ACTA PROTOZOOLOGICA

VOLUMEN XII
Fasciculi: 22–32

W A R S Z A W A 1 9 7 4

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 22

Nencki Institute of Experimental Biology, Department of Cell Biology,
00-973 Warszawa, Pasteura 3, Poland

Maria JERKA-DZIADOSZ

Cortical development in *Urostyla*. II. The role of positional information and preformed structures in formation of cortical pattern

Rozwój struktur powierzchniowych u *Urostyla*. II. Rola informacji pozycyjnej i struktur preformowanych w tworzeniu wzoru powierzchniowego

The cortical structures of ciliates consist of the kinetosomes and cilia with accompanying microtubular fibers, microfilaments and kinetodesmas. The ciliature form two functionally differentiated categories: the oral and somatic ciliatures, and usually two topographic categories: dorsal and ventral ciliatures.

The precise arrangement of all cortical organelles is usually described as the "cortical pattern". This pattern is transmitted faithfully from one generation to another during binary fission, and also is reconstructed in fragments of organisms created by microsurgical isolation.

The formation of cortical pattern may be arbitrally divided into two processess:

(1) Positioning of the primordia of ciliature, that is the process leading to the determination of the place of initial appearance of a rudiment of new group of ciliature, (e.g., AZM).

(2) Differentiation, that is development of a primordium that has already been established (proliferation of kinetosomes, ordering and morphogenetic movements).

In the literature, the problem of positioning as a process of establishing the site of formation of a primordium has been considered jointly (except Frankel 1973 b, 1974) with other aspects of cortical development.

The faithful and precise transmission of the cortical pattern, and in a sense, its independence from the genotype, were among the premises of the ideas that the cortex of ciliates is autonomous (Tartar 1962, Sonneborn 1963). According to this idea the factors responsible for positioning, orientation and differentiation of ciliary primordia are situated within the cortex itself (Wise 1965, Sonneborn 1970 a, b). These notions have been summarized by Gibson: "The precise position

This work was supported by the Polish Academy of Sciences within the project PAN-22.

and orientation of the new structures with respect to the old structure is determined by the old organelle or structure and not by the surrounding geography of the new organelle" (Gibson 1970). That this statement is not true for at least for *Hypotrichida* will be shown in this paper.

Experimental study of localization of oral primordia in different fragments of heterotrich ciliates such as *Stentor*, *Blepharisma*, *Condylostoma* and *Spirostomum* lead to the application of the gradient theory of Child 1941 to ciliate development (Weisz 1956, Suzuki 1957, Tartar 1961, 1962, Eberhardt 1962, Uhlig 1959, 1960, Schwartz 1963). It was stated that localization and orientation of the oral primordium depend on induction-inhibition interactions involving cortical fields and gradients. It was also assumed that the cortical structures themselves are responsible for the polarization of the cortex and the maintenance of gradient (Tartar 1962). Many investigations based on the gradients and field hypothesis (so called pattern hypothesis, Wise 1965) made by numerous authors yielded interesting descriptive data concerning the localization and development of the primordia of new ciliature in many ciliated *Protozoa* (for Hypotrichs to quote only Hashimoto 1961, 1963, 1964, Wise 1965, Frick 1967, 1968, Jerka-Dziadosz and Frankel 1969, Tuffrau 1970). They, however, did not supply any testable hypothesis as to the mechanisms by which the primordia are positioned.

In order to analyse the formation and regulation of pattern in multicellular systems Wolpert 1969 formulated the conception of "positional information". According to this a cell differentiates in accordance to its position in the developing system. This position is specified according to a coordinate system which often is topographic in character. The system of coordinates is formed by a gradient of some parameter and by boundary values of that gradient. Regeneration considered in these terms (Wolpert 1971) means reconstruction or formation anew of positional information in a disturbed system — that is the reconstruction of boundary values and/or the form of the gradient. A gradient which gives positional information could be provided by a "sink" at one boundary and a "source" at the other (Crick 1970), the morphogenetic substance diffusing between them. The formation of pattern is a two step process, where (1) a cell specifies its positional information in the coordinate system, (2) the interpretation of positional information leads to molecular differentiation.

If one passes now from the multicellular system to unicellular system — such as a ciliate — then one may speak about parts or regions of a cell which acquire positional information in a coordinate system, which in turn covers the whole cell.

As far as differentiation is concerned, it is well known that the development of the ciliary primordia is under the influence of preformed organelles. "Ordering and arranging of new cell structure under the influence of preexisting cell structure" — Sonneborn 1964 defined as "cytotaxis". That means, that the organization and selection of gene products are determined by preexisting organelle (Sonneborn 1970 a, b). This conception was supported by the observations on the maintenance

of polarity by reversed kineties in *Paramecium* (Beisson and Sonneborn 1965), and by observations on the formation of new kinetosomes in *Paramecium* and *Tetrahymena* (Dippel 1968, R. D. Allen 1969, Chen-Shan 1969). The study on the representatives of other groups of ciliates revealed, that kinetosomes may appear in any orientation with respect to preexisting ones (Grimes 1973 a). Grimes (1973 c) found that the complete cortical pattern of single and double forms of *Oxytricha fallax* can be reconstructed from encysted cells — in which he failed to detect any kinetosomes. It seems therefore that the positioning of primordia and their further development may proceed without the "help" of preexisting ciliary organelles.

The first part of this study (Jerka-Dziadosz 1972 b) dealt with the study on morphogenesis, i.e., formation and development of ciliary primordia in two hypotrichous ciliates *Urostyla grandis* and *Pseudourostyla cristata*. The present paper contains an experimental analysis of positioning of ciliary primordia in longitudinal fragments of *Urostyla grandis*, *Paraurostyla weissei* and *Pseudourostyla cristata*. In the Discussion an attempt is made to describe the formation of the cortical pattern in *Hypotricha* in the general framework of positional information in terms formulated for developing systems of *Metazoa* by Wolpert (1969, 1971), Lawrence (1966, 1971 a, b) and Crick (1970).

A sand hill model has been constructed to provide a formal explanation of the positioning of ciliary primordia in hypotrichs.

Material and methods

Observations presented in this paper were performed on the same organisms as that described in the first part of this study (Jerka-Dziadosz 1972 b) and in an earlier paper (Jerka-Dziadosz and Frankel 1969).

Borror 1972 renamed two of the species of *Urostyla*. *Urostyla weissei* belongs now to the genus *Paraurostyla* Borror, 1972 (*Urostylidae*) and *Urostyla cristata* belongs to genus *Pseudourostyla* Borror, 1972 (*Holostichidae*). The organisms used in this investigations were clones of *Urostyla grandis* (Ehrbg.), *Pseudourostyla cristata* (Jerka-Dziadosz) and *Paraurostyla weissei* (Stein). *Urostyla grandis* was collected in pond on Sadyba (Warsaw, Poland). *Pseudourostyla cristata* was collected in Zaborów near Warsaw. The culture method for both species were described in the first part of this study (Jerka-Dziadosz 1972 b). *Paraurostyla weissei* was collected in October 1968 from Swan Lake (Johnson County, Iowa, USA).[1] *U. grandis* and *P. weissei* were kept in sterilized Pringsheim's solution and fed daily with washed *Tetrahymena pyriformis* growing axenically in 2% proteoso peptone. *P. cristata* was

[1] Part of the experiments with longitudinal fragments of *P. weissei* was performed during my stay in Iowa University Zoology Department, Iowa City, Iowa USA.

fed every other day with dried lettuce infusion incubated with *Aerobacter aerogenes* diluted with Pringsheim's solution.

The organism were operated by hand under a stereoscopic dissecting microscope, at 60 × magnification. Cells were cut with microknife made from a dental needle. Ciliates were transected in the Petri culture dish and then immediately passed by micropipette to a depression slide containing the culture medium. The fragments were kept in a moist chamber and observed under the dissecting microscope at intervals. Small groups of fragments were fixed at various times after operation.

The protargol method after Dragesco (1962) with the modification described earlier (Jerka-Dziadosz and Frankel 1969, Jerka-Dziadosz 1972 b) was used in this study. The slides were observed under the Leitz Ortholux microscope. Most of the data presented in this paper are based on stained slides. The observations on living material were carried out on fragments isolated into small drops of culture fluid and covered by paraffin oil to prevent evaporation. The observations of wound healing were made this way. Each stained fragment was drawn in the paper chart, and photographed at different focal levels. The drawing and the photographed material where later analysed.

The sand hill model was prepared from grains of river sand. The sand was sifted out through double sieve, then washed in running tap water an dried thoroughly. The diameter of grains was from 1 to 2 mm. In the wet-sand model, small amount of water was added to the dry sand and "kneaded", so the moisture was put uniformly among the sand grains. The models were prepared from the dry sand as well as of the wet sand.

Results

Before proceeding to the description of regeneration of fragments obtained by longitudinal transection, the brief description of morphology and morphogenesis of the studied species will be given. Detailed descriptions have been published previously (Jerka-Dziadosz and Frankel 1969, Jerka-Dziadosz 1972 b).

The ventral surface of the studied hypotrichs in covered by the oral and somatic ciliature. The oral ciliature consists of the adoral zone of membranelles (AZM), which bounds the (animal's) left anterior portion of the cell, and two parallel undulating membranes (UM) which mark the (animal's) right margin of the peristome. The somatic ciliature contains meridionally arranged rows of cirri, which form three topographic groups: frontal, ventral and transverse (so called FVT complex); both the right and left edges of the ventral surface are covered by two groups of marginal cirri. The dorsal surface is ciliated by several longitudinally arranged dorsal kineties. Several caudal cirri are situated on the posterior tip of *P weissei* only.

In all three studied species, as in other hypotrichs, the processes of formation of the ciliary primordia are very similar in three developmental situations — i.e., binnary fission, reorganization, and regeneration of fragments obtained by transverse

operations. The anterior fragments (promers) and reorganizers form the primordia in a very similar fashion to proters (except the oral primordium, which forms as in the opisthe), whereas the posterior fragments — opimers, form oral and cirral primordia similarly to the posterior product of division. The short description of the localization of primordia in the reorganizers and regenerating promers or opimers is given, in order to provide a "control" for regenerating lateral (longitudinally transected) fragments. All the references to "left" and "right" correspond to the left and right sides of the ciliate — not to the right and left side of the viewer.

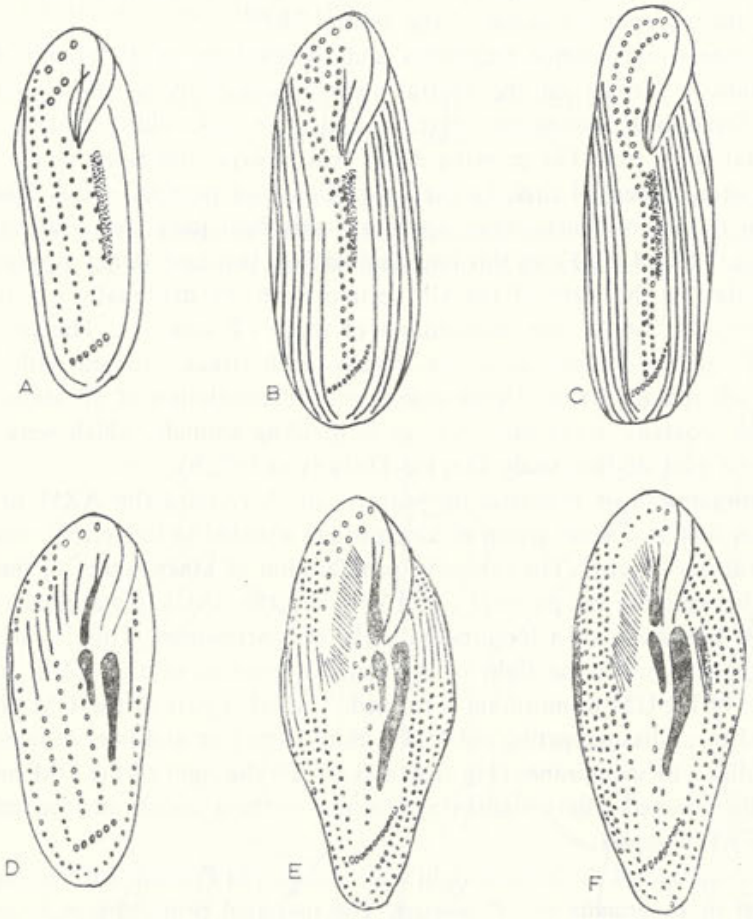

Fig. 1. Drawings of the ventral surface of the studied species A and D — *Paraurostyla weissei*, B and E — *Urostyla grandis*, C and F — *Pseudourostyla cristata*, A, B, C — specimens in first stage of reorganizational morphogenesis. Open circles represent the frontal, ventral and transverse cirri, longitudinal lines represent the marginal cirri. The longitudinal fields of fine dots represent the AZM primordium, located to the left of the postoral ventral row of cirri. D, E, F — cells in stage 2–4 of reorganization. The preexisting ciliature is indicated by open circles, while the streaks which will form the new cirri are shown as thin solid lines. The black fields represent oral primordia

In reorganizing *P. weissei* (Fig. 1 A) the oral primordium (Jerka-Dziadosz and Frankel 1969) appears on the ventral surface between the first left postoral ventral row of cirri and left marginal cirri. The primordia of the FVT complex (Fig. 1 D) are formed with participation of old frontal and ventral cirri. They appear as a longitudinally oriented ciliary streaks. There are usually 6 streaks (Pl. I 1). The marginal primordia are formed within old marginal rows (Pl. II 8). In this species the preexisting ciliature participates extensively in the formation of the primordia of cirri and dorsal cilia, whereas the oral primordium (AZM) at first forms without visible participation of preexisting cirri, and later on the growing primordium incorporates some of the ventral cirri.

In regenerating anterior fragments and reorganizers of *U. grandis* the AZM primordium is formed on the ventral surface posteriorly to the original AZM. The proliferation of kinetosomes starts around some of the old ventral cirri from the left ventral cirral row. The growing AZM field incorporates some of the more posteriorly situated ventral cirri. To the right of anterior portion of this field, another longitudinal field of kinetosomes appears — this field joins the disaggregated old UMs (Fig. 1 B and E). From this longitudinal field two undulating membranes later differentiate. To the right of the UM primordium the diagonally oriented ciliary streaks appear. This is the primordium of the FVT complex. The primordia of marginal cirri are formed as ciliary streaks, each streak situated within one old right or left marginal row. Development and differentiation of all kinds of ciliary primordia proceeds in the same way as in dividing animals, which were described in the first part of this study (Jerka-Dziadosz 1972 b).

In reorganizers or regenerating promers of *P. cristata* the AZM primordium appears at first as a small group of kinetosomes situated to the left of cirri from the left ventral row of cirri. The extensive proliferation of kinetosomes in the meridian situated between the left postoral cirral row and the first left marginal row brings about the formation of a longitudinal field of kinetosomes. This is the elongated AZM primordium. To the right of the anterior portion of the AZM primordium a portion of the UM primordium is formed. The other part of the UM primordium is derived from disaggregating old UM's. Both parts join and later differentiate into two parallel UM membranes (Fig. 1 C and F). To the right of the UM primordium diagonally oriented ciliary streaks are formed — these constitute the primordium of the FVT complex.

The formation of the left and right marginal cirri in *P. cristata* differs significantly from that in *U. grandis* and *P. weissei*. The marginal primordia in *P. cristata* are formed as a kinetosomal fields primarily situated within one of the seven old marginal rows on each side of the cell. The primordia of left marginal cirri in reorganizing cells originate from dispersion of several old marginal cirri from the first (closest to AZM) left marginal row. The primordia of the right marginal cirri originate from dispersion of several old cirri from the furthest right (furthest from the AZM) row of marginal cirri. New kinetosomes are subsequently added in both fields, and each

differentiate into seven ciliary rows giving rise to the new rows of left and right marginal cirri.

In the three species studied, in posterior fragments which lack the original AZM and UM, the UM primordium appears entirely from a single primordium situated to the right of and parallel to the AZM primordium (Pl. I 3). In small anterior fragments of *U. grandis* and *P. cristata* the AZM primordium is formed from the posterior portion of the kinetosomal field derived from disaggregation of the old UMs (Pl. I 2, 4) (compare the regeneration patterns in *Keronopsis rubra* described by Jerka-Dziadosz and Janus 1972).

Wound healing and cortical deformations after longitudinal transections

The fragments obtained by longitudinal transections may behave in different ways, causing several kinds of disturbances in arrangement of cortical structures. The differences are not species specific, and seem to depend only on the size of a

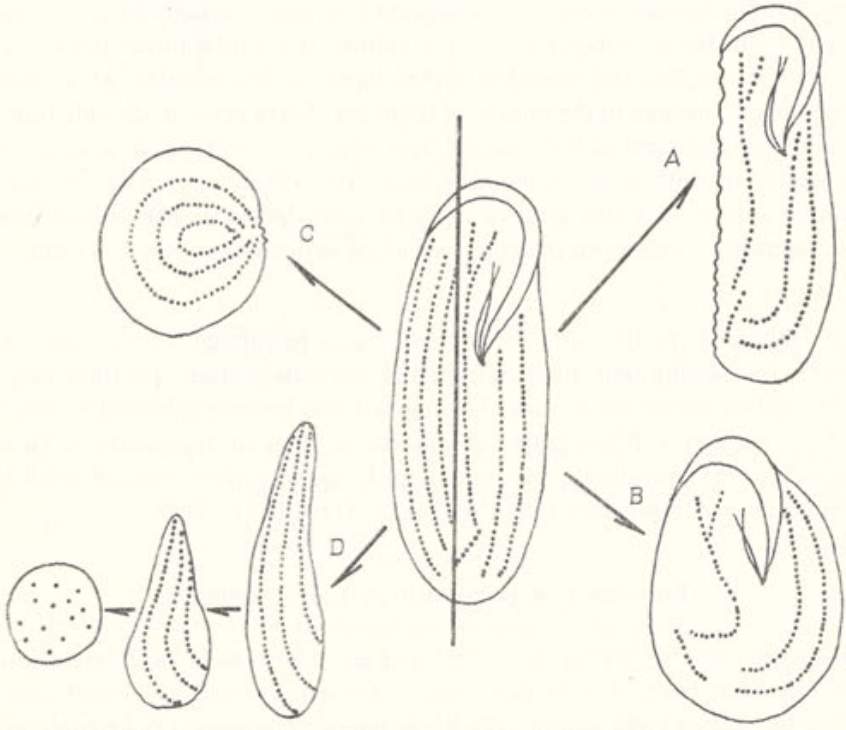

Fig. 2. Drawing of the ventral surface of the studied kinds of fragments derived from longitudinal transection. The dotted lines represent arrangement of ciliature in fragments. For explanation see the text

particular fragment. The same kinds of cortical deformations were observed in all three studied species.

Wound repair "in situ" (Fig. 2 A)

This mode is realized by large fragments obtained by excision of a long narrow slice on the lateral side of a ciliate. Such fragments do not change their shape significantly during healing. The longitudinal, meridional arrangement of cirral rows remains unchanged (at least in the anterior region, where the new primordia are going to be formed). There is a slight contraction of the wounded margin, as a result the posterior-most cirri can be shifted to the margin (Pl. II 5).

Slow constriction of the wound (Fig. 2 B)

This mode of wound repair occurs in most of the lateral, slightly oblique fragments. During the 2 h between the operation and the formation of the oral primordia, the wounded margin slowly contracts, and as a result some translocations of ciliature take place. Usually the fragments round up the undamaged margin.

Wound repair by folding two parts of the wound (Fig. 2 C)

This mode of healing is realized by long thin lateral fragments which fold together two parts of the damaged margin. This happens very fast — usually during the passing of fragments in the micropipette from the culture dish to the three spot depression slide. That means that the wound is healed during a few minutes. As a result the wounded part is located in the middle of fragment. There are considerable transpositions in the arrangement of the ciliature. The frontal and transversal cirri can come to be located in close neighbourhood to each other (Pl. II 6, 7). Very often a fragment may possess only one kind of marginal cirri (left or right) on both margins of the fragment as well as on the anterior and posterior ends of the fragment.

Rotating

This mode of reaction after operation is also performed by thin longitudinal fragments (possessing only marginal cirri). Immediately after operation they start a rapid rotating movement around the long axis and become spherical during 15–20 min. Such fragments desintegrate without visible sings of regeneration. This kind of reaction to the operation occurs mainly in *U. grandis* where most of small lateral fragments do not regenerate (see also Jerka-Dziadosz 1964).

Formation of primordia on lateral fragments

In this section the formation of oral and cirral primordia in different kinds of longitudinal fragments will be described in following order: (1) Large fragments with excised left or right margin, (2) Right lateral fragments, (3) Left lateral fragments. In each subsection fragments of *P. weissei*, *U. grandis* and *P. cristata* will be subsequently described.

Regeneration after lateral excisions

The aim of the performed operations was to describe the formation of oral and cirral primordia (particularly the marginal primordia) after excision of this old row (rows) of marginal cirri, which normally participate in formation of the primordia of marginal cirri. In this section fragments repairing the wound "in situ" will be considered.

Paraurostyla weissei

In fragments of this species, after excision of the left margin, the formation of oral primordia starts about 2.5 h after operation. The AZM primordium appears in the same place as in reorganizers (Fig. 3 R) as a longitudinal field of kinetosomes, situated close to the left postoral row of ventral cirri. Anteriorly and to the right

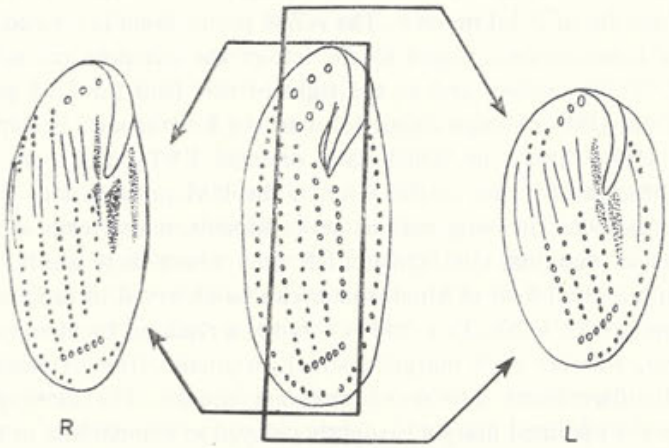

Fig. 3. Drawings of the ventral surface of *Paraurostyla weissei*. In the center — arrangement of ciliature in interdivisional specimens. L — left, R — right longitudinal fragments in stage 3–4 of regeneration. The old ciliature is indicated by open circles, while the streaks which will form new cirri are shown as solid lines and fields are represented by fine dots

of this field, another longitudinal field is formed, which later joins the field of kinetosomes originated from dispersion of the original UMs. This compound field constitutes the UM primordium.

The FVT primordia appear as meridional ciliary streaks. The first three streaks originate from disaggregation of frontal cirri No. 4, 5 and 6, the next three form within the rows of ventral cirri situated to the right of the frontal cirri.

The primordium of the right marginal cirri is formed within the old marginal row, in the mode described for the reorganizers (see Pl. II 8). The organized primordium appears as a ciliary streak (Pl. III 9).

In the same time, on the left wounded margin, where there are no old marginal cirri left the primordium is nevertheless formed anew at the topographical edge

of the animal. The primordium appears as a longitudinal field of kinetosomes — instead of a ciliary streak (Pl. III 9, 10). The development of both left and right marginal primordia proceeds almost synchronously.

In fragments of *P. weissei* with the right marginal row of cirri removed, the oral and FVT primordia are formed in a similar way as in reorganizers and promers (Fig. 3 L). Also the left marginal primordium appears in usual way as a streak situated, in the old left row of marginal cirri. On the right margin where there are no marginal cirri left — the primordia form anew without visible participation of old structures, on the topographic right margin of the fragment. In later stages of regeneration both left and right marginal primordia look much alike (Pl. III 12).

Urostyla grandis

Fragments of *U. grandis* with removed left margin, form the oral, FVT and right marginal primordia in usual manner. The AZM primordium is formed as a longitudinal field of kinetosomes situated to the left of the left postoral row of ventral cirri (Pl. III 13). Anteriorly and to the right of this field the UM primordium is formed. The old UMs undergo dispersion and the kinetosomes become incorporated into the UM primordium. Diagonally oriented FVT streaks are situated in a ladder-like form parallel and to the right of the UM primordium. The primordia of the right marginal cirri originate as seven kinetosomal streaks — each situated in an old row of marginal cirri. On the left side, where there are no old marginal cirri remaining a small field of kinetosomes can be observed in early stages of regeneration (compare Pl. V 20). This field is very soon replaced by kinetosomal streaks. It is not clear, whether each marginal streak originates from separate small field, or one field differentiates into seven marginal streaks. The development of the primordia on the operated margin is slightly delayed in comparison to the primordia formed on the unoperated right margin.

The fragments of *U. grandis* in which the right margin was cut off, are deprived of one to several right rows of marginal cirri. The oral, FVT and left marginal primordia originate in a usual way. Depending on how many of the old right marginal rows of cirri remained in the fragment, the same number of new right marginal streaks form primarily within the old row of cirri on the wounded margin. The rest of the streaks are formed on the marginal surface lacking old marginal cirri.

In fragments completely lacking the right marginal cirri a kinetosomal field was observed next to the posterior right portion of the FVT primordium (Pl. VIII 30, 32). This field probably gives rise to the primordia of the right marginal cirri. All fragments fixed after the end of regeneration possess the complete ciliature.

Pseudourostyla cristata

Operations described above were performed also in *P. cristata*. In this species, as has already been mentioned, the primordium of the seven left marginal rows of cirri originates as a single kinetosomal field derived from disagregation of a few

cirri from one (closest to AZM) row of old marginal cirri. The primordia of right marginal cirri appear also as a single kinetosomal field, which is situated at the right margin, within the right most old marginal row (compare Fig. 1 C and F).

In fragments with the left margin excised in such a way, that the first row of left marginal cirri, which participate in morphogenesis, is not destroyed or missed — the whole process of morphogenesis proceeds as in reorganizers. All primordia are located in usual places (Pl. III 11) which means that removal of an uncompetent region of the cell does not cause any shift in the position of primordia.

When the ciliates were operated in such a manner that the right margin was removed the primordium of the right marginal cirri is formed in the right most row of the old marginal cirri, which was left on the fragment. That means that the position of the right marginal primordium is slightly shifted toward the FVT primordium. (These two primordia instead of being separated by 6 right rows of marginal cirri — may be separated by two or three rows only). The oral, FVT and left marginal primordia are formed at their usual places (Pl. IV 14, 15, 16).

In *P. cristata* the primordium formed on the wounded right margin develops slightly later (or slower) than the primordium on the left margin. Photograph 15 on Pl. IV represents a fragment fixed in stage III of primordia formation. The left primordium is seen as a longitudinal field of kinetosomes, whereas the right marginal primordium is seen as a delicate kinetosomal row (Pl. IV 14). In a fragment (Pl. IV 16) fixed in a later stage of development, the large, left marginal field is in the beginning of differentiation of streaks. In the same cell on the right (wounded margin) the kinetosomal field is much smaller, however, the beginning of streak organization can be noticed.

The experiments described above, repeated several times with similar results, clearly indicate that the primordia of marginal cirri are formed on the topographic margin of the fragments, no matter which cirri, (if any) are present there.

The second important observation, is the delay in development of the marginal (and also dorsal Jerka-Dziadosz unpubl.) primordia on the wounded margin.

The third conclusion is that the site of formation of the oral and FVT primordia are not shifted in fragments with a removed margin.

Formation of primordia in right fragments

In this section the right fragments of *U. grandis* and *P. cristata* will be described. By right fragments are meant the animal's right part resulting from longitudinal transection. The right fragments of *P. weissei* do not contain nuclear apparatus, and therefore do not regenerate.

Urostyla grandis

In the right fragments obtained after logitudinal section along the central meridian of the ventral surface, in which the wound repair occurs in situ (Fig. 4)

the AZM primordium is formed near the wounded margin (Pl. V 17). The primordium first appears as a small group of kinetosomes located near to the cirri from the postoral part of the ventral cirri. On the preparations made in later stages of regeneration it can be seen that such fragments are able to form all of the kinds of ventral primordia (and dorsal primordia, Jerka-Dziadosz unpubl.). The arrangement of new structures after stage V does not differ from the arrangement of new structures in reorganizers. Presumably the left marginal primordia are formed on the territory which previously belonged to the dorsal side. However, a regulative growth of the ventral surface to form of a new left margin is not ruled out.

In smaller right fragments of *U. grandis*, in which the line of operation runs more laterally — that is in fragments devoid of ventral cirri (Fig. 4 A R), the oral primordium is formed near the wounded margin. The primordium of the AZM appears close to the left most row of marginal cirri, on its left side. The development of the primordium is generally similar to that in normal development.

The fragments of *U. grandis* which fold two parts of wounded margin will be described now (Fig. 4 B R). In right folded fragments of *U. grandis* which arise from longitudinal transection along the central meridian, the row of ventral cirri present in the fragment form an arc which is convex toward the right side (Pl. VI). This deformation in arrangement of old ciliature is caused by the folding up the two parts of the wounded margin.

The formation of oral primordia begins with the appearance of a kinetosomal field located to the left of the curved ventral cirri. At the same time the disaggregation of old UMs and frontal cirri close to them occur (Pl. VI 21, 22). Those two groups of kinetosomes form the AZM and UM primordia. To the right of them the primordia of FVT subsequently appear. As can be observed on the phot. 21 and 22, the primordia follow the curved ventral cirri. The primordia of the right marginal cirri form within old right marginal cirri, and in the first stages of regeneration they also follow the curved line of their predecessors. Later on — both oral, FVT primordia and right marginal primordia straighten up — according to the new shape of the cell.

The primordia of the left marginal cirri are formed in similar mode as in large fragments in which the left margin was removed (sse previous section). A kinetosomal field appears to the left of the AZM primordium (Pl. V 20). From this field the left marginal streak originates. The primordia formed at the wounded margin develop slightly later, than those on the intact right margin. Fragments fixed after regeneration possess all elements of the ciliature. One particular fragment of *U. grandis* will be described now. This fragment possess all seven right rows of marginal cirri, both ventral rows, and also one row of the left marginal cirri (Pl. VI 23). The fragment has folded two parts of wounded margin in such a way, that the transverse cirri appear on the left side of the original AZM, and the ventral row of cirri form U-shape angle. The fragment was fixed in the stage III–IV of regeneration. Localiza-

tion of two kinds of primordia are conspicious: First, the oral primordium is situated parallel to the first postoral cirral row of ventral cirri. The primordium follows the U-shape of this cirral row. Considering the location topographically, the oral primordium runs posteriorly from the original AZM, then turns and runs anteriorwards parallel to the original AZM. The first row of left marginal cirri is situated to the left of the AZM primordium. The second striking observation is that the primordium of the left marginal cirri is situated within this row. That means that the primordium of the left marginal cirri is not situated on the topographic margin of the fragment but is situated between two parts of the AZM primordium, in the place marked by the old marginal row. The UM, FVT and right marginal primordia are also located within appriopriate old ciliature. It should be pointed out here that the fragment is spherical, so the "preexisting" topography is grossly destroyed. The preexisting AZM may mark the anterior part of the anterior-posterior axis of the animal from before the operation. During later development the fragment reacquire the dorso-ventral flattening and all the cirral rows straighten up to form the normal meridional arrangement. The photograph 24 represents a fragment fixed in a slightly later stage of development. It can be noted that the posterior part of the oral primordium has moved anteriorwards replacing the original AZM.

The small right lateral fragments of *U. grandis* deprived of ventral cirri will be now described. In studies published earlier (Jerka-Dziadosz 1964) it was observed that small lateral fragments of *U. grandis* were not able to regenerate. This problem has been restudied. The following experiments were performed. Small lateral fragments (possessing only a few marginal rows of cirri) were cut off from large interfission animals and then isolated into depression slides. Each depression contained about 10 to 15 fragments. Most fragments did not folded two parts of the wounded margin, but instead rotated rapidly around their long axis. As a result they appeared spherical within 15 min, and usually did not regenerate. A few fragments had rounded up and were fixed 2–3 h after operation. Among well stained fragments only two having ciliary primordia were found. Both fragments will be described now.

The photograph 25 on Pl. VII shows a small right fragment, which possesses only a few of the old right marginal cirral rows. The fragment rounded in such a way, that the cirral rows bound all sides of the fragment. In the middle of the ventral side a group of kinetosomes can be observed. The anterior part of the kinetosomal field is directed toward the left. In this part of the primordium the beginning of membranelle formation can be observed. To the right of this group, a thin longitudinal field of kinetosomes appears which on the basis of its location can be considered as the primordium of UM. To the right of that field, a line of kinetosomes is in the beginning of formation of the FVT primordium. The primordia of marginal cirri are not yet seen.

Photograph 26 on Pl. VII represents another small right lateral fragment of *U. grandis* in which the primordia are more advanced in development. The distribu-

tion of the cirral fibers speaks for the statement that it really is a right fragment[2]. In this fragment three kinds of primordia can be seen in the middle of the ventral surface. The longitudinal, wide field constitute the AZM primordium. Parallel to it is situated a thin field of kinetosomes forming the UM primordium with the characteristic fork-like branch in its anterior region. To the right from the oral primordia the kinetosomal streaks of the FVT primordium are seen.

From the data presented above it can be concluded that small lateral fragments of *U. grandis* are able to form oral and cirral primordia. That means that the very

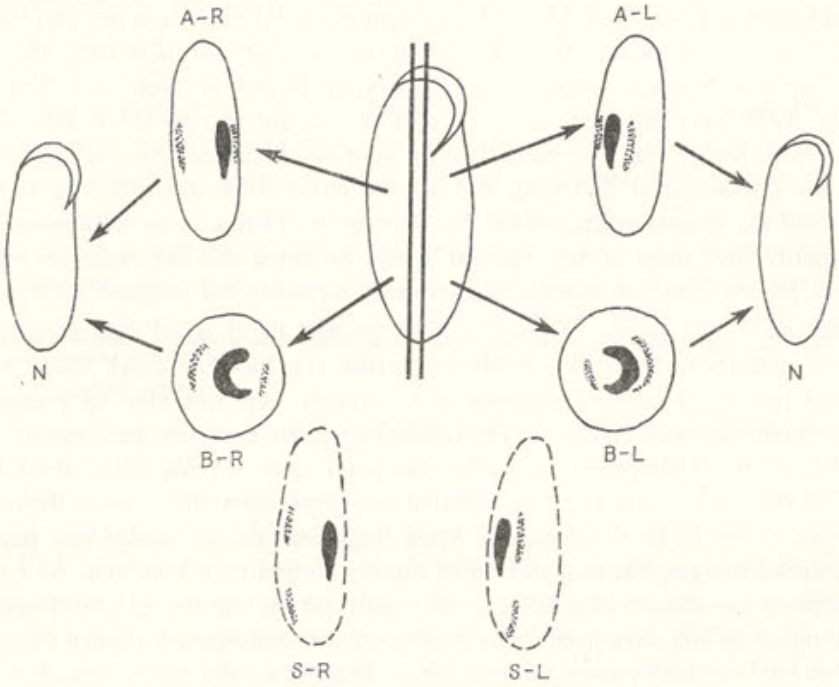

Fig. 4. A schematic representation of the position of primordia in different types of lateral fragments of the studied species. The black fields represent the oral — FVT primordia, the dotted fields represent the mariginal primordia. A — left (A–L) and right (A–R) fragments which heal the wound in situ. B — left (B–L) and right (B–R) fragments which folded on the two parts of the wound. S–R and S–L — theoretically deduced placement of primordia in folded fragments which have been "straightened". Note the position of the two marginal primordia at the same meridian. N — normal pattern

limited capacity of small lateral fragments to regenerate is not due to the inability of such fragments to form primordia. It should be recalled here, that all lateral fragments of this species possess nuclear apparatus.

[2] Each marginal cirrus of *U. grandis*, *P. cristata* and *U. weissei* possess three kind of fibers, the biggest and most prominent is the tangential fiber which is directed anteriorwards and to the animals left. Compare phot. 35, see also Tuffrau 1965, and Grim 1970.

Pseudourostyla cristata

The right fragments of *P. cristata* obtained after longitudinal section along the central meridian usually fold up the two parts of the wound, and therefore the transverse cirri come to be located right next to the frontal cirri. Such fragments usually possess a rounded shape. The middle of the ventral side is occupied by the folded U-shaped rows of ventral cirri. The margins of the fragments are covered by only one kind of marginal cirri, namely the right ones.

In the first stage of morphogenesis small groups of kinetosomes appear to the left of the cirri of the ventral row, which are located on the middle of the fragment. The proliferation of kinetosomes proceeds further and as a result the oral primordium elongates and follows the curvature of the ventral cirri row. Photograph 33 on Pl. IX represents a fragment with well developed oral primordium. In the anterior portion of this primordium the membranelles of the new AZM differentiate. It should be stressed here that the direction of curvature of the AZM primordium is opposite to the direction of its curvature in newly divided cells (or in cells which have finished regeneration). (Fig. 4 B R).

The primordium of the FVT cirri in the fragments in question are formed parallel to the oral primordia. They also are curved according to the oral primordium.

During development the "straightening" of primordia takes place. The old cirri situated on the path of developing primordia are incorporated into the primordium or resorbed.

The primordia of marginal cirri are formed on the topographic margins of the fragment, in the same position in relation to the position of the FVT primordia as in reorganizers. The primordium of the right marginal cirri is formed on the right, unoperated margin, within the old most right marginal row. The primordium follows, the old row while developing. The straightening of new marginal rows occurs in later stages of morphogenesis, when the left margin of the cell grows. The primordium of the left marginal cirri originates as a kinetosomal field, situated to the left of the AZM primordium). The left marginal primordia are usually slightly delayed in development in comparison to the right marginal primordia. They originate and develop according to the reconstituted anterio-posterior axis of the animal, parallel to the straightened primordium of the AZM (Fig. 4 B–R).

In the small right lateral fragments of *P. cristata* deprived of ventral cirri the ventral side is ciliated by only one category of cirri — namely the right marginal cirri. These fragments usually fold on two parts of the wound, and start the formation of AZM primordium about two hours after operation. In such fragments primordia of ciliature are formed in a fashion similar to that described above.

At the first stages of morphogenesis small groups of kinetosomes appear in approximately the middle of the ventral side, close to old cirri from the left-most marginal row of the old right marginal cirri which are present in the fragment (Pl. IX 37). During development the kinetosomal groups grow and form a field, which

occupies almost the whole ventral side of a very small fragment. The AZM, UM and FVT primordia later differentiate from this field. The marginal cirri are formed on the topographic marginal parts of the fragment.

It follows then that small lateral fragments of *P. cristata*, which possess only one category of old cirri — the right marginal ones — are capable of forming the complete set of primordia and reconstructing the whole cortical pattern of the ventral and also dorsal side (Jerka-Dziadosz, unpubl.).

Formation of primordia in left fragments

In this section the left fragments of *U. grandis*, *P. cristata* and *P. weissei* will be described. By left fragments are meant the animals left parts resulting from longitudinal transection.

Similarly, as in the preceeding section, the fragments which repair the wound in situ will be first described. In such fragments, the longitudinal arrangement of cirral rows remains undisturbed. In fragments where the line of operation runs through the central meridian, the left ventral row of cirri is situated near the wounded margin of the fragment. Most of the fragments possess the proximal end of the original AZM (Fig. 4 A–L and B–L).

Urostyla grandis

The left fragments of *U. grandis* which possess a portion of the original AZM usually round up very extensively and undergo some deformations. In order to obtain a left lateral fragments with old cirral rows arranged meridionally, the cells were first cut longitudinally, along the central meridian (approximately) and then the AZM was cut off. In this way a few left lateral fragments with almost no deformations in the arrangement of the old ciliature were obtained.

The AZM primordium in such fragments is formed as a longitudinal field situated to the left of the row of cirri closest to the wounded margin (Pl. IV 19). This field is situated between the first and second (counting from the fragment's right) row of cirri. The line of operation does not always run exactly between two rows of cirri, and may run slightly diagonally. What is important, however, that some old cirri are usually seen to the right of the oral primordium, which means that the AZM primordium is not formed at the "bald" margin, but to the left of the right most row of cirri. Later stages of morphogenesis were not studied.

In left fragments of *U. grandis* which possess the left part of the AZM, extensive regulation of shape during the 2 h which pass from the operation to the beginning of formation of oral primordia, causes some deformations in arrangement of the old ciliature in fragments. As a result of wound healing and of the slow constriction of the wounded margin — the left marginal cirri encircle the posterior part of the fragment, and the cirri may be situated perpendicularly to the AZM. This happens very often in oblique fragments, in which the line of operation runs slightly diagonally

(where the left fragment does not possess the transverse cirri). Such fragments usually possess a large portion of the AZM and UMs (Pl. VIII). In some of the diagonal fragments in which most of the midventral postoral region is missing the AZM primordium is formed within the posterior portion of the UM-field. The formation of oral primordia in small left diagonal fragments of *U. grandis* starts from a disaggregation of old UM's. Kinetosomes from both membranes form a longitudinal field (Pl. VIII 29, 30). The field shows a widening in its posterior part (the AZM primordium), by which it joins the original AZM. At the same time the proximal end of the AZM becomes resorbed. In larger fragments, which possess a part of the postoral portion of the cell, in addition to the kinetosomal field (UM primordium) derived from disaggregation of old UMs, on the ventral surface posteriorly to the old AZM another kinetosomal field (AZM primordium) originate. The orientation of this field is difficult to establish (Pl. VIII 31). Usually the AZM primordium is parallel to the UM primordium, but the orientation may be slightly modified by the neighbouring marginal cirri.

The FVT primordium arises as a loosely packed kinetosomal field, situated parallel to the UM primordium. From this field, the diagonal FVT streaks later differentiate (Pl. VIII 30, 32).

The primordia of right marginal cirri appear as a kinetosomal field adjoining the posterior part of the FVT primordium (phot. 32). During the regulative growth of the right margin the marginal streaks differentiate. The primordia of the left marginal cirri in diagonal fragments of *U. grandis* are formed to the left of the original AZM, within the original left rows of marginal cirri. They appear as kinetosomal streaks.

The formation of primordia in left lateral fragments which possess both: proximal part of the AZM and portion of the transverse cirri will be now described. Such fragments usually fold on two parts of wounded margin together, which often brings about a shift of the transverse cirri to the left side of the fragment.

The formation of the AZM primordium starts with the proliferation of kinetosomes close to left ventral cirri situated posteriorly to the AZM. This kinetosomal field grows covering the whole midventral portion of the fragment (Pl. VII 27). In several fragments the incorporation of the transverse cirri into the primordium has been observed. The UM and FVT primordia develop to the right of the AZM primordium. The primordia of the left marginal cirri originate as kinetosomal streaks, one in each old marginal row whereas the right marginal cirri are formed in continuation to the FVT primordium on the right side the fragment (Pl. VII 28).

Pseudourostyla cristata

The left fragments of *P. cristata* almost always fold two parts of the wound, which causes considerable translocations in cortical structures (Fig. 4 B–L). The transverse cirri may appear at the level of the original AZM. The fragments usually acquire a rounded or oval shape. They possess only one category of marginal cirri —

the left ones which cover both the left and right sides as well as the posterior of the cells (fragments).

As in all the above described fragments of *P. cristata*, the formation of the AZM primordium starts from the proliferation of kinetosomes around and to the left of the left ventral row cirri (Pl. X 37, 38, 39). The AZM primordium grows along the old ventral row of cirri, following its arc-like curvature. As a result the AZM primordium may be situated perpendicularly to the original AZM (Pl. XI 41, 43).

The primordium of UM starts with the disaggregation of the old UM (if it is present in the fragment). The proliferation of new kinetosomes occurs in the region occupied by old UM's. The FVT primordia are formed parallel and close to the UM primordium (Pl. XI 40). It may happen then, that the AZM primordium may be perpendicular to the UM–FVT primordia (Pl. XI 41, 43). During later development the old AZM is progressively resorbed and replaced by the new one.

The primordium of the left marginal cirri originates at normal position in relation to the FVT primordia. It appears as a longitudinal field of kinetosomes within the first left marginal row (Pl. X 36, Pl. XI 41). The left marginal field follows the curved old marginal row until stage IV.

The primordium of the right marginal cirri originates on the right topographic margin of the fragment, no matter what kind of cirri is present there (Pl. XI 40). In a few left fragments of *P. cristata* the formation of the right marginal primordium from the old left marginal row of cirri, situated at the right topographical margin has been observed. The photograph 36 on Pl. X shows a fragment of *P. cristata* in which the primordium of the right marginal cirri is formed in the same old marginal row (first-left) as the primordium of the left marginal cirri. In fragments fixed in later stages of regeneration it can be observed that the differentiation of kinetosomal streaks within the marginal field occurs in the same order as in normal (dividing) animals, or as on the left, undamaged margin. The kinetosomal streaks differentiate starting from the right, anterior part of the marginal field. This means that the fact that primordia form primarily in an "upside down" row of old cirri, does not influence further orientation of the new ciliature (compare Fig. 4 B–L and S–L).

The left fragments of *U. cristata*, which possess only left marginal cirri, are thus, able to form all kinds of primordia.

The mode of formation of the AZM primordium in small left fragments is very interesting. Photograph 37 on Pl. X shows a left fragments of *P. cristata* in which the AZM primordium is formed with participation of the cirri of the first left row of marginal cirri (arrows). It should be stressed here that the same cirri form in division and regeneration of transverse fragments the primordium of the left marginal cirral rows. This fact again indicates clearly that as in *U. grandis* the new kinetosomes at first are formed from or closest to the remained preexisted structure situated in the nearest vicinity of removed ventral cirri.

Paraurostyla weissei

The formation of primordia of the ciliature in left fragments of *P. weissei* will be described now. The left fragments of this species do not fold two parts of wound, but heals, and constrict the wounded margin slowly, which causes some translocations of the ciliature.

The oral primordium usually originates as a longitudinal field situated posterior to the old AZM, between the left-most ventral row and the left marginal row of cirri. The primordium of the UM originates from two parts, one of which is derived from disaggregation of the old UM's, the other part is formed parallel to the anterior portion of the primordium of the AZM. Both parts join and later differentiate into two parallel undulating membranes.

The formation of the FVT primordia is very interesting. As has been shown in the previous publications (Jerka-Dziadosz and Frankel 1969, Jerka-Dziadosz 1972 a, b), the primordia of FVT in promers, proters and reorganizers are formed with the participation of three frontal cirri, and from some of the ventral cirri, which are situated to the right from the previous ones. The FVT primordia form as 6 longitudinally oriented ciliary streaks.

In left lateral fragments of this species — that is in fragments lacking frontal and right ventral cirri the FVT primordia are formed in the fashion more similar to that described for *U. grandis* (Pl. XII 44). The FVT primordium originates as a loosely packed kinetosomal field (Pl. XII 44, 45). Kinetosomal streaks later differentiate from this field (Pl. XII 46, 47). Very often the orientation of streaks is not longitudinal, but slightly diagonal. During later development the straightening of the FVT streaks takes place (Pl. XII 48).

On the basis of observations on the pattern of formation of the oral and cirral primordia in left and right lateral fragments of *Urostyla grandis*, *Pseudourostyla cristata* and *Paraurostyla weissei* the following conclusions can be drawn:

(1) In lateral fragments of all three species the primordium of the AZM originates first, and is situated along the left postoral row of ventral cirri, or when this row is absent to the left of the ventral or marginal row, which is closest to the left postoral ventral row. In diagonal fragments, in which the postoral region is missing the AZM primordium forms from the posterior part of the UM primordium.

(2) The primordia of UM are formed with participation of the old UM's, or in fragments lacking old UM to the right and parallel to the AZM primordium.

(3) The primordia of FVT and marginal cirri are formed at the same level at the antero-posterior axis as the UM primordium.

(4) In fragments with deformations in the longitudinal arrangement of cirral rows, the primordia of ciliature follow the deformations in early stages of development. The final orientation of differentiating ciliature corresponds to the new antero-posterior axis and reconstructed polarity.

(5) In all cases in which primordia usually appear as a kinetosomal streaks with participation of preexisting ciliature when formed at places lacking old ciliature, they appear as kinetosomal fields, which later differentiate into ciliary streaks.

(6) Development of primordia formed on the injured margin is usually delayed in comparison to development of primordia on the uninjured margin.

(7) All lateral fragments of studied species which possess the nuclear apparatus are capable of forming the primordia of new ciliature.

Discussion

The most important conclusion coming from the study on lateral fragments of urostylids is, that both the location of primordia and the mode of the formation of a primordium is closely related to the position of the forming structure within the new totality.

The oral primordium can be formed at almost any point along the longitudinal axis in transverse fragments, but in longitudinal fragments the primordia are formed close to (actually to the left of) the row of cirri which is situated in the competent region. Figure 5 is a schematic representation of the presumptive sites of the formation of oral primordia. The scheme presents two features of the placement of primordia: (1) the place of the formation of oral primordium undergoes an uniform shifting along the antero-posterior axis (Fig. 5 I), (2) whereas it undergoes a stepwise shifting along the transverse axis (Fig. 5 II).

