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Krystyna GOLIŃSKA and Maria JERKA-DZIADOSZ

The relationship between cell size and capacity for division in *Dileptus anser* and *Urostyla cristata*[1]

Badania nad zależnością między wymiarami komórki
a jej zdolnością do podziału u *Dileptus anser*
i *Urostyla cristata*

Relationships between the dimensions of a cell and its ability to divide have been ascertained by numerous authors who have studied the processes of division control. Observations concerning the relationship between the size of protozoan cell and its division capacity are included in many studies dealing with such questions as growth, nutrition, and action of temperature on cell cycle. There are many hypotheses concerning the initiation of division and conditions necessary for the division to occur. They can be divided into two large groups. In the first group there are these concepts in which the initiation of division or the necessary requirements for the cell to divide are related to some geometrically measurable elements of the cell. The second large group of concepts binds the division to the defined quantity or concentration of some substance within the cell.

To the first group of hypotheses the nucleo-cytoplasmic ratio concept of Hertwig 1903 can be included. The author thought that exceeding the critical ratio switches on the division processes. This view has undergone several modifications over time. Phelps 1926 considered that in amebas division is initiated by exceeding a defined volume of cytoplasm. Weisz 1956 thought that division in *Stentor* could be induced through overstepping a critical amount of endoplasm. Causin 1931 concluded from his study on *Stentor* that upsetting an equilibrium between the volume of nucleus and the volume of cytoplasm brings about reorganization activity, which then appears as division or reorganization. Recently De Terra (1960, 1969, a, b) developed the "critical

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[1] Recently a paper of A. Borror 1972 (J. Protozool., 19, 1-23) appeared in which *U. cristata* is placed in a new genus *Pseudourostyla*.

oral-somatic ratio" hypothesis. From her experiments on *Stentor* she concluded that the formation of oral primordia is initiated through overstepping a critical value of the ratio between the amount of oral fibrillar protein and the amount of somatic fibrillar proteins. According to this concpet the proportion of the oral field to the lengths of the cell defines the ability for formation of oral primordia. Division occurs when oral primordia develop in a large cell, when the cell is small physiological reorganization takes place.

The concepts which can be included in the second group of hypotheses are based on assumption that the initiation of division is geared to the defined amount of some specific substance (review in M a z i a 1961). Consequently the occurrence of division would be rather loosely connected with the measurements and the size of a given cell. P r e s c o t t 1956 repeated and confirmed the experiments of H a r t m a n n 1928, in which the amputation of cytoplasm inhibited division. Although he did not consider doubling of the cell mass as a trigger for division, since division occurs about 4 h after growth has terminated (P r e s c o t t 1955, 1956). The same author observed (P r e s c o t t 1960) that syntheses of DNA, RNA and proteins are not directly coupled to the initiation of division. He made an interesting supposition that the preparation for a given division may take place one or several cell cycles before that division.

The Swann's "reservoir hypothesis" (S w a n n 1954, 1957) postulated the existence in a cell of a special pool of substances, which may be exhausted only through the process of division. The overfilling of the pool — i.e., exceeding of a critical amount of some substance would be the trigger for division.

Study on the components of a cell connected with the initiation of division were carried out by many workers who dealt with synchronized *Tetrahymena*. Owing to the fact that neither a definite rate of growth nor a determined size initiated division (W i l l i a m s and S c h e r b a u m 1959), the existence of special "division proteins" was postulated by R a s m u s s e n and Z e u t h e n 1962. Such temperature — sensitive proteins, necessary for division, have to be synthesized anew after the termination of heat shocks. Since two or three divisions may occur after synchronization without food supply (H a m b u r g e r and Z e u t h e n 1957) the division proteins can be synthesized at the expense of amino acids pool (C r o c k e t t et al. 1965). The "division proteins" are connected to the phenomena of cytokinesis rather than to the process of oral primordia formation, since stomatogenesis may occur without cytokinesis during temperature treatment (F r a n k e l 1964).

Watanabe and Ikeda 1965 reported that they isolated the "division proteins", however, Lowe-Jinde and Zimmerman 1971 failed to reproduce the experiments.

The studies presented in this paper represent an attempt to change experimentally the size of a dividing cell by cutting off smaller or larger parts of a cell and by supplying a large amount of food. The material chosen for the experiments seems to be very convenient for such study on several respects. *Dileptus* and *Urostyla* belong to a very distant groups of *Ciliata*, they differ substantially in the pattern of morphogenesis, way of life, nutrition and so on. Because of this, we consider that the regularities found for the two species may have a general significance.

The main conclusions of our study are: (1) That in a given ciliate cell there is a minimal size which when achieved makes the cell able to divide. This size although not itself being a trigger for division, is its necessary prerequisite. (2) There are two unequivalent kinds of interphases in the cell cycles of ciliates. (3) The proportions between different parts of cortex (oral and somatic) seem to play no role in the initiation of divisional processes.

Material and methods

Dileptus anser (O. F. M.) used in these experiments was collected in 1971 from pond in Tleń, district Świecie (Poland). The cells were maintained in Petri dishes with modified Pringsheim solution as a medium. The food was *Colpidium colpoda* cultivated separately in Erlenmayer flasks of 100-150 ml volume. Before inoculation of *Colpidium* into the Pringsheim solution several grains of oats were put into the flasks and 3-5 drops of anaerobic sulphur bacteria were added. To such a prepared medium *Colpidium* was transferred. Two times a week the culture medium was enriched with egg yolk suspension. Before being fed to *Dileptus*, the *Colpidium* culture was filtered through non absorbent cotton, washed with Pringsheim solution, condensed and collected geotactically in a flask with the narrow neck.

The cultures of *Dileptus* were fed daily. *Colpidium* was added in an amount such that 24 h after feeding there was no food left in the medium.

Experiments were performed with predividing specimens and early dividers. The dividing specimens were of different size depending on the amount of food in cultures. Exceptionally large dividing cells ("large dividers") were produced as follows: the culture in which there were many dividing cells was left without feeding until the next day, and then a large amount of *Colpidium* was added — so that for every individual *Dileptus* there were about 30-50 individual *Colpidia*. 5-6 h after extensive feeding, very large dividing animals were found in the culture.

The ability to divide in *Dileptus anser* was studied in the following groups of organisms:

— "regular dividers" — dividing cells isolated from standard cultures 24 h after feeding. Such cells did not contain visible food vacuoles with indigested food in it:

— "large dividers" — dividing cells isolated 5-6 h after heavy feeding. The cells often contained large food vacuoles.

— "operated dividers" — dividing specimens diminuted by amputation of a portion of a "regular" or "large" divider.

— "regular predividers" — cells isolated from standard cultures 24 h after feeding, of a size similar to regular dividing cells.

