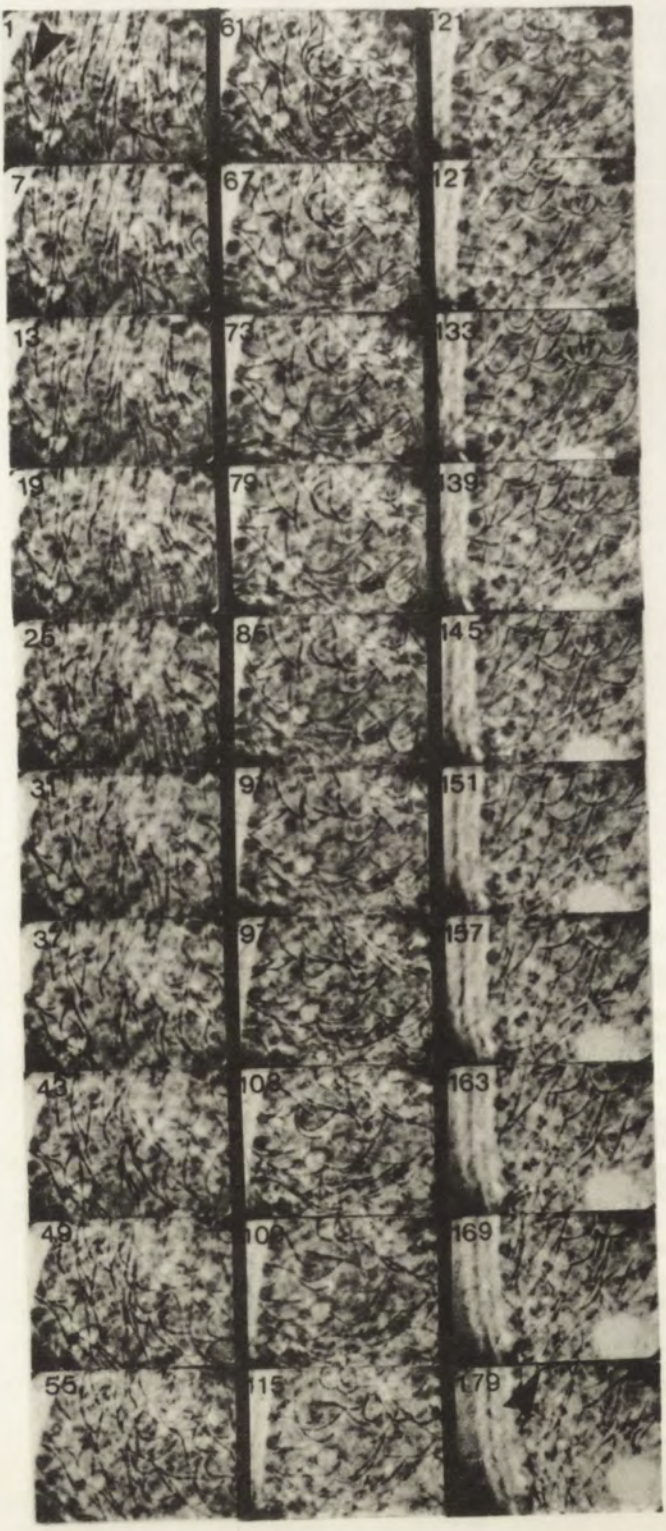

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EXPLANATION OF PLATES I–VI

PPlate I: Two successive frames showing ciliary metachrony as observed on the surface of freely swimming *Colpidium* striatum. The metachrony is "dexioplectic". The direction of the helical ciliary beat (H) is 30° to the right from the direction of the metachronal waves (M). The metachronal waves move anterior to posterior and the helical ciliary beat progresses base to tip on each cilium. The net result is a FLS form of movement. The organism is moving upward (indicated by the arrow). The cinemicrographs were taken at 400 FPS with Nomarski differential interference contrast microscopy (200× original magnification)

PPlates II and III: Eighteen successive frames showing the form of the ciliary beat in a swimming *Colpidium* as observed on the cell surface. The arrow in frames 1 and 10 indicates the direction of movement of the organism. The frame rate was 200 FPS and the original magnification was 650× (phase contrast microscopy). The cilia are beating at about 25 cycles per second (CPS). About $2^1/_2$ cycles of beating are shown in frames 1–18. Notice that a return stroke is never observed and that the undulatory wave proceeds from base to tip

PPlate IV: Twelve successive frames of the same group of cilia as observed on the periphery of a freely swimming *Colpidium*. The frame rate was 200 FPS and the original magnification was 650× (phase contrast microscopy). Approximately two cycles of beating are shown. A typical cilium (indicated by the arrow) can be followed through each of the 12 successive frames. As in plate II and III, the ciliary beat appears as an indulatory wave, and no return stroke is ever observed

PPlate V: The conicoidal beat of the body cilia of a stationary *Colpidium*, as observed near the cell periphery. Alternate frames of 29 successive frames are shown. The cilium indicated by the arrow can be followed through one entire rotation (frame 1 – frame 27). The cilium is rotating in a counter-clockwise fashion at about 7 rotations per second. (This rotation is shown diagrammatically in Fig. 2). As the cilium rotates parts of it go out of focus and the cilium never obtains a straight line appearance in any plane. The cinemicrographs were taken at 200 FPS with phase contrast microscopy (650× – original magnification)

PPlate VI: Ciliary reversal in *Colpidium* as observed on the cell surface. Every sixth frame of 179 successive frames are shown. The arrows in frame 1 and 179 indicate the direction of movement of the organism just prior to and after reversal has occurred. The framing rate was 200 FPS, and the original magnification was 650× (phase contrast microscopy)

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Institute of Cytology, Academy of Sciences of the USSR, Leningrad 190121, Prospekt Maklina 32, and All-Union Institute for Poultry Diseases, Leningrad 196006, Moskovsky Prospekt 99, USSR

T. V. BEYER, T. A. SHIBALOVA and L. P. OVCHINNIKOVA

A Comparative Cytophotometrical Investigation of the Dynamics of Cytoplasmic RNA in the Endogenous Development of *Eimeria tenella* Grown *in vivo* and in Tissue Culture II. Macrogametogenesis

Сравнительное цитофотометрическое исследование динамики цитоплазматической РНК в эндогенном развитии *Eimeria tenella in vivo* и в культуре ткани. II. Макрогаметогенез

In the previous paper the authors (Beyer et al. 1972) reported that the RNA content in the cytoplasm of first generation schizonts of *Eimeria tenella* increases progressively with the parasite's growth. The dynamics of this process appeared to be similar and independent of the parasite's locality: in chick caeca, in the tissue culture, or in the chorioallantoic membrane of chick embryo. The present communication is a continuation of the earlier study extended to include macrogametes grown in the natural host and in the tissue culture condition.

Material and Method

The technique employed in this study has been described more in detail elsewhere (Beyer and Ovchinnikova 1964, 1966, Shibalova 1968, 1969, Beyer et al., 1972, Gaĭbova 1972).

In vivo experiments

14–20 day old "Russkaya Belaya" chickens were used in the experiments. The birds were fed fractionated doses of sporulated oocysts (10,000–15,000) administered at 20–24 h intervals, so that the various phases of macrogamete maturation could be seen in the same preparation.

Second generation merozoite suspension

Birds after administration of 20,000–25,000 sporulated oocysts were sacrificed at 120 h, their caeca were removed for obtaining 2nd generation merozoites. For this, the caeca cut into pieces were placed for 30 min into a sterile bacterial flasks containing nutritive medium heated up to 40–41° C. Merozoites were then isolated from the infected caeca by shaking in

the same medium. To obtain a pure mass of merozoites the whole content of the flask was filtered through a double cheesecloth filter and centrifuged. A counted portion of merozoites was suspended in a definite volume of the nutritive medium with 100 units of penicillin and streptomycin per 1 ml.

Inoculation of tissue culture

A primary cell culture of chick embryo fibroblasts used in the experiments was handled as previously reported (Beyer et al. 1972). 2nd generation merozoites were inoculated in concentration of 500,000–1, 000,000 per 1 ml of the nutritive medium. 4–5 h after inoculation, the medium containing merozoites was removed, the test-tubes with glass pieces covered with monolayered cells were rinsed with a fresh medium at 40°, and then 2 ml of the medium was added to each test-tube.

Histological examination

The caeca of infected birds were removed 5–7 days after oocyst administration when macrogametes of various maturity were seen: from small trophozoites to large female gametes shortly before fertilization. Paraffin 5 μm thick sections were prepared from the material fixed by the Carnoy fluid. The sections were stained with gallocyanin-chromalum for 48 h (Pearse 1960, see also Einarson 1951, and Ovchinnikova and Selivanova 1964).

