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Electron microscopic study of microgametogenesis in two species of coccidia from rabbit *(Eimeria magna* and *E. intestinalis)*

Электронномикроскопическое исследование микрогаметогенеза у двух кокцидий кролика (*Eimeria magna* и *E. intestinalis*)

The fine structure of different stages of development in the genus *Eimeria* had been recently studied (Holz 1954, Cheissin i Mosevitch 1961, Cheissin 1964, Scholtyseck 1962 and 1963, Scholtyseck und Weissenfels 1956, Scholtyseck und Schäfer, Scholtyseck und Voigt 1964, Scholtyseck und Spiecker 1964).

Results of these studies revealed some peculiar ultrastructures of merozoits and microgametes in *E. intestinalis* and *E. perforans*. Nevertheless, the present data on this subject are not exhaustive. The number of coccidia representatives examined by the electron microscope is too restricted to allow general conclusions. Even in *E. perforans* and *E. intestinalis* far not all the stages have been examined completely. Electron microscope provides the possibility to reveal such essential structural features of merozoits, gametes and oocysts which failed to be detected in the light microscope. Besides, a problem arises as to the course of morphogenesis of various structures in the complex development cycle of coccidia. How occurs e.g. the formation of the peculiar conoid, the "paired organelle" or the subpellicular fibrils in merozoits, whereas the schizont — which gives origin to merozoits — is deprived of corresponding structures? A similar question arises as to formation of flagella in microgametes which are formed from microgametocyte with no flagella. On the other hand, during transition from one stage to another, some structures should evidently disappear or become transformed into some others. It is not clear e.g. what is the fate of the structures of merozoit after its penetration into the epithelial cell and its transformation into a gamont.

In the present study only the morphogenesis of microgametes was followed, paying most attention to formation of the basal corpuscles and of flagella. No one development stage of coccidia — except the microgametes — posesses flagella. Consequently a question arises whether the flagella with their basal corpuscles are formed in microgametes from some pre-existing homologous structures present in all preceding development stages (e.g. from centrioles), or is the whole kinetic apparatus of the microgamete arising de novo from some ultrastructures of the microgametocyte existing permanently or temporarily in the cell.

Material and methods

For the study of this problem, two species of coccidia from the rabbit intestine: *Eimeria intestinalis* and *E. magna* were chosen.

Several rabbits, 12—16 days of age were infected with a considerable amount of oocysts of *E. intestinalis* or *E. magna*. In the first case rabbits were dissected on the 8th—9th day (Cheissin 1948) of the pre-patent period, in the second case dissection was made on the 6th day (Cheissin 1947). Samples of the ileum were fixed in OsO_4 1% after the method of Sjöstrand 1956. Material was embedded in Araldit M and the ultrathin sections were examined in the electron microscope JEM 5g.

For control of the electron microscope study sections were stained after Feulgen, preceded by hydrolysis in HCl, or with iron hematoxylin after Heidenhein, and observed under oil immersion (90 ×) in light microscope.

Results

Examination in the light microscope

Microgametogenesis of the species studied occurs as follows. A young gametocyte, formed from the merozoit, is of ovoid shape, its dimensions are not exceeding 5—6 μ. It localizes in the epithelial cell over its nucleus. The subsequent development of *E. intestinalis* occurs in the epithelium whereas the microgametocytes of *E. magna* penetrate into tunica propria (Cheissin 1948). The process of microgametogenesis may be divided into two phases.

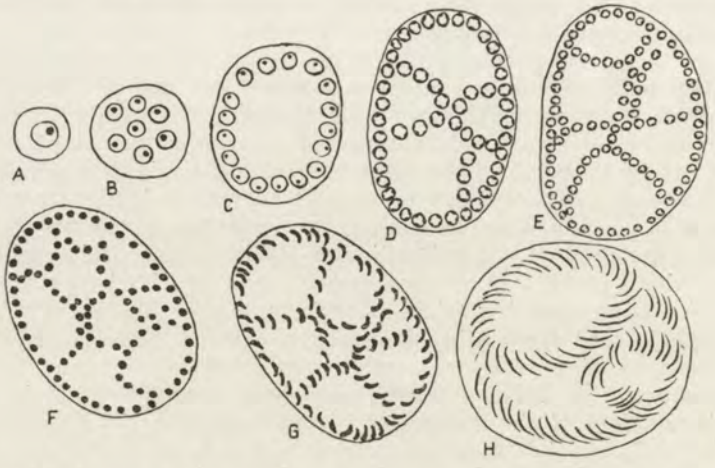

Fig. 1. Scheme of microgametogenesis in *Eimeria magna*. A—E. The first period of gametogenesis. F—H. The second period of gametogenesis

1. Period of growth of gametocyte and multiplication of its nuclei (Fig. 1). At this time an intense synthesis of protein occurs and the dimensions of gametocyte increase. It retains its ovoid form. The size of such gametocytes in *E. intestinalis* is 20—27 × 18—20 μ, and in *E. magna* 25—40 × 20—30 μ.

Microgametocytes of the last species consist of several cytomeres i.e. of cytoplasm regions with numerous nuclei on their periphery. In *E. intestinalis*, usually only one similar cytoplasmic region is found. Rarely occur individuals with two or three cytomers. At an early stage of growth, the nuclei are dispersed at random in the central part of gametocyte. In both species, the diameter of individuals with about 16—24 nuclei is 10—12 μ. After the fourth or fifth division, nuclei become located on the periphery of the gametocyte. Here they continue dividing. Simultaneously the growth of the gametocyte occurs. The size of nuclei decreases with their divisions. So e.g. in *E. magna* at the early stages of its gametogenesis the nucleus diameter was 3 μ, whereas in the large gametocyte with approx. 150—200 nuclei, it decreases down to 1.7 μ. Nuclei have a shape of vesicles with a peripheral disposition of chromatin and a central nucleolus, which is visible distinctly at the early stage of microgametogenesis and disappears towards the conclusion of growth period when the nuclear chromatin produces considerable accumulations under the nuclear membrane.

2. Differentiation period of microgametes. At that time the nuclear divisions cease, their volume slightly decreases and chromatin spreads all over their mass occupying approximately two thirds of their volume. Subsequently the nucleus assumes the feature of a compact body which since that time becomes similar to a short comma. Such a nucleus elongates gradually so as to form a long comma-shaped microgamete. All the microgametes arise on the surface of the gametocyte. The major part of the microgametocyte cytoplasm is not consumed for formation the gametes and is wasted away as a residual body. It comprises a considerable amount of glycogen and of RNA (C h e i s s i n 1958, 1961). The length of gametes is 3—4 μ, their width — 0.5 μ, the length of each of their flagella — 5—7 μ. Each microgametocyte of *E. magna* produces about 750—1000 microgametes, and in *E. intestinalis* 250—500 of them.

Examination in the electron microscope

The cytoplasm of binucleated microgametocytes contains a great number of small mitochondria with short internal tubules. The few membranes of the endoplasmic reticulum are seen in different regions of cytoplasm (Pl. I 1). In every nucleus, a big osmiophilic agglomeration is seen, possibly corresponding to the nucleolus (Pl. I 1). Separate dense granules are scattered all over the nucleus. Among them, still smaller and less dense granules are seen corresponding to the cytoplasm ribosomes by their dimensions.

The examination of numerous sections failed to reveal the presence of any structures reminding centrioles or basal bodies of flagella.

Microgametocytes assume subsequently a peripheral position of nuclei. Plates II and III show different stages of growth of microgametocytes with numerous nuclei of a characteristic structure. At the earlier development stages, concentration of osmiophilic granules under the nuclear membrane is seen (Pl. II 2 and III 3). Separate osmiophilic agglomerations are also seen in the centre of nucleus. At more advanced stages, a certain decrease of the nucleus volume and a relative increase of the peripheral dense osmiophilic granulation is observed (Pl. V 8). The study in the light microscope reveals distinctly that the Feulgen-positive chromatin is located as a rule — on the periphery of the nucleus. Consequently it may be considered as evident that the peripheral dense granules producing the agglomerations in the nucleus

and seen in electron photomicrograms — correspond to chromatin. Besides, in the subsequent differentiation of the gamete, the dense accumulations concentrate in its nucleus which is always Feulgen-positive.

In the cytoplasm of multinucleated microgametocytes, numerous mitochondria are seen which — as a rule — occupy a peripheral position (Pl. II 2 and IV 7). In the central part of the gametocyte they usually fail to appear. Mitochondria are usually located near the nucleus (Pl. IV 7 and V 8).

Ribosomes are scattered at random in the gametocyte cytoplasm or are concentrated upon the membranes of the endoplasmic reticulum (Pl. IV 7 and V 8). In some areas of the gametocyte considerable accumulations of rough membranes of the endoplasmic reticulum may be observed. Their disposition is concentric (Pl. IV 7). Observations in the light microscope show that microgametocytes are rich in RNA (Cheissin 1958, 1961). Besides the ribosomes, more voluminous granules occur in cytoplasm; they possibly contain glycogen (Pl. VI 13 and X 22).

In microgametocytes of a diameter 12—16 μ, with nuclei in peripheral position, the anlage of basal bodies of future flagella of microgametes may be observed on many sections (Pl. II 2 and III 3—6). They are always located next to the nucleus under the surface membrane. They are short tubular fibrils about 900 Å long, with the diameter about 200 Å, oriented vertically to the surface membrane of the gametocyte (Pl. III 3—6). In cross sections, 9 tubular fibrils in concentric disposal may be observed (Pl. III 4). This is the earliest stage of formation of basal bodies found in ultrathin sections of multinucleated microgametocytes. Subsequently, small buds or protuberances of a homogenous structure, coated by a dense membrane appear. Those are anlage of flagella (Pl. VI 9—10). In the short buds the tubular fibrils are not observable, but they are present in the long ones (Pl. V 8 and VI 9, 11—13). The fibrillar elements of the flagellar anlage are a continuation of the basal bodies fibrils. In the longer flagellar anlage besides the paired peripheral fibrils, two central ones may be observed. They arise later than the paired ones (Pl. VII, 14—16 and IX 18). Consequently, the formation of flagella occurs in the first phase of the gametocyte formation. They become disposed on the surface of gametocyte prior to the formation of gametes. The process of formation of flagella proceedes not quite synchronously since some long flagellar anlage may be observed simultaneously with the most early stages of their formation (Pl. VII 14—16, and VIII 17). The fine structure of fully formed flagella fails to differ from that in various representatives of flagellates.

In the first period of gametogenesis, following changes occur in the gametocyte.

In the nucleus, a concentration of a dense osmiophilic granular structure — representing chromatin — is observed in the area oriented towards the surface of the gamont (Pl. VI 13 and VII 15—16). Since the nuclear volume is diminishing at that time — when compared to an earlier stage — whereas the amount of chromatin remains unchanged, the nucleus is to be filled with chromatin (Pl. VIII 17). Next to every nucleus, a big mitochondrion appears (Pl. VII 16 and IX 18—19) (Cheissin 1964). It may be presumed that this big mitochondrion arises as result of fusion of several small ones which were numerous at earlier stages of development. Such a big mitochondrion reminds the "Nebenkern" of spermatide in many insects (Wilson 1925).

Numerous folds are formed on the surface of the microgametocyte. They are especially well marked in *E. magna* (Pl. VII 14—16 and VIII 17).

The beginning of the microgametes formation is connected with appearence of big protuberances on the surface of microgametocyte. They include the nucleus, mitochondrion and anlage of flagella (Pl. VI 13, VII 15—16 and IX 18—19). Later on, the nucleus coated by the cytoplasmic membrane of the gametocyte, begins to elongate. Its proximal segment, devoid of chromatin, remains connected with the cytoplasm of gametocyte (Pl. IX 20—21). The growth of microgamete occurs at expense of elongation of the distal segment of nucleus (Pl. IX 20—21). Simultaneously the flagella continue growing, retaining their connection with the cytoplasm of gametocyte. Till the last moment of separation from the residual body of gametocyte, gametes remain associated with it by their anterior ends. Their separation from the gametocyte cytoplasm occurs only then when the flagella have attained their maximal size and the perforator has been formed (Pl. X 22). Flagella of the gametes which are not yet detached from the residual body of the gametocyte, closely adhere to their surface. In the free moving gametes the distal parts of flagella are not stick to the body of the gamete.

Discussion

The electron microscopic examination of the successive stages of the microgametes development in *E. magna* and *E. intestinalis* revealed that in the growth period of the macrogametocyte and of multiplication of its nuclei, formation of the kinetic apparatus of future microgametes initiates as well. This process runs in two phases: 1. the phase of synthesis which is characterized by the formation of fibrillar components of the base of flagella, i.e. of their basal bodies 2. the phase of organization when formation of flagellum itself occurs (Schuster 1963). In the growing microgametocyte, not only the first but also the second phase of formation of the kinetic apparatus of the microgametes is accomplished. In the period of differentiation and of growth of the microgametes all their structural elements which have arisen in the microgametocyte, coordinate and assume their final position.

Nevertheless, a question arises whether the fibrillar components of the kinetic apparatus of the microgamete arise really de novo or they have some structural predecessors in the preceding stages.

In coccidia only the microgametes have flagella. No other motile stage of development is endowed with a kinetic apparatus. Besides, in the merozoits and sporozoits not only the flagella are absent but they fail to have basal bodies and their homologues — centrioles. It should be mentioned that the elements of the kinetic apparatus were not found not only by means of the light microscope investigation but even in the electron microscopic study. It seems possible that the investigators payed no attention to the study of fibrillar elements in merozoits and sporozoits. Those elements have characteristic structure and position and might be easily overlooked. Nevertheless, such structures as centrioles — homologues of basal bodies — should have been noticed by the authors (Cheissin and Mosevitch 1961, Scholtyseck and oth. 1956, 1962, 1963, 1964). Those structures were not found after a detailed analysis of merozoits and sporozoits of the malaria parasite and in *Lankesterella* (Garnham and oth. 1960, 1962). Evidently centrioles

and basal bodies are absent at those stages of development cycle in coccidia which precedes the formation of the microgametocyte. As follows from the figures of the Plate III, those structures become discernable only then when the microgametocyte reaches the size 12—15 μ and its nuclei occupy a peripheral position.

Surely, it is not easy to provide convincing evidences of absence of the kinetic apparatus elements in all the development stages which precede the microgametocyte. The opponent may always keep some doubt as to the accuracy of investigation. However in the ultrathin sections of multinucleated microgametocytes studied in the electron microscope, different stages of formation of the basal bodies are fairly often found. In contrast to this, in the early stages of microgametogenesis or in merozoits the authors failed to reveal any structure which could be recognized as a basal bodies or centriole, although quite a number of microphotograms were examined in order to find such structures. Results of such investigation may be accounted for by the absence of the kinetic apparatus elements at those stages of development.

Consequently, it may be assumed that the fibrillar elements of basal bodies of flagella in the microgametes of coccidia have no submicroscopic predecessors at early stages of development and their synthesis is accomplished de novo in the microgametocyte. This conclusion is in full agreement with the observations of Schuster 1963 who studied the formation of flagella in *Naegleria gruberi* during the metamorphosis of the amoeboid form — dominating in the development cycle of this amoeba — into the flagellate stage which is temporary and short-lasting. Schuster failed to reveal in the amoeboid form any kinetic centre (or centriole) which might give origin to basal bodies or fibrillar structures adhering to them, in the following stage of development. Formation of the kinetic apparatus is associated with the membranes of the endoplasmic reticulum of the amoeboid stage.

It may be postulated that in some cases formation of centrioles in the cells occurs de novo as well. This is suggested by the data about formation of cytasters in the activated but not fertilized eggs of sea-urchin. Cytasters may be induced in the enucleated eggs as well, which speaks in favour of their formation de novo. Dirksen 1961 showed in electron microscopic study that in every cytaster of the activated parthenogenetic eggs of sea-urchin a typical centriole exists which arises from the pre-existing membranous structures which are dispersed in cytoplasm, i.e. it arises de novo. This problem however cannot be considered as definitely solved: according the opinion of Mazia 1961, the old view that eggs contain no centrioles should be reconsidered. At the same time Mazia calls attention to the fact that the reproduction of the new centriole by fission of the old one can hardly be assumed. In his opinion the "generative mechanism" of its formation is rather acceptable: the old centriole gives origin to the anlage which determines the subsequent growth up to formation of the mature copy of the original. Formation of the anlage may be considered as a mechanism of reduplication on the molecular level (Mazia 1961).

Many investigators consider that the basal bodies of cilia and flagella, as well as the centrioles of the animal and plant cells do not arise de novo but are formed as result of differentiation of a primarily existing centriole. This is especially clearly shown on the development of the spermatozoon tail piece.

Its basal bodies develop always from one of the centrioles which are present not only in the spermatides but even at earlier stages — in spermatogonia (Fawcett 1961, Gall 1961, Sleigh 1962). The formation of basal bodies of cilia from pre-existing centrioles in the epithelial cells of chick embrio occurs in a similar manner (Sotelo and Trujillo-Cenóz 1958). Renaud and Swift 1964 studied in electron microscope the process of formation of the flagellar basal bodies in the gametes of fungus *Allomyces arbusculus*. The basal bodies proved to arise from the centriole pre-existing in the hyphae. The centriole is located at the end of the hypha, in close association with the nucleus. Subsequently, in the development of the gametangium another centriole arises. One of them increases in size, and 9 pairs of fibrils become distinctly observable in its wall. They represent the anlage of the basal bodies of the gamete flagellum. Those data however do not exclude the possibility of formation de novo of the fibrillar elements of the centriole (basal bodies), as it is observed in some representatives of *Protozoa*. This seems probable, the more so as even in ciliates in which the transmission of basal bodies was recognized (Lwoff 1960), the finding of Randall and Hopkins 1963 revealed that formation of the basal corpuscles occurs not by fission of the old one, but by synthesis of material which is indispensable for development of the new basal bodies. The course of this process is at present time unknown. At any rate the findings of Randall and Hopkins 1963, as well as the study of Ehret and Haller 1963 on the development of the basal bodies in *Paramecium*, fail to support the former view of Lwoff 1950 about the formation of new basal bodies by the fission of the old ones. Randall and Hopkins 1963 elucidated — by following the regeneration in *Stentor* — that the new basal bodies is primarily formed as "thick walled quasi-spherical object". Subsequently vesicles appear in this rudiment and the fibrills are formed; finally a cylindrical basal corpuscle arises. Its synthesis is presumably associated with the reduplication of the globular protein molecules in a definite place below the pellicle. According to Randall and Hopkins this process occurs under the control of a morphogenetic code. This problem is not quite clear presently but — no doubt — formation of the new basal bodies as observed in the microgametocytes occurs de novo in the direct proximity of the nucleus.

The problem of the origin of kinetic apparatus in different protozoa needs fundamental revision. It should be finally decided whether the basal bodies in protozoa (in ciliates and flagellates) are self-reproducing organelles, or else they arise de novo, always or in some cases, from the cytoplasm ultrastructural components, under the control of the genetic information.

The second, organization phase of the development of flagellum initiates possibly as result of induction of the just formed fibrillar component of the basal bodies upon the membrane of the microgametocyte. A bud or a protuberance appears over the rudiment of basal bodies. It subsequently elongates and includes the newly formed fibrils. This process reminds somewhat the formation of flagella in *Allomyces*. Nevertheless, in this species the primary flagellar bud is formed around the rudiment of flagellum which grows into the bud (Renaud and Swift 1964). This difference is involved by the fact that in *Allomyces* the flagellum is formed inside the gametangium and in coccidia on the surface of the microgametocyte.

The process of microgametes formation in coccidia is characterized by the fact that the gametes are connected with the cytoplasm of the gametocyte for a prolonged time. This may be accounted for by the necessity of providing a considerable amount of structural and energetic material for producing the long flagellum of the gamete. Possibly the protein synthesis is secured by a considerable amount of RNA in the cytoplasm of gametocyte while the energetic demand is fulfilled by glycogene stored in the gametocyte. The transformation of energy is effected by the big mitochondrion which is present in the anterior part of the microgamete.

Summary

Different stages of microgametogenesis in *Eimeria intestinalis* and *E. magna* have been investigated by electron microscopy. In the first period of gametogenesis growth of the gametocyte and multiplication of its nuclei occurs. When the nuclei of gametocyte shift from their central position to the periphery, formation of the basal bodies anlagen of flagella of the microgametes is observed. They arise near the nucleus under the membrane of gametocyte in the form of nine short tubules, 200 Å in diameter, oriented vertically to the surface of the gametocyte. Somewhat later on the surface of gametocyte, protuberances and buds appear; they represent the rudiments of flagella. Tubular fibrils penetrate from the basal corpuscles into their inside. Towards the conclusion of the first growth period of the gametocyte, on its surface a multitude of short flagella are present, with typical 9 + 2 pattern. Fibrillar elements of the basal corpuscle arise de novo, and later on, in the organization phase flagellum itself is formed.

In the second period of gametogenesis, the nucleus elongates. It is coated with a thin cytoplasm layer and membrane of gametocyte. Displacement of the big mitochondrion (Nebenkern) into the proximal part of the gamete is seen. Growing flagella adhere to the body of gamete. The latter separates from the cytoplasm of the gametocyte (residual body) only after its flagella had reached their maximal length.

РЕЗЮМЕ

С электронным микроскопом были исследованы разные стадии микрогаметогенеза у *Eimeria intestinalis* и *E. magna*. В первый период гаметогенеза происходит рост гаметоцита и размножение его ядер. В то время когда ядра гаметоцита из центрального положения перемещаются к его периферии, наблюдается образование зачатков базальных телец жгутиков микрогамет. Они возникают рядом с ядром под мембраной гаметоцита в виде девяти коротких трубочек диаметром 200 Å, ориентированных перпендикулярно к поверхности гаметоцита. Несколько позже на поверхности гаметоцита появляются бугорки, или почки, представляющие собой зачатки жгутов. В дальнейшем происходит рост этих зачатков и проникновение в них трубчатых фибрилл из базальных телец. К концу первого периода роста гаметоцита на его поверхности имеется множество коротких жгутов обладающих 9 парными периферическими фибриллями и двумя центральными. Процесс формирования жгутиков и их базальных телец разделяется на две фазы: синтетическую, когда происходит образование фибриллярных элементов базального тельца de novo и организационную — когда происходит формирование самого жгутика.

Во второй период гаметогенеза происходит дифференцировка микрогамет путем сильного вытягивания ядра, покрытого тонким слоем цитоплазмы и мембраной гаметоцита, над его поверхностью, перемещения в проксимальный отдел гаметы крупной митохондрии (Nebenkern) и роста жгутов, соединенных с телом растущей гаметы. Она отделяется от цитоплазмы гаметоцита (остаточное тело) только после достижения максимальной длины её жгутов. Их рост осуществляется за счет синтеза белков и других материалов, находящихся в цитоплазме гаметоцита.

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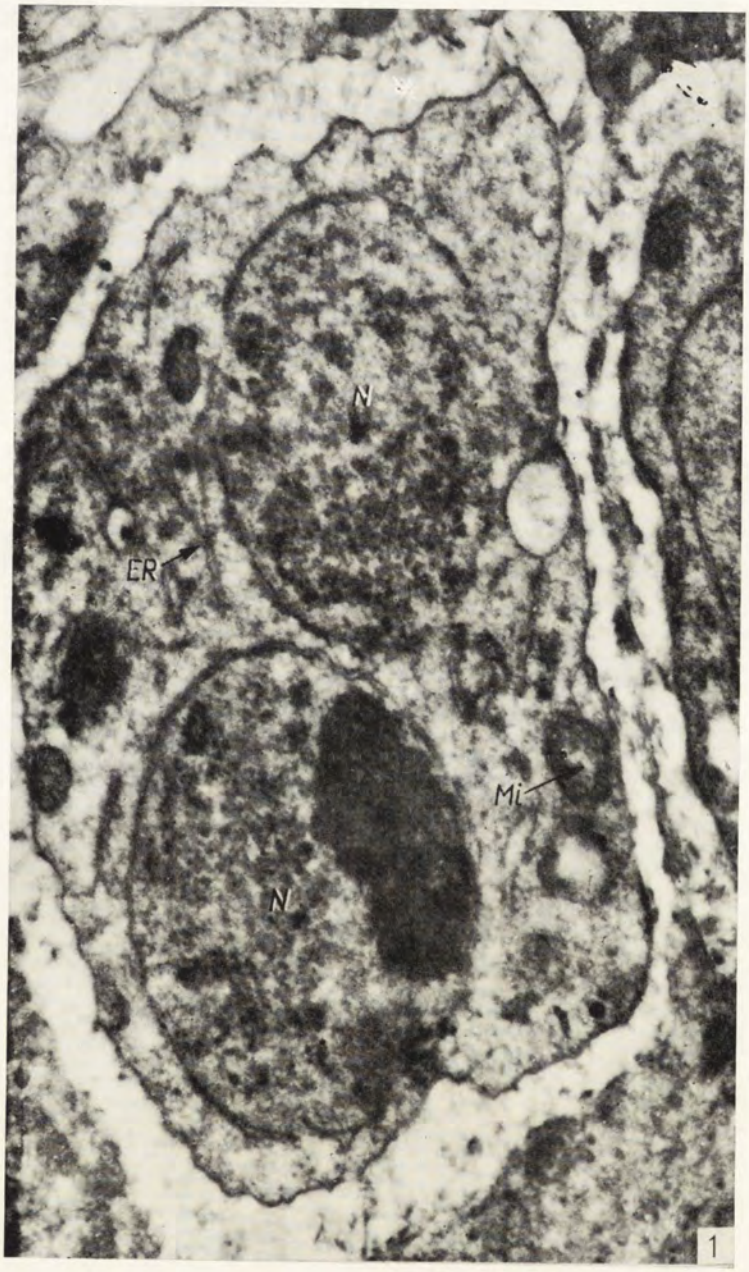
EXPLANATION OF PLATES I—X

1: Binucleated microgametocyte of *E. instestinalis* (×30 000)
2: Multinucleated microgametocyte of *E. magna* with a peripheric disposition of nuclei. Anlage of basal bodies are seen (× 15 000)
3: Fragment of a multinucleated microgametocyte of *E. intestinalis*. Anlage of basal bodies are seen (× 20 000)
4: Anlage of the basal bodies in cross section in a multinucleated microgametocyte of *E. magna* (× 45 000)
5—6: Fragments of multinucleated microgametocytes with nuclei and anlage of basal bodies in longitudinal section (× 45 000)
7: Multinucleated microgametocyte of *E. magna*. Section area near the surface. Accumulation of rough membranes of the endoplasmic reticulum (×15 000)
8: Fragment of a superficial section of a multinucleated microgametocyte at a more advanced stage of development than in the preceding photomicrogram, with anlage of flagella (× 35 000)
9—10: Anlage of flagella in cross and longitudinal sections on the surface of the gametocyte of *E. magna* (×35 000)
11—12: Longitudinal sections of flagellar anlage at more advanced stages than in the preceding photomicrograms (approx. ×60 000)
13: Formation of microgametes at the beginning of the second period of gametogenesis. Flagellar anlage are shown (× 55 000)
14: Anlage of flagella in longitudinal and cross sections at the conclusion of the first period of gametogenesis in *E. magna* (×75 000)
15—16: Formation of microgametes on the surface of microgametocyte at the second period of gametogenesis. Sections at different planes of the developing microgamete (×60 000)
17: General view of a fragment of microgametocyte of *E. magna* at the onset of the second period of gametogenesis (×35 000)
18—21: Various stages of microgametes formation in *E. magna* in longitudinal and cross sections. 18 and 19 are the earliest stages, 20 and 21 are subsequent stages of development (×60 000)
22: Fully formed microgametes of *E. magna* not yet detached from the residual body (×40 000)
[EF — fibrillar elements of the basal bodies or of flagella, FR — endoplasmic reticulum, F — anlage of flagellum, M — anlage of microgamete, Mi — mitochondria, N — nucleus, P — perforatory, RB — residual body of the gametocyte; arrows indicate the basal bodies anlage (micrograms 2—6) or flagellar anlage (micrograms 9—14)]



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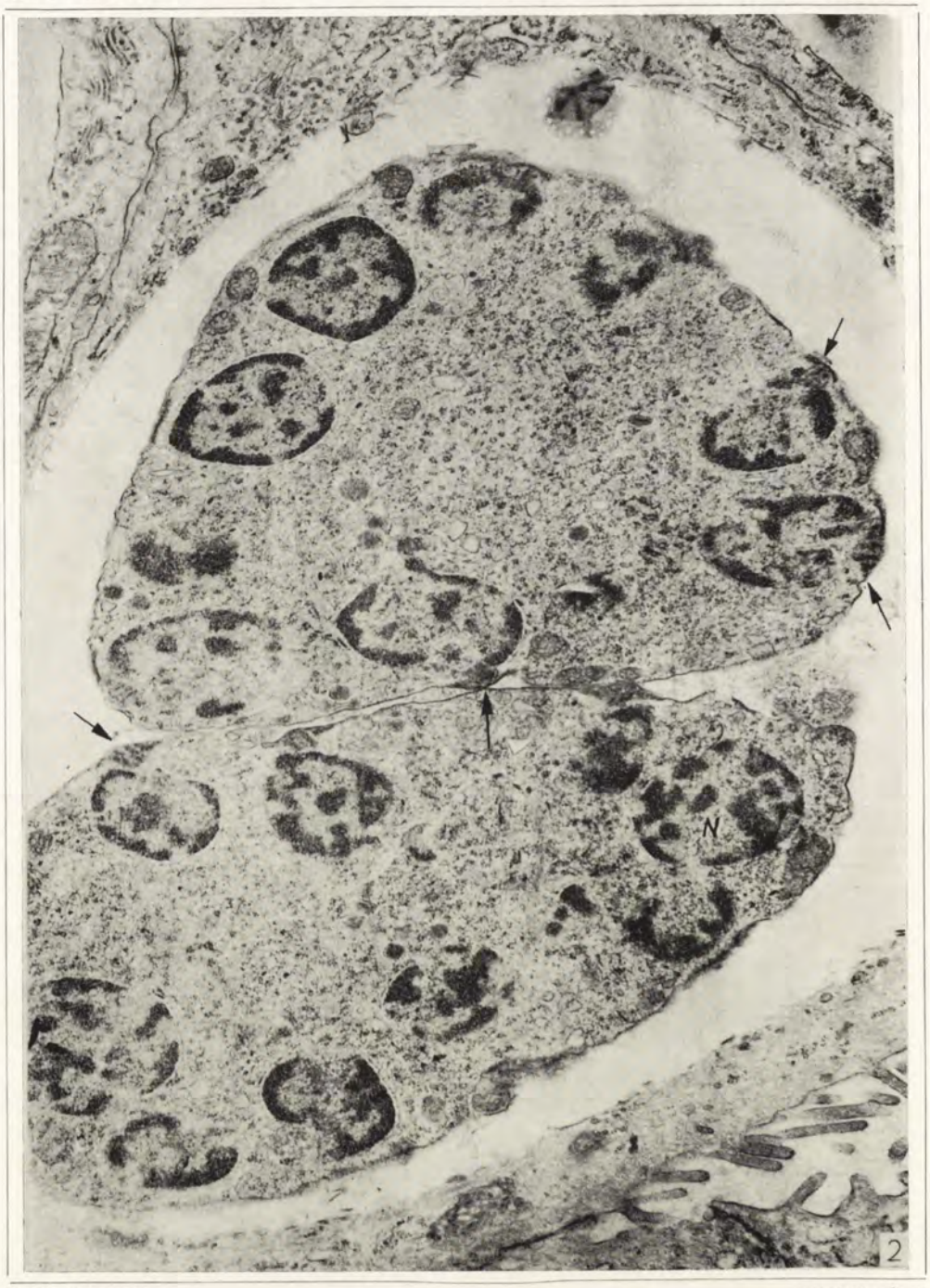

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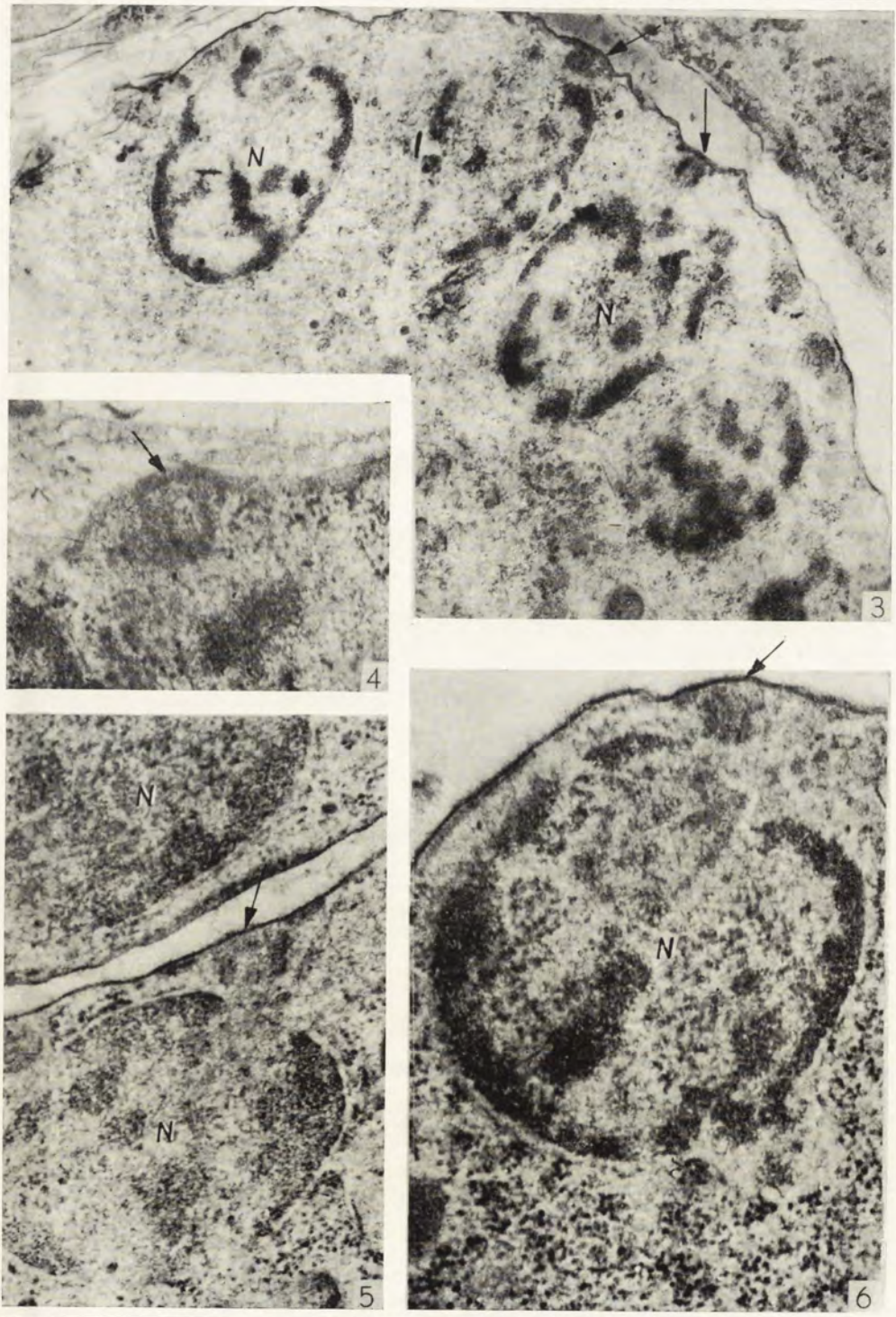

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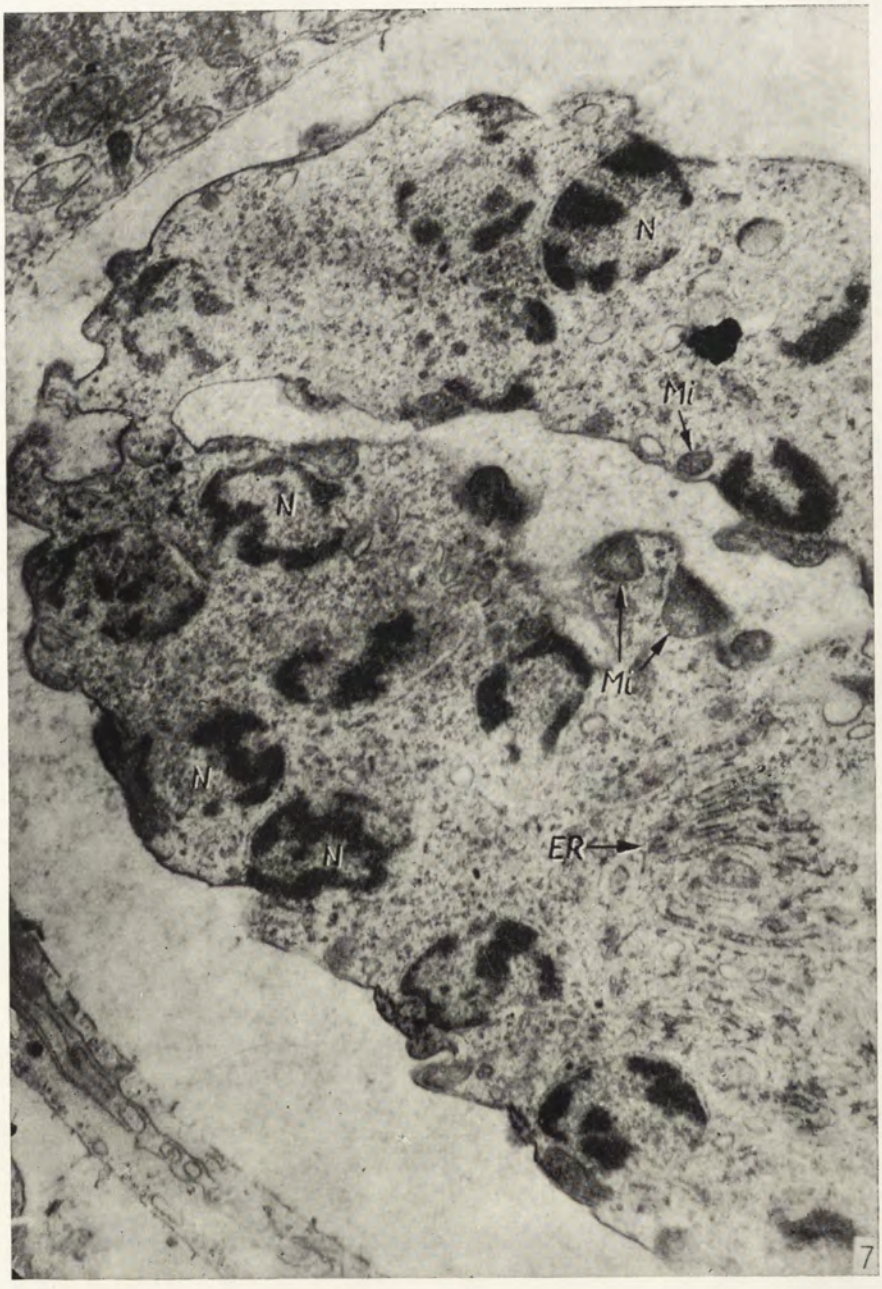