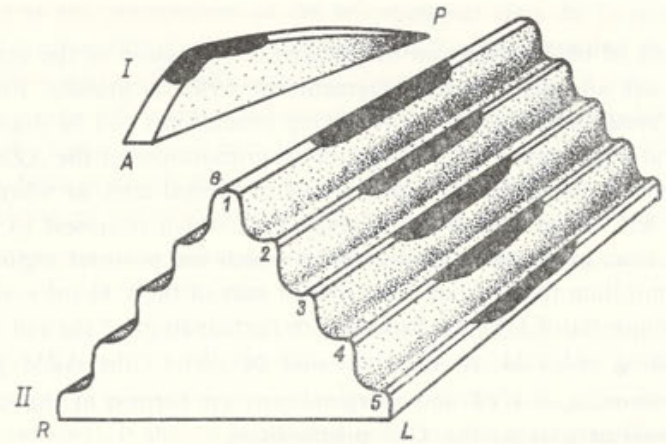

Fig. 5. The localization of the presumptive sites of the formation of the oral primordia of the studied species. A — anterior, P — posterior, R — right, L — left. I — a profile of any meridian along which the site of primordium formation can be shifted, II — the possibility of the transverse stepwise — shifting of the site of primordium formation. The place 1 is preferentially utilized when it is present, when it is absent the site 2 is utilized and so on. The edges (e) represent the rows of old cirri, the depressions other cortical structures oriented longitudinally

The development of the primordia of new cortical structures usually initiate at cortical sites already occupied by preformed structures. Therefore the presence of the old structures itself may influence the formation of new ciliature. This influence, however, has two aspects — the preformed cortical structures may help the development of primordia (structural guidance), or they may limit the development. That the cirri themselves do not control the positioning of a particular primordium follows from many observations that the primordia may arise "de novo" in case of the lack of old cirri (Jerka-Dziadosz and Frankel 1969, Grimes 1973 a).

Two main factors involved in the control of the cortical development in ciliates will be discussed — namely the preformed structures and the positional information.

Role of preformed structures in positioning of primordia

The four methods, by which the primordia of new ciliature can be formed are schematically summarized on Fig. 6.

The first method (Fig. 6 I) is realized by the AZM primordium in *P. weissei* (Jerka-Dziadosz and Frankel 1969) and usually in *U. grandis* (Jerka-Dziadosz 1972 b). New kinetosomes appear in a space between two rows of cirri, and they most probably are formed de novo — that means without any visible participation of old neighbouring cirri.

The second manner of the formation of the AZM primordia (Fig. 6 II) occurs in *P. cristata* (Jerka-Dziadosz 1972 b), *Keronopsis rubra* (Jerka-Dziadosz and Janus 1972), *Kahiella* and *Hypotrichidium* (Tuffrau 1970) In these species small groups of kinetosomes appear very close to the left of the old cirri from the left postoral ventral row.

The third method of primordia formation (Fig. 6 III) is realized by the primordia of somatic cirri in *P. weissei U. grandis* and *P. cristata*. The primordia are formed with an active participation of some old cirri. The kinetosomes from basal plates lose their close packing and undergo a transformation into an anarchic field or kinetosomal streak. (Jerka-Dziadosz and Frankel 1969, Jerka-Dziadosz 1972 b).

The fourth method of the formation of a primordium (Fig. 6 IV) occurs in fragments with the old cirri which normally participate in development removed. In this way the marginal primordia of *P. weissei* and *U. grandis* develop in the fragments with excised old marginal rows (Pl. III 9, 12).

The observations on regeneration of transverse fragments of *P. weissei* (Jerka-Dziadosz and Frankel 1969) and longitudinal fragments of *P. weissei*, *P. cristata* and *U. grandis* presented in this paper showed that the mode by which particular primordium is formed is not fixed — that is — there is not one stable pattern by which any primordium (oral or cirral) must originate.

In the absence of preformed cirri, which normally are used in the formation of primordia, the rudiments can appear with the participation of other cirri, which find themselves in the competent region (Fig. 6 arrow from II to III), or they can form without participation of the old cirri (IV), if they are not present in the competent region.

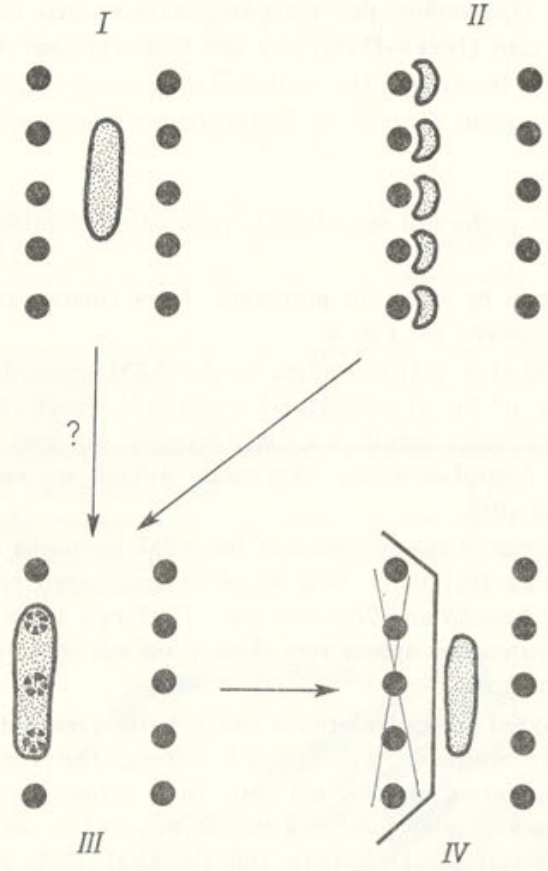

Fig. 6. The interrelations between the mode of the formation of primordia and preexisting cirri of the studied species. Black circles — the cirri dotted fields — the primordia. For explanation see the text

The passing from mode II to III is of particular interest. In opisthes and opimers of *P. cristata*, the AZM primordium originates in the form of small groups of kinetosomes localized to the left from old unchanged ventral cirri. In the left lateral fragments (Pl. X 37) in which the ventral rows of cirri are removed, the AZM primordium forms within the first left marginal row of cirri (the row, in which the primordium of the left marginal cirri is normally formed).

The kinetosomes of the old marginal cirri disperse and first form small fields, later on the fields fuse and form longitudinal AZM field. So in this case the AZM primordium originates in the same way as the marginal primordium usually does.

The mutual interrelation of the method of primordia formation and preexisting cirri might indicate, that the spatial organization of old cirri determines the mode of the formation of a primordium. Actually, the ultrastructural study on the morphology of the ventral and marginal cirri in *P. cristata* shows certain differences in the arrangement of kinetosomes and their accompanying fibers in the basal plates (Jerka-Dziadosz unpubl.). Also the fact, that primordia which normally originate with the participation of preexisting ciliature as ciliary streaks (like marginal cirri in *P. weissei*) — develop as less neatly ordered kinetosomal fields when deprived of the "help" of the old structures, indicates some role of the preexisting ciliature. That the preformed cortical structures strongly influence the formation and development of ciliary primordia can be concluded also from the observation on "curved" oral and cirral primordia in folded lateral fragments. All these phenomena show the character of the features classified by Frankel 1972 as "structural guidance".

The other, aspect of the role of preformed cortical structures present in the cortex of the studied species will be considered now. In *P. cristata* the spaces between rows of cirri (Pl. XIII 49) are filled with trichocysts (Jerka-Dziadosz 1970). The anterior and posterior edge of each cirrus is occupied by a thick microtubular fiber running under the pellicle (Pl. IX 35). Therefore the only "free" places where new kinetosomes may appear on the surface along the central meridian in the postoral region — are small areas to the left (Pl. XIII 51) of each cirrus of the left postoral row of ventral cirri.

Considering now the marginal cirri — they are surrounded by trichocysts from the right and left side with the anterior and posterior sides occupied by fibers running from each cirrus (compare Tuffrau 1965, Grim 1970). It seems, that there is not much room left for new kinetosomes to appear — unless the old cirri disperse and lose their subpellicular fibers. It is worth mentioning here that in *U. grandis* the mucocysts show very similar arrangement to that of the trichocysts of *P. cristata* (Pl. XIII 48–50).

As a result of the study of the distribution of trichocysts of *P. cristata* during cortical development it has been stated that the trichocysts disappear from the regions of the cortex where the primordia of new ciliature differentiate (Jerka-Dziadosz 1970 and unpublished EM study), but this process takes place after the initiation of the formation of primordia.

It seems therefore that the arrangement of cortical structures such as the trichocysts or mucocysts may limit the space available for appearance of the new kinetosomes in regions of the cortex which are morphogenetically active.

The "guiding" role of preformed cirri and the space-limiting role of other cortical structures may possibly explain why any primordium of new ciliature is always formed close to the preformed cirri — if they are present within the competent area.

The problem of what determines the site of the formation or primordium will be discussed in the next chapter.

Positioning of oral and cirral primordia

On the basis of observation on the formation of ciliary primordia during division in three species of lower *Hypotrichida* (Jerka-Dziadosz 1972 b, Jerka-Dziadosz and Frankel 1969) as well as on the basis of analysis of formation of the cortical pattern in transverse fragments (Jerka-Dziadosz 1972 a, Jerka-Dziadosz and Frankel 1969, Jerka-Dziadosz and Janus 1972) and in longitudinal fragments (Jerka-Dziadosz 1966 and this paper) it can be concluded that a region privileged with respect to morphogenetic abilities exists.

The privilege of this region is expressed by the capability for formation of new oral primordia during division, reorganization and regeneration of transverse fragments. The privileged region has been defined as "presumptive organization area" (Jerka-Dziadosz 1964) or as the stomatogenic meridian (Jerka-Dziadosz and Janus 1972). The position of this region can be defined as a meridian covering the undulating membranes and left postoral row of ventral cirri if one takes the elements of the ventral ciliature as cortical markers.

If one specifies this region topographically (taking the overall shape of the cell as coordinates) — it covers approximately the central meridian of the ventral surface. On the basis of the above observations a following hypothesis is postulated:

(1) It is assumed that on the ventral surface of hypotrich ciliate there is an organization area which covers the stomatogenic meridian.

(2) This organization area reflects the highest edge of the mediolateral gradient of some property. The highest point on that edge reflects the highest point of the antero-posterior gradient. It is located slightly posteriorly to the original AZM, at the place where the AZM primordium in reorganizers and promers first appear. The gradient slopes down towards anterior and posterior ends of the cell and also from the rim to both left and right margins. The highest and lowest values of the gradient establish the boundary values which specify the coordinates for the position of any point within a surface of the cell. This has been defined after Wolpert 1969 as positional information.

(3) In different developmental situations the oral primordia are positioned in places corresponding to the high point of the polarity potential, and the cirral primordia consequently at lower values of the potential.

(4) The system of gradients establishing the positional information possess a capacity for regulation, when it is disturbed. In fragments of cells, the gradient under-

goes a regulation, establishing a new coordinate system. According to the new positional information the primordia are positioned.

(5) The final orientation of new ciliary organelles in regenerated fragments is in conformance with the regulated gradient system and not with the orientation of the preexisting ciliature.

The sand hill model

In the first part of the Discussion it has been shown (Fig. 5) that the morphogenetic potentialities of the ventral surface show gradient-like properties, with the central meridian being the "top" of the gradient. A formal analog of such gradient system will be shown in order to provide a model in which positional information can be used to explain the placement of primordia.

The cellular gradient shares properties with a sand hill gradient, in that it has a maximum stable slope resulting from an equilibrium between two forces (gravity, and friction between the sand grains) Lawrence 1971 a, b). This model provides a formal explanation of experiments that set up unstable gradient situations (the transverse and longitudinal transections) which resulted in flow of the gradient itself until new gradients were set up at the maximal steepness that was stable.

If we pour out a hill of dry sand in such manner that the crest forms distinguishable edge then this edge will reflect the high rim of the mediolateral gradient (Pl. XIV 52).

When we insert a glass plate transversaly into the sand hill, dividing the hill into two "fragments", and then remove the posterior part, the following properties can be observed in the anterior fragment: (a) If the line of operation runs close to the equatorial part of the hill or posteriorly to it — then the highest point of the gradient in the anterior part is not moved (Pl. XIV 53, 54). This situation corresponds exactly to the observed result in regenerating promers. In reorganizers and large promers the oral primordium forms in the same place — actually close to the same old cirri from the left ventral row of cirri (Jerka-Dziadosz and Janus 1972 and this paper).

If the glass plate is inserted anteriorly to the highest point and the posterior part of the hill removed with the glass — then a new high point is formed at some distance from the anterior margin of the hill. That corresponds to the localization of the AZM primordium in small promers in *Keronopsis rubra*, *P. cristata* and *U. grandis* — where it is formed within the old UM (Pl. I 2).

A new high point of the antero-posterior gradient is also reconstructed in the posterior part of the sand hill (after removal of anterior part) (Pl. XIV 53, 55). Again the point is localized in some distance from the anterior end. A beautiful example of this situation is provided by experiments on regeneration of "large opimers" of *P. weissei* (Jerka-Dziadosz and Frankel 1969).

The three most left streaks of FVT in *Urostyla weissei* in reorganizers and promers (also proters) arise from disaggregation of three frontal cirri, Nos. 4, 5 and 6. But if the cell is operated near the anterior end, so that the frontal cirri 1, 2 and 3 are excised, but 4, 5 and 6 remain, then the FVT streaks develop not from cirri 4, 5 and 6, as they would in reorganizers, but in a bare patch immediately posterior to these cirri (see Jerka-Dziadosz and Frankel 1969, Fig. 20). The cirri 4, 5 and 6 later are resorbed, as are 1, 2 and 3 in reorganizers. This experiment demonstrates that the basal bodies of cirri No. 4, 5 and 6 are neither necessary nor sufficient as sources of streak initiation, the streaks appear to be formed at a position determined relationally with the respect to the highest point of the gradient.

Another example of the regulation of the gradient in transverse fragments is the localization of oral primordia in small opimers in *K. rubra* and *U. grandis* where the oral primordium is formed in the middle of the ventral surface of the fragments with participation of some of the transverse cirri.

To summarize: the position of the oral primordia is not obligatory linked to any preexisting structure, but is determined relationally with respect to the reconstructed gradient system.

Let us consider now the longitudinal transection of the sand hill. If a glass plate is inserted in the hill of sand (Pl. XV 59) parallel to the crest and the glass then moved laterally, removing one part of the hill, the following properties can be observed: after removal of the margin of hill a cliff is created down which the sand flows. That causes a shift of the crest in the direction opposite to the cut off margin (Pl. XV 59, 60, 61). The distance of shifting corresponds to the size of cut off side. (a) When only a small margin of the hill is removed, the crest remains in the same place, and only the grains from the cut side flow down. (b) When a large portion of the margin of the sand hill is removed — then the crest is shifted. (c) When a large lateral portion of hill, including the crest is removed — a new crest is formed in the small lateral fragment. The position of that crest corresponds to the relative position of the crest in untouched sand hill (Pl. XV 61).

It follows then that regulation of gradient in the dry sand hill proceeds in the same way along both antero-posterior and transverse axes. However, the observations of the regulation of gradients within the cell showed that the regulation proceeds at different rates along the two axes, with a greater tendency to stick to pre-set values along the right-left axis. It seems that the final regulation of the gradient in the lateral fragments takes place some time after the primordia differentiate. Therefore the initial positioning of the oral primordia in lateral fragments does not correspond to the dry sand model.

Let us introduce a model in which a regulation of gradients proceeds in a lower rate (Pl. XVI 62–65). If the sand hill is made from wet sand, then after longitudinal division of the sand hill the cliff thus created does not flow down until it is dried out (Pl. XVI 53, 64).

If a glass plate is inserted in the hill of wet sand, parallel to the crest and the glass then moved laterally so that the two parts of the hill are separated, the following properties can be observed. The coherence of the sand prevents the grains from flowing, therefore the crest in the larger part of the hill remains in the same place. In experiments with urostyls this situation corresponds to the regeneration of large fragments with excised marginal cirri in which all primordia (except on the wounded margin) appear close to and /or with participation of the same cirri as in reorganizers (compare Pl. XVI with III and IV).

Now, let us consider the second experimental situation, that is the reconstruction of the edge in lateral part of the hill, deprived of the crest. In the wet sand model (Pl. XVI 64 viewer's right) the new ridge is situated at the top of the cliff.

In *U. grandis* and *P. cristata* this situation corresponds to the localization of the oral primordia in small lateral fragments which heal the wound in situ. In such fragments the AZM primordium is formed at the margin, to the left of the row of cirri closest to the stomatogenic meridian (Pl. V 19).

The lateral fragments which fold on two parts of wounded margin will be discussed now. Let us consider the situation on the wet sand model. In a reconstructed "folded" sand hill (Pl. XIV 56) the top of the gradient comes to be situated near the middle of the hill. Indeed in regenerating fragments, proliferation of kinetosomes of the AZM primordium starts close to these cirri which are situated in the middle of the fragment (Pl. IX 34).

In all studied lateral fragments in the course of regeneration, by the time the primordia on the wounded margin differentiate, the position of all primordia become regulated so that the final position of well-development primordia in stage V shows relatively the same positions as in reorganizers or transverse fragments. That means that although the process of regulation of gradient proceeds at different rates along the antero-posterior and transverse axes — the final results is the same — the gradient has regulated and so has regulated the new cortical pattern.

The gradient, the properties of which have been discussed above provides a coordinate system establishing positional information (Wolpert 1969). It has been proposed above that this system of positional information is reponsible for the positioning and orientation of the ciliary primordia in ciliates belonging to lower hypotrichida (see also Frankel 1973 a). This hypotheses has testable consequences.

(1) If the initial positioning of primordia is determined by positional information and not by the elements of preexisting ciliature then it should occur in accordance to the preexisting structures only if they are present at appriopriate ("induced") places in the gradient system. This requirement is satisfied by all primordia which form in different kinds of transverse fragments of *U. grandis*, *P. cristata*, *P. weissei* and *K. rubra*.

A comparable demonstration has been made for the primordia of the right marginal cirri of *P. cristata* in longitudinal, left fragments, which folded on the two parts of the wound. In some such fragments the left margin, posterior end and

right margin are occupied by but one category of the old marginal cirri — namely the left ones. The primordia of marginal cirri of such fragments originate at relatively the same places as in normal fragment (promer). The left marginal kinetosomal field originates within the left-most old marginal row of cirri. Yet the right marginal kinetosomal field appear also within the same "left" marginal row of cirri, which after the folding on the two parts of fragments appeared at the right margin of the fragment, in an "upside down" orientation. This experiment confirmed by other observations on formation anew of marginal cirri demonstrated that the marginal cirral primordia form at the geometrical margin of the ciliate, regardless of what (if any) preexisting ciliary material is present there.

(2) If the initial positioning of primordia is determined by the gradient system and not by the preexisting organelles the position should correspond to the gradient system if the preexisting ciliary structures are not present.

This requirement has been satisfied by the FVT and marginal primordia in *P. weissei*. As has already been mentioned the three left FVT streaks of *P. weissei* originate from disaggregation of preexisting frontal cirri. When a preformed "disaggregating" old cirrus was removed by UV irradiation (Jerka-Dziadosz 1972 a) a new kinetosomal streak formed nevertheless at the normal place, where the old cirrus had been before irradiation.

Another example is provided by the regeneration of large fragments of *U. weissei* with excised left or right rows of marginal cirri. Normally the first primordia of the streaks that will later develop into new marginal cirri appear as a result of relignment of the constituent cilia of preexisting marginal cirri. If a longitudinal cut is made such that a lateral portion of the cell including the old row of cirri is excised, the primordia of the cirri form at the new margin despite the absence of preexisting marginal cirri at this site.

(3) If the orientation of new structures is relevant to the regulated gradient system, and not to the preexisting structure, the deformations in arrangement of preexisting structure should not influence the final cortical pattern after regeneration has ended.

This requirement has been satisfied by the mode of pattern formation in longitudinal fragments of *U. grandis*, *P. cristata* and *P. weissei*. In fragments of *U. cristata* where both margins are covered by one category of old cirri, and the cirri in the wounded portion of the margin have a direction opposite to normal the primordia of new cirri, although formed within the inverted row exhibit normal orientation.

In many folded lateral fragments of *U. grandis* and *P. cristata*, the primordia of early stages of development follow the old ciliature. As a result a curved AZM primordium appears. However, during differentiation of membranelles and cirri the straightening of new structures takes place (Pl. XII 47). Careful analysis of the orientation of the new structures in deformed fragments has revealed, that the orientation of new cortical pattern (by the end of resorption of old ciliature) is related to the reconstructed antero-posterior axis of the cell.

From the above presented analysis it follows that both the positioning and orientation of the primordia of new cortical organelle of the ventral side of a hypotrich ciliate are not controlled by the preexisting ciliature. This point of view I have first expressed as a diagram shown on Figure 7 (Jerka-Dziadosz 1966). This scheme in an abstract and simplified mode represents the comparison of old and new cortical patterns in different fragments of three species of *Hypotrichida*.

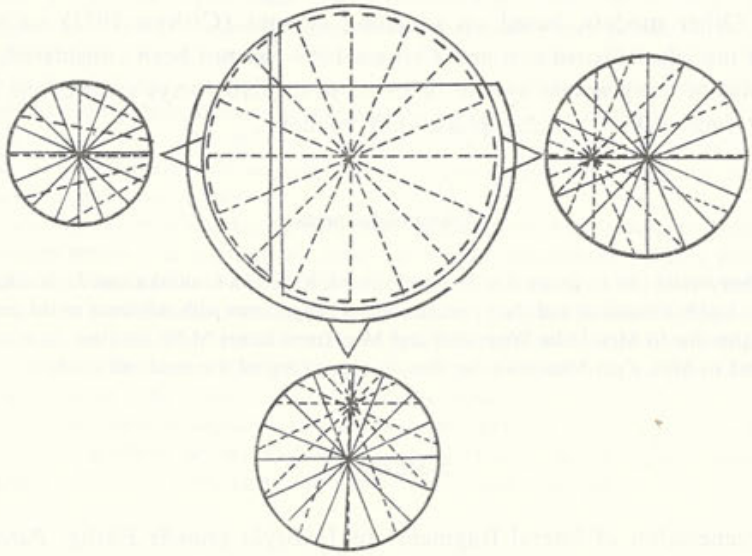

Fig. 7. The scheme representing the comparison between old and new cortical pattern in different types of fragments in hypotrichs. The dotted lines represent preexisting pattern, the solid lines represent the new pattern

The primordia of new ciliature are positioned through a gradient system which provides a positional information for the surface of a ciliate.

The physical or chemical properties which obey the formal character of the sand model of gradient remain to be discussed. Lawrence, the creator of the sand model as applied to the formation of the pattern in the insect segment (Lawrence 1966, 1971 a, b) suggested the existence of a diffusible substance (defined by Crick 1970 as a morphogen), a concentration gradient of which would correspond to the gradients in the sand model. The diffusible model developed by Lawrence has been fruitfully applied to the development of amphibian eggs (Cooke 1972) and to regeneration of *Hydra* (Wolpert et al. 1971, 1972).

The phenomena which occur during pattern regulation in regeneration of fragments in hypotrichs are consistent with the "source" and "sink" hypothesis (Crick 1970). The central meridian of the ventral surface with organization center situated posteriorly to the original mouth could be either a "source" or a "sink".

The differences in the rate of the process of gradient regulation along the two axes could be interpreted as a different rate of diffusion of morphogen along antero-posterior and transverse axes. The "transverse" diffusion could be limited by longitudinally oriented microfibrillar fibers running from the bases of cirri (Pl. IX 35) and also by the trichocysts or mucocysts (Pl. XIII 48, 49) being a mechanical barier for the diffusing substance (Fig. 6). The nature of the morphogen, and also the mechanism by which the positional information is read remains completely unknown. Other models, based on signaling systems (Cohen 1971) such as the phase-shift model of Goodwin and Cohen 1969 has not been considered, yet the highest point of the gradient system in *Urostyla* in many ways satisfies the requirements of "pace-maker" of the phase-shift model.

Acknowledgements

The author would like to thank drs. Joseph Frankel, Krystyna Golińska and Janina Kaczanowska for the valuable discussion and their comments and suggestions with reference to the manuscript. Thanks are also due to Mrs. Lidia Wiernicka and Miss Irena Janus M.Sc. for their careful technical assistance and to Mrs. Zyta Stawińska for the photographing of the sand hill models.

Summary

The regeneration of lateral fragments of *Urostyla grandis* Ehrbg. *Paraurostyla weissei* (Stein) and *Pseudourostyla cristata* (Jerka-Dziadosz) was studied. The locations of primordia and their development were observed. In cells with a removed margin the site of formation of the oral and FVT primordia are not shifted. In lateral fragments of all three species the primordium of the AZM originates first and is situated along the left postoral row of ventral cirri, or when this row is absent to the left of the ventral or marginal row, which is closest to the left postoral ventral row. In fragments with deformations in the longitudinal arrangement of cirral rows, the primordia of ciliature follow the deformations in early stages of development. The final orientation of differentiating ciliature corresponds to the new antero-posterior axis and reconstructed polarity.

The positioning of primordia possess a gradient properties. The regulation of the gradient proceeds at different rates along the antero-posterior (faster) and transverse axes (slower). Two main factors involved in cortical development are discussed: the preformed cortical structures and the positional information. The preferential utilization of the close vicinity of old cirri in competent regions as a places for proliferation of new kinetosomes is interpreted by the guiding role of preformed cirri and space-limiting role of other cortical structures.

It is postulated that the primordia are positioned through a positional information

provided by a gradient of some parameter. A sand hill model has been constructed to provide a formal explanation of the positioning of ciliary primordia in hypotrichs.

STRESZCZENIE

Badano regenerację fragmentów bocznych u *Urostyla grandis* Ehrbg., *Paraurostyla weissei* (Stein) i *Pseudourostyla cristata* (Jerka-Dziadosz). Obserwowano lokalizację i rozwój zawiązków. W komórkach z obciętym bokiem ciała miejsce powstawania zawiązków oralnych i kompleksu FVT nie ulega przesunięciu. W bocznych fragmentach u wszystkich trzech gatunków zawiązek AZM powstaje zawsze pierwszy i jest usytuowany wzdłuż lewego postoralnego rzędu cirri brzusznych, a kiedy ten rząd jest odcięty — powstaje na lewo od rzędu brzusznego lub marginalnego, który jest położony najbliżej od południka stomatogennego. We fragmentach z deformacjami w południkowym ułożeniu rzędów cirri, zawiązki orzęsienia powtarzają te deformacje we wczesnych stadiach rozwoju. Końcowa orientacja różnicującego się orzęsienia odpowiada nowej osi przodo-tylnej i zrekonstruowanej polarności komórki.

Pozycjonowanie zawiązków wykazuje właściwości gradientowe. Regulacja tego gradientu odbywa się w różnym tempie wzdłuż osi przodo-tylnej (szybciej) i poprzecznej (wolniej). Dwa czynniki zaangażowane w kontroli procesów rozwojowych orzęsków są dyskutowane, są to preformowane struktury rzęskowe i informacja pozycyjna. Preferencyjne wykorzystywanie bliskiego sąsiedztwa preformowanych cirri jako miejsca proliferacji nowych kinetosomów w rejonach morfogenetycznie kompetentnych jest tłumaczone z jednej strony przez "przewodnią" rolę preformowanych cirri i ograniczającą miejsce, rolę innych utworów powierzchniowych.

Postuluje się, że zawiązki rzęskowe są pozycjonowane poprzez system informacji pozycyjnej ustanawianej przez gradient pewnej właściwości. Jako formalny analog systemu gradientowego ustanawiającego informację pozycyjną zaproponowano model piaskowy.

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EXPLANATION OF PLATES I–XVI

Protargol stained fragments of *Urostyla grandis* Ehrbg., *Paraurostyla weissei* (Stein) and *Pseudourostyla cristata* (Jerka-Dziadosz), engaged in cortical morphogenesis. All photographs represent the ventral surface. The photographs were printed so that the anterior end is up and animals left correspond to the viewers right

1: A promer of *P. weissei* fixed in the 3rd stage of regeneration. The longitudinal streaks of FVT and marginal cirri are seen. The upper arrow indicate the UM primordium, the lower arrow indicate the AZM primordium

2: Small promer of *P. cristata*. The AZM primordium appear as a widening of the kinetosomal field formed within the UM field. Parallel to the oral primordium the FVT primordium is seen

3: An opimer of *P. cristata* in the 2nd stage of regeneration. The right arrow indicates the AZM primordium, the left arrow the UM primordium

4: A small promer of *U. grandis*. The AZM primordium (arrow) is formed from the widened posterior part of the UM primordium

5: Regenerating *P. weissei* with excised left margin. The primordia in stage 3–4 of regeneration. Note the longitudinal arrangement of the ventral cirri in the region close to primordia

6: A longitudinal, anucleate fragment of *P. weissei* after folding of two parts of wound. Note the position of UM, to the left of the transverse cirri

7: The longitudinal right fragment of *P. weissei* with curved left marginal and ventral cirri

8: The formation of right marginal primordial streak in *P. weissei*. The arrows indicate successive steps in disagregation of old cirri

9: A specimen of *P. weissei* regenerating after excision of the left margin. Note the normal arrangement of the FVT and right marginal streaks. The arrow indicates the primordium of the left marginal cirri

10: The enlargement of the left lateral margin of the same fragment as on phot. 9. Note the kinetosomal field on the margin. The kinetosomal field seen in the middle of the photograph represents the AZM primordium

11: A specimen of *P. cristata* regenerating after excision of left margin, The arrow indicates the left marginal primordium, situated within the first left marginal row of cirri. Note also the position of the AZM primordium formed close to the ventral row of cirri

12: A fragment of *P. weissei* regenerating after excision of the right margin. The arrow indicates the new right marginal row formed without participation of old cirri

13: A fragment of *U. grandis* regenerating after excision of the left margin. The arrow indicates the groups of kinetosomes forming the AZM primordium, close to the postoral row of ventral cirri

14 and 15: A longitudinal fragment of *P. cristata* with excised right margin. On phot. 14 arrow points to the thin row of kinetosomes on the right margin. The arrow on phot. 15 indicates the left marginal field in the same fragment. Note the difference in size of those two fields

16: A longitudinal fragment of *P. cristata* with excised right margin. Photograph is composed from two prints. On the left, uninjured margin a wide and long field of kinetosomes originated (right arrow). A small delicate field of kinetosomes appeared on the wounded margin (left arrow). Note, that in both fields the differentiation of ciliary streaks in the right part of the field have started. In the middle of the photograph the oral and FVT primordia can be seen

17: A regenerating right fragment of *U. grandis*. Arrow points to the kinetosomal field situated to the left of the ventral row of cirri. This is the AZM primordium.

18: A regenerating left lateral fragment of *U. grandis* with developmed AZM, UM and FVT primordia

19: The regenerating right lateral fragment of *U. grandis* in the 1st stage of development. The arrow indicates the AZM primordium situated near the wounded margin of the fragments. Note some cirri to the right of the primordium

20: A regenerating right lateral fragment of *U. grandis*. The arrow points to the kinetosomal field situated near the anterior part of the AZM. This is the primordium of the left marginal cirri

21: A regenerating right lateral fragment of *U. grandis* which folded two parts of wound. Note the curvature of the ventral cirri, and the kinetosomal field close to them. The arrow points to a small kinetosomal field incorporating some of the transverse cirri

22: The same fragment as on phot. 21. The arrow indicate the disaggregating old frontal cirri, which lay close to the UM

23: The regenerating right lateral fragment of *U. grandis* which folded two parts of wounded margin. The upper black-white arrow indicates the out-of-focus original AZM, the lower black — white arrow indicates the primordium of the new AZM. The black arrows indicate the right (lower) and left (upper) primordia of marginal cirri

24: A regenerating right lateral fragment of *U. grandis* which folded two parts of the wound. The arrow indicates the primodrium of the new AZM

25: A regenerating small right lateral fragment of *U. grandis* which folded the two parts of wounded margin. The lower right arrow indicates the AZM primordium. The lower left arrow points to the UM primordium. The upper arrow indicates the FVT primordium
26: A regenerating small right lateral fragment of *U. grandis* which folded two parts of the wounded margin. The right indicates the AZM primordium, the center arrow indicates the UM primordium, and the left arrow points to the FVT primordium
27: A regenerating left lateral fragment of *U. grandis* which folded two parts of the wound. The AZM and FVT primordia are located in the middle of the ventral surface
28: A regenerating left lateral fragments of *U. grandis* which folded the wounded margin. The lower arrow indicates the AZM primordium, the upper arrow indicate the UM primordium. Note the curved line of the UM primordium
29: An oblique left anterior fragment of *U. grandis*. The row of left marginal cirri can be seen, encircling the fragment
30: The same fragment as on phot. 29. The arrow indicate the FVT field
31: An oblique left anterior fragment of *U. grandis*. The UM and FVT primordia are seen on the left side of the photograph. Note the kinetosomal field to the animals left of the posterior part of the AZM
32: An oblique left anterior fragment of *U. grandis*. The oral and FVT primordia are well developed. The two arrows point to the localization of left and right marginal kinetosomal field
33: A right folded fragment of *P. cristata*. In the middle the curved primordium of the AZM is seen. The upper arrow points to the anterior part of AZM primordium. The lower arrow indicates the posterior end of the AZM primordium. The black threads on both sides of the primordium represent the stained trichocysts
34: A small right lateral fragment of *P. cristata* which folded the two parts of the wound. The arrow points the AZM primordium. The black threads represent stained trichocysts
35: A left row of marginal cirri in a protargol stained fragment of *P. cristata*. The arrow indicate the longitudinal fibers running from the basal plate of the cirrus
36: A regenerating left fragment of *P. cristata*, which folded two parts of the wound. The arrows point to the left marginal row of cirri in which the marginal primordia are seen, on both sides of the fragment
37: A left lateral fragment of *P. cristata* which possess only left marginal cirri. The arrows point to the kinetosomal field formed closed to cirri from the first left marginal row. This is the AZM primordium
38: A left lateral fragment of *P. cristata* which folded two parts of the wounded margin. The oral primordia are seen in the middle. The black threads represent the trichocysts
39: The same fragments as on phot. 38. The right arrow points to the AZM primordium. The upper left arrow points to the "anterior" part of the preformed ventral row of cirri. The direction of "straightening" of the primordia can be deduced
40 and 41: A left lateral fragment of *P. cristata* which folded two parts of the wound, on two focal planes. The arrow on phot. 40 points to the right marginal cirri. The upper arrow on the phot. 41 points to the left marginal cirri. The lower arrow indicate the AZM primordium. Note the position of the AZM primordium in relation to the original AZM and to the primordium of FVT
42 and 43: A left lateral fragment of *P. cristata* in two focal planes. The arrow on phot. 42 points to the UM and FVT primordium. The lower arrow on the phot. 43 marks the AZM primordium, the upper arrow point to the left marginal primordium
44: A left lateral fragment of *P. weissei* in early stage of regeneration. Note the loosely arranged kinetosomes on the frontal areas
45: The same fragment as on phot. 44. The arrow points to the FVT kinetosomal field
46: A regenerating left fragment of *P. weissei*. Note the disorder in the arrangement of FVT primordia
47: A regenerating left fragments of *P. weissei*. The straightening of the primordia can be observed
48: A normal interdivisional specimen of *U. grandis* stained with silver nitrate. Black dots represent the stained mucocysts. The arrow indicates the central meridian lacking the mucocysts
49: A normal interdivisional specimen of *P. cristata*, fixed in saturated mercuric chloride and stained with Protargol. The black threads represent the stained toxicysts. The arrow points to one of the rows of trichocysts separating the rows of marginal cirri
50: A fragment of the ventral surface of the interdivisional specimen of *U. grandis* stained with iron hematoxyline. The mucocysts and cirri are see. The lower arrow points to the mucocysts located between the rows of cirri. The upper arrow points to a cirrus surrounded by mucocysts
51: The middle of the ventral surface of *P. cristata*. The lower arrow indicates the AZM primordium in very early stage of development. The upper arrow indicate the first left marginal row of cirri. Note that the spaces between the rows of cirri are filled with the trichocysts

The sand hill model

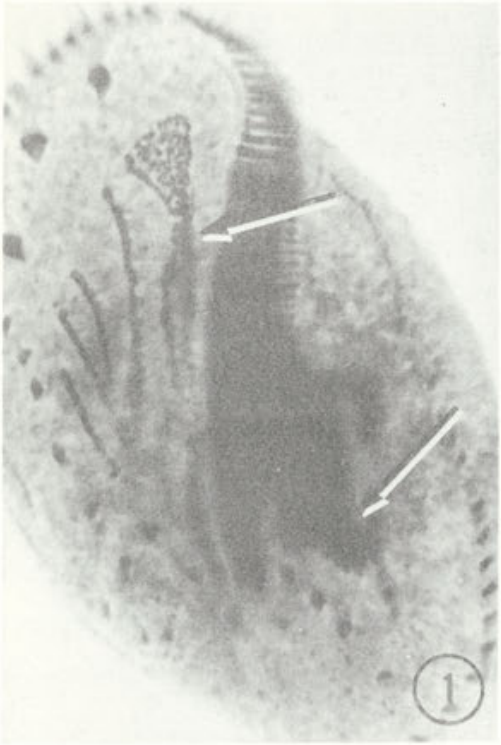
52: The hill made of dry sand. The arrow indicate the highest point of the rim
53: A glass plate is inserted in the hill of sand posteriorly to the highest point (arrow)
54: After the glass plate has been moved posteriorwards and the sand removed with the plate, the sand flows down posteriorwards moderately, so that the highest point remains in the same place as previously
55: The result of shifting of the glass plate from 53 anteriorwards and removal of the anterior portion of the sand hill. It can be seen that a new highest point appeared at some distance from the place where the glass had been, and at some distance to the anterior margin of the hill
The hill on photographs 52 to 55 was photographed from lateral view
56: A reconstruction of gradient in folded lateral fragment. The model is made from wet sand. The arrow is inserted in the highest point
57: The same hill after the sand have dryied out and fell down. The position of the high point is slightly shifted to the viewers left. The hill is photographed from its posterior

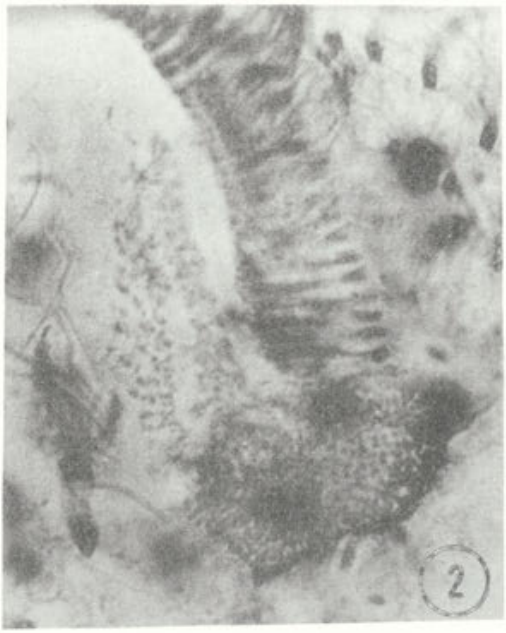
The experiments with dry sand hill models

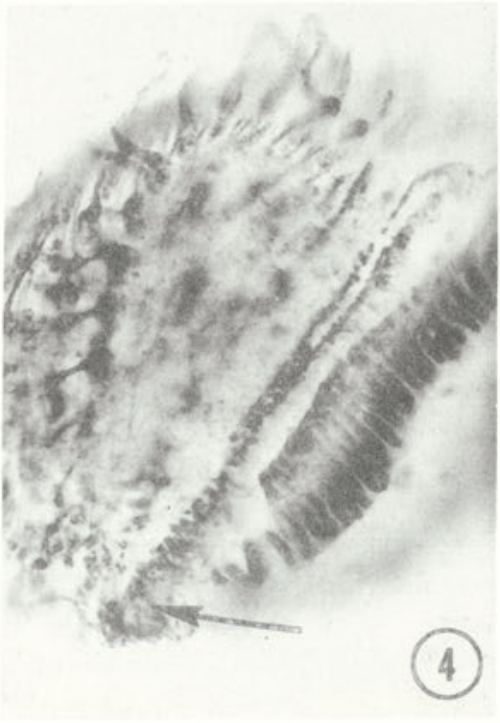
58: The hill is made from dry sand. The glass is put in front of the hill in order to clearly see the profile of the hill. The black arrow mark the rim of the hill
59: The same hill as on phot. 58 with a longitudinally inserted glass plate (the "scalpel"). The thick arrow point to the edge of the hill
60: The hill after removal of the smaller longitudinal part. The thick arrow indicates the previous edge. The thin arrow indicate the new edge after the sand have flowed down
61: The small hill after removing of the larger part (with the edge). The thick arrow indicate where the old edge had been. The thin arrow points to the new edge in the lateral fragment of the hill. The hill on photographs 58–61 was photographed from its anterior

The wet sand model

62: The sand hill is built in similar way as in previous photograph. The glass in front of the hill is put in the front of the hill in order to see clearly the profile of the hill, the arrow indicates the high edge of the hill
63: A glass plate is inserted longitudinally into the hill, parallel to the rim
64: The two parts of wet hill model after the lateral part have been shifted to the viewers right. Note that in left part the highest rim is located in the same place (thick arrow) in small lateral fragment the highest rim is localized on the top of hill (thick arrow)
65: The same hill as on phot. 64, after 1 h, when the sand has dried out. The arrows have not been moved — they mark the position of high points in wet models before falling. The sand has flowed down and the edged in both hills moved apart







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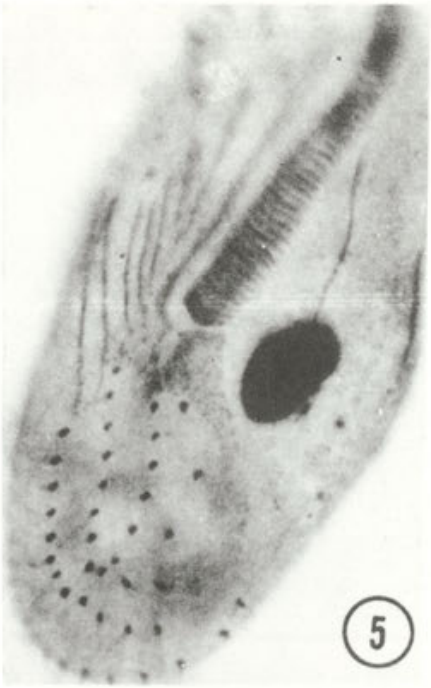

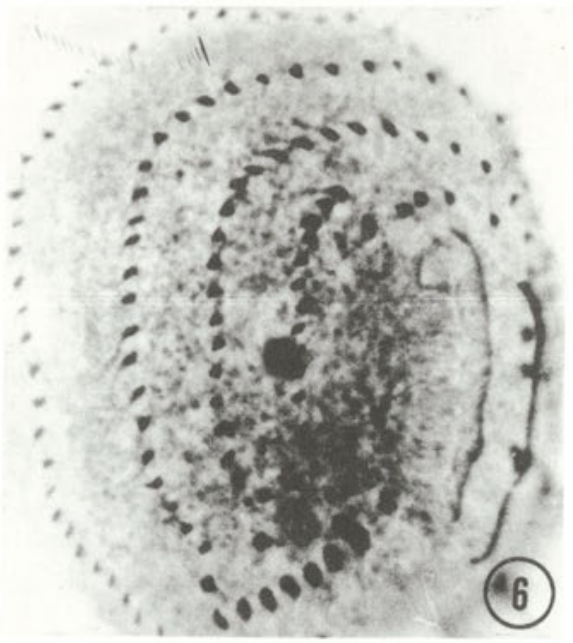

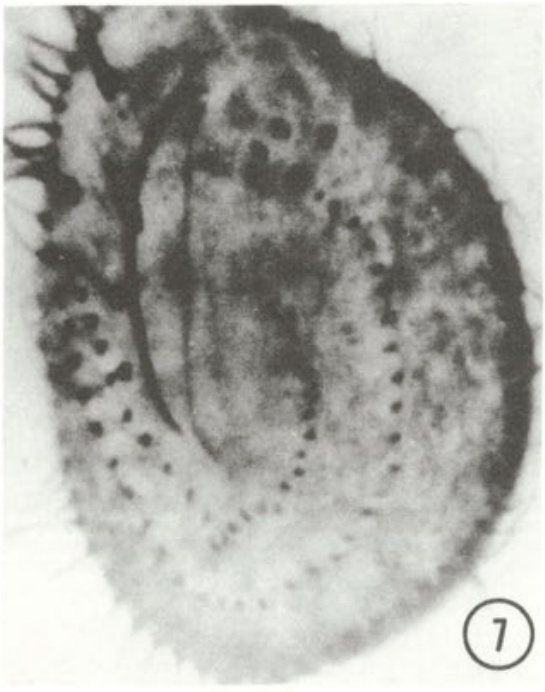

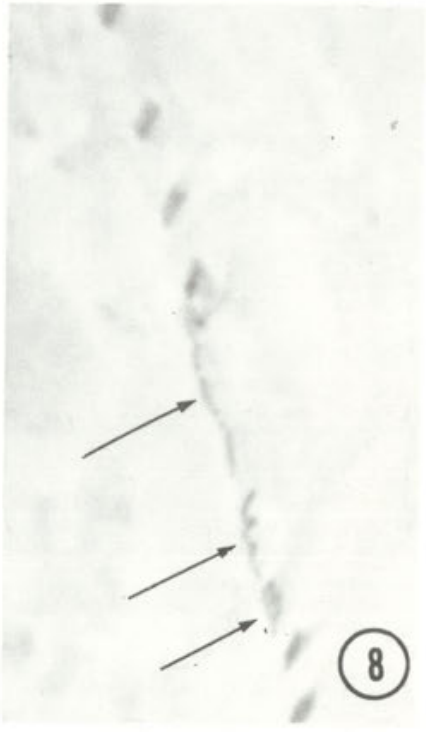

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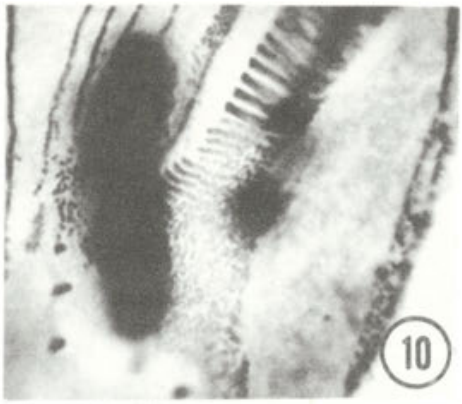

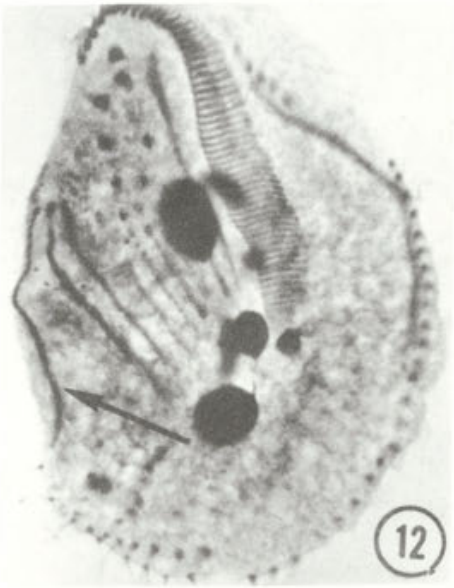

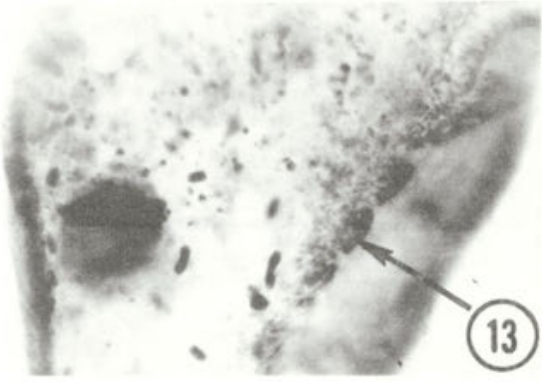

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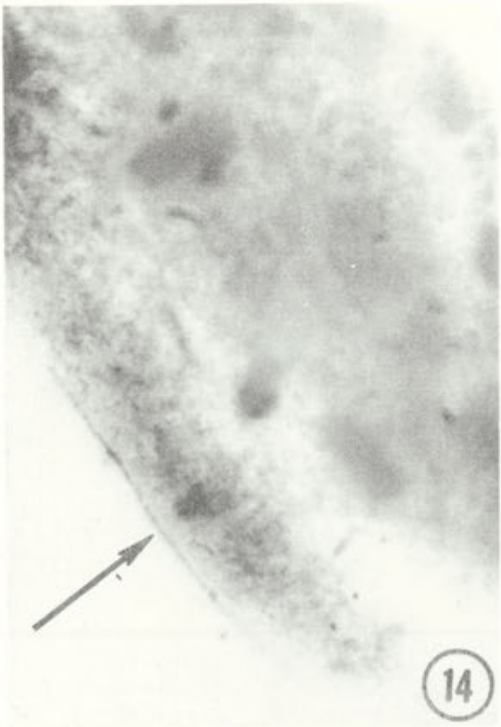