At 36–52 h, the cover slips with inoculated cell cultures were taken off the test-tubes and fixed with methanol. The material was stained with gallocyanin-chromalum for 48 h.

Preparations treated at 37° for 1 h with crystalline ribonuclease (1 mg/ml) served as a control.

Cytophotometrical examination

The cytoplasmic RNA content in macrogametes grown in the chick caeca was measured in sected cells, only the macrogametes whose nuclei displayed a distinct central nucleolus were examined (Beyer and Ovchinnikova 1964). This enabled the maximal cross-sected square of the cell cytoplasm to be measured, thereby equalizing the compared macrogametes and minimizing the errors that would inevitably occur if random segments of macrogametes were compared. The cytoplasmic RNA content in macrogametes grown in tissue culture was calculated on total preparations, i. e., on the intact cells.

For measurements, the photographical method was applied using a cytophotometer MUV-6 by scanning in $\lambda = 579$ nm. The cell squares were measured planimetrically. The quantity of RNA was calculated in relative units according to the formula (Brodsky 1956, Ovchinnikova et al. 1963): $Q = DS$, where Q is the quantity of cytoplasmic RNA, D – optimal density, and S – square.

All the cells measured were then distributed into three "age" (size) classes according to the values of their total squares, for either compared case separately (Pl. I 1–3). It is to be pointed out that unlike the schizonts, that drastically alter their shape and size while growing in tissue culture, the macrogametes, under similar condition keep as a rule their configuration unchanged, as in the preparations from the chick caeca.

This enabled us to carry out a comparison between the corresponding age groups of each of the two cases, i.e., *in vivo* and *in vitro*, since their macrogametes appeared to have actually equal equatorial squares (Table 1).

Results and Discussion

The results obtained from the quantitative measurement of macrogametes of *Eimeria tenella* are summarized in Table 1.

As seen from the Table 1, the RNA amount in macrogametes tends to increase as these grow, both *in vivo* and *in vitro*. Differences in Q values in

Table 1
Results of the cytophotometric estimation of the cytoplasmic RNA in macrogametes of *Eimeria tenella* growing in the chick caeca and tissue culture (in relative units)

"Age" classes of macro-gametes	Cross-sected squares (S)	RNA quantity (Q) calculated for macrogametes (M∓m)	
		in chick caeca	in tissue culture
I	10–12	28.00∓1.91	30.70∓3.12
II	12–16	32.15∓1.90	38.00∓1.08
III	16–20	37.80∓1.00	47.00∓2.45

the two cases compared may be accounted for by technical reasons: with equal diametrical squares (*S*), macrogametes from tissue culture will have invariably higher densities than do those from caecal preparations, the superiority which every total cell has over a cross-sected one.

However, the aim of the present study was a comparison of the cytoplasmic RNA dynamics in developing macrogametes, under strictly diverse growth conditions, rather than measurements of absolute quantities of this RNA. The evidence obtained suggests the identity of the dynamics.

The gallocyanin-chromalum staining procedure is known to detect both RNA and DNA (Pearse 1960). The treatment with ribonuclease indicated that no DNA was available in the cytoplasm of macrogametes of *E. tenella*, and that the performed investigation was confined to the cytoplasmic RNA only.

The quantitative technique was earlier applied to measuring cytoplasmic RNA in macrogametes of some mammalian coccidia – *E. magna* and *E. intestinalis* from rabbits (Beyer and Ovchinnikova 1964, 1966) and *E. schamchorica* and *E. gliris* from hibernating and non-hibernating rodents (Gaïbova 1972). In all these studies, a steady increase in RNA was followed as female gametes grew and maturated.

The similar tendency was found in the present study. But it is to be noticed that in this case a comparison was made between macrogametes of the same species (*E. tenella*), rather than of two different species of the same host (Beyer and Ovchinnikova 1964) or of two hosts with different ecology (Gaïbova 1972). Macrogametes of *E. tenella* were provided different conditions for their growth, i.e., inside or outside the host body. Despite some discrepancies in the absolute values of RNA obtained (Table 1), a general increasing tendency was also followed.

It is true that conditions found by the parasite in chick caeca do actually differ from those it finds in tissue culture. Nevertheless the evidence so far available, though not yet numerous, suggests a keen similarity in metabolic patterns of *E. tenella* grown in host body and in tissue culture (Hare and Strout 1972, Beyer and Shibalova 1973).

A comparison of the above facts suggests that the developing parasite may follow some definite pattern of metabolism, presumably similar for all the eimerians examined, provided that the conditions for its growth are satisfactory though not identical.

That the intracellular parasite lives at the expense of the host cell hardly needs reminding; however, it is to be noted that the intracellular existence is far from being a passive process. On the contrary, it involves certain activities of both the parasite and the host. The infected cell is getting altered not only morphologically. It is seen to change its metabolic pattern thus providing optimal conditions for the developing parasite (Fletcher and Maegraith 1962, Beyer and Sidorenko 1973, Beyer and Shibalova 1974).

The assumption that the satisfactory environment is stimulated by the parasite due to specific changes in the metabolic machinery of the host cell may account for the ability of some intracellular parasites to grow equally well in the cell types having little, if any, in common; for instance in different cell cultures (Shibalova 1973) and under quite different conditions such as the chick caeca and the primary cell culture of chick embryo fibroblasts, as has been demonstrated in the present communication.

Summary

A comparative quantitative study of the cytoplasmic RNA content during macrogametogenesis of *Eimeria tenella* was performed using a microspectrophotometer MUV-6 by scanning in $\lambda = 579$ nm. Growing macrogametes were compared from chick caecal epithelial cells and from primary cell culture of fibroblasts derived from 10–11 day chick embryos. In growing macrogametes their cytoplasmic RNA content was found to increase progressively, the pattern being similar, in essence, in the two cases compared.

In addition, some peculiarities of host cell–parasite relationships are discussed.

РЕЗЮМЕ

Методом сканирования в длине волны 579 nm на микроспектрофотометре МУФ-6 проведено сравнительное исследование динамики цитоплазматической РНК в процессе макрогаметогенеза *Eimeria tenella* при развитии в эпителии слепых отростков кишечника и в культуре фибробластов. Показано, что в растущих макрогамтеах происходит неуклонное возрастание количества РНК, причем наблюдаемая картина в основном идентична *in vivo* и *in vitro*.

Обсуждаются некоторые особенности взаимоотношения паразита с клеткой хозяина.

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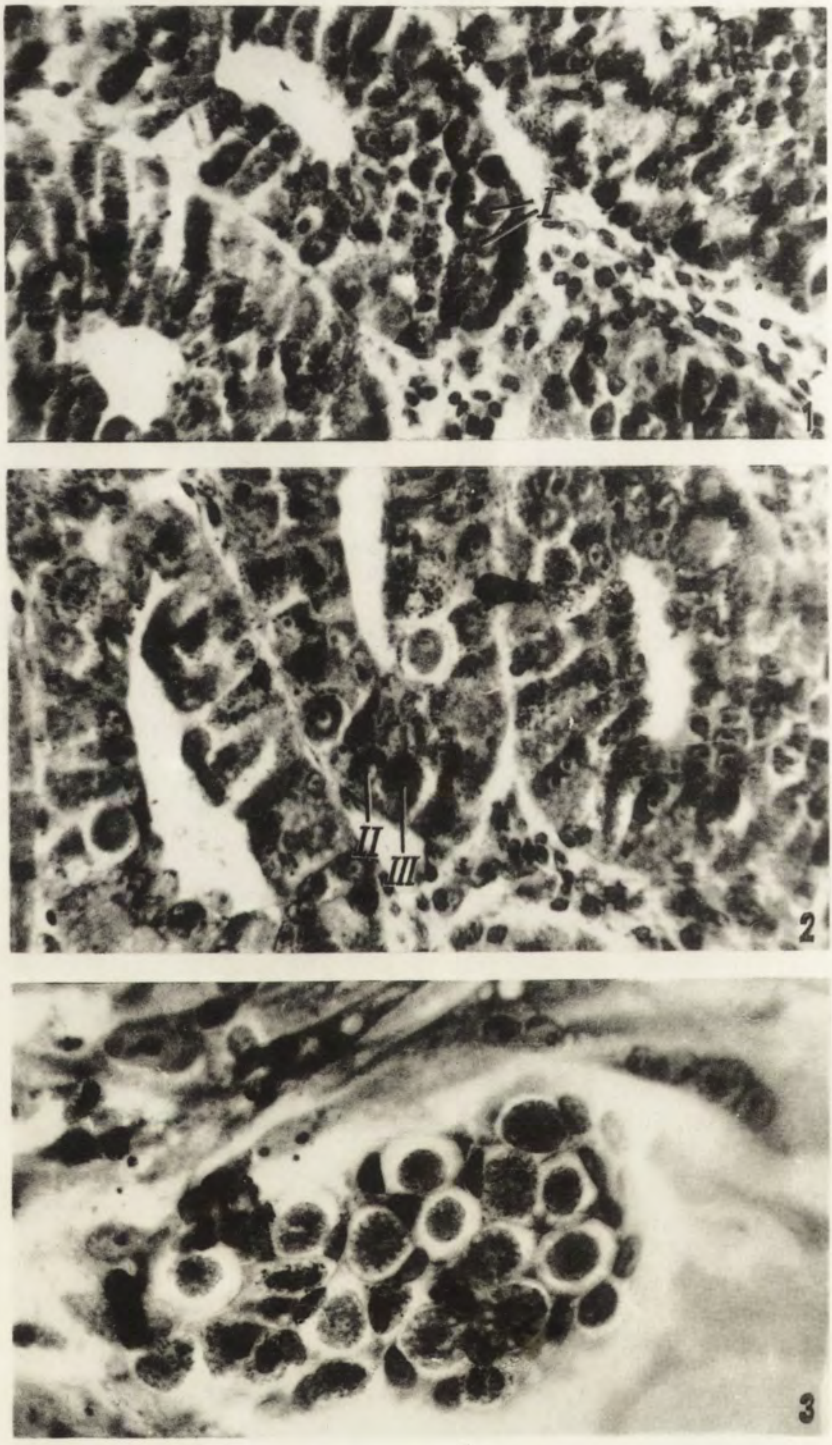
EXPLANATIONS OF PLATE I