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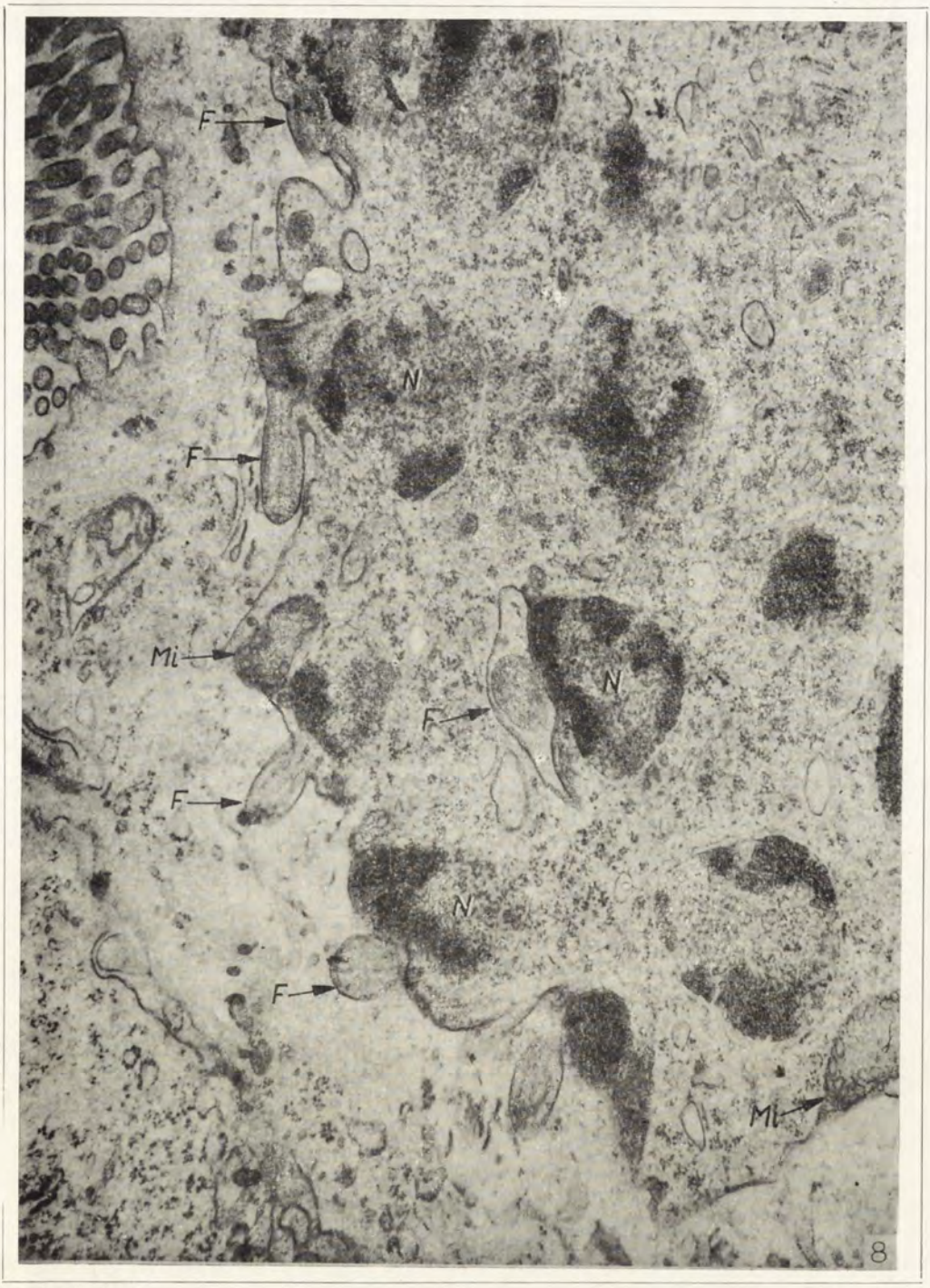

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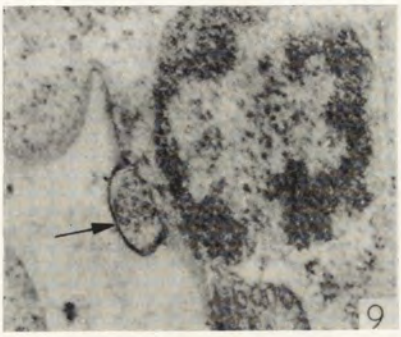

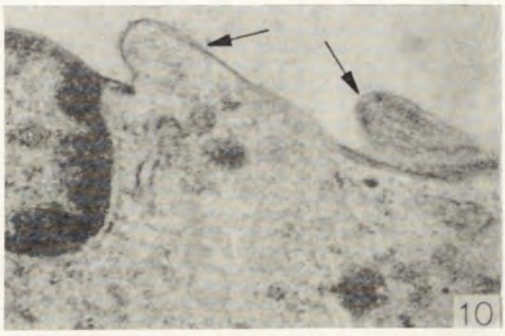

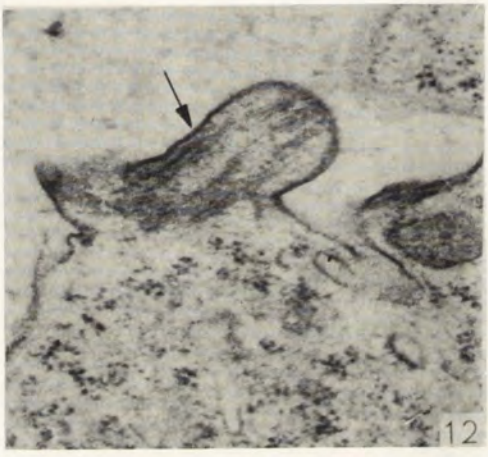

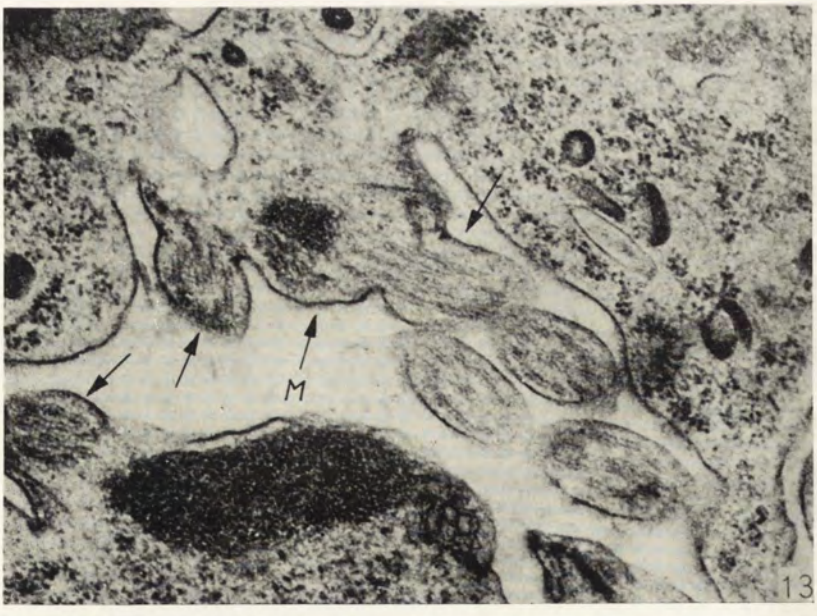

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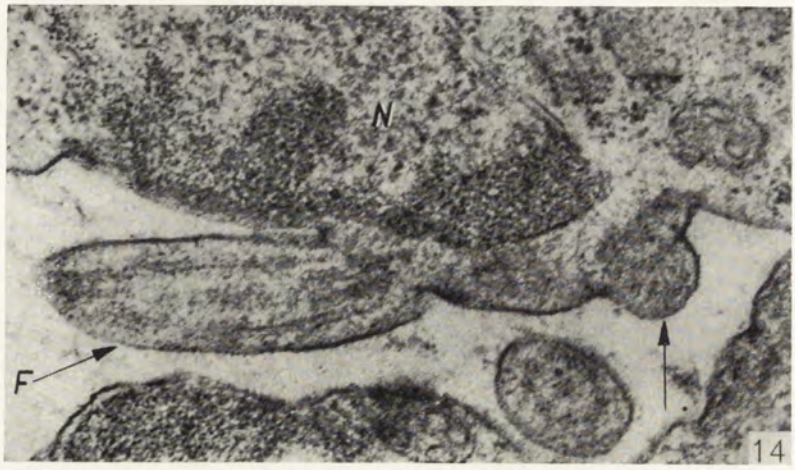

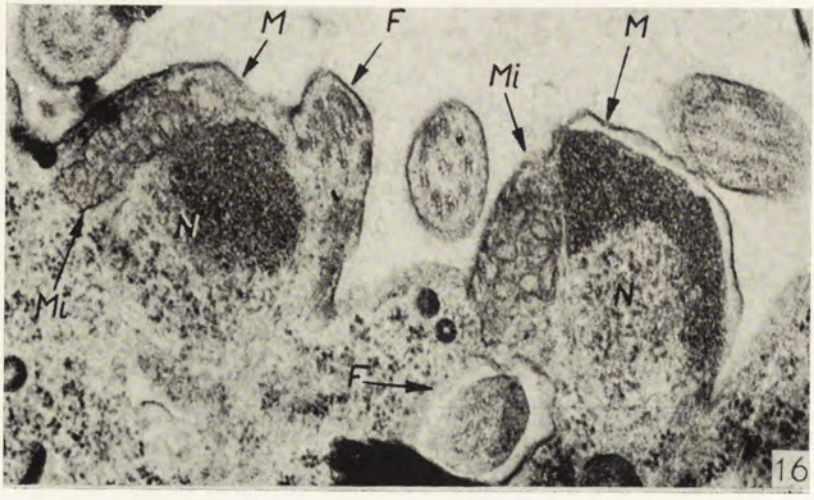

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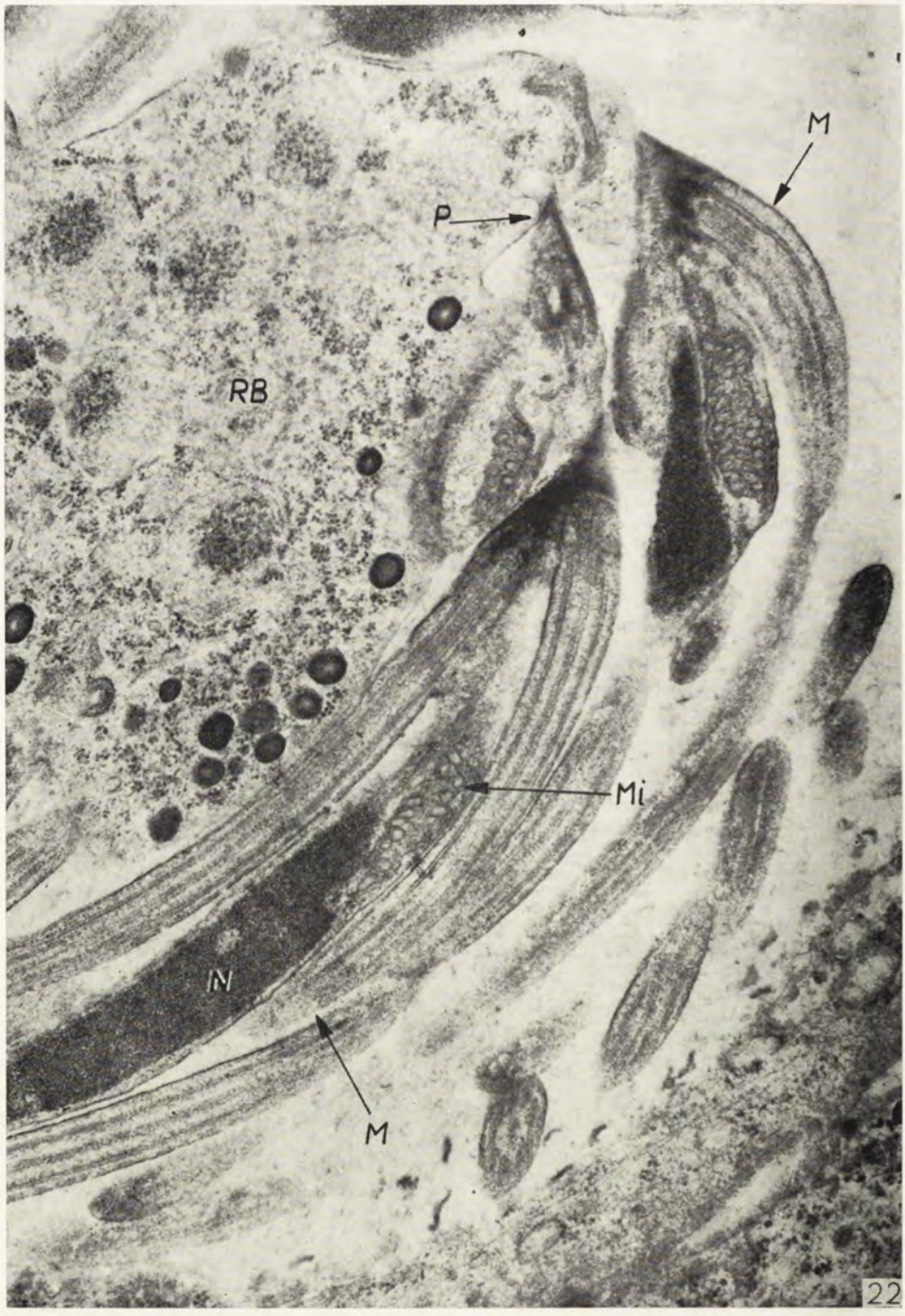

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About a presumable phylogenetic link between *Mesnilella (Astomata)* and *Conchostoma longissimum* Fauré-Fremiet *(Trichostomata)*

O możliwym związku filogenetycznym pomiędzy *Mesnilella* (*Astomata*) a *Conchostoma longissimum* Fauré-Fremiet (*Trichostomata*)

The polyphyletism of *Astomata* has been repeatedly discussed in litterature (Raabe 1947, Corliss 1956, Puytorac 1954, 1957). This question remains open even after excluding from this group several species which were included to the order *Apostomea*. Attention is most often payed to the great distinctness of *Haptophryidae* and to the position of the mouthless ciliate *Curimostoma renalis* Kozloff, with no doubt nearly related to *Tetrahymena* (Kozloff 1954).

On the other hand, the phylogenetic integrity of the main families of *Astomata*, especially of the family *Hoplitophryidae* (Puytorac 1954, 1957), has rarely been objected. Only Raabe 1947 postulated the possibility of polyphyletism in this group. He payed attention to the essential structural differences occurring between *Radiophryinae* on one and *Mesnilellinae* on the other side.

Radiophryinae are characterized by a large flattened body. One of its surfaces shows in its anterior part a characteristic concavity being the thigmotactic area of the ciliate. This concavity is associated with the occurrence of the superficial cytoskeleton. Its fibers run concurrently to the kineties of the body. Flattening on the *Radiophryinae* body corresponds to that of the *Hysterocinetidae*, if the concept of phylogenetic link between these families would be accepted. Therefore the surface with the thigmotactic area might be called the left side, according to the orientation of the body postulated by Raabe 1949; it may also be accepted as the inferior one, when basing on the orientation of body suggested by Kozloff 1960. Then the opposite side should be recognized as the right or the upper one if the same orientation as in *Hysterocinetidae* has been applied.

The plane of symmetry in *Radiophryinae* is perpendicular to the body surfaces (sides) and crosses the middle of the ciliate. Another relationship is found in *Mesnilellinae*. Their vermiform elongated body shows a distinct flattening in its anterior part. This flattening is regular on both sides. In this way, both body sides are symmetric to each other. The symmetry plane crosses the body margins and is vertical to that plane which would be the symmetry plane of *Radiophrya*. The diagram (Fig. 1) represents the differences of struc-

ture in *Radiophryinae* and *Mesnilellinae* which had been described above. Besides, the cytoskeleton of *Mesnilella* has the form of a spicule, with an axial position inside the cytoplasm, and not of numerous subkinetal fibrils.

The arguments put forward by R a a b e 1949 are in some extent attenuated by the research of P u y t o r a c 1954, and 1957 who pointed out the possibility of transitory forms between the flat species of *Radiophryinae* and some elongate vermiform ones which may occur even within the same family, e.g. *Juxtaradiophrya* and *Hoplitophrya*. Moreover his ultramicroscopic investigations on

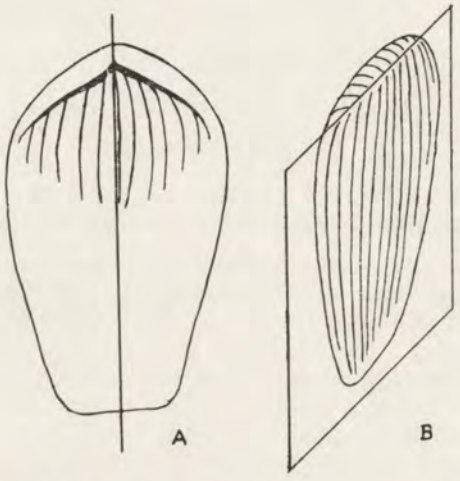

Fig. 1. Comparison of the structure of *Radiophrya* (A) and *Mesnilella* (B)

Mesnilella trispiculata showed that in this species the axial fiber arises by fusion of tiny fibrils into bigger complexes and that the most elementary cytoskeletal fibrils in *Mesnilella* (P u y t o r a c 1963) are of the same character as in *Radiophryinae* (P u y t o r a c 1959, 1961) since they both arise in connection with the kinetosomes. R a a b e 1949 indicated however the possibility of relations of some of the *Astomata* — especially of *Mesnilella* — with *Protoanoplophrya* which has been included by C o r l i s s 1961 into the *Thigmotricha*. *Protoanoplophrya* — despite the distinct membranes in its buccal apparatus — shows some essential similitudes to *Astomata*. Those characters are as follows:

1. Elongation of the whole body. The most striking difference is between *Protoanoplophrya bithyniae* Raabe and *Protoanoplophrya stomata* Miyashita (Fig. 2). In different species of the genus *Mesnilella*, a strikingly similar tendency exists to a considerable gradual elongation of the body.

2. The single lenticular micronucleus as charàcteristic for the *Astomata*.

3. Two rows of contractile vacuoles located on the body sides.

4. The uneven division of the body which is connected with the tendency to separate small opisthes, leading in consequence to catenular divisions.

5. Asymmetry of each of the surfaces of the body, and a simultaneous symmetry of these surfaces in relation to each other (symmetry of the body

Fig. 2. A. *Protanoplophrya stomata* Miyashita (acc. Miyashita 1929). B. *Protoanoplophrya bithyniae* (acc. Raabe 1933). C. *Conchostoma longissimum* Fauré-Fremiet (acc. Fauré-Fremiet 1963). D. *Mesnilella clavata* (orig.). E. *M. depressa* Rossolimo (acc. Cheissin 1930). F. *M. trispiculata* (modified acc. Puytorac 1963). G. *M. fastigata* Mob. (orig.)

related to the plane crossing the body margins) which is achieved here owing to the marginal location of the peristome. This type of symmetry and of structure plane remind those occurring in the genus *Mesnilella*.

*

As it seems, there exists another possibility of putting forward one more concept concerning the origin of the genus *Mesnilella* from the stomatous forms.

Fauré-Fremiet 1963 described a new genus of psammon ciliate under the name of *Conchostoma longissimum*. This ciliate shows, at a first glance, a considerable similitude to many of *Mesnilella* species. Its body is vermiform and much elongated; its anterior end is cut off obliquely in a characteristic

manner like in many species of *Mesnilella*. The only element which disturbes the lateral body symmetry is a small infundibulum, characteristic of *Trichostomata* (Figs. 2, 3, 4). The unusually elongated Ma assumes the form of a thin rod. On both body sides, two rows of contractile vacuoles are located. At last,

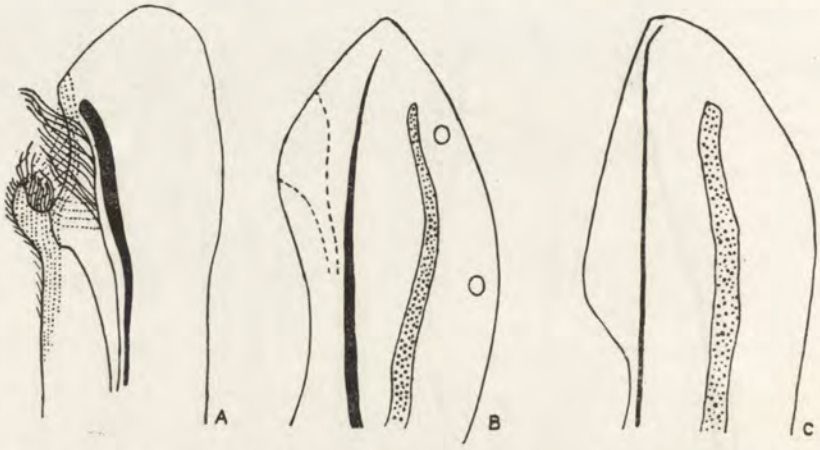

Fig. 3. A. Anterior body part of *Conchostoma longissimum* (acc. Fauré-Fremiet 1963). B. Same in *M. fastigata* (orig.). C. Same in *M. depressa* (acc. Cheissin 1930).

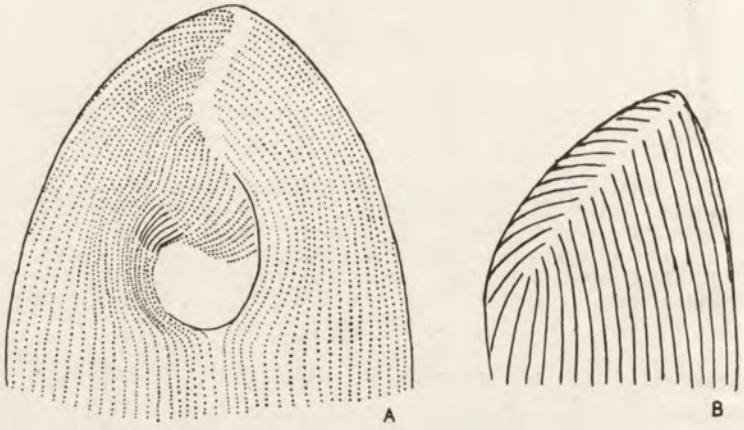

Fig. 4. A. Ciliature in *Conchostoma longissimum* (acc. Fauré-Fremiet 1963). B. Ciliature in *Mesnilella* (scheme)

in the anterior body part a straight thick fibril occurs, of a cytoskeletal character, lying inside the cytoplasm along the cytopharynx as its supporting structure (according to Fauré-Fremiet 1963).

Numerous tiny micronuclei are — besides the oral apparatus — another essential feature distinguishing *Conchostoma* from *Mesnilella* which like all *Astomata* is mono-micronuclear. Fig. 4 shows the similitude of the general aspect of *Conchostoma* and of different species of *Mesnilella*. Fig. 3 presents

the similar pattern of the anterior body region and similar disposition of cytoskeletal fibrils in *Conchostoma* and in two species of *Mesnilella*: *M. fastigata* and *M. depressa*. The enlarged anterior body region in *M. depressa* resembles much the feature of the same body part in *Conchostoma longissimum*. It should be also remarked that the fresh water psammon, as well as the sea water one, are the medium inhabited by numerous *Oligochaeta*, and in conditions of a full salinity — by *Polychaeta*. Those animals may be the hosts of *Astomata*.

Considering that *Conchostomata* are marine animals, the information concerning the occurrence of *Oligochaeta* infected by *Astomata* in fresh water might be of interest. So Frolova 1957 reported the occurrence of *Oligochaeta* infected — among others — by *Mesnilella fastigata* and *Radiophrya prolifera* in the Barents' sea. These data are in full agreement with the earlier observations of Rossolimo 1926 as well as with the author's own observations from the Bay of Puck in the Baltic Sea. There was a possibility of finding among others: *Helella rhizodrili*, *Mrazekiella* sp., *Inthoshellina* sp., *Radiophrya prolifera* in the *Oligochaeta* of the family *Tubifecidae*, and *Radiophrya prolifera* in *Naididae*.

It seems possible to imagine the transition of some psammon ciliates occurring in the brackish water and in salt water to the intestinal parasitism in the *Oligochaeta* or *Polychaeta*. Such ecological remarks may serve as additional arguments in favour of the view of the phylogenetic connection between *Conchostoma* on one, and *Mesnilella* — on the other side.

The eventual transition from the psammon to the internal intestinal parasitism in the *Oligochaeta* may be promoted by the fact that the alimentary tract of those animals is always filled with sand and with other particles originating from the external medium. This is connected with the manner of feeding characteristic of these ciliates. This transition from the psammon to the internal parasitism in *Oligochaeta* might possibly occur — as it could be imagined — by intermediate forms, i.s. the facultative parasites. Since the *Oligochaeta* live in the media rich in organic debris and deficient of free oxygen, it might be postulated that the adaptation to the anaerobic conditions of the host's internal medium was possible. The *Oligochaeta* are mostly burried in silt, with a part of their body projecting above the surface and moving freely in water. The deficiency of oxygen does not concern themselves but it may concern the ciliates living in the same medium.

The hypothesis presented here differs from the former attempts of phylogenetic linking *Astomata-Hoplitophryidae* with the oral ciliates because it relates them to the completely free-living animals.

*

The earlier hypotheses looked for common features in the stomatic structures of *Thigmotricha*. Raabe 1949, Puytorac 1957, Kaczanowski 1961 assumed the existence of an essential relationship between *Hystérocinetidae* and *Radiophryidae*. Puytorac 1954 stressed the relationship of *Conchophtiridae* and *Anoplophryidae*. The hypothesis of Raabe 1949 about the connection of *Protoanoplophrya* and *Mesnilella* is of the same character.

Both hypotheses about the independent descendence of the astomatic *Mesnilella* from the stomatic ciliates — on one side from the *Protoanoplophrya*

and on the other side from the ciliates of the *Conchostoma* type — are not compatible. The structure of the oral apparatus is in both cases different. This fact suggests a remote systematic position of *Protoanoplophrya* and *Conchostoma*. In *Protoanoplophrya* the hymenial apparatus is composed of two distinct ciliary membranelles, parallel to each other. This reminds somewhat the buccal apparatus of *Hysterocinetidae* and still more *Cochliophilus* (K o z l o f f 1945). *Conchostoma* has a typical funnel (infundibulum) characteristic of *Trichostomata* in which the distinguished kineties, forming the buccal ciliature, fail to exist.

Fig. 5. Morphology of *Protoanoplophrya bithyniae* (orig.)

So it may be stated that two ciliates, so remote from each other as to the taxonomic position, developed independently numerous features which are characteristic of *Astomata-Hoplitophryidae*. The vermiform elongated body occurring in *Hoplitophrya* and *Mesnilella* appears in *Protoanoplophrya* and *Conchostoma* with no regard to the phylogenetic relationship of any of those ciliates to *Mesnilella*. The above feature appears also in the case of *Juxtaradiophrya*. P u y t o r a c 1954, 1957 documented that the cytoskeleton of *Juxtaradiophrya* is similar to that in *Radiophrya*, being nevertheless reduced. For that reason this author considers that *Juxtaradiophrya* derives from *Radiophrya*. As it seems, this hypothesis is justified. It does not exclude a different origin of other elongated vermiform ciliates as *Hoplitophryidae* (e.g. *Mesnilella* or perhaps even *Hoplitophrya*). The elongation of the body in *Conchostoma* and in *Protoanoplophrya* is of the same character as in *Astomata* because it is accompanied by the enormous elongation of macronucleus which extends all along the body, by the occurrence of contractile vacuoles in rows, by the single lenticular micronucleus in *Protoanoplophrya* and by the cytoskeletal rod in *Conchostoma*.

On the other hand those characters are not occurring in *Astomata* of the family *Haptophryidae* (e.g. *Steinella* or *Sieboldellina*). Perhaps *Astomata* are polyphyletic in a much higher degree than it is imagined, i.e. not only the

different femilies but even akin forms considered as belonging to the same family, might arise directly of the stomatic forms. It seems also possible that the considerable elongation of the body may efface the essential primitive structural characters.

Summary

A new hypothesis is put forward concerning the phylogenetic relationship of *Mesnilella* from the family *Hoplitophryidae* (*Astomata*) and *Conchostoma longissimum* (*Trichostomata*). Some other hypothetical concepts are discussed: the relationship between *Mesnilella* and other *Hoplitophryidae* which would prove the monophyletism of this family (Puytorac 1954, 1957), as well as the phylogenetic links between *Protoanoplophrya* (*Thigmotricha*) and *Mesnilella* (Raabe 1947). Some essential morphological similitudes are pointed out in *Protoanoplophrya, Conchostoma, Mesnilella,* and, at last, *Juxtaradiophrya* (*Hoplitophryidae*).

The possibility of suggesting independent hypotheses concerning the origin of *Mesnilella* from various groups of ciliates speaks in favour of the polyphyletic character of *Hoplitophryidae* and of an independent development of the characteristic features in this family.

STRESZCZENIE

Autor wysuwa nową hipotezę pokrewieństwa filogenetycznego pomiędzy *Mesnilella* z rodz. *Hoplitophryidae* (*Astomata*) i *Conchostoma longissimum* (*Trichostomata*). Autor omawia również hipotezę o pokrewieństwie filogenetycznym między *Mesnilella* i innymi *Hoplitophryidae* zgodną z tezą o monofiletyczności tej rodziny (Puytorac 1954, 1957) oraz hipotezę o powiązaniach filogenetycznych między *Protoanoplophrya* (*Thigmotricha*) i *Mesnilella* (Raabe 1947). Autor wskazuje również na szereg istotnych podobieństw morfologicznych między *Protoanoplophrya, Conchostoma, Mesnilella* i wreszcie *Juxtaradiophrya* (*Hoplitophryidae*). Możliwość wysuwania niezależnych hipotez dotyczących pochodzenia *Mesnilella* od różnych grup wymoczków przemawia na korzyść tezy o polifiletycznym charakterze *Hoplitophryidae* i niezależnym wykształcaniu cech charakterystycznych dla tej rodziny.

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Changes in the heteromeric macronucleus in division of *Chilodonella cucullulus* (Müller)

Przemiany heteromerycznego makronukleusa w czasie podziału u *Chilodonella cucullulus* (Müller)

The previous research on *Chilodonella cucullulus* (Müller — cited after Kahl 1930—1935, Fauré-Fremiet 1950, Ivanič 1933) has been confined to morphology and morphogenesis of this ciliate. No data were reported concerning the division of Ma. Changes occurring during the division process are the subject of the present study.

Material and methods

Material for the study was provided from the Municipal Sewer Clensing at Otwock. Samples were taken from the trickling filters.

Ciliates were cultivated on a liver homogenate nutrient containing: NaCl — 4 g., H_2SO_4 — 0.01 g., KH_2PO_4 — 1 g., gum arabic — 10 g., water — 1000 ml. and the liver homogenate of frog, rabbit or rat. The pH of the nutrient was adjusted to 7.1. Schaudinn and Carnoy fluids were used for fixation. The Feulgen reaction for desoxyribonucleic acid was performed on smears, as well as the Unna's pyronine—methyl green test for the ribonucleic acid. Observation of the living material was made by the aid of a light microscope as well as of the phase contrasting optics.

The nuclear apparatus of *Chilodonella cucullulus* is composed of the big Ma and of small Mi adjacent to it. Ma — of an ellipsoidal shape — occupies the central part of the cell; it slightly deviates from the long body axis. This deviation is possibly caused by the trichits of the "basket" and by pushing aside by food vacuoles. Ma consists of two contiguous parts which are coated by a common nuclear envelop. One of them — the orthomer — is highly basiphil, contains a granular mass and surrounds another part of Ma — the achromatic paramer. The latter contains the karyolymph and the basiphil endosome which is a structure of disputable function. This type of Ma is determined by Fauré-Fremiet 1957 as heteromeric centrical in contrast to the homogenous type which has been called homeomeric.

Results

As the interdivision Ma, this one was recognized which remains with no division changes for a longest period of time. In this phase Ma is ellipsoidal, the orthomer presents a faintly granular mass with slightly refractile bodies. The observation in the phase contrast reveals the presence of small granules and more voluminous globules. After fixation and the Feulgen reaction, the granules are stained intensely and the globules remain stainless. After the method of Unna, granules stain with methyl green whereas the globules bind pyronine. It may be concluded therefore that DNA is present most probably in the granules on the territory of orthomer, and RNA is contained in the globules closely adhering to the nuclear membrane. The globules seem to correspond to the nucleoli. This is also indicated by the result of digestion with ribonuclease prior to the Feulgen procedure.

Nucleoli fail to show granulations after staining with pyronine as well as in observation in the phase contrast. In Ma two forms of nucleoli occur: the polar ones on the poles and the spherical on the sides of nucleus (according to K a n e d a 1960). It may be stated after the observation of the living paramer that it consists of karyolymph in which the endosome is present. Endosome is a spherical central body, composed of tiny and fairly refractile grains. After the Feulgen test, karyolymph is only moderately stained whereas endosome shows an intense staining. Similarly after the method of Unna, methyl green stains mostly the endosome and the karyolymph remains only slightly stained. Most probably an insignificant amount of dispersed DNA occurs on the karyolymph territory, and its high concentration exists in the endosome (Pl. I 1—2). The interdivision phase lasts for about 17—18 hrs.

First signals of the approaching nuclear division are the changes occurring in the Ma. The cortical changes are delayed when compared to the nuclear transformations. An increase of the nuclear mass may be observed in the living material. Both types of nucleoli, the spherical and the polar ones increase in size, the boundary of endosome becomes slightly effaced (K a n e d a 1961 b proved in the electron microscope that the endosome has no membrane). A slightly staining zone round the endosome becomes visualized after the Feulgen test (Pl. I 3—6).

The next stage is a period of a very intense increase in size of the peripheric and polar nucleoli (similarly as it was ascertained by K a n e d a 1960 b for *Chlamydodon pedarius*). This increase reaches such a level that a layer of nucleoli arises which separates completely the granular part of the orthomer from the nuclear membrane. In the orthomer territory a translocation of DNA containing grains towards both poles is observable. In this way "caps" are formed (Pl. I 6). The feature of the paramer is changed when compared to the preceding phase. On the whole karyolymph territory, around the endosome a radiate zone extends. At this stage the Feulgen reaction reveals a distinct staining of the endosome and of the radiate zone. A distinct increase of the Ma size is consistent with the findings of S e s h a c h a r 1950 in *Chilodonella uncinata* and of K a n e d a 1961 a in *Chlamydodon pedarius*.

In the subsequent period an abrupt decrease in size of the peripheral and polar nucleoli is observed (Pl. I 7). Ma becomes rounded, in the orthomer occurs a confluence of chromatin grains, which form rods. Those are concentrically disposed around the paramer (Pl. I 7—9).

Their number amounts about 350—400. Kaneda 1961a observed the occurrence of similar structures in *Chlamydodon*. They were also found by Raikov 1962 in *Nassula*. In the territory of the paramer, the endosome becomes less and less visible (the karyolymph territory reveals a weaker Feulgen staining than the rods). In this stage the nuclear membrane is distinctly present, the volume of Ma diminishes as result of extrusion of RNA, the shape remains regular. It lasts about 8—10 min. This stage is followed by a period in which the nucleoli are nearly in atrophy, except the polar ones. The chromatin grains are formed again from the rods. Similar pictures were observed by Tuffrau 1953 in *Spirochona*. Ma assumes an ellipsoidal shape, the endosome diappears completely (Pl. II 10). The duration of this period is about 15—20 min.

The next stage is characterized by elongation of Ma and a marked constriction in its median part (Pl. II 11). In this part, a transitory occurrence of a residuary body is found, which is in some individuals intensely basiphil (Pl. II 12) after the Feulgen reaction, and in the others shows no basiphilia — (Pl. II 15) (c. f. Kidder 1933 — *Conchophtirus mytili*). The chromatin grains from the orthomer territory are segregated to both poles of Ma. Nucleoli increase in size in this period as well (Pl. II 13). The territory of the paramer shows no distinct changes. The duration of this period is about 5—10 min.

Subsequently Ma elongates and becomes gradually constricted which leads to its complete fission. The residuary body becomes extruded outside or joins one of the filial individuals — the proter or the opisthe. It may be also divided into two equal parts by the division furrow (Pl. II 14) or dissolved entirely.