— "large predividers" — cells isolated from culture after 5-6 h after heavy feeding, of the same size as dividing cells in the same culture.

Only early dividers were studied. Specimens were considered as early dividers which posessed oral primordia in the stages of development from the appearance of a ventral band primordium on the dorsal side to the appearance of a protrusion on the ventral side indicating the onset of formation of cytopharyngeal complex (Golińska and Doroszewski 1964, Golińska 1972). In this period of development there are no changes in the nuclear apparatus yet, and no signs of constriction of the fission furrow.

The moment of isolation always marked the beginning of starvation of studied animals. Isolated specimens of *Dileptus* were placed individually into small drops of culture medium, mounted in paraffin oil, or into single spot of three depression slides containing 0.5 ml culture medium. The individuals mounted in small and flat drops covered by paraffin oil were cut with a glass needle by means of the De Fonbrune micromanipulator. Observations were made using the phase-contrast microscope. The experiments were performed in uncontrolled room temperature ranging from 24-27°C.

Urostyla cristata Jerka-Dziadosz used in this study was collected in June 1971 in the pond of Sadyba in Warsaw (Poland). The cells were maintained in Petri dishes. Every day half the amount of culture medium was removed and new food diluted 1 : 1 with Pringsheim solution was added. The food was *Aerobacter aerogenes* incubated for 24 h in 0.15% dry lettuce infusion in 27°C.

24 h after feeding a semi-synchronous burst of division occurred in the cultures. The dividing and predividing animals were then isolated. Cells corresponding to "regular" and "operated" dividers and predividers of *Dileptus* were investigated. Predividers and dividers of *U. cristata* were isolated individually into spots in three depression slides with about 0.5 ml of sterile Pringsheim solution. Starvation began with the moment of isolation. The operations were carried out by hand in the culture dishes using sharp microscalpel. The fragments to be studied were transferred to a depression slide, whereas the portions removed by amputation were transferred on to a slide and immediately fixed with the saturated mercuric chloride. The existence of a replication band in the distal half of macronuclei, and large micronuclei, indicated that the operated cells had been in late S phase of the cell cycle (cf. Jerka-Dziadosz and Frankel 1970, Jerka-Dziadosz 1972). Individuals without replication band, or with less than half of each Ma replicated were rejected. In this way each operated predivider had a cytological control of the stage in cell cycle at the moment of operation and isolation into the mineral medium.

Celss of *U. cristata* in which the replication bands were located at the distal

end of each Ma, or the macronuclei were rounded up, but still not undergoing coalescence, were considered as early dividers. At this time one may observe the first two stages of ciliary primordia formation on the surface. The kinetosomes for the oral and cirral primordia for proter and opisthe are formed. The details of morphogenetic processes were described elsewhere (J e r k a - D z i a d o s z 1972).

Results

Observations on division of unoperated specimens

Before the beginning of the actual experiments, the observations on the length of the generation time in the studied species in the presence of food were carried out. Late dividers with a well developed fission furrow were isolated from cultures and observed until the next division.

15 samples of synchronously dividing specimens of *U. cristata* with 10 individuals in each, were observed. The micropopulations were maintained in culture medium (1 : 1 mixture of Pringsheim solution and *A. aerogenes* in dry lettuce infusion). Under these conditions the shortest generation time was 10.5 h and most cells divided about 18-20 h after isolation.

15 samples of synchronously dividing cells of *D. anser* with 10 individuals in each sample were observed. The cells were kept in Pringsheim solution and washed *Colpidium* were added, so that for each specimen of *Dileptus* there were about 15 cells of *Colpidium*. The shortest generation time was 12 h, most cells divided about 20 h after isolation, the longest generation time was about 36 h.

Preliminary observations of isolated single dividing cells in medium lacking food (sterile Pringsheim solution) were performed. It was observed that isolated dividers of *Urostyla* and *Dileptus* were able to go through a second division in spite of the lack of food. 30 dividing cells of *Urostyla* were isolated into single spots in three depression slides and observed for 48 h. From that 100% furrowed about 1 h after isolation and 75% of the group divided second time within 24 h (Fig. 4 control). It was also observed that the division of offspring of isolated dividers in *Urostyla* is synchronous, when both proter and opisthe are able to divide second time. In about 40% of the group only one descendant was capable for division. The other sister cell did not divide at all, and could have reorganized several hours later. In 43% of the group both proter and opisthe divide synchronously (the generation time was about 22-24 h for that group). These observations served as a control for operated early dividers in *Urostyla*.

The observations on control dividing cells of *Dileptus* revealed that the descendants of a dividing *Dileptus* cell do not divide synchronously,

with the interval between division of proter and opisthe lasting several hours. Very often in the absence of food only the proter divides a second time. Careful observations have revealed that the division of *D. anser* is very frequently unequal, i.e., the fission furrow is shifted posterior-wards, and the descendant opisthe is much smaller than the proter. Opposite situations, where the proter would be smaller than the opisthe, were not noticed.

Observations of predividing specimens of *Dileptus* serve as controls for corresponding observations on operated specimens. Since *Dileptus* does not exhibit such visible markers of the cell cycle as the existence of the replication band in the Ma of *Urostyla*, it was necessary to obtain
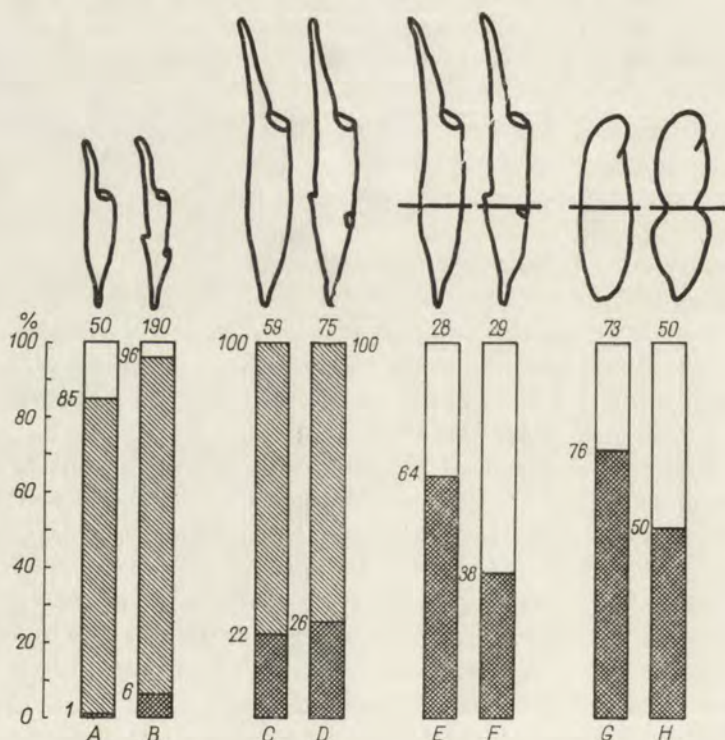
Fig. 1. Comparison of division ability in unoperated and operated cells. Transection in experiments E-H is considered as first division. A-F — *Dileptus*, G-H — *Urostyla*, A — regular predivider, B — regular divider, C — large predivider, D — large divider, E — operated large predivider, F — operated large divider, G — operated predivider, H — operated divider