Photomicrographs of *Eimeria tenella* macrogametes belonging to different "age" classes

1: Cross-section of the infected chick caecal epithelium (I class). ×600
2: The same (II and III classes). ×600
3: Macrogametes of various maturity in tissue culture. All stained with gallocyanin-chromalum. n. ×900



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Department of Zoology, Karnatak University, Dharwar 580003, India

S. D. AMOJI and S. S. RODGI

On the Nature of Polysaccharide Reserve in the Gregarine, *Stylocephalus mesomorphi*. A Cytochemical Study

Sur la nature de la reserve des polysaccharides chez la Grégarine *Stylocephalus mesomorphi*. Une étude cytochimique

A number of cytochemical studies with the light microscope (Daniels 1938, Ganapati and Narasimhamurti 1955, Stein 1960, Schrével and Fouguet 1968 and Loubes and Bouix 1970) and electron microscope (Grassé and Théodoridès 1959, Vivier and Schrével 1966, and Schrével 1969, 1970) have shown that sporozoans store their carbohydrate in granular form. However, due to lack of information on its exact biochemical nature, this substance was designated as "paraglycogen" (Bütschli 1885), "zooamylon" (Maupas 1886), "vegetative grains" (Horning 1929), "glycogen" (Edgar et al. 1944), "paraplasmic granules of paraglycogen" (Théodoridès 1959) and "coccidienglycogen" (Scholtyseck 1964). Though there is general agreement that the polysaccharide reserve of gregarines is paraglycogen, the results of the cytochemical tests reported by several investigators (Daniels 1938, Dutta 1962, Stein 1963, Bobyleva 1963, Costa and Lage 1964, and Loubes and Bouix 1970) are at variance. The present study was undertaken to throw light on the biochemical nature of the polysaccharide reserve of the gregarine, *Stylocephalus mesomorphi* (Devdhar, thesis 1962) using cytochemical techniques.

Material and Methods

The intestines of the *Mesomorpha velliger* heavily infected with gregarine *Stylocephalus mesomorphi* were isolated and fixed separately in absolute alcohol, Mota et al. fixative and in Carnoy's fluid for the preservation of polysaccharides, mucopolysaccharides and proteins, respectively. The paraffin blocks were prepared after embedding the material in benzene-paraffin (equal volumes) mixture followed by pure paraffin. The sections were cut at 5–6 μm thick. Unfixed fresh smears were used whenever found necessary.

The cytochemical procedures followed herein were essentially those described by Pearse (1968). Periodic acid-Schiff (PAS) followed by acetylation and saponification, Best's carmine and Lugol's iodine were used for staining polysaccharides. Metachromatic reactions with 0.02% Azur A (Spicer 1960), acid Alcian blue 8GS (Mowry 1960) and critical electrolyte

concentration technique (Scott et al. 1964) were carried out to differentiate mucopolysaccharides. Mercury bromophenol blue was used for the recognition of proteins in general. For detecting NH_2-proteins, chloramine-T-Schiff (Burstone 1955) and ninhydrin-Schiff (Yasuma and Itchikawa 1953), and for SS and SH-proteins, performic acid-Alcian blue (Adams and Sloper 1956) and modified alkaline tetrazolium reaction (using nitro-BT) (Deguchi 1964) were adopted. The lipids were demonstrated by staining the fresh smears with Sudan black B in propylene glycol.

Sections incubated at 37° in enzymes such as: (1) 1% malt distase, 1% β-amylase, 1% α-amylase in phospahate buffer (pH 7.0) and human saliva for digesting glycogen (2) 0.5% papain in phosphate buffer (pH 7.4) and rat pancreatic homogenate for digesting proteins, served as controls. Extraction of lipids was done by treating sections with chloroform-methanol (2:1, v/v) at 60° C. Acid hydrolysis with 2 NH_2SO_4 at 98° C was carried out to find the hydrolizability of the granules. Sections treated with methanolic thionyl chloride for 30 min to 24 h or with methyle iodide for 18 h, followed by saponification with 1% KOH in 80% ethanol for 30 min served for blocking and unblocking the reactions for proteins and sulphated mucopolysaccharides.

Results

The observations on the fresh gregarines *Stylocephalus mesomorphi* revealed the presence of refractile granules measuring 0.25–3.0 μm in diameter. These were insoluble in cold as well as hot (60° C) water and were found to be soluble after acid hydrolysis for an hour.

Both cytoplasm and granules were stained intense purple with PAS, indicating the probable presence of polysaccharides and lipids. The granules were not distinct (Pl. I 1) due to deep colouration. The acetylation followed by PAS showed the absence of colour while, intense colour after deacetylation decisively prooved the presence of 1:2 glycol groups. The treatment with hot chloroform-methanol (for 10 h) prior to PAS reaction yielded equally dense colour consequently indicated that the lipids were ineffective in changing the intensity of PAS colour. Test with Sudan black B showed the presence of fat globules of various sizes both in protomerite and deutomerite. However, the granules yielded negative results.

Best's carmine stained the granules more densely than the cytoplasm (Pl. I 3). The positive findings with this stain exibited the probable presence of glycogen.

When sections were treated with PAS and Best's carmine after the malt distase-digestion, the granules were found to be more deeply stained than the cytoplasm with PAS, thereby indicating their non-glycogen carbohydrate nature. The complete absence of the cytoplasmic colour with Best's carmine displayed the localization of glycogen in this area, whereas, deep red colouration of the granules evidenced the presence of other polysaccharides associated with them.

Digestion with β-amylase followed by PAS and Best's carmine (Pl. II 8) reduced the stainability of granules and cytoplasm. The granules were persistant in both the cases. On the contrary, α-amylase or saliva-digestion followed by PAS (Pl. II 7) yielded faint positive results only with the cytoplasm while, with the Best's carmine both cytoplasm and granules were found negative, clearly indicating complete digestion of granules. These results predicted the presence of branched chain polysaccharides.

Insolubility with water and undigestability of the granules with malt distase lead to conjecture the possible presence of proteins. The blue stainability of the cytoplasm and granules with mercury bromophenol blue revealed the deposition of proteins in these sites. Ninhydrin-Schiff and chloramine-T-Schiff (Pl. II 11) rendered pink colour to both these areas, whereas performic acid-Alcian blue and modified alkaline tetrazolium reaction (Pl. II 12) produced faint blue and violet colour to the cytoplasm, respectively, and the granules were stained only at the peripheries. These results indicated uniform distribution of NH_2-proteins and SS-proteins in the cytoplasm. The NH_2-proteins appear to be spread in the granules while, SS and SH-proteins located at the peripheries. All the reactions for proteins were blocked by methylation and reversed after saponification, which further indicated the existence of proteins in cytoplasm and granules.

Digestion with papain and staining with PAS (Pl. I 5) and Best's carmine resulted in relatively less dense colouration of both cytoplasm and granules, whereas pancreatic homogenete-digestion only for 2 h resulted in comparatively negligible staining. The central regions of the granules were almost unstained (Pl. I 6). During this process only some portion of the proteins were removed allowing certain carbohydrates to be present in sections. This showed the association of the proteins with polysaccharides. Further, the digestion in this homogenate for 10 h, followed by treatment with respective stains yielded absolutely no colour as a result indicated depletion of proteins and associated carbohydrates.