Similar observations were made by Dobrzańska-Kaczanowska 1963, in *Allosphaerium*. All over the time of the Ma fission the nuclear membrane persists. The granular substance is entirely shifted to the filial nuclei. The nucleoli keep increasing their volume. This stage is accompanied by the fission of the filial individuals. This stage lasts about 10—15 min.

After the fission of the individuals, Ma is smaller than its exit size. Spherical nucleoli are near the nuclear membrane, the polar ones near the poles. The territory of the orthomer is occupied by the chromatine grains. In this period the endosome is still not observed on the territory of the paramer (Pl. II 16). After one hour Ma assumes its exit feature (Pl. II 17). Shift of the nuclear substance occurs simultaneously in the opisthe Ma, so that it assumes the feature of the Ma of proter. A similar process was observed by Dobrzańska-Kaczanowska 1963 in *Allosphaerium*. According to the data of Kaneda 1961 for *Chlamydodon pedarius* and of Fauré-Fremiet 1957 for *Dysteria monostyla*, in those ciliates a reversion of the opisthe Ma of about 180° occurs, which was not observed in this study in *Chilodonella cucullulus*.

Discussion

The characteristic behaviour of the polar nucleoli might be connected with some special characters of their structure, in spite of the lack of direct evidences. It should however be reminded that Kaneda 1960 found two types of nucleoli differing by constant structures. According to this author the polar nucleoli remain present for all the time of Ma division, whereas

the spherical ones become evacuated to the cytoplasm entirely. The period in which the increase of size of the spherical and polar nucleoli occurs, is probably the time of an intensified RNA synthesis on the Ma territory. A rythmic synthesis of RNA was observed by Elliot 1962, and Ruthmann 1963.

A similar increase of the nucleolar size was observed by Seshachar 1950 in *Chilodonella uncinata,* and by Kaneda 1960 b in *Chlamydodon pedarius.* Fauré-Fremiet 1957 and Elliot 1962 observed a similar process in the homeomeric Ma. The short period when nucleoli are diminished, as observed in the present study, is possibly connected with the penetration of RNA from Ma to the cytoplasm. For all that time the nuclear membrane persists. Kaneda 1961 reported two different ways of RNA penetration into the cytoplasm: by budding and by disruption of the nuclear membrane, which is not supported by the observations of the present study.

Mirsky, Allfrey and Frenster 1960 observed by means of the electron microscope some structures on the territory of nucleus, resembling the ribosomes. Those structures were sometimes found in the pores of the nuclear membrane also. This fact seems to speak in favour of the suggestion that during the emission of RNA from the nucleoli, the nuclear membrane undergoes changes which enable RNA to penetrate into the cytoplasm without necessity of disrupting it or of a visible budding.

Simultaneously with the RNA emission from the nucleoli to the cytoplasm, visible changes occur also on the paramer territory.

Endosome which persisted over the whole interdivision period, becomes dissolved during division. The substance contained in the endosome passes over to the karyolymph. It may be observed after the Feulgen and Unna tests that the paramer territory is stained whereas the endosome itself becomes less visible. Tuffrau 1953 stated in *Spirochona* that the whole karyolymph of the paramer is distinctly Feulgen positive but its staining is diffuse. Consequently, it may be stated that the endosome becomes completely dissolved in division.

In many ciliates a residual body is temporarily formed in the equatorial region of Ma during division. It contains chromatin or is deprived of it sometimes and is dissolved or eliminated outside in the subsequent stage of division (Kidder 1933, Dobrzańska-Kaczanowska 1963). In *Nassulopsis lagenula* (Tuffrau 1962) the residual body is of a chromatin character as well although its fate is quite different. If dissolving of the residual body is delayed, then the division furrow may split it by half, and the fragments are transmitted to each of the filial individuals, or the fission may omit this body. In this case it is herited by one of the filial cells, most frequently by the proter.

Kaneda 1961 a suggests that in the filial individuals, remnants of the endosome occur and are resorbed by cytoplasm where they perform the role of activators of enzymic processes. This would be of special importance in ciliates in which conjugation rarely occurs and the cytoplasm contains no chromatin reserve originating from the dissolved Ma of the exconjugants.

The nuclear membrane persists in the course of division in *Chilodonella cucullulus* and the residual body is not extruded to the cytoplasm. Possibly it is the part of chromatin which was not segregated and remained in the median zone.

Summarizing the above data, it may be stated that the fate of the residual body depends on the ratio of the rate of Ma transformations to the velocity of cortical changes. It means that if the division of Ma is delayed when compared to the division of the whole cell — the residual body is not divided and becomes located casually in one of the filial individuals (only in this case when it was not dispersed earlier).

The period in which separate chromatin grains elongate forming rods and subsequently separate again to produce grains — is possibly the period of segregation of chromatin grains in Ma. The period of DNA synthesis would coincide with the interdivision phase. Similar phenomena were observed by Raikov 1962 in *Nassula ornata*.

Summary

The ribonucleic acid contained in the nucleoli is not connected with the chromatin elements. Before the division of Ma, RNA possibly penetrates into cytoplasm. This process occurs through the nuclear membrane and is not associated with budding or disruption of this structure. In division and in the interdivision phase nucleoli grow which seems to suggest the occurrence of rythmical waves of RNA synthesis. In division, on the paramer territory, the endosome dissolves completely. After division it is formed again and shows the chromatic character. The subsequent fate of the residual body depends on the rate of segregation and on cortical transformations which divide the cell. The residual body in *Chilodonella cucullulus* fails to penetrate to the cytoplasm. The increase of chromatin content occurs — as it seems — in the early interdivision phase. In the first stage of division, grains form rods and subsequently the segregation occurs.

STRESZCZENIE

Kwas ribonukleinowy zawarty w nukleolach nie jest związany z chromatynowymi elementami strukturalnymi. Przed podziałem Ma prawdopodobnie wydostaje się on do cytoplazmy. Proces ten zachodzi przez błonę jądrową i nie jest związany z jej pączkowaniem, ani rozerwaniem. W czasie podziału i w okresie międzypodziałowym nukleole rosną, co wskazuje na występowanie rytmicznej fali syntezy RNA. Na terenie parameru podczas podziału endosom rozpuszcza się całkowicie. Natomiast po podziale wytwarza on się ponownie i ma charakter chromatynowy. Losy ciała resztkowego zależą od tempa segregacji i przemian kortikalnych dzielących komórkę. Ciało resztkowe u *Chilodonella cucullulus* nie wydostaje się na teren cytoplazmy. Powiększenie się ilości chromatyny zachodzi, jak się wydaje, we wczesnym okresie międzypodziałowym. Natomiast w czasie pierwszego stadium podziału z ziaren tworzą się pałeczki, po czym następuje segregacja.

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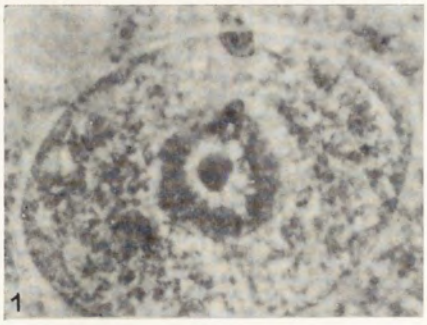
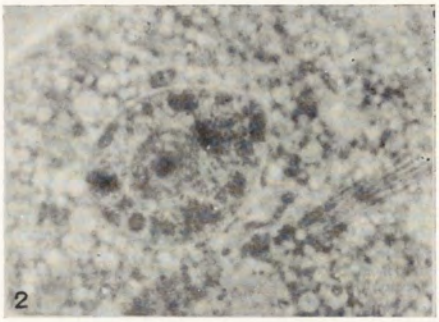
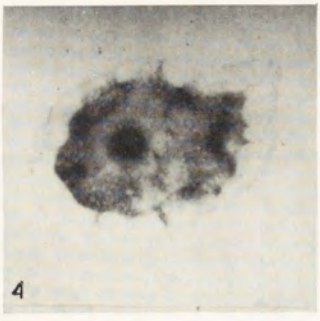
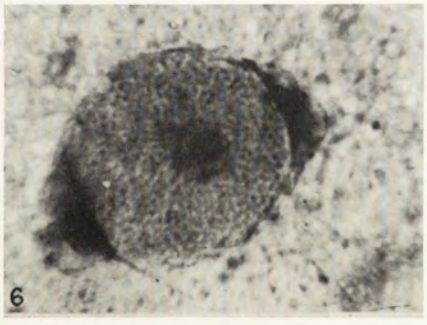
EXPLANATION OF PLATES I—II

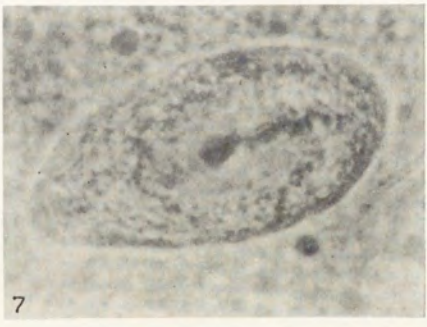
1: Interdivision Ma of *Chilodonella cucullulus* (living material)
2: Initial stage of the increase in size of nucleoli (living material)
3—5: Diffusion of the endosome border and the further increasing of nucleoli (Feulgen)
6: Formation of caps (Feulgen)
7: Decrease in size of nucleoli (living material)
8: Rounding of Ma and formation of the chromatin rods (living material)
9: Chromatin rods (living material)
10: Elongation of Ma and further reduction of the endosome (living material)
11: Constriction of Ma and disappearing of the endosome (living material)
12: Initial stage of formation of the residuary body (Feulgen)
13: Secondary increase in size of nucleoli; division of the residuary body (living material)
14—16: Further stages of division (Feulgen)
17: Ma after division (living material)

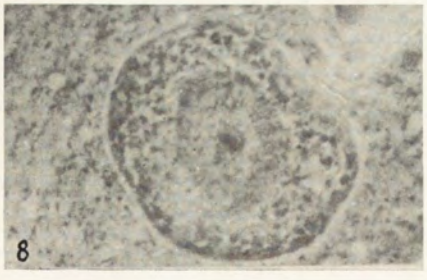


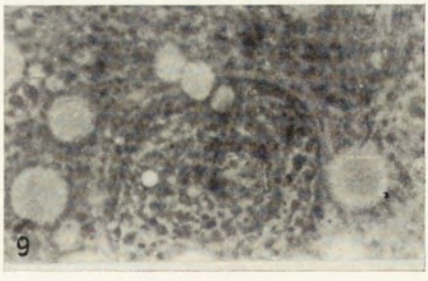












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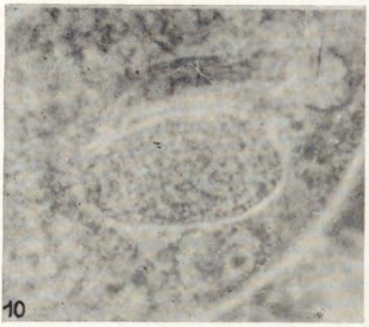

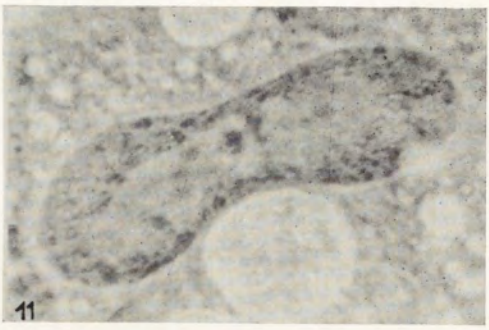

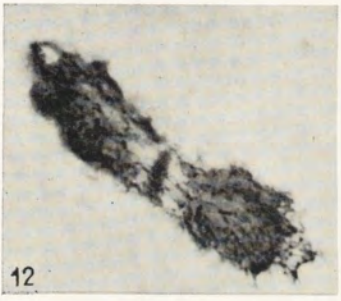

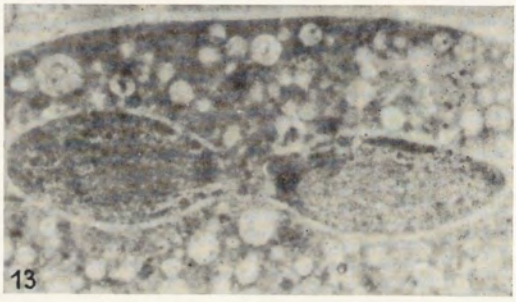

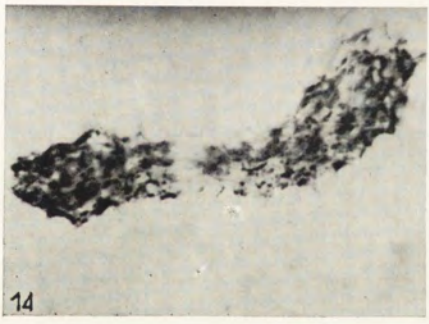

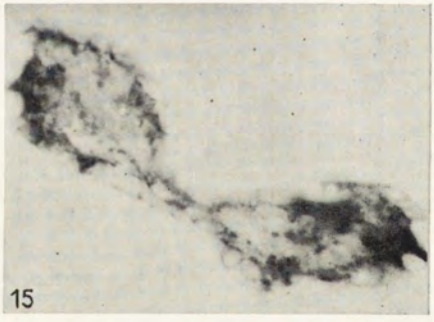

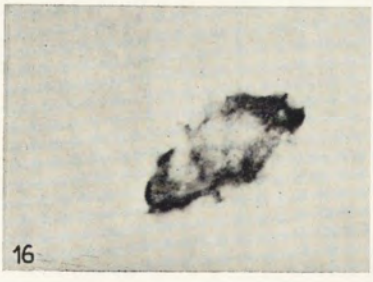

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Процессы конъюгации *Paramecium putrinum* Clap. et Lachm. VII. Ядерные процессы при внеконъюгационной автогамии, индуцированной новым методом „множественного спаривания"

Conjugation processes in *Paramecium putrinum* Clap. et Lachm. VII. Nuclear processes at autogamy in singles induced with a new technique — multiple mating

Некоторые виды инфузорий реорганизуют ядра не только при конъюгации, но и путем автогамии; последняя имеет место в одиночных особях, без контакта — даже кратковременного — с особью иного типа спаривания. Процесс этот очень редок: регулярная внутриклональная автогамия известна ныне только у 4 видов плёнчаторотых — *Paramecium aurelia* (Diller 1934), *P. polycaryum* (Diller 1954), *P. jenningsi* (Mitchell 1962), *Tetrahymena rostrata* (Corliss 1952). Вимала Деви (Vimala Devi 1961) считает доказанной автогамию у *Frontonia leucas*, но эти данные пока неубедительны и нуждаются в проверке и подтверждении. Достоверно известно, что автогамия отсутствует у 6 (из 10 ныне известных) видов парамеций — *Paramecium putrinum, P. calkinsi, P. bursaria, P. caudatum, P. woodruffi, P. multimicronucleatum*, возможно также у *P. arcticum*.

При этом у целого ряда видов внеконъюгационная автогамия может быть вызвана опытным путем. Наиболее эффективны 2 метода: искуственное разъединение партнёров конъюгирующей пары (метод Провазека — Prowazek 1895), и спаривание двух линий, в одной из которых инфузории убиты формалином (метод Хиватаси — Hiwatashi 1949). Диллер (Diller 1948) смог индуцировать автогамию у *P. calkinsi* при смешивании двух линий с комплементарными типами спаривания; мы не нашли в литературе иных сообщений о возможности применения этого метода к иным видам; за весь период работы с *P. putrinum* (1959—1964 гг), спаривая в самых различных комбинациях множество линий в 3 сингенах, мы не нашли на препаратах почти ни одного автогамонта. Четвёртый метод индукции автогамии описан Метцом и Фоули (Metz and Foley 1949); это также частный метод, применимый лишь к одной линии (СМ) *P. aurelia*. Особи этой линии способны к реакции спаривания (агглютинации), но не к конъюгации; после короткого контакта партнёры разъединяются, причем в нормальном партнёре имеет место автогамия.

Природная автогамия у изучаемого нами вида, *P. putrinum*, отсутствует, опытным же путём она еще не была вызвана. Индукция и изучение этого процесса важны в связи с тем, что у *P. putrinum* чрезвычайно распространены нетипичные способы реорганизации ядер при конъюгации (Янковский 1960—1965); применив метод индукции автогамии, мы получим возможность вести дальнейшие исследования на гомозиготном материале.

Новый метод индукции автогамии, позволивший выполнить данное исследование, был разработан нами в марте — июле 1964 г. До марта 1964 г., изучая ядерные процессы у *P. putrinum*, мы всегда сливали по 2 линии комплементарных типов спаривания; весной 1964 г. были впервые слиты вместе инфузории не 2, а 7 линий, принадлежащих к 4 типам спаривания сингена 4. За сутки до слияния, в фазе активного размножения, инфузории были лишены питания и поэтому находились в состоянии, благоприятствующем спариванию. Последовала, как и при слиянии 2 линий, активная реакция спаривания — образование групп слившихся своими ресничками особей; спустя 0.5 часа они распались на отдельные пары. Среди массы конъюгирующих пар мы отметили (а) тройные конъюгирующие группы (подробнее см. Янковский 1965 b), и (б) одиночные особи странной формы: уже при малом увеличении на правой стороне тела, вентрально, виден „околоротовой конус" — гомогенный выступ, лишённый расничек. Эти инфузории отличаются от нормальных, не принявших участия в конъюгации, ещё и тем, что в цитоплазме их нет пищевых вакуолей; минеральные кристаллы не рассеяны по всему телу, а собраны у заднего конца. Отличаются они и особенностями движения: это не равномерное вращение вокруг продольной оси тела, а какое-то некоординированное рыскание во все стороны. Если потревожить таких особей микропипеткой, они некоторое время плавают, затем вновь оседают на дно чашки Петри и надолго прекращают движение. Цитологическое изучение таких особей показало, что в них происходит внеконъюгационная автогамия.

Как выяснилось позднее, появление этих форм в опыте от 8.III.64 не было случайным. В марте — июле 1964 г. мы провели ещё 4 аналогичных опыта, и автогамонты обнаруживались всегда, в неизменно низком (2—4%) проценте. В апреле число спариваемых линий было уменьшено до 6 (с изъятием селфинг-линии MR-8), затем до 4; и в этих случаях на препаратах обнаруживаются нормальные конъюгирующие пары, триплеты и автогамонты. Автогамия никогда не индуцируется при спаривании 2 линий, принадлежащих к 2 типам спаривания, а также в тех случаях, когда спариваются 3—6 линий 2 типов спаривания. Следует заключить, что для индукции автогамии у *P. putrinum* нужно иметь минимум 3—4 линии, принадлежащие к 3 и более типам спаривания.

Метод „множественного спаривания" или, точнее, „мультиагглютинации" стал стандартным методом индукции автогамии у *P. putrinum;* при изучении автогамии основное внимание было уделено выяснению 3 вопросов: (а) прослеживание нормального хода ядерных процессов; (б) определение характера и частоты аберраций в поведении ядер; (в) выяснение влияния автогамии на жизнеспособность инфузорий.

Материал и методика

Для опытов использованы следующие линии *Paramecium putrinum:* M-6 (тип спаривания I), MR-14 (т. сп. I), M-23 (т. сп. III), MR-11 (т. сп. III), IR-6 (т. сп. IV), IR-4 (т. сп. V) и селфинг-линия MR-8, ведущая себя как т. сп. IV. Линии

расы M изолированы в 1961 г. в Сестрорецке, линии расы MR в 1960 г. в Ломоносове, линии расы IR в 1960 г. в оз. Хеппо-ярви; эти 3 пункта находятся в 30—45 км от Ленинграда, по различным направлениям. Все указанные линии принадлежат к сингену 4 (по типу спаривания), к миксотипу A (по способу ядерной реорганизации); это облигатные амфимикты. Инфузорий культивировали на среде Лозина-Лозинского в пробирках емкостью 13 мл, с ежедневной подкормкой взвесью дрожжей.

Для приготовления препаратов инфузорий фиксировали смесью Буэна, приклеивали к предметным стёклам по превосходному методу Чена (Chen 1944), с помощью целлоидина, и окрашивали на ДНК по Фёльгену без докраски лихтгрюном; изучение и фотосъёмку препаратов проводили с зелёным фильтром. Все приведённые в статье рисунки выполнены с рисовальным аппаратом при увеличении 60×10. Фотографии выполнены с помощью установки МБИ-6 на плоских плёнках низкой светочувствительности в кассетах формата 9×12 см и отпечатаны на нормальной, не контрастной фотобумаге.

Результаты

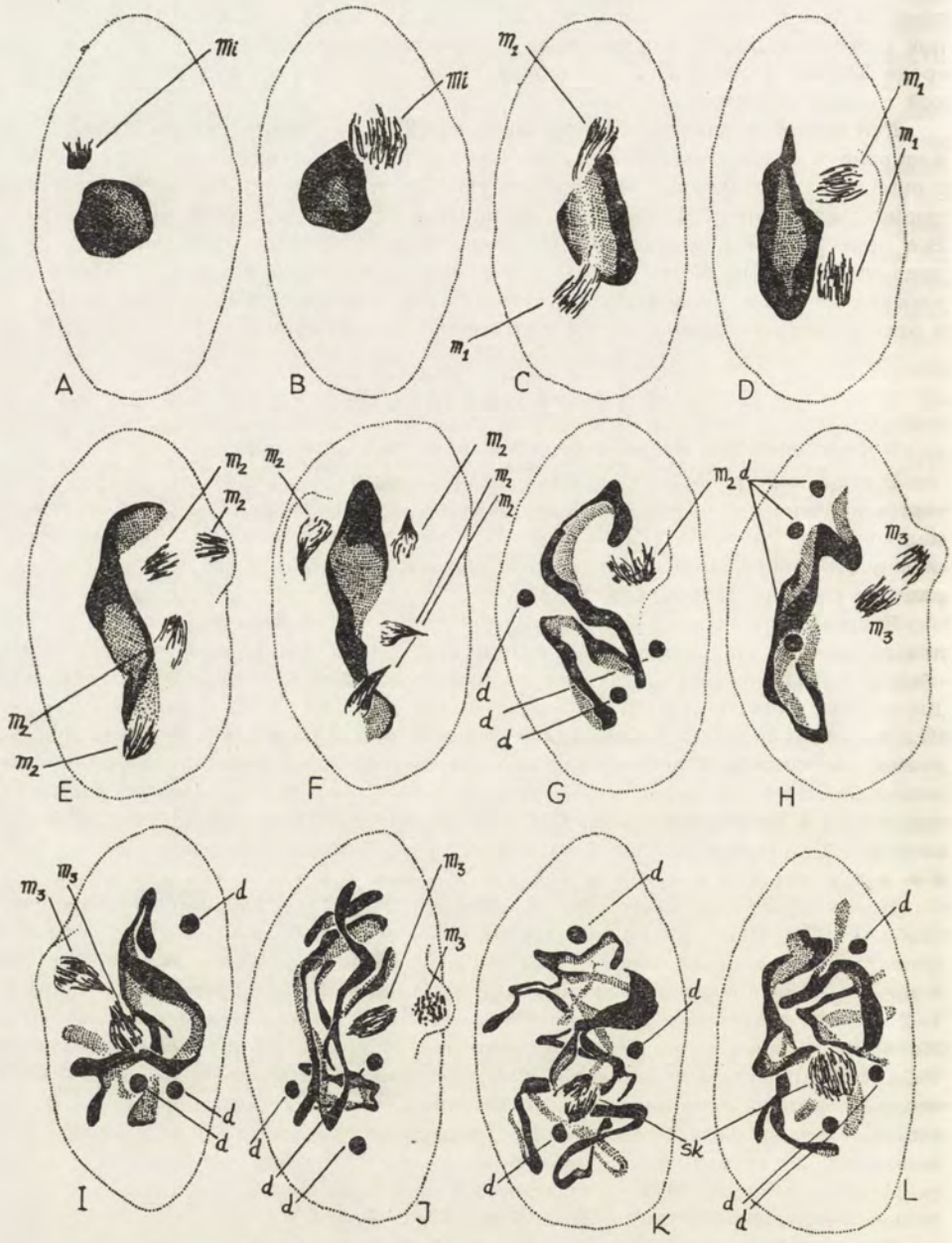
Нормальный ход ядерной реорганизации при автогамии

Всего в ходе работы окрашено несколько тысяч пар конъюгантов, среди которых найдено и зарисовано 583 автогамонта; ядерные процессы оказались нормальными у 451 особи (75.2%, т. е. 3/4 особей); у остальных 132 автогамонтов обнаружены аберрации в поведении микронуклеуса (Ми), у 156 особей — абберрации в распаде макронуклеуса (Ма).

Ядерные процессы при автогамии у *P. putrinum* протекают в той же последовательности, что и при конъюгации; отсутствует лишь, естественно, стадия обмена пронуклеусами. Амфимиктическая конъюгация у *P. putrinum* описана Диллером (Diller 1934, 1948) Вихтерманом (Wichterman 1937) и автором (Янковский 1960); различимы 3 деления созревания Ми, обмен пронуклеусами, кариогамия, 3 деления синкариона; партнёры расходятся на различных стадиях метагамной фазы, в зависимости от расы — в сингене 4 партнеры разъединяются в промежутке между II и III делениями синкариона. Эксконъюгант получает 8 диплоидных ядер, 3 из которых резорбируются, 1 превращается в Ми, 4 — в зачатки Ма.

Нормальный ход автогамии показан на Рис. 1, и на микрофотографиях (Табл. I и II). Наиболее ранная стадия автогамии изображена на Рис. 1 A: Ми непохож на овальный, компактный Ми вегетативных особей и напоминает Ми в профазе деления клетки, но в наших опытах парамеции голодают в течение 1—2 суток и делящихся особей на препаратах нет. По мере увеличения размеров Ми в нем становятся видны многочисленные удлиненные хромосомы (Рис. 1 B; Табл. I 2). Профаза I деления Ми и при автогамии, и при конъюгации занимает около 3/4 времени реорганизации; эта стадия особенно часта на препаратах. Учитывая, что в профазе I деления Ми аберрации в поведении Ми еще не обнаруживаются, мы старались изолировать автогамонтов на поздних стадиях процесса. Из общего числа 583 изученных особей на стадии про-, мета- и анафазы I деления обнаружено поэтому всего 199 особей (34.4%).

Веретено I деления Ми вытянуто вдоль тела; одно из дочерних ядер остается в задней части тела, другое отходит в околоротовой конус (Рис. 1 C-D; Табл. I 2-3). Околоротовой конус образуется у автогамонтов задолго до I деления Ми; этот выступ отчётливо виден при рассматривании автогамонтов сбоку



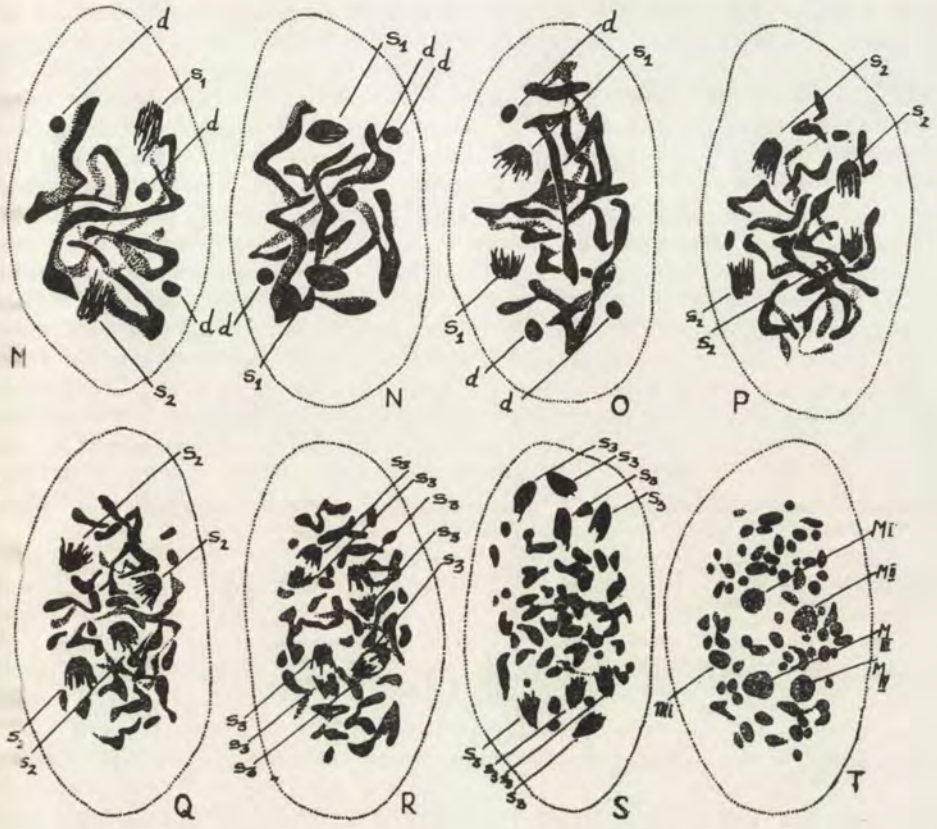


Рис. 1. Нормальный ход реорганизационного процесса при экспериментально индуцированной автогамии у *Paramecium putrinum*. С препаратов, окрашенных по Фёльгену; рис. апп., объ. × 60, ок. × 10. Показаны только Фёльген-позитивные структуры. Обозначения: Mi — микронуклеус; m_1, m_2, m_3 — продукты I, II и III делений микронуклеуса; d — ядра, дегенерирующие после I деления микронуклеуса; d_2 q ядра, дегенирирующие после II деления микронуклеуса; d_3 — ядра, дегенерирующие после III деления микронуклеусов; sk — синкарион; p — пронуклеусы, не слившиеся в синкарион; p_1, p_2, p_3 — продукты I, II и III делений гаплоидного ядра; M_I — M_{IV} — зачатки макронуклеусов. A — C. Профаза и I деление Ми. D — E. II деление Ми. F — I. III деление Ми. J. Интерфаза. K. Кариогамия. L — M. I деление синкариона. N — P. Интерфаза и II деление синкариона. Q — S. Последнее, III деление синкариона. T. Эксавтогамонт с зачатками макронуклеусов

Fig 1. Normal course of the nuclear reorganization during autogamy experimentally induced in *Paramecium putrinum*. Feulgen staining, camera lucida drawings (ob. ×60, eye piece ×10). Only the Feulgen positive material is marked. Explanations: Mi — micronucleus, m_1, m_2, m_3 — products of the 1st, 2nd and 3rd Mi division; d — nuclei degenerating after the 1st Mi division; d_2 — nuclei degenerating after the 2nd Mi division; d_3 — nuclei degenerating after the 3rd Mi division; sk — synkaryon; p — pronuclei not fused in the synkaryon; p_1, p_2, p_3 — products of the 1st, 2nd and 3rd division of haploid nucleus; M_I—M_{IV} — macronuclear anlagen. A—C. Prophase and the 1st Mi division. D—E. 2nd Mi division. F—I. 3rd Mi division. J. Interphase. K. Karyogamy. L—M. 1st division of synkaryon. N—P. Interphase and the 2nd division of synkaryon. Q—S. 3rd division of synkaryon. T. Ex-autogamont with macronuclear anlagen

(Рис. 1 E, H, J; Табл. I 4, 6, 7) и менее заметен, когда вершина его обращена к наблюдателю (Рис. 1 D, F, G). После I деления Ми следует очень короткая интерфаза; ядра не сжимаются в тёмные удлиненные ярко красящиеся тела, как это происходит при всех делениях; не дегенерируя, оба ядра делятся вновь (Рис. 1 E-F; Табл. I 4-5). Далее из 4 мелких (гаплоидных) ядер 3 ядра дегенерируют, одно (в ротовом конусе) делится в третий раз (Рис. 1 G-H; Табл. I 6-7). После III деления Ми образуются 2 пронуклеуса, один — в ротовом конусе, другой в глубине тела, близ ротового конуса (Рис. 1 J; Табл. I 7, II 10), но не у заднего конца тела, как у конъюгантов. В этот момент ротовой конус развит особенно отчётливо: при просмотре живых и тем более окрашенных автогамонтов создаётся впечатление, что „мигрирующий" пронуклеус лежит вне тела (Табл. I 7, II 10). При иммерсионном увеличении видно, что пронуклеус лежит в околоротовом конусе; прослойка цитоплазмы здесь незначительна, и своей вершиной пронуклеус упирается в пелликулу у вершины конуса (Табл. I 9). На Табл. I 8 видно, что пронуклеус лежит вблизи вестибулярного отверстия. Интерфаза после III деления Ми (Табл. I 7) очень часта на препаратах и, очевидно, довольно длительна. К моменту кариогамии „мигрирующий" пронуклеус покидает конус, высота последнего резко уменьшается и с этого момента конус перестаёт различаться.

Слияние пронуклеусов происходит не в околоротовой зоне, как при автогамии *P. aurelia* и *P. polycaryum* (Diller 1936, 1954), а в задней трети тела (Рис. 1 K; Табл. II 11-12). Пронуклеусы, образовавшие синкарион, долгое время сохраняют свою индивидуальность и перестают различаться в профазе I деления синкариона (Табл. II 13). Синкарион делится трижды, образуя в итоге 8 мелких ядер; I деление синкариона показано на Рис. 1 L-M; два дочерних ядра принимают в интерфазе вид заострённых овалов, в профазе вид щеток (Рис. 1 N-O; Табл. II 14). II деление синкариона показано на Рис. 1 P; профаза и анафаза последнего, III деления — на Рис. 1 Q-R и Табл. II 16. Экс-автогамонт получает в итоге 8 мелких овальных ядер (Рис. 1 S), быстро округляющихся (Табл. II 17); они дифференцируются далее так же, как и у экскоңъюгантов — 3 ядра резорбируются, 1 превращается в Ми, 4 в зачатки Ма (Рис. 1 T; Табл. II 18). Различить большинство экс-автогамонтов от экскоңъюгантов на препаратах невозможно.

Аберрации в поведении Ми

Линии *P. putrinum*, использованные для наших опытов, отличаются от иных культивируемых нами линий чрезвычайно низким — порядка 2—3% процентом аномалий в поведении Ми при конъюгации. При внеконъюгационной автогамии процент аномалий резко повысился до 24.8%. На разных стадиях процесса количество аномальных особей различно: процент аберраций возрастает от момента I деления Ми до момента кариогамии, затем несколько снижается (Таблица 1).

1. Профаза и I деление Ми. Эта стадия очень часта на препаратах, но процент аберраций минимален (7%). Чаще всего это ненормальная форма веретён деления: в анафазе хромосомы распределяются не по полюсам удлиняющегося веретена, а по всему веретену (Рис. 2 A-B); что может привести к неправильным разрывам веретена и к потере хромосом (Рис. 2 C-D; Табл. III 20). При конъюгации тех же линий такая аберрация отмечена не была, но сходные картины в массе вызываются рентгеновским облучением конъюгантов (Ковалева и Янковский 1965).

Таблица 1

Количество особей с нормальным ходом ядерных процессов и с аберрациями в поведении Ми на разных стадиях внеконъюгационной автогамии

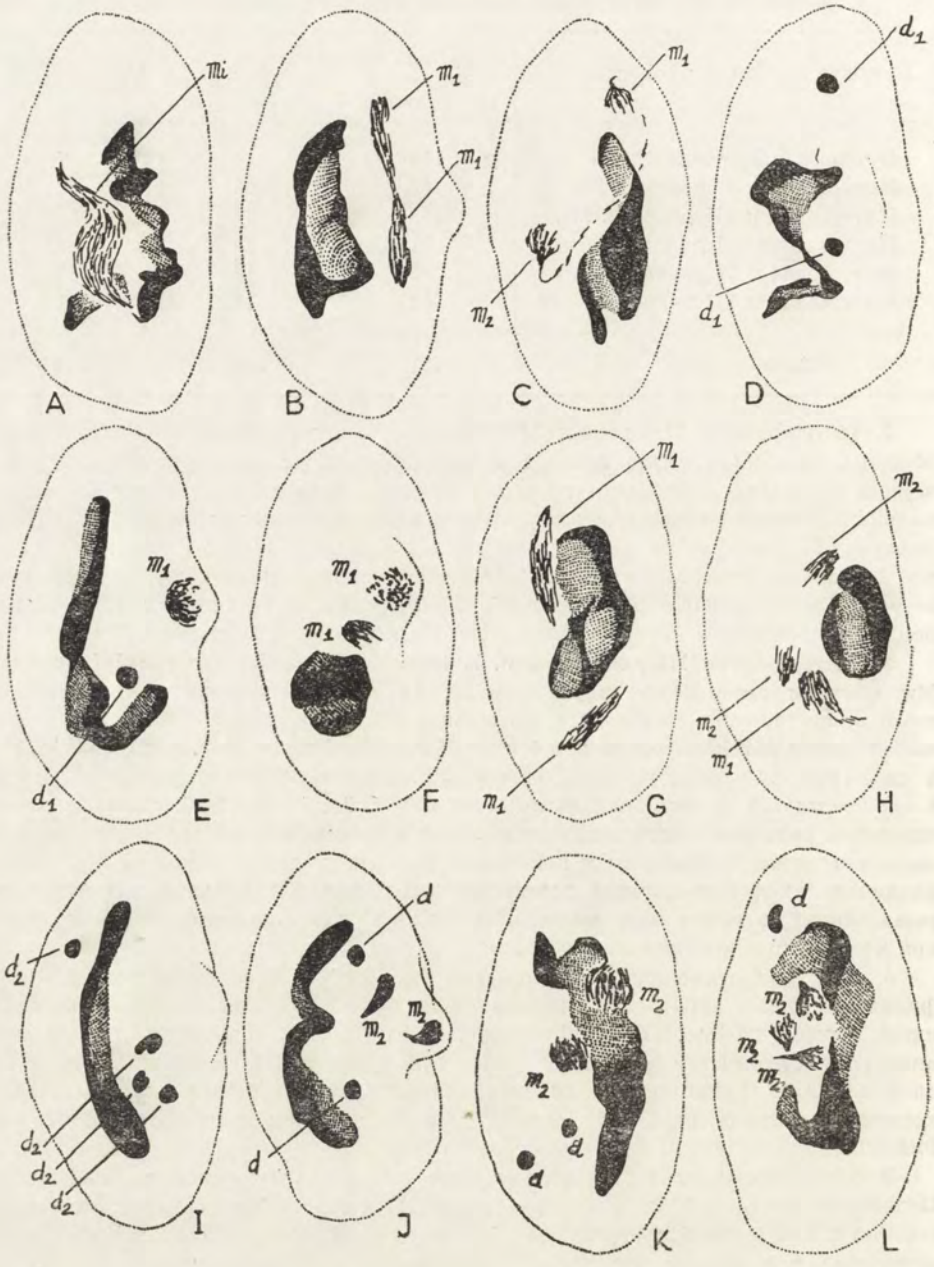
Фаза ядерных процессов	количество особей		% аномалий
	нормальных	аберрантных	
Профаза и I деление Ми	185	14	7
Интерфаза и II деление Ми	66	15	22
Интерфаза и III деление Ми	112	56	33
Кариогамия и I деление синк.	21	12	36
Интерфаза и II деление синк.	32	15	32
Интерфаза и III деление синк.	35	16	31
Итого	451	132	24.8

2. Интерфаза и II деление Ми. На этой стадии аберрации более разнообразны. Непосредственно после 1 деления Ми могут дегенерировать оба дочерних ядра (Рис. 2 D) или 1 из них (Рис. 2 E). Если ядра остаются функциональными, может наблюдаться асинхрония их дальнейших делений (Рис. 2 F-H). Веретёна II деления Ми могут иметь столь же ненормальную форму, что и после I деления (Рис. 2 G: ср. Рис. 2 A-B). В зависимости от того, сколько ядер вступило во II деление Ми, могут образоваться 2 или 4 дочерних ядра; II деление не произойдёт, если дегенерируют оба ядра после I деления Ми.