The numbers on the bar tops indicate the number of studied cells in each group. The striped portion of the bars indicate the proportion of cells which divided once during 24 h observation, in percentage marked next to the upper margin of striped area. The cross-hatched parts of the bars indicate the proportion of cells which divided twice during 24 h, in percentage marked next to the upper limit of cross-hatched areas. The results presented in bars A and B are the control observations for the experiments from Fig. 4 (F—J) and Fig. 5.

data which then will make possible estimation of actual predividers in mass cultures. Specimens of the same size as specimens actually dividing in a given culture were regarded as predividers. The time of observation of isolated specimens was 24 h from the moment of isolation.

190 predividing specimens of *D. anser* were observed. During 24 h 84.7% of individuals divided once, 0.6% divided two times and 14.7% did not divide (Fig. 1 A). Among 50 studied regular dividers — i.e., small cells at early stage of division — 90% divided within 24 h, 6% divided two times and in 4% of the specimens the division is blocked (Fig. 1 B).

Among 63 large predividers all (100%) divided once during 24 h, and 22% of specimens divided two times (Fig. 1 C). 78 large dividers were observed. 100% divided once and 25.6% of individuals divided two times (Fig. 1 D).

61 specimens of *Dileptus* were observed longer time (48 h). Among them 97% divided within the first 24 h, and only 3% divided later than 24 h.

Therefore, the division capability of different fragments during 24 h of isolation and starvation were studied.

Morphogenetic response after operation in *Urostyla* and *Dileptus*

Before describing the influence of different kinds of operation on the ability of *U. cristata* and *D. anser* to divide we will provide some information as the fate of different fragments of early dividers of the species in question. Observations on this matter were partially published in previous publications (G o l i ń s k a 1966, J e r k a - D z i a d o s z 1967).

The fragment of *Dileptus* initiates regeneration of an excised part almost immediately after operation, equally after loss of the mouth as of the tail. This occurs independently of whether the division furrow (closely connected with the primordia of the new mouth) is resorbed or not. After operation of predividers the simultaneous initiation of regeneration and furrow formation occur. The formation of a division furrow — i.e., the formation of oral primordia for the opisthe — begins after the excised original mouth has regenerated.

Inhibition of the development of the division furrow as a result of operation is always coupled with the resorption of oral primordia. This inhibition may be permanent or division can be reinduced after few hours and a new fission line is formed in a new place (Fig. 2 A). Approximate measurements of the time elapsed between resorption and reinitiation of the division furrow in different kinds of fragments in which the division furrow initially regresses were made. They amount to respectively: after transection in the middle of the body — 3.5 h;

after excision of the proboscis — 5 h, 5 h, 5.5 h, 4.5 h, after excision the whole mouth with the nemadesma basket — 5 h, 5.5 h, after excision a large posterior part of the body — 3 h, 5.5 h, after incision — 4 h. As it follows from these limited measurements the site of injury does not influence the duration of blockage of furrowing.

When the division furrow of *Dileptus* is not resorbed after operation, the division processes proceed independently from regeneration of mouth or tail. This results in one of the products of division being smaller than the other (Fig. 2 A).

The mode of reaction of early dividers of *U. cristata* is represented in Fig. 2 B. In our experiments the inhibition of furrowing in operated early dividers of *Urostyla* occurred very rarely. In most cases the divisional processes proceeded undisturbed until completion of constriction, and afterwards (about 4 h after cleavage) the regeneration of injured offspring began.
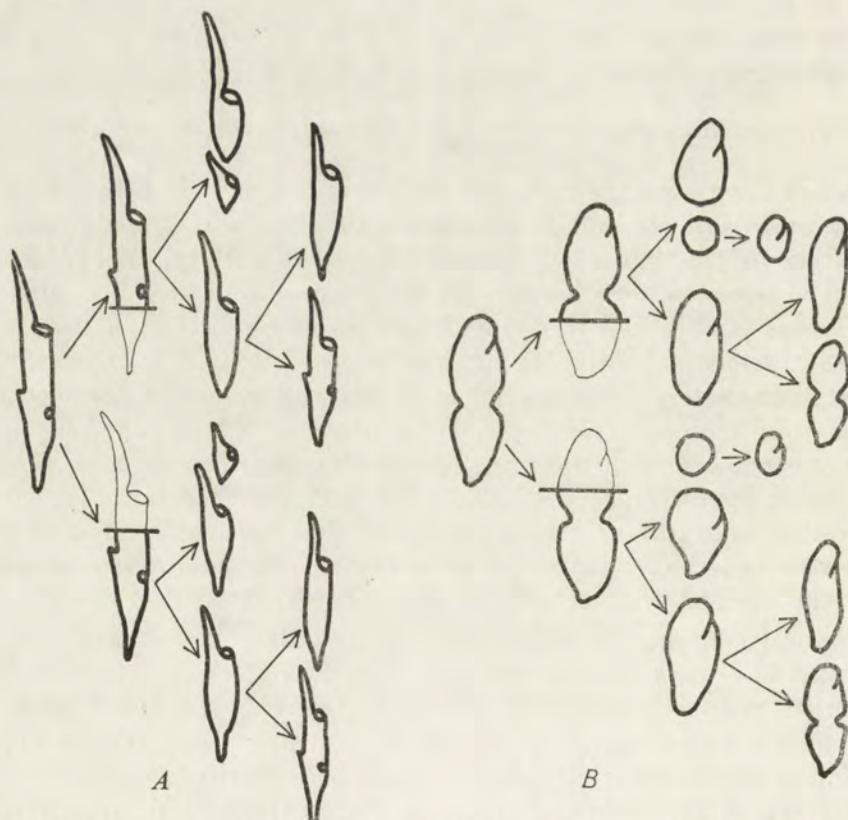
Fig. 2. Morphogenetic response after operation of dividers of *Dileptus* (A) and *Urostyla* (B). Heavy lines represent the observed fragment, thin lines indicates the excised fragments

In some (infrequent) cases the divisional morphogenesis and formation of the furrow is inhibited, the divisional primordia become resorbed, and after several hours regeneration primordia are formed. Reinitiation of new divisional processes also can occur. In this case — as in *Dileptus* — the furrow is located in new place, proportionally to the size of the fragment.