The sections predigested either with papain (24 h)/pancreatic homogenate (2 h) and with distase (24 h) when tested with PAS and Best's carmine (Pl. II 9) though showed considerable reduction in colour intensity, the granular structure was unaltered, thereby clearly showed that the proteins were not interfering during malt distase-digestion and also in dissolving it in water.

With the Lugol's iodine the granules were stained purple brown and the cytoplasm purple (Pl. I 2). This showed the probable presence of amylopectin and glycogen. After digestion with papain or pancreatic homogenate followed by iodine test rendered faint purple colour to both cytoplasm and granules (Pl. II 10). Hence, the granular polysaccharide appears to be amylopectin in nature.

The granular polysaccharide was found to be negative to all the tests for mucopolysaccharides, with and without methylation and followed by saponification. On the contrary, the cytoplasm and cell membrane showed the presence of various mucopolysaccharides (Amoji and Rodgi, unpublished).

Discussion

The shape and size of the polysaccharide granules appear to be specific for a given species. They are fusiform or ovoid (0.3–06 μm × 0.15–0.2 μm) in *Seledinium hollandei* and *Eimeria tenella*, reniform (1.5–2.0 μm × 1.0 μm) in *Leucudina tutzetae* (Schrével 1970) and spherical (5–10 μm in diameter) with a central black region in *Diplocystis minor* and *D. clerci* (Loubes and Bouix 1970). These granules in *Gregarina blabrae* are reported (Mercier et. al. 1973) to measure only 6 μm. In *S. mesomorphi* they are also spherical (0.25–3.0 μm in diameter) with a central black spot.

The PAS and Best's carmine staining revealed the presence of polysaccharides. Further, the results of acetylation and saponification showed that they are composed of 1 : 2 glycol groups. The partial digestion of the cytoplasmic and granular polysaccharides with the β-amylase and also their complete destruction by human saliva/α-amylase confirm their branched nature consisting of α-1,4 glucose residues joined by α-1, 6-glucosidic interchain linkages. This type of structure is found only in glycogen, amylopectin and starch. However, the presence of starch is ruled out as there was no blue colour with iodine. Moreover, the purple brown colour of the granules and the purple colour of the cytoplasm with the Lugol's iodine further evidenced that these polysaccharides are of branched chain (Oser 1965). However, the differential staining of the granules on the one hand, and that of cytoplasm on the other, indicated differences in polysaccharide properties.

The negative staining of the cytoplasm with Best's carmine after distase-digestion, and also its less stainability with iodine test suggest that non-granular polysaccharide to be glycogen. This finding is in conformity with the observation of Dobell (1925) who reported the presence of glycogen in *Aggregata eberthi.*

Though amylopectin and glycogen are having similar glucosidic linkages, the former due to its longer unit chains (Manners 1962) than the latter, exibits more open structure and as a result shows differential physical properties. The properties of the polysaccharide granules of *S. mesomorphi* such as, insolubility in water, close association with proteins, purple brown staining with iodine and only faint purple staining after removal of proteins, removal after acid hydrolysis, suggest their similarities with the polysaccharide

reserve of *E. tenella*, *E. brunetti* (Ryley et al. 1969), *S. hollandei* and *L. tuzetae* (Schrével 1970) and also of *G. blaberae* (Mercier et al. 1973) which has been reported as amylopectin.

Hence, from the foregoing discussion it can be inferred that the polysaccharide reserve of *S. mesomorphi* consists of (1) non-granular, distase-labile, glycogen and (2) granular, α-amylase/saliva-labile, but distase-stable, amylopectin.

Acknowledgements

The authors are grateful to Dr. M. Appaswamy Rao, Professor and Head of the Zoology Department, Karnatak University, Dharwar for providing laboratory facilities and to Dr. J. C. Frandsen, Acting Director, United States Department of Agriculture, Auburn, Alabama USA, for his valuable suggestions in writing this manuscript. Appreciation is extended to Mr. P. R. Patil, for his assistance in microphotography.

Summary

The cytochemical reactions were followed for finding the nature of the reserve polysaccharide of *Stylocephalus mesomorphi*, a gregarine parasite of the beetle, *Mesomorpha velliger*. The findings revealed that the non-granular, cytoplasmic polysaccharide was glycogen and the granular polysaccharide was amylopectin. The amylopectin granules were 0.25–3.0 μm in diameter. This substance was found to be associated with NH_2, SS and SH-proteins.

RÉSUMÉ

Les réactions cytochimiques ont été employées pour déterminer la nature du polysaccharide de reserve chez *Stylocephalus mesomorphie*, une Grégarine parasite du Coléoptère, *Mesomorpha velliger*. Les résultats nontrent que ce polysaccharide agranulaire est la glycogene, et le polysacharide granule est l'amylopectine. Les granules de l'amylopectine méasuraient 0.25–3.0 μm de diamètre. On a trouvé cette substance en association avec les protéines comportant les groupes NH_2, SS et SH.

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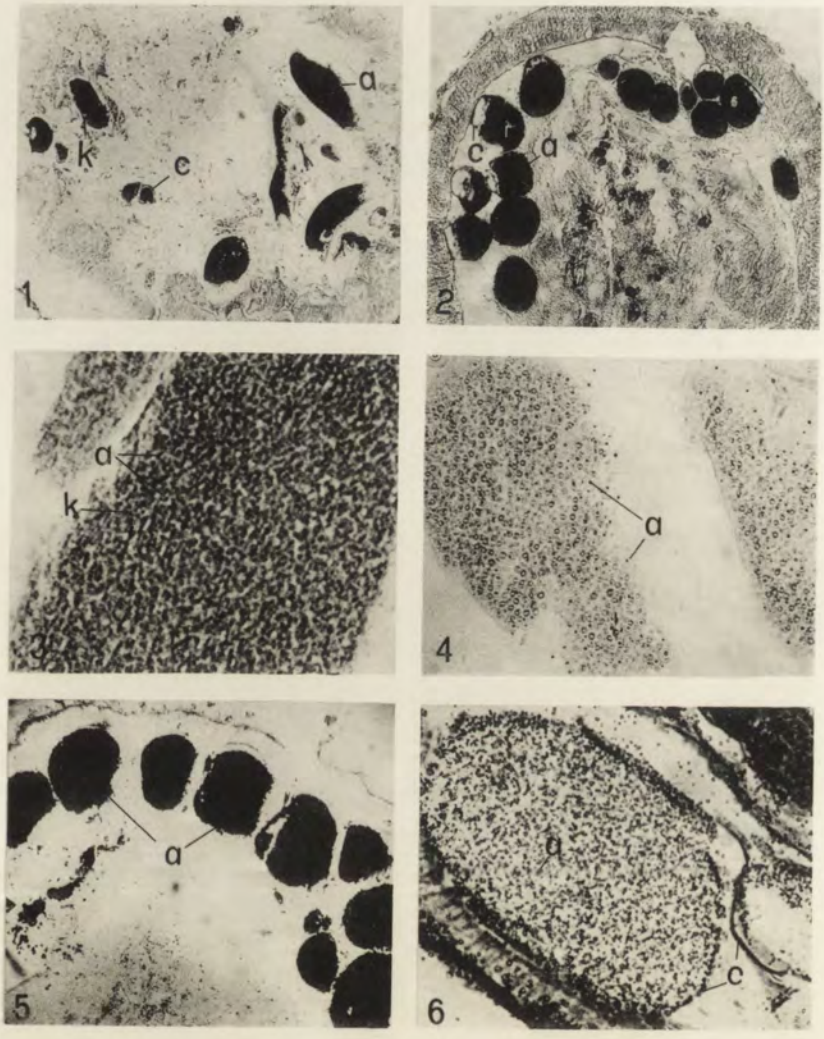
Received 14 January 1974

EXPLANATION OF PLATES I–II

Cytochemical study on *Stylocephalus mesomorphi* parasitizing with *Mesomorpha velliger*

1: PAS. 310×
2: Lugol's iodine. 150×
3: Best's carmine. 800×
4: Best's carmine after malt distase-digestion. 1200×
5: PAS after papain-digestion for 24 h. 300×
6: PAS after rat pancreatic homogenate for 2 h. 660×
7: PAS after α-amylase-digestion for an hour. 360×
8: Best's carmine after β-amylase-digestion for 24 h. 770×
9: Best's carmine after digestion with papain (24 h) and malt distase (24 h). 690×
10: Lugol's iodine after papain-digestion for 16 h. 165×
11: Chloramine-T-Schiff. 1060×
12: Modified alkaline tetrazolium reaction. 920×