3. Интерфаза и III деление Ми. В норме в интерфазе после III деления Ми должны дегенерировать 3 ядра из 4; при конъюгации отклонения от этого правила очень редки. При автогамии, напротив, такие отклонения очень часты; могут дегенерировать все 4 ядра (Рис. 2 I), 2 ядра (Рис. 2 J; Табл. III 24), 1 ядро (Рис. 2 L) либо, наконец, дегенерация ядер вообще не происходит и все 4 ядра остаются функциональными (Рис. 2 P; Табл. III 22). Наблюдаются, далее странные разрывы дегенерирующих ядер: в норме форма их округлая или овальная, реже С-образная; у некоторых же автогаментов видно по 4—5 резко различающихся по величине дегенерирующих ядер (Рис. 2 Q-R). Момент разрыва одного из таких ядер виден в особи на Рис. 2 Q. Аберрации такого типа при конъюгации исключительно редки.

4. 3 других отклонения показаны на Рис. 2 M-O. В особи на рис. 2 M оба функциональных ядра резко отличаются по размерам и несомненно гетероплоидны. В особи на Рис. 2 N оба функциональных ядра находятся на разных стадиях роста: верхнее в интерфазе, нижнее в профазе III деления Ми. В особи на Рис. 2 O за функциональным ядром тянется нить, состоящая из отдельных хромосом; видимо, веретено деления имело здесь неправильную форму (ср. Рис. 2 C).

В зависимости от того, сколько ядер осталось функциональными после II деления Ми — 0, 1, 2, 3, или 4 — после III деления Ми образуется соответственно 0, 2, 4, 6 или 8 пронуклеусов. Все эти теоретически возможные варианты встречены и в нашем материале. Образование 2 пронуклеусов показано на Рис. 2 S, 3 A; 4 пронуклеусов — на Рис. 2 T, 3 D; 6 пронуклеусов — на Рис. 3 E-F; 8 пронуклеусов — на Табл. III 21. Само деление Ми может также протекать ненормально. В особи на Рис. 2 S оно протекает почему-то вне ротового конуса.



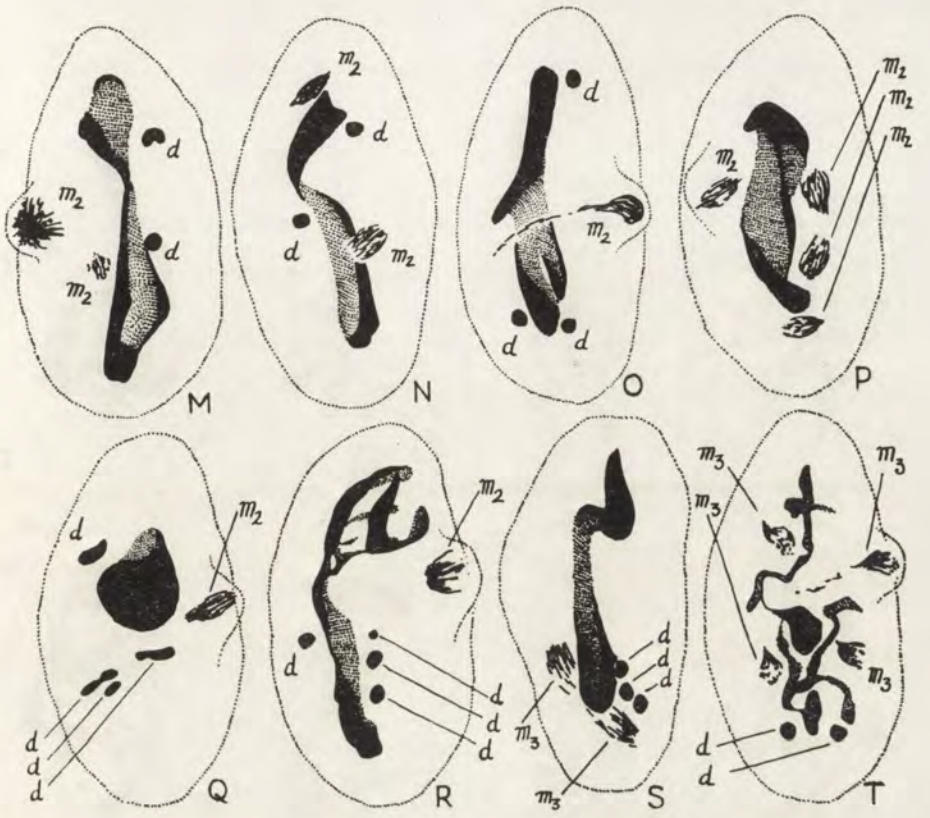
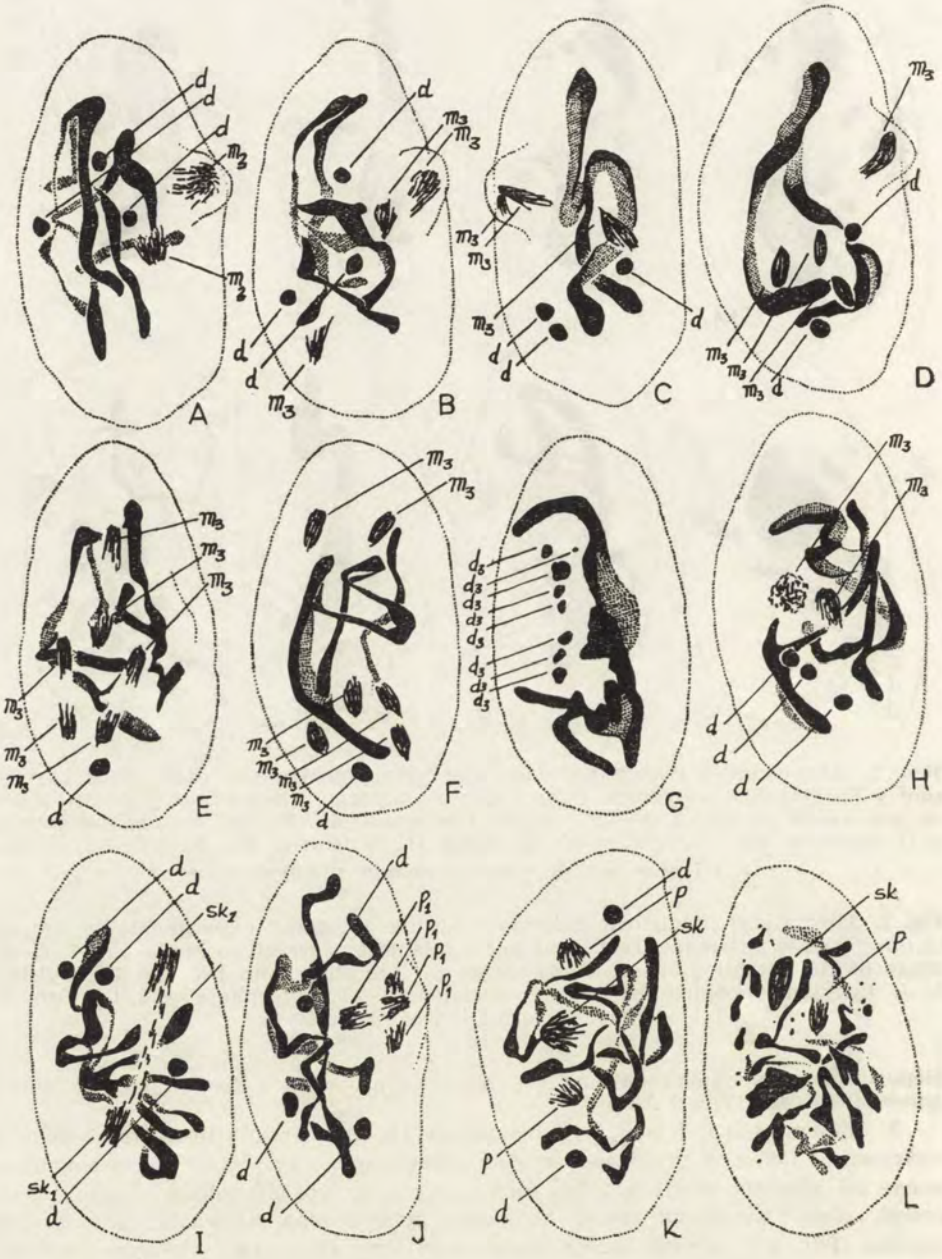


Рис. 2. Аберрации в поведении ядер при экспериментально вызванной автогамии у *Paramecium putrinum*. С препаратов; окраска, увеличение и обозначения те же, что и на рис. 1. A — C. стадия I деления Ми. D — H. стадия интерфазы и II деления Ми. I — J. Интерфаза перед III делением Ми. K — T. Интерфаза и III деление Ми; формирование пронуклеусов

Fig. 2. Aberrations of nuclear behaviour during autogamy experimentally induced in *Paramecium putrinum*. Technique and explanations are given in the Fig. 1. A—C. Stage of the 1st Mi division. D—H. Stage of interphase and the 2nd Mi division. I—J. Interphase before the 3rd Mi division. K—T. Interphase and the 3rd Mi division; formation of pronuclei

Нередок неправильный разрыв веретен, с образованием 2—3 ядер различной длины и формы (Рис. 3 A-C).

5. Кариогамия и I деление синкариона. На этой стадии процент аберраций максимален (36%). В некоторых особях дегенерируют все ядра, образовавшиеся после III деления Ми (Рис. 3 G). Вновь, как и на предыдующих стадиях автогамии, обнаруживаются такие аномалии, как потеря хромосом делящимися ядрами (Рис. 3 I), асинхрония в созревании ядер (Рис. 3 H). Пронуклеусы могут не слиться в синкарион и делиться самостоятельно (Табл. IV 28—29; Рис. 3 J). Избыточные пронуклеусы, по-видимому, не дегенерируют, и в ряде особей мы видим не только синкарион, но и 1—2 ядра вдвое меньших размеров (Рис. 3 K-L).



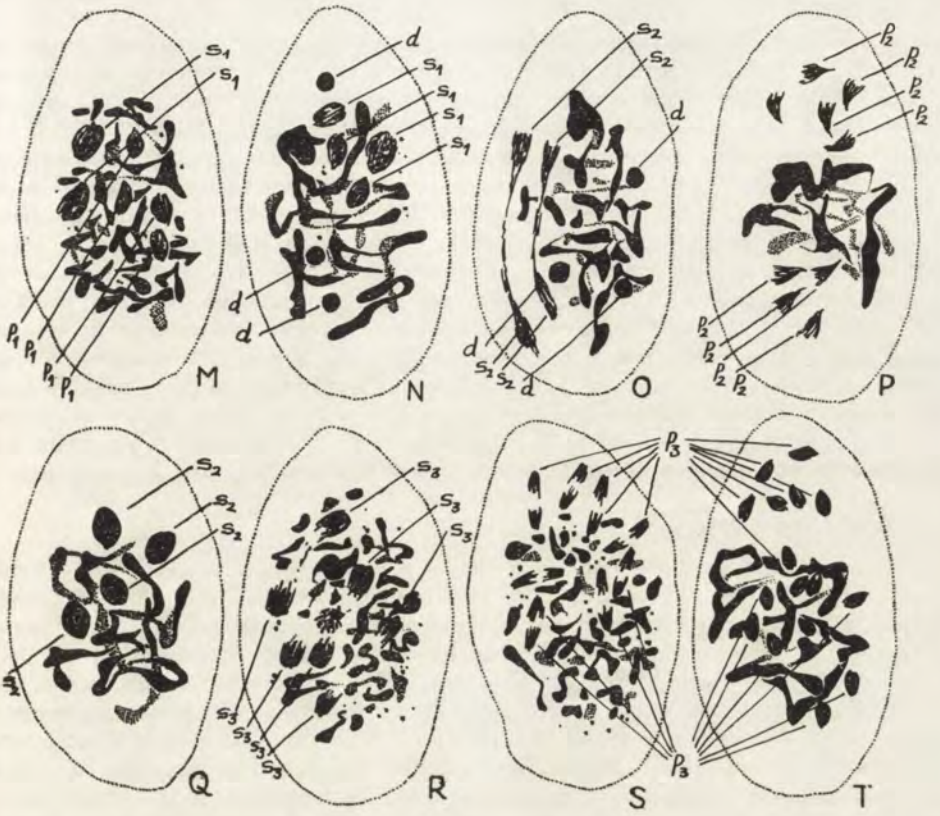


Рис. 3. Аберрации в поведении ядер при экспериментально вызванной автогамии у *Paramecium putrinum*. С препаратов; окраска, увеличение и обозначения те же, что и на рис: 1. A — H. Интерфаза после III деления Ми; III деление Ми. I. Деление синкариона. J. Деление пронуклеусов без слияния в синкарион. K — N. Интерфаза после I деления синкариона. O. II деление синкариона. P — Q. Интерфаза после II деления синкариона. R — T. III деление синкариона

Fig. 3. Aberrations of nuclear behaviour during autogamy experimentally induced in *Paramecium putrinum* (continuation). A—H. Interphase after the 3rd Mi division. I. Division of synkaryon. J. Division of pronuclei without their fusion in synkaryon. K—N. Interphase after the 1st division of synkaryon. O. 2nd division of synkaryon. P—Q. Interphase after the 2nd division of synkaryon. R—T. 3rd division of synkaryon

Начиная с момента I деления синкариона мы часто встречаем автогамонтов, ядра внутри которых отличаются по своей плоидности.

6. Интерфаза и II деление синкариона. После I, II и III делений синкариона ядра уже не дегенерируют, поэтому процент аберраций несколько снижается. Во II деление в норме могут вступить также и избыточные пронуклеусы; после I деления синкариона и избыточных пронуклеусов автогамонт может получить 2 диплоидных и 4 гаплоидных ядра (Рис. 3 M), соответственно после II деления он получит 4 диплоидных и 8 гаплоидных ядер. Веретена II деления могут вы-

глядеть при этом столь же ненормальными, как и при делениях Ми (Рис. 3 O, Табл. IV 32).

7. Интерфаза и III деление синкариона. Аберрации, обнаруживаемые на этой стадии, сводятся в основном к различиям в числе и плоидности ядер у разных особей. Дивергенция здесь поразительна. В нормальных особях видны 4 овальных, несколько заострённых ядра размерами около 5.5 × 3 мк (Табл. II 15). Могут встретиться, однако, особи с 8 мелкими, несомненно гаплоидными ядрами (5.5 × 2 мк, Рис. 3 P). Это скорее всего результат аномалии, показанной на Рис. 3 I, когда оба пронуклеуса делятся, не сливаясь в синкарион. Соответственно, все 8 гаплоидных ядер должны разделиться ещё раз; в итоге образуется 16 гаплоидных ядер вместо 8 диплоидных (Рис. 3 S-T) Нередки особи с 4 крупными, около 5.5 × 6 мк, несомненно гиперплоидными ядрами (Рис. 3 Q; Табл. IV 35); в образовании их могли принять участие избыточные пронуклеусы. Все 4 ядра вступают в III деление, образуя в итоге 8 гигантских ядер (Рис. 3 R; Табл. IV 36, ср. контроль — Табл. IV 30). Встречаются и особи с крошечными, очевидно гипоплоидными ядрами (Табл. IV 34). Наконец, очень часты особи с гетероплоидией ядер; так, в особи на Табл. IV 33 видны 4 крупных и 8 мелких (часть не в фокусе) ядер.

Аномалии в распаде Ма

Параллельно делениям Ми у автогамонтов, как и при конъюгации, протекает распад Ма; у 427 особей (из 583) этот распад протекает без видимых отклонений, у 156 особей (26.9%) обнаружены отклонения, причем часты именно такие отклонения, которые при конъюгации не наблюдаются. Нормальный процесс распада Ма показан на Рис. 1: Ма ветвится, принимает сетевидную форму; к моменту III деления синкариона сеть распадается на отдельные ветви, делящиеся далее на 50—90 овальных телец диаметром от 1,5 до 4 мк, чаще всего 2 мк. У конъюгантов (в контроле) наблюдается изредка задержка распада Ма в одном из партнёров по сравнению с другим; у отдельных партнёров Ма может остаться округлым, и распад его произойдёт лишь после разъединения пары. Явление задержки в распаде Ма отмечено нами и у многих автогамонтов; так, в особи на Рис 2 Q Ма ещё не принял удлиненной формы; в особи на Рис. 2 S Ма ещё не ветвится. Крайне редко среди автогамонтов попадаются особи с разрывом Ма на 2—3 части — гемикты (Табл. III 19); столь же редки такие особи и среди конъюгантов.

Среди автогамонтов часты особи с нетипичным распадом Ма: распадается не весь Ма, а только часть его, оставшийся кусок не ветвится и сохраняет аморфные, глыбовидные очертания (Рис. 2 T; 3 M, N, O; Табл. III 25—27; IV 30). Среди конъюгантов данных рас такая аномалия никогда не отмечалась. Частичная фрагментация Ма приводит к тому, что на поздних стадиях автогамии среди ветвей или фрагментов Ма мы часто видим крупную глыбу, не подвергшуюся фрагментации (Рис. 3 R; Табл. IV 31, 34). В отдельных случаях размер глыб достигает 12 мк.

Наблюдается и процесс иного характера: среди автогамонтов на поздних стадиях процесса нередки особи, у которых вся цитоплазма усеяна массой Фёльген-позитивных телец. Размеры их мы с достоверностью определить не могли, поскольку их диаметр составляет доли микрона. Эти тельца не отмечаются в особях, находящихся на стадии I, II и III делений созревания Ми, кариогамии и I деления синкариона, и начинают появляться на стадии II деления синкариона. Изучение препаратов при максимальном увеличении микро-

скопа (× 1800) показало, что эти частицы образуются при неправильных разрывах ветвей Ма: ветвь не делится на 2 равные части, как в норме; середина или основание резко утоньшается, рвётся на несколько частей, с образованием мелких, едва различимых фрагментов; последние сначала удлинены, имеют вид палочек или нитей, затем округляются и выглядят как точки.

Процесс образования мельчайших фрагментов при неправильных разрывах ветвей Ма поразительно напоминает нам описанное выше явление „потери хромосом" при неправильных разрывах веретен делений Ми.

Жизнеспособность автогамонтов

Установленный выше факт резкого повышения частоты аномалий в поведении Ма и Ми при автогамии ставит вопрос о том, могут ли все автогамонты дать жизнеспособное потомство. Опыты (предварительного характера) по выяснению жизнеспособности после автогамии были проведены 8—13. 7. 1964 г.;

Таблица 2

Выживаемость и темп деления эксконъюгантов и экс-автогамонтов

Отсаженные особи	№	8.7	9.7	10.7	11.7	12.7	13.7
Эксконъюганты	1	1	1	1	2	2	4
	2	1	1	2	2	3	6
	3	1	1	2	2	3	5
	4	1	1	1	2	2	4
	5	1	1	1	2	4	7
Экс-автогамонты	1	1	1	2	2	2	4
	2	1	1	1	1	2	4
	3	1	1	1	2	4	7
	4	1	—	—	—	—	—
	5	1	1	1	2	4	6
	6	1	1	2	2	2	4
	7	1	—	—	—	—	—
	8	1	1	1	2	3	5
	9	1	1	1	2	2	3
	10	1	—	—	—	—	—
	11	1	1	—	—	—	—
	12	1	—	—	—	—	—
	13	1	1	2	2	3	7
	14	1	1	—	—	—	—
	15	1	1	1	2	4	6
	16	1	1	2	2	3	5
	17	1	1	2	2	2	4
	18	1	2	2	2	4	8
	19	1	1	—	—	—	—
	20	1	1	1	1	2	3
Смертность среди автогамонтов		—	4	3	—	—	—

в 25 солонок было отсажено 5 эксконъюгантов (для контроля) и 20 автогамонтов. Для достоверности опытов, чтобы не спутать эксконъюгантов с экс-автогамонтами (внешне они неотличимы без окраски) мы изолировали конъюгирующие пары, готовые к разъединению, и в солонки отсаживали только-что разошедшихся особей. Автогамонтов отсаживали до моментта кариогамии, когда их можно безошибочно узнать по наличию ротовых конусов. Наблюдения над темпом деления отсаженных особей вели в течение 5 дней.

Эти данные, конечно, носят ориентировочный характер; обращает на себя внимание отсутствие смертности среди эксконъюгантов, более или менее одинаковый темп их деления: I деление эксконъюганта происходит на 2—3 сутки после разъединения пары, следующиее на 3—4 сутки, далее ежесуточно. Из 20 отсаженных автогамонтов 4 особи погибли не разделившись в переделах 1 суток после отсадки, еще 3 — также не разделившись, но на 2-е сутки. 3 особи делились в замедленном темпе: их I деление произошло лишь на 3—4 сутки, 7 особей делились с такой же частотой, как и в контроле. Только 13 особей (65%) дали жизнеспособное потомство.

Обсуждение результатов

Литературные данные по внеконъюгационной автогамии инфузорий

Как отмечалось во введении, природная автогамия с достоверностью обнаружена пока лишь у 4 видов, принадлежащих к 2 родственным семействам отряда *Hymenostomatida: Paramecium aurelia* (Diller 1934, 1936, 1940; Sonneborn 1939; Beale 1954), *P. polycaryum* (Diller 1954, 1957), *P. jenningsi* (Diller and Earl 1958; Mitchell 1962) и у *Tetrahymena rostrata* (Corliss 1952, 1956; Corliss and Dysart 1959, 1960).

Некоторые авторы допускают возможность регулярной внутриклональной автогамии у иных видов — *Stylonychia mytilus* (Fermor 1913); *P. caudatum* (Erdmann and Woodruff 1916; Chejfec 1930); *P. bursaria* (Erdmann 1925); *P. multimicronucleatum* (Straghöner 1932); *P. nephridiatum* = *P. woodruffi* (Gelei 1938). В основном такие указания ничем не подтверждаются; позднее выяснилось, что у *Stylonychia* в цистах происходит слияние двух Ма, но не Ми (Sokoloff 1930; Kay 1945); указания на возможность автогамии у перечисленных видов парамеций не были подтверждены последующими авторами (*P. bursaria* — Egelhaaf 1954; *P. multimicronucleatum* — Giese 1957; *P. caudatum* — Gilman 1959; *P. woodruffi* — Янковский 1961). Столь же маловероятны появившиеся в последние годы указания на возможность автогамии у *Pyxicola* sp. (Finley and Bacon 1963) и у *Homalozoon vermiculare* (Diller 1960, 1964).

Еще реже, чем внеконъюгационная автогамия, у инфузорий встречается автогамия при конъюгации. По Вихтерману (Wichterman 1940, 1953) автогамия при конъюгации („цитогамия") свойственна отдельным расам *P. caudatum;* по Диллеру (Diller 1957, 1958) и Митчелу (Mitchell 1963) это видовой признак: предполагается, что амфимиктических рас у *P. jenningsi* и *P. polycaryum* нет. Нельзя не отметить, что ни Вихтерман, ни Митчел не опубликовали сколько-нибудь подробных цитологических данных; Вихтерман, более того, вообще не проверял наличие „цитогамии" на препаратах и пользовался только живым материалом (!). Метод микросжатия, использованный этим автором, приводит (согласно Портеру — Porter 1963) к самым разнообразным аномалиям в функционировании различных органелл, при длительном сжатии могут возникнуть

и морфологические уродства. Диллер (Diller 1950) указывает, что обмен пронуклеусами легко прослеживается у окрашенных конъюгантов *P. caudatum;* Пенн (Penn 1937) и Вивье (Vivier 1960) показали, что конъюгирующие парамеции обмениваются пронуклеусами не только при межклональном, но и при внутриклональном спаривании (в селфинг-линиях). На наших препаратах процесс обмена пронуклеусами был прослежен у различных рас *P. caudatum* и *P. bursaria,* также у *P. woodruffi* и *P. multimicronucleatum.* Все это заставляет нас скептически отнестись к открытию Вихтермана.

Теоретически возможность автогамии при сохранении типичной прогамной фазы (3 деления Ми) очень маловероятна. Единственный достоверный случай автогамии при конъюгации описан у *P. putrinum* (Янковский 1960, 1962), но прогамная фаза сокращена здесь до 2-х делений; нет III деления Ми, дифференцирующего пронуклеусы на мигрирующий и стационарный; сливаются 2 сходных ядра, расположенных в одном и том же участке клетки. Здесь как бы преждевременно образуются пронуклеусы á III деление Ми наследственно выпало.

Мы убеждаемся теперь, что автогамия (при конъюгации и вне конъюгации) — это процесс крайне редкий (5 видов) и в целом для инфузорий нетипичный. Изредка же автогамия может произойти случайно; такие отклонения наблюдаются обычно у незначительного числа конъюгирующих форм и описываются в большинстве работ, посвященных конъюгации. Достаточно сослаться, к примеру, на изменчивость ядерных процессов у *P. bursaria,* детально изученную Гамбургер, Вихтерманом, Ченом и Эгельхааф.

Экспериментальным путем автогамия при конъюгации не была ещё вызвана ни у одного вида, автогамия вне конъюгации индуцирована, по литературным данным, у 8 видов: *Stylonychia mytilus, Paramecium caudatum, P. aurelia, P. calkinsi, Euplotes eurystomus, E. harpa, Bursaria truncatella* (Prowazek 1899; Иловайский 1917, 1926; Полянский 1938; Metz 1947, 1954; Diller 1948; Hiwatashi 1949; Metz and Foley 1949; Katashima 1952, 1959).

Большинство перечисленных авторов воспользовались методом Провазека (искусственное разъединение конъюгирующих пар); Метц и Хиватаси спаривали живых и мёртвых парамеций. В обоих случаях автогамии предшествовал контакт партнёров двух комплементарных типов спаривания, с образованием коротко существующей конъугирующей пары. Такой контакт, по Нэнни (Nanney 1954) и Метцу (Metz 1957), совершенно необходим для активации микронуклеуса партнеров и для снятия „блокирующего" механизма; все же дальнейшие процессы (образование ротового конуса, мейоз, распад Ма) произойдут автоматически, независимо один от другого. В этом положении мы видим ключ к решению вопроса о том, почему при мультиагглютинации у части особей индуцируется процесс автогамии.

Возможная причина автогамии в наших опытах

В нашем материале внеконъюгационная автогамия была вызвана новым методом — при одновременном смешивании линий, принадлежащих к 3 и более типам спаривания. Интенсивность агглютинации при таких спариваниях отнюдь не выше, иногда даже ниже, чем при двойном спаривании: не в каждом опыте нам удавалось точно определить момент, когда инфузории всех линий способны к моментальной агглютинации. В каждом опыте среди массы спаренных инфузорий мы находили и тройные конъюгирующие группы (триплеты), и автогамонтов.

Подробно процесс тройного спаривания описывается отдельно (Янковский 1965 b); здесь же для нас важно отметить лишь то, что каждый партнёр присоединяется одновременно к двум другим, так, что их вентральные стороны соприкасаются и партнёры располагаются параллельно один другому. Это особый способ мультиконъюгации, известный у *Tetrahymena* (Nanney 1953; Corliss 1960), у *Colpidium* (наши препараты) и неизвестный ранее у *Paramecium*. Такой способ соединения говорит о том, что все три партнёра, составляющие триплет, имеют разные типы спаривания. Действительно, все это, в отличие от хорошо изученных видов типа *P. aurelia*, *P. caudatum* — виды со множественной системой типов спаривания. У *P. putrinum* тройная конъюгация и автогамия не могут быть вызваны спариванием 2 комплементарных линий; скрещиваемые инфузории должны относиться по крайней мере к 3 типам спаривания.

Важно и следующее: во всех проведённых нами опытах число автогамонтов намного превышает число триплетов; в период март — июль 1964 г. мы изолировали и окрасили 583 автогамонта и всего 75 триплетов. Учитывая приведённые факты, мы можем предположить следующий механизм активации в наших опытах.

При одновременном сливании инфузорий нескольких типов спаривания происходит как процесс образования двойных пар (A + B), так и процесс образования триплетов (A + B + C). Далеко не всегда последний процесс завершается именно образованием тройной группы; чаще третий партнёр отделяется от двух других. Причиной может быть то, что в некоторых триплетах контакт двух партнёров оказывается прочнее, чем с третьим. Время, в течение которого третий партнёр был в контакте с двумя другими, оказывается достаточным для активации Ми и автогамии.

Не следует забывать о том, что реакция спаривания, как бы интенсивна она не была, не является сама по себе активирующим механизмом. Реакция спаривания (агглютинация особей при случайном контакте) — это всего лишь непрочное слипание ресничек партнёров; на этой стадии партнёров легко отделить друг от друга, к примеру встряхиванием или подогревом, и контакт ресничек к автогамии никогда не приводит. В методах Провазека, Хиватаси, Диллера и Метца обязательным является непродолжительный, но достаточно прочный контакт партнеров своими вентральными сторонами; судя по вышеизложенным соображениям, это правило справедливо и в нашем случае.

Аберрации в поведении ядер

По указанию Диллера (Diller 1957) „...мы знаем, что автогамия, генетически, имеет своим следствием гомозиготность. Возможно, это имеет определённое значение для повышения жизнеспособности вида". В литературе описывается обычно именно такой, повышающий жизнеспособность эффект автогамии (Fauré-Fremiet 1953, Sonneborn 1954; Corliss and Dysart 1960); автогамия имеет летальный эффект лишь в старых клонах (Sonneborn and Schneller 1955, 1960), у облучённых линий (de Haller 1964).

В нашем случае наблюдается резкое повышение процента аномалий в поведении Ма и Ми; предварительные опыты показывают значительную смертность после автогамии. Можно ли объяснить повышение процента аномалий старением линий? В старых линиях *P. aurelia* летальной оказывается не только автогамия, но и конъюгация (Sonneborn and Schneller 1955); установлено также, что по мере старения линии конъюгация приобретает все более

патологический характер, и нередко гибнут все партнёры, не разъединяясь, либо все эксконъюганты, не делясь (Jennings 1944; Chen 1951; Byrd 1959; Diller 1959; Sonneborn and Schneller 1960). В свете этих данных логично ожидать что если повышение процента аберраций при автогамии вызвано старением линий, то столь же ненормально должна протекать и конъюгация, что не наблюдается. Напротив, процент аберраций при конъюгации линий сингена 4 поразительно низок (порядка 3%) и эти линии были отобраны именно ввиду стабильности контроля. Нет, поэтому, доводов в пользу того, что патологическое течение автогамии вызвано старением линий.

Представляют интерес исследования Диллера (Diller 1962) и де Халлера (de Haller 1964), показавших, что эксконъюганты и автогамонты у *P. putrinum* и *P. aurelia* могут в массе гибнуть из-за отсутствия стоматогенеза: в процессе конъюгации и автогамии, как известно, старый ротовой аппарат резорбируется, новый образуется заново после завершения метагамной фазы; если клетка лишена Ми или произошла от облученной УФ-лучами особи, стоматогенеза может не произойти. Выше мы отмечали, что при автогамии у *P. putrinum* выражена тенденция к дегенерации функциональных ядер, и часть экс-автогамонтов оказывается амикронуклеарной. Мы не располагаем достаточным экспериментальным материалом, чтобы обсуждать возможные причины смертности после автогамии; нельзя не отметить, однако, что аномалии в поведении ядер — это показатель глубоких патологических нарушений в клетке, а не причина ненормального хода реорганизационного процесса.

В литературе двух последних десятилетий нередко описываются случаи, когда инфузории двух линий (A и B) совершенно нормально конъюгируют между собой, при спаривании же их с третьей (C) наблюдается ненормальное течение реорганизационного процесса; конъюгация приводит к гибели партнёров. Так, аберрации в поведении ядер и стопроцентная смертность эксконъюгантов наблюдались при спаривании нормально-конъюгирующих линий со старыми клонами (Chen 1951), с линиями, продуцирующими антибиотики (Siegel 1954), с инфузориями, облученными лучами Рентгена (Kimball and Gaither 1955), с линиями, инфузории которые имеют гаплоидный или гипоплоидный Ми (Clark and Elliot 1956), с линиями иного сингена (Jennings and Opitz 1944; Chen 1946; Levine 1953), с линиями иного вида (de Garis 1935), с линиями, имеющими несовместимую цитоплазму (Ray 1955 a, b) и т. д. В нашей статье впервые описано явление противоположного характера, когда линии способны к нормальной конъюгации, автогамия же их оказывается патологическим процессом и для многих особей имеет летальный исход. Для нормального хода процесса ядерной реорганизации оказывается необходимым постоянный контакт партнеров, при отсутствии контакта процесс нарушается. Это неожиданный и пока еще необъяснимый вывод.

Применимость метода; некоторые перспективы

Поскольку для индукции автогамии нужны особи минимум трёх типов спаривания, метод применим только к видам со множественной системой типов спаривания; таковыми являются *P. putrinum* (Янковский 1962 b), *P. bursaria* (Jennings 1939), *Tetrahymena pyriformis* (Elliott and Hayes 1953; Gruchy 1955), возможно также близкий к тетрахимене вид *Colpidium truncatum* (Sonneborn 1957 b). Множественная система широко распространена у *Hypotrichida*, к примеру у *Euplotes* (Kimball 1939), *Oxytricha* (Siegel 1956), *Stylonychia* (Downs 1959). И у этих видов, однако, возможны определённые пре-

пятствия к применению метода: наличие суточной ритмичности в способности к спариванию (*P. bursaria* — Cohen 1964), неспособность к агглютинации у ряда видов гипотрихид, отсутствие реакции спаривания в сингене 5 *P. putrinum*, и т. д. По-видимому, данный метод может быть применен и к видам типа *P. multimicronucleatum*, дважды в сутки меняющим свой тип спаривания на противоположный (Sonneborn 1957; Sonneborn and Barnett 1958). Дальнейшее изучение этого метода целесообразно вести прежде всего на двух видах — *P. putrinum* и *T. pyriformis* [1].

Ядерные аномалии и смертность после автогамии у *P. putrinum* не являются стопроцентными, и метод „множественного спаривания" может применяться для самых различных целей, главным образом для получения гомозиготных (диплоидных), гаплоидных и амикронуклеарных линий без воздействия таких повреждающих факторов, как УФ-лучи (в опытах Кларка), лучи Рентгена (в опытах Кимбалла), высокие температуры (в опытах Соннеборна). Линии, выведенные от экс-автогамонтов, теоретически являются гомозиготными. Если у автогамонта удалить ротовой конус (и расположенный в нём пронуклеус), потомство должно быть гаплоидным. Если ротовой конус удален на более ранней стадии — перед III делением Ми, когда внутри конуса расположено единственное функциональное ядро клетки, — такие особи должны дать начало амикронуклеарным линиям. Можно ожидать, что у таких клеток произойдет регенерация Ма из фрагментов; по Диллеру (Diller 1962) у *P. putrinum* такая регенерация невозможна, поскольку амикронуклеарный эксконъюгант не образует рта и гибнет от голодания; однако процесс регенерации Ма у тех же рас *P. putrinum*, которые использованы для опытов по индукции автогамии, был вызван рентгеновским облучением конъюгантов (Ковалёва и Янковский в печати).

Чрезвычайно интересные результаты должно дать применение разработанного нами метода индукции автогамии в случае не-амфимиктических рас *P. putrinum* (сингены 1, 2, 5; миксотипы B, C и D), и особенно в случае факультативно-амфимиктических рас, где клетки способны к переключению амфимиктического способа реорганизации на апо- и автомиктический (в зависимости от миксотипа партнёра). Неясно, по какому пути пойдет внеконъюгационная ядерная реорганизация у таких рас.