Division ability of the fragments from the predividers and dividers of *Urostyla*

The results of experiments with operation of predividers and dividers of *Urostyla* are represented on Fig. 3 and 5. Each type of operation was repeated on at least 30 individuals. On the diagrams the shaded part indicates the percentage of individuals which divide one or two times within 24 h after operation. The general conclusion of this experiment is that the ability for division decreases proportionally with the diminution of the size. Since no measurements were made of fragments the size was estimated according to the outline of the cell. This is very rough estimation, and the "size" corresponds rather to the surface of the fragment, than to the volume of the studied fragment.

The observations on operated predividers can be divided into three groups: 1 — observations on promers (Fig. 3, E, F, H), i.e., fragments with the posterior part of the cell removed. 2 — observations on opimers (Fig. 3, A, C, D) i.e., fragments with the anterior part of the cell removed, and 3 — middle fragments with removed anterior and posterior portions of the cell removed (Fig. 5 A, B, C, D).

In *Urostyla* no difference in the ability for division has been observed between promers and opimers of similar size. However, middle fragments of the same size differ from them (cf. Fig. 3, A, H, Fig. 5, D). Their ability for division is much greater. This result indicates, that the presence, injury or lack of the oral apparatus does not influence the ability of the given fragment of *Urostyla* to divide. The most prominent feature of the middle fragments, which may be responsible for their relatively great ability for division, in their significant width — they contain much more endoplasm than small promers and opimers.

All fragments of predividers after operation formed regeneration primordia, later on they formed divisional primordia. The replacement of regeneration by division was never observed.

Two fragments of the same cell almost never divide synchronously. Even when the line of section corresponds to the division furrow the resulting promer and opimer did not divided synchronously, and often only one of the fragments divide. This is in sharp contrast to the control,
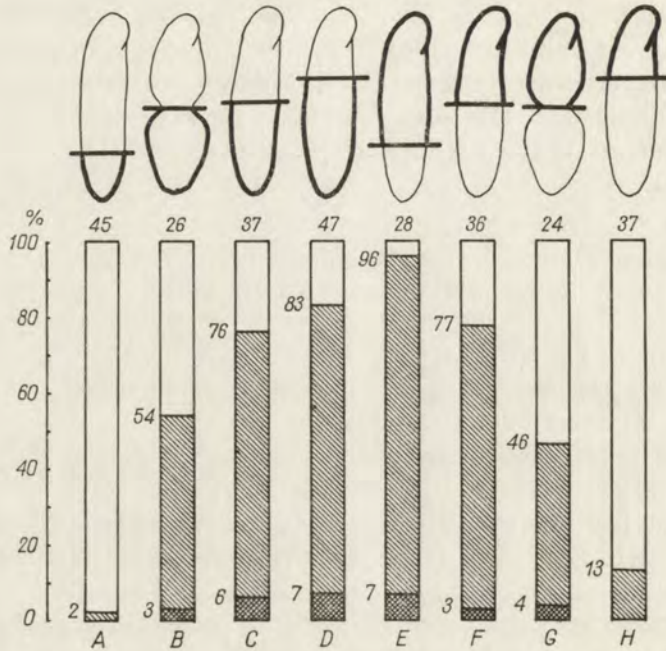
Fig. 3. Division ability of the fragments of *Urostyla*. A, C, D, E, F — predividers, B, G — dividers. The conventions are the same as on Fig. 1. The studied fragments are marked by heavy lines

unoperated dividers in which offspring of one cell mostly divide synchronously.

As it follows from the figures, small fragments which constitute only 1/3 of a normal predividing cell can regenerate and divide. Half of the predividing cell can not only regenerate but can occasionally perform two succesive divisions without food supply. In order to get a complete picture several operations were performed in which fragments smaller than 1/3 of the cell were obtained. Also some of the young G1 cells were transected and followed. Such small fragments were not able to divide.

A similar series of experiment as described above for predividing cells were performed also on early dividers (Fig. 3, B, G, Fig. 4). The results lead to the same general conclusion that the capability for division increases with the increase of the fragment size.

Closer comparison of the results of experiments on predividers and dividers reveals some interesting differences. The corresponding fragments of predividers showed in comparison to divider fragments higher percentage of division (cf. Fig. 3 B and C, F and G) in case when the line of cut follows the division furrow.

The descendants of dividing fragment did not form a uniform group and therefore can not be simply compared with the fragments from dividing cells of *Dileptus*. The group of descendants of divider fragments of *Urostyla* contains the fragments which resumed the process of division initiated before the operation and those in which cytokinesis was inhibited and division later induced anew. As it follows from the results presented on Fig. 4 a cell which is already dividing almost invariably completes that division in progress after the transection (100%, 97%, 96% respectively). A true test of its division capacity is whether it will divide again. This is showed on the second part of the Fig. 4 (A', B', C'). A newly divided cell is thus comparable to a fragment of similar size cut from a predivider (Fig. 4, D, H).
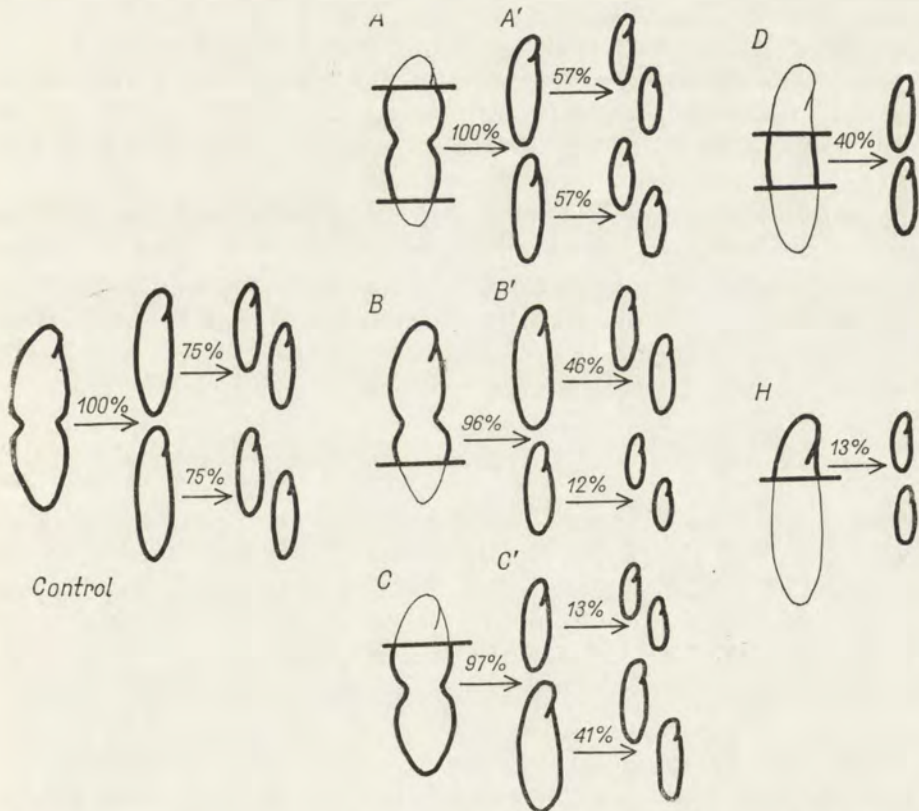
Fig. 4. Comparison of division ability of control dividing specimens of *Urostyla* and fragments from dividers (A, B, C). The outlines D and H are the same from Fig. 3 and 5. The studied cells are marked by heavy lines. The number on the arrows indicate the percentage of divided specimens in each group. Each group contains about 30 individuals