Abbreviations used: a – amylopectin, c – cell membrane, k – cytoplasm



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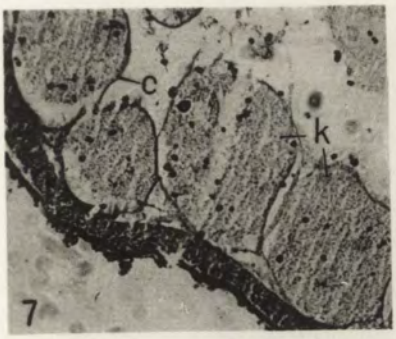

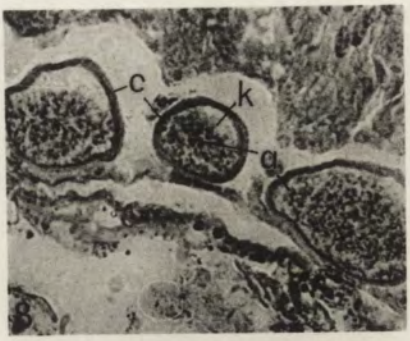

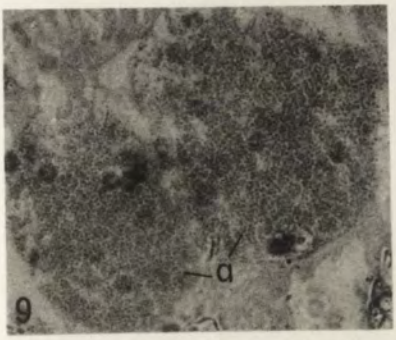

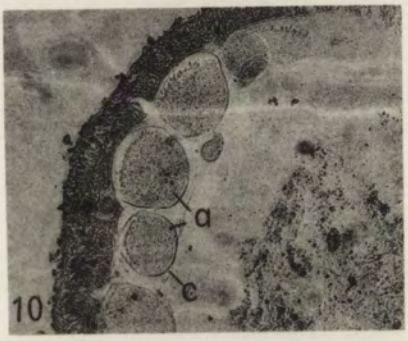

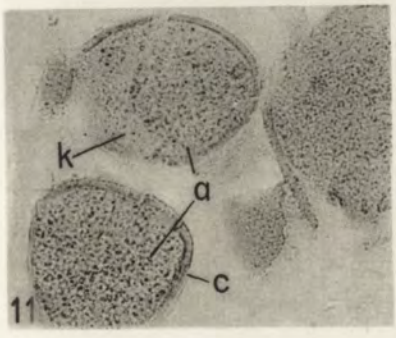

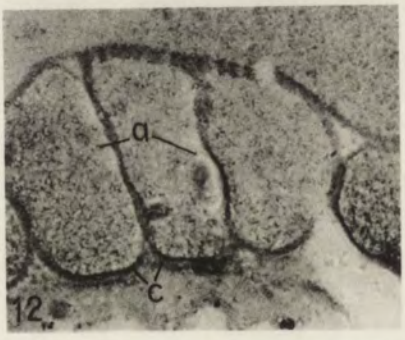

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VOL. XIII WARSZAWA 31.X.1975 FASC. 37

Department of Biology, Semmelweis University of Medicine 1094, Budapest, Tüzoltó 58, Hungary

György CSABA and Tibor LANTOS

Effect of Amino Acid and Polypeptide Hormones on the Phagocytosis of *Tetrahymena pyriformis*

Aminósav és polypeptid hormonok hatása a *Tetrahymena pyriformis* phagocytosisára

There has been little known so far on the fine mechanisms representing the basis of the control-system of *Protozoa*. Kostojanc (1955) assumes that the impulse reactions of *Protozoa* and the nervous function of multicellular organisms have very similar physiological basis. In his opinion the basic characteristics of the cellular function are present also in the *Protozoa* and these general and basic characteristics of the cell develop the neurophysiological mechanism, undergoing evolution and selection. Investigations of last years indicated actually, that the hormone-like mediator substances functioning in the transmission of nerve impulses, might be detected in the *Protozoa* too. Janakivedi et al. (1966) isolated adrenalin and noradrenalin from *Tetrahymena* and observed that these protozoan incorporated labelled phenylalanine, thyrosine and dopamine, constituents of catecholamines. Iwata and Kariya (1973) demonstrated the presence of monoamine-oxydase in *Tetrahymena*, and Lantos (1967) described earlier the function of acetylcholine-cholinesterase system in this species. All these examinations refer to, that hormone-mediators characterizing the higher organisms, occur also in the *Protozoa*.

Studies on the efficacy of hormones introduced in protozoan, throw light upon this problem from another side. Acetylcholine and triiodothyronine influence the protozoan according to certain data (Blum 1967, Karlson 1972) and our earlier investigations (Csaba and Lantos 1973) show, that simple hormones may also have specific effect on them, corresponding to that exerted in higher organisms. Histamine, a hormone influencing the phagocyte cell in higher organism (Jancsó 1955, Lison 1949, Kuschinsky 1955) was found to enhance the phagocytosis of *Tetrahymena* significantly and the same effect was displayed by the physiologically similar serotonin. On the other hand indole acetic acid, a plant hormone which is related to the latter, was found ineffective. These experiments suggested that protozoan possess defined receptors of significant specificity. The aim of our present experiments was to obtain new information on the specificity of these receptors.

Materials and Methods

Two days old organisms of the *Tetrahymena pyriformis* GL strain, cultured in 0.05% yeast extract-containing 1% Bactotrypton medium, were used. Twenty four hours previous to the experiment, the animals were separated by centrifugation from the medium and kept hence in Losina-Losinsky (1931) solution.

Experiments were carried out identically on six different occasions. The vacuole-free *Tetrahymenae* have been treated for 3 min in solutions of different molarities of the hormone preparations tested, subsequently they were fed with India ink and smears were prepared after quick drying. Vacuoles were counted in 50 *Tetrahymena* organisms in each experiment and each concentration, under microscope. Mean values were calculated, the phagocytic index (average number of India ink containing vacuoles in one animal) and phagocytic coefficient were determined, the latter being the ratio between the phagocytic index in experimental and control animals.

The following hormones were tested: thyroxine hormone-group (Thyroxine-Roche, solutions diluted from 1 M to 10^{-20} M) mono- and diiodothyrosine as well as triiodothyronine (Reanal dilutions from 10^{-2} to 10^{-16} M), adrenalin (Tonogen-Richter – dilutions from 10^{-1} to 10^{-21} M) and insulin (Richter – dilutions from 10^{-1} to 10^{-18} M, which correspond to 40 IU/ml to 4^{-14} IU/ml concentrations.

Results

All the hormones inhibited in high concentration the phagocytosis of *Tetrahymena*. Out of the iodine hormones (Fig. 1) the thyroxine caused the strongest and longest inhibition. At 10^{-9} M concentration the phagocytic

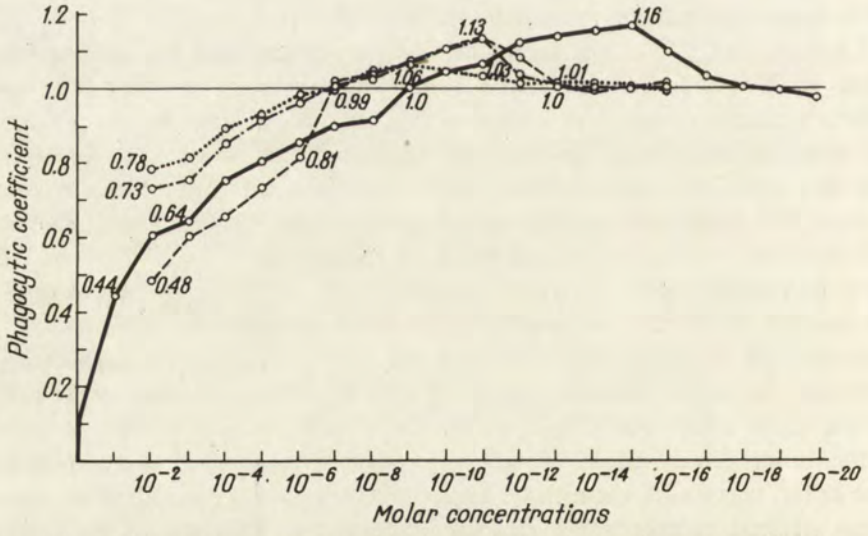

Fig. 1. Effect of iodine-hormones on the phagocytosis of *Tetrahymena pyriformis*. ——— Thyroxine, —·—· 3-Mono-iodo-L-Tyrosine, ... 3,5-diiodo-L-Tyrosine, – – – – – – 3,3'5-Triiodo-L-Thyronine

capacity rose to the control level. Subsequently its enhancing effect was increasing up till the concentration 10^{-15} M, then it started to decrease slowly. The inhibition of mono- and diiodothyrosine was slighter, it reached sooner the control level and displayed maximum effect at 10^{-11} M concentration. The inhibiting effect of triiodothyronine rose parallel with that of the mono- and diiodothyrosine up till the level of control, its enhancing effect was, however, very slight.