Наличие своеобразной „системы миксотипов" (внутривидовых групп с различными способами ядерной реорганизации) делает изучаемый вид очень перспективным в генетическом отношении, и метод „множественного спаривания" должен стать одним из основных методов генетической работы с *P. putrinum*.

Резюме

Методом одновременного сливания инфузорий 3—7 линий *P. putrinum*, принадлежащих к 3—4 типам спаривания сингена 4 миксотипа A (облигатные амфимикты) у изучаемого вида впервые индуцирован процесс внеконъюгацион-

[1] Судя по недавней сводке Гекмана и Зигеля (Heckmann and Siegel, 1964) к моментальной агглютинации при смешивании линий неспособны не только гипотрихиды, но и тетрахимены; в любой момент эти инфузории имеют вещества спаривания, но в крайне незначительных количествах, и лишь после случайного контакта двух комплементарных особей начинается ускоренный синтез таких веществ (длящийся минимум 40 мин.). Остается заключить, что единственными видами, к которым приложим метод мульти-агглютинации, являются *P. putrinum* и *P. bursaria*.

ной ядерной реорганизации (автогамии). На препаратах, окрашенных по Фёльгену, изучены ядерные процессы у 583 автогамонтов; из них у 451 особи (75,2%) ядерные процессы протекают нормально — различимы I, II и III деления Ми, кариогамия, I, II и III деления диплоидного синкариона. У 132 особей (24,8%) отмечены разнообразнейшие отклонения в поведении Ми: дегенерация всех функциональных ядер после I, II или III делений созревания Ми; аномальные веретена деления; потеря хромосом делящимися ядрами; образование от 1 до 8 пронуклеусов либо их полное отсутствие; асинхрония в делении ядер внутри особи; гетероплоидия ядер особи. У 156 особей (26,9%) Ма распадается нетипичным образом. Основная часть наблюдавшихся отклонений не отмечена в контроле — при конъюгации тех же линий, когда процент отклонений незначителен — 3% при двойной конъюгации, 5.7% при тройной конъюгации. Предварительные опыты по определению жизнеспособности автогамонтов показывают, что значительная часть особей выживает и дает начало жизнеспособному потомству.

SUMMARY

Autogamy in singles occurs periodically in 4 related hymenostome species (*Paramecium aurelia, P. polycaryum, P. jenningsi, Tetrahymena rostrata*) and may be induced experimentally in other species by the techniques of Prowazek and Hiwatashi, in 2 particular cases — by these of Metz and of Diller. Autogamy is absent in *P. putrinum*; it was first induced by a new "multi-agglutination technique": a simultaneous mixture of 3 or more lines belonging to 3 or more mating types. All paramecia must be in condition favourable for mating. All selected for experiments lines belong to races M, MR and IR, syngen 4, subspecies *P. putrinum vernalis*, mixotype A (obligate amphimixonts). Autogamic process was never induced at mixtures of 2 complementary lines, or 3 to more lines belonging to 2 mt's only. The "multi-agglutination" yields a number of pairs, a low percent of autogamonts and a still lower percent of tri-conjugants (described separately, in Jankowski 1965 b).

In sum, 583 autogamonts were isolated and stained (Feulgen); 97 ones are figured in the article. The nuclear behavior is normal in 451 cell (75.2%); 132 animals exhibit aberrations in Mi behavior (24.8%) and 156 — in Ma breakdown process (26.9%). A description of the normal reorganization pattern is presented; it includes 3 fissions of Mi, paroral cone formation, caryogamy, disappearence of a cone, 3 fissions of synkaryon, nuclear differentiation.

Aberrations in Mi behavior reach 3% at control (double mating), 6% at tri-conjugation and 24.8% at autogamy; the relative number of aberrations is lowest at I Mi fission (7%), increases at II one (22%), further at III one (33%), reaches a peak at synkaryon formation and its I fission (36%), then become slightly lowered to 32 and 31%. A number of anomalies occurs which were never recorded in a control, e. g. degeneration of all daughter nuclei after I, II or III fissions of Mi; formation of 0—8 pronuclei; atypical fission spindles; ruptures of such spindles with lack of chromosomes; ruptures or too rapid resorption of degenerated nuclei. Asynchrony in nuclear fissions is common. A synkaryon may or may not be formed; pronuclei may behave as hemikarya; supernumerary nuclei, haploid or hypoploid, may be revealed in addition to synkaryon; heteroploidy is peculiar for a large ratio of cells; atypical nuclear differentiation in ex-autogamonts commonly occurs.

Mi anomalies are concerned frequently with anomalies in Ma breakdown; considering the latter, we find again those distributed in a control (a delay in caryorrhexy) as well as peculiar pictures never recorded for control pairs: a partial Ma breakdown, with conservation of a voluminous amorphous piece of Ma; atypical ruptures of macronuclear branches yielding a number of smallest dust-like Feulgen positive particles filling the cytoplasm. Ma is much more resistant to deleterious agents than Mi and it must be noted that such changes were not induced in Ma even by X-rays (Kovaleva i Jankowski, in press) in doses evoking 100% anomalies in Mi behavior (at double conjugation of the same lines).

Some preliminary data on the viability and fission rate show that near 30% of cells die after autogamy without cell fissions. This lethality is not possibly a result of the ageing of lines used for experiments (3—4 years in laboratory cultures) since double conjugation of the same lines is practically normal phenomenon.

It seems probable that autogamy at "multi-agglutination" is a result of an uncompleted process of a tri-conjugating group formation. A series of data indicates that all three mates composing a triplet belong to three different mt's (A, B, C). If a third mate is not joined firmly and separates from two other mates after a time sufficient for resorption of the cilia in a contact area, its Mi may be activated. According to Nanney-Metz "activation theory", all other phenomena (Ma breakdown, paroral cone formation, nuclear and cortical reorganization) will automatically begin. It is a high probability that in the "multi-agglutination" technique, here proposed, like in all pre-existing techniques, a preliminary union of cells with different mt's is inevitable condition of an induced autogamy.

We may expect that the "multi-agglutination" technique is appliable to some other ciliates that are capable for an intensive agglutination and possess multiple mt systems. *Paramecium bursaria, Tetrahymena pyriformis* and *Colpidium truncatum* satisfy to both conditions, but hypotrichs seem to lack the first one. The induction of autogamy in *P. putrinum* may be possibly used for obtaining homozygotes; for haploidization (at removal of the paroral cone with a nucleus after II fission of Mi); for obtaining cells with no Mi and regenerating Ma (at removal of the cone after III fission of Mi, well seen in vivo, before caryogamy). All this will be inevitable steps to solve the problem of the origin of so puzzling "mixotype system" in a species under investigation.

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ПОДПИСИ К ТАБЛИЦАМ I—IV

Нормальный ход процесса реорганизации ядер при экспериментально индуцированной автогамии у *Paramecium putrinum*. Реакция Фёльгена

1—3: I деление микронуклеуса
4—5: II деление микронуклеуса
6—7: Интерфаза после II деления микронуклеуса
8: Пронуклеус в околоротовом конусе, вершина его обращена к вестибулярному отверстию
9: Пронуклеус в околоротовом конусе, вид сбоку
10: Интерфаза после III деления микронуклеуса: начало ветвления Ма
11: Кариогамия; синкарион виден сбоку
12: Кариогамия; синкарион виден с полюса
13: Ранняя профаза I деления синкариона
14: 2 ядра после I деления синкариона
15—16: 4 ядра после II деления синкариона
17: 8 ядер после III деления синкариона
18: Зачатки макронуклеусов у экс-автогамонта

Аберрации в поведении ядер при экспериментально индуцированной автогамии у *Paramecium patrinum*. Реакция Фёльгена

19: Фрагментация макронуклеуса — гемиксис
20: Потеря хромосом (стрелка) при I делении микронуклеуса
21: Образование 8 пронуклеусов
22: Все ядра после II деления Ми остаются функциональными
23: После II деления Ми нет ядра в околоротовом конусе
24: После II деления Ми остаются функциональными 2 ядра
25—27: Процесс частичного распада микронуклеуса
28—29: Деление пронуклеусов без их сливания в синкарион
30: Нормальные размеры ядер после III деления синкариона (для сравнения с 33—36)
31: Частичный распад микронуклеуса: стадия I деления синкариона
32: Аномальные веретена деления; стадия II деления синкариона
33: Гетероплоидия ядер у экс-автогамонтов
34: Экс-автогамонт с крайне мелкими (гипоплоидными?) ядрами
35: Экс-автогамонт с 4 гигантскими (гиперплоидными) ядрами
36: Деление 4 гиганских ядер

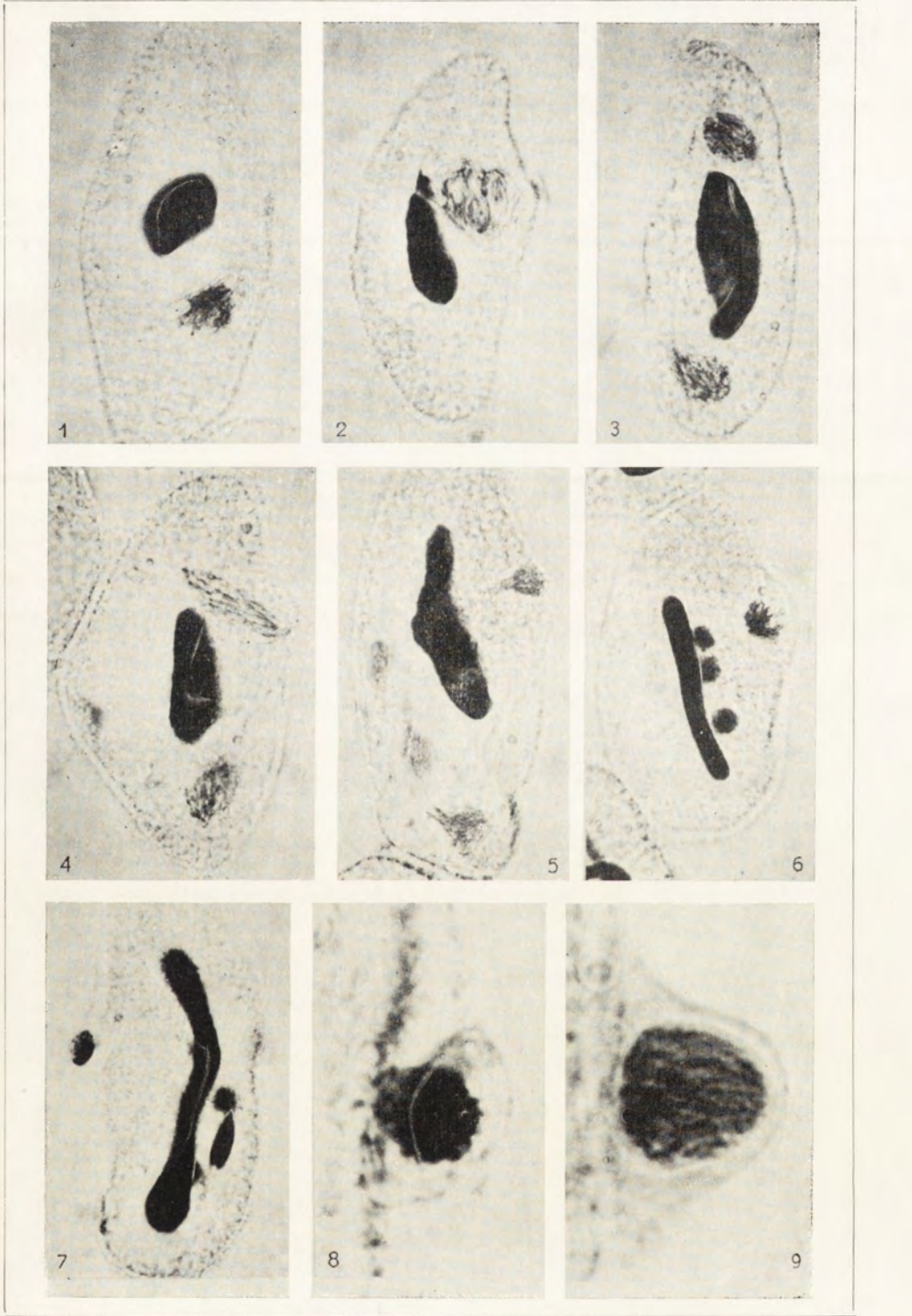
EXPLANATION OF PLATES I—IV

Normal course of nuclear reorganization during autogamy experimentally induced in *Paramecium putrinum*. Feulgen staining

1—3: 1st division of micronucleus
4—5: 2nd division of micronucleus
6—7: Interphase after the 2nd Mi division
8: Pronucleus in the paroral cone its upper part is directed towards vestibulum
9: Pronucleus in the paroral cone, side view
10: Interphase after the 3rd Mi division, Ma branching begins
11: Karyogamy; synkaryon in a side view
12: Karyogamy; synkaryon seen from the pole
13: Early prophase of the 1st division of synkaryon
14: Two nuclei after the 1st division of synkaryon
15—16: Four nuclei after the 2nd division of synkaryon
17: Eight nuclei after the 3rd division of synkaryon
18: Macronuclear anlagen in an ex-autogamont

Aberrations of nuclear behaviour during autogamy experimentally induced in *Paramecium putrinum*. Feulgen staining

19: Fragmentation of macronucleus — hemixis
20: Chromosome deficiency (arrow) during the 1st division of Mi
21: Formation of 8 pronuclei
22: All nuclei after the 2nd Mi division remain in functional state
23: Lack of nucleus in the paroral cone after 2nd Mi division
24: Two functional nuclei remain after the 2nd Mi division
25—27: Partial dispersion of macronucleus
28—29: Division of pronuclei without their fusion in synkaryon
30: Typical size of nuclei after the 3rd division of synkaryon (for comparison with 33—36)
31: Partial dispersion of Ma; stage of the 1st division of synkaryon
32: Atypical fission spindles; stage of the 2nd division of synkaryon
33: Nuclear heteroploidy in ex-autogamonts
34: Ex-autogamont with extremely small (hypoploid?) nuclei
35: Ex-autogamont with 4 enormous (hyperploid) nuclei
36: Division of 4 enormous nuclei



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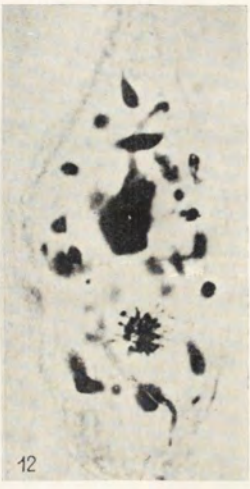
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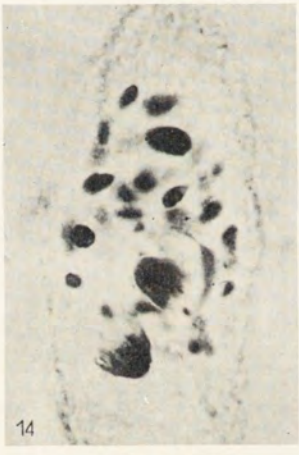
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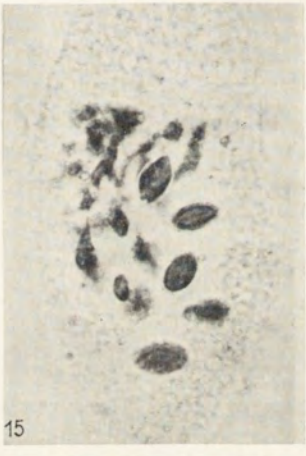
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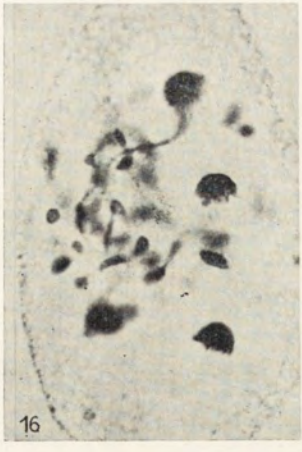
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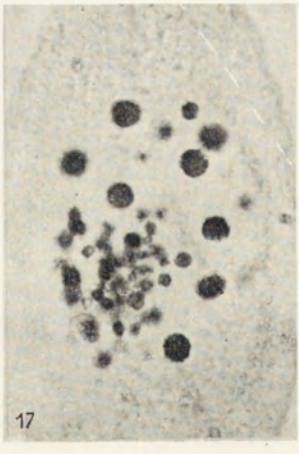
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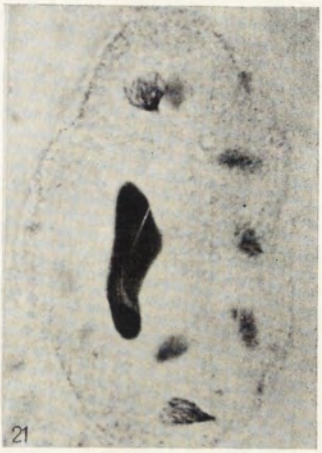

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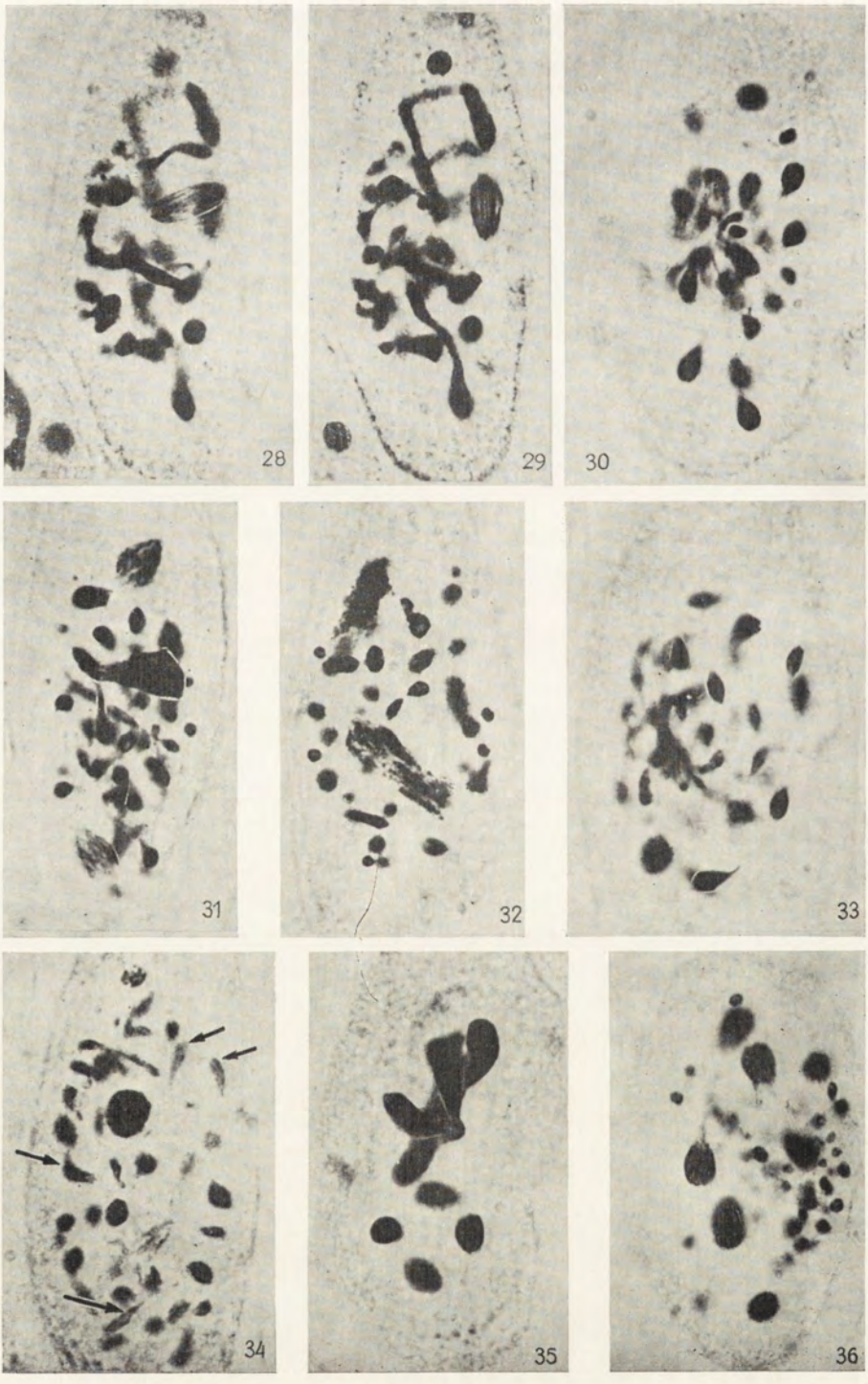

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ACTA PROTOZOOLOGICA

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Izabela BIERNACKA

Ausscheidung gehäusebildender Substanzen durch reife Formen gewisser Arten der Gattung *Tintinnopsis* Stein

Wydzielanie substancji pancerzykotwórczej u osobników dojrzałych niektórych gatunków rodzaju *Tintinnopsis* Stein

Während meiner Untersuchungen der innerhalb der Form im Teilungsstadium der Gattung *Tintinnopsis* Stein (Biernacka 1952) vorgehenden Prozesse habe ich festgestellt, dass sich im Moment der Erscheinung des Kernbandes und des ersten Peristomumrisses am aboralen Ende der Form zahlreiche kleine Körnchen bilden, die sich nach der Mann'schen Methode rot färben. Charakteristische Verlagerungen dieser Körner bei den untersuchten Arten vom aboralen Ende der Form bis in die Peristomgegend der oberen nachfolgenden Form (anterior daughter), wo sie die ganze Periode der neuen Gehäusebildung verbleiben, lassen vermuten, dass sie mit dem Bau eines neuen Gehäuses im Zusammenhang stehen. Sobald der Bau des neuen Gehäuses beendet ist, verlagern sich die Körner erneut in Richtung des aboralen Endes und verschwinden allmählich. Sie treten erneut erst im Vorteilungsstadium des gegebenen Wimpertierchens in Erscheinung. Man könnte demnach vermuten, dass der Gehäusebildungsprozess einmalig ist und der Aufwuchs der Form bis zur Grösse der Muttergestalt von ihm begleitet wird, wonach er aufhört und in der Vegetationsperiode nicht mehr auftritt.

Zahlreiche Formen der Gattung *Tintinnopsis* Stein scheinen jedoch darauf hinzuweisen, dass die Wimpertierchen in manchen Fällen zur Ausscheidung gehäusebildender Substanzen auch in der Vegetationsperiode fähig sind und sich einen Teil des Gehäuses nachzuerarbeiten imstande sind. Derartige „nachgearbeitete" Gehäuse habe ich bei zwei Arten feststellen können, nämlich bei *Tintinnopsis lohmanni* Laack. (Abb. 1 A) und *Tintinnopsis campanula* Ehrbg. (Abb. 2 A), insbesondere bei der letzteren. Die Bildung des fehlenden Gehäuseteiles erfolgte, wenn das bestehende Gehäuse eine Beschädigung erlitt und „ausgebessert" werden musste, oder auch wenn in der Zeit des Aufwuchses des Wimpertierchens nach der Teilung im Bau des Gehäuses eine Hemmung eintrat.

Die Gehäusestrukturen dieser beider Arten unterscheiden sich etwas voneinander. Die Gehäusewände der *Tintinnopsis lohmanni* Laack. sind von 3.2 μ bis 3.5 μ dick und besitzten ausgeprägte konvexe, aufeinander übersgreifende Verdickungen. Die Gehäusewände der *Tintinnopsis campanula* Ehrbg. dagegen sind nur 1.55 μ bis 2.2 μ dick und haben dünn verteilte Verdickungen (Biernacka 1952).

Die Wimpertierchen der Gattung *Tintinnopsis* Stein bilden ihre Gehäuse derart, dass die obere Nachfolgeform (anterior daughter) sich nach seiner Loslösung vom unteren durch eine schnelle progressive Drehbewegung von diesem entfernt und in der Peristomgegend eine gehäusebildende Substanz ausscheidet, die nach unten abrinnt. Im Erstarren bildet es einen weiteren verschmälerten Teil des Gehäuses in der Form eines Beckchens. Der weitere Zufluss der ausgeschiedenen Substanz führt zu einer Verlängerung des Gehäuses in Richtung des Peristoms. Die Gehäusebildung hört auf, wenn das Wimpertierchen mit einem noch nicht voll ausgebildeten Gehäuse in seiner progressiven Drehbewegung durch ein Hindernis an dem es hängen geblieben ist, behindert wird. In diesem Fall ist der Wuchs des Wimpertierchens selbst normal, jedoch sein Gehäuse erreicht nicht die normale Grösse. Erfolgt nach einem gewissen Zeitraum die Loslösung des Gehäuses und das Wimpertierchen erlangt seine progessive Drehbewegungsfähigkeit wieder, so erfolgt der Weiterbau des Gehäuses, bis zwischen der Grösse des Wimpertierchens und der Länge des Gehäuse Gleichgewicht eintritt.

Bei *Tintinnopsis lohmanni* Laack. habe ich meistens verlängerte Gehäuse angetroffen. Der „nacherarbeitete" Teil unterscheidet sich immer vom alten Teil und zwar dadurch, dass er durchsichtiger und zarter ist (Abb. 1 B—D). In seinen späteren Vegetationsperioden ist das Wimpertierchen scheinbar nur

Abb. 1. Gehäuse *Tintinnopsis lohmanni* Laack. A. Normales Gehäuse. B—D. „Nachgearbeitete" Gehäuse. E—F. „Ausgebesserte" Gehäuse

Ausscheidung einer geringeren Menge gehäusebildender Substanz fähig. Bei dieser Art habe ich auch „ausgebesserte" Gehäuse angetroffen. In dem auf Abb. 1 E und F gezeigten Fall erlitt das Gehäuse an seinem Fuss eine Beschädigung und wurde „ausgebessert". Dieser Teil unterscheidet sich deutlich von dem ganzen übrigen Gehäuse, ist glatt, durchsichtig und nicht, wie normalerweise, geschlossen, sondern besitzt am Ende eine runde Öffnung.

Bei der Art *Tintinnopsis campanula* Ehrbg. ist die „Ausbesserung" der beschädigten Gehäuse weit öfter anzutreffen als bei der vorher genannten Art, was vielleicht darauf zurückzuführen ist, dass die Gehäuse *T. campanula* Ehrbg. zarter sind und daher öfter eine Beschädigung erleiden können Art, was vielleicht darauf zurückzuführen ist, dass die Gehäuse *T. campa*-Teils des deutlich unbeendeten Gehaüses, das wahrscheinlich in seiner Gestaltungsperiode auf ein Hindernis gestossen ist, angetroffen (Abb. 2 F).

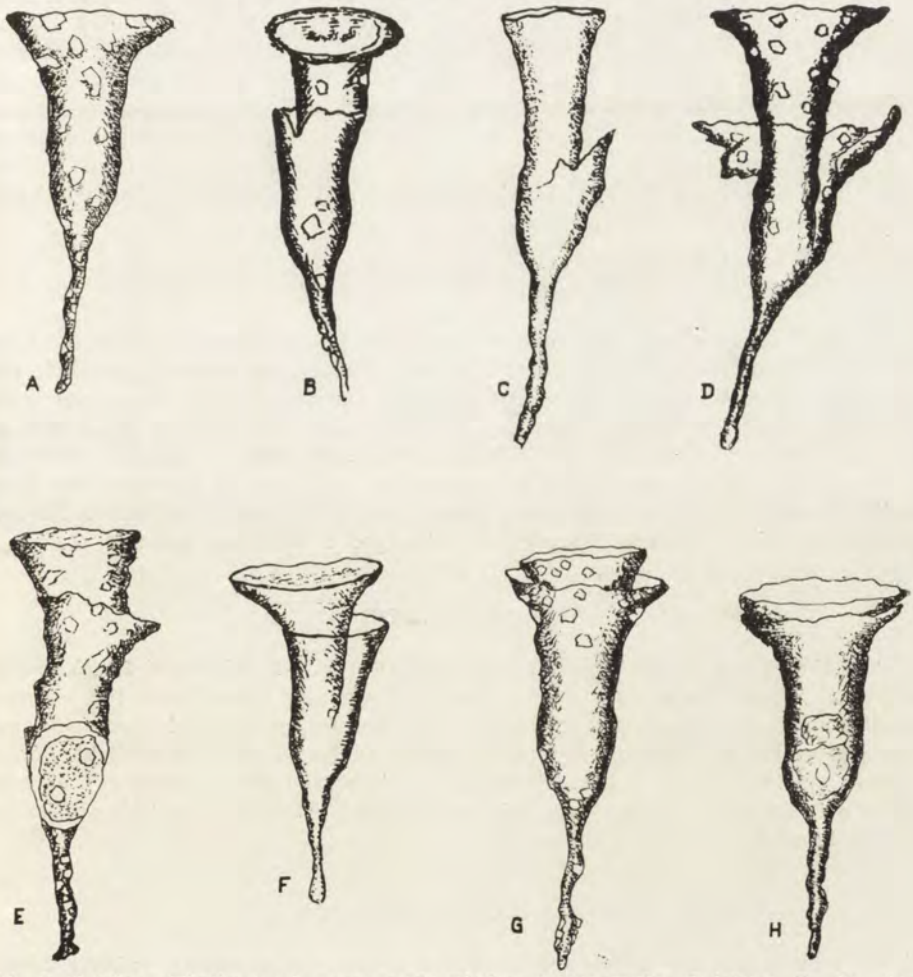

Abb. 2. Gehäuse *Tintinnopsis campanula* Ehrbg. A. Normales Gehäuse. B—D. „Ausgebesserte" Gehäuse. E. „Nachgearbeitete" Gehäuse. F. Ergänztes Gehäuse. G—H. Normale Gehäuse mit „nachergearbeitetem" Ansatz

In einem Fall erfolgte die Bildung des ursprünglichen Gehäuses scheinbar etappenweise, wodurch es nicht eine gerade Gestalt erhielt wie ein normales Gehäuse, sondern eine auf eine Seite gekrümmte Form (Abb. 2 E). Das Wimpertierchen hat sich einen auf die entegengesetzte Seite gekrümmten Ansatz nachgearbeitet. Eine ganze Reihe von an verschiedenen Stellen der mittleren Ostsee gefangenen Gehäusen hatte im oberen Teil des normalen Gehäuses einen nacherausgearbeiteten Ansatz.

Diese Beobachtungen lassen die Vermutung zu, dass die Ausscheidung der gehäusebildenden Substanz bei *Tintinnopsis campanula* und *Tintinnopsis lohmanni* nicht ausschliesslich mit der Teilungsperiode und dem Aufwuchs des Wimpertierchens verbunden ist. Sie kann auch in der trophischen Periode zwischen zwei aufeinanderfolgenden Teilungen stattfinden.

Laackmann 1908 berichtet, er hat Gehäuse *Tintinnopsis campanula* Ehrbg. mit doppelten Ansatz ziemlich häufig angetroffen, gibt aber keine Aufklärung dieser nicht sehr seltenen Erscheinung. Zur Klärung dieses Phänomens wurden an *T. campanula* Untersuchungen unternommen, ob nicht vielleicht das Verhältnis der Länge des Schwänzchens zur Länge des ganzen Gehäuses eine Rolle spielt und ob die „Hinzumauerung" des zweiten Kelches nicht eine „Verbesserung" dieses Verhältnisses darstellt. Zahlreiche Messungen ursprünglicher und verlängerter Gehäuse führten jedoch zu keinem ausschlaggebenden Ergebnis. Das Phänomen des doppelten Ansatzes bleibt infolgedessen weiterhin ungeklärt.

Zusammenfassung

Es wurden Gehäuse mit „nacherausgearbeitetem" beschädigtem Teil des normalen Gehäuses oder mit bereits in der trophischen Periode deutlich verlängerem Gehäuse angetroffen. Aus den Untersuchungsergebnissen der anormalen Gehäuse wird die Folgerung gezogen, dass die Ausscheidung der gehäusebildenden Substanz von *Tintinnopsis campanula* und *T. lohmanni* nicht ausschliesslich mit der Teilungsperiode und dem Aufwuchs des Wimpertierchens verbunden sein muss, sondern auch in der trophischen Periode zwischen zwei aufeinander folgenden Teilungen stattfinden kann.

STRESZCZENIE

Autorka spotykała *Tintinnopsis campanula* Ehrbg. i *T. lohmanni* Laack. o pancerzykach z „dorobioną" uszkodzoną częścią pancerzyka normalnego, lub wyraźnie przedłużonych już w okresie troficznym. Z wyników przebadania tych anormalnych pancerzyków wysnuto wniosek, że wydzielanie substancji pancerzykotwórczej u *Tintinnopsis campanula* i *T. lohmanni* nie jest związane wyłącznie z okresem podziału i wzrostu orzęska, lecz może występować również w okresie troficznym między dwoma kolejnymi podziałami.

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Cytoplasmic streaming in *Paramecium caudatum* exposed to electric field

Prąd cytoplazmatyczny u *Paramecium caudatum* w polach elektrycznych

Out of the two main types of the cytoplasmic streaming — the cyclosis and the amoeboid movement — the last one has been looked upon as a manifestation of the contractile activity of ectoplasm since the beginning of the protozoological research. According the theory of Goldacre 1961, this movement is a consequence of contraction and solification of the ectoplasmic gel in the posterior body part of amoeba, i.e. a result of reaction of an actomyosin-like protein with ATP.

A movement of a linear type occurs also in *Paramecium caudatum* when placed in the field of direct current. This movement directed towards the cathode was observed by Carlgren 1905, Coehn und Barratt 1905, Fabre 1942, and Grębecki 1962. These authors considered that the linear stream of endoplasm occurring in the ciliate in electric field is evoked by the action of the electrokinetic forces. However, a similitude of this movement to the amoeboid one suggests that the ectoplasmic contraction which occurs in *Paramecium* under the influence of the electric current, propels the endoplasm towards the cathode. Consequently, not the electrokinetic forces but the active contraction could be assumed as the source of the endoplasm movement.

In order to verify the above hypothesis, the behaviour of *Paramecium* endoplasm was investigated in the direct and alternating currents as well as the influence of the electric field upon the decalcified ciliates.

Material and methods

The material of the experiments was *Paramecium caudatum* Ehrbg. Ciliates were rinsed in distilled water and fed with the suspension of India ink; 15—20 min. after feeding they were placed in a cuve and exposed to the action of the current; then the movement of the endoplasm was observed in the cell.

In some cases the ciliates were fixed with osmium and photographed. Then the distribution of the food vacuoles, filled with India ink, served as indice of endoplasm displacements. Ciliates fixed immediately after feeding with ink, not exposed to the action of electric or chemical stimuli, were used as control (Pl. I 1).

Results

Effect of the direct current

Ciliates were exposed to the action of direct current (15.7 mA/cm²). The circuit was broken after 30 secs. In this moment the typical deformations of the ciliate body were yet observable. In the individuals which were in the homodrome position in the moment of switching on the current, a strong ectoplasm contraction occurs and simultaneously an endoplasmic streaming starts flowing towards the cathode. Fig. 1 shows schematically the source and the

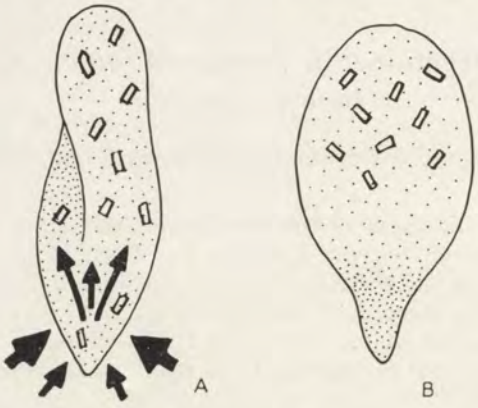

Fig. 1. Ectoplasm contraction and endoplasm movement in *Paramecium* exposed to direct current. A. Scheme representing the course of reaction. B. Its final result

direction of the endoplasmic streaming in a ciliate in the field of the direct current, as well as the change of the body shape evoked by the contraction. Pl. I 2 demonstrates the food vacuoles pushed towards the body part which is turned to the cathode. This shift is the result of the endoplasm flow in this direction.

Effect of the alternating current

Ciliates were exposed to the action of alternating current of the same density as in the preceding experiment. The current was switched out after 30 secs. In the individuals oriented in parallel to the direction of the electric field in the moment of making the current, a strong contraction of ectoplasm on both body ends and two endoplasmic streamings were observable.

The scheme of this process is represented in Fig. 2. In the Pl. I 3 the food vacuoles pushed off to the middle of the ciliate body are seen. This shift has been evoked by the streaming of endoplasm flowing from both body ends, in this case.

Effect of calcium and EDTA

Before experiment the ciliates were rinsed several times with distilled water. Chelating of calcium by means of EDTA (0.5—2.5 mM) was performed. Subsequently the ciliates were placed in a cuve and exposed to the action

of the direct current of the same intensity as in the previous experiments. Ciliates were not deformed and endoplasmic streams were not visible in them. In higher concentrations of EDTA (2.0 and 2.5 mM), deformations failed to occur even when the flow of the current lasted 3 min.

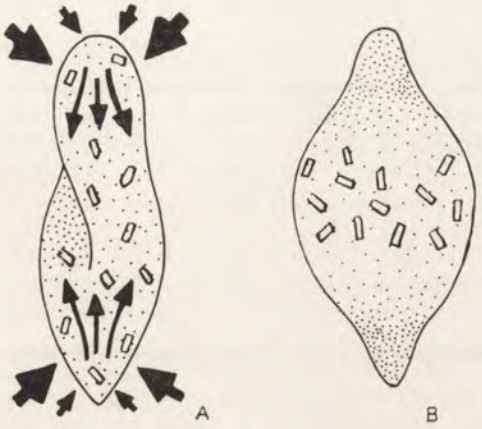

Fig. 2. Ectoplasm contraction and endoplasm movement in *Paramecium* exposed to alternating current. A. Scheme representing the course of reaction. B. Its final result

Similar but less striking effects were obtained after application of sodium citrate (5.0 mM): a slight deformation occurred only after a prolonged electric stimulation.