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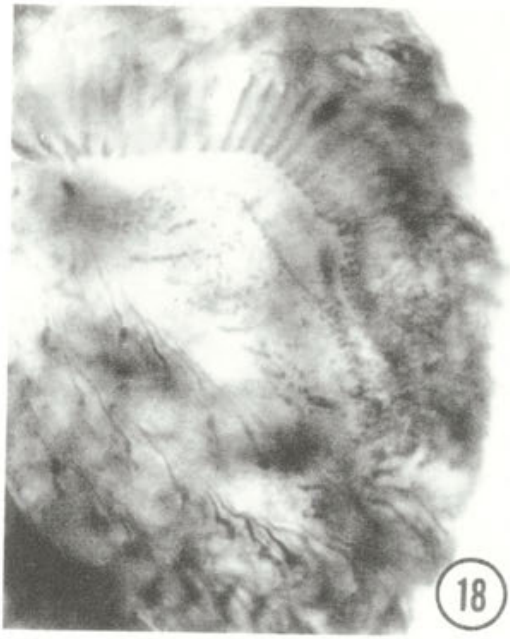

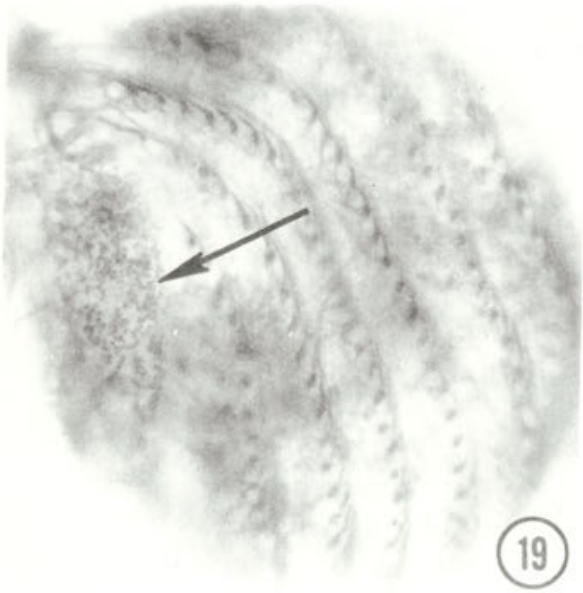

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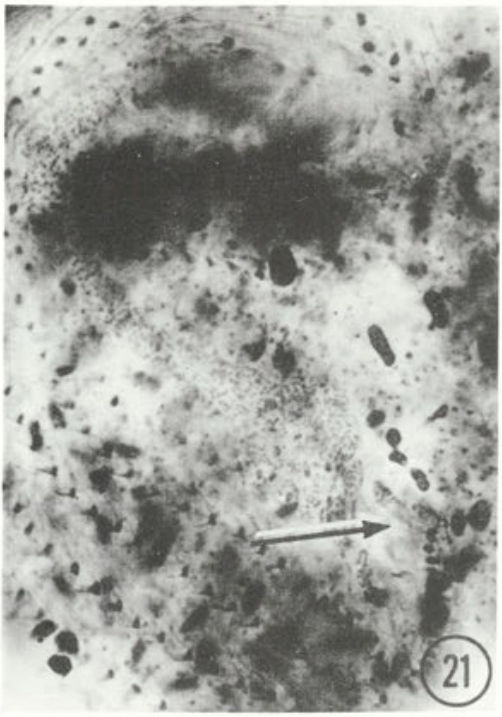

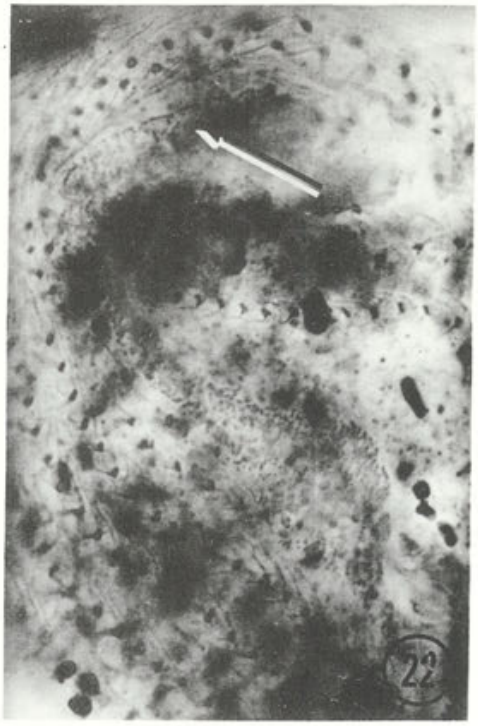

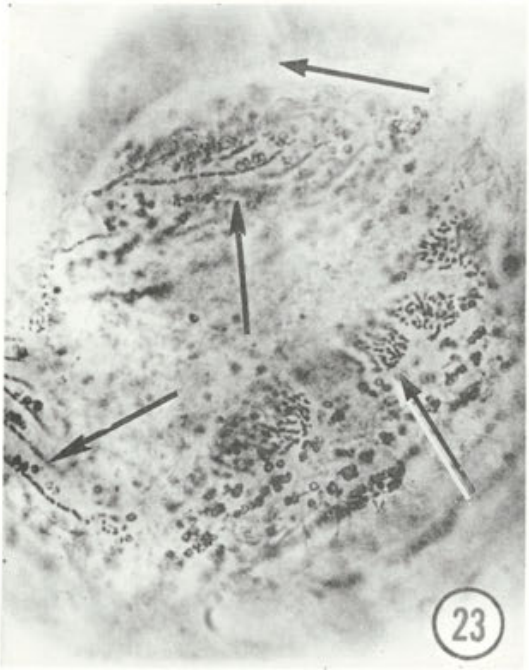

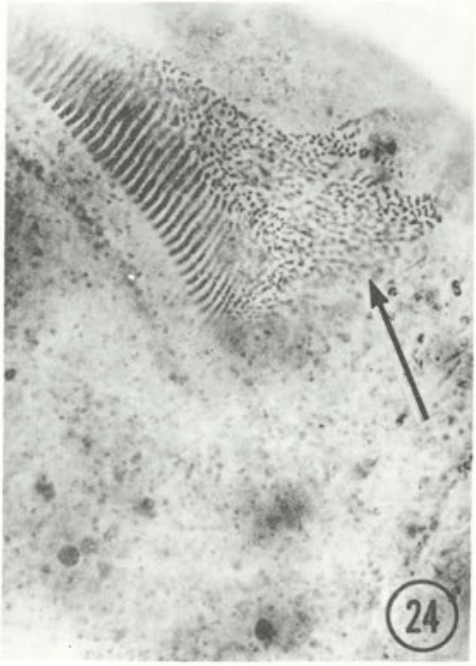

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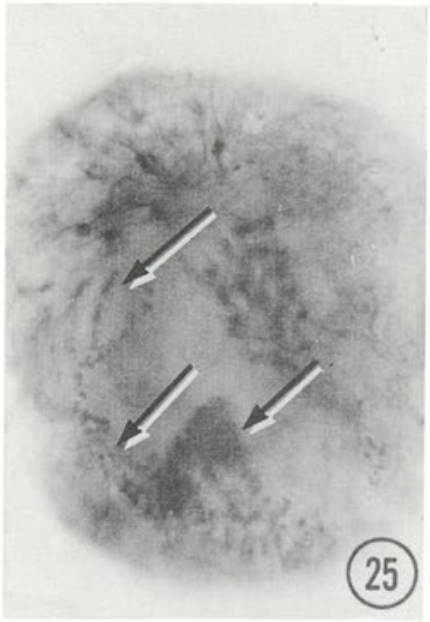

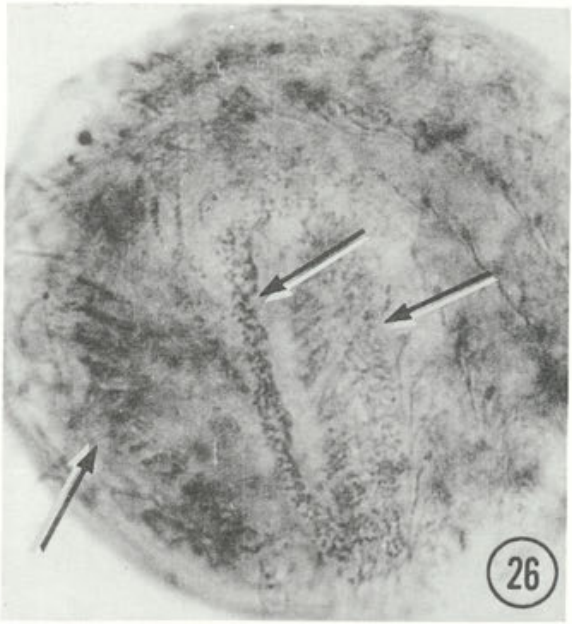

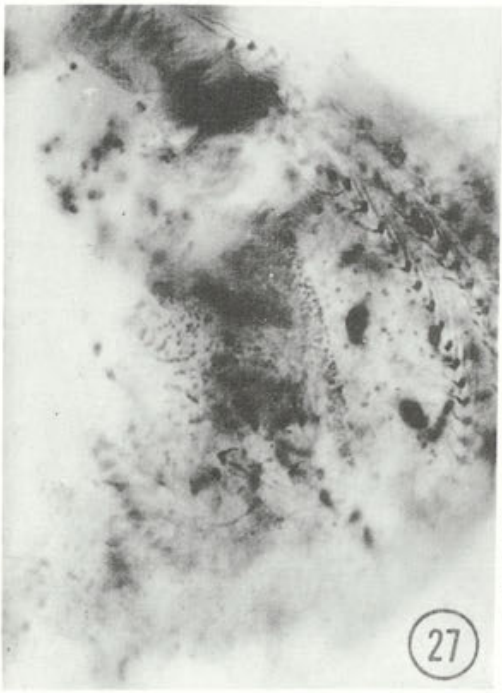

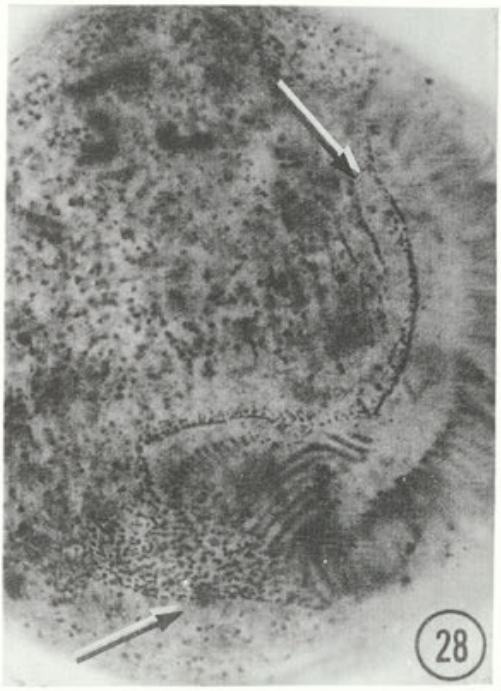

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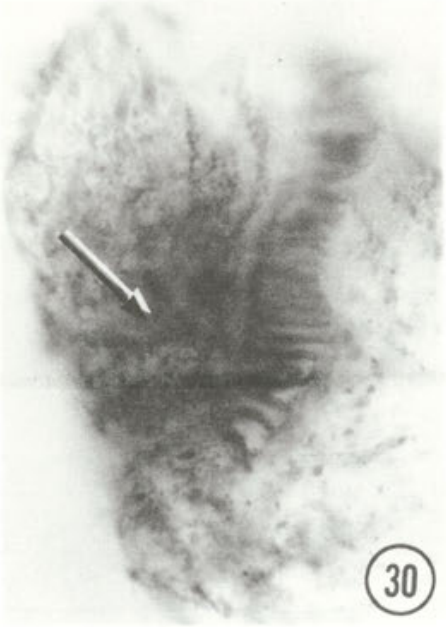

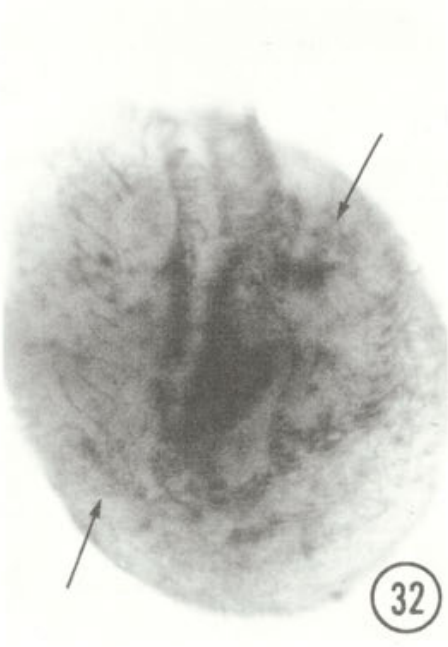

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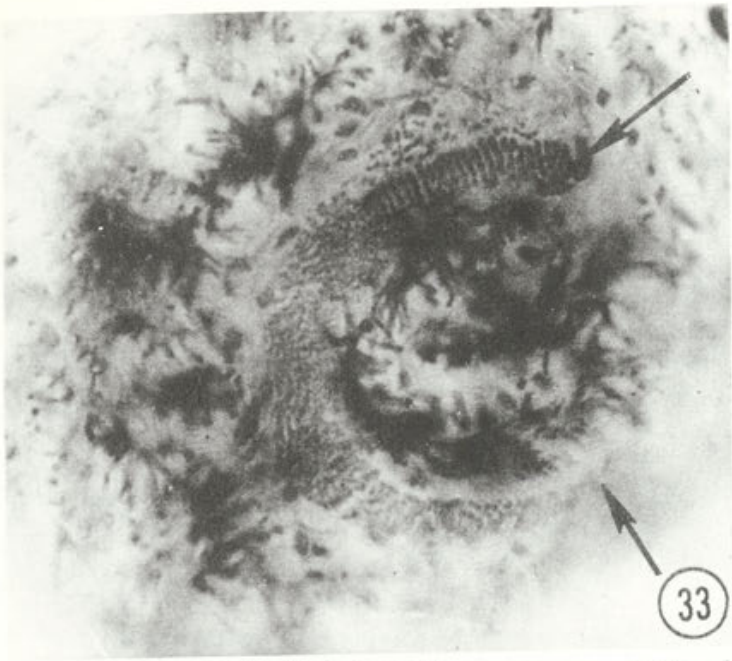

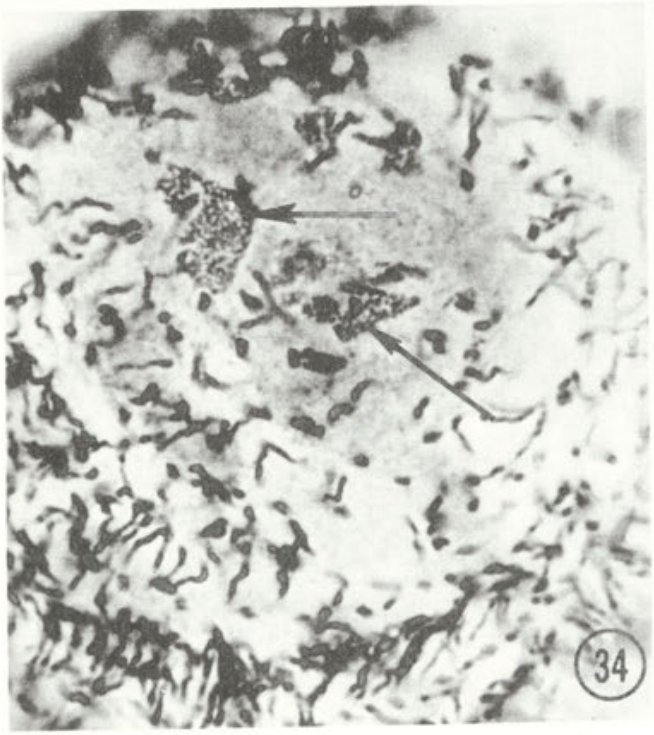

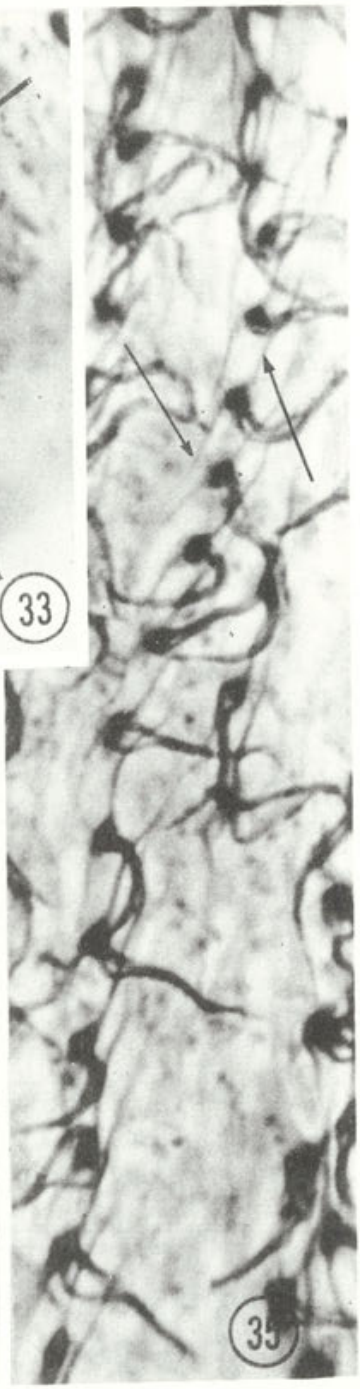

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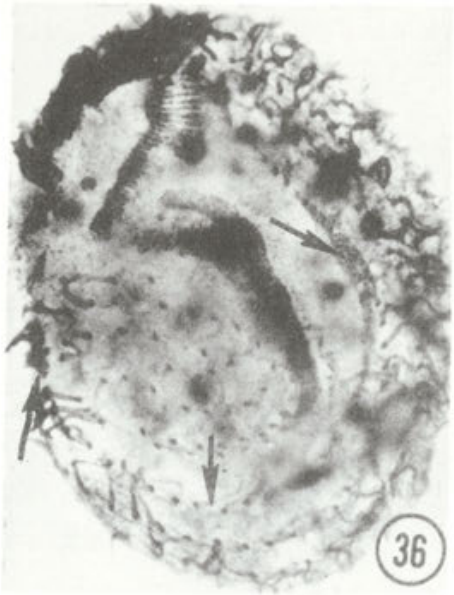

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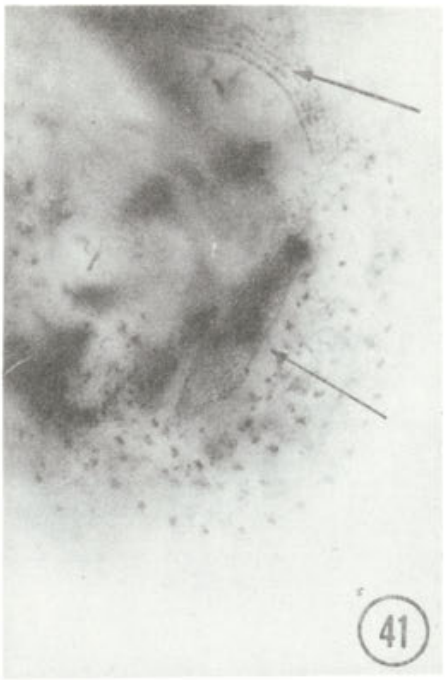

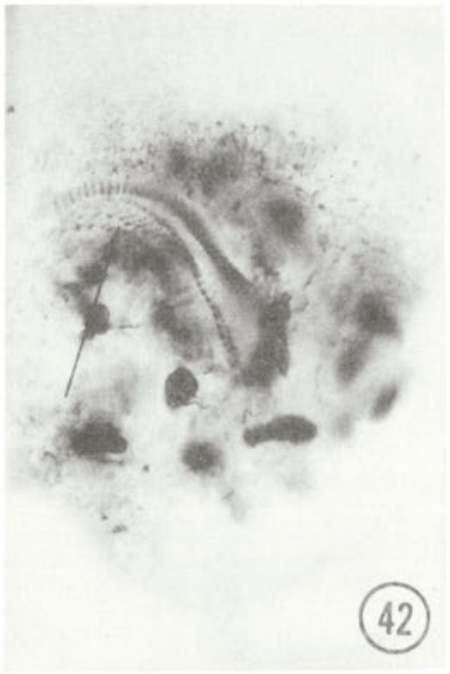

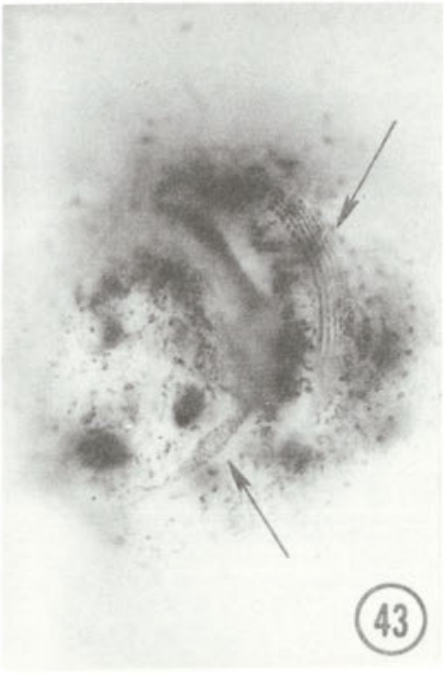

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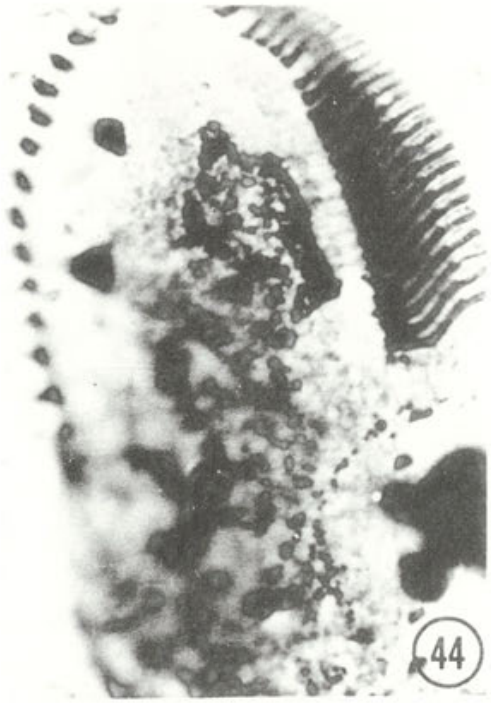

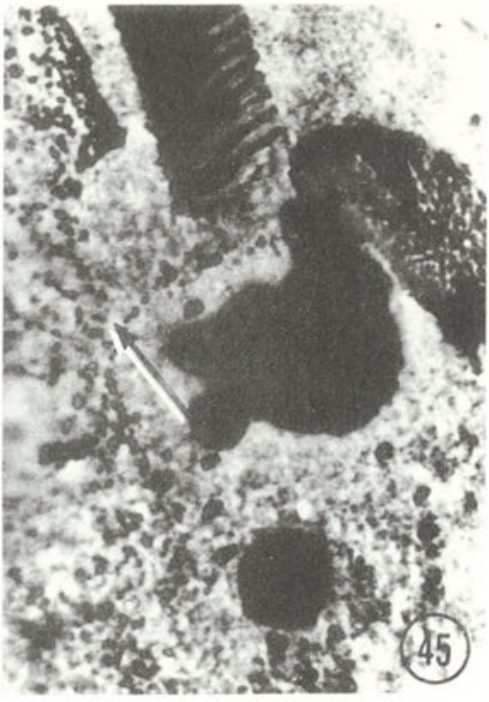

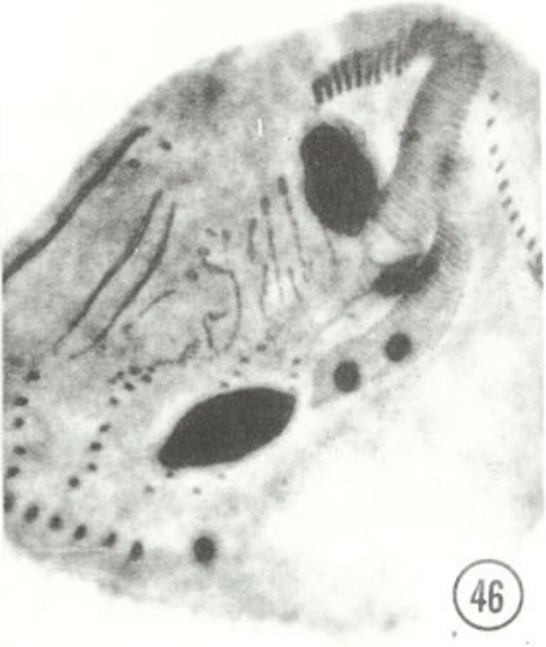

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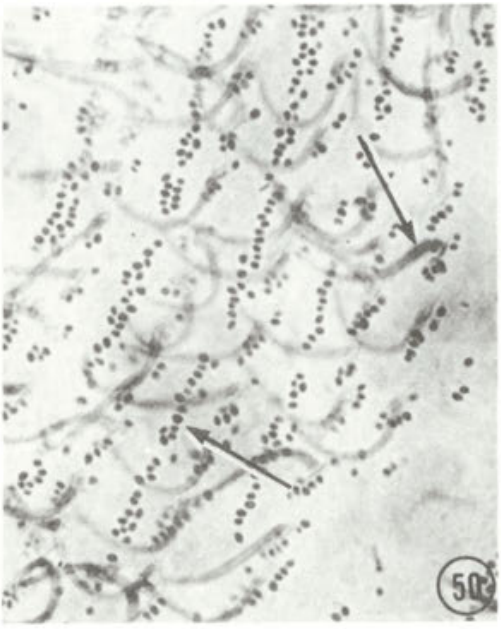

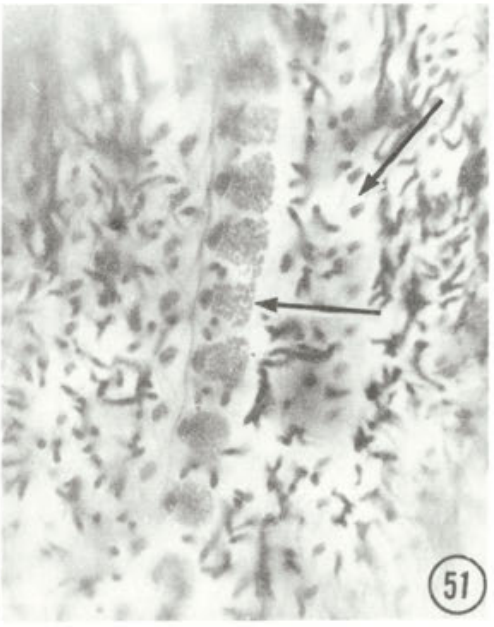

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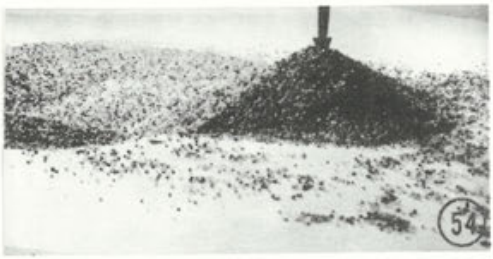

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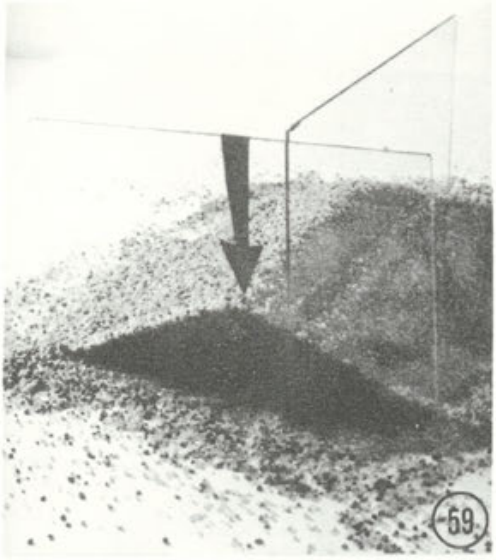

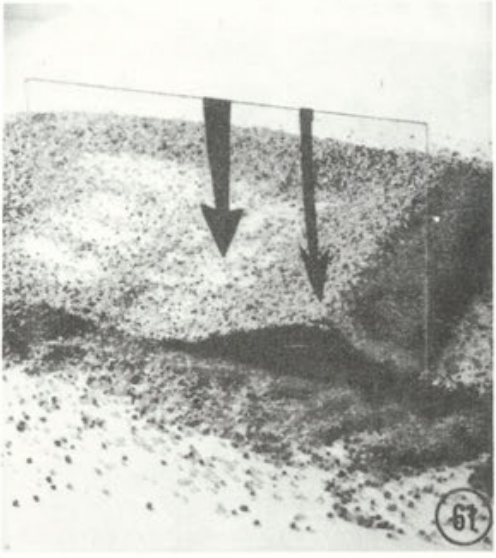

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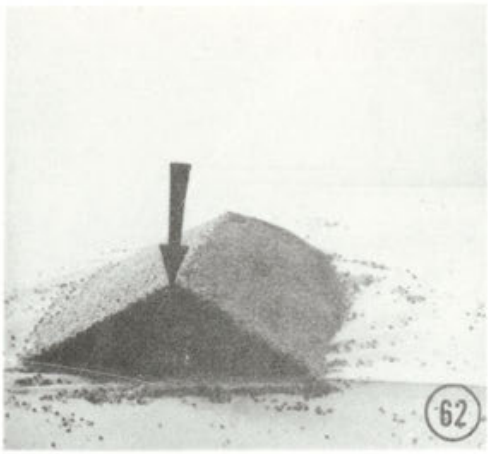

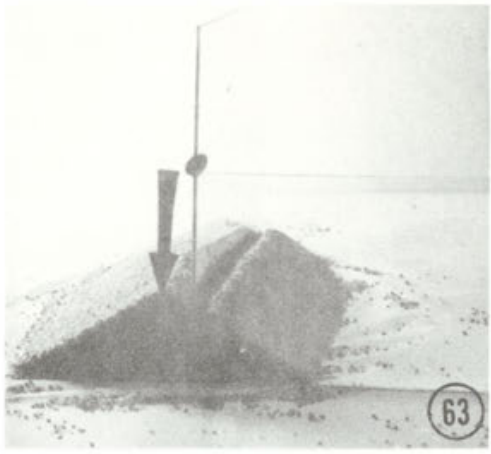

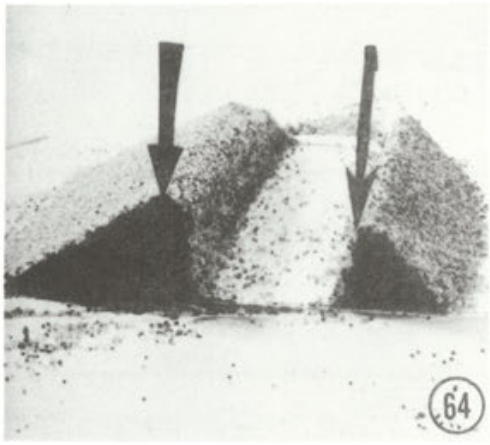

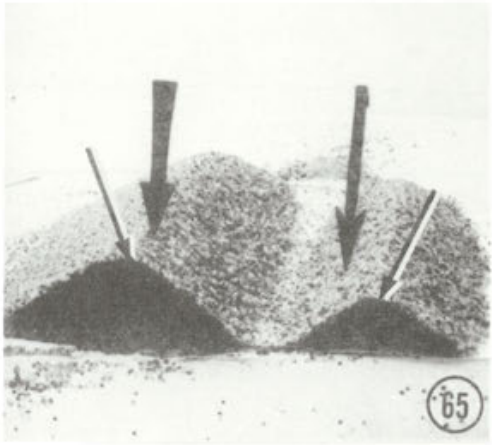

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 23

Лаборатория цитологии одноклеточных организмов, Института цитологии Академии наук СССР, Ленинград Ф-121, Проспект Маклина 32, СССР
Laboratory of Cytology of Unicellular Organisms, Institute of Cytology of the Academy of Sciences of the USSR, Leningrad F-121, Prospekt Maklina 32, USSR

Н. В. ВИННИКОВА
N. V. VINNIKOVA

Конъюгация *Dileptus anser* (O. F. M.) (*Gymnostomatida, Tracheliidae*)

Conjugation in *Dileptus anser* (O. F. M.) (*Gymnostomatida, Tracheliidae*)

Изучение конъюгации у *Dileptus anser* (O. F. M.) представляет интерес с точки зрения эволюции форм полового процесса у инфузорий и необходимо для последующей генетической работы с этим объектом. Литературных данных по конъюгации этого вида немного. Конъюгация *Dileptus* "*gigas*" (*anser*) индуцированная голоданием, была получена Гертвигом (Hertwig 1904). По Гертвигу, конъюгации всегда предшествуют два "голодных" деления (Hungerteilungen), большое значение имеет также фактор сезонности. Никаких сведений о поведении ядер во время полового процесса Гертвиг не приводит.

Лишь в работе Вишера (Visscher 1927) по *D. anser* ("*gigas*") имеются сведения по этому вопросу. Автор также уделяет внимание фактору сезонности и считает, что конъюгация встречается главным образом весной. Вишер подтверждает данные Гертвига о том, что всегда происходят два преконъюгационных деления особи, в результате которых размеры инфузорий уменьшаются. Эти деления не сопровождаются делениями ядер, в результате простого распределения макро- и микронуклеусов число тех и других снижается. Склеивание будущих конъюгантов происходит навстречу друг другу с помощью вентральных отделов хоботов. Ротовые отверстия никогда не сближаются. Лишь один из присутствующих микронуклеусов, по мнению Вишера, принимает участие в 1-м делении созревания, тогда как все остальные ядра мигрируют в задний конец особи. Второе деление созревания дает начало четырем ядрам, три из которых дегенерируют, а одно делится и дает пронуклеусы. Затем следует переход мигрирующих пронуклеусов из одного партнера в другой, после чего конъюганты расходятся. Синкарион образуется в эксконъюганте и делится на два неравных ядра, меньшее — это микронуклеус, а большее — макронуклеус. Далее происходит одно, два лии три

деления этих ядер, до образования четырех макро- и четырех микронуклеусов. Каким образом происходят эти деления в работе не указано. Далее, образовавшиеся макронуклеусы (очевидно, зачатки макронуклеусов) начинают делиться амитозом до образования 32 или 64 ядер, затем наступает фрагментация последних на дефинитивные макронуклеусы. Микронуклеусы делятся митозом, давая 16, 32 или 64 ядра. Деление экскоиъюгантов начинается не раньше, чем через 4 дня после конъюгации.

В настоящей работе изучена конъюгация у двух клонов *Dileptus anser*.

Материал и методика

Работа выполнена на двух клонах *D. anser* (O.F.M.) комплементарных типов спаривания, клон L и клон S (Винникова и Тавровская 1973). Культивирование инфузорий производилось по методике, описанной Николаевой (Николаева 1968). Через 2–3 часа после сливания культур этих клонов образуются пары. При температуре 22 ± 0.5° процесс конъюгации занимает 24–25 час (до момента разъединения пар). Все указания возраста конъюгантов и экскоиъюгантов, приводимые ниже, относятся именно к этой температуре.

Материал фиксировался каждые 15–20 мин, начиная с момента образования пар, смесями Буэна, Бенда, Щаудина и сулемой с уксусной кислотой. Ядерные процессы были изучены на срезах, так как из-за большого количества ядер и слабой окрашиваемости микронуклеусов тотальные препараты оказались для этой цели малопригодными. Заливка велась в парафин на целлоидиновых пластинках по методу Петерфи. Срезы толщиной 5 μm окрашивали железным гематоксилином Гейденгайна, по Фельгену и метиловым зеленым — пиронином по Унна–Паппенгейму. Общая картина той или иной стадии получалась с помощью графических реконструкций. Для более детального изучения хромосом в мейозе использовалась фиксация осмием по электронномикроскопической прописи с последующей заливкой в эпон. Срезы толщиной в 1 μm, готовились на ультромикротоме LKB и окрашивались толуидиновым синим.

Результаты

После образования пар *D. anser* не питаются и почти не двигаются. Особи соединяются друг с другом вентральными сторонами хоботов (Табл. I 1). Объединяются инфузории, имеющие приблизительно однородный небольшой размер тела, тогда как вегетативные особи в среднем значительно крупнее (Табл. 1). Этот факт свидетельствует о том, что парочки образуют клетки, которые предварительно претерпели особые преконъюгационные деления. Последние не являются у *Dileptus anser* обязательными, но встречаются довольно часто.

У другой части конъюгантов преконъюгационные деления отсутствуют, но зато наблюдается чрезвычайно своеобразное явление — деление партнеров уже после спаривания. Такое деление во время конъюгации начинается с появления едва заметной поперечной перетяжки, которая затем углубляется.

Таблица 1

Table 1

Длина тела у вегетативных особей и у конъюгантов *Dileptus anser* при скрещивании клонов L и S

Body length of the trophonts and the conjugants of *Dileptus anser* during mating of strains L and S

	Количество измеренных особей Number of specimens measured (n)	Длина тела Length of the cell		
		Пределы изменчивости, μm Limits of distribution (lim)	Среднее арифметическое, μm Arithmetical mean (M±m)	Критерий Стюдента Student's test (t)
Вегетативные клетки Trophonts	300	78.5–235.5	162.4±1.7	8.8
Конъюганты Conjugants	100	47.1–251.2	130.8±3.2	

Клоны в данном опыте не маркировались, измерялась длина клетки без хобота на тотальных препаратах с помощью окуляр-микрометра при увеличении 10×10.

In this case strains are not marked; length of the cells is measured withouth the proboscis in magnification 10×10, using eye-micrometer.

Процесс деления занимает около часа, причем последние 15–20 мин происходит формирование у задней особи короткого хобота. Делятся конъюганты не на равные половины. Обе передние дочерние особи остаются соединенными в пару. Отделившиеся задние дочерние особи представляют собой маленьких дилептусов грушевидной формы, с коротким хоботом (Табл. I 2). Через несколько часов отделившиеся дилептусы приобретают нормальную форму и в свою очередь способны вступать в конъюгацию. Наибольший процент делящихся в соединенном состоянии наблюдается через три часа после слияния клонов.

Мы столкнулись с большим разнообразием процесса конъюгационного деления. Конъюганты могут вообще не делится, могут делиться оба партнера, и, наконец, может делиться только один конъюгант (Табл. I 3).

Ядерный аппарат преконъюгантов и конъюгантов

Ядерный аппарат вегетативных особеи *D. anser* состоит из 17–20 плотных микронуклеусов (Mi) небольшого размера (0.3 μm) и около 500 макронуклеусов (Ma) размером 2–4 μm (Dragesco 1963). Преконъюгационные деления начинаются с митотических делений всех Mi, Ma принимают форму от палочковидной (3–5 μm) до лентовидной (10–34 μm) (Табл. I 4). В это время Ma имеют неодинаковую ширину и неравномерно красятся по Фельгену. Ко

времени цитокинеза часть Ma перетягивается надвое и распределяется между клетками. Образовавшиеся в результате деления дилептусы имеют приблизительно одинаковые размеры, исключая длину хобота. У передней дочерней особи хобот почти в полтора раза длиннее тела, тогда как у задней вместо хобота имеется маленький отросток. Приблизительно через час у дочерней особи образуется нормальный хобот.

Ядерный аппарат только что разделившихся дилептусов представлен 17–20 Mi (1μm) и порядка 300 округлых Ma (2–4 μm). Возможно, нормальное количество макронуклеусов у *D. anser* в дальнейшем достигается путем делений отдельных Ma.

Вегетативные и преконъюгационные деления друг от друга не отличаются, за исключением размеров Mi. После преконъюгационного деления микронуклеусы немного крупнее, чем после вегетативного деления. Соединяются друг с другом особи, ядерный аппарат которых морфологически сходен с таковым у вегетативных клеток.

Во время конъюгационных делений макронуклеусы и микронуклеусы делятся так же, как и при преконъюгационных делениях. Отличаются эти деления друг от друга лишь тем, что во время конъюгационных делений место перетяжки клетки не всегда проходит по середине тела. Довольно часто задняя особь гораздо меньше передней.

Конъюгация в парах типа A

В связи с тем, что было выявлено большое разнообразие в путях реорганизации ядерного аппарата у *Dileptus anser*, мы выделяем три типа пар: A, B, C. Парами типа A будем называть такие пары, в которых ядерные изменения происходят в обеих клетках. Ядерные изменения в парах типа B затрагивают только одного партнера и наконец, в парах типа C ядерные изменения не происходят вовсе. Необходимо отметить, что ядерные процессы могут протекать как синхронно, так и асинхронно. При асинхронном развитии процессов Mi одного из партнеров проходят, например, стадию профазы I деления созревания, а Mi другого партнера — стадию метафазы I деления созревания.

Деления созревания Mi. Первое деление созревания претерпевают от 4 до 12 ядер. Профаза I деления созревания начинается проблизительно через 4 часа после образования пар и длится около 8 часов [1]. В начале профазы Mi вздуваются (диаметр около 8–10 μm), а внутри них образуется очень тонкая фельген-положительная спирема (Рис. 1 a). Далее нити спиремы становятся отчетливо заметными (Рис. 1 b).

С помощью 1-микронных эпоновых срезов удалось обнаружить диплотеновую стадию. Хромосомы в это время выглядят длинными и рыхлыми,

[1] В дальнейшем, говоря о продолжительности конъюгации и эксконъюгационного цикла и о "возрасте" конъюгантов мы будем вести отсчет от момента образования пар.

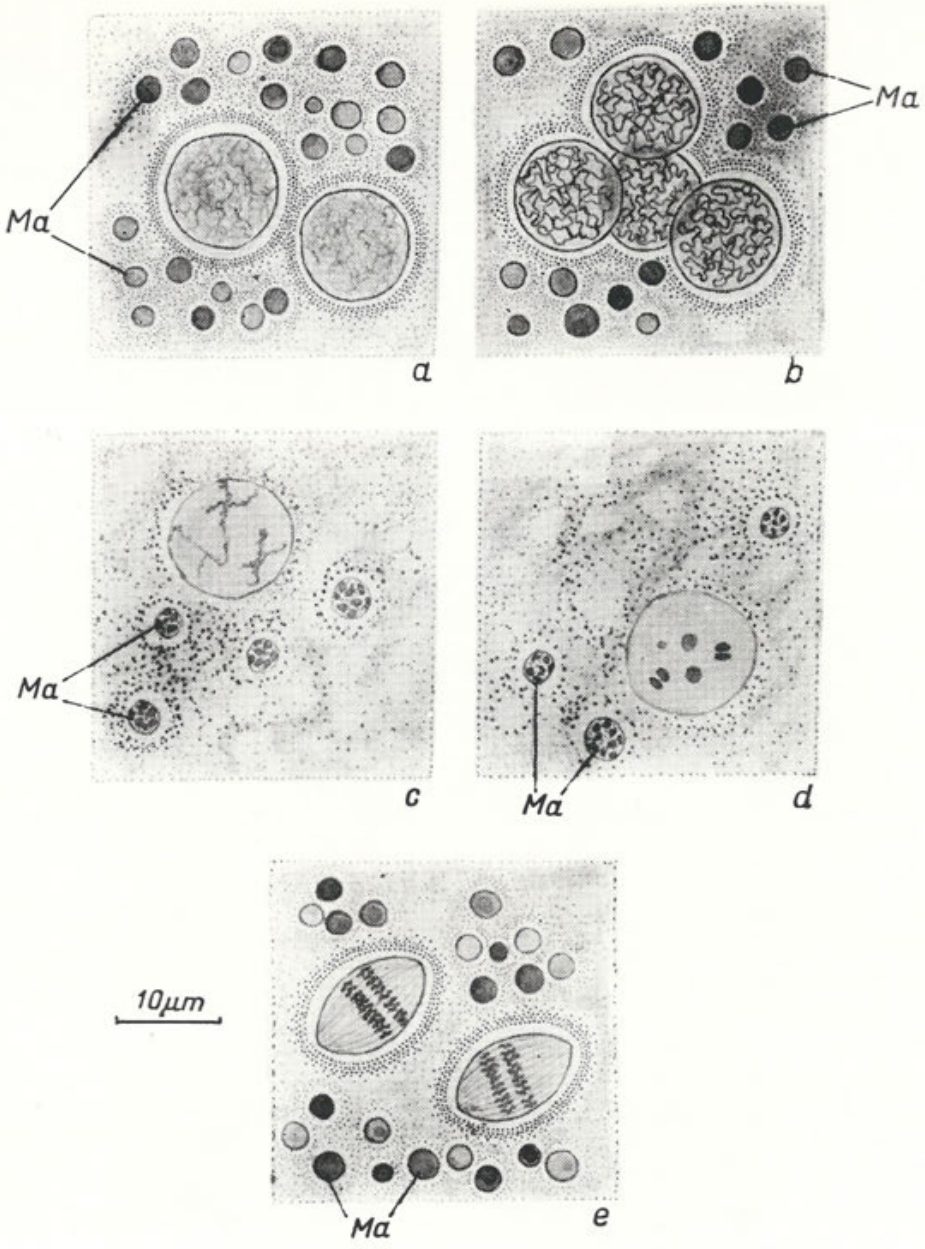
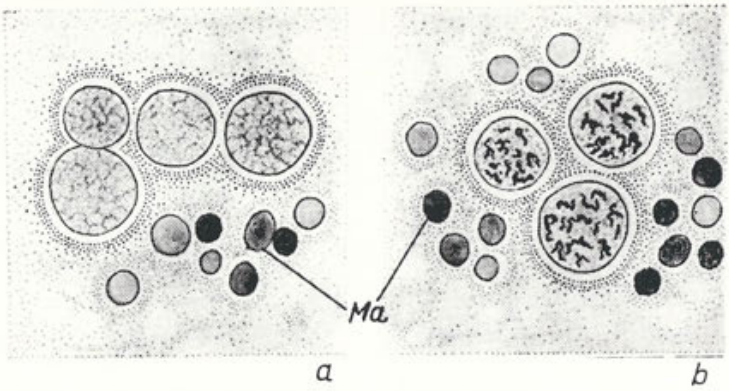


Fig. 1

Рис. 1. Конъюгация *Dileptus anser*. Первое деление созревания микронуклеусов (a, b, e — срезы 5 μm, железный гематоксилин, c, d — срезы 1 μm, заливка в эпон, толуидиновый синий); a — ранняя профаза, b — утолщение нитей спиремы, c — диплотена, d — диакинез, e — анафаза, Ma — макронуклеусы

Fig. 1. Conjugation in *Dileptus anser*. First maturation division of micronuclei (a, b, e — 5 μm sections, iron haematoxylin, c, d — 1 μm epon sections, toluidine blue); a — early prophase, b — thickening of the spireme threads, c — diplotene stage, d — diakinesis, e — anaphase, Ma — macronuclei



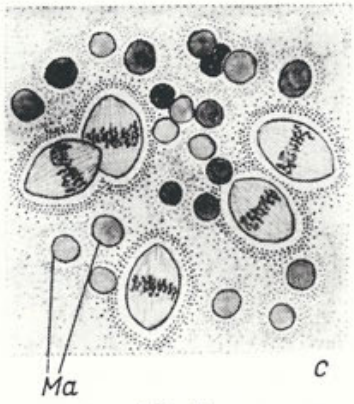


Fig.2

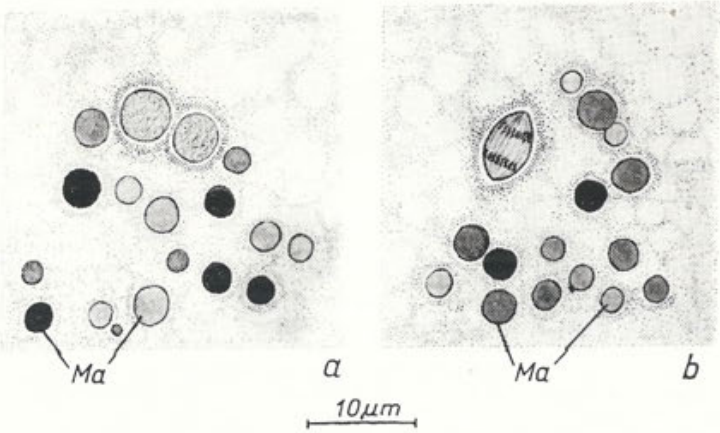


Fig.3

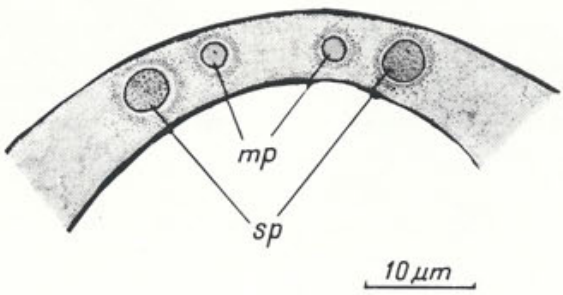


Fig.4

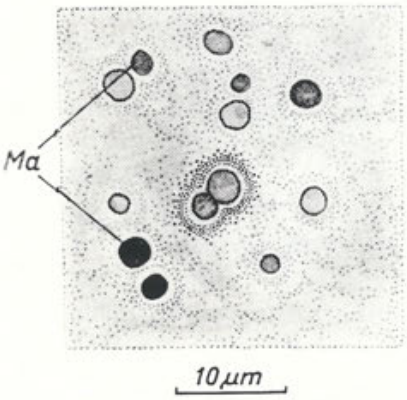


Fig.5

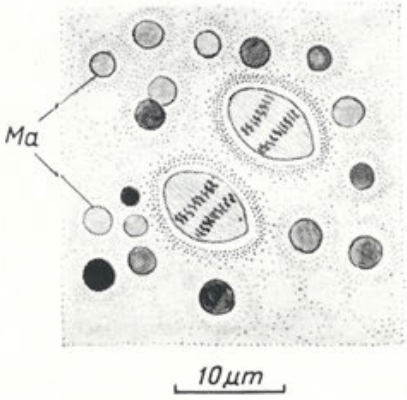


Fig.6

Рис. 2. Второе деление созревания микронуклеусов (срезы 5 µm, железный гематоксилин); a — ранняя профаза, b — поздняя профаза, c — метафаза, Ma — макронуклеусы

Fig. 2. Second maturation division (5 µm sections, iron haematoxylin); a — prophase, b — late prophase, c — metaphase, Ma — macronuclei

Рис. 3. Третье деление созревания микронуклеусов (срезы 5 µm, железный гематоксилин); a — профаза, b — анафаза, Ma — макронуклеусы

Fig 3. Third maturation division (5 µm section, iron haematoxylin) a — prophase, b — anaphase Ma — macronuclei

Рис. 4. Пронуклеусы в перемычке между партнерами (тотальный препарат, Фельген); sp — стационарный, mp — мигрирующий пронуклеус

Fig. 4. Pronuclei in the cytoplasmic bridge between the conjugants (whole mount, Feulgen); sp — stationary pronucleus, mp — migratory pronucleus

Рис. 5. Образование синкариона (срез 5 µm, железный гематоксилин); Ma — макронуклеусы

Fig. 5. Synkaryon formation (5 µm section, iron haematoxylin); Ma — macronuclei

Рис. 6. Второе деление синкариона (срез 5 µm, железный гематоксилин), Ma — макронуклеусы

Fig. 6. Second synkaryon division (5 µm section, iron haematoxylin); Ma — macronuclei

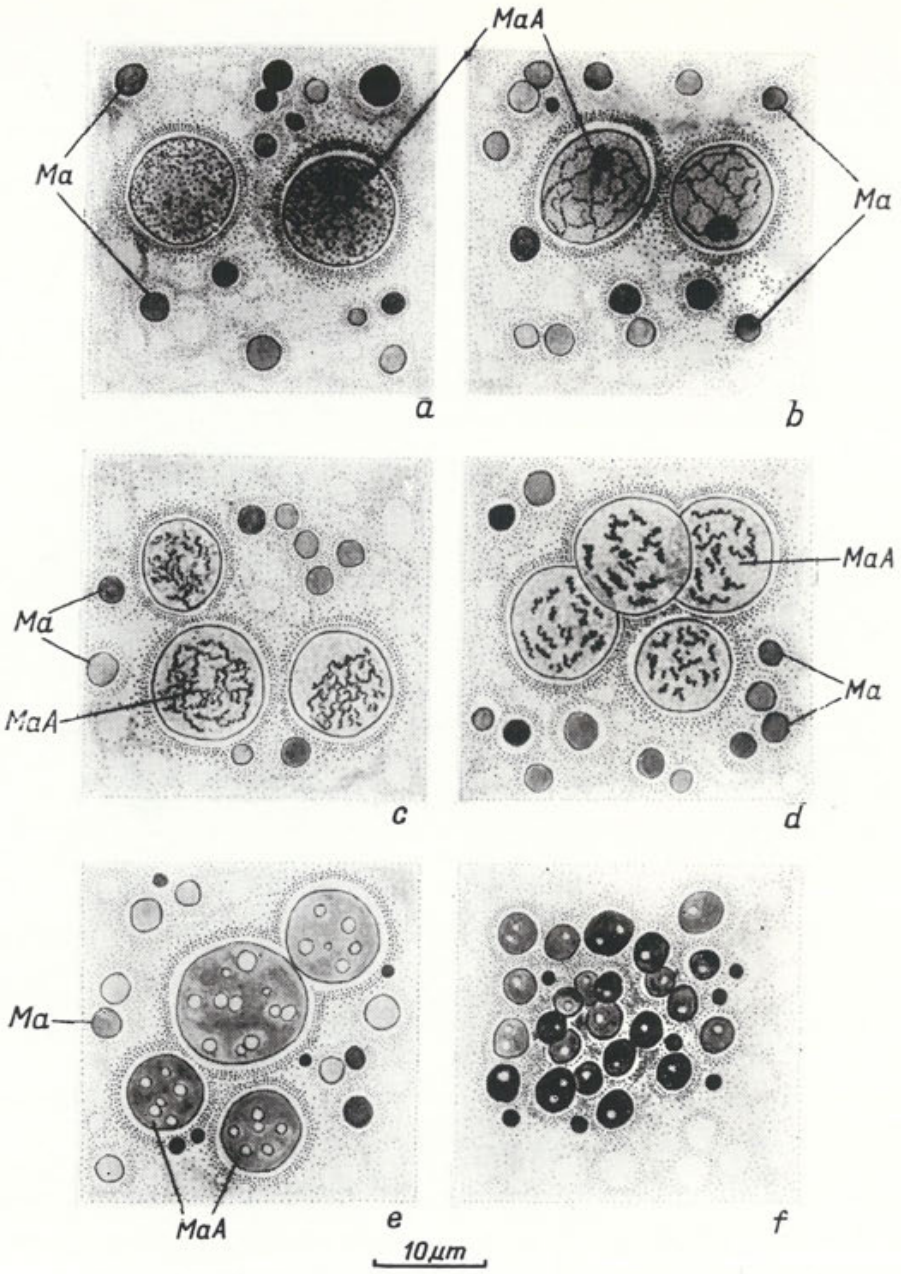


Fig.7

Рис. 7. Развитие зачатков макронуклеусов (a–d — срезы 5 µm, железный гематоксилин, e, f — тотальные препараты, Фельген); a — стадия мелкозернистого хроматина, b — тонкая хромосомная сеточка, c — стадия укорачивания хромосом, d — стадия коротких хромосом, e — фельген-положительная стадия, f — формообразовательная стадия. Ma — старые макронуклеусы, MaA — зачатки макронуклеусов

Fig. 7. Macronuclear anlagen development (a–d — 5 µm section, iron haematoxylin, e, f — whole mounts, Feulgen); a — stage of fine granular chromatin, b — thin chromosome net, c — chromosome shortening, d — condensed chromosome, e — Feulgen-positive stage, f — formative stage. Ma — old macronuclei, MaA — macronuclear anlagen

образуют тетрады с одной хиазмой (У-образные и крестообразные) (Рис. 1 c). На следующей стадии, очевидно соответствующей диакинезу, хромосомы выглядят компактными и принимают бобовидную форму. Хромосомы соединены в биваленты, между гомологами в некоторых бивалентах заметна щель (Рис. 1 d), хиазм больше не видно. В метафазе первого деления созревания хромосомы располагаются в экваториальную пластинку. Веретено I деления овальное, с хорошо выраженными ахроматиновыми нитями (Рис. 1 e). В анафазе наблюдается сильное удлинение веретена. Во время первого деления созревания все элементы ядерного аппарата сосредотачиваются в середине тела инфузории. Задняя четверть клетки и область от хобота до ротового отверстия остаются свободными от ядер.