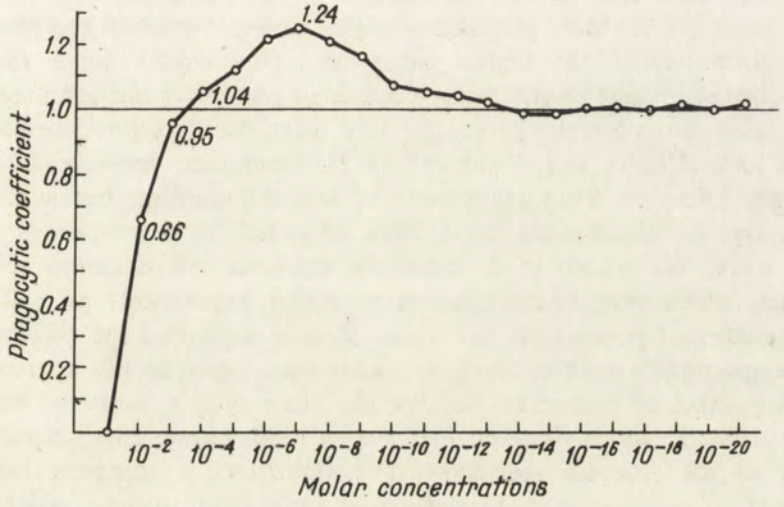

Fig. 2. Effect of adrenalin on the phagocytosis of *Tetrahymena pyriformis*

Adrenalin inhibited phagocytosis only (Fig. 2) in the highest concentrations, while stimulated it between 10^{-4} and 10^{-10} M concentrations. It displayed the strongest stimulation at 10^{-8} M concentration.

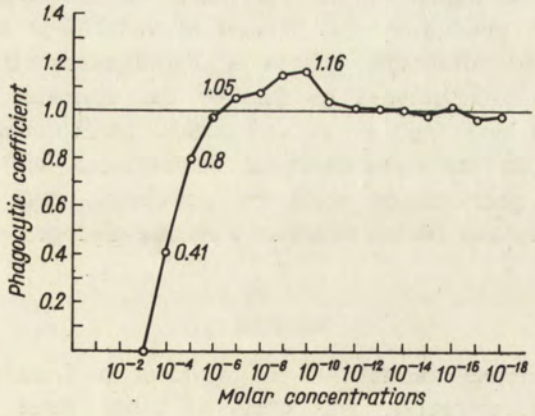

Fig. 3. Effect of insulin on the phagocytosis of *Tetrahymena pyriformis*

The effect of insulin (Fig. 3) reached after the initial strong inhibition the control level at 10^{-6} M concentration, exerting the strongest stimulation at 10^{-10} M dilution.

Discussion

Findings mentioned in the introduction (Csaba and Lantos 1973) led to the assumption, that protozoan might possess specific receptors for certain hormones of the higher organisms. This would mean that the receptor, being present originally as the defined pattern of the cell membrane or cytoplasm, shows receptor properties only, when the corresponding hormone attaches to it (Csaba and Bierbuer 1973). Hormones, considered to have no specific effect on the phagocytosis of higher organism, but stimulating significantly the metabolism, have been subjected to investigations in the present work. We wished to continue the examinations of amino acid-type hormones, which were found effective in earlier experiments as well as to study the effect of polypeptide hormones. Results supported the possibility of receptor specificity, since none of the hormones displayed the approx. 40% enhancing effect of histamine, the specific phagocytosis hormone tested in earlier studies. Findings obtained with the iodine-hormone group pointed out likewise to the receptor specificity. The thyroxine, a complete hormone form with 4 iodine atoms, proved to be only slightly more potent than the mono- and diiodothyrosine, which deviated markedly from the other three iodine-thyrosines in toxicity and effective concentration range. It showed maximum effect in a concentration as low as containing only a few hormone molecules. As to whether this effect was due to the increase of metabolism, no answer could be obtained from the present experiments.

The effect of adrenalin was stronger than that of the iodine-hormones, which might be explained by the increase of metabolism as well as by the stimulation of the adrenergic system of *Tetrahymena* (Blum 1967). The effect of insulin corresponded to that of the thyrosine, being effective, however, only in very high doses and within small concentration ranges. Further studies on the metabolism of *Tetrahymena* will probably throw light upon this phenomenon with the possibility, that as a secondary effect the phagocytosis occurs together with the glycogen storage.

Summary

Thyroxine increases the rate of phagocytosis in *Tetrahymena pyriformis* even in low concentration. The effect of other three iodine-hormones (monoiodothyrosine, diiodothyrosine and triiodothyronine) is somewhat

slighter and requires essentially higher concentration. Adrenalin and insulin have also enhancing effect on the rate of phagocytosis. The fact that the enhancing effect of these hormones fails to reach the intensity of the histamine refers to the presence of specific cell receptors.

ÖSSZEFOGLALÁS

A Tetrahymenák phagocytozisa thyroxin hatására igen alacsony koncentráció alkalmazásakor is fokozódik. A másik három jód hormon – a mono és dijódtyrosin és trijódthyronin – esetében a hatás valamivel csekélyebb, és lényegesen nagyobb koncentrációkat igényel. Az adrenalinnak és inzulinnak ugyancsak van serkentö hatása. Mindezek a hatások nem érik el a hisztamin korábban kimutatott intenziv serkentö hatását, ami specifikus receptorok jelenlétére utal.

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Received 27 September 1973

ACTA PROTOZOOLOGICA
VOL. XIII WARSZAWA 31.X.1975 FASC. 38

Department of Zoology, Parasitology Section, University of Edinburgh, Great Britain

O. O. DIPEOLU[1]

The Infectivity to Tsetse Flies of Different Antigenic Variants of *Trypanosoma brucei*

Die Infektivität zu Tsetsefliegen der verschiedenen Antigenvarianten von *Trypanosoma brucei*

Although much is known of many of the factors which influence the susceptibility of tsetse flies to trypanosomes, such as temperature (Ford and Leggate 1961, Kinghorn and Yorke 1912, Kinghorn, Yorke and Lloyd 1913); age of the fly (Wijers 1958 a, 1958 b); morphology of trypanosomes in the infective feed (Wijers 1959, Wijers and Willett 1960); and the strain of the trypanosomes species (Robertson 1912); it is not yet known whether different antigenic variants of trypanosomes are different in their infectivity to tsetse flies. This factor could be of importance because results of the previous workers are based on the experimental procedure in which flies were infected by feeding them on the same animals at different times during an infection and the results of the different feeds were compared with one another.

Gray (1962) and Wilson (1968) had shown that different antigenic variants of *T. brucei* are produced at intervals of about 5–6 days in an animal host. The tsetse flies fed at different intervals during the course of infection in the animal hosts are therefore likely to be exposed to different antigenic variants. Hence, the results of different feeds may not be strictly comparable.

This investigation was conducted to ascertain whether the different antigenic variants of *T. brucei* influence the infection rates of this species of trypanosomes in the tsetse.

Materials and methods

The experiments were conducted with derivatives of *Trypanosoma brucei* TREU 667. This strain was isolated by the Edinburgh Veterinary Expedition (EVE) to East Africa in 1966 (Reid et al. 1970). On receipt of the stabilates at the Centre for Tropical Veterinary

[1] Present address: Department of Veterinary Pathology, University of Ibadan, Nigeria.

Medicine (CTVM) of Edinburgh University, 2 rats were infected and the stabilates made from them designated TREU 667. There were four other passages through mice and at the 5th passage the infected blood was preserved and designated 667-A. The concentration of trypanosomes per ml of blood calculated through haemocytometer counts before preservation was 5×10^8 for 667-A.

Glossina morsitans orientalis Vanderplank were used. These were maintained at 26° C and relative humidity of 65–70%. The experimental flies after sexing, were placed in individual polystyrene cages of 1/2 inch diameter (Mews 1969). The first feed of the flies was the infective feed given within 36 h of eclosion. The following day female flies were mated. Thereafter, flies were offered food on alternate days but flies that did not feed within 10 min were removed and offered food daily until a meal was taken.