In another series of experiments the incubation in EDTA (0.5 mM) was followed by addition of $CaCl_2$ (2.0 mM); ciliates regained their contraction capability, i.e. they were deformed similarly as the control material, and showed the endoplasmic streaming again.

Discussion

In normal conditions, in *Paramecium caudatum* a circular endoplasmic streaming occurs — the cyclosis. The linear endoplasmic streaming in *Paramecium* exposed to the action of a direct current — observed first by Carlgren 1905 — was looked upon as an electrokinetic phenomenon. According to Carlgren this is an electroosmotic stream of the liquid endoplasm fractions. It follows from the investigations of Ludloff 1895, Statkevitch 1904, Kamada 1928, Kamada and Kinosita 1945, and Hisada 1952 that a strong ectoplasm contraction and deformation of the body occurs in *Paramecium* under the influence of a strong electric current. As consequence of this deformation, in *Paramecium* placed in the homodrome position, the anterior (cathodal) part becomes thick and round while the posterior (anodal) contracts and shortens. When the alternating current had been used (Hisada 1952), contraction on both body ends occurred being slightly stronger in the posterior part. Contraction in *Paramecium* is accompanied by electric hyperpolarization of the membrane (Kinosita, Dryl, and Naitoh 1964).

The above facts allow to consider the movement of endoplasm observed in the present experiments as consequence of the ectoplasm contraction evoked by the current. This postulation has been ascertained in this study by following findings:

1. It was stated that the ectoplasm contraction and the flow of the endoplasmic streaming are synchronous which seems to indicate a causal relation between both phenomena.

2. The alternating current should have evoked no streaming if only the electrokinetic forces had acted. Nevertheless, it causes a flow of endoplasm from both body ends towards its middle. The endoplasmic stream might consequently be the result of ectoplasm contractions on both ends of the body.

3. This conclusion is supported also by the lack of the endoplasm movement in decalcified protozoa placed in the field of electric current.

As commonly known, calcium has a decisive influence upon the activity of the muscle fiber and decalcification fully anihilates the contractility. Calcium is also the factor influencing the contraction activity of protozoan cells. Addition of Ca^{2+} ions to the medium brings about the ectoplasm contracture in *Paramecium* (K a m a d a and K i n o s i t a 1945). According the findings of S a t o 1962 reduction of the calcium content in the medium evokes slowing down the flow rate of the endoplasmic streaming in *Acetabularia calyculus*.

In *Paramecium* decalcified by means of EDTA or by sodium citrate and placed subsequently in the direct current, neither the ectoplasm contraction nor flow of the endoplasmic streaming could be obtained in this study. The recalcification abolished this inhibition and renormalized the response of ciliates which appear under the influence of the current.

It follows from the above experiments that an analogy exists between the amoeboid movement and the linear endoplasmic stream occurring in ciliates in the field of the direct current. Photograms (Pl. I 4) demonstrate the similitude in localization of the contraction site and of the so called hyaline cap in *Paramecium caudatum* exposed to the direct current and in the monopodial form of *Amoeba proteus*. In both cases the active contraction of ectoplasm seems to be the source of the movement of endoplasm.

S u m m a r y

In the direct current field, endoplasm starts streaming towards the cathode. This stream was considered earlier as electrokinetic one. However, it cannot be due to the electrokinetic forces, but results from an active ectoplasm contraction because: 1. the ectoplasm contraction and the endoplasm streaming are strictly correlated in time; 2. a bidirectional stream arises in alternating current; 3. the contraction and the streaming are simultaneously and reversibly blocked by decalcification.

STRESZCZENIE

W polu prądu stałego strumień endoplazmy płynie w kierunku katody. Zjawisko to uważane było za wynik działania sił elektrokinetycznych. Wydaje się jednak, że jest ono rezultatem aktywnego skurczu ektoplazmy ponieważ: 1. skurcz

ektoplazmy i prąd endoplazmatyczny są ze sobą zsynchronizowane; 2. prąd zmienny powoduje przepływ endoplazmy z obu końców ciała pierwotniaka ku jego środkowi; 3. skurcz ektoplazmy i prąd endoplazmatyczny są hamowane przez czynniki odwapniające.

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EXPLANATION OF THE PLATE I

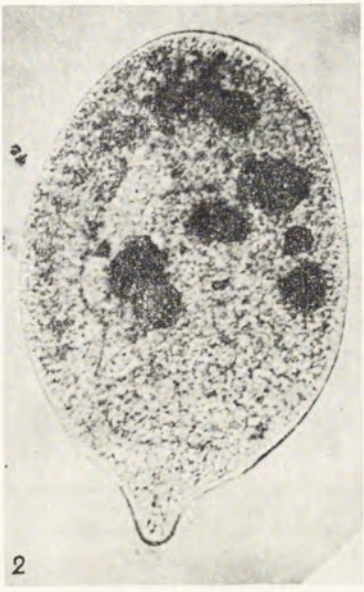
1: Random dislocation of food vacuoles in a control specimen of *Paramecium caudatum*

2: Food vacuoles pushed forwards by the shift of endoplasm towards the cathode during DC stimulation

3: Food vacuoles pushed into the middle of the body by two polar shifts of endoplasm occurring during AC stimulation

4: Comparison of the form assumed by *Paramecium caudatum* in a DC field and the monopodial form of *Amoeba proteus*


1

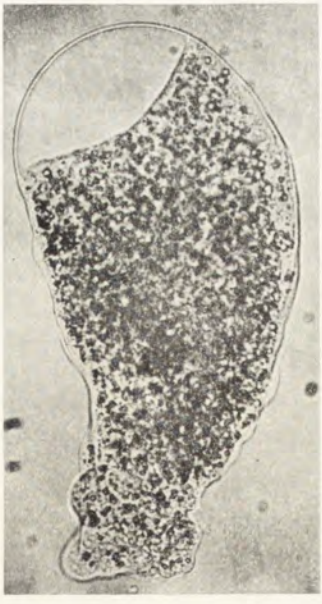

2


3

HYALINE CAP

4

CONTRACTING TAIL



L. Czarska auctor phot.

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Role of Ca^{2+} ions in the excitability of protozoan cell. Decalcification, recalcification, and the ciliary reversal in *Paramecium caudatum*

Znaczenie jonów Ca^{2+} w pobudliwości komórki pierwotniaczej. Odwapnianie i nawapnianie, a rewersja rzęskowa u *Paramecium caudatum*

The essential role of calcium in excitation and response phenomena is conspicuously emerging in the recent physiological research. Its hyperpolarizing effect upon the resting cell membrane, and significant influence upon the action potential, are commonly revealed in various excitable cells. The extensive biochemical research made evident the necessity of presence of Ca ions for producing the reaction of contractile protein, i.e. the motor response. Numerous studies support the view that in the muscle Ca is a coupling agent which links the electrical events with the mechanical ones, i.e. the excitation with the motor response: a depolarization wave, brought about by the action potential, liberates Ca ions from their binding sites in the membrane structures (mainly from the endoplasmic reticulum); then, Ca reaches the contraction site and promotes the response of the contractile protein.

Experimental data suggest important role of Ca in excitation and motor response of protozoan cells. Calcium component of the resting membrane potential, revealed first in *Opalina* by Kinosita 1954, was confirmed subsequently in the same organism by Ueda 1961, Naitoh 1964, and in *Paramecium* by Yamaguchi 1960 and by Kinosita, Dryl and Naitoh 1964 b. Calcium role in inducing contraction of the ciliary contractile protein was investigated by Hoffmann-Berling and later on by Seravin 1961. Many various facts summarized by the author previously (Grębecki 1963) allow to suggest an analogy between the external Ca action and the effect of inward current (anodal stimulation), and between the external K action and the cathodal effect. This analogy is based on the findings of various authors and it was demonstrated for the ciliate cell in similar way as it was done by Tobias 1960 for the axon. External Ca^{2+} stimulates the normal ciliary stroke and antagonizes the action of agents which promote the ciliary reversal, i.e. a gradual decrease of external Ca^{2+} makes the reversal much easier. It remains, however, a puzzling paradox that a strong decalcification exerts an opposite effect, namely the reversal of ciliary beat could not be induced at all, by chemical factors (Kamada and Kinosita 1940)

or by stimulation with cathodal current (F a b r e 1947); under these conditions the ectoplasmic contraction in *Paramecium* also was inhibited (C z a r s k a 1965).

It was calculated by J a h n 1962 (basing on experimental data of K a m a d a and K i n o s i t a 1940) that the calcium-potassium antagonism should follow the Donnan ratio. As a matter of fact, it was lately confirmed that at a constant $[K^+]/\sqrt{[Ca^{2+}]}$ ratio in the medium the duration of ciliary reversal in *Paramecium* remains largely constant with no regard to the absolute ions concentration (G r ę b e c k i 1964). At the same ionic equilibrium the pulsation rate of contractile vacuoles in *Paramecium* may be also stabilized (C z a r s k a 1964). It follows from the Gibbs equation and from the empirical evidence given by Donnan that a constant $[K^+]/\sqrt{[Ca^{2+}]}$ ratio in the medium brings about a constant and definite density of calcium ions upon the adsorption sites (provided that only these two ions are considered and the other essential factors remain also unchanged). It means presumably that active state of protozoan cell remains constant at constant calcium level in its membrane or in its cortical layer, and the excitation-reaction processes start when the density of bound calcium become changed.

At present, four different concepts may be applied for explanation of the part taken by Ca in the ciliary reversal:

1. Theory of d i r e c t a c t i o n was accepted by the major part of earlier workers (e.g. M a s t and N a d l e r 1926, O l i p h a n t 1942). According to this view, various chemical agents evoke the reversion of ciliary beating directly, by their specific action. Under such conditions calcium would be simply an antagonist of potassium ions, not involved in the essential mechanism inducing the ciliary reversal.

2. C a l c i u m u p t a k e concept is not a theory expressed explicit in physiology of ciliates; it represents rather a possibility of direct analogy between the ciliary reversal and the muscle contraction. It might be now indentified with the hypothesis of Ca-coupling in muscle. According to this postulation, it should be assumed that the stimuli liberate calcium ions bound to the membrane and/or to the cortical structures, these ions enter the cell and promote the inversed response of cilia. Consequently, it could be conceived that after stronger stimulation more Ca is depleted and the ciliary reversal lasts longer.

3. C a l c i u m l o s s theory postulates that the ciliary reversal is evoked only by decrease of Ca content inside the cell (K a m a d a 1938 and 1940), or decrease of Ca density upon the membrane (J a h n 1962). It means that the ciliary reversal would occur when Ca is desorbed, with no regard to the subsequent fate of liberated ions.

4. C a l c i u m c o n t e n t hypothesis is another possible explanation of the available data, which is considered by the author in the present report. This concept is based on the assumption that the behaviour of ciliate depends not on the loss or uptake of liberated Ca^{2+} ions, but it is related to the amount of calcium resting on the adsorption sites and influencing the state of the cell membrane. Consequently, it should be conceived that the degree of excitation corresponds to the gradual changes of the amount of bound calcium. Of course, the available experimental methods allow only to change the Ca content in any direction, but its effective value cannot be quantitatively determined.

An attempt of evaluation of the above concepts is the aim of this study.

The author wishes to express his hearty thanks to Doc. S. Dryl for reading the manuscript and for the stimulating discussion.

Methods

Paramecium caudatum, grown in the milk-fed culture, served as test organism. All experiments were carried out in 20—22°C.

In some experiments the duration of ciliary reversal was taken as an index of induced response. It was recorded in the same manner as described in an earlier report (Grębecki 1964). Most experiments concerned, however, only the kind of ciliary response manifested approx. within the first minute following the chemical stimulation, since the renormalization of ciliary behaviour is beyond the scope of this study. Some responses were recorded by means of the photomacrographic registration technique of Dryl 1958.

Recognition of the main types of ciliary responses in *Paramecium* is very essential for the present study, and their brief definition seems to be useful for further considerations.

1. Normal movement (NM): animals swim forwards tracing an elongated spiral path (see Pl. I 1); metachronal ciliary waves originate in the caudal region and are propagated forwards (Párducz 1954).

2. Continuous ciliary reversal (CCR): animals swim backwards following an apparently straight, slightly spiralized path (Pl. I 2); metachronal waves are propagated backwards (Párducz 1954), beginning from the cytostome (Grębecki 1965 a).[1]

3. Periodic ciliary reversal (PCR): animals periodically change the forward movement for the backward one (Pl. I 3); this behaviour was described by Eisenberg-Hamburg 1932 and by Grębecki i Kuźnicki 1955 in paramecia exposed to $SrCl_2$ or $BaCl_2$ solutions, and its study was greatly advanced by Dryl 1961 and Kinosita, Dryl and Naitoh 1964 a, c; recently the evidence is brought by Kuźnicki (in preparation) that PCR may be induced by quite a number of inorganic cations[1].

4. Partial ciliary reversal (PaCR): animals describe circles or loops on the spot (Pl. I 4); this is involved by the fact that only a part of cilia beat inversely, while movement of others is normal in the same organism (Párducz 1959), and the dislocation of normal and reversal areas follows the dorso-ventral asymmetry of the ciliate (Grębecki 1965 a).

It is worth to be noted that so far no other types of ciliary behaviour were reported in paramecia exposed to the uniform chemical changes in the surrounding medium.

Decalcification during normal movement

Effect of Ca dilution

Paramecia were incubated in $CaCl_2 + KCl$ solutions, mixed in ratios which still did not change the normal direction of ciliary movement (cf. the first column of the Table 1). After about 1 hr. the $[K^+]/\sqrt{[Ca^{2+}]}$ ratio was

[1] The terms "continuous ciliary reversal" and "periodic ciliary reversal" were introduced by Dryl 1961 in his report on the effects of $BaCl_2 + CaCl_2$ solutions on the ciliary activity in *Paramecium.*

considerably increased up to the values giving the ciliary reversal. However, it was not obtained — as commonly done — by rising the K content, but by reducing the Ca concentration (see the second column of the Table 1). It means that the intensity of factor commonly considered as inducing CCR (K^+) remained unchanged. Nevertheless, clear ciliary response was obtained (the third column in the Table 1).

Table 1

Effect of reducing the external Ca concentration without changing the K concentration. Response is given in sec. of duration of CCR

Initial medium		Stimulation medium		CCR in sec.
$CaCl_2$ mM	KCl mM	$CaCl_2$ mM	KCl mM	
4	8	0.25	8	80
4	8	1	8	20
8	16	0.5	16	140
8	16	2	16	50
16	24	1	24	170
16	24	4	24	70

Manifestation of a well pronounced CCR induced by dilution of calcium, without any change in the concentration of potassium, cannot be explained by the theory of direct action of reversing agent, unless some additional assumptions concerning Ca antagonism would be introduced. On the other hand, it seems evident that decrease of Ca^{2+} concentration in the liquid phase (external medium) brings about a new ionic equilibrium between K and Ca on the adsorption sites (Gibbs-Donnan principles), i.e. a part of bound calcium must be depleted. This process allows to explain the ciliary reversal induced by Ca dilution in the medium, by Ca uptake or by Ca loss, as well as by changed Ca content in the membrane (or in the cortical layer) of the ciliate.

Effect of Ca chelation

Paramecia were incubated, 24 hrs. prior to the experiment, in 1, 0.1 or 0.01 mM $CaCl_2$. K^+ was omitted and no other ions were present in the medium. After this preliminary incubation period Ca^{2+} was partly chelated by addition of different amounts of EDTA-sodium salt. The immediate motor response of ciliates was observed.

As shown in the Fig. 1, a slight Ca chelation involves no visible change in the swimming direction. With a greater concentration of EDTA — PCR is clearly manifested. With somewhat stronger decalcification the ciliary reversal becomes continuous. When EDTA concentration is raised still more, CCR changes for PaCR. Further Ca chelation inhibits all reversal phenomena and the normal ciliary work (NM) appears again.

This sequence of different types of ciliary responses appears regularly in all the experiments. Data presented in the Fig. 1 prove that this series of responses are distinctly manifested in various Ca concentrations, provided the EDTA concentration was properly adjusted.

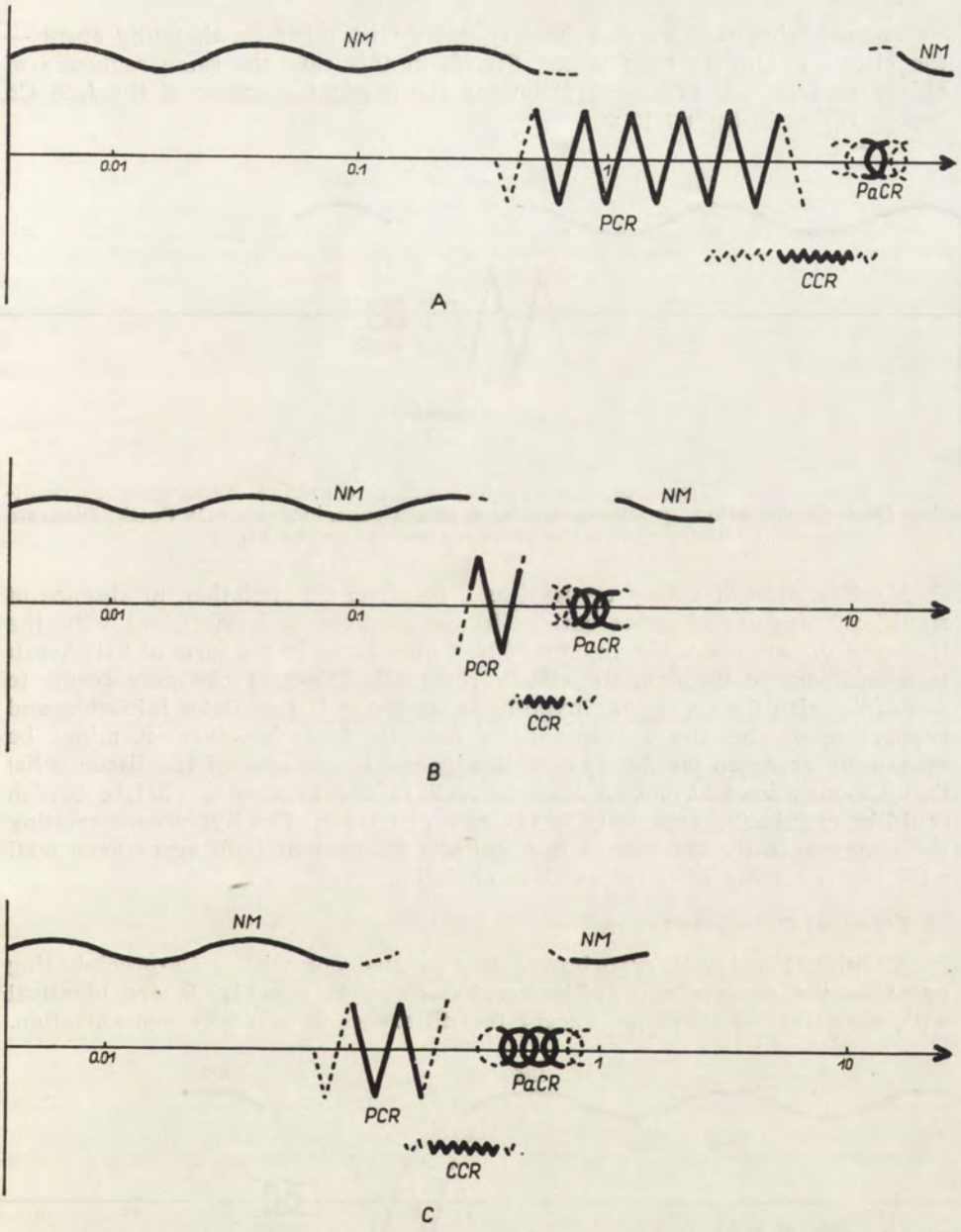

Fig. 1. Sequence of ciliary responses corresponding to gradual decalcification resulting from Ca chelation by EDTA. Concentration of EDTA is marked on the horizontal axis. The wavy line above the axis represents the normal movement (NM), the notched line below the axis — the continuous ciliary reversal (CCR), the zig-zag line transgressing the axis upwards and downwards symbolizes the periodic ciliary reversal (PCR), and the circles on the axis — the partial ciliary reversal (PaCR). A. Responses obtained when 1 mM $CaCl_2$ was present in the initial medium. B. Same when initial medium contained 0.1 mM $CaCl_2$. C. Same whith 0.01 mM $CaCl_2$ in the initial medium

The experiment in question was repeated with other Ca chelating agent — the citrate anion. As seen in the Fig. 2, in this case the same sequence of ciliary responses is produced, following the gradual decrease of the free Ca concentration in the medium.

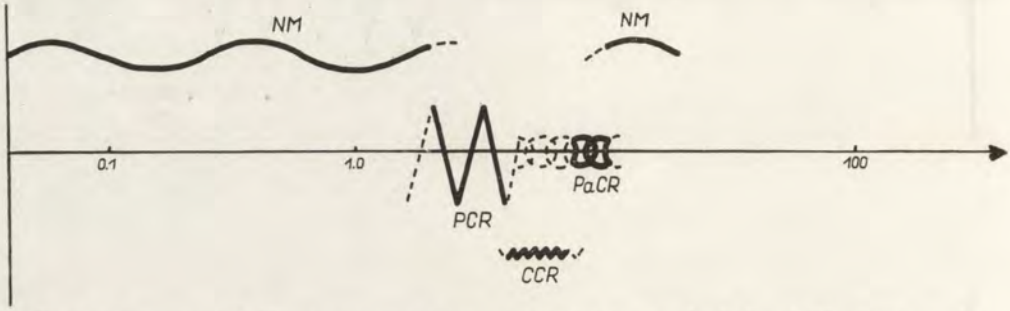

Fig. 2. Sequence of ciliary responses corresponding to gradual decalcification resulting from Ca chelation by citrate. Initial medium contained 0.1 mM $CaCl_2$. Diagram constructed in the same manner as in the Fig. 1

Manifestation of ciliary reversal as a result of Ca chelation, in absence of significant amount of other cations in the medium, is inconsistent with the theory of direct action (the amount of Na^+ introduced in the form of EDTA-salt is insufficient to promote the ciliary reversal). Effect of chelators seems to contradict also the Ca uptake hypothesis, as far as it postulates initiation and supporting of the motor response by free Ca ions; however, it might be eventually accepted (as R u b i n 1963 suggests in the case of the tissue cells) that Ca may be transported into the cell in the form of a chelate (which could be convincing especially in the case of citrate). The hypotheses relating the response to the Ca loss or to a definite Ca content both agree very well with the reversing effect of calcium chelation.

Effect of Ca precipitation

Additional series of experiments was carried out with a Ca-precipitating agent — the oxalate anion. The results, given in the Fig. 3, are identical with the effect of chelators, except the difference in effective concentration.

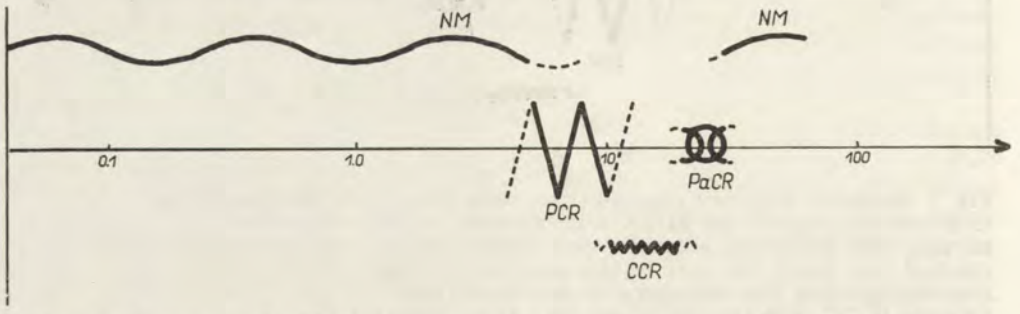

Fig. 3. Sequence of ciliary responses corresponding to gradual decalcification resulting from Ca precipitation by oxalate. Initial medium contained 0.1 mM $CaCl_2$. Diagram constructed in the same manner as in the Fig. 1

The reversing effect of a simple Ca precipitation is hardly compatible not only with the theory of direct action, but also with the Ca uptake concept. Both other theories explain this result well.

Stimulation of paramecia pretreated with EDTA

Potassium effect

Paramecia were incubated for 24 hrs. in 0.1 mM $CaCl_2$ and afterwards the different amounts of EDTA were added. Immediately after EDTA administration (i.e. after inducing the definite kinds of behaviour demonstrated in the Fig. 1 B) the animals were stimulated with KCl, which is a classical agent causing ciliary reversal.

In ciliates remaining still in the NM condition, K ions introduced in sufficient concentration induce a typical CCR.

Paramecia slightly decalcified with EDTA, so as to evoke the PCR, change it for CCR even after addition of small amount of potassium. Still higher concentration of KCl solution may involve only PaCR, or even NM appearing again.

In animals exhibiting EDTA-induced CCR, KCl evokes PaCR when added in a small quantity. A stronger action of potassium brings about a full renormalization of the movement. The potassium-induced renormalization of ciliary reversal is perhaps the most striking phenomenon, which speaks against the direct reversing action of K.

Ciliates decalcified with EDTA in concentration sufficient to induce PaCR, are also renormalized by administration of KCl.

At the highest EDTA concentrations, when paramecia recover the forward movement (NM), potassium is ineffective. It is the same phenomenon which was stated by Kamada and Kinosita 1940, in their report on the impossibility to induce ciliary reversal in strongly decalcified paramecia.

All experiments described in this section demonstrate that the addition of K ions strengthens the effect of EDTA, and shifts the ciliary responses more and more in the direction: NM → PCR → CCR → PaCR → NM. It may be infered that K^+ presumably shifts the ionic equilibrium in the membrane or in the cortical structures towards stronger decalcification[2].

Calcium effect

As it is known, Ca addition induces no changes in the direction of movement in paramecia swimming forwards. Consequently, addition of $CaCl_2$ in present experiments has also no effect when EDTA-pretreatment was so slight that the ciliates manifested still the NM response.

In paramecia decalcified to the degree promoting PCR, addition of free Ca to the medium brings about the renormalization of ciliary work.

Also paramecia pretreated with somewhat higher concentration of EDTA, so as to induce CCR, recover the forward swimming when sufficient amount of Ca^{2+} has been added. It means that the EDTA-induced ciliary reversal may be renormalized by both opposite factors — external Ca or external K addition, i.e. by recalcification as well as by further decalcification.

[2] It could be eventually explained as K-Ca competition for the same adsorption sites, or as an after-effect of the K-induced depolarization of the membrane. It seems also possible that both mechanisms are linked together.

Ciliates decalcified still more and manifesting PaCR, react to administration of $CaCl_2$ by CCR (very little Ca added), by PCR (moderate recalcification), or by full renormalization (an intense recalcification).

Paramecia very strongly decalcified, up to disappearence of all kinds of ciliary reversal, can be gradually recalcified and then manifest step by step all types of behaviour. As shown in the Fig. 4, the sequence of ciliary responses corresponding to the gradual recalcification is an exact although inversed replication of the analogous sequence previously obtained by gradual decalcification. Appearance in this series of the ciliary reversal evoked by Ca administration seems to be a significant fact that contradicts directly the theory of the Ca loss as universal mechanism of inducing the ciliary reversal.

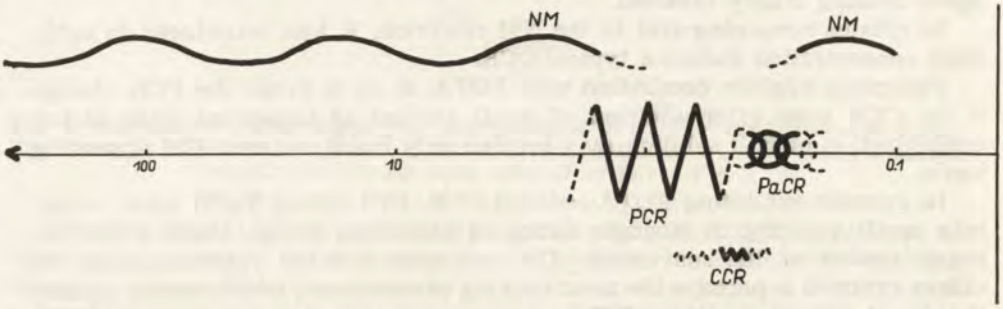

Fig. 4. Sequence of ciliary responses exhibited by paramecia strongly decalcified previously (0.1 mM $CaCl_2$ + 2 mM EDTA) and subsequently exposed to gradual recalcification by addition of $CaCl_2$. Diagram constructed in the same manner as in the Fig. 1

All experiments of this section suggest that after decalcification the ionic equilibrium may be shifted towards recalcification again, and it results in producing all typical ciliary responses in a recurrent sequence: NM → PaCR → → CCR → PCR → NM.

Effect of other factors

Besides KCl and $CaCl_2$, some other chemical agents were applied for stimulating the EDTA-pretreated paramecia. They were: barium chloride, acetylcholine chloride, caffeine sulphate and heparin. Ba ions, ACh and caffeine are known to liberate Ca^{2+} adsorbed on the fibre membrane, when inducing the muscle contraction (Bianchi 1961, Frank 1962, 1963). Heparin affects the membrane properties and induces clear motor responses in amoebae (Bell and Jeon 1962); its antagonism with Ca ions was demonstrated in the muscle fibre (Horvath and Csaba 1963) as well as in the ciliate cell (Miyake 1958). In ciliates, all these factors promote some forms of ciliary reversal, the PCR at least.

All agents mentioned above are comparable to K ions in their motor effect on paramecia treated previously with EDTA, i.e. presumably they bring about their further decalcification. As a result, they shift also the ciliary responses in the direction: NM → PCR → CCR → PaCR → NM (to the right in the Fig. 1 B).

This might explain some known effects of accetylcholine upon the ciliary movement, e.g. its ability to induce the ciliary reversal in *Spirostomum*

(Seravin 1962) and to prolonge the K-induced CCR in *Paramecium* (Müller and Toth 1959).

In accordance with all data presented above, acetylcholine may promote or increase the reversal responses, only when a sufficient quantity of Ca^{2+} is present in the medium. Paramecia pretreated in high EDTA concentrations become insensitive to ACh, caffeine, heparin and Ba^{2+}, in the similar degree as to the action of K^+. This was registered as the chemotactic behaviour. ACh, like other decalcifying factors, produces a strong chemotactic response in normal paramecia — they respond with short-lasting reversal of ciliary beat (avoiding reaction) when meeting ACh on their way. On the contrary, paramecia which swim forwards in the condition of strong decalcification, show no motor responses when coming in contact with ACh. Both different modes of behaviour were registered, as shown in the Pl. II 5—6.

Of course, the effect of ACh (and of some other specific stimulants and inhibitors) upon Ca equilibrium, membrane polarization and ciliary reversal in ciliates, deserves more extensive study[3].

Calcium shifting during the K-induced CCR

Effect of recalcification

In paramecia incubated in 0.1 mM $CaCl_2$ (24 hrs. prior to the experiment) CCR was induced with 2 mM KCl. It lasts in this case 20 sec. During this period $CaCl_2$ concentration in the medium was raised (with no change in the K content). Several stronger KCl concentrations promoting longer CCR, were also tested.

A slight increase in external Ca content shortens the duration of CCR, and higher increase of Ca may interrupt this response. This latter effect was registered by photographic technique. Pl. II 7—8 demonstrate a K-induced reversal manifested all over the field, except a circular area where $CaCl_2$ was mixed with the initial solution.

The shortening or interrupting of CCR by recalcification may be easily explained by the hypothesis that the kind and intensity of reversal response depends on the content of Ca on binding sites. It seems obvious that addition of external Ca may shift the equilibrium of adsorbed Ca ions out of this range which promotes the reversal effects. On the contrary, this experiment seems hardly compatible with the Ca uptake theory. If the reversal would be induced by intracellular action of Ca ions liberated formerly, the subsequent addition of Ca^{2+} to the medium should exert no inhibitory influence.

Effect of decalcification

Under similar conditions as in the preceding experiment, instead of $CaCl_2$ the Ca-chelating or Ca-precipitating agents were introduced.

As it is seen in the Table 2, the moderate "subtreshold" concentrations of EDTA, citrate or oxalate may prolonge CCR induced previously with KCl. A stronger decalcification shortens the response, or it can even remove the

[3] A study on the effect of heparin on motor responses in *Paramecium* is simultaneously published by Lantos, Müller and Csaba 1965; the antagonism between heparin and calcium is also clearly demonstrated, although the technique and experimental conditions were different than in the present research.

Table 2

Intensification of K-induced CCR when small amounts of decalcifying agents are administered during its course (0.1 mM $CaCl_2$ present in the medium)

Concn. of stimulating agents	Duration of CCR in sec.
2 mM KCl	20
2 mM KCl + 0.2 mM Na-EDTA	55
2 mM KCl + 1.0 mM Na-citrate	70
2 mM KCl + 2.5 mM Na-oxalate	45

reversal effects completely. Disappearance of K-induced CCR in the area where sufficient amount of EDTA was added, is presented in the Pl. II 9.

Both experiments described in last two sections prove that K-induced CCR may be interrupted as well by further decalcification as by recalcification. This supports the view that the character of ciliary response may be connected with the density of adsorbed Ca ions in the cell membrane (or in the cortical layer). Shifting the equilibrium in either direction abolishes the response in similar degree. Ca uptake theory does not explain the renormalizing effects of recalcification, whereas the Ca loss theory seems to contradict the renormalization produced by further decalcification.

Concluding remarks

The experiments described and discussed above allow to choice the most promising concept among different views on the ionic mechanism of ciliary reversal. Principal evidences supporting or contradicting these hypotheses are summarized in the Table 3. Direct action theories are incoherent with all reversal phenomena induced by adjusting the external Ca content only, without other changes in the cations concentration. The most important phenomena speaking against the Ca uptake concept are: the ciliary reversal caused by Ca chelation or precipitation, and inhibition of previously induced CCR by subsequent addition of external Ca^{2+}. The Ca loss theory fulfills almost all requirements, but it seems unable to explain the reversing effect of recalcification and renormalizing effect of decalcification, occurring under some experimental conditions.

The principal conclusion of this research is the assumption that the kind and intensity of ciliary reversal correspond to density of calcium upon the adsorption sites, probably in the cell membrane. Above this decisive range of Ca content and beneath it — cilia beat in the normal direction. All results reported in the present study and numerous facts revealed by earlier workers are in agreement with this suggestion.

The decisive role of the amount of calcium remaining on the adsorption sites was visualized by means of the photomacrographic method of registration of movement (Pl. III 10). A shallow cuve was filled with 2 mM $CaCl_2$ solution on one side and with 2 mM EDTA on the other. Both solutions diffused mutually forming a gradient of free Ca ions. Paramecia (incubated previously

Table 3

Consistency of various data on the ciliary reversal with four different concepts of its ionic mechanism. The facts considered are characterized as: accounted for by a theory (+), contradictory to it (—), or eventually compatible with it if some additional assumptions are made (?)

Responses considered	Direct action concept	Ca-uptake concept	Ca-loss concept	Ca-content concept
All monovalent and some divalent inorganic cations, and many organic cations induce ciliary reversal	+	+	+	+
Ciliary reversal is connected with membrane depolarization	+	+	+	+
External Ca antagonizes the reversing factors in Donnan ratio	?	+	+	+
External dilution of Ca may promote the ciliary reversal	?	?	+	+
External Ca chelation induces the ciliary reversal	—	?	+	+
Intracellular injection of Ca chelating agents induces the ciliary reversal	—	?	+	+
External Ca precipitation induces the ciliary reversal	—	—	+	+
Previously induced ciliary reversal is abolished by recalcification	—	—	+	+
Previously induced ciliary reversal is abolished by further decalcification	—	?	—	+
Strong decalcification makes induction of ciliary reversal impossible	—	+	—	+
Moderate recalcification of strongly decalcified paramecia induces the ciliary reversal	—	?	—	+
Ciliary reversal is self-renormalized after longer exposure to stimulation medium	?	+	?	?

in 0.1 mM $CaCl_2$) were introduced on both sides. As clearly seen in the picture, they swim normally as well in Ca-rich as in Ca-defficient medium. However, a quite distinct zone remains free of ciliates between both media. Paramecia cannot enter this intermediate area, because a ciliary reversal interferes when an animal has reached its boundaries (the avoiding reaction). It is the ciliary reversal zone, where the concentration gradient corresponds to this range of Ca content which induces the inverse work of cilia.

When accepting the view that the degree of excitation of ciliate corresponds to the density of Ca upon the adsorption sites, it should be however, stressed that this fails to explain the reason why the ciliary reversal may be self-renormalized, without changing the external Ca^{2+} concentration. In this respect the Ca uptake concept could be eventually considered to be even more promising. Problem of the self-renormalization and duration of ciliary rever-

sal remains open for a further study. The role of intracellular Ca was also not taken into consideration in the present research.

Present experiments seem to offer a partial explanation why the protozoan cell seems to be entirely inexcitable after a strong decalcification (although after a slight decalcification its excitability is higher than normal). It might be concluded that in strongly decalcified paramecia Ca density is shifted to the opposite side of the reversal range and — in fact — the cell becomes not insensitive but responds only to the opposite factors: to recalcification instead of decalcification.

Another conclusion of this research is ranging all known types of ciliary behaviour in *Paramecium* and an attempt to relate them to gradual shifting of the membrane Ca content, in the following sequence: NM — PCR — CCR — PaCR — NM.