Второе деление созревания Mi начинается через 12–15 час и продолжается 1.5–2 часа. В нем участвуют от 2 до 6 продуктов I деления Mi. В профазе ядра набухают (диаметр около 5–7 μm), а внутри них образуется тонкая спирема (Рис. 2 a). Довольно скоро нити спиремы становятся короткими и толстыми (Рис. 2 b). В метафазе второго деления образуется типичная экваториальная пластинка, с хорошо выраженными нитями веретена (Рис. 2 c). Форма ядер более округлая и длина веретена меньше, чем в метафазе I деления. На стадии анафазы и телофазы второго деления ядра имеют вытянутую форму. Эти стадии протекают довольно быстро.

Третье деление созревания и последующие стадии вплоть до делений синкариона следуют очень быстро друг за другом и протекают приблизительно за час. Количество Mi, начинающих III деление, колеблется от 1 до 3, но заканчивает его, по-видимому, одно ядро. Диаметр Mi очень мал — 3–4 μm (Рис. 3 a). В метафазе и анафазе хромосомы настолько мелки, что едва тонкая экваториальная и дочерние пластинки (Рис. 3 b).

Пронуклеусы и деления синкариона. Образовавшиеся во время III деления созревания ядра морфологически отличаются друг от друга. Одно из них мелкое, плотное, диаметром около 2 μm. Другое более крупное (около 4 μm), зернистое, оно слабее окрашивается по Фельгену. Оба ядра были обнаружены в районе глотки или слившихся хоботов, то есть в перемычке между партнерами, причем в последнем случае более крупное ядро находилось проксимальнее (Рис. 4). Иногда более крупное ядро лежало в основании хобота. Очевидно, эти два ядра являются пронуклеусами.

Следующая отмеченная стадия — образование синкариона. Пронуклеусы лежат рядом друг с другом в передней части тела дилептуса (Рис. 5). В это время они находятся и интерфазном состоянии. В месте их соприкосновения ядерные оболочки сливаются и затем сливается содержимое пронуклеусов. Образовавшийся синкарион почти сразу начинает делиться. В результате этого деления образуются два ядра, которые приступают ко второму делению синкариона (Рис. 6). Веретена второго деления лежат параллельно друг другу. Число делений синкариона очень изменчиво и варьирует от 2 до 4, изредка

встречается одно деление (Табл. II 5). В результате делений синкариона образуются ядра, часть которых начинает расти и превращается в зачатки макронуклеусов, а часть дифференцируется в микронуклеусы.

Изменения зачатков макронуклеусов. Зачатки макронуклеусов образуются через 16 час, в условиях нашего опыта, и в начале имеют небольшие размеры (5 μm), затем они набухают и достигают 7–10 μm в диаметре. Сначала хроматин выглядит мелкозернистым и довольно равномерно распределен по всему ядру (Рис. 7 a). Затем становятся видимыми тонкие хромосомы (Рис. 7 b), которые укорачиваются, утолщаются и равномерно распределяются по всему ядру (Рис. 7 c, d). Вслед за этим, на стадии 24–25 часов, партнеры расходятся. Дальнейшие ядерные изменения протекают в эксконъюгантах.

Эксконъюгационный период. У просмотренных эксконъюгантов было выявлено большое разнообразие в соотношении числа Mi и зачатков Ma (Табл. II 5–7, Рис. 8). Наиболее часто встречающееся соотношение — один Mi и четыре зачатка Ma (Табл. II 6). В эксконъюгантах продолжается развитие зачатков. Последние становятся отчетливо фельген-положительными (Рис. 7 e). Отдельных хромосом уже более не видно. В каждом зачатке появляются мелкие вакуоли, не красящиеся по Фельгену. Приблизительно в это время происходит несколько последовательных делений зачатков надвое

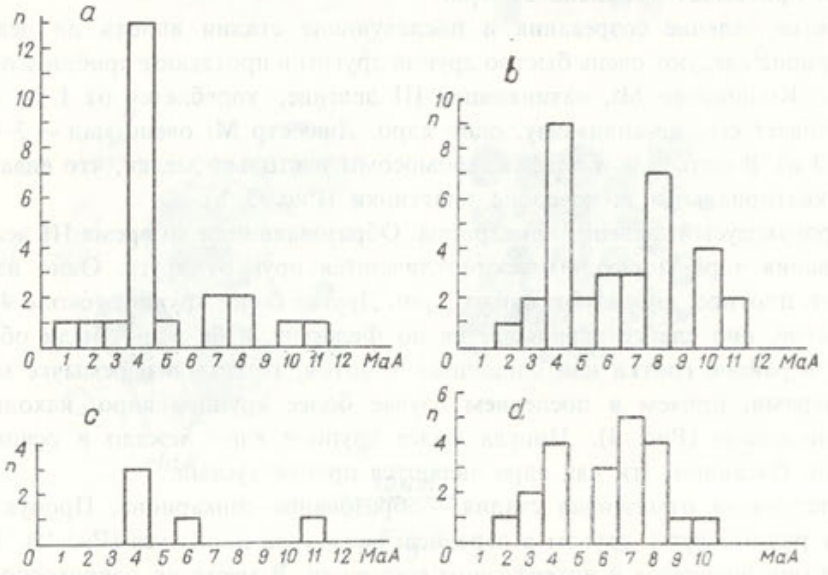


Рис. 8. Зависимость между числом зачатков макронуклеусов (по оси абсцисс) и числом микронуклеусов у эксконъюгантов. На оси ординат отложено число случаев (n), в которых наблюдалось определенное количество макронуклеусов: a — при 1 Mi, b — при 2 Mi, c — при 3 Mi, d — при 4 Mi

Fig. 8. Correlation between the number of macronuclear anlagen (abscissae) and the number of micronuclei in exconjugants. Ordinates: frequencies for each number of macronuclei in cases with one Mi (a), with two Mi (b), with three Mi (c), and with four Mi (d)

(Табл. II 8) пока из них не образуются многочисленные тельца — дефинитивные макронуклеусы (Рис. 7 f), число последних превышает 300 (Табл. II 9). Нормальное число Mi восстанавливается в результате митотических делений имеющихся Mi. Реорганизация ядерного аппарата *D. anser* не сопровождается метагамными делениями экскоңъюгантов.

Старые макронуклеусы могут не изменять свою форму до конца конъюгации, только со временем бледнее красятся по Фельгену и исчезают совсем уже у экскоңъюгантов через 50–60 часов. Некоторые макронуклеусы через 4–5 час после образования пар приобретают форму неправильно изогнутых тяжей. Далее эти тяжи распадаются на мелкие фрагменты, часть которых дегенерирует. Оставшиеся фрагменты сливаются в более крупные, неправильной формы, которые постепенно все бледнее и бледнее окрашиваются по Фельгену и исчезают совсем.

Конъюгация у пар типа B

В некоторых случаях при изучении конъюгации у *Dileptus anser* было обнаружено отсутствие ядерных изменений в одном из партнеров, тогда как в другом эти изменения происходили. По внешнему виду партнеров невозможно сказать, какого типа ядерная реорганизация у них происходит. Морфологических различий в стадиях реорганизации ядер у пар A и пар B практически нет. Отличаются лишь количества ядер, участвующих в реорганизации.

Первое деление созревания начинается через 6–12 час и в нем принимают участие 5–8 Mi. Через 12–14 час 2–3 микронуклеуса приступают ко II делению, а затем следует III деление, в котором участвует лишь одно ядро. В результате этого деления, как и у пар типа A, образуются два морфологически различных ядра, пронуклеусы. По-видимому в том партнере пары типа B, где идет процесс ядерной реорганизации, синкарион образуется путем слияния двух сестринских пронуклеусов, то есть путем автогамии. Однако самый момент слияния пронуклеусов нами не наблюдался. Образовавшийся синкарион делиться 2–4 раза. Дальнейшее развитие дериватов деления синкариона сходно с таковым в парах типа A. Через 24–25 час происходит разъединение парочек. В экскоңъюганте, где происходит ядерная реорганизация, развитие зачатков макронуклеусов и микронуклеусов происходит таким же образом, как и у экскоңъюгантов типа A. Ядерный аппарат партнера, в котором не происходили ядерные изменения, остается аналогичным таковому у вегетативных клеток.

Наряду с конъюгацией, происходящей по типу A и B мы обнаружили в 27% случаев пары, где отсутствовали ядерные изменения в обоих партнерах (пары типа C). Такие клетки оставались соединенными друг с другом в течение 10–15 час, а затем расходились.

Обсуждение

Преконъюгационные и конъюгационные деления. Преконъюгационные деления среди инфузорий, для которых известны типы спаривания, описаны только у *Stentor coeruleus* (Webb and Francis 1969). Особые деления перед началом конъюгации среди свободноживущих инфузорий описаны у *Didinium nasutum* (Prandtl 1906), *Stentor polymorphus* (Mulsow 1913), *Loxodes* (Bogdanowicz 1930), *Fabrea salina* (Ellis 1937). У *Dileptus* особые деления перед конъюгацией были отмечены еще Гертвигом (Hertwig 1904). Гертвиг пытался связать их с голоданием инфузорий и назвал их "голодными делениями". Вишер (Visscher 1927) также отмечал два деления перед конъюгацией, причем во время преконъюгационного деления им не было обнаружено ни одного делящегося ядра. Таким образом он считал преконъюгационные деления плазмотомией, не сопровождаемой размножением ядер. По нашим данным, прконъюгационные деления у *Dileptus anser* встречаются довольно часто и представляют собой нормальное деление надвое, а не плазмотомию. Mi во время преконъюгационного деления делятся митозом, а Ma становятся удлиненными и перешнуровываются. Дилептусы, образующие пары, отличаются от вегетативных клеток только меньшими размерами, в отличие от паразитических и ряда низших инфузорий, у которых ядерный аппарат вегетативных клеток и преконъюгантов различен (Dogiel 1912, Полянский и Стрелков 1938, Райков 1958, 1963, Raikov 1972, Kovaleva 1972 и др.).

Обнаруженные у *Dileptus anser* деления после образования пар (конъюгационные деления) были до сих пор описаны только для инфузорий отряда *Apostomata* (Minkiewicz 1912, Chatton et Lwoff 1935). У этих инфузорий после соединения конъюгантов начинается палинтомия обоих партнеров, в результате которой образуются цепочки особей. Последние затем распадаются на мелкие конъюгирующие пары. Этот процесс назван синдесмогамнией или зигопалинтомией. У *Dileptus anser* поведение ядерного аппарата при приконъюгационном и конъюгационном делении одинаково. В связи с этим можно предположить, что конъюгационные деления, по сучшеству, являются преконъюгационными, но смещенными во времени и происходящими после образования пар.

Ядерная реорганизация при конъюгации. Необходимо отметить, что описанный нами ход конъюгации у *D. anser* существенно отличается от данных Вишера (Visscher 1927). Очевидно можно сравнивать нашу конъюгацию у пар типа A с конъюгацией, описанной Вишером. Нам не встретился ни один случай, чтобы к I делению созревания приступал только один Mi, как это описано Вишером. Число веретен первого деления созревания не обязательно равно числу Mi, но их всегда несколько. Также не было отмечено миграции всех ядерных элементов во время первого деления созревания в задний ко-

нец тела дилептуса, а, наоборот, была отмечена их концентрация в середине тела. Из работы Вишера не ясно, каково число делений синкариона у *Dileptus* "*gigas*" (*anser*?). По Вишеру, в результате первого деления синкариона образуются два неравных ядра. Маленькое ядро он обозначает как Mi, а большое — как Ma. Далее следует одно, два, или три деления этих ядер. Каким образом делятся эти ядра, наблюдался ли митоз — из работы Вишера не ясно. Очевидно, можно слитать что, по Вишеру, у *Dileptus* имеет место одно деление синкариона. По нашим данным, число делений синкариона очень изменчиво, изредка бывает одно деление, а чаще от 2 до 4. По Вишеру, деления синкариона происходят в экскоиъюганте. Мы считаем, что эти деления имеют место до разъединения парочек (через 15–16 час в условиях нашего опыта). Поскольку наши данные существенно отличаются от данных Вишера, сказанное выше дает возможность предполагать, что последний работал с каким-то другим видом.

В этой связи важно, что в систематике рода *Dileptus* долгое время была путаница (Dragesco 1963). Вишер называл инфузорию, с которой работал, *Dileptus gigas*. В дальнейшем Хаес (Hayes 1938) и Дражеско (Dragesco 1968), используя описания Вишером ядерного аппарата, его формы, высказали мнение, что последний имел дело с *D. anser*. Однако это не более, чем предположение. В настоящее время трудно сказать, с каким видом *Dileptus* работал Вишер и почему наши и его данные столь сильно отличаются друг от друга.

Первому делению созревания у инфузорий предшествует мейотическая профаза. Обычно она включает одну из двух характерных стадий—либо "стадию серпа", либо "стадию парашюта". Несмотря на то, что нами не было отмечено ни той ни другой стадии мы считаем, что в I делении созревания имеет место мейоз. Об этом говорит наличие тетрад в диплотеновой стадии, бивалентов во время диакинеза, а также большой размер ядер, участвующих в первом делении созревания.

Пронуклеусы, стадия образования пронуклеусов и их миграция, мало изучены Вишером. Однако он пишет, что обмен пронуклеусами происходит. Основным доказательством обмена пронуклеусами могут служить непосредственные наблюдения в момент перехода пронуклеусов из одного партнера в другой. Поскольку нами были обнаружены пронуклеусы в районе хобота (Рис. 4), можно предположить, что у *D. anser* имеет место движение мигрирующих пронуклеусов по классической схеме. Однако, не исключено, что, находящиеся в районе хобота пронуклеусы не переходят в клетку партнера и что синкарионы у пар типа А образуются в результате двойной автогамии. Тем не менее, значительно более вероятно, что у пар типа А ядерная реорганизация протекает по типу амфимиктической конъюгации. В литературе примеры двойной автогамии в основном известны в пределах рода *Paramecium* (Wichterman 1940, Diller 1948, 1958, Янковский 1960).

Своеобразная ядерная реорганизация наблюдается у пар типа В. Ссылок в литературе на подобные варианты (отсутствие ядерных изменений в одном из партнеров и наличие их в другом) нам не удалось найти. У пар типа В возможны два варианта ядерных изменений. Если мигрирующий пронуклеус переходит в партнера, в котором ядерные изменения не происходят, то новый ядерный аппарат у клетки-донора образуется из одного пронуклеуса (гемикариона). В литературе подобные примеры известны (Chen 1940 a, b, d, e, f и др.). У *Paramecium bursaria* односторонний переход мигрирующих пронуклеусов иногда имеет место при конъюгации, где ядерные процессы идут в обоих партнерах. У *P. caudatum* и *P. bursaria* развитие одного гемикариона в каждом партнере может происходить при конъюгации нормальной особи с безмикронуклеусной (Chen 1940 a, b, e, f, Скобло 1969, Скобло и Осипов 1972).

Второй вариант ядерных изменений у пар типа В возможен, если оба пронуклеуса клетки, в которой происходят ядерные изменения, сливаются между собой в синкарион. Такой тип ядерных преобразований можно назвать односторонней конъюгационной автогамией. Эта возможность нам кажется более реальной, но пока нельзя исключать и существование первого варианта.

В литературе есть примеры автогамии, индуцированной путем искусственного или естественного преждевременного разъединения конъюгирующих пар (Полянский 1938, Vivier 1960, Осипов 1966, Ossipov and Skoblo 1968, Скобло и Осипов 1966, Skoblo and Ossipov 1968, Скобло 1972 и др.). Для нормального течения ядерных процессов здесь достаточно довольно короткого времени соединения партнеров в отличе от нашего варианта.

Наконец, по каким-то причинам в парах типа С ядерные процессы вовсе не происходят, что, по-видимому, можно рассматривать как пример псевдоконъюгации.

Период реконструкции ядерного аппарата у *Dileptus anser* отличается столь большой вариабельностью, что трудно выделить один из способов реконструкции и считать его нормой. В большинстве случаев новые макронуклеусы образуются из нескольких зачатков. Поскольку метагамных делений экс-конъюганта у *D. anser* нет, то, возможно что Ма в пределах клона могут происходить из разных зачатков. Это существенно осложнит последующую генетическую работу с этим объектом.

Резюме

Перед образованием пар особи *D. anser* (O. F. M.) могут испытывать преконъюгационные деления. Инфузории могут делиться также и в первые часы после соединения (конъюгационные деления). Во время тех и других делений Mi делятся митозом, а Ма становятся лентовидными или палочковидными и перешнуровываются надвое не сливаясь между собой.

Почти все микронуклеусы участвуют в 1 делении созревания. Ко второму делению созревания приступают от 2 до 6 продуктов первого деления. Третье деление начинают от 1 до 3 ядер, но заканчивает его, по, видимому, одно ядро. Образовавшиеся в результате третьего деления ядра морфологически отличаются друг от друга, очевидно — это пронуклеусы. Далее следует образование синкариона и его последующие деления. Число делений синкариона изменчиво (от 1 до 4). Часть дериватов делений синкариона превращается в зачатки Ma, а часть в Mi. Зачатки Ma позже последовательно делятся надвое и принимают форму, характерную для Ma вегетативных клеток. Нормальное число Mi восстанавливается в результате их дополнительных митотических делений. Метагамные деления экскоиъюгантов (с распределением зачатков Ma) отсутствуют.

Было обнаружено большое разнообразие в путях реорганизации ядерного аппарата во время конъюгации *D. anser* (O. F. M.) Выделено три типа пар: пары A — ядерные изменения происходят в обеих клетках, пары типа B — ядерные изменения происходят только в одном партнере и пары типа C — ядерные изменения не происходят ни в одном из партнеров.

SUMMARY

The ciliates *Dileptus anser* (O.F.M.) can undergo a pre-conjugation division. They can also divide during the first two hours after pairing (the so-called conjugation division). During both pre-conjugation and conjugation divisions, micronuclei divide by mitosis. Macronuclei become ribbon-like or stick-like and divide too without fusing with each other.

Almost all micronuclei undergo the first maturation division, which is preceded by a typical meiotic prophase. Two to six derivatives, of the first division take part in the second maturation division. One to three nuclei begin the third maturation division, but only one of them brings this division to the end. Two pronuclei are formed which differ from each other. Synkaryon formation follows. The number of synkaryon divisions varies from one to four. Macronuclear and micronuclear anlagen are formed. The macronuclear anlagen later divide into definitive macronuclei.

The normal number of micronuclei is restored after several additional mitotic divisions. No metagamic divisions of exconjugants (segregating the macronuclear anlagen) exist.

There are three ways of nuclear reorganization during conjugation of *Dileptus anser*: (1) pairs of type A — nuclear reorganization takes place in both conjugants , (2) pairs of type B — nuclear reorganization takes place only in one conjugant, (3) pairs of type C — no nuclear changes are present in either conjugant.

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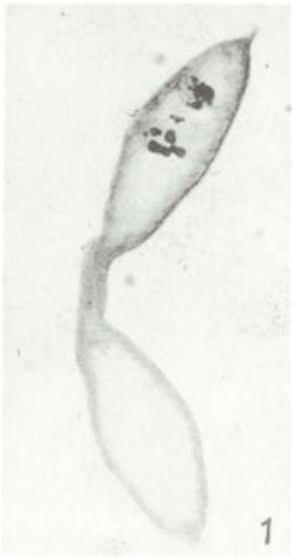
ПОДПИСИ К ТАБЛИЦАМ I–II

1: Конъюгирующие особи *Dileptus anser* O. F. M. клонов L и S (прижизненно, зерна туши маркируют клетку клона L) 100×
2: Отделившийся задний продукт конъюгационного деления (прижизненно). 300×
3: Деление во время конъюгации одного из партнеров (прижизненно, зерна туши маркируют клетку клона L). 100×
4: Вытягивание макронуклеусов при преконъюгационном делении (срез, железный гематоксилин). 1000×
5: Эксконъюгант (тотальный препарат, Фельген). В результате одного деления синкариона образуется 1 Mi и 1 MaA. 1200×
6: Эксконъюгант с 1 Mi и 4 MaA (тотальный препарат, Фельген), 1200×
7: Эксконъюгант с 1 Mi и 5 MaA (тотальный препарат, Фельген). 12000×
8: Деление зачатков макронуклеуса (MaA) надвое (тотальный препарат, Фельген). 1200× Mi — микронуклеусы
9: Преобразование зачатков Ma в дефинитивные ядра (тотальный препарат, Фельген). 1200×

EXPLANATION OF PLATES I–II

1: Conjugation in *Dileptus anser* O. F. M. between cells of L and S clones (living cell, the L animal marked by India ink inclusions). 100×
2: Posterior product of the division during conjugation. In vivo, 300×
3: Division during conjugation of only one partner (in vivo, the L cell marked by India ink inclusions). 100×
4: Stretching of macronuclei during preconjugation division (section, iron haematoxylin). 1000×
5: Exconjugant showing one micronucleus and one macronuclear anlage as a result of a single synkaryon division. (Whole mount, Feulgen). 1200×
6: Exconjugant with 1 micronucleus and 4 macronuclear anlagen. (Whole mount, Feulgen). 1200×
7: Exconjugant with 1 micronucleus and 5 macronuclear anlagen (Whole mount, Feulgen). 1200×
8: Binary division of macronuclear anlagen (Ma A) (Whole mount, Feulgen). 1200×. Mi-micronuclei
9: Transformation of macronuclear anlagen into definitive macronuclei (Whole mount, Feulgen). 1200×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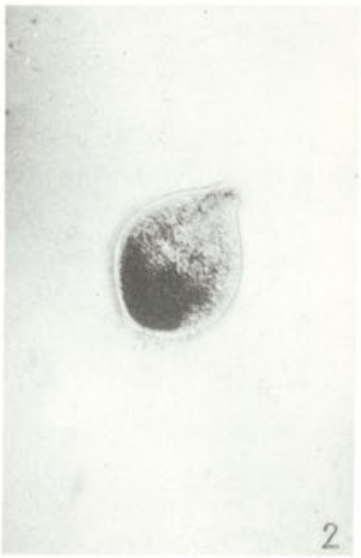
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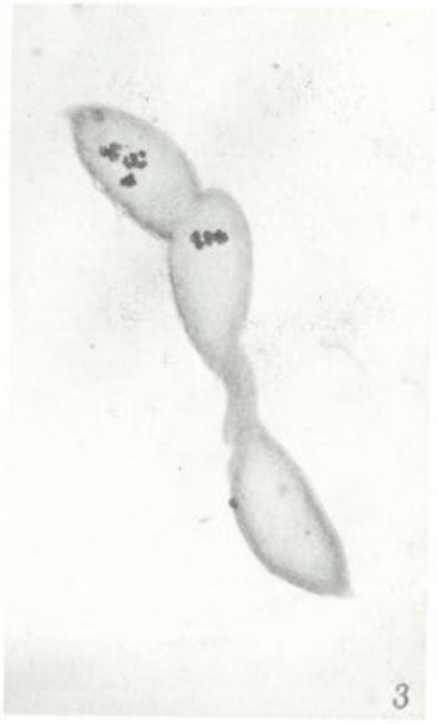
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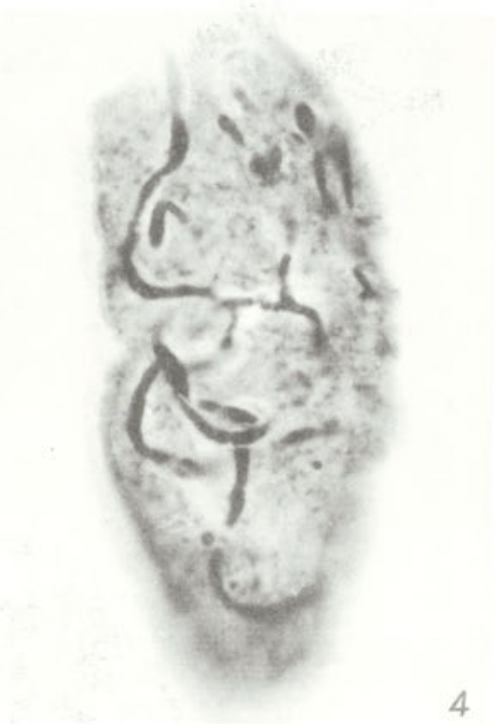
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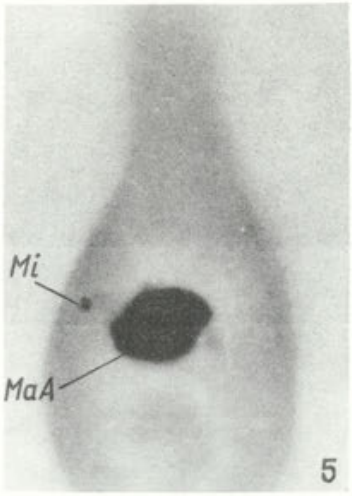

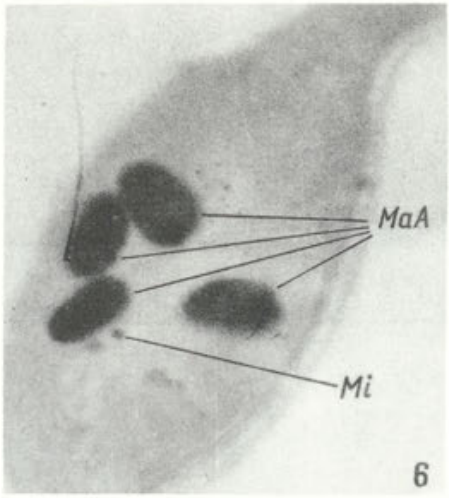

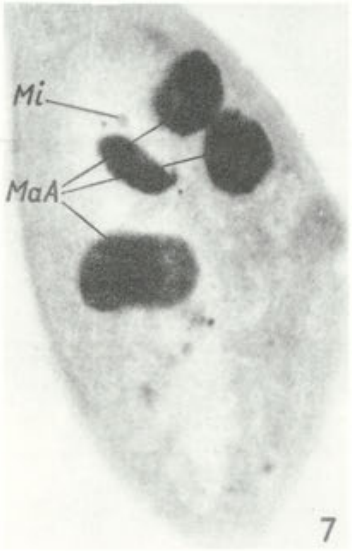

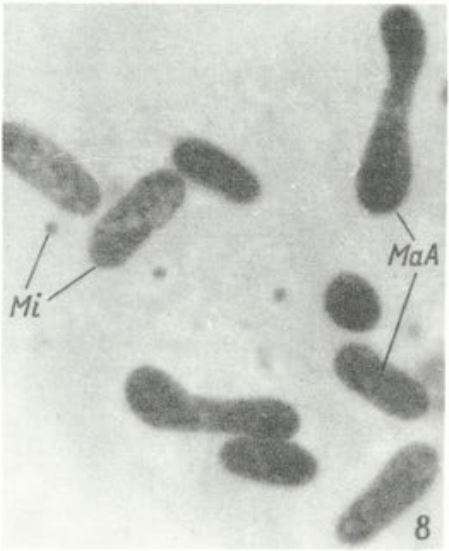

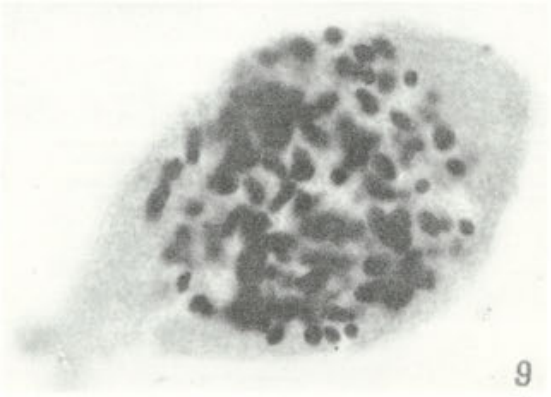

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 24

Department de Zoologie, Universite de Clermont,
Complexe Scientifique des Cezeux, B.P. 45, 63-Aubiere, France

Krystyna GOLIŃSKA[1]

Effect of puromycin on regeneration processes in *Dileptus anatinus* Golińska, 1971

Wpływ puromycyny na procesy regeneracyjne u *Dileptus anatinus* Golińska, 1971

In the following studies the effects of puromycin upon the stomatogenesis and on the processes of proboscis and tail formation in regenerating fragments of *Dileptus anatinus* were observed.

The puromycin inhibits the synthesis of proteins (Yarmolinsky and De La Haba 1959) binding with the peptide chain and causing its detachment from the ribosome (Nathans 1964 a, b, also review of Sprin and Gavrilova 1969).

The effects of puromycin on the regeneration of ciliates was studied in *Stentor* and *Lacrymaria*. The observations concerned mainly the process of stomatogenesis. The exposure to puromycin after operation may inhibit the regeneration of the mouth in stentor, with that the resorption of early primordia occurs, or their development becomes blocked in late stages (Burchill 1968, Ellwood and Cowden 1966, James 1967). James (1967) administrated puromycin for long time before the operation and stated that in this case the later stages of stomatogenesis are blocked — the regeneration may initiate in the presence of puromycin, but it can not pass the stage 2 of regeneration. Burchill (1968) supposed that the final, essential protein synthesis for regenerational stomatogenesis of stentor takes place during the stage 5. Burchill (1968) and James (1967) observed also the slowing down and delay of the regeneration of the mouth of stentor caused by puromycin. A similar phenomena stated Bohatier (1972) in *Lacrymaria*. Ellwood and Cowden (1966) observed the influence of puromycin upon the regeneration of the AZM in stentor — they stated that the inhibition of regeneration is complete and irreversible. However, they used very high concentrations of puromycin.

The divisional processes of ciliates can also be inhibited by action of puromycin. Frankel (1966) observed a significant prolongation and inhibition of division in *Tetrahymena*, and found the transition point for the action of puromycin (Fran-

[1] Permanent address: Department of General Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, 00-973 Warszawa, Pasteura 3, Poland.

kel 1967). He also observed in higher concentrations of puromycin a delay in the rate of resorption of early oral primordia. The study on the effects of puromycin on the divisional processes in *Paramecium* revealed that the cytokinesis is inhibited to a greater extent than the processes of stomatogenesis (Golińska and Hanson 1973).

The data obtained in the studies on the action of puromycin indicate, that the extent of the inhibition of a morphogenetic process depends not only on the concentration and the time of action of the inhibitor, but also on the kind of observed process (e.g., stomatogenesis, cytokinesis, resorption of primordia, remodeling of the shape).

The regeneration of opimers only was described in *Dileptus anser* and *Dileptus cygnus* by Golińska and Doroszewski (1964), Golińska (1966), Golińska and Grain (1969). The proliferation of kinetosomes for the new mouth starts 1/2 h after the operation (Golińska and Grain 1969). All parts of the mouth are formed in the same rate both in nucleated and anucleated opimers of *D. cygnus* (Golińska 1966, Golińska and Grain 1969). The ability of the anucleate fragments of *Dileptus* to regenerate distinguishes this species from other ciliates such as stentor or paramecium, and allows to presume that in *Dileptus* a permanent pool of substances necessary for the development of the mouth exists. In order to study the nature of that cytoplasmic store and in order to ascertain whether the reserve contains precursors which have to be synthesized on the ribosomes, or they are in a form already synthesized — the study on the action of puromycin on the regeneration of *Dileptus* was carried out.

On the basis of the performed experiments it can be presumed that only the oral proteins are present in the cytoplasmic reserve (store) in a ready form, after being synthesized on the ribosomes. The rest of the cytoplasmic reserve which is necessary for the growth of the surface and the shapening of the fragment is sensitive to the action of puromycin. The role of the ecto-endoplasmic microfibrillar layer in the remodeling of a shape, and the interrelations between the functions of the layer and the accompanying vesicular elements are discussed.

Material and methods

The ciliate *Dileptus anatinus* Golińska, 1971, belongs to *Gymnostomata*, *Rhabdophorina*. It is about 900–1200 μm long, the nuclear apparatus containing about 200 Ma and less Mi, is dispersed. The anterior part of the cell, over the cytostome is elongated into appendix — the proboscis. On the ventral side of the proboscis the ventral band containing the toxicyst is located. This is a specialized part of the cytostomal lip. The posterior end of the cell is tapered into small tail.

The culture methods for *D. anatinus* are identical with these for other species of *Dileptus* and have been described for *D. anser* by Golińska and Jerka-Dziadosz 1973. The only difference was the composition of culture medium. In this study instead of Pringsheim solution, natural spring water (Volvic water) was used.

The operation were carried out under the bisecting microscope by hand, using the microscalpel. The cells were always transected in the middle of the body. Therefore, the promer (anterior fragment) posessed the whole oral apparatus and regenerated only the posterior tail-part. The opimer (posterior fragment) was deprived of the oral apparatus and proboscis. For the experiments the interphasal cells from well — fed cultures were chosen, 24 h after feeding.

For the observations in the light microscope, the cells were stained with iron hematoxylin after Parducz 1952. The stained cells were used for the establishing of the stages of the processes of regeneration. Also the observations on the course of regeneration in the presence of the puromycin were mostly based on the preparations stained with hematoxylin.

The preparations for the electron microscope were prepared as for *D. cygnus* (Grain and Golińska 1969). The material was fixed in osmic acid dissolved in phosphate buffer. The slices were stained with uranyl acetate and lead citrate after Reynolds (1963). The material was observed, and photographed under Siemens Elmiscop 1, and partly under Jem 7a.

The inhibitor used in the study was puromycin dihydrochloride (Nutritional Biochemical Corporation, Cleveland, Ohio). The inhibitor was dissolved in the culture fluid. The final concentrations used in the study were: 100, 200, 300, 600, and 1000 µg/ml. The cells were operated after the transferring into the inhibitor solution and kept there until fixation. The possibility of recovery and resuming of regeneration were not studied.

Results

Regeneration of untreated fragments

The three developmental processes were observed: the formation of oral primordia, shapening of the proboscis and shapening of the posterior end of the cell — the tail.

That the processes of the formation of oral primordia and shapening of proboscis were considered separately followed from the observation that a particular stage of the development of the oral primordia does not correspond to a particular length of the proboscis. Both processes differ in the rate, sometimes the formation of the proboscis occurs faster, another time the oral primordia develop faster. The difference in phase of those two processes never exceeded one stage. The stages of formation of the oral primordia and shapening of the anterior and posterior parts are represented schematically on Fig. 1. The schemes of the opimers represent the most often found fragments with the developing oral primordia and proboscis. The graphic designation of the stages inserted in the squares on the lower part of the drawing is further used in all the following figures.

The regeneration stomatogenesis of *D. anatinus* is very similar to that described in *D. cygnus* by Golińska and Grain (1969). In the opimers as a result of the healing of the wound the anterior pole of the fragment become rounded and uniformly covered by cilia. As the first stage of stomatogenesis was assumed the formation of the non-ciliated area on the anterior pole (Fig. 1 I, Pl. I 1). This field grows and elongates toward the ventral side of the fragment (Pl. I 2). In this time the resorption of the cilia situated on the field takes place. The proliferation of kinetosomes and formation of the future feeding cilia occur on the periphery of the field. 30–40 min after operation the trichocysts start to plug into the primordium of the ventral band.

During the second stage of stomatogenesis, as observed in the light microscope, the elongation of the primordium takes place. On the right side of the non-ciliated field the kinetosomes come to be arranged into double row of feeding cilia (Fig. 1 II, Pl. I 3 arrow). The preparations fixed 55–60 min after operation, sectioned and

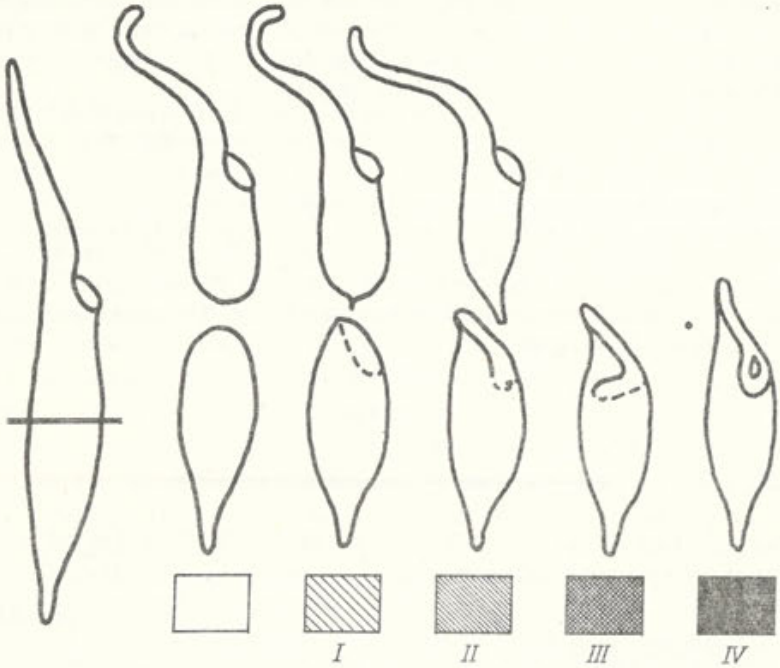

Fig. 1. The schematic representation of the subsequent stages of regeneration of *Dileptus anatinus*. The upper row of drawings shows the promers — fragments which regenerate the tail. The lower row of drawing shows the stages of the formation of mouth and the shapening of proboscis. Below the drawing the squares represent the graphic designation of the stages

observed in the EM showed the well-developed ventral band and the first appearance of the differentiating mouth.

The third stage of development is characterized by the formation of the paracytostomal elements. In the light microscope it can be observed that the posterior part of the non-ciliated field elongates and grows to the animal's right. Owing to that the field appears in a shape of reversed letter L (Fig. 1 III, Pl. I 4). The part of the field which is perpendicular to the antero-posterior axis of the body represents, similarly as in division (Golińska 1972) the future paracytostomal lip. The sections of this stage observed under the EM, showed the presence of the nemadesms of both the external and internal baskets, the bundles of the transverse fibers and the typical phagoplasm with the characteristic smooth vesicles. In the stage in question the ventral band on the proboscis is long. On its left side the ordering of kinetosomes takes place, the left paracytostomal kinety and short oblique rows are formed (Pl. IV 4). The EM pictures of the band in this stage do not differ from that of a normal specimen.

During the fourth stage of stomatogenesis (Fig. 1 IV, Pl. I) the cytostom is formed. This is probably achieved by curving to the left of the elongated primordium of the ventral band. At the end of this stage all parts of the oral apparatus are formed, but neither the ventral band, nor the paracytostomal lip have yet reached the final size. The formation of the feeding cilia and the nemadesms is not full ascertained.

The second developmental aspect studied was the shapening of the proboscis. Three stages were discriminated in this process. Immediately after operation and during the healing of the wound the operated pole of the posterior fragment achieves a shape of hemisphere (Pl. II 6). As the first stage of the formation of the proboscis the change of this shape into conical-like one (Pl. II 7) was assumed. The apex of this irregular cone is shifted slightly toward the dorsal side, this is the future apex of the proboscis. The second distinct stage represents a short proboscis, with equal length and width (Pl. II 8, Fig. 1 II). During the third stage the proboscis eleongates (Pl. II 9, Fig. 1 III).

The third developmental process taken into consideration is the formation of the tail parts of *Dileptus*. Two stages were distinguished in this process. During the healing of the wound in the anterior fragments the posterior, operated part of the fragment appears as a hemisphere (Pl. II 12). As the first stage of the formation of the tail, the appearance of tiny appendix on the rounded posterior part, was assumed (Pl. II 11, 13, Fig. 1 I). During the second stage the tail appears to be completed. The part of the cell adjoining the tail forms a regular cone (Pl. II 14, Fig. 1 II).

The appearances of the wound healing in the EM pictures are identical with those observed in *D. cygnus* (Golińska and Grain 1969). The healing proceeds in the same way in anterior and posterior parts of the cell. The wound is hauled by the whole ectoplasm. The surface of the wounded region is covered by cilia with their kinetosomes, attached to the microfibrillar layer, as well as other ectoplasmic elements as the mucocysts, microtubular fibers attached to the kinetosomes and different types of vacuolar structures. The edge of the microfibrillar layer which draw onto the wounded portion, is surrounded by the characteristic smooth vacuoles similar to that found in the proximity of the division furrow of *D. anatinus* (Golińska 1972). During the wound healing and later the proliferation of kinetosomes during formation of the tail was not observed.

The timing of normal regeneration of *Dileptus anatinus*

In order to designate the timing of particular stages of regeneration, the preparation stained with iron hematoxylin after Parducz (1952) were used. Each slide contained about 100 fragments operated during 15 min and fixed at known time after operation. 11 samples were fixed, they contained fragments fixed 15–30 min after operation, 30–45 min, 45–60 min, and so until 165–180 min after operation, consequently. On each slide about 50–70 fragments were studied, and the number of cells found in particular stages of stomatogenesis (Fig. 2), shapening of the proboscis (Fig. 3), and shapening of the tail (Fig. 4) were scored.

Stomatogenesis of opimers

The results of observations on stomatogenesis, summarized in the bars on Fig. 2 allow to make some remarks. The dispersion in the rate of regeneration is remarkable. It should be recalled that all fragments were of similar size — the posterior halves of the interphasal cells.

The number of fragments with completed stomatogenesis increases between 1.5 and 2.5 h after operation. Between 2.5 to 3 h (and longer — not included in the bars) the number of completely regenerated cells remains on the same level (about

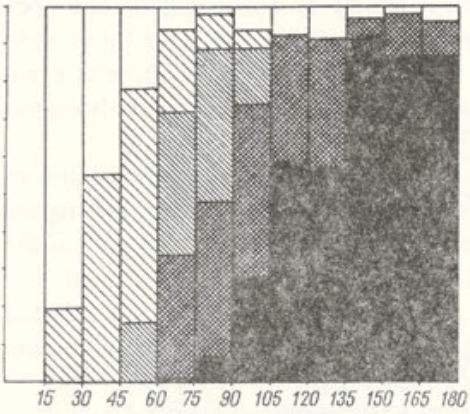

Fig. 2

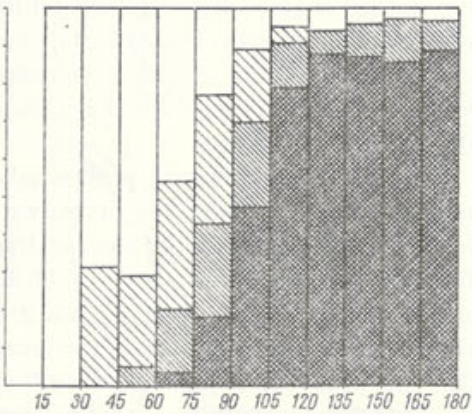

Fig 3

Fig. 2. The formation of oral primordia of *Dileptus anatinus*. Each bar represent a sample of 50–70 fragments. The graphic designations show the precentage of fragments in particular stage of regeneration as shown on Fig. 1. The numbers below the bars represent the time after operation
Fig. 3. The formation of the proboscis of *Dileptus* anatinus. The conventions applied as on Fig. 2

85–90%). In each sample there are always some fragments which are not able to regenerate. There is also in each sample a group of fragments which are arrested in the stage III of stomatogenesis — they are not able to form the cytostome. It is known whether these fragments are able to renormalize after longer time — the samples fixed 4–5 h after operation (not included in the bars) also contained a certain number of fragments arrested in the stage III of stomatogenesis. Two hours and later after operation fragments in stages I and II of stomatogenesis were not found.