A semi-lop eared rabbit was inoculated intraperitoneally with a high dose of stabilated population of 667-A (5×10^6 trypanosomes). On the 5th, 10th, 15th and 20th days after infection as many flies as were available were allowed to feed on the rabbit. Immediately afterwards, about 2 ml of blood was bled from the rabbit's ear and 0.3 ml of this was injected intraperitoneally into each of 3 mice. A tiny drop of the blood was examined under the microscope for the presence of trypanosomes. When the parasitaemia in the mice was high, they were killed and stabilates prepared from their blood. Stabilates were prepared in lymph tubes as described by Cunningham, Lumsden and Webber (1963) and haemocytometer counts of the concentration of trypanosomes were made before preservation. Some of the stabilates prepared from the mice which had been infected with rabbit blood bled on the 5th and 20th days after infection, were used to infect further groups of flies through artificial membrane feeding technique. Stabilates were diluted with defibrinated ox blood at a ratio of 1 : 100. This technique had been described by Dipeolu (1972).

In each experiment flies were separated into groups of ten. From the 3rd to the 12th day after the infective feed, one fly from each group was killed each day and examined for trypanosomes. Initially, flies in which trypanosomes were found in the midgut were recorded as being "infected". Later, a distinction was made between flies that were "infected" and those with "established" infection. The criteria for this distinction had been described (Dipeolu 1972). Results were compared by calculating X^2 by means of a 2 by 2 contigency table. Because the total number for comparison was less than 200 in each case, Yates correction was applied (Fisher 1941).

Results

On each occasion when the rabbit was bled not more than one trypanosome was seen in 150 microscope fields but the mice into which a portion of the blood was injected became parasitaemic 3–4 days later. A similar observation was made by Gray (1962). The infection rate among flies which fed directly on the rabbit 5 days after infection was significantly higher than those of the flies which fed 15 and 20 days after infection (Table 1).

No significant difference was observed in the proportion of flies that were infected or carried established infections when stabilates prepared from the parasitaemic mice of the 5th and 20th days rabbit inoculation were used. (Table 2).

Table 1
Comparison of Infection Rates in Flies that Fed on Different Days Directly on Infected Rabbit

No. of days after infec.	Total flies	Total infected	% infected	Values of X^2 and P
5	30	8	26.7	
10	60	8	13.33	*3.430 P < 0.10
15	40	4	10	*4.628 P < 0.05
20	70	9	13	*3.901 P < 0.05

* Value of X^2 is obtained by comparing the infection rate with that of flies that fed on rabbit 5 days after infection.

Table 2
Comparison of Infection Rates in Flies that Fed on Stabilated Populations of 667-A

Designation of stabilate	Conc. of trypano. per ml. infective feed after dilution with ox blood	Total flies	Total infected	Total estab.	Values of X^2, P
5th day mice population of 667-A	5.12×10^6	50	19	6	
20th day mice population of 667-A	5.36×10^6	50	16	3	*1.953 P < 0.20

* Value of X^2 is obtained by comparing the number of established infections with that of the 5th day mice population of 667-A.

Discussion

From the conclusions of Gray (1962) and Wilson (1968) it can be assumed that the flies which feed directly on the rabbit, 5, 10, 15 and 20 days after infection had ingested trypanosomes of different antigenic strains. Among these, it appears that the 5th day variant has the highest rate of infection to flies. However, the infection rates in flies which had fed through the membrane on the stabilated populations of the 5th and 20th day variants

are similar. Wilson (1968) showed that when blood containing a particular antigenic variant of *T. brucei* is injected into a susceptible animal host, the antigenicity of the trypanosomes of the first parasitaemia in the susceptible host is the same as that of the inoculated blood. Hence, the flies which had fed directly on rabbit 5 days after its infection and those that had fed on the stabilated populations of the 5th day blood injection into mice ought to have probably fed on trypanosomes of similar antigenic variants. The same could be said of flies which fed on the rabbit 20 days after its infection and those that fed on the 20th day blood injection into mice. It would therefore have been expected that the infection rate among flies that fed on the stabilated population of the 5th day mice inoculation will be significantly higher than that of those that fed on the stabilates of the 20th day mice inoculation since such was the observation made when flies were fed directly on the rabbit 5 and 20 days after its infection.

The disparity between the results is probably due to the difference in the method used in infecting the flies. When they were fed directly on rabbit variables such as the fluctuation in the number of trypanosomes in the peripheral blood and the rabbit's antibody response could not be controlled. These factors were eliminated when the flies were fed on the stabilated populations through the membrane. It appears therefore that the results obtained when the flies were fed directly on the rabbit were influenced by factors other than the difference in the antigenicity of the trypanosomes.

Summary

Glossina morsitans were fed directly on a rabbit 5, 10, 15 and 20 days after it had been infected with *Trypanosoma brucei*. The results showed that the infection rate was highest among flies which fed on the rabbit 5 days after it was infected. On each occasion after the flies had fed 2 ml of blood was bled from the rabbit's ear and 0.3 ml of these was injected into each of 3 mice. Stabilates were prepared from the parasitaemic blood of these mice. Some stabilates of the 5th and 20th day rabbit to mice blood innoculations were diluted 1 : 100 with defibrinated ox blood and fed to flies through the chicken skin membrane. The rates of infection among flies that fed on the stabilated variants were similar. It was concluded that the latter are more reliable and the factors which affected the former results are discussed.

ZUSAMMENFASSUNG

Ein Kaninchen wurde mit *Trypanosoma brucei* artifiziel infiziert und 5, 10, 15 und 20 Tage danach liess ich *Glossina morsitans* an dem Kaninchen saugen. Das Ergebniss zeigte, dass das Infektionsrate mit Trypanosomen am höchsten innerhalb der *G. morsitans* ist, die an dem

Kaninchen 5 Tage nach Infektion gesaugt hatten. Gleich nach dem Saugen wurde jedesmal 2 ml. Blut von einem Ohr genomen und 0.3 ml der Menge in drei Mäuse eingespritzt. Nachdem viele Trypanosomen in Blut der Mäuser erschienen hatten, wurden sie getötet und ihr trypanosomreiches Blut in flussigem Stickstoff konserviert. Dabei wurden vier Antigenvarianten von *T. brucei* konserviert. Die funftage Variante wurde mit defibriniertem Rinderblut in Verhaltnis 1 : 100 vermischt und an dieser Suspension wurden *G. morsitans* durch Huhnhautmembran saugen. Dasselbe wurde fur die zwanzigtage Variante gemacht. Die Infektionsraten der Tsetsefliegen, die an den beiden Antigenvarianten gesaugt haben, waren nicht von bedeutsamer Abweichung. Es ist geschlossen, dass die letzte Ergebnisse mehr zuverlässig sind, und die Faktoren, die die ersten Ergebnisse beeinflüssten, sind diskutiert.

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Received 13 February 1974

ACTA PROTOZOOLOGICA
VOL. XIII WARSZAWA 31.X.1975 FASC. 39

Department of Cell Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, 02-093 Warszawa, Poland

Jerzy SIKORA

A New Method of Locomotion Arresting in some Ciliates without Ciliary Immobilization

Une nouvelle méthode d'arrêter la locomotion chez quelques Ciliés sans immobilisation des cils

Many ciliates physiologists have been searching for a method suitable to prevent swimming, or to hold the moving cell (Wichterman 1953, Sonneborn 1970, Vivier 1974). There was specially great demand for a method which would allow observations of the single specimen at the time of exposing it to the experimental solution, or soon after the exposure. The use of light microscope for examining alive immobilized specimen was possible by means of a variety techniques like: physical compression in microcompression chamber (Wichterman 1940), increase of medium viscosity (Marsland 1943, Brown 1944), anesthetics (Bills 1922, Sonneborn 1970), nickel ions (Kuźnicki 1963), antiserum against ciliary surface antigens (Kuźnicki and Sikora 1971). All these techniques did not allow an easy exchange of the medium surrounding observed specimen. The insertion of microneedle by means of the micromanipulator (de Fonbrune 1949) seem to be the best choice for that purposes (Kinosita et al. 1964), however, this device is expensive, time-consuming and the use of it needs some experience. The simple method for locomotion arresting of some ciliates has been worked out. It allows observation of the chosen subject by means of light microscope even under high magnification with simultaneous exchange of the surrounding medium. The advantage of proposed method is its simplicity, low expense and wide applicability. High proportion of holded animals by means of the proposed method survives without any visible signs of pathological changes.