The fragments from dividers differ from the predivider ones also with respect to the time required for regeneration. The fragments derived from dividing cells revealed a time of regeneration significantly longer than fragments from the morphostatic cells (J e r k a - D z i a- d o s z 1967). In such a fragment the regenerating primordia were initiated about 6 h after operation (2 h in morphostatic cells) and the process of regeneration was completed about 9 h after operation.

Comparison between unoperated descendants from operated dividers and control dividers shows that the ability for two divisions is almost two times larger in the control, unoperated animals (45% and 75% respectively). This would indicate that even in operated dividers in which the division is not inhibited by operation, the fission line can be shifted toward the injured offspring, and/or there is rearrangement of endoplasm. As a result the unoperated descendant from operated early divider could be of smaller size than the control. However, there is no direct proof for that supposition.

From above it follows, that despite the differences in reaction to operation in operated dividers in *Urostyla* and *Dileptus*, in both species in dividers as well as in predividers the capability for division is proportional to the size of fragment.

In conclusion to this section it is worth pointing out that in these experiments the process of physiological reorganization was not taken into consideration. This process frequently occurred in specimens in which division was inhibited. Also the occurrence of the physiological reorganization between regeneration and division was occasionally observed.

Division capacity in regular fragments of *Dileptus*

The experiments with the operation of predividers and early dividers in *Dileptus anser* showed that division ability decreases parallel to the diminution of size. The results of experiments are shown in diagrams on Fig. 5 E-I, and Fig. 6. As in *Urostyla*, each diagram represents more than 30 fragments after similar operation. The shaded part represents the percentage of individuals which divided during 24 h after operation.

Unoperated, regular specimens of *Dileptus* exhibited the little capacity to divide two times successively in the absence of food (cf. Fig. 1 A and B). Any kind of operation resulted in the fragments being able to divide only once. It seems that the limit of size of the fragment capable of division is about half of a "regular" individual — predivider as well as divider. The lack of ability for division of the microsurgically separated opisthe (0% — Fig. 5 B), and considerably higher ability for division

of the microsurgically separated proter (25% — Fig. 6 N) may be explained by the fact, that the division furrow in regular dividers is almost always located slightly posterior of the middle of the cell. As a result the two fragments are of different size. The lack of differences between both halves of predividers (Fig. 6 A and M) when the operation plane run actually in the middle of the body supported this explanation.
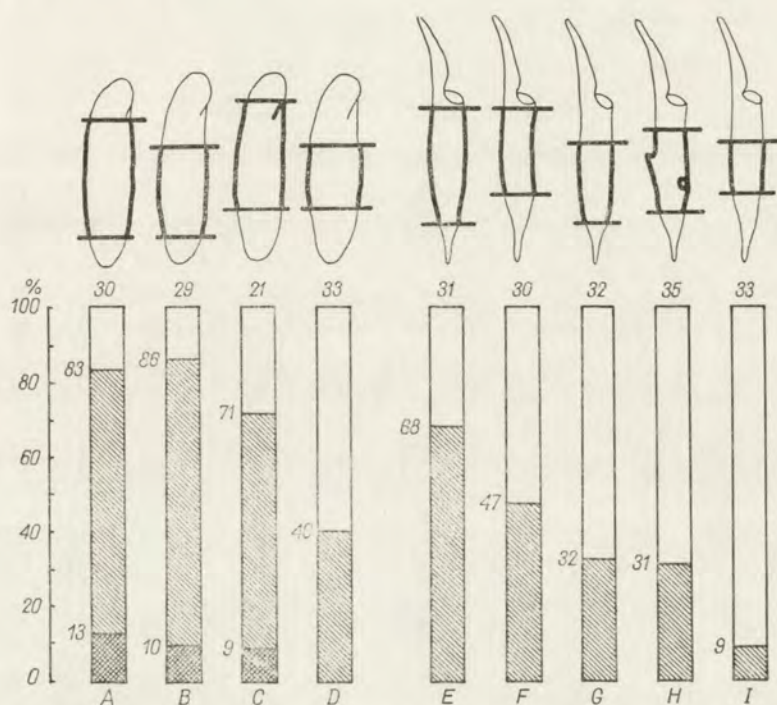

Fig. 5. Comparison of division ability of middle fragments in *Urostyla* (A-D) and regular *Dileptus* (E-I). A, B, C, D, E, F, G, I — predividers, A, H — dividers. The studied fragments are marked by heavy lines. The conventions are the same as applied on Fig. 1

In *Dileptus* as in *Urostyla* no relationships were found between the existence of any particular structures and the capability for division. The fragments with proboscis removed (Fig. 6 G, H) did not differ significantly from the fragments with posterior part of the body removed (Fig. 6 I, J). Furthermore the excision of the proboscis with the damage of cytopharyngeal complex (Fig. 6 E, F) seems to be equivalent with the removal of large portion of posterior part (Fig. 6 K, L). It seems that the process of regeneration of the oral apparatus involving the formation of every part of mouth plus proboscis (Golińska and Grain 1969) exhausted the resources necessary for support of division to the same

extent as the regeneration of the posterior part of the body which does
not involve proliferation of kinetosomes (Golińska unpubl.). The
removal of the whole cytopharyngeal complex (Fig. 6, C, D) in "regular"
specimens established a damage which required equal reorganization
(restitution of mouth plus proboscis) as the transection in the middle of
the body (Fig. 6, A, B). However, the difference in the ability for division
of these fragments is evident, and it most probably is a result of the
difference in size of two kinds of fragments.