It should be stressed that all fragments which are capable to regenerate, complete the process in time shorter than 2.5 h.

The shapening of the proboscis in opimers

The process of the formation of the proboscis is represented in the bars on Fig. 3. Comparing the results of this series with the previous one, it can be observed that the shapening of the proboscis initiates later than the stomatogenesis — the formation of the non-ciliated field for the oral primordia very often takes place

in rounded fragments, before the stage I of the formation of proboscis. Similar as in the preceeding series in some fragments of each sample (5%) the proboscis does not appear. The arresting of the formation of the proboscis was usually observed in some fragments only in stage II — when proboscis can be discriminated as small protrusion of the equal length and width. Two hours after the operation, the fragments arrested in stage II of the formation of the proboscis, are not able to enter the next stages of the process — that is the elongation of the proboscis and narrowing of the anterior part of the fragment. It seems that this arresting of the formation of the proboscis corresponds to the arresting of the stomatogenesis in stage III.

The process of the shapening of the proboscis comes to the end about 2 h after operation.

The formation of the tail in promers

The bars on Fig. 4 represent the data concerning the process of the shapening of the posterior part of the fragments.

In this case also the process of regeneration is finished about 2 h after operation. In each studied sample a small number of fragments can be found which is not

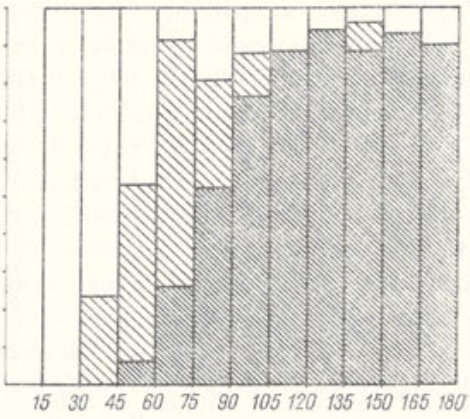

Fig. 4. The formation of tail of *Dileptus anatinus*. The conventions applied as on Fig. 2

able to form the tail parts. The fragments arrested in the stage I of the formation of the tail were not found in each sample and it is not certain if such arresting is possible. It seems rather that the process of the formation of the tail once started is always finished.

The process of regeneration of *Dileptus anatinus* with its three aspects: stomatogenesis, formation of proboscis and tail, involves the regions immediately adjoining the wounded part only. After the end of the regeneration of the tail a cell having the large proboscis and cytopharyngeal complex is obtained (Pl. II 14). After regene-

ration of the anterior part, the tail remains unproportionally large. The reasuming of the normal proportions of the cell parts must take place later on.

It should be stressed that all the studied regeneration processes of *Dileptus* are finished in the period of time shorter that 3 h.

The course of regeneration in the presence of puromycin

The preliminary experiments revealed that in the presence of puromycin the fragments may react in either of three ways: the initiation of the regeneration may be inhibited and regeneration does not occur. The regeneration may initiate and later may be stopped. Regeneration starts at normal time and proceeds untill completion. The delay of the initiation of regeneration was never observed in *D. anatinus*, and also the prolongation of any stage did not occur. The arresting of development can happen at any stage and under the continuous exposure to puromycin this process proved to be irreversible.

In the experiments with puromycin all fragments were always fixed 3 h after the operation — when all possible regenerational processes could be completed.

Two series of experiments were carried out. In the first series the cells were transferred to the puromycin immediately before the operation. The concentrations of the inhibitor were as follows: 100, 300, 600 and 1000 μg/ml. In the second series the cells were incubated in puromycin for 5 h before the transection. The concentration of the inhibitor was 100, 200, 300, 600 and 1000 μg/ml. The results of the first series of experiments are presented in bars on Figs. 5 A, 6 A and 7A separately for each regeneration process. In all concentrations puromycin to some extent inhibited both the formation of the oral primordia and the shapening of the proboscis and tail. This clearly follows from the comparison with the control bars — the upper ones on Figs. 5, 6, 7 which were taken from the control observations inserted on Figs. 2, 3, and 4. The great individual variability in the resistance to puromycin should be pointed out; in each used concentration certain number of fragments was able to complete the regeneration. It is also clear that none stage of regeneration is particularly sensitive to the action of puromycin.

The process of the formation of proboscis is always more inhibited by puromycin than the process of stomatogenesis. In the extreme case it may lead to the formation of the apically situated cytopharyngeal complex (Pl. II 10), without proboscis. Such regenerated fragments resembled simple *Gymnostomatida*, as *Prorodon* for instance. In most cases the mouth was accompanied by more or less developed ventral band. Very often the fragments posessing a part of the ventral band posessed also an outline of the proboscis — but this was not a rule.

The diagrams for 1000 μg/ml of Figs. 5 A and 6 A contain, exceptionally, the results of scores of two different samples, therefore it should not be presumed that

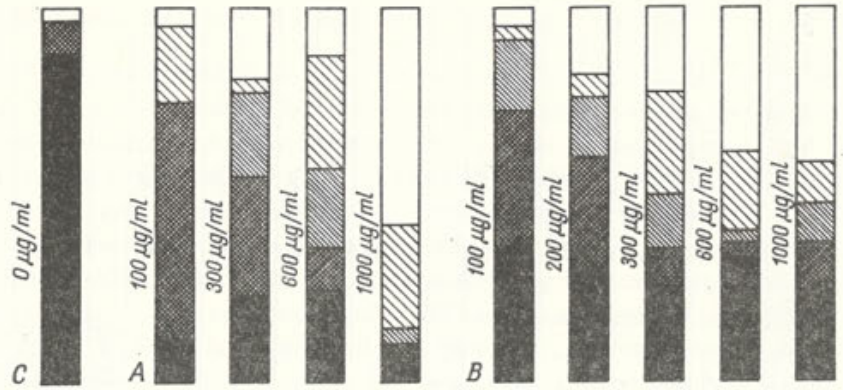

Fig. 5. The effect of puromycin on the formation of the oral primordia of *Dileptus anatinus*. Each bar represent a sample of 50–70 fragments fixed 3 h after the operation. C — the control untreated sample, A — the experiments in which puromycin was administrated immediately before the operation, B — the experiments in which the cells were incubated for 5 h before the operation. The concentrations of inhibitors of each sample are shown next to the bars. The graphic designation of stages as on Fig. 1

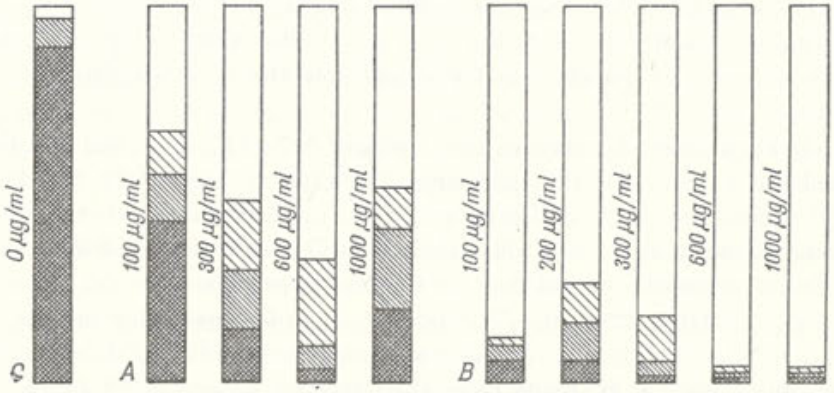

Fig. 6. The effect of puromycin on the shapening of proboscis of *Dileptus anatinus*. Each bar represents a sample of 50–70 fragments which were fixed 3 h after the operation. C — the control, untreated fragments, A — the experiments in which puromycin was introduced immediately before the operation. B — the experiments in which the cells were treated for 5 h before the operation. The concentrations of the inhibitor are shown next to each bar. The graphic designation of the stages as on Fig. 1

the formation of proboscis without the mouth is possible. The specimens with the regenerated proboscis and lacking the mouth were never observed.

The formation of the tail parts appeared to be relatively more resistant to a short exposure to puromycin and only the highest concentration used distinctly inhibited this process. (Fig. 7 A).

The results of the second series of experiments, where the cells were exposed to puromycin 5 h before operation, are represented in the bars on Figs. 5 B, 6 B, and

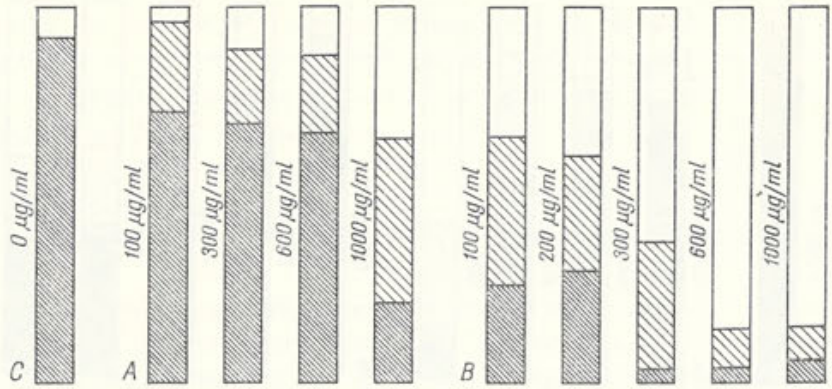

Fig. 7. The effect of puromycin on the shapening of tail in *Dileptus anatinus*. Each bar represents a sample of 50–70 fragments, fixed 3 h after operation. C — the control, untreated sample, A — puromycin was added immediately before the operation, B — the cells were incubated in puromycin for 5 h before the operation. The concentrations of inhibitor are shown next to the bars. The graphic designations as on Fig. 1

7 B. In this series the difference of the extent of inhibition of the stomatogenesis and the shapening was stronger (compare 5 B and 6 B). More often than in the previous series the fragments with the apically stiuated cytopharyngeal complex appeared.

A longer exposure to puromycin (compare B and A form Figs. 5, 6, and 7) distinctly inhibited the processes of the shapening of the tail and proboscis, but did not arrest the formation of the oral primordia. Also the dependence between the concentration of the inhibitor and the extent of inhibition was more distinct for the formation of proboscis and tail than for the stomatogenesis.

The independence of the degree of inhibition of the regeneration process, upon the concentration of inhibitor and time of exposure is greater for the shapening of the tail than the proboscis. It should be recalled, that the process of formation of the proboscis is closely connected with the formation of ventral band — being the part of the oral primordium, whereas the shapening of the tail involves the remodeling of preexisting structures only.

The exposure longer than 5 h before operation in the concentration above 200 µg/ml leads to a gradually stronger inhibition of all the regeneration processes. A high percentage of the fragments incubated for 24 h before the operation, died. The observations under the light microscope revealed, that the individuals which survived 24 h in the presence of the inhibitor and than were transected — are able only to close the wound by the ciliated surface. No other regeneration processes were observed. When the operated and unoperated specimens of *D. anatinus* were left in the puromycin for longer time, all of them died about 36 h after the beginning of the experiments.

The ultrastructural changes of fragments regenerating in the presence of puromycin

All fragments studied in the EM were fixed 3 h after the operation — that means that the EM pictures do not reflect the regeneration process going on, but the processes arrested or finished. The fragments were incubated before the operation for 1, 2, 3, 5 and 24 h. The concentrations of the inhibitor were the same as in the previous series. The promers and opimers were kept and fixed in separate samples.

The pictures of the fragments incubated for 1, 2 and 3 h before the transection were very similar, both for the preparations differing in time of incubation and in the concentration. The changes found in the endoplasm were the same for promers and opimers. In the endoplasm of all the observed fragments numerous autophagic vacuoles were observed. They contained mitochondria, toxic trichocysts, lipids and some finely granulated material. The toxicysts found in the endoplasm were almost exclusively located in the autophagic vacuoles (Pl. VI 28).

The changes of the boundary between endo and ectoplasm were observed. In normal cell the microfibrillar layer separating the ecto and endoplasm is surrounded by canals of rough endoreticulum, and by vacuoles with smooth walls, round in the cross-section with the diameter about 1000–1200 Å (Pl. III 15). The smooth vacuoles accompanying the microfibrillar layer are less numerous in *D. anatinus* in comparison to analogous systems in *D. cygnus* (see Grain et Golińska 1969), but always can be found. After the treatment with puromycin the changes in the structure of the endoplasmic reticulum adjoining the layer can be observed. The canals posses very spaced ribosomes, or the ribosomes are located only on one side of the canal (Pl. III 16). The smooth vesicles accompanying the microfibrillar layer remain unchanged.

The primordia of the ventral band, which can be formed in the posterior fragments may posses normal toxic trichocysts. The arrangement of kinetosomes and microtubular fibers on the ventral band is normal. The pictures of the cytopharyngeal complex revealed that all structures of the mouth — both microfibrillar and microtubular, may develop in the presence of the puromycin (Pl. V 22, 23). The proliferation of somatic kinetosomes was observed in the superficial regions of many fragments both in promers and opimers.

The fragments exposed to puromycin for 5 h before the operation also did not reveal significant differences in the ultrastructures depending on the concentration. However, the time of exposure seems to influence the extent of the changes.

In the endoplasm of the promers and opimers numerous autophagic vacuoles are seen. They are similar to those found in the previous series, however, they do not contain the toxicysts. Also the systems of the rough endoplasmic reticulum which normally accompanies the mitochondria disappears (Pl. VI 29), but some elements of this reticulum can be seen in the endoplasm. Around the microfibrillar layer the detachement of the ribosomes from the elements of the endoplasmic reticulum occurs. In this series also whole cells, or large parts of one cell are found, in which

the microfibrillar layer was deprived of the elements of the rough endoreticulum and only smooth vesicles were present there (Pl. III 17).

The preparations containing opimers from this series showed that all the microtubular and microfibrillar elements of the oral structures have developed. However, the ventral bands which developed in the presence of the puromycin are completely lacking the toxic trichocysts (Pl. VI 27). On their place mucocysts are found between the bundles of transverse fibers.

The last series of the fragments observed in the EM concerned the cells incubated in the puromycin for 24 h before the operation and fixed, as usually, 3 h after operation. The differences in the concentration did not influence the changes in the ultrastructures. After such long incubation approximately 50% of cells died in each concentration used, and the survivers were able to form neither the proboscis or the tail, nor the mouth.

In the endoplams of the promers and opimers the autophagic vacuoles are still present, and also large spaces filled with a finely granulated material are seen (Pl. VI 30). These structures probably correspond to those in *Paramecium* after treatment with various factors (Golińska and Hanson 1973). In *Dileptus* they can also be identified as an ultrastructural pendant to the "vacuolized" cytoplasm, which can be observed in the light microscope.

The EM study of the wounded region of the promers and opimers of this series showed, that the wound is healed and completely covered by the cytoplasm. Equally often fragments can be found in which the microfibrillar layer bounding the wounded region is very delicate, and runs deeply in the endoplasm — taking the shortest run — not along the border between ecto- and endoplasm (Pl. IV 19). In many fragments an interruption of the microfibrillar layer is observed in the wounded region. In such cases the margin of the layer if often broadend and is accompanied by numerous vesicles. Such pictures are interpreted as an arresting or slowing down of the wound healing process. Similar pictures were seen in *D. cygnus* (Golińska et Grain 1969).

In many fragments of this series images reflecting the denaturation of the microfibrillar layer were found (Pl. III 18). In large spaces instead of the fibrillar material appeared a granular material, which seems to "disperse" in the cytoplasm. Fragments with the whole microfibrillar layer changed in this way were not found, since this probably would be equal to the death of a fragment.

In few cases on the preparations containing opimers pictures of early stages of the regeneration were found (compare the data for *D. cygnus*, Golińska et Grain 1969). The images interpreted as resorption of cilia were seen (Pl. IV 21). Also places were found where the microfibrillar layer was probably pushed apart — it looks like it was mixed up with the smooth vesicles (Pl. IV 20). In this region newly formed kinetosomes were observed. That they were new ones — outcomes from their location deep under the surface, lack of the cilia, and the lack of any depression of the surface above them. The kinetosomes possessed only the transverse fibers (Pl. IV

20), in that they are similar to the kinetosomes found in early stages of the formation of the ventral band. It seems, therefore, that fragments maintained for 24 h in the solution of puromycin are still able to initiate the process of stomatogenesis, although they are not able to complete the process.

Discussion

As a result of the performed study, it was observed that puromycin inhibits in different way the process of formation of the oral primordia and the processes of shapening of the tail and proboscis. The inhibition of the process of shapening depends on the concentration and the time of action of the agent. The process of the formation of oral primordia, however, is more strongly inhibited in higher concentrations, but does not show any relation with the time of exposure (lack of the decrease of the number of regenerated fragments in series B Figs. 5, 6, 7, in comparison with series A). The number of fragments which are able to develop the oral primordia in the presence of puromycin is relatively stable. It should be recalled, that the cytoplasmic reserve the existence of which is deduced from the behaviour of anuclear fragments (Golińska and Grain 1969) should contain the proteins which are necessary for the development of the oral apparatus as well as those used in the processes of the formation of the proboscis and tail — during the growth of the surface.

In anucleated fragments both processes — the stomatogenesis and the formation of the proboscis — occur parallel, similar as in the normal cells. Moreover, the cytoplasmic reserve which is necessary for the regeneration of *Dileptus* is present in the cytoplasm all the time during the cell cycle: there is no such stage in which the removing of nuclear apparatus prevents the regeneration (Golińska 1966).

The mode by which puromycin inhibits the regeneration allows to precise the considerations of the hypothetic cytoplasmic reserve. It seems that the proteins which are necessary for the shapening of the proboscis and tail are present in the pool in the form of precursors, and have to be synthesized on the ribosomes, but the proteins which are necessary for the stomatogenesis may exist in the form of building blocks (after synthesis on the ribosomes) at least in some of the stages of the cell cycle. This supposition is consistent with the studies of Frankel (1967) who found that puromycin inhibits the divisional morphogenesis when administered before the "transition point" in *Tetrahymena*. The numerous studies on the action of different inhibitors of metabolic syntheses and their influence on the stomatogenesis of *Tetrahymena*, also speak in favor of the existence of the cytoplasmic pool of proteins required for the development of the mouth (Frankel 1969, see also discussion in Rannestad and Williams 1971). In *Tetrahymena*, the material needed for the building of the microtubules is finally synthesized in particular moment of the cell cycle (Rannestad and Williams 1971).

The non-uniform development of the oral primordia and proboscis may lead, as it has been observed in this study, to the appearance of fragments with apically situated cytostome and completely lacking the proboscis with the ventral band. Such a cytopharyngeal complex posess its own nemadesms connected with the kinetosomes which are specially formed. It is interesting that in the case when the process of the shapening of the proboscis is arrested, the part of the oral primordia which is normally formed on the ventral side of the proboscis does not develop. However, the elements of the cytopharyngeal complex do develop, in spite that in the course of the normal development the ventral band forms as first, then the cytopharyngeal complex follows in the later steps of the morphogenesis. It seems therefore, that the process of the formation of the proboscis somehow decides that the originating structures organize into the system of the ventral band, or the system of the cytopharyngeal complex.

The inhibition of the formation of the proboscis and the tail depends on time with the disappearance of the ordered systems of the rough endoplasmic reticulum situated around the microfibrillar layer separating the ecto- and endoplasm, and situated around the mitochondria. The deterioration of the rough endoreticulum after the treatment of the puromycin was observed in metazoan cells by Gersh and Ude (1971). The breakdown of polyribosomes was observed by Gambetti, Gonatas and Flexner (1968), Zambrano and De Robertis (1967). The detachment of the ribosomes from the reticular membranes was observed after the inhibition of protein synthesis caused by actinomycin D (Gersh and Ude 1970). It seems therefore that the detachment of the ribosomes from the reticular membranes is related to the inhibition of protein synthesis.

The inhibition of the shapening of the fragment by puromycin is probably not related to the arresting of the synthesis of the microtubular elements. This supposition is based on the fact that after exposure to puromycin for 24 h the opimers are able to form new kinetosomes. It seems therefore that the building blocks for the microtubules are present in fragment and are ready to be used. The promers kept in puromycin for 24 h are not able to form even a little tail. Fragments exposed to puromycin for 24 h are still able to form the microfibrillar layer, which separates the wounded part from the rest of the cytoplasm. This suggests that the fragments are also able to synthesize the microfibrillar elements, or that there is possibility of an extensive regrouping of the microfibrillar material in the fragments which have the ability of the formation of the proboscis or tail completely blocked.

The inability of the fragments to form the proper shape is probably also not due to the inhibition of the synthesis of the membranes, The fragments with the apically located cytostome — which are not able to form the proboscis — can form a normal phagoplasm containing the smooth, elongated vesicles. Moreover, the autophagic vacuoles, very often surrounded by multimembraneous walls, can be found in the fragments exposed for 24 h to puromycin. The possibility of the inhibition of specific kind of membranes can not be ruled out. Friedman and

Cardell (1972) observed, that puromycin inhibits specifically the synthesis of membranes of the Golgi apparatus.

In the process of shapening of the fragment, besides the growth of the surface, a factor responsible for the changes of the curvatures of the existing surface is necessary, because in case of *Dileptus* the regeneration occurs without growth of the volume of the fragment. Since the lack of the formation of the proboscis and tail is associated with the disappearance of the rough endoplasmic reticulum around the microfibrillar layer, it is postulated that the system of microfibrillar layer together with acompanying rough endoreticulum is responsible for the ability of the cell to change the curvatures of its surface. In this case the puromycin would inhibit the synthesis of a substance necessary for the microfibrillar layer to be able to remodell the shape of the fragment. It seems that the process of modeling of the existing surface is more sensitive to the action of puromycin than the process of the growth of the surface. This is supported by the study on *Paramecium* (Golińska and Hanson 1973) where the shapening of the narrow ends of the offspring during division is more inhibited by puromycin than the elongation of kineties.

Around the microfibrillar layer, besides the elements of the rough endoreticulum, vesicles having smooth walls with diameter approximately 1000–1200 Å exist. Such vesicles are also present in the vicinity of the microfibrillar layer of other species of *Dileptus*. They are particularly numerous around the microfibrillar bundles in the contractile proboscis of *D. cygnus* (Grain and Golińska 1969), close to the edges of the microfibrillar layer during the healing of the wound (Golińska and Grain 1969), in the region of the division furrow — also closely to microfibrillar ring (Golińska 1972). It was stated that these vesicles do not disappear after exposure to the puromycin and are seen in the vicinity of the microfibrillar layer even after 24 h treatment with the inhibitor. It should be recalled that the fragments in which the ability to form the proboscis and tail is blocked, are yet for long time able to heal the wound. It seems that probably such functions as the healing the wound, constriction of the divisional furrow, the twisting of the proboscis can be performed by the structural system containing the microfibrillar layer accompanied by the smooth vesicles.

On the basis of the obtained results a hypothesis of the double role of the microfibrillar layer has been postulated. It is suggested that the microfibrillar layer with the rough endoplasmic reticulum is responsible for the shapening of the fragment, whereas the microfibrillar layer with the smooth vesicles may function as contractile element. Allen (1971) suggested that in *Paramecium* the alveoli situated in the close vicinity of the microfibrillar system are considered as an analog of the sarcoplasmic reticulum of the muscles — that is the structure which accumulates the calcium (Allen and Eckert 1969, Allen 1971). The smooth vesicles could play a similar role in *Dileptus*.

The hypothesis of the dependence of the function of the microfibrillar layer on the kind of accompanying ultrastructure elements is under further experimental study by the author.

Acknowledgements

I wish to express my gratitude to Professsor P. de Puytorac and members of his laboratory for their helpful discussions and technical assistance during the course of this work. Thanks are due also to Dr M. Jerka-Dziadosz for helpful comments concerning the preparation of this manuscript.

Summary

The course of three different regeneration processes in normal *D. anatinus* and in cells affected by the action of puromycin was studied. It was stated, that the shapening of the narrowed parts of the cell is more strongly inhibited by puromycin than the stomatogenesis. The ultrastructural changes parallel to the diminution of the ability of the fragment to form the proboscis and tail were studied. The deterioration of the elements of the rough endoreticulum around the microfibrillar layer was observed. The function of the microfibrillar layer in morphogenesis and the correlation between the inhibition of protein synthesis and arresting of regeneration processes are discussed.

STRESZCZENIE

Badano przebieg różnych procesów regeneracyjnych w komórkach normalnych *Dileptus anatinus*, oraz w komórkach zmienionych działaniem puromycyny. Stwierdzono, że kształtowanie zwężonych części komórki jest hamowane silniej przez inhibitor niż stomatogeneza. Badano zmiany ultrastrukturalne równolegle do zaniku zdolności fragmentu do ukształtowania proboscis czy ogona. Stwierdzono zanik elementów szorstkiego endoreticulum przy warstwie mikrofibryllarnej. Dyskutuje się funkcję warstwy mikrofibryllarnej w morfogenezie, oraz związek między hamowaniem syntezy protein a zahamowaniem procesów regeneracyjnych.

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EXPLANATIONS OF PLATES I–VI

Regeneration processes in *Dileptus anatinus* Golińska, 1971

Stages of the formation of the oral primordia

1: The non-ciliated field, formed on the anterior pole of the opimor
2: An elongated primordium of the ventral band (arrow). The feeding cilia on both sides are not orderly arranged
3: The beginning of ordering of kinetosomes on the right side of the ventral band (arrows)
4: The stage III of stomatogenesis. The primordium of the cytopharyngeal complex is seen (c). The double row of feeding cilia begin to organize on the right side of the ventral band (r), and single row and short oblique rows on the left side (l)
5: The fragment after the stomatogenesis has completed

The shapening of proboscis

6: An opimer after the healing of the wound, the anterior part is rounded up
7: An opimer in the stage I of the formation of proboscis
8: An opimer in the stage II of the formation of proboscis

9: An opimer in stage III of the formation of proboscis
10: An opimer, exposed to puromycin for 5 h before operation, a concentration 300 μg/ml, fixed 3 h after operation. The apically located cytostome (c) with well developed cytostomal lip (arrows) is seen

The shapening of the tail

11: The formation of the tail arrested in the stage I. A small tail (t) is formed on the rounded posterior end
12: A promer after the healing of wound. The posterior end of cell is rounded
13: A promer in stage I of the formation of tail. A small tail (t) is seen on the rounded posterior end
14: A promer after regeneration. The posterior part is formed. The proboscis has not diminuted the size to the normal proportions of cell
1–14: The photographs of fragments stained with iron hematoxylin and observed under the light microscope

The ultrastructural changes in the ectoplasm of *D. anatinus* caused by treatment with puromycin

15: The normal ectoplasm of *D. anatinus*. The microfibrillar layer is indicated by the arrows, er — the rough endoplasmic reticulum, v — smooth vesicles. The mucocysts are seen as large dark organelles in the ectoplasm
16: A section through the ectoplasm of a fragment of *D. anatinus* incubated for 1 h before the operation in the 600 μg/ml of puromycin, fixed 3 h after the operation. The canal of endoreticulum, lacking the ribosomes on one side is seen (er)
17: A section through the ectoplasm of a fragment of *D. anatinus* incubated for 5 h in the 300 μg/ml of puromycin, fixed 3 h after the operation. Around the microfibrillar layer only the smooth vesicles are seen (v)

The processes of regeneration in fragments of *D. anatinus* incubated for 24 h in puromycin

18: The destruction of the microfibrillar layer. Concentration of inhibitor was 300 μg/ml. The arrows point to the finely granulated part of the layer. A small autophagic vacuole is seen (a). The mitochondria are not surrounded by the endoreticulum
19: The microfibrillar band (arrows) formed by a fragment, fixed 3 h after operation. The concentration of inhibitor was 300 μg/ml. An autophagic vacuoles is seen (a)
20: An opimer fixed 3 h after operation. The concentration of inhibitor was 600 μg/ml. A new kinetosomes are seen (k) two of them posess the transverse fibers (arrows). The broadened margin of microfibrillar layer contains the smooth vesicles (v)
21: An opimer fixed 3 h after operation (1000 μg/ml). The microfibrillar layer is continuous. The arrow points to the resorbed cilium. A smooth vesicles seen around the layer

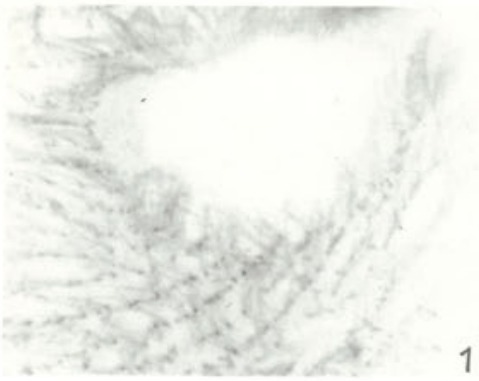
The microtubular structures formed in the presence of puromycin

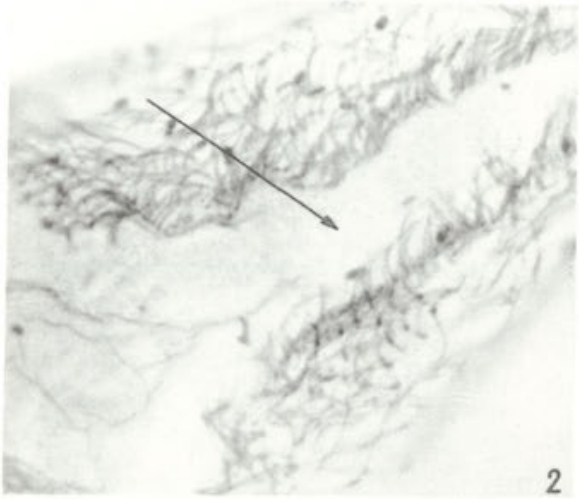
22: An opimer 3 h after operation, incubation for 5 h before the operation in 300 μg/ml of puromycin. The thick nemadesms of the external basket of the cytopharyngeal complex are seen (n). The proboscis is not regenerated
23: The same cell as on phot. 22. The thin nemadesms of the internal basket (n) are attached to the bundle of microfibrills (arrows)
24: A promer, 3 h after the operation. Proliferation of kinetosomes — arrow points to the new kinetosome. The fragment was incubated for 3 h before the operation in 300 μg/ml of the inhibitor
25: An opimer fixed 3 h after the operation, incubated for 24 h in 600 μg/ml of the puromycin. The new kinetosome (arrow) with its tubular fiber is situated deeply under the surface

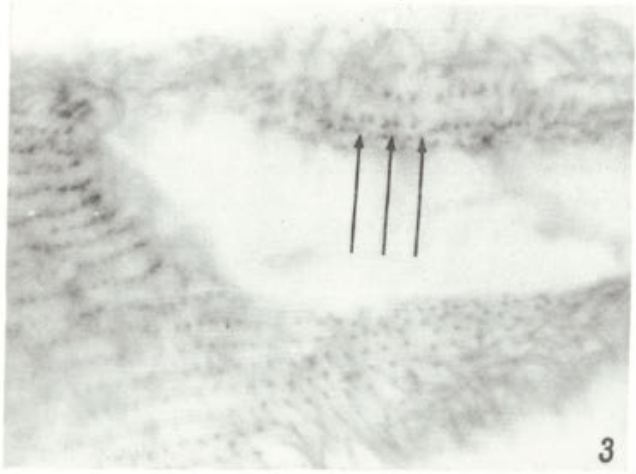
The changes in the toxicysts and endoplasm after the treatment with puromycin

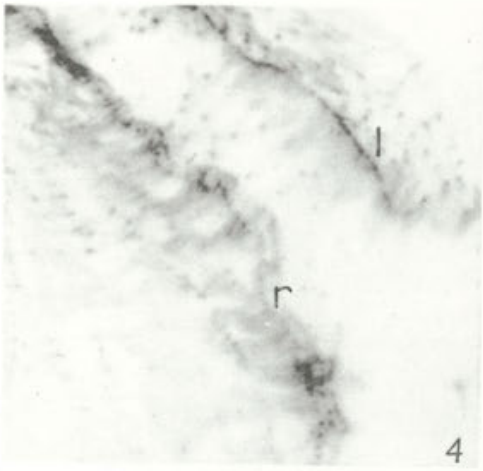
26: A toxicyst in the endoplasm of the normal cell of *D. anatinus*. The vesicle surrounding the toxicyst is filled with a finely granulated material (arrow)
27: An opimer fixed 3 h after the operation incubated for 5 h in 600 μg/ml of the inhibitor. Between the strands of the transverse fibers (t) the mucocysts only are seen
28: A fragment fixed 3 h after the operation, incubated for 3 h in 300 μg/ml of puromycin. The toxicyst is surrounded by multimembraneous wall (arrows) and is located within an autophagic vacuole
29: The endoplasm of a normal cell of *D. anatinus*. The mitochondria are surrounded by a system or endoreticulum (arrows)
30: A fragment 3 h after the operation, incubated for 24 h in 600 μg/ml of puromycin. The spaces filled with the granulated material (the right side of the photograph) correspond to "vacuolization" of a fragment

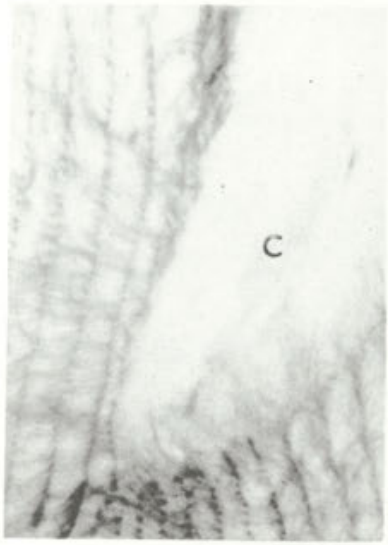
The photographs 15–30 represent pictures from the electron microscope

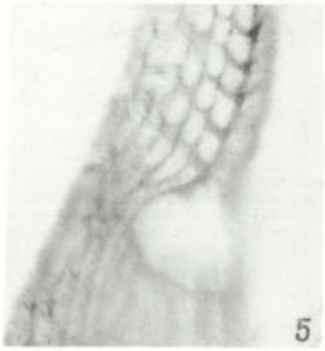












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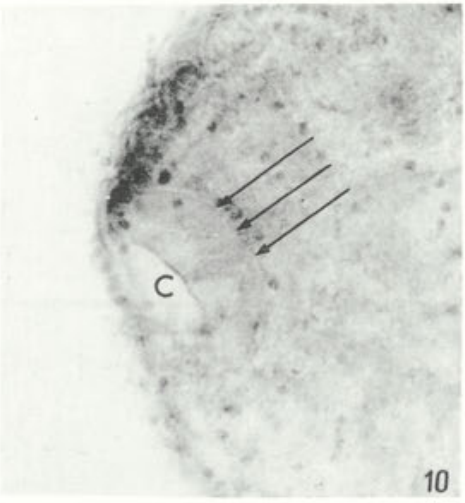

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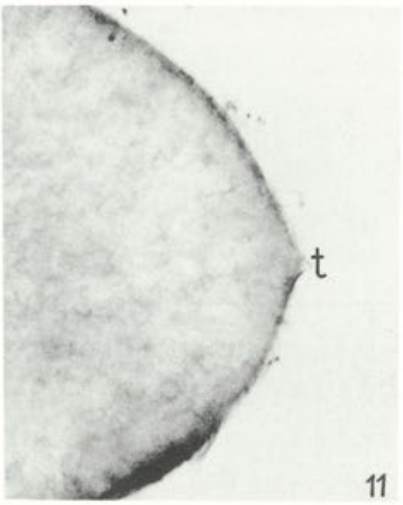

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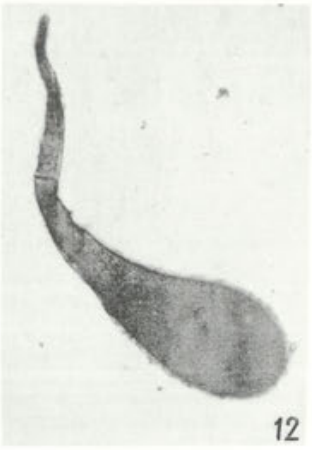
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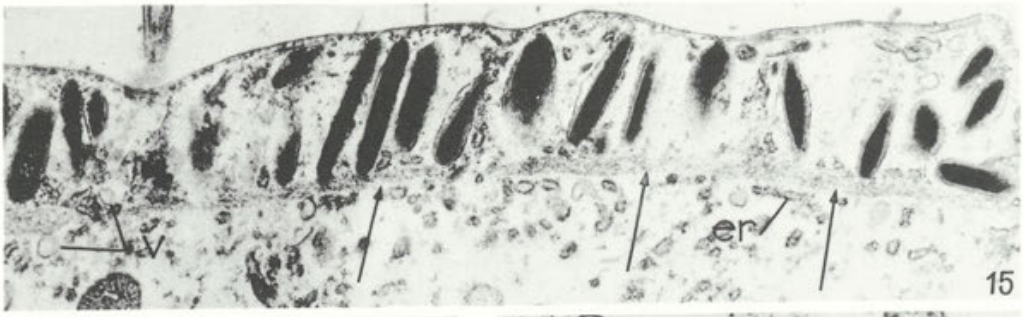

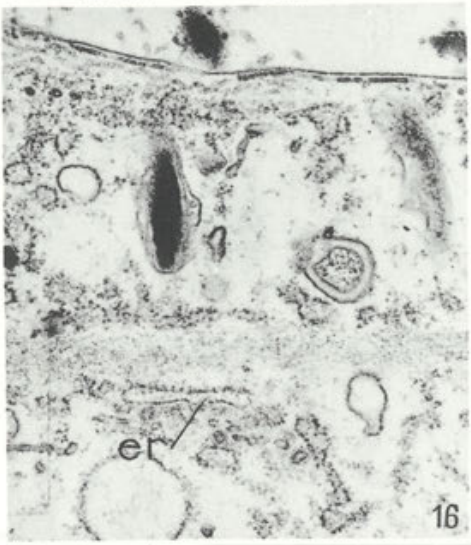

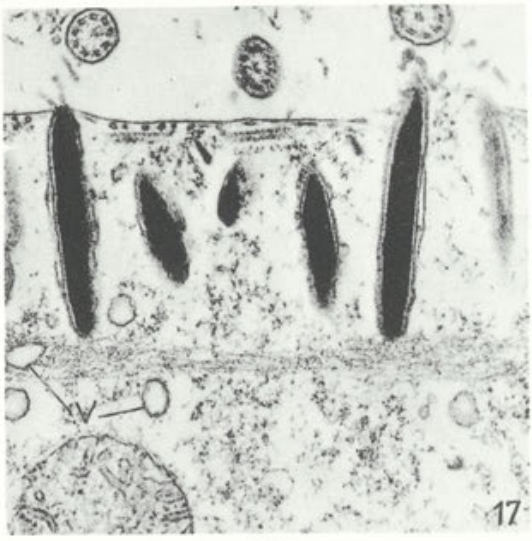

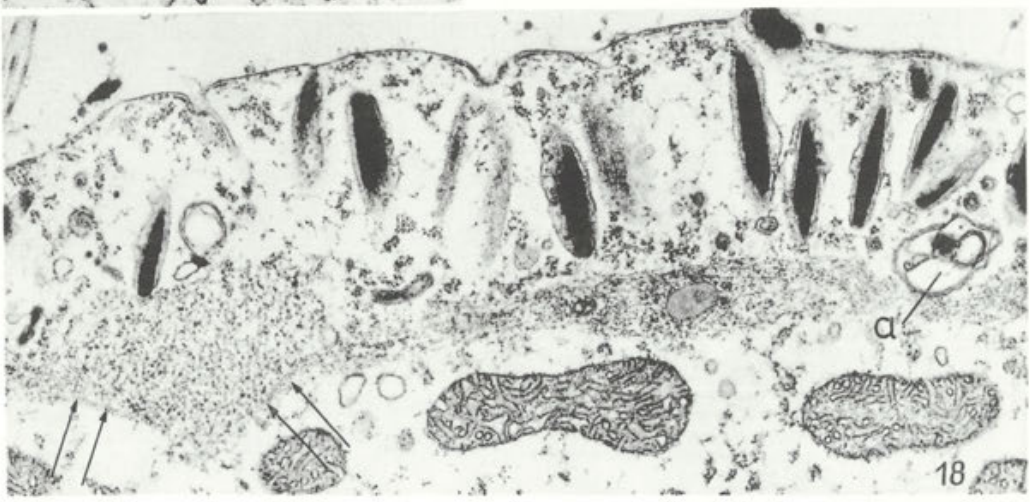

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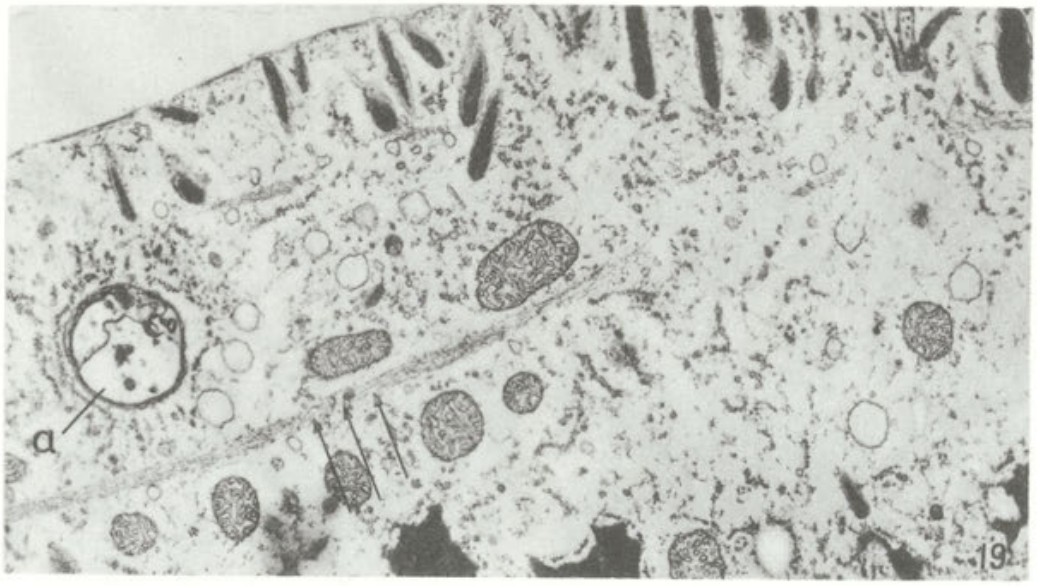

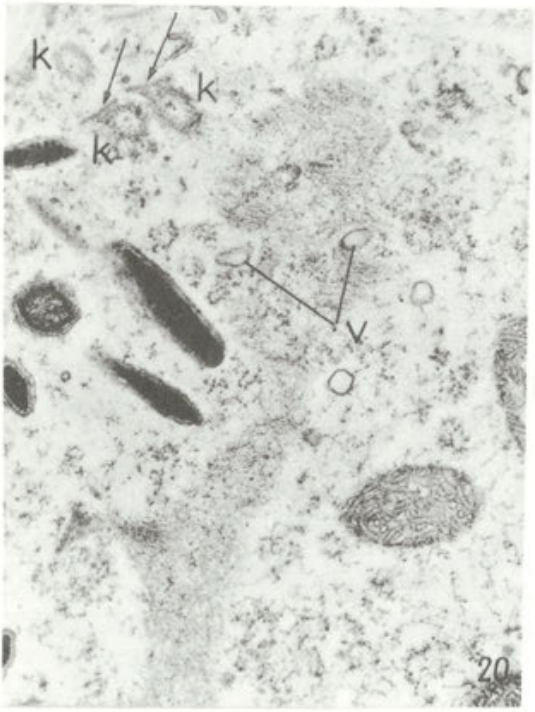

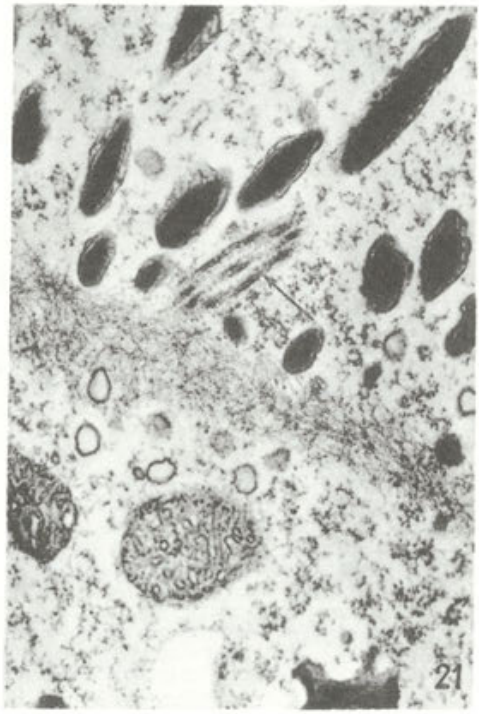

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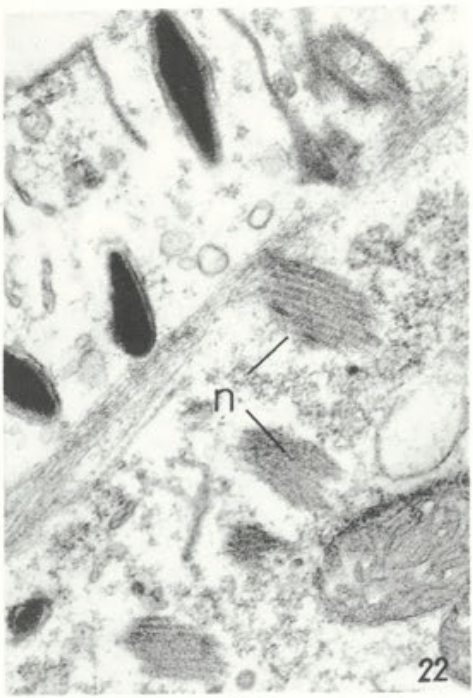

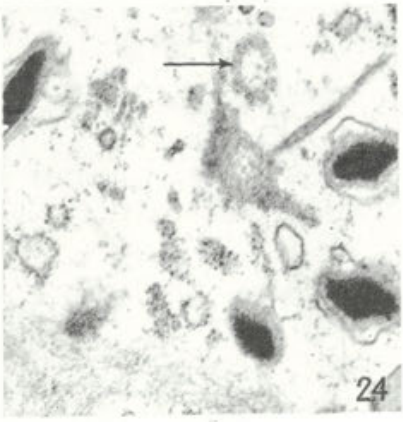

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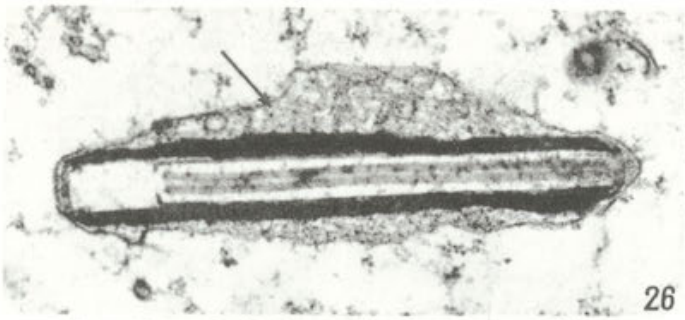

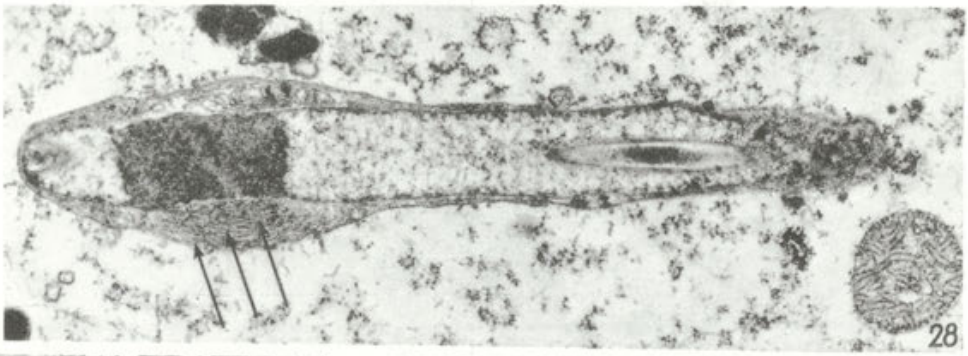

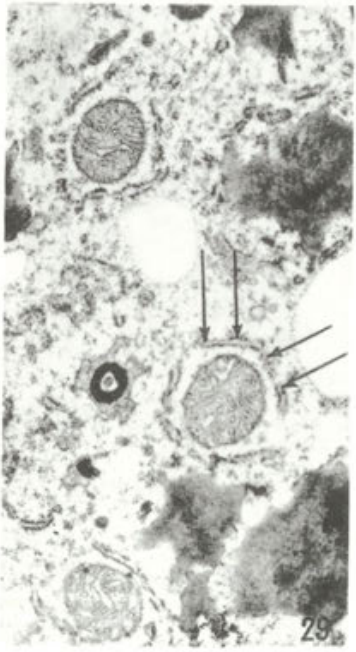

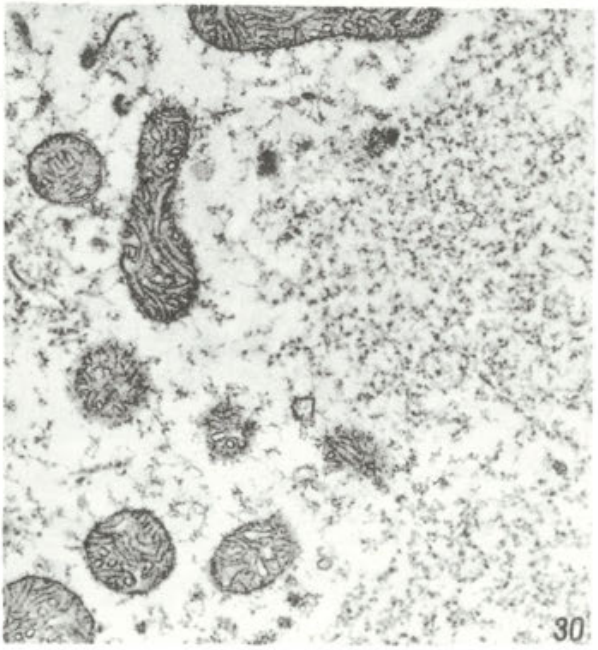

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 25

Institute of Animal Genetics, West Mains Road, Edinburgh EH9 3JN, Scotland, U.K.