This clear series of responses might serve as an indication of decalcifying action of different factors, especially when the after-treatment with $CaCl_2$ gives a recurrent sequence. Such a work was just initiated in this laboratory and the results concerning the reversing action of some inorganic cations are to be published (Kuźnicki, in prepration).

It may be supposed that the ciliary reversal induced by electric field is also connected with Ca behaviour — Ca loss (as postulated by Jahn 1962) or rather establishing the critical range of Ca content. The first result in this field is simultaneously presented by the author elsewhere (Grębecki 1965 b). It was found that the anodal galvanotaxis (i.e. the ciliary reversal induced by anodal current instead of cathodal one) appears in paramecia after their appropriate decalcification; it is connected with the inversed sensitivity, since the anodal galvanotaxis was found in those animals which were sensitive to Ca, but not to K. The possible decalcifying action of cathodal current in ciliates needs an experimental approach.

The attractive analogy between the ciliary reversal and muscle contraction looses its validity when Ca uptake theory is postponed and the concept of a critical range of Ca content is accepted, as suggested in this study. As a matter of fact, the ciliary reversal is a rather more complex behaviour, not comparable to a single twitch of the muscle fibre. It consists of thousands of inversed ciliary strokes, synchronized by the inversely propagated metachronal waves. Perhaps the analogy with the muscle contraction (Ca uptake links excitation with response) should be seen not in the ciliary reversal but in the single stroke of each cilium when an individual metachronal impulse reaches it on its way; it may concern each stroke, with no regard of its direction — either normal or inversed. As yet, consideration of such eventuality is impossible, since we do not know: 1. what is the electrical nature of metachronal wave in protozoa, 2. what happens with membrane calcium near or upon a single cilium when metachronal wave passes over, 3. what is a response of individual cilium and its contractile structures to changing calcium concentration.

Summary

A gradual decalcification produces the series of ciliary responses in the following sequence: NM → PCR → CCR → PaCR → NM. Gradual recalcification brings about a recurrent sequence.

The decalcification was obtained by external Ca chelation, Ca precipitation, or by introducing ions competing with Ca^{2+}, probably for the same adsorption sites. Recalcification was induced by raising the external $CaCl_2$ concentration.

Numerous data are discussed which speak against the hypotheses that the ciliary reversal is involved by direct action of various chemical agents, that it is caused by uptake of Ca liberated from its binding sites, or that it is a result of simple Ca loss. The view is put forward that the degree of excitation corresponds to the gradual changes of the amount of Ca remaining on the adsorption sites and influencing the state of the cell membrane. There exists some decisive range of Ca content involving the ciliary reversal, but above it and below cilia beat in the normal direction.

Insensitivity of paramecia after sufficiently intense decalcification is explained by passing beneath the decisive range of Ca content, which results in sensibilization of cell to the opposite action — to recalcification instead of decalcification.

Presented data speak against the direct analogy between the ciliary reversal and muscle twitch, since the ciliary reversal seems to involve multiple excitation-contraction cycles.

STRESZCZENIE

Stopniowe odwapnianie wywołuje kolejne reakcje rzęskowe zgodnie z następującą serią: NM → PCR → CCR → PaCR → NM. W następstwie stopniowego ponownego nawapniania otrzymuje się serię odwrotną.

Odwapnianie uzyskiwano drogą chelacji zewnętrznego Ca, jego wytrącania, lub poprzez wprowadzanie jonów prawdopodobnie współzawodniczących z Ca^{2+} o te same miejsca adsorpcji. Ponowne nawapnianie uzyskiwano zwiększając stężenie $CaCl_2$ w środowisku.

Rozważono liczne dane przemawiające przeciwko hipotezom głoszącym, że rewersja rzęskowa jest następstwem bezpośredniego działania różnych czynników chemicznych, że wywołuje ją pobieranie Ca uwolnionego z miejsc adsorpcji, albo że wynika ona ze zwykłej straty Ca. Wysunięto pogląd, że stopień pobudzenia może odpowiadać kolejnym zmianom ilości Ca pozostającego w miejscach adsorpcji i wpływającego na stan błony komórkowej. Istnieje pewien decydujący zakres zawartości Ca, który wywołuje rewersję rzęskową, poniżej zaś i poniżej rzęski uderzają w kierunku normalnym.

Niewrażliwość pantofelków po ich dostatecznym odwapnieniu jest tłumaczona jako spadek zawartości Ca poniżej tego decydującego zakresu, co powoduje uczulenie komórki na bodźce przeciwne — na nawapnienie zamiast odwapnienia.

Przedstawione dane przemawiają przeciwko wysuwaniu bezpośredniej analogii pomiędzy rewersją rzęskową a skurczem mięśnia, tym bardziej że na rewersję rzęskową raczej składa się wiele powtarzających się cykli pobudzenia i reakcji.

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EXPLANATION OF PLATES I—III

1: Photomacrographic registration of paths covered by paramecia within 5 sec. during NM (1 mM $CaCl_2$ and no other ions present in the medium); the inset mounted in the left upper angle presents the analogous registration taken at higher magnification

2: Same during CCR (1 mM $CaCl_2$ and 32 mM KCl in the medium)

3: Same during PCR (induced by 1 mM ACh in the medium)

4: Same during PaCR (1 mM $CaCl_2$ and 10 mM EDTA-Na salt in the medium)

5: Strong negative chemotactic response on the boundary of a drop containing 1 mM ACh (central area), as manifested by paramecia moving normally in Ca-containing medium (0.1 mM $CaCl_2$)

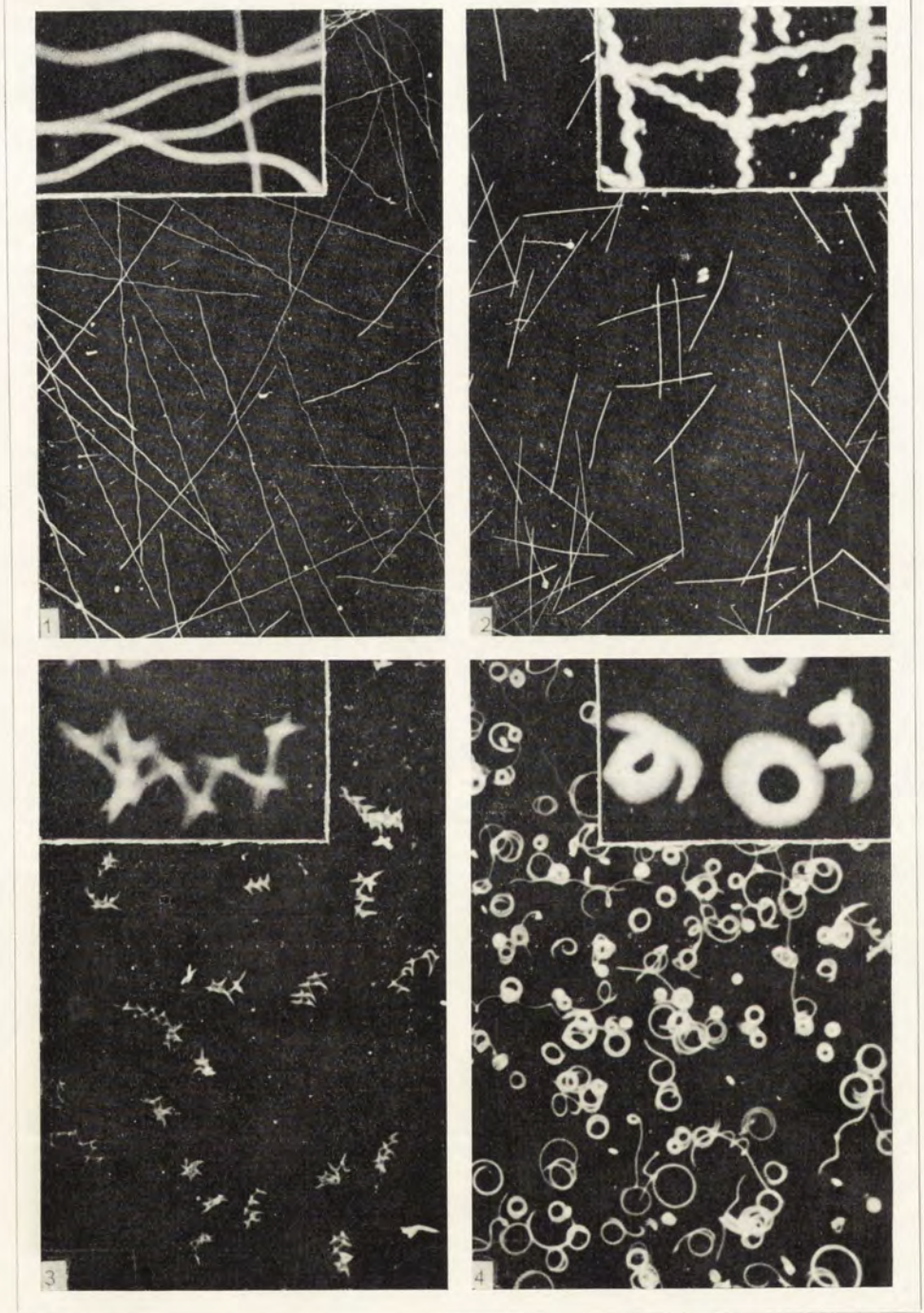
6: Lack of response on the boundary of central area containing 1 mM ACh, in paramecia moving normally after strong decalcification (0.1 mM $CaCl_2$ + 10 mM EDTA)

7: CCR induced by 8 mM KCl (marginal field) and recovery of NM when 4 mM $CaCl_2$ has been added to the medium (central area)

8: Same registered at higher magnification to show the character of reversed and renormalized movement; a segment of the Ca-rich area is seen in the left upper angle

9: CCR induced by 8 mM KCl (marginal field) and recovery of NM when 2 mM EDTA has been added to the medium (central area)

10: Behaviour of paramecia along a linear gradient of Ca concentration produced by gradual diffusion of $CaCl_2$ against EDTA; intermediate zone of ciliary reversal (an inaccessible region free of ciliates) is clearly seen, as well as NM in the Ca-rich and Ca-defficient media



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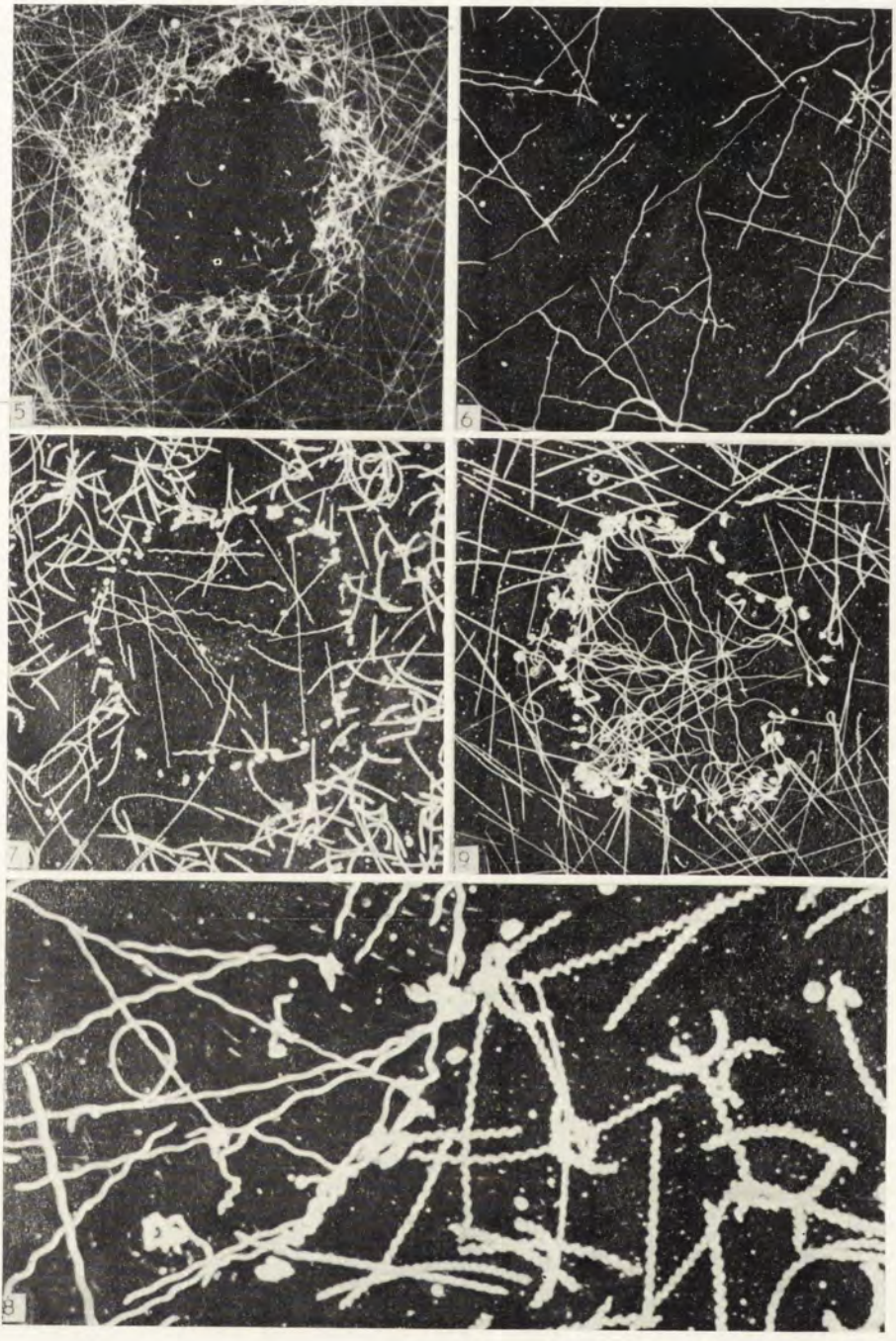

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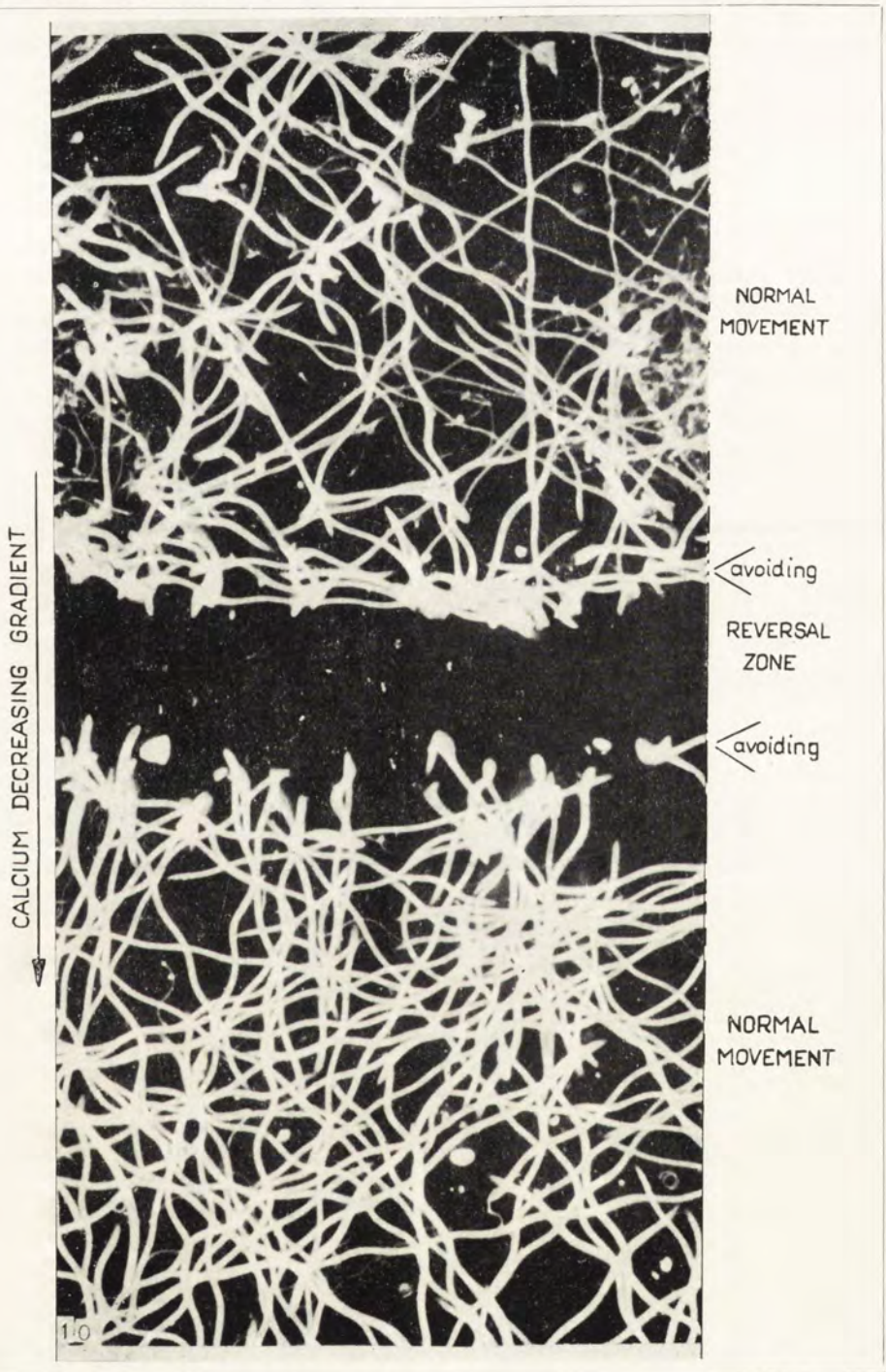

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Effect of heparin on locomotion and potassium reversal in *Paramecium multimicronucleatum*

Heparin hatása *Paramecium multimicronucleatum* mozgására és kálium reverziójára

Heilbrunn in a long series of communications (see Heilbrunn 1955) proposed the idea that a system eliciting protoplasmic clotting or liquefaction is active in all kinds of tissues and cells. He assumes furthermore that this system plays a major role in special cell functions, e.g. in the contraction of muscle cells or in the excitation of nerve. Studies performed by the Heilbrunn group and among others by Csaba and co-workers (Csaba and oth. 1962, Horváth and Csaba 1963, Csaba and Horváth 1963) in this Department revealed that heparin exerts marked effects on widely different biological objects.

The effect of heparin on protozoa has been studied only in a small number of papers. Miyake 1958 observed induction of conjugation in *Paramecium* by heparin and the lack of this reaction in presence of calcium ions. Bell and Jeon 1962 demonstrated stimulation of pseudopod formation in *Amoeba proteus* by heparin and subsequently also a membrane depolarization was shown in the same objet (Bingley, Bell and Jeon 1962). In view of the paucity of information authors endeavoured to obtain some data on the effect of heparin in *Paramecium*. In this paper its effect on locomotion and reversal will be presented.

Materials and methods

A *Paramecium multimicronucleatum* strain isolated by Dr. B. Párducz was cultured agnotobiotically in manure infusion and washed repeatedly 24 hours prior to the experiments in a balanced salt solution (Losina-Losinsky medium — LL) of the following composition: NaCl — 0,01%, KCl — 0,991%, $MgCl_2$ — 0,001%, $CaCl_2$ — 0,001%, $NaHCO_3$ — 0,002%). Heparin (Heparin pulvis Richter, 100 i.U./mg.) or/and calcium chloride were dissolved in the same solution. Equal amounts of LL solution with the washed animals and the solution of the substance to be tested were mixed. All concentrations given are final concentrations of this mixture.

The swimming of the animals was recorded photomacrographically in a device assembled according to Dryl 1958. Each value representing the speed of paramecia is the mean of 50 tracks measured in one record. Excitation

was induced by quick addition of a 50—100 fold quantity of 15 mM KCl in LL solution to few animals in a very small drop (placed on a depression slide). The animals were observed under a dissecting microscope and the time elapsed until the cessation of backward swimming was recorded. Each value is the mean of data obtained in 6 to 12 animals. Standard deviation of the value is also included in the tables.

Results

A marked variability in responsiveness as observed in different experiments does not permit direct comparison of the results of all experiments. Accordingly, only some typical experiments are presented here. We wish to add, however, that in all experiments the trend of changes was the same, the difference being only in the efective range of concentrations.

Deleterious effect of heparin was produced only when present in concentrations of 0.1 per cent or more. In such solutions the animals settled down and swam slowly within 30—60 minutes and died within one day. No animals died or exhibited changes indicating damage in more diluted solutions within this time.

Effects of 30 min. treatment with heparin on swimming and reversal are shown in Fig. 1 and Plate I. As the actual speed values do not give a full

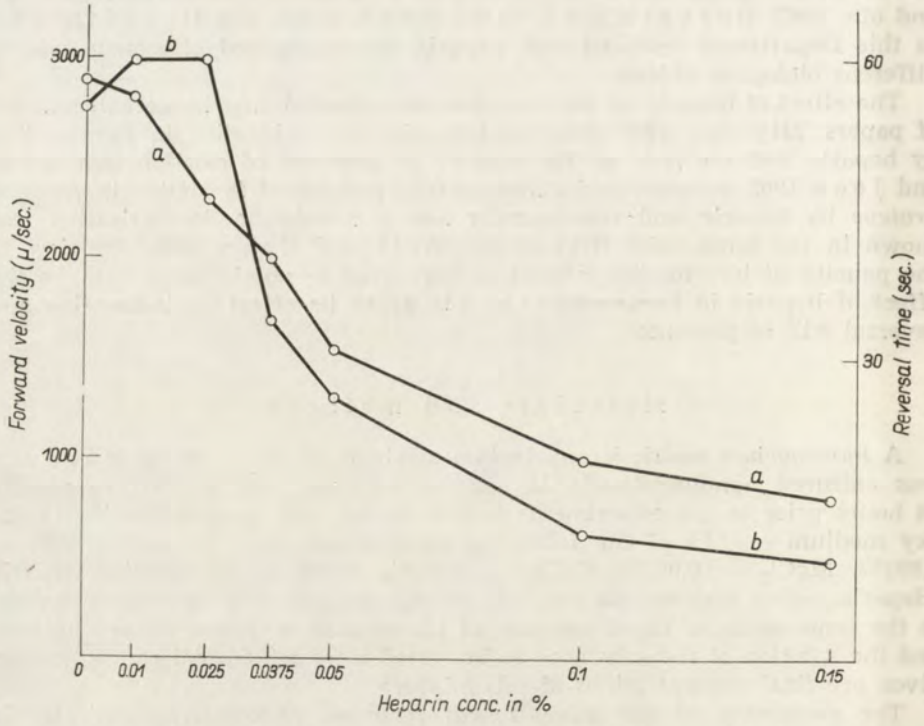

Fig. 1. Effect of 30 min. heparin treatment on *Paramecium multimicronucleatum* (a — speed; b — duration of reversal)

picture of the swimming behaviour, some typical tracings of animals are also shown. A depression of the swimming speed was elicited by almost all concentrations used. Higher, harmful concentration (0.1 per cent and higher) led to a sharp depression and other disturbances preventing the animals in swimming freely, and so caused them to settle down soon. In more diluted solution (0.037 per cent and less) the swimming behaviour of the animals was almost normal in spite of a certain decrease in their speed. A similar depression is observed for reversal. High concentrations elicit sharp changes, lower concentrations have a weaker effect. It is interesting to note, however, that in all experiments certain intermediate concentrations which produced no changes in swimming behaviour (although led to decreased speed) evoked a very strong depression of the reversal. Still weaker solutions, on the contrary resulted in a slight slowing down of the swimming without affecting the reversal. Thus the effects on the two processes do not show parallel changes, i.e. certain medium concentrations seem to affect rather the reversal, whereas weaker concentrations affect only the swimming.

Table 1

Effect of 30 min. heparin or heparin + calcium treatment on reversal (in sec.) in *Paramecium multimicronucleatum*

Exp. No.	Control	Heparin	Heparin + Calcium
1	74.5±5.8	0.15% 17.5±5.27	0.15% 5 mM 67.1±8.0
2	54.5±5.6	0.037% 37.0±5.2	0.037% 33 mM 57.5±8.2

Table 1 presents the effect on reversal of heparin and calcium ions acting simultaneously. In these experiments heparin concentrations were used which were even alone, highly depressing. If, however, small amounts of calcium ions were also present in the medium, no effect of heparin on reversal could be observed. Calcium ions in similar concentration rapidly abolished the changes already developed in swimming and reversal due to higher heparin concentrations. The speed decreased in 0.1 per cent heparin to 16 per cent of its original value after 30 min. and quickly returned to its original level after the addition of calcium ions. Fig. 2 shows the time course of changes in reversal essentially exhibiting similar features. Control experiments showed that the same amount of calcium ions does not produce any changes in control animals.

Discussion

In the present work two physiological phenomena served to demonstrate the effect of heparin in paramecia. The swimming speed gives certain information on the function of cells not excited, since in paramecia normal forward movement can be regarded as the resting state. The reversion elicited by potassium is a process of excitation and reflects the ability of the cell to

become excited and to return to its normal state (Mast and Nadler 1926). The end point of our timing, i.e. the cessation of backward swimming is not identical with the complete disappearance of excitation but represents only a transitory phase where the still excited and the normalised part of the cell are in equilibrium (Párducz és Müller 1958, Párducz 1959). The choice of this end point was found, by several authors, to be useful also in experiments directed at the elucidation of the effect of different substances on paramecia.

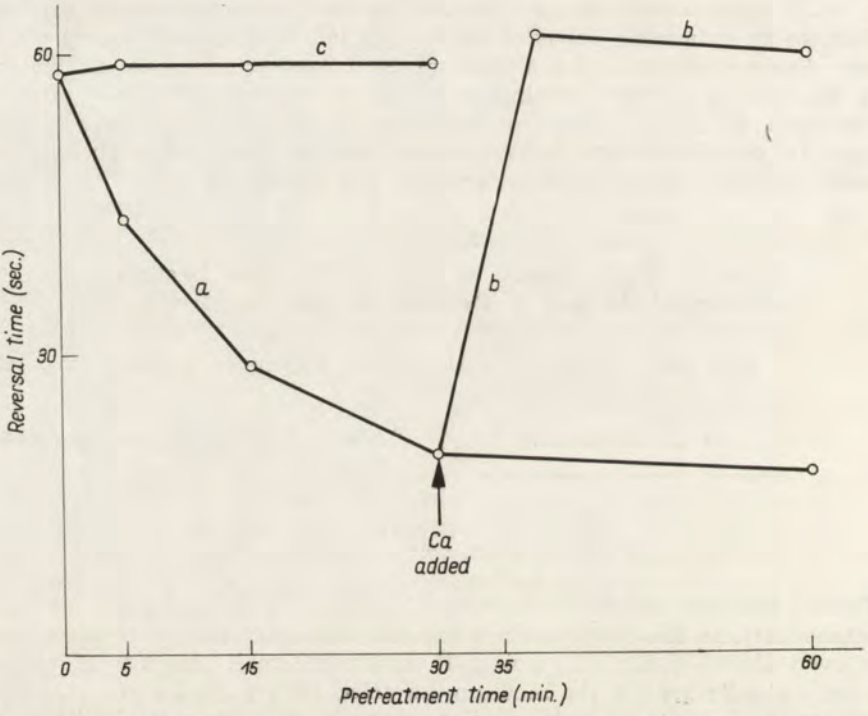

Fig. 2. Effect of 0.1% heparin and subsequent transfer into medium with increased (+ 5 mM) calcium content on reversal in *Paramecium multimicronucleatum* (a — development of heparin effect; b — abolishment of heparin effect after transfer into medium with increased calcium content, indicated by the arrow; c — effect of the same amount of calcium alone)

In this work a gradually developing strong effect on locomotion and reversal was observed when heparin was applied in concentrations which caused pronounced cell damage. Marked effect was, however, induced also by concentrations which did not lead to impairments of cell viability within the duration of the experiments. As no other cell functions were registered we cannot exclude the possibility of heparin affecting other processes as well. The unimpaired viability suggests, however, that these functions may be ranked among those which are first modified by the action of heparin. Heparin, accordingly, elicits certain changes in cellular structures responsible for the movement and/or response of the ciliate cell.

Similar depressing effects have been observed under the influence of a wide variety of other substances (see Wichterman 1953). The action of heparin thus cannot be regarded as specific. The mode of action is difficult to explain on the basis of the available evidence. Most probably the effects can be interpreted as elicited by some disturbances, perhaps of colloidal nature, in the cortical region of the cell. In the case of muscle tissue an interaction of heparin with proteins leading to their liquefaction is assumed (Heilbrunn 1956, Horváth and Csaba 1963, Csaba and Horváth 1963, etc.). In *Amoeba proteus* weak heparin solution which diffused from the tip of a pipette elicited pseudopod formation (Bell and Jeon 1962) interpreted as dependent on local membrane depolarisation (Bingley, Bell and Jeon 1962). The possible role of increased solification in the ectoplasm cannot be excluded in this case. A cortical solification under heparin effect is held by Miyake 1958 to be responsible for the induction of conjugation in *Paramecium.* Another possible mechanism of the changes in reversal could be the decrease of intracellular calcium (Kamada and Kinosita 1940) under heparin effect, which is assumed to take place also in mammalian striated muscle (Caporro, Marro and Valzelli 1958). These last results, however, have been disproved by Thomason and Schofield 1959 who used a different material. The problem cannot be solved before further evidence is available.

It has been repeatedly shown that the effect of heparin on contractile tissues is counteracted by calcium ions (Heilbrunn 1956, Csaba and Horváth 1963, Horváth and Csaba 1963). Miyake 1958 also could find no heparin effect in *Paramecium* if calcium ions were present in the medium. In our experiments calcium ions inhibited the development of changes when present from the beginning of the experiments and abolished when added after the appearance of these changes. Slight increase of calcium content in the medium sufficed to eliminate completely the deletorious effects of higher heparin concentrations. The prolonging effect of intracellular calcium ions on ciliary reversal, and especially the shortening of ciliary reversal by external Ca, are well known (Mast and Nadler 1926, Kamada and Kinosita 1940). In the present work, however, calcium acted in concentrations not affecting the reversal and movement. These findings suggest that in these experiments a rather specific antagonism of heparin and calcium was found as observed in other systems too, and that the observed effects were not due to the less specific protective action which could be assumed on the basis of the work of Seravin 1958.

Summary

Heparin in concentrations of 0.15 to 0.01 per cent exerts a marked depressing effects on the speed of locomotion and the duration of potassium induced reversal in *Paramecium multimicronucleatum.* The presence of excess calcium ions in a concentration of 2 to 5 mM in the medium prevents the development of heparin effect. When calcium of the same concentration is added to animals showing marked changes due to heparin treatment, quick normalisation of movement and reversal result. The results obtained show that heparin evokes certain changes in protoplasmic structures responsible for normal locomotion or reaction and that calcium can compensate for these changes as it does in other cases of heparin action.

ÖSSZEFOGLALÁS

Heparin 0.15—0.01% koncentrációban csökkenti a *Paramecium multimicronucleatum* mozgásának sebességét és a káliumionok által létrehozott reverzió tartamát. Kalcium ionok jelenléte fokozott koncentrációban (2—5 mM) meggátolja a heparin hatásának kifejlödését. Ha a hatás kifejlödése után adunk kalciumot a fenti koncentrációban, mind a mozgás, mind a reverzió gyorsan normalizálódik. Az eredmények arra mutatnak, hogy a heparin bizonyos változásokat idéz elö a normális mozgást vagy más reakciókat biztositó protoplazmastrukturákban, továbbá, hogy kalcium ezeket a változásokat ki tudja védeni, éppugy, mint a heparinhatás más eseteiben.

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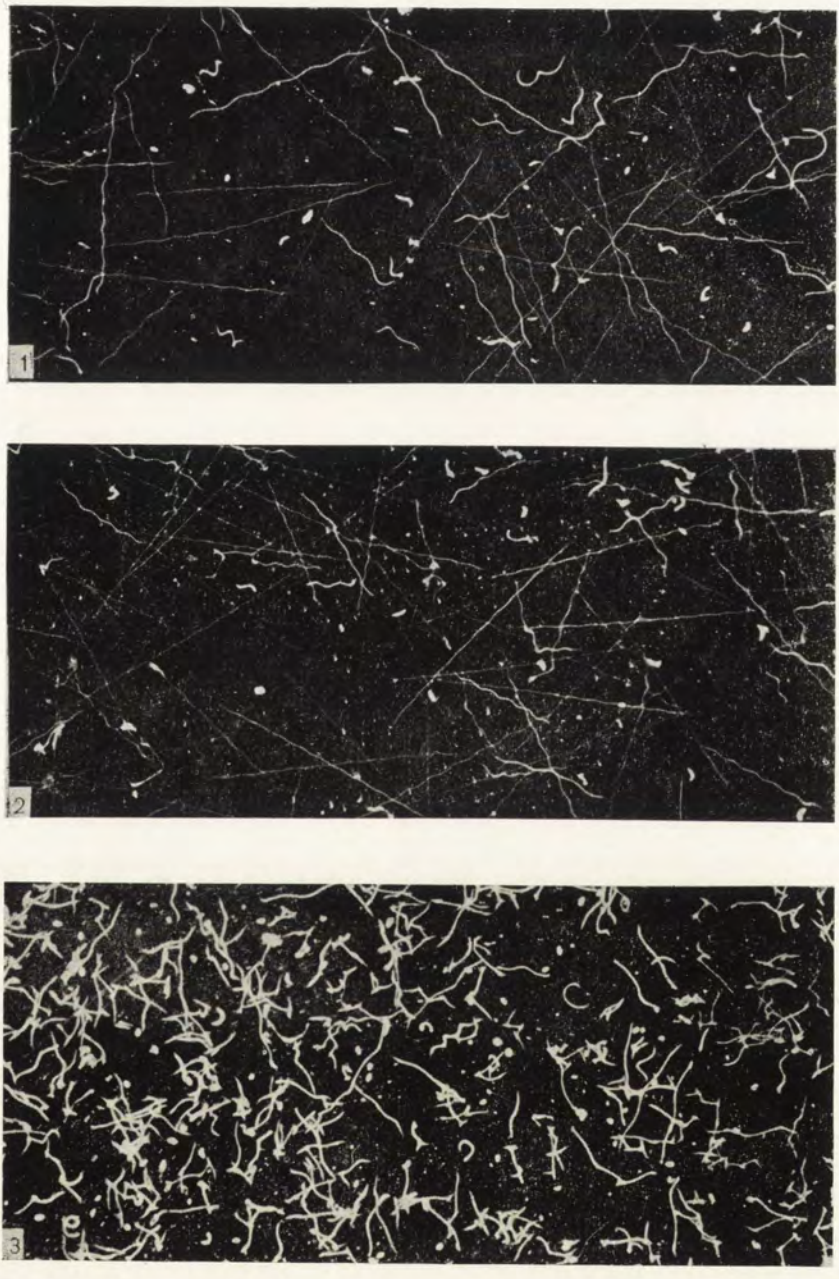
EXPLANATION OF THE PLATE I

Dark field tracings of animals swimming in a flat horizontal vessel

1: Untreated animals

2: Animals treated with 0.037% heparin for 30 min.

3: Animals treated with 0.1% heparin for 30 min.



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Hydrobiologische Station des Institutes für Ökologie der Polnischen Akademie der Wissenschaften, Mikołajki

Forschungsstelle für Limnologie der Deutschen Akademie der Wissenschaften, Jena-Lobeda

Wilfried SCHÖNBORN

Die sedimentbewohnenden Testaceen einiger Masurischer Seen

The sediment-inhabiting Testacea from some Masurian Lakes

Im Rahmen eines Studienaufenthaltes an der Hydrobiologischen Station in Mikołajki war es mir vergönnt, Untersuchungen über die Testaceenfauna des masurischen Gebietes durchzuführen, deren systematische Ergebnisse mit der vorliegenden Arbeit publiziert werden.

Die Masurische Seenplatte, erst in jüngster Zeit ein Gegenstand intensiver limnologischer und faunistischer Forschung, ist in der Testaceenliteratur weitgehend unberücksichtigt geblieben. Abgesehen von einigen früheren Untersuchungen an einigen Mooren, hat erst Moraczewski 1961, 1962 einen beachtenswerten Anfang in der Erforschung der Testaceenfauna der Masuren gemacht. Seine Untersuchungen beziehen sich auf den Mamry-See und den Kisajno-See. Während Moraczewski das flache Litoral untersuchte, beziehen sich meine Ergebnisse auf die profundalen Zonen einiger Seen. Mit Ausnahme des oligotrophen Hańcza-Sees im Suwałki-Gebiet stammen die aufgeführten Testaceenarten aus dem Śniardwy-See und einigen benachbarten Seen (Abb. 1). Diese Seen sind mesotroph bis eutroph; teilweise haben sie eine dystrophe Komponente.

Ich bin der Forschungsgemeinschaft der Deutschen Akademie der Wissenschaften zu Berlin für die Ermöglichung des Studienaufenthaltes und dem Leiter der Hydrobiologischen Station in Mikołajki, Herrn Dr. A. Szczepański, für einen Arbeitsplatz in seiner Station und die großzügige und verständnisvolle Unterstützung meiner Untersuchungen zu großem Dank verpflichtet. Weiterhin danke ich allen anderen polnischen Kollegen, die mir mit Rat und Tat zur Seite gestanden haben.

Liste der gefundenen Arten

Es wurden folgende Masurische Seen untersucht: Śniardwy-See (eutroph), Mikołajskie-See (eutroph), Tałty-See (mesotroph), Tałtowisko-See (mesotroph), Flosek-See (eutroph und dystroph), Lisunie-See (eutroph und dystroph), Bełdany-See (mesotroph). Ausserdem wurde auch der Hańcza-See (oligotroph) untersucht. Die Tiefe, aus der die Proben stammen, ist in Abb. 1 eingetragen. Die Proben aus dem Hańcza-See wurden aus 104 m Tiefe entnommen.