Operated large specimens of *Dileptus*

Two series of experiments were performed on large individuals of
Dileptus. The first involved the transection in the middle of the body
of predividers, the second involved transected early dividers. Each
operation, as before, was repeated more than 30 times.
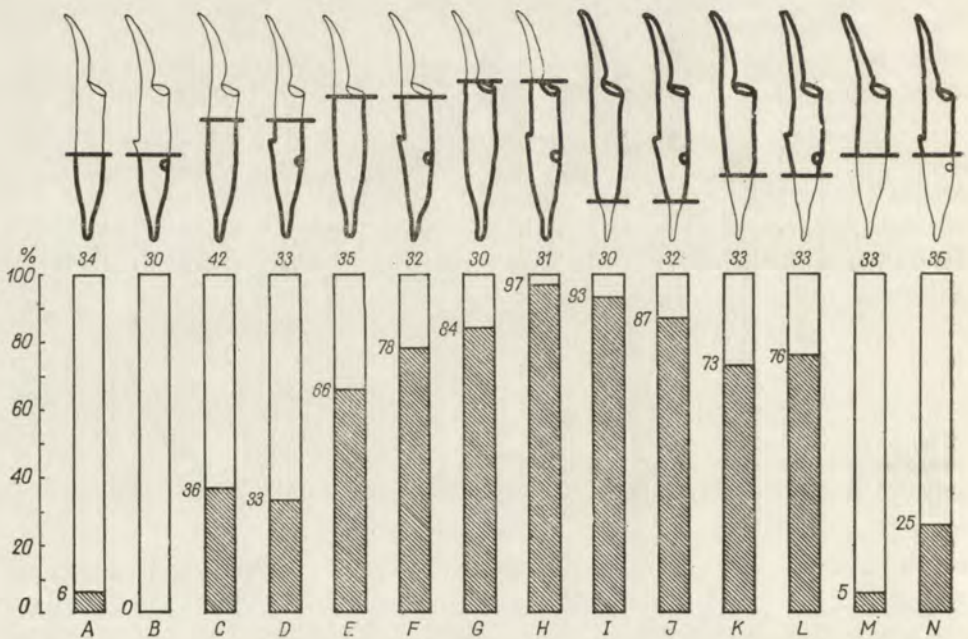
Fig. 6. Division ability of fragments of regular *Dileptus*. A, C, E, G, I, K, M —
predividers, B, D, F, H, J, L, N — dividers. The studied fragments are marked by
heavy line. The conventions as on Fig. 1

The transected large predividers and dividers (Fig. 1, E, F) were able
to divide only once during 24 h — as observed for the regular pre-
dividers and dividers — but the percentage of fragments dividing was
much higher. There is also a difference between the ability for division

of large predividers and dividers. The operation seems to increase the capability to division in large predividers. This is very conspicious, if one compare the percentage of divided cells of operated large predividers (Fig. 1 E) with the percentage of two successive divisions of unoperated large predividers (Fig. 1 C). It is interesting, that similar results were obtained in *Urostyla*, where also the fragment from predividers had a higher percentage of division than fragment from dividers.

Discussion

The results presented above yielded some interesting questions which will be discussed subsequently. These are: (1) The problem of the minimal size of a cell fragment able to divide. (2) The existence of two different kinds of interphases in the cell cycles in ciliates. (3) The role of the oral apparatus in the division processes.

Minimal size requirement for division

The results of experiments performed on *Dileptus* and *Urostyla* showed clearly that the ability for division decreases parallel with the size of the specimen. It is possible to obtain a group of fragments in which the size is close to the lower limit necessary for division to occur. It is also possible to isolate a group of moderately fed dividers (small dividers) which are able to divide twice only in a low percentage. It follows than that there exists a constant, species characteristic minimal size of a ciliate which when achieved makes the cell able to divide. This size although not itself beeing a trigger for division is its necessary prerequisite. It is worth pointing out here that the size of the dividing cell in its natural condition almost always exceeds the minimal size.

The experimentally observed size limit of specimens which are capable of dividing is very similar for both studied species. The "minimal size" corresponds to a slightly smaller fragment than 1/3 of a normal specimen of *Urostyla* and half of a regular *Dileptus* which corresponds to 1/4 of large specimen of *Dileptus* — comparable with *Urostyla*. From the study of H a m b u r g e r and Z e u t h e n 1957 it follows that in synchronized *Tetrahymena* the minimal size ranges also between 1/2 and 1/4 of a cell, since in their experiments the population can divide twice (some cells divide three times), after washing at the end of synchronization treatment. Heat-shock synchronized *Tetrahymena* is larger than normal and might be compared with the large specimens of *Dileptus*.

The results obtained in experiments on *Urostyla* allow one to consider the relationship between the division ability and the volume of endoplasm. If we compare the small fragments — anterior, central and posterior we can see that the anterior and posterior fragments show a significantly smaller ability for division (Fig. 3, A, H, Fig. 4 and 5 D), than the meridional fragment which posesses approximately the same surface as the anterior and posterior ones respectively but contains more endoplasm. Similarly among operated dividers of *Urostyla* the removal of a large portion from the anterior or posterior portion of the cell inhibits division to a greater extent than removal of two smaller fragments from anterior and posterior part (Fig. 4, A, B, C). In the last case very "flat" portions of the cells are removed.

There is one more fact which need attention. Transection in the middle of the body of *Urostyla* predivider and large predividing *Dileptus* inhibits division in significantly lower percentage than the same operation does on dividers. Moreover, the percentage of division of halves produced by operation is significantly higher than the percentage of second division of large dividers and predividers of *Dileptus* (Fig. 1). There are several possible explanations of this phenomenon. The most probable seems to be the supposition that operation somehow stimulates division in the overfed specimens. In favor to this explanations speaks the observation, that such regularities were not observed in regular predividers and dividers of *Dileptus*.

Not entering into the nature of the factor initiating the division, it can be said, that this factor is formed by the specimen which exceeds the minimal size and that this factor is present in endoplasm of predividers and early dividers.

Two different kinds of interphases

The next problem to be discussed now is the existence of two unequivalent types of interphases. After applying the appriopriate culture conditions one can obtain a cell cycle in which the cell grows, or one which lacks growth. Interphase without growth, i.e., one in which there is no increase of dry mass, took place when we had two successive divisions after isolation in medium lacking nutrients. It is reasonable to assume that the diversity among the interphases caused by changes in the food supply can exist also in natural conditions. The observations on giant forms in such ciliates as *Dileptus, Blepharisma, Stylonychia* (J a n o v y 1963, Gi e s e 1938, G i e s e and A l d e n 1938, P a d-m a v a t h i 1961) support this conclusion. The giant forms can perform several fast divisions after transferal into different medium, even

though this medium lacks food (G i e s e 1938), as do well fed pre-dividers and dividers of *Urostyla* and large specimens of *Dileptus* in our experiments.