Supported by Polish Academy of Sciences (PAN-22)

Description of the Method

The method of locomotion arresting is applicable to ciliates capable to form food vacuoles filled with ferromagnetic particles, and consist in applying the source of magnetic force as close as possible to the specimens. Minute spherical iron particles (from 0.5 to 5 μm in diameter) were fed by ciliates, which had been previously starved for 2–3 h in maintenance solution (4 mM Na-phosphate buffer of pH 6.4). Few minutes after such feeding, some of food vacuoles were filled by iron particles. Animals with "iron" food vacuoles and some particles in excess (Pl. I 1) were put on microscopic slide and sealed under cover glass, and only two opposite edges of the cover glass have to be covered by vaseline (Fig. 1). When the weak

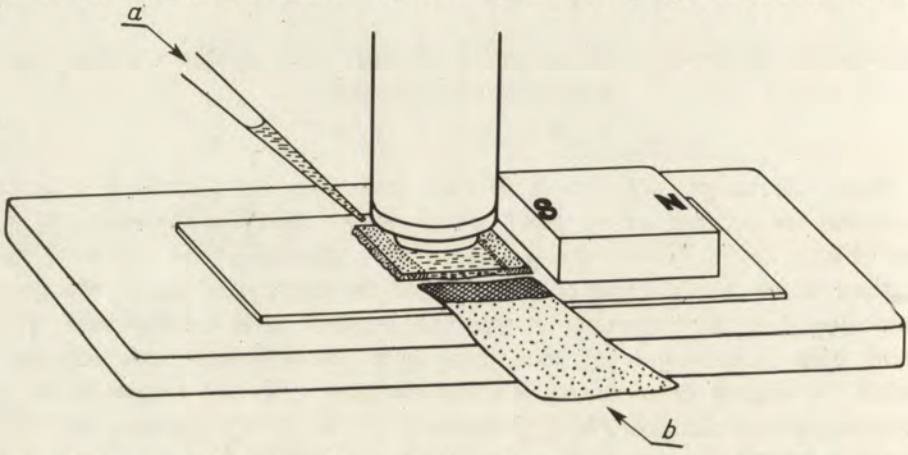

Fig. 1. Diagram of ciliates locomotion arresting device allowing to wash the specimen with experimental solution. The cover glass with the two edges covered with vaseline. Stage of microscope and objective made of brass: a – pipette with the experimental solution, b – stripe of filter paper

magnetic force is applied (5.5–8.5 mT), the iron particles were set along the magnetic field lines. After short period of time the animals possessing "iron" food vacuoles attach to the strands of combined iron particles which became magnets in the presence of magnetic field (Pl. I 2). Anchored animals could be easily washed by experimental solution and observed by means of light microscope even under high magnification (Pl. II 3). Excess of given solution have to be filled in at the one open side of cover glass and sucked out carefully with hard filter paper at the other side (Fig. 1). Provided, that washing has been done little by little, it could be repeated several times. Number of stocks of ferromagnetic particles had been used to feed *Paramecium aurelia, Paramecium caudatum, Paramecium bursaria* and *Tetrahymena pyriformis*. According to the presented above method for

holding ciliates, the reduced iron particles (E. Merck AG, Darmstadt, pro analysis), proved to be most proper (Pl. II 4). There were no signs of the pathological changes of ciliates anchored at the rear side of the body, for several hours, as well as no toxic influence of reduced iron put into culture of ciliates for few days.

The changes in physiology of the ciliates exposed to the magnetic field reported by (Brown 1962, Kogan and Tikhonova 1965, Kogan et al. 1968, Rostkowska and Moskwa 1968, Isquith and Bobrow 1973), might be neglected in the case, because the range of the used intensity of magnetic field induction never excessed 10 mT.

The proposed method of locomotion arresting in some ciliates seems to be useful in studies on: uptake and digestion of food vacuoles, permeability of membrane and water exchange, mechanism of ciliary beating, intracellular cytoplasmic streaming, electrophysiology, microinjection etc.

Summary

In the described method low intensity magnetic field is applied to "anchor" ciliates with ingested ferromagnetic particles to the ferromagnetic particles which became magnets in the surrounding medium.

RÉSUMÉ

Une méthods très simple est décrite d'immobilisation des certais Ciliés. En utilisant le champ magnétique assez faible on fait "ancrer" les Ciliés qui ont formé vacuoles digestives remplies particules ferromagnétiques. Les particules ferromagnétiques qui restent dans le milieu ambiant agissent comme des aimants sur ceux qui sont contenues dans les vacuoles.

Acknowledgement

The author wishes to express his special thanks to Dr. Noburo Kamiya for help in obtaining the stock of ferromagnetic particles from: Kantokagaku Co., Toda Kogyo Co., Junsei Kagaku Co., Nippon Kemiraito Kogyo Co. The cooperation of the staff of the Laboratory of Electron Microscopy, M. Nencki Institute of Experimental Biology is highly appreciated.

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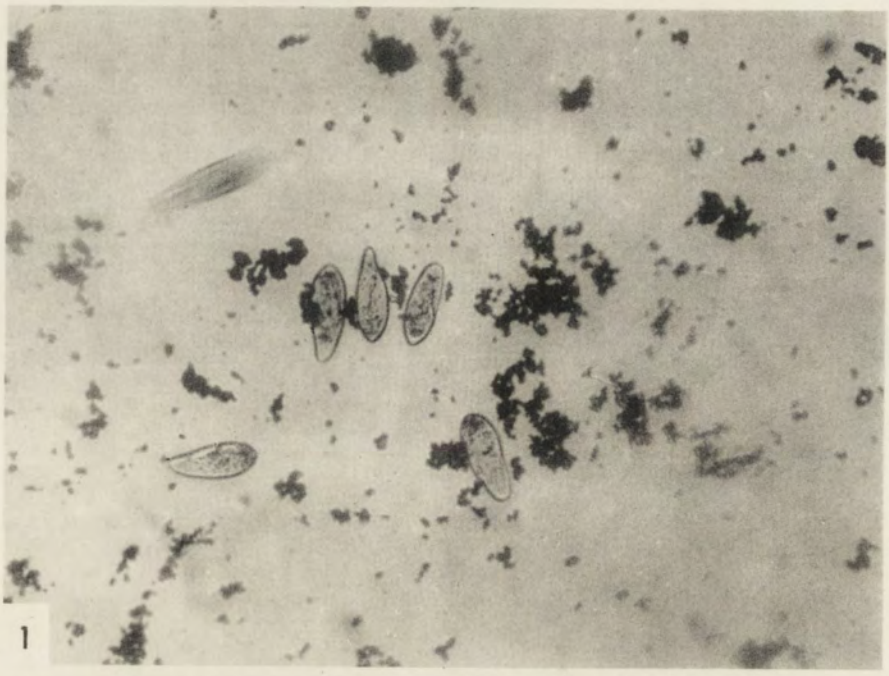
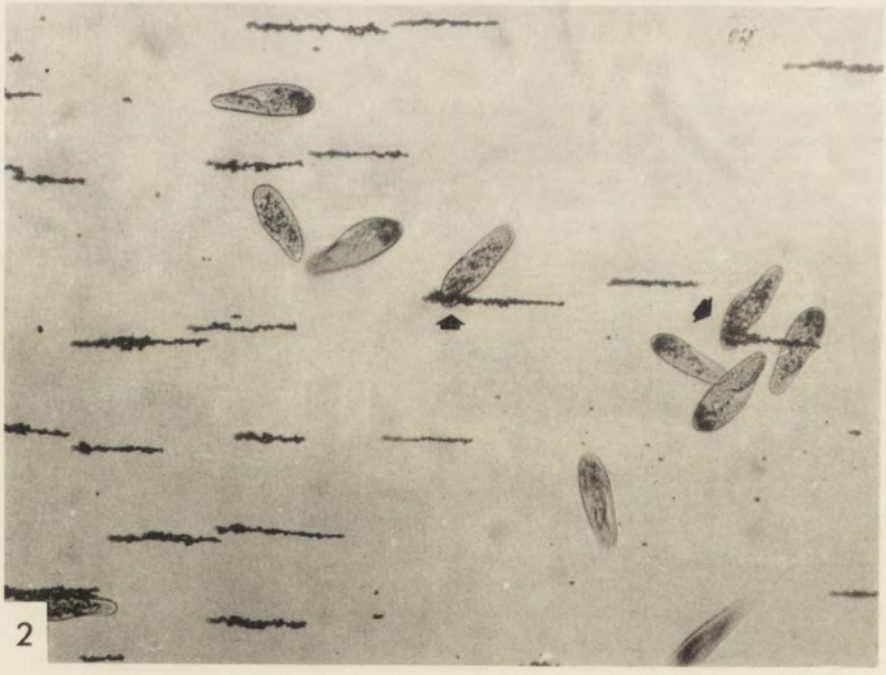
Received 2 September 1974

EXPLANATION OF PLATES I–II

1: Randomly distributed ion particles in medium with freely swimming *Paramecium aurelvlia* before applying magnetic field
2: Iron particles set along the magnetic field lines of 5.5–8.5 mT induction. Some ciliatetes (arrows) anchored to the strands
3: *Paramecium aurelia* with iron particles which became magnets in the presence of externnal magnetic field
4: Scanning electro microscope image of the reduced iron particles used for ciliates feedining





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