Eine Liste der gefundenen Arten wurde in der Tabelle 1 dargestellt.

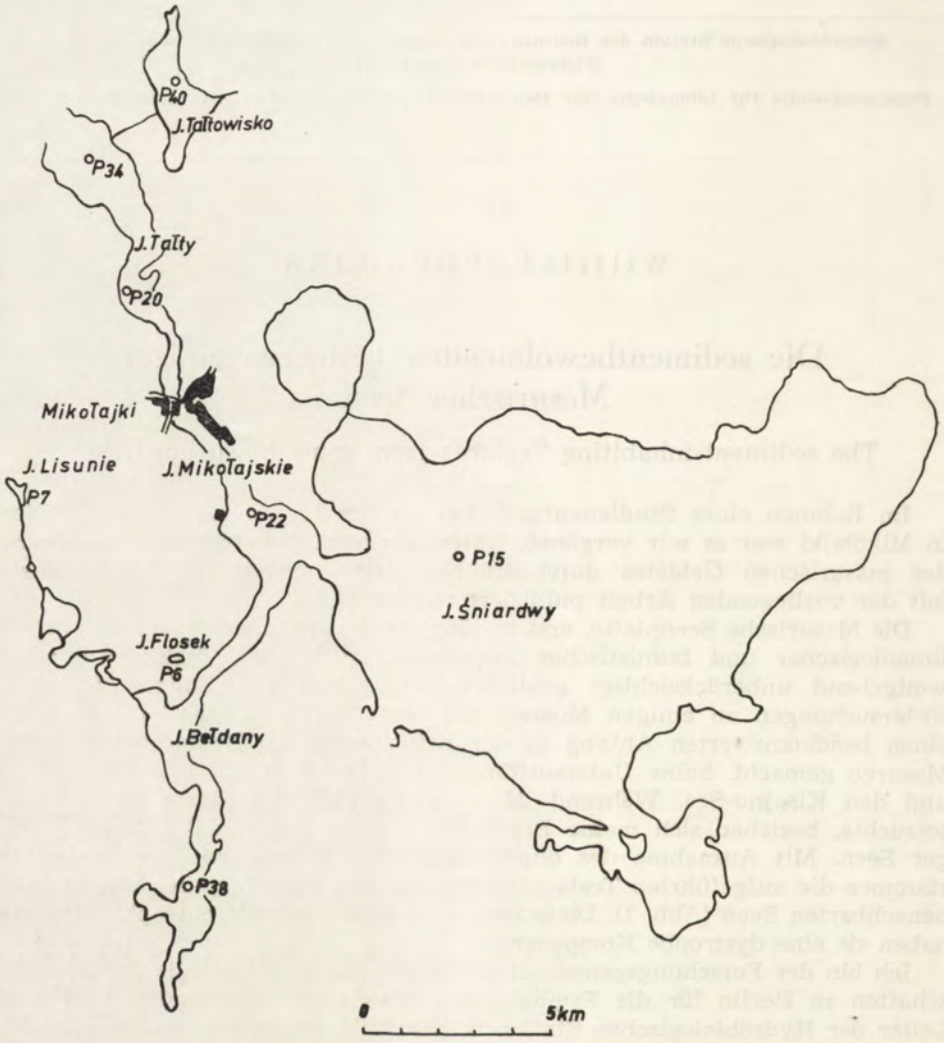

Abb. 1. Die untersuchten Seen (mit Ausnahme des Hańcza-Sees). Das P zeigt den Ort der Entnahmestelle der Proben und die nebenstehende Zahl gibt die Tiefe der Entnahmestelle in m. an

Besprechung einzelner Arten

Centropyxis deflandrei Rampi, 1950

Diese von Rampi gefundene Art (Abb. 2) stellte ich mehrmals im Lisunie-See fest. Der sehr hohe Fundus-Teil der Schale steht fast senkrecht zum Pseudostom; Dornen sind nicht vorhanden. Die Kittmasse der Schale ist hyalin; sie ist *Difflugia*-artig fast lückenlos mit Quarzsteinchen belegt. Der Plasmakörper ist mit Hilfe von Epipodien an der Schalenwand befestigt. Im Kern befinden sich mehrere Nukleolen. Maße: 200 μ hoch; 80—100 μ

Tabelle 1

Liste der gefundenen Arten. H. — Hańcza-See, S. — Śniardwy-See, M. — Mikołajskie-See, TG. — Tałty-See, T. — Tałtowisko-See, F. — Flosek-See, L. — Lisunie-See, B. — Bełdany-See

Gefundene Arten	H.	S.	M.	TG.	T.	F.	L.	B.
Arcella hemisphaerica Perty, 1852	+	−	+	+	+	+	+	+
Arcella hemisphaerica var. *undulata* Defl., 1928	−	−	+	−	−	−	−	+
Arcella hemisphaerica var. *intermedia* Defl., 1928	−	−	−	−	−	−	−	+
Arcella gibbosa Penard, 1890	−	−	−	−	−	−	+	−
Centropyxis discoides (Penard, 1890) Deflandre, 1929	+	−	−	+	−	+	+	−
Centropyxis aculeata (Ehrenberg, 1838) Penard, 1902	+	+	+	−	+	+	+	+
Centropyxis hirsuta Deflandre, 1929	−	+	−	−	−	−	−	−
Centropyxis deflandrei Rampi, 1950	−	−	−	−	−	−	+	−
Centropyxis platystoma (Penard, 1890) Deflandre, 1929	+	−	−	−	−	−	−	−
Centropyxis aërophila Deflandre, 1929	+	−	−	−	−	−	−	−
Centropyxis aërophila var. *sphagnicola* Defl., 1929	+	−	−	−	−	−	−	−
Centropyxis constricta (Ehrenberg, 1838) Deflandre, 1929	−	+	−	+	−	−	−	−
Centropyxis cassis (Wallich, 1864), Deflandre, 1929	+	−	−	−	−	−	+	−
Oopyxis cyclostoma Thomas, 1958	+	+	−	−	−	−	−	−
Geopyxella aquatica n. sp.	−	−	−	−	−	−	+	−
Difflugia oblonga Ehrenberg, 1838	+	+	−	+	−	+	+	−
Difflugia oblonga var. *parva* Thomas, 1954	+	−	−	−	−	−	+	−
Difflugia oblonga var. *nodosa* Leidy, 1879	−	−	−	−	−	+	+	−
Difflugia capreolata Penard, 1902	−	−	−	−	−	−	+	−
Difflugia lebes var. *masurica* n. v.	−	+	−	−	−	+	−	−
Difflugia viscidula Penard, 1902	−	+	−	−	−	−	−	−
Difflugia penardi Hopkinson, 1909	−	−	−	−	+	−	−	−
Difflugia histrio Penard, 1908	−	−	−	+	−	−	+	−
**Difflugia limnetica* (Levander, 1900) Penard, 1902	+	+	+	+	+	+	−	+
Difflugia lobostoma Leidy, 1879	+	−	−	−	−	+	+	−
Difflugia gramen Penard, 1902	−	−	−	−	−	−	+	−
Difflugia tuberculata (Wallich, 1864) Archer, 1897	−	−	−	−	−	−	+	−
Difflugia baculosa n. sp.	+	−	−	−	−	−	−	−
Difflugia elegans Penard, 1890	+	+	+	+	+	+	+	−
Difflugia elegans var. *teres* Penard, 1899	+	+	+	−	−	+	+	−
Difflugia varians Penard, 1902	+	+	−	−	−	−	+	−
Difflugia acuminata Ehrenberg, 1838	+	+	+	+	−	+	+	−
Difflugia acuminata var. *inflata* Penard, 1899	+	+	−	−	−	+	−	−
Difflugia lithophila (Penard, 1902) Gauthier-Lièvre et Thomas, 1958	+	−	−	−	−	−	+	−
Difflugia urceolata Carter, 1864	−	+	−	−	−	+	+	−
Difflugia amphora var. *africana* van Oye, 1931	−	−	−	−	−	+	+	−
Difflugia amphora var. *acollis* n. v.	−	−	−	−	−	+	+	−
Difflugia brevicolla Cash, 1909	−	−	−	+	−	−	+	−
Difflugia solowetzkii Mereschkowský, 1879	−	−	−	−	−	−	+	−

* Leere Schalen aus dem Pelagial.

Gefundene Arten	H.	S.	M.	TG.	T.	F.	L.	B.
Difflugia solowetzkii var. *stepaneki* n. v.	–	–	–	–	–	–	+	–
Difflugia corona Wallich, 1864	–	+	+	–	–	–	+	–
Difflugia lithoplites Penard, 1902	–	–	–	–	–	–	+	–
Difflugia lemani Blanc, 1892	–	–	–	–	+	–	–	–
Difflugia lanceolata Penard, 1890	–	–	+	–	+	–	–	+
Difflugia stechlinensis Schönborn, 1962	+	–	–	–	–	–	–	–
Difflugia pulex Penard, 1902	+	–	–	+	+	–	–	+
Difflugia pulex var. *cuneata* Playfair, 1917	+	–	–	–	–	–	–	–
Difflugia minuta Rampi, 1950	–	+	–	–	–	–	+	–
Difflugia richmondiae Playfair, 1914	+	–	–	–	–	–	–	–
Difflugia mica Frenzel, 1897	+	–	–	+	–	–	+	–
Difflugia globularis (Wallich, 1864) Leidy, 1877	–	+	–	–	–	–	–	–
Difflugia avellana var. *gigas* Gauthier-Lièvre et Thomas, 1958	+	–	–	–	–	–	–	–
Difflugia szczepanskii n. sp.	+	–	–	–	–	–	–	–
Difflugia compressa var. *minima* n. v.	–	+	–	–	–	–	–	–
Pontigulasia spectabilis Penard, 1902	+	+	+	+	–	+	+	–
Pontigulasia bigibbosa Penard, 1902	–	+	+	–	–	+	+	–
Pontigulasia incisa Rhumbler, 1896	–	–	+	–	+	–	+	–
Cucurbitella mespiliformis Penard, 1902	–	–	–	–	–	+	–	–
Lesquereusia spiralis (Ehrenberg, 1840) Schlumberger, 1845	–	–	–	–	–	+	+	+
Lesquereusia modesta Rhumbler, 1896	–	–	–	+	–	–	+	–
Hyalosphenia cuneata Stein, 1857	–	–	–	–	–	–	+	–
Phryganella acropodia (Hertwig et Lesser, 1874) Hopkinson, 1909	–	–	–	+	–	–	–	–
Cyphoderia trochus Penard, 1899	–	–	+	–	+	–	–	–
Cyphoderia ampulla (Ehrenberg, 1840) Schlumberger, 1845	–	+	+	–	–	–	–	+
Schaudinnula arcelloides Awerintzew, 1907	–	–	+	–	–	–	–	–
Summe der gefundenen Arten:	27	21	15	14	10	19	36	9

breit. Nach ihrem Bautyp scheint *C. deflandrei* eine typische sedimentbewohnende Art zu sein. R a m p i 1950 fand sie in Seen Italiens.

Centropyxis aërophila Deflandre, 1929; *Centropyxis cassis* (Wallich, 1864) Deflandre, 1929; *Centropyxis platystoma* (Penard, 1890) Deflandre, 1929 und *Centropyxis constricta* (Ehrenberg, 1838) Deflandre, 1929.

Diese Arten (Abb. 3), die in der Literatur als typisch aërophil bezeichnet werden, kommen auch im Sediment der Seen vor. *Centropyxis aërophila* bildet hier Standortsvarietäten aus, die sich durch dichten Xenosomenbelag von den xerisch lebenden Individuen unterscheiden (S c h ö n b o r n 1962). Diese *Centropyxis*-Gesellschaft des Sedimentbodens kommt im wesentlichen nur in oligotrophen Seen vor, wie auch die vorliegenden Untersuchungen

wieder bestätigten. Es wurde ferner ein Exemplar einer *C. aërophila* var. *sphagnicola* gefunden; doch kann es sich auch nur um ein zufällig in die Probe geratenes Tier handeln.

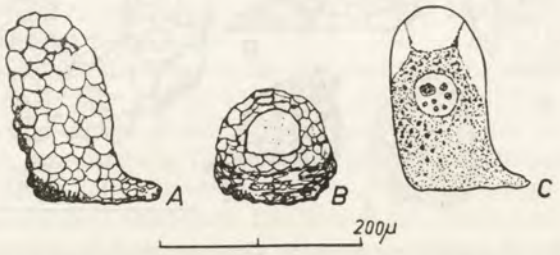

Abb. 2. *Centropyxis deflandrei*. A. Seitenansicht. B. Vorderansicht. C. Plasmakörper

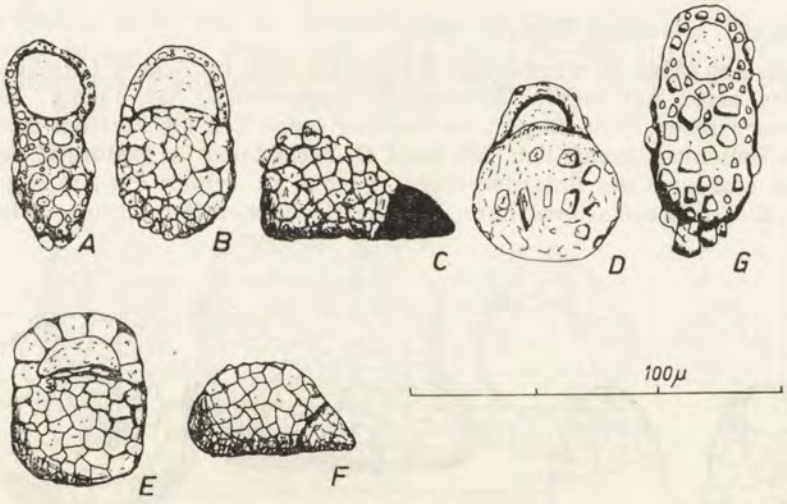

Abb. 3. Die sedimentbewohnende *Centropyxis*-Gemeinschaft des oligotrophen Sees. A. *Centropyxis platystoma* (Vorderansicht). B. *Centropyxis aërophila* (Vorderansicht). C. *Centropyxis aërophila* (Seitenansicht). D. *Centropyxis aërophila* (Übergangsform zwischen Xenosomen- und membranöser Schale). E. *Centropyxis cassis* (Vorderansicht). F. *Centropyxis cassis* (Seitenansicht). G. *Centropyxis constricta* (Vorderansicht)

Centropyxis aculeata (Ehrenberg, 1838) Penard, 1902; *Centropyxis discoides* (Penard, 1890) Deflandre, 1929 und *Centropyxis hirsuta* Deflandre, 1929.

Die drei Arten (Abb. 4) sind typische Bewohner des Aufwuchses. Auf dem Sedimentboden bilden einzelne Populationen von ihnen Standortsvarietäten aus, die sich in folgenden Merkmalen von den Aufwuchs-Tieren unterscheiden: Besatz mit Quarz-Xenosomen, Wegfall der Dornen und höhere Schalen (Schönborn 1962). Auch die vorliegenden Untersuchungen bestätigen dies. Moraczewski 1961 gibt *C. hirsuta* für den Boden des Kisajno-Sees an.

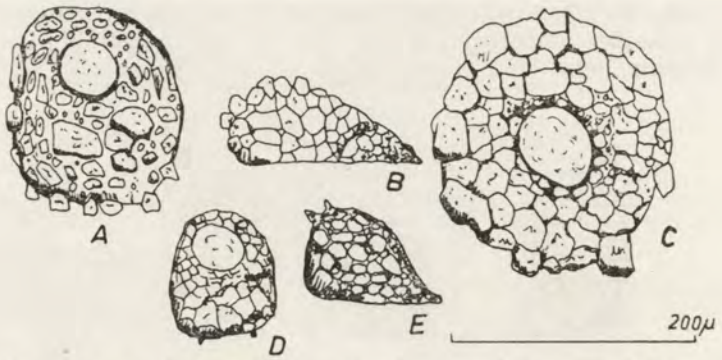

Abb. 4. Die sedimentbewohnende *Centropyxis*-Gemeinschaft verschiedener See-Typen. A. *Centropyxis aculeata* (Vorderansicht). B. *Centropyxis aculeata* (Seitenansicht). C. *Centropyxis discoides* (Vorderansicht). D. *Centropyxis hirsuta* (Vorderansicht). E. *Centropyxis hirsuta* (Seitenansicht)

Oopyxis cyclostoma Thomas, 1958.

Diese Art (Abb. 5) wurde von Thomas 1958 in Tibesti (Afrika) gefunden. Die erste Art dieser Gattung, *O. cyphostoma*, hat Jung 1942 aus Chile beschrieben. Seitdem galt die Gattung lange Zeit als verschollen. Daraus ist ihre Seltenheit ersichtlich. Ich fand *O. cyclostoma* im Sediment des Hańcza-Sees und auch des Śniardwy-Sees. Thomas erhielt die Art aus Algenproben. *Oopyxis cyclostoma* kann bei oberflächlicher Betrachtung sehr leicht

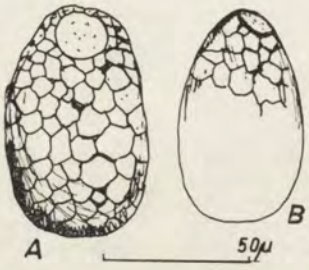

Abb. 5. *Oopyxis cyclostoma*. A. Vorderansicht. B. Seitenansicht

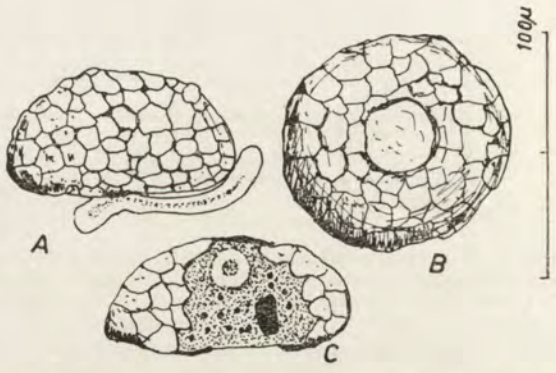

Abb. 6. *Geopyxella aquatica* n. sp. A. Vorderansicht. B. Seitenansicht. C. Plasmakörper

mit einer Difflugie verwechselt werden. Das Pseudostom ist nur sehr schwach abgeschrägt. Die Schale ist eiförmig, hyalin und lückenlos mit Quarz belegt. Das Pseudostom ist rund und zeigt keine Invagination. Alle gefundenen Schalen waren leer. Maße: 50—80 µ Länge; 40—50 µ Breite, 20—25 µ Durchmesser des Pseudostoms.

Geopyxella aquatica n. sp.

Die Gattung *Geopyxella* wurde von Bonnet et Thomas 1955 an der ausschließlich im Boden vorkommenden Art *G. sylvicola* aufgestellt. *Geopyxella* ist halbkugelig; das Pseudostom nimmt nicht die ganze Ventralseite ein und zeigt keine Invagination. In dem letzten Merkmal unterscheidet sie sich von der Gattung *Cyclopyxis*.

Geopyxella aquatica (Abb. 6) ist hemisphaerisch, das Pseudostom liegt zentral, ist kreisförmig, nimmt nicht die ganze Schalenbasis ein und zeigt keine Invagination. Nach diesen Merkmalen gehört die Art zumindest formalsystematisch zur Gattung *Geopyxella*. Die scharfe Trennung der Lebensweise beider. Arten läßt allerdings eher auf eine Analogie als auf eine natürliche Verwandtschaft schließen. Die Schale ist hyalin und dicht mit Quarzkörnchen belegt. Das Zytoplasma ist sehr durchsichtig; der Kern besitzt einen zentralen Nukleolus. Die Pseudopodien sind lobos. Maße: 50—75 µ Höhe; 90—110 µ Durchmesser; 20 µ Pseudostomdurchmesser.

Difflugia oblonga var. *parva* Thomas, 1954

Die Gestalt (Abb. 7A) ist birnenförmig und ähnelt bis auf die geringere Größe der Stammform. Maße: 100—150 µ Höhe; 40—50 µ Breite. Die Varietät war bisher aus Frankreich (Thomas 1954) und Afrika (Gauthiè-Liè vre 1958) bekannt.

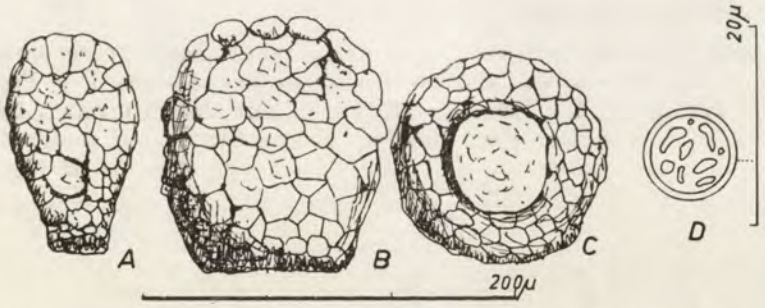

Abb. 7. A. *Difflugia oblonga* var. *parva* (Seitenansicht). B. *Difflugia lebes* var. *masurica* n. v. (Seitenansicht). C. *Difflugia lebes* var. *masurica* n. v. (Vorderansicht). D. *Difflugia lebes* var. *masurica* n. v. (Kern)

Difflugia lebes var. *masurica* n. v.

Die Varietät (Abb. 7 B—D) unterscheidet sich von der Stammform fast nur in der Größe (etwa halb so groß wie die Stammform). Sie trat in den von ihr bewohnten Seen stets konstant in Größe und Gestalt auf, so daß die systematische Kennzeichnung berechtigt ist. Die Gestalt der Schale ist fast kugelförmig. Das Pseudostom ist groß und kreisförmig. Der Kern besitzt zahlreiche unregelmäßige Nukleolen. Die Stammform war in keiner Probe anwesend. Maße: 150—200 µ Höhe; 150—180 µ Pseudostomdurchmesser.

Difflugia histrio Penard, 1908

Diese Art wurde von Penard 1908 aus dem Profundal einiger Schweizer Seen beschrieben. Später fand sie Jung 1942 in Moosen aus Chile wieder. Die Art ist sicher nicht so selten, wie man annehmen möchte. Sie ist vor allem sehr schwierig zu identifizieren. Die Schale ist breit, fast sackförmig und etwas unregelmäßig. Neben Quarzkörnchen findet man als Xenosomen überstehende Detrituspartikel (Abb. 8A). Maße: 150—180 µ Höhe; 70—100 µ Breite; 25µ Pseudostomdurchmesser.

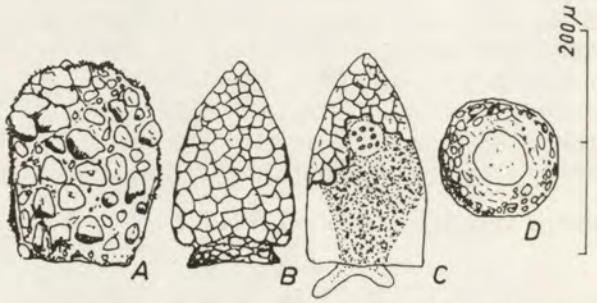

Abb. 8. A. *Difflugia histrio* (Seitenansicht). B. *Difflugia amphora* var. *africana* (Seitenansicht). C. *Difflugia amphora* var. *acollis* n. v. (Seitenansicht). D. *Difflugia amphora* var. *acollis* n. v. (Vorderansicht).

Difflugia baculosa n. sp.

Die walzenförmige Schale (Abb. 9) hat parallele Seiten, eine dorsale Rundung und nur eine schwache Abstutzung am Pseudostom. Das Pseudostom selbst ist dreilappig. Die Schale ist lückenlos mit Quarzsteinchen belegt; die Kittmasse ist hyalin. Der Kern liegt im oberen Schalenteil und besitzt eine variable Zahl von Nukleolen. Maße: 90—95 µ Länge; 40—45 µ Breite.

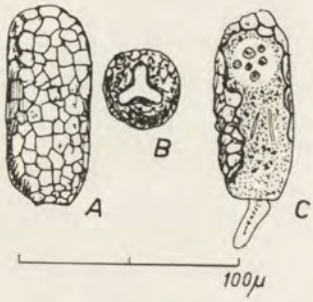

Abb. 9. *Difflugia baculosa* n. sp. A. Seitenansicht. B. Vorderansicht. C. Plasmakörper

Difflugia amphora var. *africana* van Oye, 1931 und *Difflugia amphora* var. *acollis* n. v.

Die Schale der neuen Varietät *D. amphora* var. *acollis* (Abb. 8 C—D) hat eine breite Basis und läuft dorsal spitz zu. Sie unterscheidet sich von der Form, die van Oye 1931 als *D. amphora* var. *africana* beschrieb (Abb. 8 B), durch das Fehlen eines durch eine Ringfurche abgegrenzten Kragens. Die

Schale von *D. amphora* var. *acollis* ist lückenlos mit Quarz belegt; die Kittmasse ist hyalin. Der Kern besitzt zahlreiche kleine Nukleolen. Die Pseudopodien sind lobos. Maße von *D. a.* var. *africana*: 180—200 µ Höhe; 90—100 µ Breite. Maße von *D. a.* var. *acollis*: 200 µ Höhe; 110—115 µ Breite.

Difflugia solowetzkii Mereschkowsky, 1879 und *Difflugia solowetzkii* var. *stepaneki* v. n.

Difflugia solowetzkii ist von Mereschkowsky 1879 im nördlichen Rußland entdeckt und meines Wissens seitdem nicht wieder gemeldet worden. Chardez 1956 bildet eine ähnliche Form ab, die aber die charakteristischen Merkmale „spitze Schale" und „Einschnürung vor dem Pseudostom" nicht so ausgeprägt besitzt, wie die von Mereschkowsky und von mir jetzt gefundenen Exemplare. Chardez rechnet seine Form noch zum Formenkreis der *Difflugia oblonga* var. *lacustris* Penard. Da die in den masurischen Seen gefundenen Individuen konstante Formenmerkmale besaßen, halte ich die Art für berechtigt (Abb. 11 A).

Daneben trat eine kleinere Form auf, die dem von Štěpánek 1963 aus Katanga abgebildeten Exemplar identisch zu sein scheint. Štěpánek ordnete sie provisorisch (mit einem Fragezeichen versehen) der Art *Difflugia elegans* zu. Diese Form ist morphologisch aber nahe verwandt mit *D. solowetzkii*, so daß ich sie als *D. solowetzkii* var. *stepaneki* benenne (Abb. 11 B—D).

Maße von *D. solowetzkii*: 80—100 µ Höhe; 30—35 µ Breite, 15—18 µ Pseudostomdurchmesser. Maße von *D. solowetzkii* var. *stepaneki*: 30 µ Höhe; 15—18 µ Breite, 7—8 µ Pseudostomdurchmesser.

Difflugia richmondiae Playfair, 1914

Die Schale ist klein, gedrungen, dorsal gerundet und vor dem Pseudostom etwas konisch zugespitzt, wodurch die Art ein sehr charakteristisches Aussehen erhält (Abb. 10). Die Schale ist hyalin und mit vereinzelten, amorphen, grünlich-glänzenden „Pseudoquarzen" bedeckt. Das Plasma ist völlig hyalin. Es konnte eine pulsierende Vakuole beobachtet werden, die sowohl in der Nähe des Kernes als auch nahe der Schalenöffnung liegen kann. Der Kern

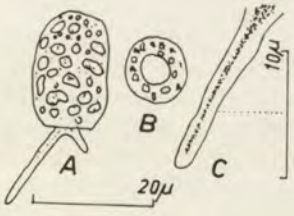

Abb. 10. *Difflugia richmondiae*. A. Seitenansicht. B. Vorderansicht. C. Pseudopodium

besitzt einen zentral gelegenen Nukleolus. Die Pseudopodien sind schmal, vorn abgerundet und in der Gestalt fast als reticulolobos zu bezeichnen. Doch ist deutlich eine endoplasmatische Achse vorhanden, so daß diese Pseudopodien zu den Lobopodien gerechnet werden müssen. Die Art ist bisher nur aus Australien bekannt (Playfair 1914). Maße: 16—25 µ Höhe; 10—13 µ Breite; 3—4 µ Pseudostomdurchmesser.

Difflugia avellana var. *gigas* Gauthier-Lièvre et Thomas, 1958

Diese Varietät (Abb. 12 A—C) ist größer und breiter und etwas stärker komprimiert als die Stammform. Das Pseudostom ist ungleichmäßig elliptisch. Die Schale ist leicht bräunlich, bedeckt mit vereinzelten Quarzen (Xenosomen) und Pseudoquarzen (Idiosomen). Bisher ist die Form nur aus Afrika bekannt (Gauthier-Lièvre et Thomas 1958). Maße: 200 µ Länge; 130—135 µ Breite; 108—110 µ Dicke.

Difflugia szczepanskii n. sp.

Die Schale (Abb. 11E—H) ist oval, komprimiert und am Pseudostom nur schwach abgestutzt. Das Pseudostom ist unregelmäßig kreisförmig. Die Schale ist hyalin und bedeckt mit vereinzelten Quarzen (Xenosomen) und grünlichglänzenden Pseudoquarzen (Idiosomen). Der Kern enthält mehrere Nukleolen. Die Pseudopodien sind lobos. Die Art wurde regelmäßig in dem oligotrophen Hańcza-See in über 100 m. Tiefe gefunden. Ich benenne die Art zu Ehren von Herrn Dr. Szczepański. Maße. 60—80 µ Länge; 30—35 µ Breite; 20—27 µ Dicke.

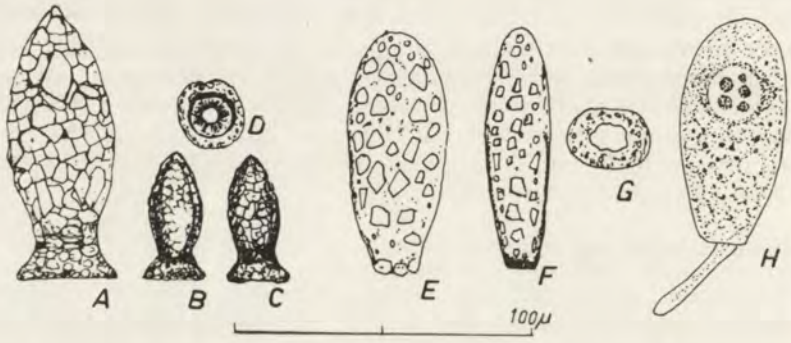

Abb. 11. A. *Difflugia solowetzkii* (Seitenansicht). B. und C. *Difflugia solowetzkii* var. *stepaneki* (Seitenansicht). D. *Difflugia solowetzkii* var. *stepaneki* (Vorderansicht). E. *Difflugia szczepanskii* n. sp. (Breitseite). F. *Difflugia szczepanskii* n. sp. (Schmalseite). G. *Difflugia szczepanskii* n. sp. (Vorderansicht). H. *Difflugia szczepanskii* n. sp. (Plasmakörper)

Difflugia compressa var. *minima* n. v.

Die Schale (Abb. 12 D—F) ist komprimiert mit stark konvergierenden Flanken. Das Pseudostom ist rund; bedeckt ist die Schale mit großen lückenlos zusammengefügten Quarzkörnchen. Die Varietät unterscheidet sich nur durch die geringere Größe von der Stammform. Die Stammform selbst war in keiner Probe anwesend. Maße: 150—180 µ Höhe; 110—120 µ Breite; 20—35 µ Dicke; 15—20µ Pseudostomdurchmesser.

Schaudinnula arcelloides Awerintzew, 1907

Diese von Awerintzew 1907 auf der Insel Waigatsch gefundene Gattung galt seitdem als verschollen. Die zusammenfassenden Werke über Testaceen führen diese Gattung nicht mehr auf. Der erneute Fund aus dem Mikołajskie-See ist also eine Wiederentdeckung dieser interessanten Form. Ob es sich bei dieser Art um einen borealen Verbreitungstyp handelt, läßt sich

vermuten, aber noch nicht beweisen. Die Gattung vermittelt, wie dies auch schon Awerintzew betont, zwischen *Cyphoderia* und *Campascus*. Awerintzew hat die Ausbildung von Pseudopodien nicht beobachten können. Nach meinen Beobachtungen handelt es sich um Filopodien, so daß die Einordnung in die *Cyphoderiidae* bestätigt wird.

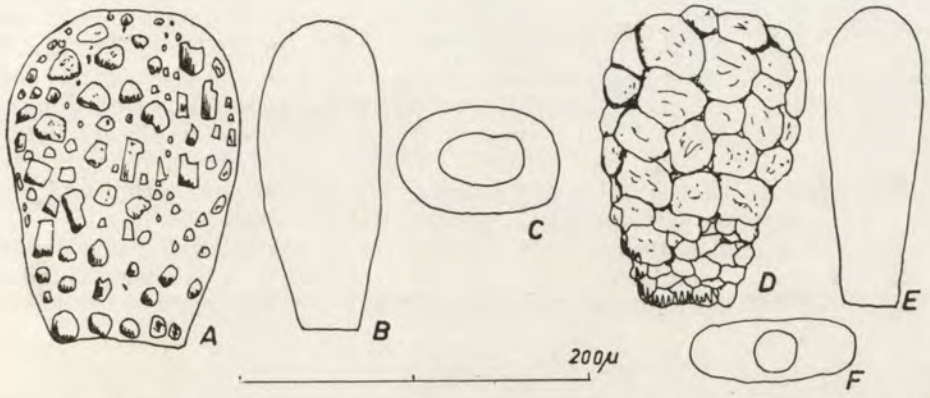

Abb. 12. A. *Difflugia avellana* var. *gigas* (Breitseite). B. *Difflugia avellana* var. *gigas* (Schmalseite). C. *Difflugia avellana* var. *gigas* (Vorderansicht). D. *Difflugia compressa* var. *minima* n. v. (Breitseite). E. *Difflugia compressa* var. *minima* n. v. (Schmalseite). F. *Difflugia compressa* var. *minima* n. v. (Vorderansicht).

Schaudinnula arcelloides (Abb. 13) hat eine retortenförmige Schale, die im Querschnitt dreieckig ist. Sie erinnert in der Gestalt an *Campascus triqueter* (die Konturen sind jedoch regelmäßiger), unterscheidet sich aber von ihr durch das Fehlen eines hyalinen Kragens und der Xenosomen. Sie besitzt

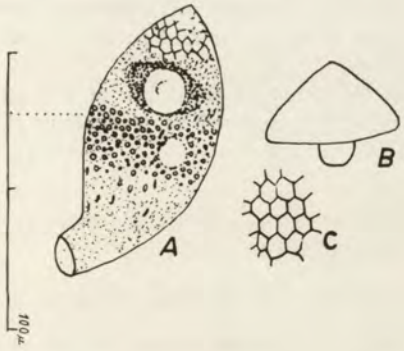

Abb. 13. *Schaudinnula arcelloides*. A. Seitenansicht. B. Querschnitt. C. Schalenstruktur

dagegen unregelmäßige hexagonale Plättchen, die entfernt an die Felderung von *Arcella* erinnern. Das Plasma ist deutlich zoniert. Der Kern mit seinem zentralen Nukleolus ist von einem Chromidium umgeben. Unterhalb des Kernes befinden sich „Phäosomen" und andere Partikel. In dieser Zone liegt auch die pulsierende Vakuole. Maße: 98—105 µ Länge; 40 µ Breite.

Zusammenfassung

Die sedimentbewohnenden Arcellen sind *Arcella hemisphaerica* und *A gibbosa* mit ihren Varietäten. Als typische *Centropyxis*-Art des Sedimentes kann *C. deflandrei* angesehen werden. Die *Centropyxis*-Arten *C. aërophila, C platystoma, C. constricta, C. discoides, C. aculeata* und *C. hirsuta* bilden sedimentbewohnende Populationen aus, die sich als morphologische Standortsvarietäten von ihren in anderen Biotopen lebenden Stammformen unterscheiden. Die charakteristische Testaceen-Gattung des Profundals ist *Difflugia*. Von den 65 identifizierten Testaceen-Formen der untersuchten Seen der Masuren gehören 39 zur Gattung *Difflugia*. Neu für die profundale Testaceenfauna sind *Oopyxis cyclostoma* Thomas, *Geopyxella aquatica* n. sp. und *Schaudinnula arcelloides* Awerintzew. Ihre Bautypen entsprechen aber den Lebensformtypen der sedimentbewohnenden Testaceen. Es konnten noch zwei neue Arten und vier neue Varietäten der Gattung *Difflugia* aufgestellt werden.

Die Vertreter der übrigen in der Artenliste aufgeführten Gattungen können nach unseren heutigen Kenntnissen ebenfalls als typisch oder zumindest obligatorisch für das Sediment der Seen gelten.

SUMMARY

The sediment-inhabiting species of *Arcella* are *Arcella hemisphaerica* and *Arcella gibbosa* and their varieties. As a typical *Centropyxis*-species of the sediment the *C. deflandrei* may be looked upon. The *Centropyxis*-species of *C. aërophila, C. cassis, C. platystoma, C. constricta, C. discoides, C. aculeata* and *C. hirsuta* are producing sediment-inhabiting populations that distinguish themselves as morphological locality-varieties from their parent forms living in other biotopes. The characteristic testacea-genus of the profundal is *Difflugia*. Of the 65 identified testacea-forms in the investigated Masurian Lakes, 39 belong to the genus *Difflugia*. What was new for the profundal fauna of testacea were *Oopyxis cyclostoma* Thomas, *Geopyxella aquatica* n. sp. and *Schaudinnula arcelloides* Awerintzew. Their structural types however correspond with the living-form types of the sediment-inhabiting testacea. What could be established in addition were two new species and four new varieties of the genus *Difflugia*.

The representatives of the remaining species listed in the table of genera may be likewise, according to our present knowledge, considered as typical or at least obligatory for the sediment of the lakes.

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