A. JURAND and D.M. SAXENA[1]

Ultrastructural features of the trichless mutant of *Paramecium aurelia*

Les particularités de l'ultrastructure du mutant de *Paramecium aurelia* sans trichocystes

In *Paramecium aurelia* Ehrbg. mutations can be easily induced, detected and analysed. The induced recessive gene mutations are brought to homozygous condition and thus to phenotypic expression by autogamy (Sonneborn 1970). X-ray irradiation and many chemical mutagens have been successfully applied to *P. aurelia.*

The trichless mutant of *Paramecium aurelia* characterized by complete absence of trichocysts was obtained by treatment of animals of the strain 51.S with 75 μg per ml of nitrosoguanidine for one hour (Pollack 1970). The mutant was isolated by Kung (unpublished, Preer 1972) and recognized by Pollack (1970) who studied its genetic aspects. Its symbol in the relevant literature is d4–105.

Trichocyst mutants are usually examined for presence and behaviour of trichocysts by treating the animals in one drop of culture medium with two drops of saturated solution of picric acid. The animals are examined at × 1000 magnification in an ordinary research light microscope or in a microscope equipped with a dark field condensor. Picric acid kills the animals and causes in wild-type stocks the trichocysts to discharge so that the trichocyst shafts are visible (Jennings 1906).

Many mutants of *P. aurelia* with abnormal morphology of undischarged trichocysts were obtained after treatment with nitrosoguanidine. In a number of mutants of this type trichocysts are unable to be discharged under the influence of picric acid solution. The trichless mutant was recognized to be without trichocysts by the fact that after treatment with picric acid it did not show any shafts of discharged trichocysts outside the cells nor were there any undischarged trichocysts visible inside the cells.

Material and methods

The trichless mutant was grown in baked lettuce infusion containing *Aerobacter aerogenes*. The animals were fixed in 1% osmium fixative (Jurand and Selman 1969). Dehydration was carried out by graded series of ethanol and cells were embedded in Araldite, using 1:2-epoxypropane

[1] Permanent address: Department of Zoology, University of Delhi, Delhi-7, India.

as transition solvent. Polymerization was accomplished at room temperature for 24 h then at 60°C overnight. Thin sections were cut on Porter Blum microtome MT1, collected on collodion-carbon coated grids, stained with 2.5% uranyl acetate in 1% potassium permagnate. The sections were examined in AE1-6B and Siemens Elmiskop 1A.

For cytochemical demonstration of acid phosphatase in Golgi complex, Gomori's technique 1952 adapted for electron microscopy by Holt and Hicks 1961 was applied. The cells were concentrated and fixed at 0°C for 1 h with 2.5% gluterealdehyde in cacodylate buffer (Plumel 1948) at pH 7.3. The cells were washed three times 5 min each in the same buffer containing 3.75% sucrose. The material was then incubated for 30 min at 37°C in a medium containing sodium glycerophosphate as substrate and lead nitrate dissolved in 0.05 M acetate buffer pH 5.2. After rinsing for 5 min each in (1) 4.8% sucrose, (2) 4.8% sucrose with 1% acetic acid and (3) 4.8% sucrose (2 changes,) the material was postfixed with 1% osmium fixative and processed as usual. The controls were fixed with 2.5% gluteraldehyde in cacodylate buffer at 0°C and processed without incubation in the substrate medium. The experimental material was stained with 2.5% uranyl acetate and the controls with 2.5% uranyl acetate followed by lead citrate (Reynolds 1963). Observation made were compared with the ultrastructural data on the development of normal trichocysts in *P. aurelia* (Selman and Jurand 1970).

Observations

In mutants neither mature or juvenile trichocysts are seen in the cortex attached to the pellicle nor are there pretrichocysts present in the endoplasm. The only structure in mutants comparable to the very early pretrichocysts stages are endoplasmic vesicles, spherical or slightly elongated in shape, about 0.4 μm in diameter (Pl. I 1). The membrane of these vesicles is a usual trilaminar unit-membrane about 6 nm thick. On the inside the mebrane is covered by an internal electron dense apparently discontinuous coat about 10 nm thick. The vesicles enclose a moderately electron dense, homogenous and amorphous mass in average 0.3 μm in diameter located in the centre. The amorphous mass remains detached from the membrane of the vesicle leaving an electron translucent zone underneath the membrane. The vesicles appear randomly distributed in the endoplasm without any topographical association with other cytoplasmic organelles. Comparing these vesicles with the primordial pretrichocysts vesicles from the non-mutant stocks (Pl. I 2) it appears that the vesicular membrane is similar in both cases. Apart from this, the mutant vesicles are smaller and the crystalline core characteristic for the non-mutant developing pretrichocyst never appears in the internal homogenous mass.

Another type of vesicles are also present in the mutant. These are numerous small, smooth vesicles, 0.25 μm in diameter, in aggregates of 20–40. As many as 8–10 such groups of vescicles are seen in one section. These vesicles are distributed throughout the endoplasm, the majority of them are completely electron translucent and are not associated with other cytoplasmic organelles (Pl. I 3, II 4). The vesicles are negative to the acid phosphatase test.

In exconjugants of non-mutant *P. aurelia* we have observed a few small, curved, smooth and flattened saccules with swollen ends and vesicles (Pl. II 5). One or two

such groups are seen in one section as compared to over eight groups of vescicles in the mutant.

Another feature of the trichless mutant is the presence of markedly numerous lipid droplets located mainly at the periphery of the endoplasm.

Discussion

The regeneration and development of trichocysts have been described in *Paramecium* (Ehret and De Haller 1963, Yusa 1963, Selman and Jurand 1970) and *Frontonia* (Yusa 1965). In trichless mutants of *P. aurelia*, we have observed the presence of endoplasmic vesicles with an amorphous matrix. These vesicle are morphologically somewhat similar to the primordial pretrichocyst vesicles in non-mutant animals. However, in mutants neither a structure similar to the crystalline core, which develops in non-mutants, is formed nor an increase in the volume of the vesicle takes place. The differentiation of the crystalline body is a very important and crucial step for the development of normal trichocysts. The subsequent steps in maturation of trichocysts are features secondary to the initiation of this major morphogenetic event (Yusa 1965). If we assume that the endoplasmic vesicles in the mutant are primordia of trichocysts which do not develop the crystalline body of the normal pretrichocysts type, they could be termed as "abortive pretrichocysts". The origin and nature of degeneraton of these vesicles remains to be investigated.

To some extent similar ultrastructural features were found in mutant clones of *Paramecium aurelia* obtained by sublethal U. V. irradiation (de Haller and ten Heggler 1969). In this case, however, the trichocysts are initially developing normally in the endoplasm. Then, at the stage of pretrichocysts, when still bounded by a membrane, they become unstable and readily discharge while located in the endoplasm. They form short striated shafts, later form irregular electron dense masses and eventually undergo resorption.

Thus the trichocysts in the U. V. mutants never mature and never reach the preformed sites in the pellicle which alternate along the kinetics with the ciliary units. In the light microscope the cell cortex of these mutants appears to be devoid of trichocysts because the preformed sites in the pellicle remain empty and in this respect the U.V. mutants resemble the trichless mutant where the trichocyst sites are also empty (Pollack 1970).

Many protozoa have a well defined Golgi apparatus consisting of small saccules with swollen ends and vesicles (Pitelka 1963). For ciliates, Golgi apparatus has been recently described in *Epistylis* (Fauré-Fremiet et al. 1962). *Campanella* (Carassso et al. 1964), *Blepharisma* (Kennedy 1965), *Colpoda* (Rudzińska et al. 1966) *Frontonia* (Kennedy 1967), *Tetrahymena* during mating (Elliot and Zieg 1968) and *Paramecium caudatum* (Esteve 1970).

In *P. aurelia*, particularly in exconjugants, we have observed Golgi as consisting of saccules and vesicles which are quite similar to those described in *Tetrahymena* (Elliot and Zieg 1968) during mating.

In trichless mutants, the saccules are not seen but groups of numerous vesicles are present. Similar vesicles in *P. aurelia* (Jurand and Selman 1969) and in salivary gland cells of the larvae of the dipteran *Smittia parthenogenetica* (Jacob and Jurand 1965) have been regarded as Golgi complex. Thus in trichless mutants from the structural point of view, these groups of vesicles are thought to be Golgi apparatus. The vesicles are acid phosphatase negative. Golgi vesicles in *Campanella* (Carasso et al. 1964) and in *Tetrahymena* (Elliot and Zieg 1968) during mating are also acid phosphatase negative suggesting that hydrolases are not produced or stored in them.

The significance of such an extensive Golgi in mutants is not clear. It remains to be elucidated whether this extensive form of Golgi is a direct result of mutation induced by nitrosoguandine or a later physiological adaptation or change in the mutant. It is feasible that such an elaborate Golgi apparatus is in functional association with the degeneration of endoplasmic vesicles in the trichless mutant.

Acknowledgements

Thanks are due to Miss M. Schneller and Dr. S. Pollack, Department of Zoology, Bloomington, for having sent us the culture of trichless mutant. One of us (DMS) was the holder of a post-doctoral fellowship of the Association of Commonwealth Universities, U.K.

Summary

In trichless mutants of *Paramecium aurelia* stock d4-105, syngen 4, no mature or juvenile trichocyst are seen. The only recognizable organelle, comparable to the non-mutant form, is the primordial endoplasmic vesicle. These endoplasmic vesicles have an amorphous and homogenous mass in the centre and they do not develop the crystalline body of the normal pretrichocyst type. The vesicles probably degenerate in the cytoplasm and can be called abortive pretrichocysts. In the endoplasm of these mutants there are also groups of smooth surfaced, acid phosphatase negative, vesicles in aggregates of 20–40. These are regarded as representing an extensive Golgi complex of the vesicular type. The exconjugants of *Paramecium aurelia*, non-mutant form, show also Golgi apparatus consisting of saccules with vesicles similar to those in the mutant.

RÉSUMÉ

Chez le mutant étudie de *Paramecium aurelia*, souche d4-105, syngen 4, aucunes trichocystes, ni jeunes, ni adultes n'étaient visibles. Les seules organelles reconnaissables, comparables à celles qui apparaissent chez les-non-mutants — ce sont les vésicules initiales d'endoplasme. Ces vésicules

ont le contenu amorphe et homogène et ne forment pas de corpuscules caractéristiques pour les protrichocystes normales. Ces vésicles dégenèrent probablement dans la cytoplasme et l'on peut les qualifier de protrichocystes avortées.

Dans l'endoplasme de ces mutants apparaissent aussi les groupes de 20 à 40 vésicles aux parois lisses et dont la réaction à la phosphatase acide est négative. Elles sont considérées comme appareil de Golgi du type vésiculaire. Exconjugant de *Paramecium aurelia*, les non-mutants, démontrent la présence de l'appareil de Golgi composé de sachets et de vésicules semblables à celles qui paraissent chez les mutants.

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EXPLANATION OF PLATES I–II

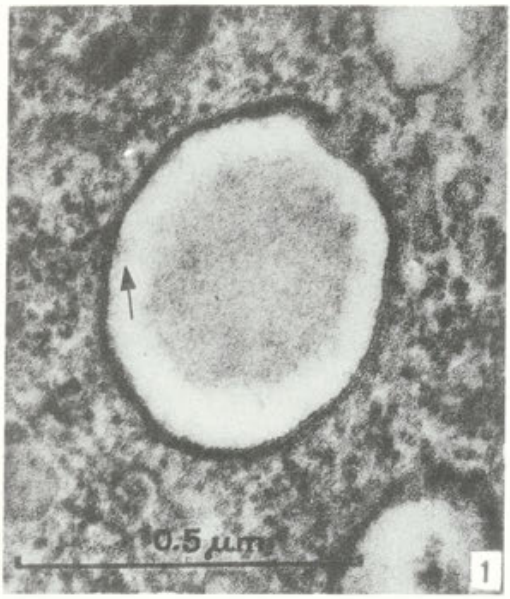
1: Electronmicrograph of a section of trichless mutant of *Paramecium aurelia* showing the endoplasmic vesicle. Notice the amorphous mass in centre and the limiting unit-membrane. Arrow indicates the electron dense coat on the inside of the membrane — 90,000×

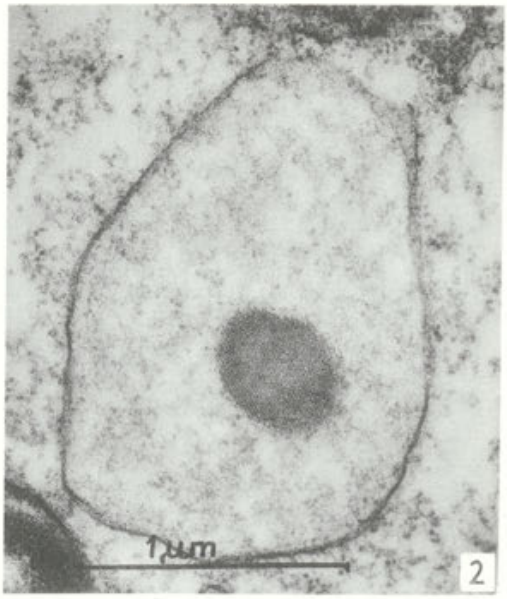
2: Electronmicrograph of a section of non-mutant *P. aurelia* showing the primordial pretrichocyst vesicle. Notice the crystalline body with periodicities located near the centre. There is no electron dense coat on the inside of the unit-membrane — 41,000×

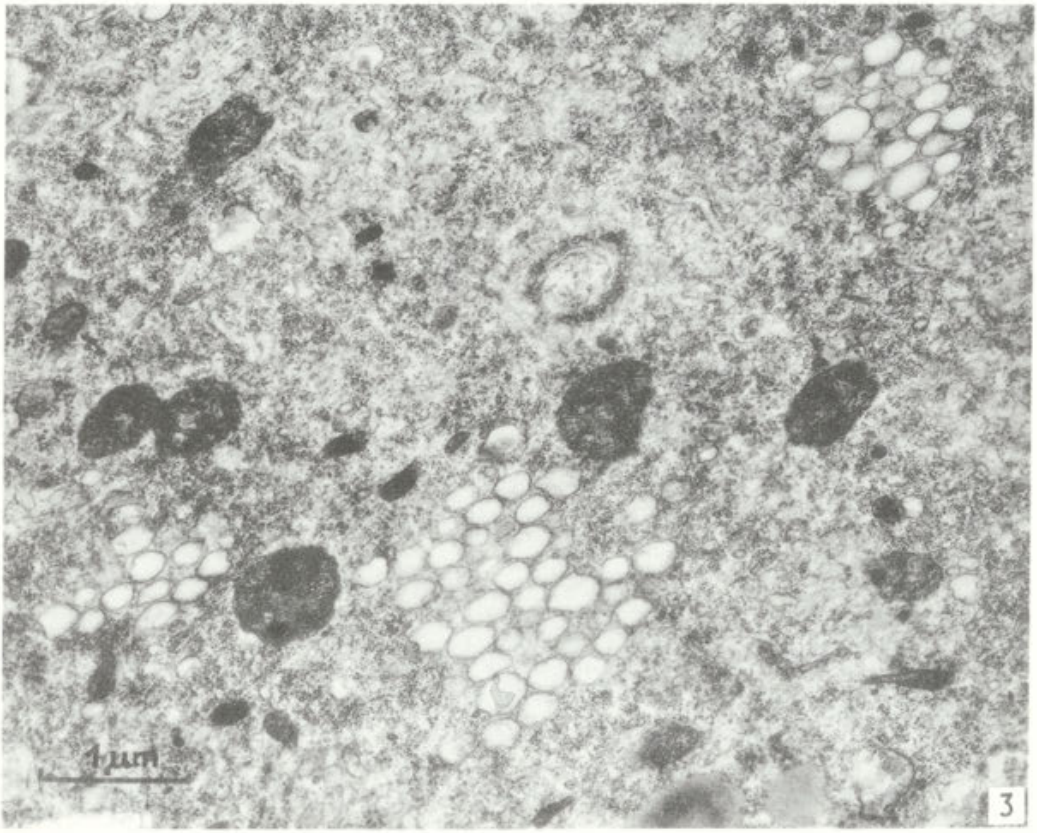
3: Low power micrograph of the endoplasm of the trichless mutant showing three groups of Golgi complex — 20,000×

4: Golgi complex in trichless mutant at higher magnification. Notice the electron translucent nature of majority of vesicles — 60,000×

5: Golgi complex in non-mutant *P. aurelia* (stock 31, syngen 8) during conjugation. Note the similarity of these vesicles to Golgi vesicles in trichless mutant (4) — 40,000×







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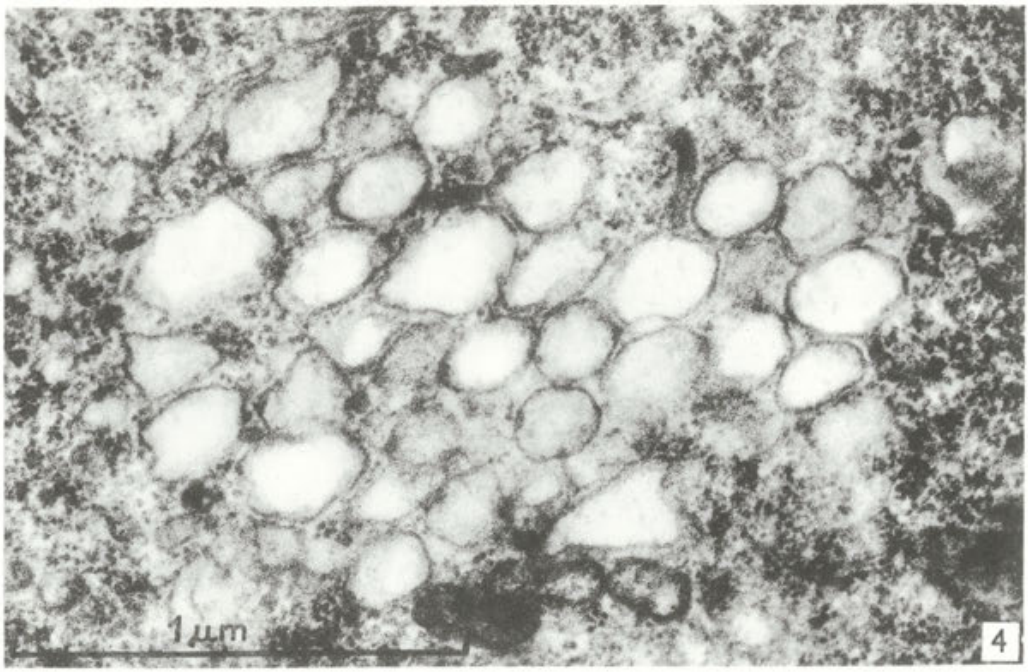

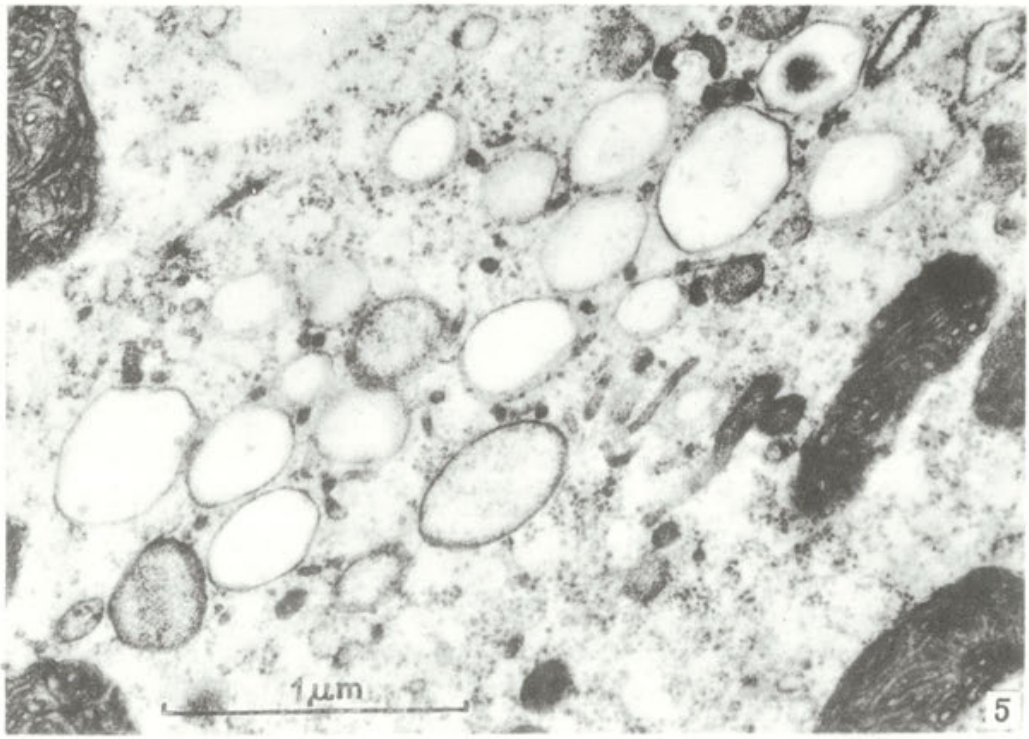

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 26

All-Union Institute of Poultry Diseases, Leningrad 199006, Moscow Prospect 99, USSR

T. A. SHIBALOVA

Electron microscope observations on the development of *Eimeria tenella* (*Sporozoa, Coccidia*) in tissue culture I. The fine structure of the sporozoite

Электронномикроскопическое исследование *Eimeria tenella* (*Sporozoa, Coccidia*) при развитии в культуре ткани. I. Ультраструктура спорозоитов

The fine structure of different stages of *Eimeria tenella* was reported by many investigators (Scholtyseck and Strout 1968, McLaren and Paget 1968, Scholtyseck 1969, Scholtyseck et al. 1969 a, b, Ryley 1969, Sénaud et Černa 1969, McLaren 1969, Seliverstova 1970 and others). Much interest to this species of avian coccidia may be accounted for, in part, by its high pathogenic effect exerted on the host, and by the associated importance in poultry farm economy.

The in vitro cultivation technique applied to the coccidian research made it possible to carry out a more detailed study into the parasite's physiology and metabolism, the results of which may be employed for purposes of the chemotherapeutic control of coccidiosis.

Since 1968, studies on cultivation of avian coccidia both in tissue culture and in chick embryo have been performed in the Laboratory of Cultivation of Protozoa of the All-Union Research Institute of Poultry Diseases in Leningrad.

The present communication deals with the results of the ultrastructural investigation of the sporozoites of *Eimeria tenella*, both free and intracellular ones.

Material and methods

Oocysts of a "pure" local strain of *Eimeria tenella* (Railliet et Lucet) were passed through coccidia-free chickens. Oocysts used in this work were obtained from the chick droppings or caeca and then allowed to sporulate in flasks under conditions of permanent aeration attained with the aid of a microcompressor, at 21–23°C.

Sporulated oocysts were treated with sodium hypochloride and washed twice with distilled water followed by a third washing in phosphate buffer. Gaining of oocysts and their further sporulation were carried out under sterile conditions.

Sporozoites were released from mechanically destroyed oocysts followed by digestion with

trypsin and chicken bile, and were separated from unbroken oocysts and oocysts debris by differential centrifugation (Doran and Farr 1961, Shibalova 1968).

Pellets of sporozoites were used for electron microscope observations and for tissue culture infection. Cells of 9–12 day old chick embryos were used for tissue culture preparations. The infected host cells were examined with light microscope, 5–24 h following sporozoite inoculation. Simultaneously, the infected cultures were examined with electron microscope using plane-parallel embedding technique on glass smears (Bykovsky 1961). The material was fixed in 3% glutaraldehyde in phosphate buffer followed by postfixation in veronal acetate-buffered 1% OsO_4, dehydrated in a graded series of ethyl alcohol, passed through aceton and then embedded in Epon 812. Sections were contrasted with lead citrate. Negative staining was carried out by mixing equal amounts of a suspension of sporozoites and a 2% solution of sodium phosphotungstate. Collodium-coated grids were allowed to touch the mixture surface, the grids were then washed and dried. Photographs were taken with an UEMV-100K electron microscope.

Results

A free extracellular sporozoite of *Eimeria tenella* is elongated in shape (Pl. I 1). After the penetration of the host cell, the sporozoite is getting oval (Pl. I 6) and then — rounded.

The sporozoite pellicle is composed of three unit membranes, each 75Å thick (Pl. I 5, III 7) with two electron opaque layers between them. The inner layer is thinner (100–120 Å) than the outer one (190–200 Å) (Pl. I 5). In well fixed specimens the membranes are seen continuous over the entire organism except at two poles of the sporozoite where two inner membranes are interrupted.

The apical pole of the sporozoite is provided with a complex of organelles serving for penetration of the host cell; among these a conoid, rhoptries ("paired organelles") and subpellicular microtubules being of primary importance.

The conoid is a cone shaped structure. Its longitudinally sectioned wall looks as a discontinuous line; a cross-sectioned conoid looks like a condensed ring. In the upper part of the conoid there are two preconoidal (apical) rings. In the lower part there is a polar ring from which subpellicular microtubules radiate downwards (Pl. I 4). The microtubules are running under the pellicle (Pl. III 7) and can be clearly seen in the negatively stained specimens (Pl. I 2). In the contact area between the tubules and the polar ring a row of plates is seen.

Behind the conoid, the cytoplasm contains numerous micronemes and rhoptries (Pl. III) 7) of different size. More numerous smaller structures (micronemes) 600–900 Å in diameter, are covered with a unit membrane (Pl. I 5) and filled with homogenous substance of an average electron density. They are evenly thick over the whole length. The larger sac-shaped organelles, tapering in the upper part (800 Å in diameter) and broadening in the lower one (1600–1900 Å in diameter), are also covered with a unit membrane and contain a homogenous to fine granulated electron transparent substance (Pl. I 5, III 7). These are rhoptries which in our material are as many as 6–9.

The presence of paranuclear bodies surrounded with amylopectin granules is characteristic of the sporozoite stage (Pl. I 1, 6, 9). Extracellular sporozoites display two-three bodies on the either side of the nucleus (Pl. I 1). Intracellular sporozoites show more commonly one paranuclear globule only, behind the nucleus (Pl. II 6). The globule is composed of loosely packed osmiophilic granules and has no limiting membrane. Round or oval amylopectin granules are seen surrounding the paranuclear body which looks, in our material, more frequently as electron transparent vacuole (Pl. I 1, IV 9). The sporozoite is getting older, the number of amylopectin granules is eventually reduced to none in rounded sporozoites.

At the level of the nucleus, a micropore (or ultracytostome) is present in the intracellular sporozoite (Pl. I 3). It is an invagination of the outer unit membrane into the interior of the cytoplasm through a thickened opening formed by two inner membranes. One micropore was found in the sporozoite and invariably near the nucleus.

The nucleus of the sporozoite (Pl. I 1, II 6, III 8) is limited with two membranes with an electron transparent layer between them. Small osmiophilic granules of chromatin are evenly dispersed. As the sporozoite maturates, the granules are accumulating in small separate groups beneath the nuclear membrane and in the nucleoplasm.

The Golgi elements ar found constantly near the nucleus (Pl. I 1, II 6). In large mitochondria filled with a fine granulated substance, cross-cut alveole of tubular cristae are seen (Pl. I 1, IV 10). In addition, the sporozoite cytoplasm contains ribosomes, polysomes, endoplasmic reticulum and vacuoles of various shape.

The intracellular sporozoite is lying within a vacuole separated from the host cell by a membrane (Pl. III 8, IV 10). The membrane of this parasitophorous vacuole makes folds, invaginations and protrusions (Pl. III 8). Within the vacuolar space there are small osmiophilic particles, membranous material, and globules lying near the parasite's surface (Pl. III 8).

In the host cell, canals of the granular endoplasmic reticulum, mitochondria and sometimes Golgi elements are seen crowding along the membrane of the parasitophorous vacuole (P. III 8, IV 10). In some cases, partial destruction of the host cell cytoplasm was observed in areas adjacent to the sporozoite (Pl. IV 9).

Discussion

The present communication deals with the ultrastructure of one stage of the eimerian life cycle — the sporozoite. Special attention has been paid to two main phases of its existence: extracellular survival outside of the host cell, and intracellular development after entering the cultured cell. The elongated shape of the free sporozoite facilitates penetration into the host cell. Inside the host cell, the sporozoite is assuming an oval to round shape.

Ryley 1969 and Seliverstova 1970 reported the presence of two membranes constituting the pellicle of the motile stages (sporozoite, merozoite) of *E. tenella*. Two membranes were found in the pellicle of merozoites of *E. magna* and *E. intestinalis* (Snigirevskaya 1969), and of *Plasmodium* (Aikawa 1966). Three membranes in the pellicle, where two inner ones were very close together, have been reported for *Toxoplasma gondii* (Vivier et Petitprez 1969), *E. larimerensis* (Roberts et al. 1970, 1971), and *E. necatrix* (Dubremetz 1971).

Our observations suggest that the pellicle of sporozoites (both extracellular and intracellular) is composed of three distinct unit membranes with two electron transparent layers between them. The inner layer is thinner (100–200 Å) than the outer one (190–200 Å). This may account for a possible confusion in counting the number of membranes since the two inner membranes being very close together, may be taken as one. The outer unit membrane limits the entire body, whereas the other two membranes are interrupted at the apical pole, in the conoid zone, in a small area of the posterior body end, and in the point of plasma membrane invagination in the micropore region.

McLaren and Paget 1968 described two rhoptries (paired organelles) in the *E. tenella* sporozoite, whereas Ryley 1969 reported four rhoptries. In our specimens at least 6–9 rhoptries were seen.

The intracellular sporozoite has a micropore (ultracytostome). This structure was originally described in sporozoites of avian malaria parasites (Garnham et al. 1960); its possible function as ultracytostome was first suggested by Cheissin and Snigirevskaya 1965. In our material, the micropore was always single with a stable position at the level of the nucleus. Intracellular sporozoites displayed the so-called functioning micropores.

The infected host cell is seen to be immediately separated from the parasite by a unit membrane producing a parasitophorous vacuole where a further development of the parasite proceeds. Thus, the parasite has no direct contact with the host cell cytoplasm, being separated by a definite barrier constituted by the parasitophorous vacuole and vacuolar space.

Immediately after the host cell gets infected, its endoplasmic reticulum canals, mitochondria and Golgi elements are seen "arranged" along the parasitophorous vacuole. This may be due to some changes in cell energy transport reported by Sampson and Hammond 1972. The Golgi elements accumulated in this particular region may be involved in formation of membranes of the parasitophorous vacuole.

Summary

A study was made on free living sporozoites of *Eimeria tenella* (Railliet et Lucet) and their following penetration of the tissue culture.

The sporozoite pellicle consists of three obvious unit membranes with two elec-

tron transparent layers between them. As many as 6–9 rhoptries were detected on the apical pole of the sporozoite, these being sac-shaped and limited with a unit membrane; 3–6 smaller segments of rhoptries were seen in the conoid region.

A single micropore was observed in the pellicle of the intracellular sporozoite; its position was fixed constant at the level of the nucleus.

РЕЗЮМЕ

В представленном сообщении включено изучение ультратонкого строения спорозоита *E. tenella* (Railliet et Lucet): внеклеточное его существование и короткий период внутриклеточного развития в культуре ткани.

Показано, что оболочка спорозоита (как свободного, так и внутриклеточного) имеет в своем составе три четко разделенные элементарные мембраны, между которыми заключены два электронноoптически светлых слоя. На апикальном полюсе выявлялось 6–9 роптрий, которые имели мешковидную форму и были ограничены элементарной мембраной. Три — шесть более узких участков роптрий, обнаруживались в области коноида.

В оболочке внедрившегося в клетку спорозоита констатировали только одну микропору — ультрацитостом, постоянное местоположение которого на уровне ядра.

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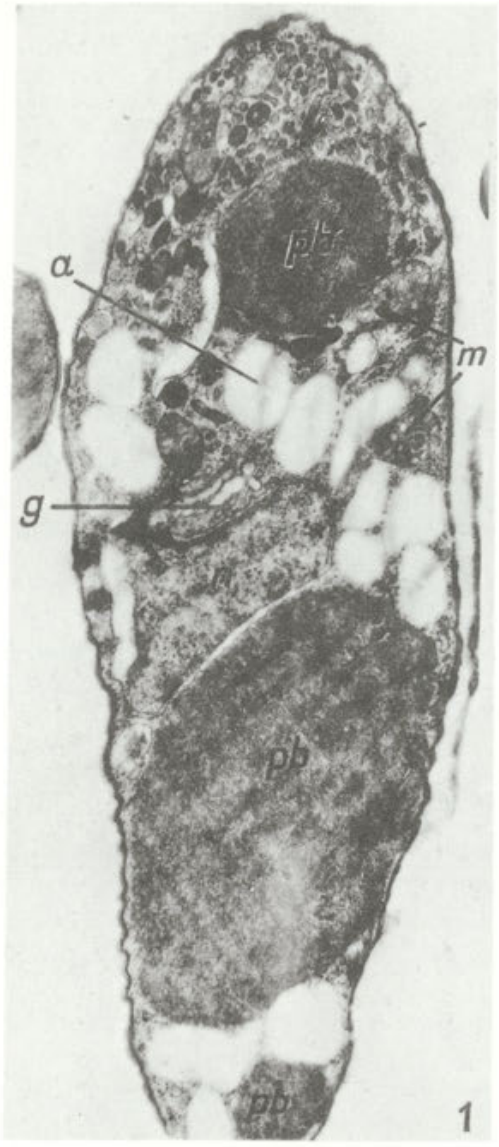
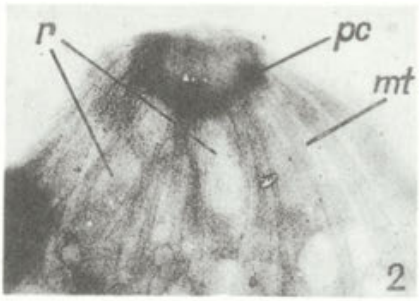
EXPLANATION OF PLATES I–IV

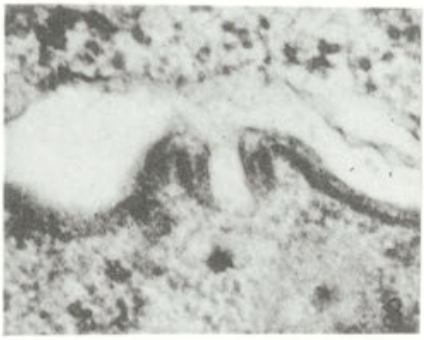
The fine structure of the sporozoite of *Eimeria tenella* (Railliet et Lucet)

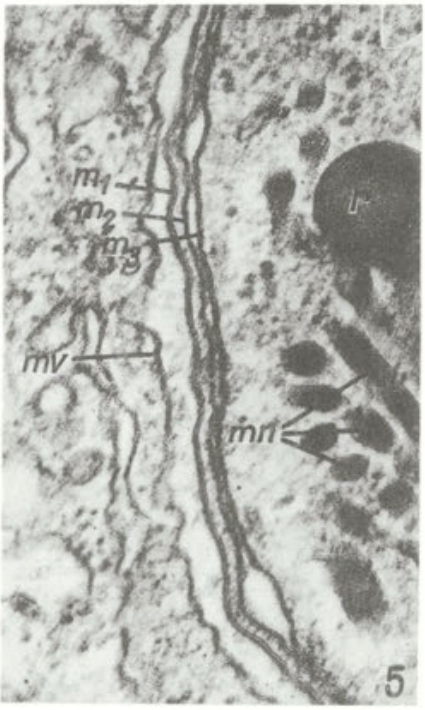
1: A longitudinal section of excysted sporozoite. ×20,000
2: Negative contrasting. The apical pole of sporozoite. Note subpellicular microtubules attached to the polar ring in the form of plates. Rhoptries are seen, on the background of microtubules, as electron transparent structures of various diameters. ×25,000
3: Micropore (ultracytostome) in sporozoite pellicle. ×80 000
5: A cross-section of the sporozoite at the level of conoid. ×50,000
5: Three-membrane pellicle of the sporozoite. Rhoptries are surrounded with a unit membrane. ×90,000
6: General view of an intracellular sporozoite. ×20,000
7: Tangentially sectioned anterior end of the sporozoite. Note nine longitudinally sectioned broadened parts of rhoptries. ×50,000
8: Accumulation of mitochondria and Golgi elements in the adjacent host cell cytoplasm. Note a row of globules near the surface of the sporozoite. ×20,000
9: Destruction of the sporozoite-infected host cell cytoplasm. ×10,000
10: Accumulation of the host cell granular endoplasmic reticulum canals limiting the parasitophorous vacuole. ×17,000

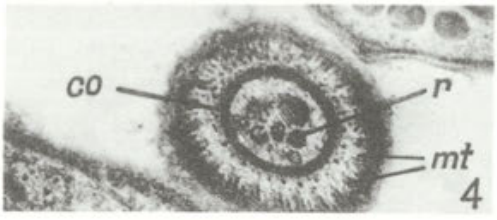
Abbreviations used:
a — amylopectin, co — conoid, er — granular endoplasmic reticulum, g — Golgi elements, m — mitochondria, m_1, m_2, m_3 — membranes constituting the pellicle of sporozoite (trophozoite), mg — microglobules, mn — micronemes, mv — membrane of parasitophorous vacuole, mt — subpellicular microtubules, n — parasite's nucleus, nhc — host cell nucleus, pc — polar ring of conoid, pb — paranuclear body, r — rhoptries (=paired organelles)











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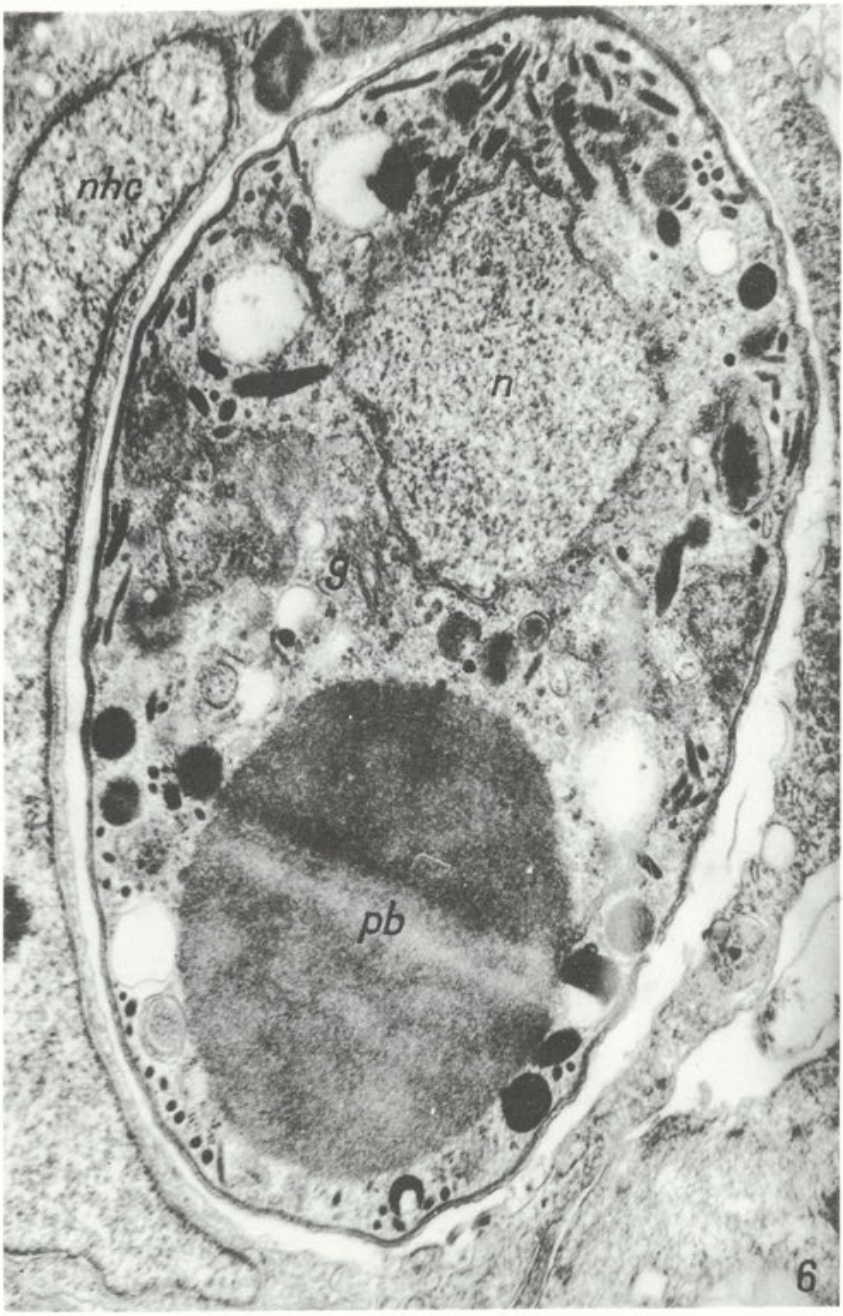

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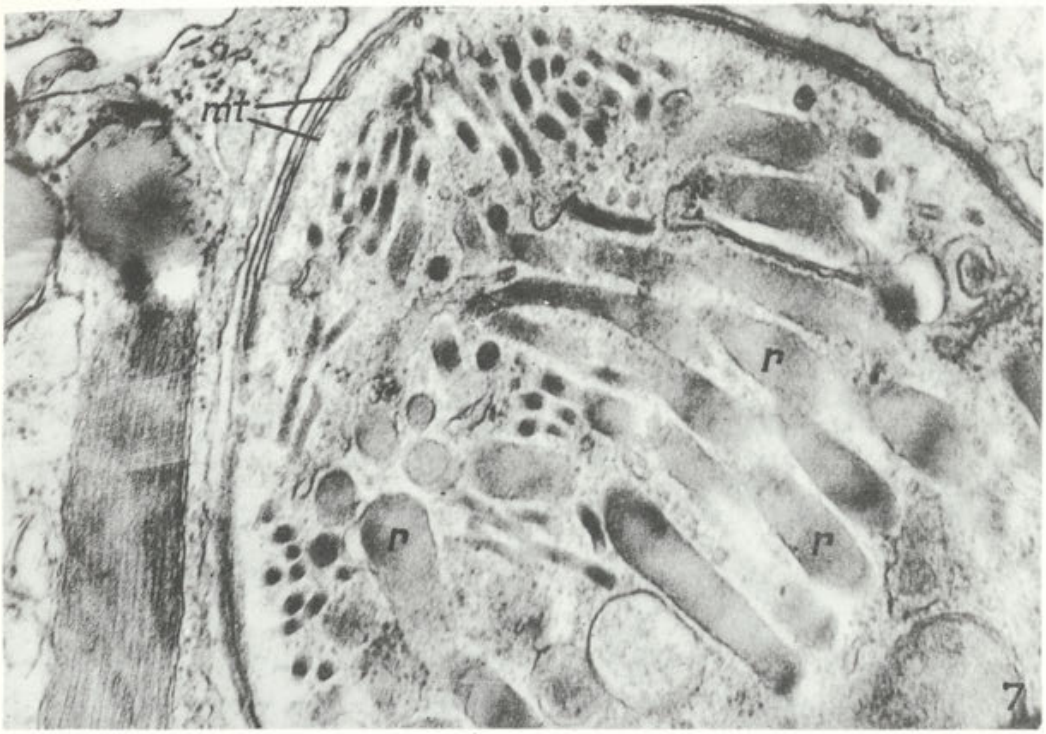

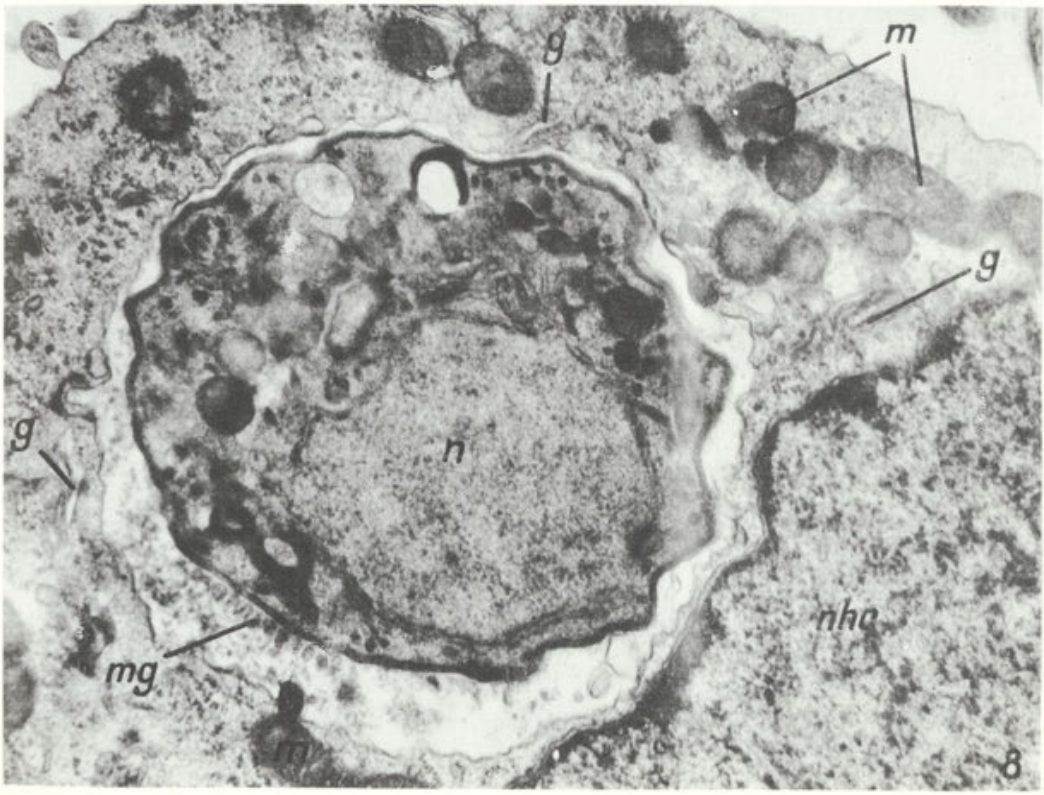

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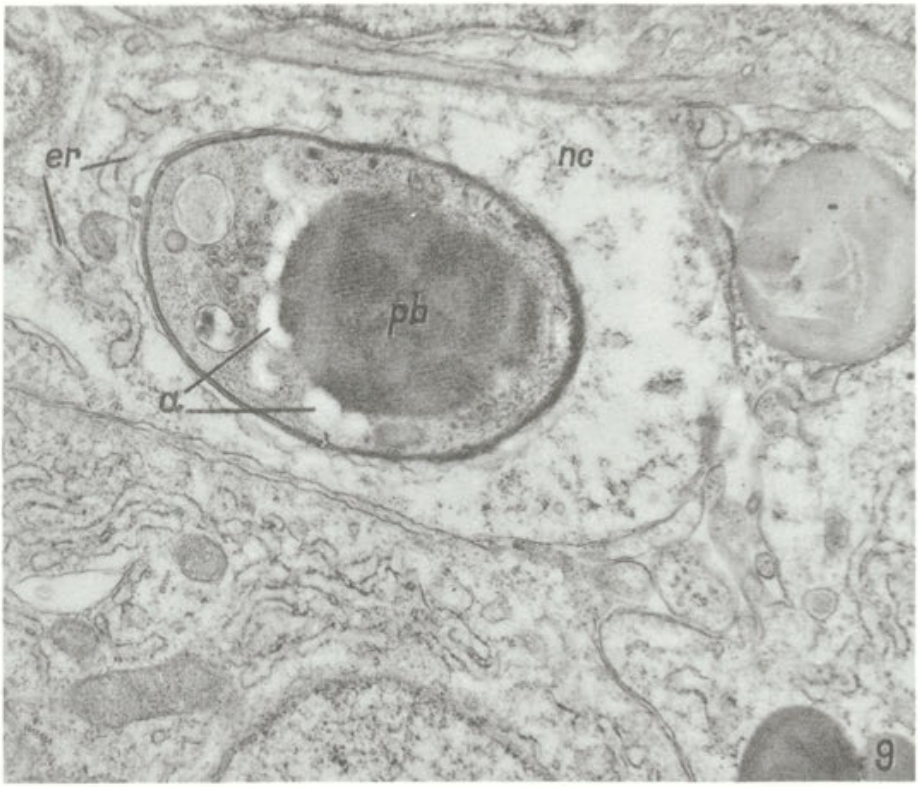

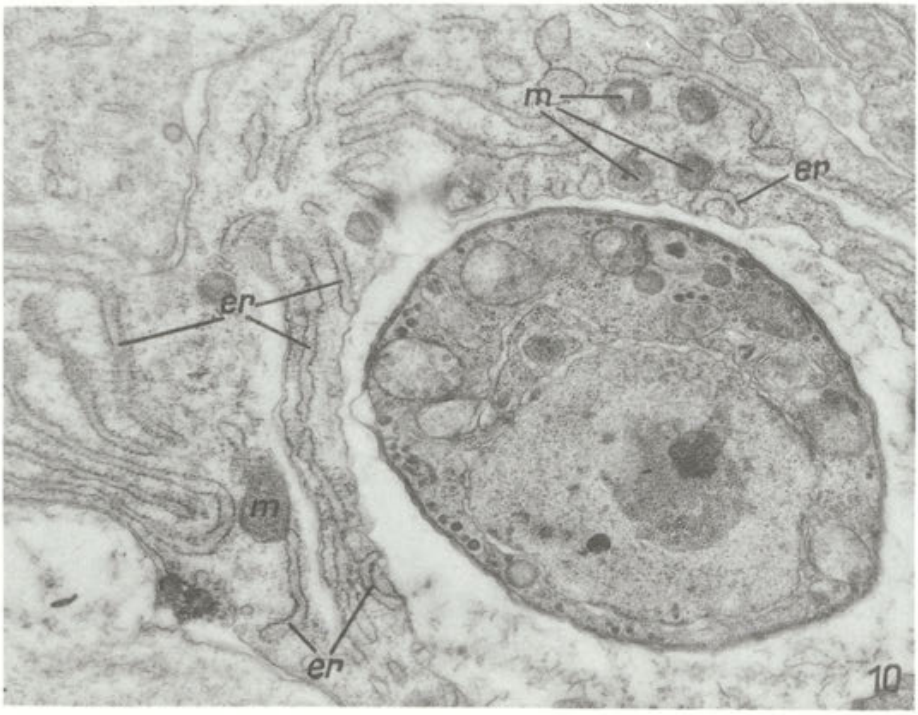

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Department of Zoology, Central College, Bangalore University, Bangalore, India

A. R. KASTURI BAI and S. V. TARA

Volume changes in *Blepharisma intermedium*

Changements de volume dans le *Blepharisma intermedium*

Growth and division are two distinct phenomena of life. These two processes are affected by many factors. The rate of growth in protozoans alters with the changes in the culture medium. One of the parameters employed to measure growth rate is by determining the volume of the cell. In this investigation the cell volume of the ciliate *Blepharisma intermedium* grown at different temperatures, different buffers of varying molarity and pH has been studied. Since traces of many heavy metals are known to promote growth, a study of the effects of copper and zinc on volume changes has also been included.