Other system in which different types of interdivisional periods occur is in multiple division within the reproductive cysts (A d o l p h 1929, W i l l i a m s 1961). Usually there are two division in the cyst. In this system interphase without growth takes place in the cysts, and the interphase with growth occurs in the free-swimming form.

It is not clear, however, how the existence or lack of the growth phase in the cell cycle influences the pattern, existence and timing of different cellular syntheses. Some conclusions can be drawn from the study on synchronized *Tetrahymena*, in which the growth-interphase is artificially prolonged, and the interphase without growth is probably changed by the synchronization treatment. It is well known, that the interdivisional period of normally cultivated cells of *Tetrahymena* differ significantly from the interdivisional period of synchronous divisions in heat-shocked cultures in respect of the pattern of DNA synthesis. Between the end of the synchronization treatment and the second synchronous division a large portion of the population (29%) may not synthesize DNA at all (J e f f e r y et al. 1970). Similarly in other unicellular organisms — like bacteria — the pattern of DNA synthesis in synchronized cells is quite different from exponentially growing cells (S c h a e c h t e r et al. 1959). In further experiments we plan to study the pattern of DNA synthesis of *Urostyla* and *Dileptus* in different types of interphases.

The relationship of the oral apparatus to division

The last problem we will discuss here is the role of the oral apparatus in the divisional processes. There are two aspects of this problem. First is the role of oral apparatus in the initiation of division as postulated by de T e r r a 1960, 1969 a, b. The second is correlation between the formation of the oral apparatus and synchronous division of the offspring.

De T e r r a in her "critical cortical ratio hypothesis" sets up two postulates: first, that when during the cell cycle the protozoan body become too large for its oral apparatus it will form oral primordia. Second, that furrowing is initiated when the oral primordia are formed in a sufficiently large cell. Our observations on operated predividers and dividers do not confirm this interpretation. The removal of the posterior part of predividers, which changed significantly the proportions between oral apparatus and the rest of the cortex, should have according to

de T e r r a' s concept, inhibited division — which is not sure. Also unexplainable would be the reinitiation of division in *Dileptus* after removal and regeneration of original mouth — unless we suppose that the new mouth formed during regeneration would be too large from the beginning in proportion to the rest of the body. In experiments on *Urostyla*, when we cut off the posterior part of predividers and left a fragment larger than "minimal" — this fragment would have divided, without preceeding regeneration, as it has been reported in *Oxytricha* by R e y n o l d s (1932). In our case we always observed regeneration first, and then division.

The reinitiation of division as observed in operated early dividers in *Dileptus* has been observed in several *Heterotrichida* by Y a g i u 1951, 1956 b, S u z u k i 1957, T a r t a r 1966. In their cases the divisional furrow is also shifted according to the new shape of the dividing fragment. What is also important is that the initial resorption of the fission line is always correlated with the resorption of the oral˙ primordia. The divisional primordia never serve as reorganization primordia (T a r t a r 1958, S u z u k i 1957, F a u r é - F r e m i e t 1910, de T e r r a 1960, Y a g i u 1956 a, W e i s z 1956).

In *Dileptus* and *Urostyla* there is no relationship between the cortical structures left on the fragment and its ability to divide. Promers and opimers of comparable sizes exhibit similar capability to division. Moreover, this ability does not seem to be exhausted by the formation of regeneration primordia before the division.

The observed difference in the synchrony of division in the two offspring of a cell of *Dileptus* and *Urostyla* need some explanation. The proter and opisthe of *Urostyla* divide synchronously in isolation, whereas in the same conditions the proter and opisthe of *Dileptus* never divide synchronously. There is always a difference of several hours. It is worth mentioning here that during division the anterior oral apparatus of *Urostyla* is formed anew, as well as a posterior one (J e r k a - D z i a d o s z 1972). In *Dileptus*, however, the anterior mouth passes unchanged to the proter. Also in *Dileptus* there is a strong tendency for the fission line to be shifted posteriorwards, especially in regular dividers. This then produces unequal offspring. It is reasonable to consider that the process of reorganization of the anterior mouth during division in some way assures the uniform distribution of cytoplasm among the offspring. T u f f r a u 1959 observed in *Euplotes*, which similarly as *Dileptus* preserve the unchanged anterior mouth in proter, that after changing the nutritional regime the animals may undergo unequal fission. It would be interesting to evaluate the correlation between the size of a

specimen and the length of its cell cycle. That would probably explain the differences in the time of division of offspring of a single *Dileptus* cell.

Summary

The ability to divide of microsurgically diminuted predividers and dividers of *Dileptus* and *Urostyla* was studied. It was observed that the division ability decreases proportionally to the size of a fragment. In both studied species there is a constant, species characteristic, minimal size of a ciliate which allows a cell to divide if (and only if) it is achieved. The ability to divide seems to depend on the volume of endoplasm rather than on any cortical structures left on the fragments.

The problems of minimal size, the existence of unequivalent interphases in cell cycle in ciliates and the role of oral apparatus in the control of division is discussed.

STRESZCZENIE

Badano wpływ operacyjnego zmniejszania komórek na zdolność do podziału u przedpodziałowców i wczesnych podziałowców *Dileptus* i *Urostyla*. Stwierdzono, że zdolność do podziału maleje proporcjonalnie do wielkości fragmentu. U obu badanych gatunków istnieje wielkość minimalna charakterystyczna dla gatunku, i tylko po jej przekroczeniu, komórka jest zdolna do podziału. Zdolność do podziału zdaje się zależeć od objętości endoplazmy a nie zależy od rodzaju struktur powierzchniowych pozostawionych na fragmencie. Dyskutuje się zagadnienia wielkości minimalnej komórek dzielących się, istnienie nierównowartościowych okresów międzypodziałowych w cyklu życiowym orzęsków oraz rolę aparatu oralnego w kontroli procesów podziałowych.

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Stefan RADZIKOWSKI

Ultrastructure of the nuclear apparatus in *Chilodonella cucullulus* (O.F.M.) with observations on number and size of nucleoli during interphase

Ultrastruktura aparatu jądrowego *Chilodonella cucullulus* (O.F.M.) z uwzględnieniem zmian ilości i wielkości jąderek w interfazie

In memory of my Teacher Professor dr Zdzisław Raabe

It was ascertained that in the interphase the nuclear apparatus of *Chilodonella cucullulus* undergoes some cyclical changes in number and size of nucleoli (R a d z i k o w s k i 1969). The present paper gives an attempt to elucidate this phenomenon with the aid of the electron microscopy.

The study was carried out on *Chilodonella cucullulus* (O.F.M.) of the stock B. The ciliates were isolated from a sevage plant and reared in spring water boiled before the use. They were fed with yeast.