Methods

Stock cultures of *Blepharisma intermedium* were grown at 26°C±2 in hay infusion fortified with Horlicks malted milk (10 mg/100 ml). These cultures were transferred to a thermostat in which incubations could be made simultaneously at two different temperatures — 32°C and 35°C. After incubation at these temperatures for seven days, the ciliates were fixed on slides for cell volume studies. Controls were maintained at 26°C±2.

From the stock culture, the ciliates were inoculated to fresh hay infusion which forms the organic medium, 1% Cramer Myers (Cramer and Myers 1952) as the ciliates did not survive in the concentrated Cramer Myers medium and this forms the inorganic medium and filtered stream water which forms the natural medium. These three media were supplemented with zinc at a concentration of 1 mg/100 ml, and with copper separately at a concentration of 1 mg/1000 ml in hay infusion, 1 mg/10 000 ml in Cramer Myers and stream water. Equal volume of a solution of Horlicks (10 mg/100 ml) was added to all these to support bacteria. The ciliates grown at 26°C±2 in these supplemented media for seven days were isolated and fixed to study the volume changes.

To test the effect of pH on growth, *Blepharisma intermedium* was grown in hay infusion at 26°C ±2 in three different buffers at different pH and molarity and fed on Horlicks malt (10 mg/100 ml). The buffer components were separately dissolved in this medium and mixed in requisite proportions to get the required pH and was diluted with the original medium when necessary, to the required molarity. The following buffer systems were used: Citrate phosphate buffer system at 1 mM molarity, the pH tried was 5 and 6. At 1 mM concentration in acetate buffer 5 and 5.6 pH were used. In tris maleate — sodium hydroxide buffer, the pH and molarity used were 5.2 and 5 mM, 6 and 1 mM,

6 and 10 mM, 9 and 5 mM. After 48 h, the ciliates were fixed for volume studies. Ciliates grown in hay infusion at 26°C±2 with a pH 6.8 were kept as controls for all the experiments in the present study.

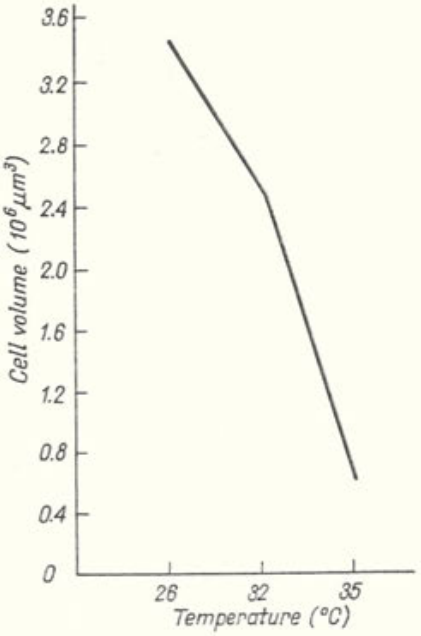
The ciliates exposed to the two temperatures, two metals and three buffers with varying pH and molarity were fixed in Carnoy's fluid, stained in Schiff's reagent and counterstained in light green. The length and the width of the stained ciliates were measured on Camera Lucida drawings in milimeters, converted to microns using a stage micrometer (8×eye piece and 10× objective) and the cell volumes in μ^3 were computed from the formula for a prolate spheriod

$$V=4/3\pi \ (A/2) \ (B/2)^2$$

in which A is the major axis and B is the minor axis. This formula has been employed by Scherbaum (1956) and Thormar (1962) for similar studies on *Tetrahymena pyriformis*.

Results

Blepharisma intermedium at higher temperature shows reduction in volume as shown in Figure 1, where the cell volume is plotted versus the incubation tempera-



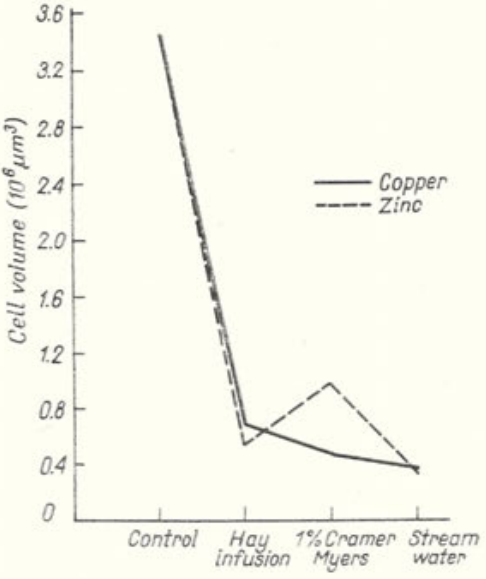


Fig. 1. Volume changes in *Blepharisma intermedium* at different temperatures at the end of 7 day
Fig. 2. Volume changes in *Blephrisma intermedium* grown in different media with copper and zinc at room temperature at the end of 7 day

ture. Figure 2 shows the volume changes with copper and zinc in the three different media, Figure 3 indicates the volume at different buffers, pH and molarity.

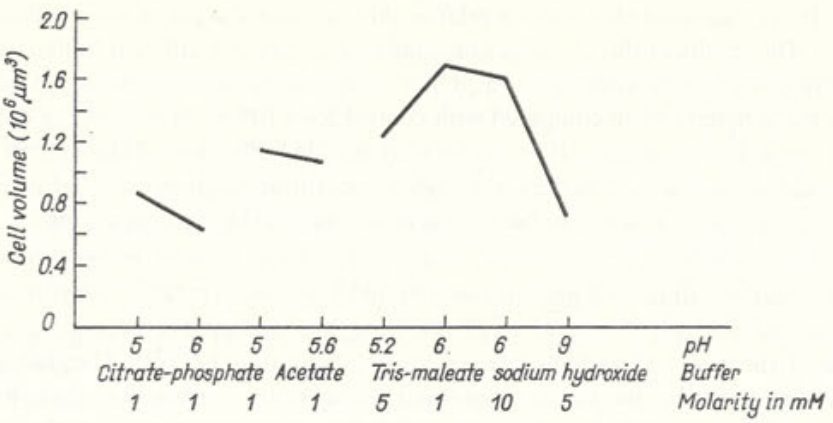


Fig. 3. Volume changes in *Blepharisma intermedium* grown in different buffers at different pH and different molarity at the end of 2 day

Discussion

The findings of several workers studying the effect of temperature (James and Padilla 1959, James and Read 1957, Popoff 1908) on the size of various protozoa indicate that there is an increase in size at low temperatures. Nemeth (1972) has found decrease in cell size in *Tetrahymena pyriformis* exposed to cold shock (—25°C) and heat shock (39°C). However, Thormar's (1962) observations are different. He has observed an increase in cell size in the ciliate *Tetrahymena pyriformis* at 34°C when compared to 10°C. Our earlier investigation (Kasturi Bai et al. 1972) showed that the number of fissions at 32°C and 35°C were the same i.e., three fissions per day whereas at 26°C there was only one fission per day. Our present experimental results show that there is a decrease in cell size in *Blepharisma intermedium* incubated at 32°C and 35°C when compared to the control 26°C. This decrease in size is more marked at 35°C than at 32°C. Growth in *Blepharisma intermedium* is temperature sensitive and it decreases with increasing temperatures.

It has been found that copper and zinc in traces are very essential for the growth of most of the protozoans (Hall 1965). Copper is necessary for the enzymes polyphenol oxidase, ascorbic acid oxidase, and tyrosinase (Prosser and Brown 1961). Zinc is found widely in higher concentrations than copper in animal tissues and is an essential component of the enzymes carbonic anhydrase, alcohol dehydrogenase. Recently it is known to play a role in stimulating RNA and protein synthesis (Wegener and Romano 1963). However, at the concentrations tried in our studies, both these metals reduce the growth rate. There is a considerable decrease in the volume of the treated ciliates (Fig. 2) at 26°C.

The effect of pH of the medium upon the growth of several protozoan species has been studied (Elliot 1933, Loefer 1935, 1938, 1942, Wingo and Anderson

1951). It is established that there is relationship between the pH of the medium and growth. The results of this investigation confirm that pH and different buffer systems at varying molarities affect growth. There is considerable decrease in volume in all the three buffers when compared with control 3.4×10^6 μ^3. However, the decrease in cell size is less in tris maleate — sodium hydroxide buffer among the buffers tried. In the acetate and citrate buffers, the higher concentrations proved lethal to the ciliates and hence cell size has been determined in 1 mM concentrations only.

Cytochemical studies on ciliates grown in hay infusion with buffers show that the lipid, carbohydrate and protein contents and enzymes — succinic dehydrogenase, alkaline and acid phosphatases of *Blepharisma intermedium* are dependent on the nature of the buffer (Shadaksharaswamy and Jyothi 1973 a). The fission rate is also dependent on the molarity, pH and nature of the buffers (Shadaksharaswamy and Jyothi 1973 b). Nemeth (1964) has observed changes in size, fission rate as well as activity of some enzymes in *Tetrahymena pyriformis* exposed to cold and heat shocks.

Our findings confirm that growth is sensitive to many factors in the medium. A slight change in any one of the factors like temperature, pH, buffer systems and increase in the concentration of trace metals bring about a considerable change in the growth rate which is reflected in the volume of the cell.

Acknowledgements

Our grateful thanks are due to Prof. E. Zeuthen, Carlsberg Foundation Biological Institute, Copenhagen, Denmark for the keen interest he showed in this investigation. Our thanks are also due to Dr K. Pampapathi Rao, Professor and Head of the Department of Zoology, Central College, Bangalore University, Bangalore, India for his encouragement and to the U.G.C. for the award of Junior Research Fellowship to one of us (Tara, S. V.).

Summary

Cultures of *Blepharisma intermedium* were grown in hay infusion at room temperature. These were exposed to different temperatures — 32°C and 35°C. Trace metals Copper and Zinc were supplemented in the following medium — hay infusion (1 mg/1000 ml of copper and 1 mg/100 of zinc), Cramer Myers and Stream Water (1 mg/10 000 ml of copper and 1 mg/100 ml of zinc) *Blepharisma intermedium* was grown in these medium at room temperature. The cultures of *Blepharisma intermedium* at room temperature were also grown in Citrate-phosphate, Acetate and Tris maleate-sodium hydroxide buffers with varying molarity and pH. The volume changes of ciliates grown under above mentioned conditions was determined. Considerable decrease in size has been observed in all ciliates when compared to the control.

RÉSUMÉ

On a cultivé des souches du *Blepharisma intermedium* dans des infusions de foin à la température ambiante. Elles ont été exposées à des températures différentes de 32°C et de 35°C.

On a ajouté des traces de cuivre et de zinc au milieu suivant — infusion de foin (1 mg de cuive/1000 ml et 1 mg de zinc/100 ml), Cramer Myers et de l'eau de rivière (1 mg decuivre/10,000 ml et 1 mg de zinc/100 ml). Le *Blepharisma intermedium* a été cultivé dans ce milieu à la température ambiante.

On a aussi cultivé des souches du *Blepharisma intermedium* dans du citrate — phosphate, de l'acétate et des solutions tampons d'hydroxyde de tri — maleate — sodium à molarité variable et avec des pH différents. Les changements de volume des ciliates cultivés dans les conditions ci-dessus mentionnées ont été déterminés.

Une diminution considérable de volume a été observée chez tous les ciliates per rapport à l'échantillon standard.

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Department of Biological Sciences, Quinnipiac College, Hamden, Connecticut 06518, and
Department of Biology, Fairleigh Dickinson University, Teaneck, New Jersey 07666, USA

Arthur J. REPAK and Irwin R. ISQUITH

The systematics of the genus *Spirostomum* Ehrenberg, 1838

Die Systematik der Gattung *Spirostomum* Ehrenberg, 1838

Since the establishment of the genus *Spirostomum* Ehrenberg, 1838, there has been an unnoticed increase in the number of described species. Considerable intrageneric confusion, if not already evident, is anticipated. This situation will most likely be compounded by a lack of familiarity of many taxonomists with the original journals coupled with relative inacessibility of these obscure journals in which many of the species were described.

The purpose of this study is to focus the attention of protozoan taxonomists and field workers on the genus *Spirostomum* by bringing all bona fide species to light.

The following criteria, when available, were used to differentiate species: body size, length to width ratios, relative peristomal lengths, shape of the anterior tip and posterior end, the number of somatic meridional rows, macronuclear shape (i.e., ovoid, moniliform, or filiform), macronuclear nodal number nad micronuclear number.

General description of the genus *Spirostomum*

Members of the genus *Spirostomum* Ehrbg., 1838 have a large, elongated, often worm like, cylindrical (or, at times, laterally flattened) appearance. The anterior end is either rounded or appears as a slight beak like projection. In most species, the posterior end is truncated. Some species, however, possess a tapered tail. Length to width ratios vary from 6–7:1 to 20:1. On the whole, these ciliates are nonpigmented, though some appear yellow or brown (Kudo, 1971). Zoochlorellae have been recently observed in the endoplasm of one species (personal observation). Well developed, subpellicular myonemes, present along the anterioposterior axis independent of the longitudinal ciliary rows, account for the high degree of contractility and body torsion. The pellicle has been described as a series of wide ridges and narrow furrows, which are derived as elevations of the ectoplasm. The somatic ciliature arises from the furrows (Finley et al. 1964).

The buccal apparatus consists of an adoral zone of membranelles (AZM) on the left, a long narrow peristome, a cytostome and cytopharyngeal fibrils. The AZM

begins at the anterior tip and progresses posteriorly parallel to the peristome. At a point across from the cytostome, the AZM curves to the right and terminates on the ventral border of the cytostome (Tuffrau 1967). Each adoral membranelle is composed of 3 membranelles originating from a lamellar base consisting of 3 parallel rows of kinetosomes (Finley et al. 1964, Tuffrau, 1967). The peristome, to the right of the AZM, is devoid of cilia. The right border of the peristome consists of a row of somatic ciliature. Fibrils, connected to cilialess kinetosomes in the middle of the floor of the peristome, extend and join each adoral membranelle. The ratio of peristome to body length per species is quite variable and ranges from 1:4 to 3:5. Cytopharyngeal fibrils, originating apparently from peristomal fibrils and those of the AZM, extend into the cytoplasm. There is no evidence of an undulating membrane (Finley et al. 1964, Tuffrau 1967).

The somatic ciliature is uniform and complete, but the number of ciliary rows appears to vary according to the species (Boggs 1962). There is one posterior contractile vacuole with a canal leading anteriorly (Kudo 1971). The cytoproct is terminal at the posterior end.

There are three general macronuclear configurations which vary according to the species: ovoid (compact), filiform (rod shaped), and moniliform (noded). The number of nodes in a moniliform macronucleus may be exceedingly large. The size, number and location of the micronuclei are also variable.

This heterotrichous ciliate is generally found in stagnated marine and freshwaters and subsists on a diet of bacteria.

Species descriptions

Spirostomum ambiguum Ehrenberg, 1838

Synonymy: *Trichoda ambiguum* Müller, 1786.
S. ambiguum var. *major* Roux, 1901.

This species, the largest of the genus, measures 1–4 mm (Kahl 1932, Roux 1901). The length to width ratio is about 10:1 (Kahl 1927). Its peristome extends 2/3 of the body length. The posterior is blunt and cylindrical with a small contractile vacuole. The cytoplasm may at times appear yellowish and is reported to contain small globules of an undetermined nature (Roux 1901). One strain has been found to contain zoochlorellae (personal observation). The macronucleus is moniliform, containing 12–50 nodes connected by tapering filaments. There is a relationship between the age of the organism and the macronucleus. The young exconjugants display small macronuclei with fewer nodes than do the more mature forms. Conjugation, however, is so infrequent that it has been difficult to establish any clear correlation (Sesachar and Padmavathi 1956). The micronuclei are numerous (12–100) and have a diameter of 1.5–1.8 μm (Roux 1901). The peristome and oral organelles are not obvious. This species is highly contractile and contains 46 somatic ciliary rows (Boggs 1962). It is found in freshwater. See Fig. 1.

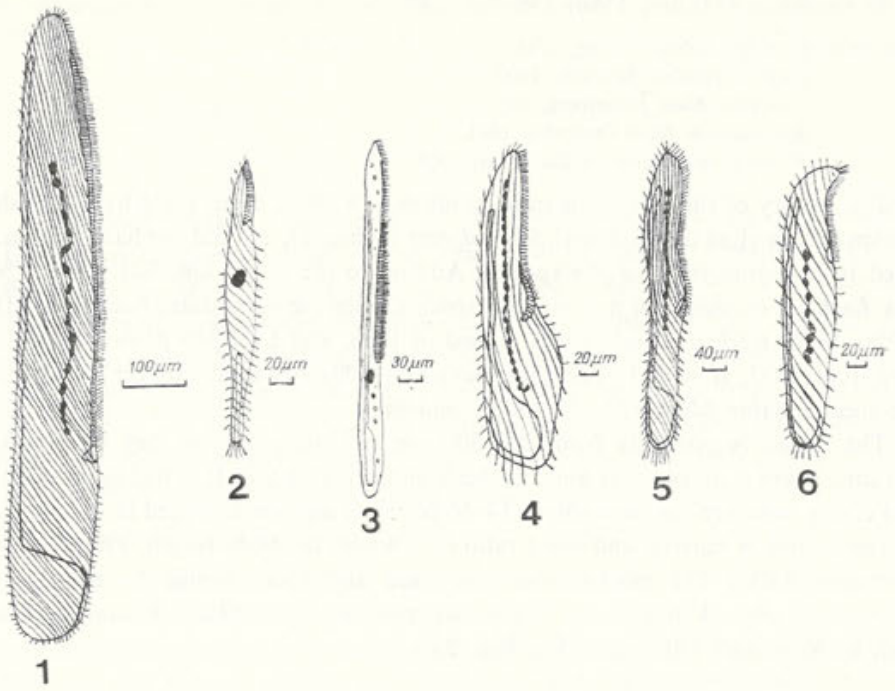

Fig. 1. *Spirostomum ambiguum*. Camera-lucida drawing from a Chatton-Lwoff silver impregated specimen. Fig. 2. *S. caudatum* after Kahl 1932. Fig. 3. *S. ephrussi* after Claparede and Lachmann 1858. Fig. 4. *S. inflatum* after Kahl 1932. Fig. 5. *S. intermedium* after Kahl 1932. Fig. 6. *S. loxodes* after Kahl 1932

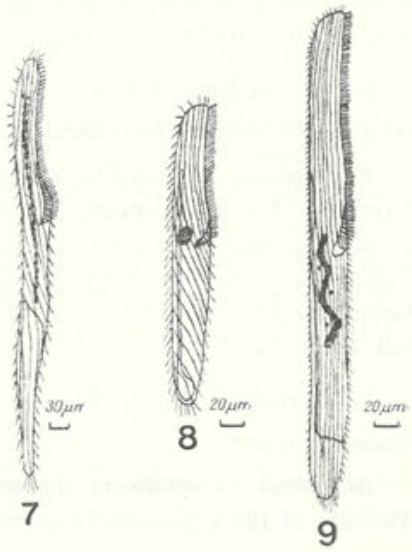

Fig. 7. *S. minus* after Kahl 1932. Fig. 8. *S. teres* after Kahl 1932. Fig. 9. *S. yagiui* after Shigenaka 1959

S. caudatum (Müller, 1786) Delphy, 1939

Synonymy: *Enchelis caudata* Müller, 1786.
Enchelis caudata Schrank, 1803.
Uroleptus filum Ehrenberg, 1833.
Spirostomum filum Dujardin, 1841.
S. teres var. *caudatum* Zacharias, 1903.

The history of this species is quite confused. In 1939, when Delphy established this species, he cited it as follows: *S. caudatum* (Schrank), but did not have Schrank listed in his synonymy for the species. Adding to the confusion, Schrank (1803) lists *Enchelis caudata*, but not as a new species, since the name dated back to Müller (1786). Since *Enchelis caudata* was named in 1786, and *Uroleptis filum* was not named until 1833, it would seem that Delphy (1939) was quite correct in replacing the species name *filum* with the older *caudatum*.

The length is generally from 200–300 μm but the organism can reach up to 700 μm. There is an obvious anterior beak and tapering tail. It is highly contractile. The ciliary rows are few in number (14–16 per side) and are arranged in tight spirals. The peristome is narrow and has a ratio of 1:4 with the body length. There is a well developed AZM. The macronucleus is ovoid and accompanied by many small micronuclei aligned in a series of parallel rows (Penard 1922). It may be found both in fresh and salt water. See Fig. 2.

S. ephrussi Delphy, 1939

Synonymy: *S. teres* Roux, 1901.
non: *S. teres Cláparéde* et Lachman, 1858.

This species has not appeared in the literature since named by Delphy (1939). The size is estimated at 450 μm and it has an ovoid macronucleus. The peristome is 3/5 of the total body length. Typically, the body is regarded as undulate when moving (Delphy 1939). See Fig. 3.

S. inflatum Kahl, 1932

Synonymy: *S. ambiguum* var. *inflatum* Kahl, 1927.

This species, as drawn by Kahl (1927), resembles a *Blepharisma* with the characteristics of a *Spirostomum*. The body length ranges from 300–400 μm and the peristome extends to over 1/3 of the body length. The length to width ratio is 10–12:1. The contractile vacuole is typical of the genus. There is a distinct vestibulum in the peristome. The macronucleus is moniliform with 25 nodes. Its general habitat is salt water. See Fig. 4.

S. intermedium Kahl, 1932

Synonymy: none.

In general characteristics, this species resembles a small variety of *S. ambiguum*. The size of the organism, however, ranges from 400–600 μm. The macronucleus

is moniliform with about 17 nodes. The number of somatic ciliary rows range from 25–30 for one side of the body (Eberhardt 1962). It is found in fresh water. See Fig. 5.

S. loxodes Stokes, 1885

Synonymy: *S. ambiguum* Dujardin, 1841.
S. ambiguum Stein, 1867.

The body length of this organism ranges from 300–600 μm. The anterior tip typically is bent into a beak like structure and the body is contorted or at times flattened with a length to width ratio of 6–7:1. The peristome is 1/3 of the body length. The contractile vacuole extends from a bulbous posterior to a tapered channel anteriorly. The macronucleus is moniliform with about 9 nodes. The number of micronuclei is unknown. This species is reported to be slow in responding to stimuli which is believed to be due to poor contractility (Delphy 1939). The somatic ciliature (number unknown) typically spirals toward the organism's left from anterior to posterior. It is found in fresh water. See Fig. 6.

S. minus Roux, 1901

Synonymy: *S. ambiguum* var. *minor*, Roux, 1901.
S. minus Kahl, 1932.

The length of this species ranges from 500–800 μm. The body is slender and often flattened and tapered toward the extremities. The peristome extends less than 1/2 the body length (Roux 1901). The number of ciliary meridians ranges from 10–12 per body side with an average of 12 (Boggs 1962). The macronucleus is moniliform with about 24 nodes. The micronuclei lie close to the macronucleus and number 4-20 with a diameter of 1.2 μm (Kudo 1971, Padmavathi 1959). Found in both fresh and salt water. See Fig. 7.

S. teres Cláparéde et Lachmann, 1858–1859

Synonymy: none.

This species measures 150–400 μm in length. It can be often recognized by the ovoid macronucleus situated close to the mouth. The peristome terminates at the middle of the body and is connected to a short, tubular cytopharynx. The somatic ciliary rows number 7–12 with an average of 9 per body side. The ciliary meridians run parallel to the longitudinal axis (Boggs 1962). It has a rounded anterior end and a truncated posterior end. The contractile vacuole and canal are typical of the genus. The number of micronuclei are unknown. This ciliate may be found in both fresh and salt water. See Fig. 8.

S. yagiui Shigenaka, 1959

Synonymy: *Spirostomum* sp. Seshachar et Padmavathi, 1956.
S. dharwarensis Desai, 1966.

This species represents how confusing various taxonomic descriptions can become. Seshachar and Padmavathi (1956) described a species of *Spirostomum*

with a filiform macronucleus without offering a species name. Shigenaka (1959) described an identical species and called it *S. yagiui*. Shigenaka's description included not only the nuclear description but also buccal and somatic observations. Desai (1966), on the other hand, loosely described what appears to be the same species and offered a different species name. In addition, Desai's description was incomplete to the extent that it is doubted that anyone would be able to duplicate his work. In comparing the work of the above investigators, it is our opinion that all three strains are representative of one species and that the credit for the species should go to Shigenaka. This species contains all the strains reported to have filiform macronuclei.

S. yagiui has a flattened, ribbon like appearance with a rounded anterior end. The average size is about 400 μm (range: 300–800 μm). The length to width ratio averages 14:1 (range 11–18:1). The peristome starts at the anterior tip and runs along the ventral margin up to the middle of the body where it terminates at a funnel like cytopharynx. The adoral membranelles number 130–140. There are about 20 somatic ciliary rows which begin at the anterior end and run parallel to the dorsal margin until they reach the posterior end. The micronuclei number 3–15. There is a large, posterior contractile vacuole with a long canal proceeding anteriorly along the dorsal margin (Shigenaka 1959). See Fig. 9.

Discussion

In this organization of the genus, *Spirostomum*, the treatment of the species has been extremely conservative. No new species have been described. An attempt has been made to clearly define each previously named species. If a more liberal approach had been taken, many of the descriptions in the literature could have been given specific status of their own based upon one characteristic or another. For example, different macronuclear nodal number ranges in various descriptions of *S. ambiguum* could lead to the consideration that these various strains are indeed different species. The filiform strain described by Seshachar and Padmavathi (1956) has 6–15 micronuclei whereas the filiform type described by Desai (1966) has 7 micronuclei which might have been considered sufficiently important by some authors to warrent the establishment of two distinctly different species. We, however, did not feel this to be the case.

There are two reasons for this conservative approach, both stemming from our extensive taxonomic studies on *Blepharisma*. From its inception, *Blepharisma* taxonomy has been chaotic. This has not been due to any especial ineptitude on the part of the taxonomists working with that genus, but rather to two features of the genus: literally every morphological feature of the numerous named species shows considerable variation; and due to the extensive collecting of the genus, numerous intergrades have now been cultured that show that each species is a part of a morphological

continuum with all other species (Isquith 1966, Repak 1967). Since the species of *Blepharisma* have been established on rather minute morphological grounds, many of the previously established species are now considered to be synonyms.

Therefore, as long as *Spirostomum* taxonomy is based solely upon morphological characteristics, broad species boundaries should be maintained. When additional data are gathered, then it may become possible to safely divide the species.

Future morphological investigations of the genus *Spirostomum* might improve the current descriptions of species by filling in the appropriate missing information. In addition, more attention might be paid to methods involving more exact measurements, e.g., it might be more appropriate to measure the organisms in the contracted state as opposed to estimating the elongated length, since it is difficult to obtain full elongated specimens in fixed preparations and equally difficult to slow living ciliates. It is further suggested that future taxonomic studies be performed and reported on larger numbers of ciliates whenever possible so as to present statistical validity.

The following key provides only that information needed to separate the species from one another. The characteristics were chosen solely for facility of use. No habitat data were used, since insufficient sampling has lead to the consideration that such data is at this time invalid:

KEY

1a. Macronucleus filiform 2
1b. Macronucleus ovoid 3
1c. Macronucleus moniliform 5
2. Length 300–800 μm. Macronuclear length 1/2 to equal that of the body . . *S. yagiui*
3a. Tail present . . . . . *S. caudatum*
3b. No tail 4
4a. Length 100–400 μm. Peristome less than 1/2 body length . . . . . *S. teres*
4b. Larger than 400 μm. Peristome about 3/5 body length . . . . . *S. ephrussi*
5a. Posterior end swollen . . . . . *S. inflatum*
5b. Posterior end not swollen 6
6a. Length 500–800 μm. Length to width radio about 20:1 . . . . . *S. minus*
6b. Length to width ratio about 10:1 or less 7
7a. Organism longer than 1 mm . . . . . *S. ambiguum*
7b. Organism significantly less than 1 mm 8
8a. Peristome about 1/3 body length; anterior beak . . . . . *S. loxodes*
8b. Peristome about 1/2 body length; no anterior beak . . . . . *S. intermedium*

Summary

Since the establishment of the genus *Spirostomum* by Ehrenberg in 1838, the number of described species has increased. The objective of this investigation is to

alleviate any potential or existing confusion by gathering all described species under one cover and clarifying their taxonomic status.

Using various morphological criteria, the following organisms have been differentiated and classified as bona fide species: *Spirostomum ambiguum*, *S. caudatum*, *S. ephrussii*, *S. inflatum*, *S. intermedium*, *S. loxodes*, *S. minus*, *S. teres*, and *S. yagiui*.

ZUSAMMENFASSUNG

Seit der Feststellung des Genus *Spirostomum* in Jahre 1838 von Ehrenberg, hat sich die Zahl der beschriebenen Spezies vermehrt. Das Ziel dieser Forschung ist die Abschaffung aller möglichen oder gegenwärtigen Verwirrung, indem alle beschriebenen Spezies unter eine Decke gebracht werden und ihr taxonomischer Zustand aufgeklärt wird.

Durch den Gebrauch morphologischer Kriteria wurden folgende Organismen differenziert und als echte Spezies klassifiziert: *Spirostomum ambiguum*, *S. caudatum*. *S. ephrussii*, *S. inflatum*, *S. intermedium*, *S. loxodes*, *S. minus*, *S. teres*, und *S. yagiui*.

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 29

Zoological Survey of India, 8 Lindsay Street, Calcutta-16, India

A. K. DAS

On the genus *Oxymonas* Janicki (*Pyrsonymphidae*:*Mastigophora*) from Indian termites

Remarques sur le genre *Oxymonas* Janicki (*Pyrsonymphidae*:*Mastigophora*) le parasite des termites Indiens

As early as 1915 Janicki erected the genus *Oxymonas* to accomodate a species *Oxymonas granulosa*. Since then Kirby 1926, Kofoid and Swezy 1926, Connell 1930, Zeliff 1930, Cleveland 1935, Nurse 1945, Cross 1946 and de Mello 1953 have established further 21 species of this genus from various termite hosts, all strictly confined to the family *Kalotermitidae*. But in India termites of this family remain practically unexplored for their flagellates. Only de Mello and de Mello 1944, de Mello 1946 and Das 1972 have examined *Cryptotermes* sp. and *Cryptotermes havilandi* (Sjösted) for their symbionts. Nevertheless, *Oxymonas* is not yet recorded from this subcontinent.

In this paper, however, four species of *Oxymonas* including two new ones, collected from the gut of *Cryptotermes havilandi* (Sjöstedt) and *Neotermes bosei* Snyder have been described. *O. parvula* Kirby and O. *grandis* Cleveland which have been recorded from the new hosts, namely, *C. havilandi* and *N. bosei* respectively have also been dealt with.

Material and methods

The specimens of *Cryptotermes havilandi* were collected from banyan trees (*Ficus bengalensis*) in Ballygunge Lake area, Calcutta and at Falta, 24-Parganas District, West Bengal, India. For the study of living flagellates 67% Locke's solution was added to the fresh gut-contents in which the flagellates remain active and less deformed for a longer period of time. For permanent preparation, smears of the gut contents were fixed in Schaudinn's fluid, Carnoy's fluid, Flemming's fluid and acetoformol and stained in Heidenhain's and Delafield's haematoxylin.

The specimens of *Neotermes bosei* which had been collected from Atiamochar beat, Jalpaiguri District and Cooch Behar forest range, Cooch Behar District, West Bengal and preserved in 70% alcohol were studied for their flagellates. The entire gut of the preserved termite was carefully dissected and kept in running water until all the alcohol was washed out. The smears of the gut-contents were then drawn on the slides and dipped slowly in 70% alcohol. The usual staining procedure for Heidenhain's and Delafield's haematoxylin was then followed.

All the measurements were taken with the aid of ocular micrometer and the drawings were made with a camera lucida.

Results

Oxymonas bengalensis sp. n.

Fig. 1, Pl. I 1

Morphology

The shape of the body is generally sub-elliptical with pointed posterior end. Length of rostellum is greatly variable. It may be shorter than or as long as or even much longer than the body length. Occasionally it bifurcates at its anterior end. Axostyle is stout and stiletto-shaped. It is broadest near the nucleus, gradually

Fig. 1. *Oxymonas bengalensis* sp. n.

pointed to the posterior end of the body and ultimately enclosed within the cytoplasm. Rostellum contains the "non-staining" portion of the axostyle, the "recurvent" portion of which could not be traced. Two blepharoplasts are located near the base of the rostellum. Each blepharoplast gives rise to two flagella. Nucleus is ovoid in shape and situated at the anterior end of the body near the shoulder of the axostyle. It contains fine chromatin granules just beneath the thin nuclear membrane and a round karyosome of 2.5 µm in diameter surrounded by a distinct halo. Endoplasm is clear but sometimes contains fragmented wood particles.

Measurements

	Range	Mean
Length of the body	42.5–98.6 µm	68 µm
Breadth of the body	15.3–17 µm	16.6 µm
Length of the nucleus	5.1–6.8 µm	5.9 µm
Breadth of the nucleus	3.4–4.2 µm	3.8 µm
Diameter of karyosome	–	2.5 µm

Ratio of body length to body width	2.3–6.4	4.2
Ratio of body length to nuclear length	15–16.5	15.4

Host: *Cryptotermes havilandi* (Sjöstedt).
Locality: Ballygunge lake area, Calcutta, India.

Remarks

Among all the species of *Oxymonas* Janicki described so far, the new species, *O. bengalensis*, resembles *O. projector* Kofoid and Swezy and *O. jouteli* Zeliff in having stiletto-shaped axostyle and in the shape and position of karyosome but is closer to the former in the shape of the body. The species under report is, of course, clearly distinguishable from the above two species by being larger in size (*O. projector* 12 to 40 μm long and *O. jouteli* 13.3 to 30.7 μm long) and in having stout axostyle always enclosed within the cytoplasm. Whereas in O. *projector* and O. *jouteli* axostyle is slender and projects out of the posterior cytoplasm to a moderate distance.

Oxymonas bosei sp. n.

Fig. 2, Pl. I 2

Morphology

The shape of the body is broadly ovoidal. Length of rostellum is considerably variable depending perhaps on the attached or motile phase of the species. (According

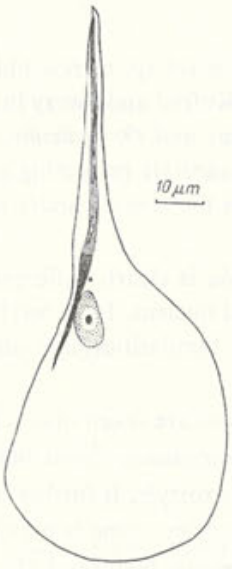

Fig. 2. *Oxymonas bosei* sp. n.

to Cross 1946 a lengthy rostellum probably results from a passive growth in adaptation to external pressures acting on plastic cytoplason). Axostyle is stiletto-shaped. Its "staining" portion projects slightly below the nucleus and hangs free in the cyto-

plasm and its "non-staining" portion is extended into the rostellum. Blepharoplasts are two in number and located in the same position as described in *O. bengalensis*. The nucleus is situated at the anterior part of the body near the base of the rostellum. It is elliptical in shape and contains numerous fine chromatin granules mostly concentrated to the peripheral zone. It also possesses an ovoid karyosome, 2 μm in diameter, situated almost at the centre of the nucleus and surrounded by a distinct halo. Endoplasm occasionally contains fragments of wood particles.

Measurements

	Range	Mean
Length of the body	172–236.2 μm	204.6 μm
Breadth of the body	31–48.6 μm	38.2 μm
Length of the nucleus	9–14.3 μm	11.9 μm
Breadth of the nucleus	4.6–8 μm	5.1 μm
Diameter of karyosome	—	2.0 μm
Ratio of body length to body width	4.3–6	5.0
Ratio of body length to nuclear length	19.6–20.2	19.8 —

Host: *Neotermes bosei* Snyder.
Locality: Atiamochar beat, Dist. Jalpaiguri, West Bengal, India.

Remarks

Oxymonas bosei sp. n. resembles *O. dimorpha* Connell, *O. barbouri* Zeliff and *O. pediculosa* Kofoid and Swezy in body-shape and in having stiletto-shaped axostyle. But O. *barbouri* and *O. pediculosa* are much smaller in size (as shown in Table 1) and have the axostyle projecting very slightly beyond the posterior extremity of the body, while in the species under report the axostyle hangs free within the cytoplasm.

O. dimorpha is clearly differentiated from this new species by its much broader size and round nucleus. Its axostyle also extends little beyond the posterior extremity of the body. Similarities and differences of these four species are tabulated in Table 1.

If dimensions are taken into account *O. bosei* also resembles *O. grandis* Cleveland and *O. megakaryosoma* Cross but distinctly differs from them by the shape of the body and the axostyle. It further differs from *O. grandis* in the nature of its nucleus. A conspicuous karyosome is present in this new species while absence of karyosome is the characteristic feature of *O. grandis*. *O. bosei* can be easily distinguished from any other species of *Oxymonas* described so far by its larger dimensions, elliptical nucleus, centrally placed ovoid karyosome and stiletto-shaped axostyle of shorter length.

Table 1

Comparison of *Oxymonas bosei* sp. n. with related species

	O. dimorpha	*O. barbouri*	*O. pedi-culosa*	*O. bosei* sp. n.
Average length of the body	120 μm	16.1 μm	34 μm	204.6 μm
Average breadth of the body	100 μm	11 μm	21 μm	38.2 μm
Shape of the nucleus	round	round	round	elliptical
Average dimensions of the nucleus	7.7 μm	4 μm	7 μm	11.9 μm×5.1 μm
Ratio of body length to body breadth in average	1.2	1.6	1.7	5
Ratio of body length to nuclear length in average	15.5	4	5	19.8
Axostyle	Resembles a stiletto in form and projects out of the posterior estremity of the body	Similar	Similar	Resembles a stiletto in form but hangs free in the cytoplasm
Type host	*Paraneotermes simplicicornis* Banks	*Glyptotermes barbouri*	*Kalotermes nigripces*	*Neotermes bosei* Snyder

Oxymonas parvula Kirby, 1926

Fig. 3

Type host: *Cryptotermes hermsi* Kirby, Fanning Island.

Morphology

Body is ovoid or slender with round or truncated posterior end. Rostellum is minute or as large as body length. Axostyle is very slender projecting sometimes far beyond the posterior part of the body. Nucleus is oval or round. Fine chromatin granules are distributed throughout the nucleus. The small spherical karyosome is not surrounded by halo. Endoplasm contains many granules or wood fragments.

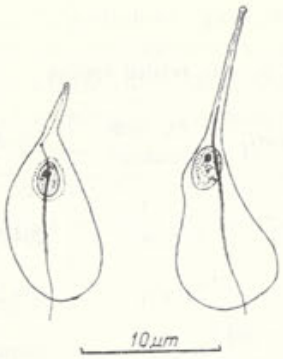

Fig. 3. *Oxymonas parvula* Kirby, 1926

Measurements

	Range	Mean
Length of the body	23.8–45.9 µm	31.1 µm
Breadth of the body	3.4–8.5 µm	5.9 µm
Length of the nucleus	3–4.2 µm	3.6 µm —
Breadth of the nucleus	1.7–2.5 µm	2.0 µm
Ratio of body length to body width	2.8–6	4.1
Ratio of body length to nuclear length	5.4–8.7	7.2 —

Host : *Cryptotermes havilandi* (Sjöstedt).
Locality: Jagannathpur (Falta), Dist. 24-Parganas, West Bengal, India.

Remarks

O. parvula is recorded for the first time from Indian termites. It is also a new record from the host *Cryptotermes havilandi*. Type specimens measure 5–13 µm in length. In the original description of Kirby 1926 as well as in the subsequent description by Cross 1946, all other measurements except length are lacking. *O parvula* from the new host is much larger in size than the specimens described from the type host species. Yet the shape, nature of axostyle, minute karyosome, nucleus with uniformly distributed chromatin granules and endoplasm with many inclusions, as observed in the specimens collected from the Indian host are in conformity with Kirby's description of *O. parvula*.

Oxymonas grandis Cleveland, 1935

Fig. 4

Type host: *Kalotermes* (*Neotermes*) *dalbergiae* Kalshoven.
Additional host: *K.* (*N.*) *tectonae* Dammernan.

Morphology

The shape of the body is ellipsoid. The rostellum is of varying size depending on the attaching or motile phase of the species. Axostyle resembles a scimitar in shape. Nucleus is ovoid in shape and situated just posterior to the shoulder of the axostyle

in the interkinetic period. It has the length of 8.5 to 17 μm and breadth of 5.1 to 6.8 μm. Karyosome is totally absent. Chromatin granules are of varying size. Endoplasm is finely granular and usually contains wood fragments.

Fig. 4. *Oxymonas grandis* Cleveland, 1935

Measurements

	Range	Mean
Length of the body	78.7–266.2 μm	158.3 μm
Breadth of the body	18.7–45 μm	30.7 μm
Length of the nucleus	8.5–17 μm	12.1 μm
Breadth of the nucleus	5.1–6.8 μm	5.9 μm
Ratio of body length to body width	3.5–8	5.7
Ratio of body length to nuclear length	10.1–12.6	11.9

Host: *Neotermes bosei* Snyder

Locality: Atiamochar beat, Dist. Jalpaiguri, Cooch Behar Forest range, Dist. Cooch Behar, West Bengal, India

Remarks

O. grandis Cleveland is a new record from the host *Neotermes bosei* Snyder and also the first record from Indian termites. The measurements of the species from *N. bosei* and from *Kalotermes* (*N.*) *dalbergiae* and *K.* (*N.*) *tectonae* as given by Cleveland 1935 and Cross 1946 respectively, are compared in the Table 2.

It is revealed from the Table 2 that the specimens of *O. grandis* collected from the local host are obviously the largest in dimensions but their nuclei are comparatively small.

Table 2

Comparison of measurements of *Oxymonas grandis* from different hosts (in μm)

From *N. bosei*			From *K.* (*N.*) *dalbergiae* and *K.* (*N.*) *tectonae*			
			by Cleveland		by Cross	
	Range	Mean	Range	Mean	Range	Mean
Length of the body	78.7–266.2	158.3	76–183	121	41–241	109
Breadth of the body	18.7–45	30.7	31–79	52	15–78.3	36.4
Length of the nucleus	8.5–17	12.1	20–23	21	8.7–24.9	16
Breadth of the nucleus	5.1–6.8	5.9	20–23	21	8–20.7	12.6

Acknowledgements

Thanks are due to Dr. A. P. Kapur, Director Zoological Survey of India for the laboratory facilities, Dr. P. K. Maiti for identifying the host specimens, Dr. S. Khera and Shri A. S. Rajagopal for encouragement. The author is also indebted to Dr. A. Choudhury, Science College, Calcutta for many useful suggestions and Mr. K. Misra for photomicrographs.

Summary

Four species of *Oxymonas* Janicki, namely, *O. bengalensis* sp. n., *O. bosei* sp. n., *O. parvula* Kirby *O. grandis* Cleveland have been described from the gut of the termite, *Cryptotermes havilandi* (Sjöstedt) and *Neotermes bosei* Snyder collected from West Bengal, India. Genus *Oxymonas* has been recorded from the Indian subcontinent for the first time.

RÉSUMÉ

La déscription de quatre espèces d'*Oxymonas* Janicki: *O. bengalensis* sp. n., *O. bosei* sp. n., *O. parvula* Kirby et *O. grandis* Cleveland vivants dans l'intestin des termites, *Cryptotermes havilandi* (Sjöstedt) et *Neotermes bosei* Snyder (Bengal d'Ouest, Indes) est presentée. Le genre *Oxymonas* est reporté de subcontinent Indien pour la première fois.

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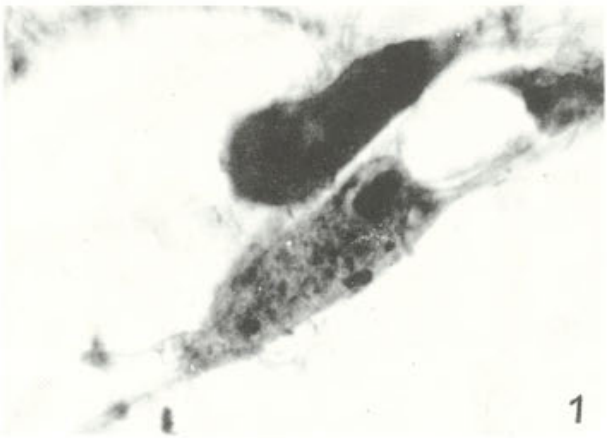
EXPLANATION OF PLATE I

1: *Oxymonas bengalensis* sp. n. ×863
2: *Oxymonas bosei* sp. n. ×400



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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 30

Department of Zoology, University College of Science and Technology,
Calcutta University, Calcutta-19, India

K. K. MISRA, N. C. NANDI, S. RAUT and A. CHOUDHURY

Haemogregarina simondi sp. n. a new haemogregarine from a river turtle, *Trionyx gangeticus* Cuvier

Haemogregarina simondi sp. n. la nouvelle hémogrégarine de la tortue *Trionyx gangeticus* Cuvier

The genus *Haemogregarina* comprises the common blood parasites of the cold blooded vertebrates. They have been described from different hosts of fishes, amphibians and reptiles. The parasite characteristically develops within the red-blood cells of the peripheral circulation and asexual reproductive stages are found in the endothelial cells of the vertebrate hosts.

Previous reports on haemogregarines were made by different authors from various hosts, and so far chelonian host is concerned Danilewsky (1885) described for the first time a haemogregarine, *Haemogregarina stepanowi* from European water tortoise *Emys orbicularis*.

In India, studies on haemogregarines from chelonia were started by Simond (1901), who described two haemogregarines, *Haemogregarina laverani* and *H. mesnili* from *Emyda granosa* and *Emys tectum* respectively, turtles of river Jamuna at Agra. Simond (1901), also observed a haemogregarine similar to that of *H. stepanowi* along with an infection of *Haemoproteus* (cited by Garnham 1966) in the soft leathered turtle *Chitra indica* (=*Trionyx indicus*). Later Patton (1908), described *Haemogregarina nicoriae* from *Emyda granosa*. In 1912, Laveran and Nattan-Larrier described a new species *Haemogregarina testudinis* from a tortoise *Testudo emys*. Besides, three more haemogregarine parasites were described from a turtle, *Lissemys punctata granosa* Schoepff from Portuguese India, Nova Goa. These are *Haemogregarina vittatae* Robertson, 1908, *H. malabarica* de Mello, 1932 and *H. xaveri* de Mello, 1932 (cited by Bhatia 1936). *Lissemys punctata granosa* Schoepff was also found to be infected with *H. nicoriae* (cited by Bhatia 1936). Since then, in India, haemogregarines of chelonia remained untouched. The present communication deals with a haemogregarine parasitizing the erythrocytes and lung endothelial cells of a soft leathered turtle, *Trionyx gangeticus* Cuvier, inhabiting the river Ganges and its estuary. *H. stepanowi* undergoes asexual reproduction in the bone marrow of the vertebrate host and thus differs from the present parasite which develops

in the lung. It may be mentioned that Ball (1958), described *H. mirabilis* from a water snake in which the lung was the main site of schizogony. The parasite under the present discussion has been given a new name *Haemogregarina simondi* after the name of Simond, who was to describe first a haemogregarine from India.

Materials and methods

The turtles were collected from the local market and examined for the parasites. Thin blood films were drawn on clean grease-free slides and stained with Giemsa after fixation in methanol. Contact smears of liver and lung were made and stained following the same procedure. Small pieces of liver and lung were fixed in Carney's fixative as well as in Bouin's fluid. Six micron sections were cut and subsequently stained with iron alum haematoxylin.

Camera lucida drawings of the parasites were made and microphotographs were taken with the help of a Leica camera using Kodak film of 32 ASA.

Results

The haemogregarine parasites in the peripheral blood were intracellular and infect only the erythrocytes. Some extracellular forms were found and identified as the mature gametocytes. Double infection of similar forms (Pl. I 3, Pl. II 16) or of different forms were also observed. Percentage of double infection fluctuates according to the intensity of parasitemea. Of five turtles examined so far three were found to be infected with the parasite under report.

Only the gametocytic stages were found in the erythrocytes of peripheral blood. Though no merozoite was found to invade the erythrocytes, many early stages of gametocytes were observed successfully. This signifies that the infections are chronic.

Development of gametocytes in the erythrocyte

Youngest form

Both the macro and micro-gametocytes were found to develop in the red blood cells. Youngest forms of both the gametocytes were minute and round to pear shaped in structure (Pl. I 1, 8). Cytoplasm is clear and transparent but sometimes is stained faint pink with Giemsa. The outer cell membrane of the parasite is distinct. Nucleus is a compact mass situated the middle of the parasite. It stains deep red when stained with Giemsa. Youngest forms of both the gametocytes are alike, but the later developmental stages differ characteristically.

Development of microgametocyte

The youngest form increases in size and becomes oval in shape, both the ends being blunt. Cytoplasm is clear and transparent with distinct cell membrane as in

the earlier forms. At this stage the nucleus becomes ribbon or band shaped and adhere to the cell membrane along the longitudinal axis of the parasite (Pl. I 9). Staining reaction is the same as in the youngest form.

As the development proceeds, parasites become elongated with both ends blunt and turn bean-shaped. Cytoplasm takes a light stain and is devoid of granules. Nucleus continues to proceed along the longitudinal axis and reaches the middle of the parasite still adhering to the cell membrane (Fig. 1 A, Pl. I 10–12, Pl. II 13). Meanwhile the parasite elongates further.

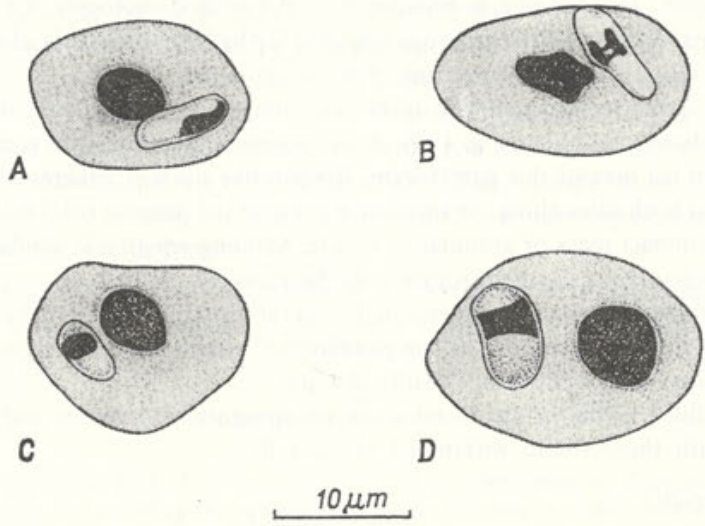

Fig. 1. Camera lucida drawings of *Haemogregarina simondi* sp. n. A — Developmental stage of microgametocyte, B — Developed microgametocyte, C — Developmental stage of macrogametocyte, D — Fully formed macrogametocyte

A fully developed microgametocyte (Fig. 1 B) is characterized by the peculiar configuration of the nucleus. The elongated bean-shaped body may be slightly curved. Cytoplasm again becomes clear and transparent, with no granules or pigment. Nucleus is not at all a compact mass but two band-shaped structure adhering to the opposite sides of the cell-membrane along the longitudinal axis. (Pl. II 14–16). It may be noted that in some cases it appears as a division of the nucleus. Careful observation reveals and justify that though the nuclear mass shares both the sides axially, chromatin threads or granules link them. At this stage the parasite measures 9.16 µm in length and 3.2 µm in breadth.

This is the last developmental stage of microgametocyte so far observed in the peripheral blood. The microgametocyte may develop in any part of the cytoplasm of the erythrocyte.

Development of macrogametocyte

As the youngest form develops it attains the typical pyriform shape with one end broad and blunt and the other end narrow but also blunt (Fig. 1 C). Cytoplasm more or less transparent and a distinct cell membrane is present. Nucleus comparatively large and triangular in shape. It is situated at the broader end of the parasite and not adhering with the cell membrane. (Pl. I 2). Nucleus stains deep red with Giemsa.

In the next phase of development the parasite increase in length. Cytoplasms stains light pink in the broader and but clear and transparent at the narrower end. Oval or "band-shaped" nucleus is situated in the middle, along the transverse plane of the parasite. The parasite is broader than the microgametocyte. Characteristic developmental feature of the macrogametocyte is that the nucleus is always at the middle and along the transverse plane of the parasite (Pl. I 3–5).

A developed macrogametocyte is broad, elongated, slightly curved and with both the ends equally blunt (Fig. 1 D). A well stained cytoplasm with some granules are the main fea ures of this gametocyte. Ribbon-like nucleus adheres with the cell membrane at both sides along the transverse plane of the parasite (Pl. I 6–7). Nucleus is either a compact mass or granular in nature. Staining reaction is similar. Position of the gametocyte in the erythrocyte may be eccentric, lateral or tangential. No further development of macrogametocyte has so far been observed in the erythrocyte. It may be mentioned that none of the gametocytes exceed the erythrocyte in length. Measurements: length 9.6 μm, breadth 4.8 μm.

Extracellular forms in the blood show no structural differences and trinctorial property with those found within red blood cells.

Pathogenicity

This intra-erythrocytic parasite has some effect on the host cell. Mostly, it has been found that the host cell nucleus is pushed to a side when the parasite is polar or tangential in position. The host cell nucleus is non attacked by the parasite. No staining differences were observed between the normal and an infected erythrocyte. Deformities of the host cell due to parasitic infestation were not observed.

Schizogony

No schizogonic stages were observed in the erythrocytes. Contact smears and tissue sections of both the liver and lung were examined carefully and some stages of schizogony were found in the lung.

The parasites were found to invade the lung parenchyma, and develop within the host cell (Pl. II 17, 18) i.e., parenchymatous tissue cells. After invasion the nuclei of merozoites divide and redivide to form multinuclear schizont. The arrangement of innumerable nuclei in the cell is irregular. The diameter of the nucleus vary between 0.75 μm to 1.0 μm. Parasitized host cell becomes slightly larger in diameter. Sometimes it has been found that the lung capillaries were blocked by the merozoites. No schizogonic stages were so far been observed in the liver.

Discussion

Many reptiles have been found to be infected with blood parasites of the genus *Haemogregarina*, which has been characterized by its schizogony taking place in the red blood cells of the vertebrate hosts (Kudo 1966). The present authors were unable to detect any schizogonic stage within the red blood cells. Ball (1958) while describing *Haemogregarina mirabilis* from a snake, referred that its schizogony occurs in lung tissue. The present parasite also performs its asexual reproduction in the lung tissue. Further observation on its asexual reproduction is going on.

The parasite under report differs from all the previous haemogregarines so far described from tortoises and turtles in measurements and in the manner of development of gametocytes. The present haemogregarine is comparatively smaller than the other haemogregarines. The peculiarity of the present parasite is that the gametocyte never exceed the size of the host cell nor bent upon itself as in the case of *Haemogregarina stepanovi Danilewsky* (Wenyon 1926). The parasite under discussion has its asexual phase in lung and thus differs from other haemogregarines so far described from chelonia in India.

So the present parasite has been assigned a new name *Haemogregarina simondi* sp. n. to accomodate the parasite in the genus *Haemogregarina* Danilewsky.

Acknowledgements

Our thanks are due to Prof. D. N. Ganguly, Head of the Department of Zoology, Calcutta University, Calcutta, for providing the necessary laboratory facilities. Authors are also indebted to Dr. M. M. Chakravarty, Retd. Scientist, L.C.A.R. Department of Zoology, Calcutta University for valuable suggestions during the course of observations.

Summary

Haemogregarina simondi sp. n. has been described from a soft leathered turtle *Trionyx gangeticus* Cuvier inhabiting the river Ganges and its estuary. Detail description of the development of the gametocytes within erythrocyte has been made as well as asexual stage in the lung has been described.

RÉSUMÉ

Haemogregarina simondi sp. n. a été décrite chez la tortue *Trionyx gangeticus* Cuvier qui habite Gange sinsi que son embouchure. On a donné une description détaillée des gametocytes résidant à l'intérieur des erythrocytes, de même que la description des stades asexuels dans les poumons.

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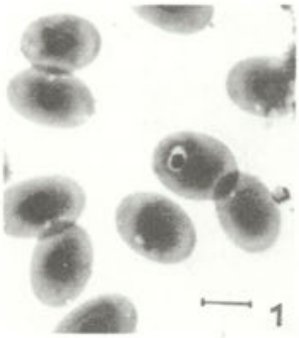
EXPLANATION OF PLATES I–II

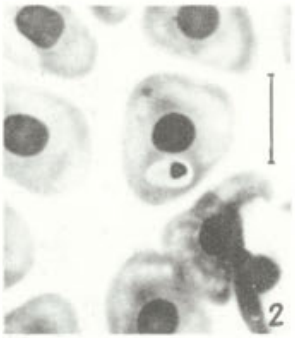
Haemogregarina simondi

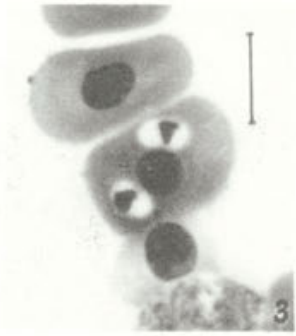
1: Youngest form
2: Early development of macrogametocyte
3: Double infection of similar forms
4 and 5: Later developments of macrogametocyte
6 and 7: Fully developed macrogametocyte
8: Youngest form of microgametocyte
9: Early development of microgametocyte, with nucleus adhering the cell membrane
10–12: Later developmental stages of the microgametocyte. Note the movement of the nucleus
13: Later development of microgametocyte
14 and 15: Fully formed microgametocyte
16: Double infection of microgametocyte
17: Section of lung. Arrow showing schizonts
18: Same as above. Higher magnification

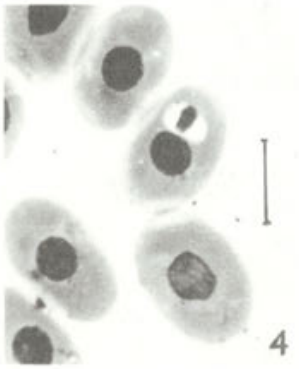
1–16: Stained with Giemsa, Figs. 17 and 18 Bouin's fixed iron- Alum-haematoxylin stain

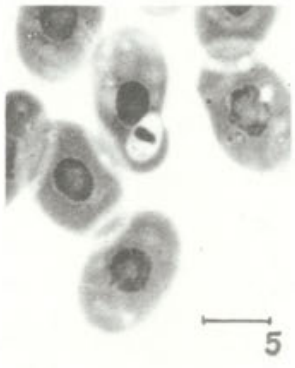
Scale represents 10 μm except stated

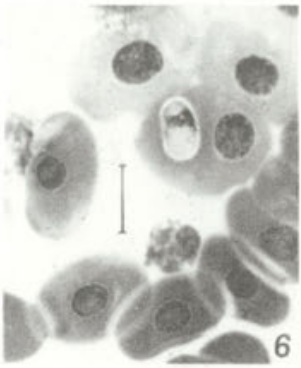


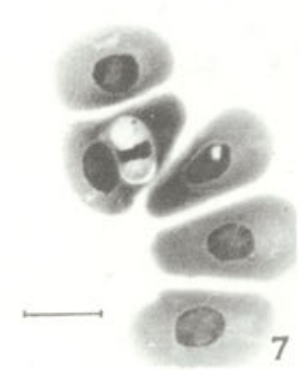


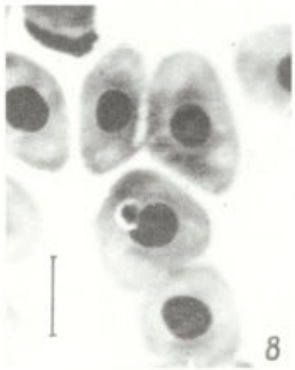


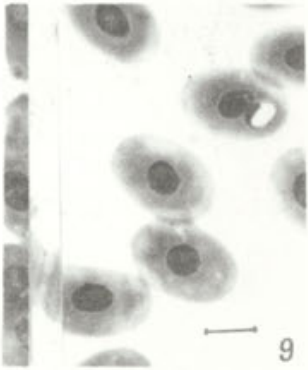


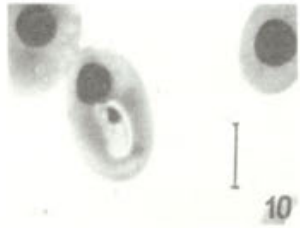


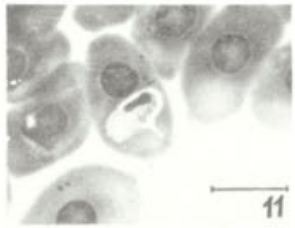


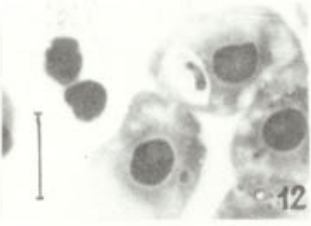












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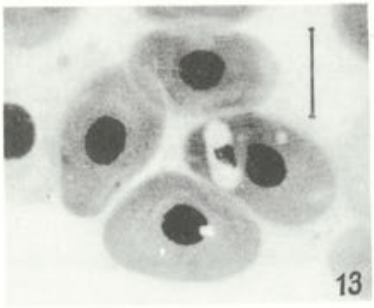

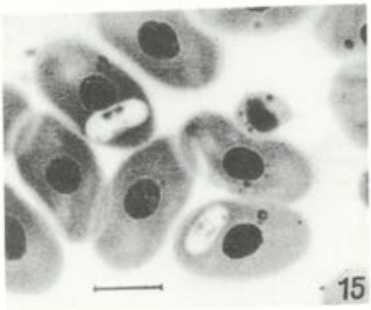

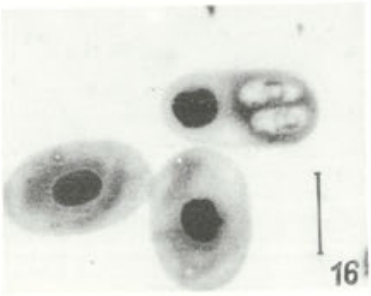

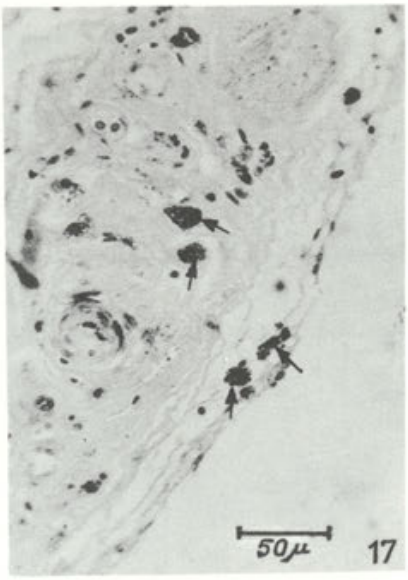

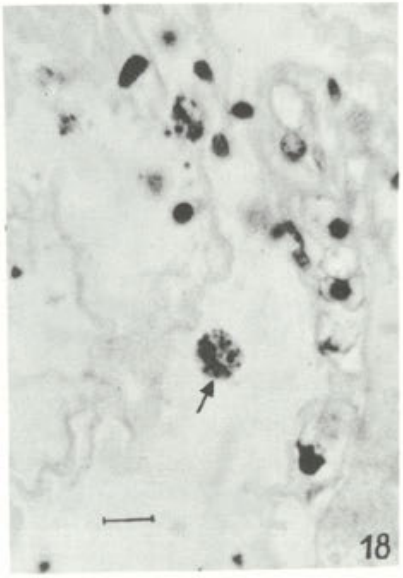

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 31

Zoological Survey of India, 8 Lindsay Street, Calcutta-16, India

A. K. MANDAL and K. N. NAIR

Clevelandella kidderi sp. n. (*Clevelandellidae*) new heterotrichous ciliate from Wood-feeding Roach (*Panesthia* sp.) of Andaman Islands, India

Clevelandella kidderi sp. n. (*Clevelandellidae*), un nouveau Cilié Hétérotriche de *Blattes xylophage* (*Panesthia* sp.) des Îles Andaman, Inde

Kidder (1938) proposed a new name *Clevelandella* for his genus *Clevelandia* Kidder, 1937, to include certain interesting heterotrichous ciliates found in the digestive tract of Wood-feeding Roaches (*Panesthia javanica* Serville and *P. spadica* Shiraki). Yamasaki (1939) unaware of Kidder's work erected the genus *Emmaninius* and described three species of ciliates from *P. angustipennis* Illiger. Corliss (1961) considered that the genus *Emmaninius* Yamasaki, 1939 is congeneric with *Clevelandella* Kidder, 1938 with a general remark that Yamasaki independently described the same interesting ciliates from the same host. However, from the available descriptions and illustrations of all the species of this genus described so far it is evident that Yamasaki's "*longicollis*" and "*plantiformis*" are conspecific with Kidder's *C. nipponensis* and *C. constricta* respectively. But there are sufficient reasons to believe that Yamasaki's another species viz. "*E. papilloris*" is a distinct form and can be treated as *Clevelandella papilloris* (Yamasaki 1939).

During the recent survey of Andaman Islands the authors encountered a species belonging to this genus inhabiting the gut of the Wood-feeding Roach (*Panesthia* sp.). This form showed considerable differences from all the known species of the genus. Hence it is described as a new species *Clevelandella kidderi* sp. nov.

The type specimens are deposited in the National Collection of Zoological Survey of India, Calcutta.

Materials and methods

The following observations are made on both the fresh specimens kept in the normal saline and fixed as well as stained specimens. Lugol's iodine was used for observing the peripheral organellae. For making permanent preparations the specimens were fixed in Schaudinn's fluid and stained in Heidenhain's iron-haematoxylin.

Description

The body is spear-shaped, greyish in colour, dorsoventrally flattened, right side moderately arched and left side more arched and forms a notch where it joins the peristomal projection. Body can be divisible into two parts — the peristomal prolongation and the body proper. Body proper terminates as a blunt point anteriorly. A distinct ectoplasmic cortical layer present all throughout. The body proper measures 61–90 μm in length (average 74 μm) and 43–60 μm in breadth (average 52 μm). The peristomal projection is eccentrically located measuring 27–33 μm in length (average 31 μm) and 13.5–25.5 μm in width (average 18.5 μm). The body ciliation is restricted to the anterior half of the body and the cilia are very fine. The ciliary lines are closely set, evenly distributed throughout and they converge to one side of the body (Fig. 1 A, B). The peristome extends up to the middle of the peristomal

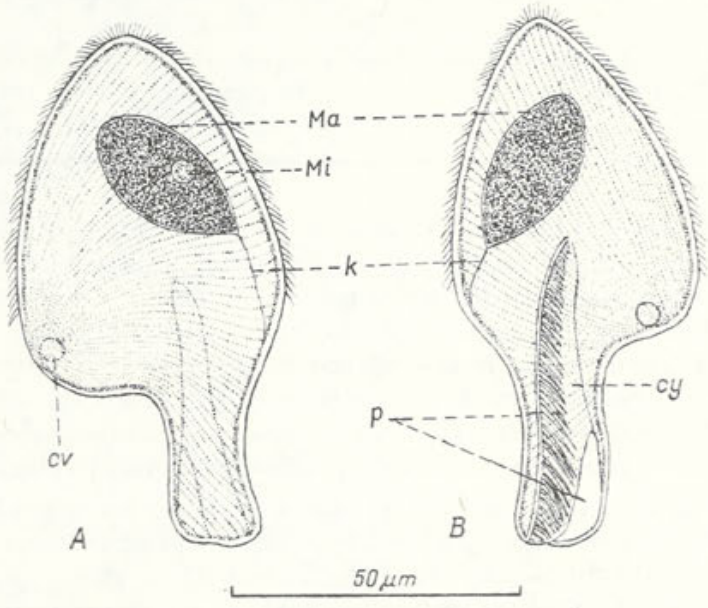

Fig. 1. *Clevelandella kidderi* sp. n. A — dorsal, B — ventral view, cv — contractile vacuole, cy — cytopharynx, k — karyophore, Ma — Macronucleus, Mi — Micronucleus, p — peristome

projection with a slightly longer dorsal flap of peristome than the ventral flap. The well developed cytopharynx extends through the peristomal projection and terminates a little distance below the macronucleus. A row of membranellae lines the left side of peristome. The contractile vacuole is single and located in the area where the ciliary lines converge. The macronucleus is broadly oval in shape, measures 27 μm by 15 μm and placed diagonally in the body proper. The kary-

ophore is formed of a single membrane and reaches the left margin of the body anteriorly. The micronucleus is single, round, measuring 5–6 μm in diameter and adheres to the macronucleus.

The individuals are sluggish displaying backward swimming locomotion.

Type host: *Panesthia* sp.
Habitat: Hind gut.
Type locality: Wright Myo, Andaman Island, India.
Date of colln.: 1–8, 1972.
Holotype: Z. S. I. Reg. No. Pt. 1590

Diagnosis of *Clevelandella kidderi* sp. n.

Body spear-shaped, dorsoventrally flattened, left margin forming a notch near proximal end of peristomal projection. Average size of body proper 74 μm (61–90 μm) by 52 μm (43–60 μm), peristomal projection prominent, average size 31 μm (27–33 μm) by 18.5 μm (13.5–25.5 μm). Peristome extends up to the middle of peristomal projection. Body ciliation closely set. Macronucleus oval, size 27 × 15 μm, with a single karyophore, micronucleus round, faintly staining with haematoxylin size 5–6 μm. Cytopharynx extends through the peristomal projection and terminates just below macronucleus.

Discussion

The species described here resembles *Clevelandella nipponensis* Kidder (=*E. longicollis* Yamasaki) in its general body shape and nuclei. But it differs in having the cytopharynx extended more into the body, closely set ciliary lines and the presence of a notch on the left side where the peristomal projection takes its origin. It also resembles *C. parapenesthiae* Kidder in body shape and the position of cytopharynx but differs from the latter in having the peristome extending up to the middle of peristomal projection, the presence of oval-shaped macronucleus and a faintly staining micronucleus. Moreover, the present from comes close to *C. hastula* Kidder in its general body shape but can easily be separated from the latter in having a distinct karyophore, small micronucleus and closely set ciliary lines. Therefore, the present species does not resemble any known species of the genus recorded so far and hence it is described here as new namely *Clevelandella kidderi* sp. n. The specific name is given after Dr G. W. Kidder, who described the genus.

Acknowledgements

The authors are grateful to Dr A. P. Kapur, Director, Zoological Survey of India, Calcutta for the facilities provided to carry out this work. Their thanks are also due to Dr S. Khera, Deputy Director and Shri T. D. Soota, Superintending Zoologist, Zoological Survey of India for encouragement and help.

Summary

The description of a new species of heterotrichous ciliate *Clevelandella kidderi* sp. n. (*Clevelandellidae*) inhabiting the hind gut of Wood-feeding Roach collected from Andaman Island, India is incorporated in this paper. Its affinities with the known species of the genus and the differences to consider it as new species are also included followed by the comments on the allied species of the genus.

RÉSUMÉ

On a décrit une nouvelle espèce du Cilié Hétérotriche *Clevelandella kidderi* sp. n. (*Clevelandellidae*) vivant dans l'intestin des *Blattes xylophage* (*Panesthia* sp.) recueillie aux Îles Andaman, Inde.

On a aussi précisé ses affinités avec les espèces du même genre, ainsi que les différences qui permettent de la définir comme une espèce toute nouvelle. On a ajouté un court commentaire au sujet des autres espèces du même genre.

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ACTA PROTOZOOLOGICA
VOL. XII WARSZAWA, 31.I.1974 FASC. 32

Department of Cytology and Histology, Biology Faculty, Lomonosov University, Moscow, USSR

A. WARTOŃ[1] and W. D. KALLINIKOVA

Cytochemistry of some *Trypanosoma* species as related to their capacity for diskinetoplastia. II. *Trypanosoma lewisi* and *Trypanosoma evansi*

Цитохимическое сравнение некоторых видов трипаносом в связи со способностью к дискинетопластии II. *Trypanosoma lewisi* и *Trypanosoma evansi*

The species of *Trypanosomidae* family, belonging to the *brucei* group — in contrast to the representatives of the other groups of this family — are characterized by the capacity of existing in the diskinetoplastic state i.e., they remain viable after an impairment of kinetoplast (more accurate kinetoplastic DNA), (Reichenov 1939, Hoare 1954, Mühlpfordt 1959, 1970 Kallinikova 1967 a, b, Trager 1970).

The results of our former investigations (Wartoń and Kallinikova 1971, Kallinikova and Wartoń 1972) suggest that unique capacity of the *brucei* group is connected with the insignificant role of kinetoplast as compared with the other trypanosomes. It became clear from our comparative cytochemical study that the kinetoplast *T. cruzi* — species not capable to diskinetoplastia — is characterized not only by big dimensions but also by high concentration of DNA and by a complex chemical composition. Around its kinetoplast, proteins and respiratory enzymes are concentrating which indicates that this structure, together with the nucleus, performs a central role in the cell metabolism. Besides, the kinetoplast of *T. equiperdum* which belongs to the *brucei* group, shows a much lower concentration of DNA and a much less complex chemical composition as well as loss of its important role in the cell metabolism.

Other *Trypanosoma* species belonging to different groups, namely: *T. lewisi* of *lewisi* group, and *T. evansi* of the *brucei* group, have been investigated cytochemically. In order to ascertain whether the difference in cytochemistry of the trypanosoma cell as a whole as well as of the kinetoplast itself are characteristic features for the groups of species under study.

[1] Present address: Department of Parasitology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 00-973 Warszawa, Pasteura 3.

Material and methods

The strains of *T. lewisi* Kent[1] were cultivated in the blood of young albino rats by subcutaneous infection at two weeks intervals. The strains of *T. evansi* Steel[2] were cultivated in the blood of white mice. In this case the strains were inoculated at 4-days intervals. Both trypanosomes were studied at the peak of infection (the 8–9th from the moment of infection with *T. lewisi* and on the 4–5th day in the case of *T. evansi*).

Similarly as in the case of *T. cruzi* and *T. equiperdum* (Wartoń and Kallinikova 1971, Kallinikova and Wartoń 1972) the following tests were applied: Feulgen (DNA), Brachet (RNA), Mazia (general protein test), Alfert and Geschwind (modification of Geyer) Amido black 10 B staining (histones), Barnett and Seligman method (SH-proteins) as well as staining with Janus Green B, method of Moog (cytochrome oxidase), Nachlass et al. (NADH- and NADPH-dehydrogenase) and benzidine method (peroxidase activity).

Results

DNA

For *T. lewisi* the presence of a rather big, rod-shaped kinetoplast with a strong reaction for DNA is characteristic. DNA is distinctly revealed in the nucleus, mostly as a peripheral ring, but here the reaction is much less intense than in the kinetoplast (Pl. I 1).

In *T. evansi*, after the reaction for DNA the result in nucleus is the same as in the case of *T. lewisi* whereas the kinetoplast is stained less intensely than in *T. lewisi*. It present a tiny pink point in some cases it fails to appear after the Feulgen reaction (Pl. I 2).

RNA

Pyronine staining is rather faint and diffuse in the cells of *T. lewisi*, being often more intense around the nucleus and rarely around kinetoplast (Pl. I 3). In single cells, between the nucleus and kinetoplast a wavy canal is observable, showing practically no basophilia. Neither volutine nor any other granules are observable. Besides cytoplasm, pyronine stains also the flagellum. In some cases the kinetoplast shows a brown shade which disappears after RNA-se. In most cells the nucleus and kinetoplast are stained after methyl green, the nucleus less intensely than kinetoplast.

The cytochemical picture of RNA revealed in *T. evansi* is very similar to that in *T. equiperdum*. The difference consists in the more intense, more diffuse and regular staining in the cells of *T. evansi* (Pl. I 4). The highest concentration of RNA and of volutine granules is observed in the cell end opposite to kinetoplast. Nucleus stains intensely with methyl green whereas kinetoplast appears as a tiny faint point only in some cells even after the treatment with RNA-se.

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[2] The strain *T. lewisi* was supplied to us by prof. W. I. Chatchayan (Inst. of Experimental Biology in Erivan).

[3] The strain *T. evansi* was supplied to us by prof. I. W. Abramov (Inst. of Experimental Veterinary in Moscow). We express our thanks to those investigators.

General protein

In *T. lewisi* a homogeneous staining appears after bromphenol blue with a disitinct concentration of stain on both sides of the rod-shaped kinetoplast, especially on the side opposite to the nucleus (Pl. I 7, 8). The nucleus itself fails to differentiate on the background of cytoplasm whereas the kinetoplast with proteins. After hydrolysis, the intensity of reaction increases especially in the nucleus. Kinetoplast is more distinctly seen and still remains stainless, on both its sides an intense staining of cytoplasm is observable. The nucleus and the point behind the kinetoplast and sometimes the kinetoplast itself show metachromazy.

Staining with bromphenol blue is less homogenous in *T. evansi* than in *T. lewisi* (Pl. I 8). Small granules and vacuoles are revealed in the cell most often on its end opposite to kinetoplast. The stainless vacuole around the kinetoplast is seen rather distinctly. Nucleus fails to differentiate on the general background of the cell. Flagellum is faintly stained. On the place of disposition of kinetoplast a bright grain is often visible. The character of bromphenol blue staining of the *T. evansi* cells reminds the same results in *T. equiperdum.*

The general intensity and homogenity of staining increases slightly after hydrolysis. The peripheral zone of nucleus and the spot of kinetoplast become metachromatic and slightly brownish. Sometimes the flagellum is intensely stained.

Histones

In the cell of *T. lewisi*, similarly as in the case of *T. cruzi*, nucleus stains intensely with Amido black 10 B (Pl. I 5, 6). In the kinetoplast the result is less intense. In the control after hydrolysis in 0.25 N HCl, neither the nucleus nor kinetoplast are stained.

In *T. evansi* and *T. equiperdum* histone staining is similar being distinctly positive in the nucleus and very often also on the kinetoplast (Pl. I 6). In *T. evansi* histones are revealed more distinctly than in *T. equiperdum.* In *T. lewisi* the positive reaction for histones in kinetoplast evokes no doubt while in the case of *T. evansi* it cannot be considered as conclusive, being, however, more definite than in the case of *T. equiperdum.*

SH-containing proteins

In the cells of *T. lewisi* the SH-proteins appear in the form of a pale diffuse reaction of the whole cytoplasm (Pl. I 9). Nucleus either fails to differentiate on this background or is slightly paler. The rodshaped kinetoplast shows negative reaction, and the cytoplasm on its both sides stains very intensely.

The picture of staining of SH proteins in *T. evansi* reminds at in *T. equiperdum.* The whole cell is very faintly stained and no one of its structures is differentiated (Pl. I 10). Despite this similitude to *T. equiperdum*, some more details could be observed in *T. evansi.* So in some cases the kinetoplast is stainless and an accumulation of stain is observed near it.

Janus Green staining

In *T. lewisi* the kinetoplast becomes stained before all. After a few minutes it appears as a rather big greenish-blue rod (Pl. II 11). Later on several granules lying near it become stained as well as more tiny granules dispersed over the whole cytoplasm. The granule behind the kinetoplast becomes nearly always distinctly differentiated.

In *T. evansi* the kinetoplast becomes stained before all, and later on also several granules without any definite topography, however, never concentrated around the kinetoplast (Pl. II 12). In some cases the accumulation of granules is even observable at the cell end opposite to kinetoplast.

Cytochrome oxidase

The intensity of reaction for cytochrome oxidase is in *T. lewisi* very high (Pl. II 13, Pl. III 19). The number of grains of indophenol blue reaches 30–40 in one cell, the majority of them being grouped between the nucleus and kinetoplast as well as at the place of the kinetoplast position.

In *T. evansi* the cytochrome oxidase reaction — although positive — is, however, limited to 6–9 grains of indophenol blue which are scattered at random over the whole cytoplasm (Pl. II 14, Pl. III 20). One of those grains is always present at the place of kinetoplast position.

NAD-H-dehydrogenase

Its activity in the *T. lewisi* cells is manifested as 8—10 granules localized in the zone around kinetoplast (Pl. II 15, Pl. III 21). In some cases the whole kinetoplast shows the activity of this enzyme.

In *T. evansi* one or several grains appear usually at the place of kinetoplast (Pl. II 16, Pl. III 22). They are much more numerous at the cell end opposite to kinetoplast.

NADPH-dehydrogenase

Its activity in *T. lewisi* is higher than in *T. evansi*. In the first species up to 15 grains are present out of which 2–3 are observable at the position of kinetoplast and the remaining ones are dispersed at random in cytoplasm (Pl. II 17, Pl. III 23). Sometimes the kinetoplast itself seems to manifest the activity of NADPH dehydrogenase. In some cells the activity of the enzyme studied fails to appear anywhere except the kinetoplast.

In *T. evansi* the granules — as a rule — are less numerous. They are usually localized at the cell end opposite to the kinetoplast (Pl. II 18, Pl. III 24). At the site of kinetoplast the granules are not always present, however, sometimes only the kinetoplast appears active in the whole cell.

Peroxidase

In the blood froms of *T. lewisi* and *T. evansi*, as well as in the blood forms of *T. cruzi* and *T. equiperdum* the peroxidase activity failed to be revealed by the cytochemic benzidine method (Pl. III 25, 26).

Discussion

The trypanosomes studied in the present work have not been investigated regularly by cytochemical methods. The data found in literature on cytochemistry of *T. lewisi* concern mostly the nucleic acids of kinetoplast, precisely its DNA (Breslau and Scremin 1924, Roskin and Ginzburg 1944), fluorescence of kinetoplast (Wotton and Becker 1963), its chemical heterogeneity (Westphal 1960) and the concentration of mitochondria around this organelle (Wotton 1940) as well as granules connected with the antigenic properties the cell (Ormerod 1958, 1959). Ris (1960) observed the mitochondrial canal of kinetoplast by the light microscopy.

In the present study on *T. lewisi* many features common with *T. cruzi* were revealed in the cytochemical characteristic of this species. The kinetoplast of *T. lewisi* is characterized not only by considerable dimension but also by a high concentration of DNA. Besides DNA, RNA is revealed, general protein of histone type. Around it the proteins of the cell are concentrating, among them the SH-proteins and the respiratory enzymes. In this species, the mitochondrial canal could be observed, running from the kinetoplast toward the opposite cell end and being most distinct between the nucleus and kinetoplast. It is distinctly seen also in *Leishmania tropica* major (Kallinikova and Nasyrov 1972).

The informations about cytochemistry of *T. evansi* are very scarce and concern mainly the DNA of kinetoplast (Hoare 1924, Baker 1961), and the presumed content of RNA in it (Baker 1961).

It has been ascertained in the present study that the cytochemical characteristic of *T. evansi* and of its kinetoplast coincide with those of *T. equiperdum*. The kinetoplast of *T. evansi* is also characterized by small dimensions, insignificant content of DNA, while the content of the histone type proteins was more definite.

Although in the majority of cases the activity of respiratory enzymes is manifested at the site of kinetoplast position, they never concentrate around it, similarly as the cell proteins.

In this way, the study of those two species of trypanosomes supports the results of cytochemical investigation of *T. cruzi* and *T. equiperdum* (Wartoń and Kallinikova 1971, Kallinikova and Wartoń 1972). Distinct cytochemical differences are also observed between *T. evansi* and *T. lewisi*. They mostly concern kinetoplast and indicate its different role in the metabolism of both species: its important role in metabolism of *T. lewisi* and its much less essential one in *T. evansi*. Consequently the cytochemical differences between *T. cruzi* and *T. equiperdum* — concern not

only the species but even group of species and speak in favour of a distinct role of kinetoplast in the metabolism of trypanosomes of groups *lewisi* and *brucei*.

The four above mentioned species of trypanosomes, distinctly differ by their cytochemical characteristics connected with their belonging to the *lewisi* or *brucei* groups. A similar cytochemical character show the species *T. cruzi* and *T. lewisi*, belonging to the *lewisi* group. They sharply differ from the species *T. equiperdum* and *T. evansi* which belong to the group *brucei*.

These groups differences are very essential and concern mostly the kinetoplast of trypanosomes. Both species of the group *lewisi*, not capable for true diskinetoplasty, are characterized by a big kinetoplast. Inside of it or also around it there accumulate important biochemical components of the cell: DNA, RNA, histones, SH-proteins and respiratory enzymes. The reaction for DNA is very intense in the kinetoplast of both species. The SH-groups reaction is very distinct in both species, this concerns before all the zone around the kinetoplast.

On the other hand, the species *T. equiperdum* and *T. evansi* of the *brucei* group differ from the former pair of species group *lewisi* by the lack of concentration of biochemical components around the kinetoplast and — possibly — by a less complex composition of this structure. In *T. equiperdum* as well as in *T. evansi* the reaction for DNA in kinetoplast being more intense than in the nucleus of those species, is, however, much weaker than in the kinetoplast of *T. cruzi* and *T. lewisi*.

The essential difference in the species studied, concerning the kinetoplast, is expressed by a different topographic relation of the most important biochemical components of the cell to this organelle. The concentration of those components around the kinetoplast is evidently not the common feature of all trypanosomes, being characteristic for the group *lewisi* only. This indicates the important role of kinetoplast in the metabolism of this species group. This explains also why the impairment of kinetoplast becomes in this case destructive for the cell.

Nevertheless the cytochemistry of trypanosomes of the group *brucei* capable of diskinetoplasty, indicates an insignificant share of kinetoplast in the metabolism of those cells. The more important biochemical components of the cell do not concentrate around the kinetoplast, a part of them distinctly gravitates toward the opposite cell end, the DNA content is lowered in kinetoplast and its chemical composition is evidently less complex. The loss of important role of kinetoplast in the metabolism of the group *brucei*, involves essential differences of cytochrome oxidase activity in both groups. The species of the *brucei* group show much lower cytochrome oxidase activity which is in conformity with their belonging to the group of non-cytochrome trypanosomes, suggesting, however, that they have lost their cytochrome system not entirely. The loss of the leading metabolic role of kinetoplast concerns not only the respiratory activity but also the protein-nucleic turnover and metabolism in general. This explains why the species of *brucei* group may endure so easily an impairment of kinetoplast.

In this way the cytochemical characteristic of the trypanosome cell as a whole, and of kinetoplast itself is actually tightly connected with the capacity for diskinetoplasty of the species and explains the different degree of the last one in different trypanosomes.

The study of four species permitted to reveal the common features of the cytochemical characteristic of the trypanosome cell independently of their systematic position. A higher DNA concentration in kinetoplast than in the nucleus of the same cell, as well as the absence of peroxidase activity were characteristic for all four species.

To the group differences should be also attributed the different activity of cytochrome oxidase and an uneven content of the SH-proteins. Both are less conspicuous in the *brucei* group. Histones or rather the alkaline proteins, were reliably revealed by us only in the kinetoplast of *T. cruzi* and *T. lewisi*. This permits to state the presence of those proteins in the kinetoplast of the group *lewisi*.

Species difference of cytochemical character were also observed within each of the two groups. This concerned mostly the species of the group *lewisi*, and less those of the group *brucei*. Those differences concerned the details and were not of an essential character. It may be concluded that the cytochemical differences of trypanosomes are before all of a species group character.

Summary

Comparative cytochemical study of two trypanosome species (*Trypanosoma lewisi* and *T. evansi*) belonging to different groups was carried out. The object of study were: DNA, RNA, general proteins, histones, SH-proteins, activity of cytochrome oxidase NADH and NADPH-dehydrogenases, stainability with Janus Green and peroxidase activity. A conclusion has been put forward on the important role of kinetoplast in the metabolism of *T. lewisi*, and its insignificant share in the metabolism of *T. evansi*. The results obtained permit to explain the different capacity of diskinetoplasty in the species of the groups *lewisi* and *brucei*.

РЕЗЮМЕ

Работа посвящена цитохимическому изучению двух видов трипаносом (*Trypanosoma lewisi* и *T. evansi*), принадлежащих к разным группам. Изучались ДНК, РНК, общий белок, гистоны, SH-содержащие белки, активность цитохромоксидазы, НАД-Н- и НАД-Ф-Н-дегидрогеназ, окрашиваемость янусом зеленым и пероксидазная активность. Рассматривалась клетка в целом, но особое внимание было уделено кинетопласту изучаемых видов. Делается вывод о важной роли кинетопласта в метаболизме *T. lewisi* и его незначительном участии в обмене *T. evansi*. Полученные данные позволяют понять разную способность к дискинетопластии видов группы *lewisi* и *brucei*.

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EXPLANATION OF PLATES I–III

1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 — Cytochemical reactions on *Trypanosoma lewisi*
2, 4, 6, 8, 10, 12, 14, 16, 18 — Cytochemical reactions on *Trypanosoma evansi*
1, 2 — Feulgen reaction for DNA
3, 4 — Staining of RNA after Brachet
5, 6 — Histone reaction
7, 8 — Reaction for general proteins, a, b — before hydrolysis, c — after hydrolysis
9, 10 — Reaction for SH-proteins
11, 12 — Janus Greeen staining
13, 14, 19, 20 — Cytochrome oxidase reaction
15, 16, 21, 22 — NADH-dehydrogenase reaction
17, 18, 23, 24 — NADPH-dehydrogenase reaction
25, 26 — Peroxidase reaction

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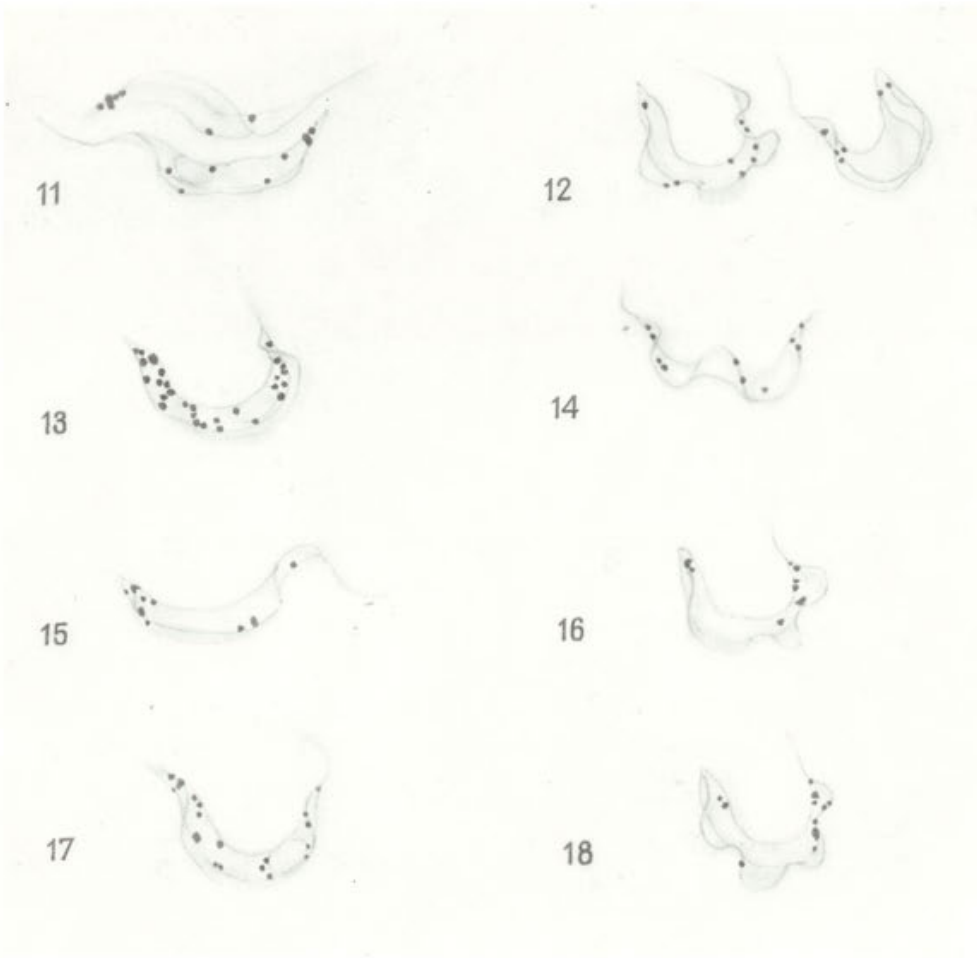

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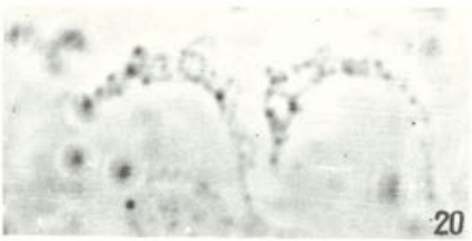

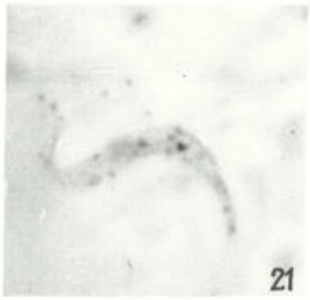

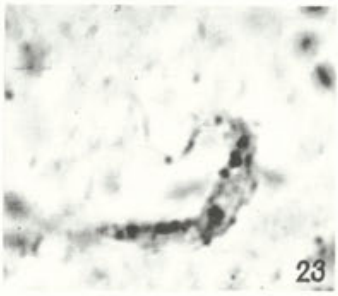

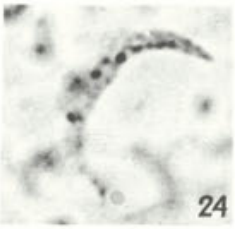

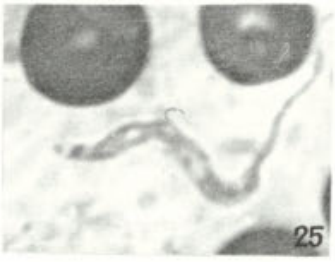

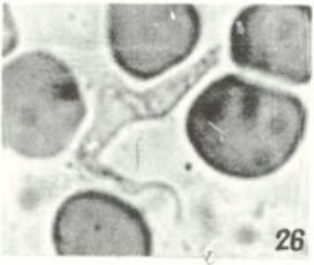

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THE JOURNAL OF PROTOZOOLOGY

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PROTOZOOLOGY

A Volume in the CELL BIOLOGY series
Karl G. GRELL, Universität Tübingen
437 figs. 15 tables. VIII, 554 pages, 1973.
Cloth DM 107,—; US $ 43.90
Berlin–Hidelberg–New York: Springer-Verlag
ISBN 3-540-06239-4

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(PWN — Polish Scientific Publishers)
Warszawa

Warszawska Drukarnia Naukowa, Warszawa, ul. Śniadeckich 8. Zam. 400/73.
Wklejki światłodrukowe wykonały Zakłady Graficzne „Ruch", Warszawa, ul. Ludna 4

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