For study on ultrastructure of the nuclear apparatus the specimens with visible division furrow, observed for at least 30 min were isolated. In order to find the differences in ultrastructure of the nucelar apparatus in the interphase the protozoa were divided into two groups; one was maintained during 2 h while the other one — during 8-9 h in culture medium. The cultures of both groups were maintained in the same conditions. Protozoa of both groups were fixed in 2% glutaraldehyde buffered to pH 7.2 with phosphate buffer for 1 h at 0°C, then in 1% osmium tetroxide with the same buffer, temperature and time. For better differentiation of particular structures in the nuclear substance a part of protozoans was fixed only in 2% glutaraldehyde, without post-fixation in osmium tetroxide. Moreover, the Feulgen reaction for DNA detection in the nuclear apparatus was applied. The changes in Ma surface were calculated planimetrically.

Morphology

The nuclear apparatus of *Chilodonella cucullulus* (O.F.M.) is composed of one macronucleus (Ma) of heterometric-centric type, and one micronucleus (Mi) (Pl. I 2). Ma is ellipsodial in shape, 10-16 μm long; its surface is irregular and folded. Two zones may be distinguished in Ma: orthomer and paramer (Pl. I 1). The orthomer zone is characterized by the occurrence of chromatin granules of various size. Numerous nucleoli of various shapes, ellipsodial, sphaerical or lobed (Pl. I 1 and 3), occur in the orthomer beneath the nuclear membrane. The paramer zone, situated near the nucleus centre, does not show the presence of chromatin granules. In the central part of Ma occurs an aggregation of chromatin, about 2 μm in diameter (Pl. I 1), called endosome (R a d z i-k o w s k i 1965). Similar structure was observed by K a n e d a (1960, 1961) in *Chlamydodon padarius* and by F a u r é - F r e m i e t (1957) in *Dysteria monostyla*.

Micronucleus is round, about 3 μm in diameter, and does not show any differentiation into zones. Nucleoli are not present in it (Pl. I 2).

Ultrastructure

Macronucleus is surrounded by a typical nuclear membrane with numerous pores, about 35 per 1 μm² (Pl. V 13), connected with endoplasmic reticulum. At the external side of the membrane, in cytoplasm, the ribosomes are situated. The zone corresponding to orthomer is filled with great quantity of irregular, electron dense corpuscules corresponding to DNA granules; they react also with Feulgen reagent. (P. II-IV 7-11). Fairly contrasting nucleoplasm occurs among these corpuscles. In this zone of Ma some electron dense, sphaerical corpuscles, fibro-granular in structure, are also present; they correspond to nucleoli. Usually inside the nucleoli two less dense spots are visible. They are ellipsoidal in shape and show some fibrillar structures inside (Pl. II 7, III-IV 9-12) arrows. Similar structures were observed by C h a r e t (1969) and G o r o v s k y (1970) in *Tetrahymena pyriformis* and by N i l s s o n and L e i c k (1970) in *T. pyriformis* GL.

Median part, corresponding to paramer, is deprived of any electron dense particles except of the central part in which the endosome is situated (Pl. II-III 7-9, 11). The endosome is composed of electron dense substance of spongy structure.

Micronucleus situated in the distance of about 0.1 μm from Ma and is covered with typical nuclear membrane. It is filled with electron dense substance corresponding to chromosomes and a karyoplasm similar

in structure to that in Ma. No structures corresponding to nucleoli have been found in Mi (Pl. III 10).

During interphase im Ma of *Ch. cucullulus* the changes in number and size of nucleoli occur (Pl. I 4-6). Two hours after division the structures corresponding to nucleoli are small and set back from the nuclear membrane (Pl. V 14). In 7-8 h after division these structures are large and adhere closely to the nuclear membrane. In this time fairly numerous bodies, similar to ribosomes, occur in the whole orthomer (Pl. VI 15). In the cytoplasm, at the external surface of the nuclear membrane, numerous ribosomes are situated exactly against the places of adherence of nucleoli. In this zone of cytoplasm an aggregation of polisomes is also observed (Pl. VI 15).

Discussion

In the structures corresponding to nucleoli, described in the present paper, two less dense spots of fibrillar appearance are usually visible. C h a r e t (1969), N i l s s o n and L e i c k (1970), and G o r o v s k y (1970) found in *Tetrahymena pyriformis* the structures corresponding to chromatin of the nucleolus. According to C h a r e t (1969) this chromatin has a retarded replication cycle in comparison with the chromatin occurring in remaining parts of the macronucleus. Then, the chromatin with the retarded replication cycle would be regarded as a component of heterochromatin parts of chromosomes. As it is generally known the heterochromatin replicates later than euchromatin (L i m a de F a r i a 1969). It seems that in *Chilodonella cucullulus* the structures seen in nucleoli correspond to the parts of chromosomes responsible for r-RNA synthesis.

As concerns the problem of changes in number and size of nucleoli the following explanations may be considered:

(1) Increase of matrix activity of segments responsible for r-RNA synthesis resulting in the increase of nucleolus substance greater then the possibility of its migration to the cytoplasm.

(2) The nuclear membrane would be impermeable for nucleolus substance causing its accumulation in Ma. In another periods the nuclear membrane becomes permeable and the quick decrease in number and volume of nucleoli occurs. Such situation is observed before division.

(3) Cyclic increase and decrease of the number of pores in nuclear membrane. Such phenomenon was observed by M e r r i a n (1962) in mutation of amphibian oocytes.

Despite numerous cytological studies the mechanism of migration of nucleolus substance to cytoplasm is not yet quite clear. According to

Elliot et al. (1962) in *T. pyriformis* the nucelolus substance accumulates near the membrane of Ma and then passes to depressions formed in the membrane. These depressions separate from the Ma and pass into the cytoplasm. But the study by Flickinger (1965), also on *T. pyriformis*, did not confirm these observations. Flickinger supposes. that the nucleolus substance passes through the pores in the membrane. Passing out of the nucleoli from Ma was observed also by Jankowski (1966) in *Paramecium putrinum* and by Kaneda (1961) in *Chlamydodon pedarius*.

Passing out of the material, not only of the nucleolus origin, through the pores in the nuclear membrane was ascertained by many authors on various objects. Migration of "cytonucleoproteins" from cytoplasm to the nucleus and vice versa may be an example (Goldstein 1965). But only a few information on morphology ascertain univocally these investigations. According to Clirot (1969) the electron dense material in oocytes of *Rana temporaria* passes from the nucleus to cytoplasm. "RNA-springs" passing through the pores in nuclear membrane were observed by Stevens (1967) as well as the passage of structures originating from Balbiani rings in the nuclei of salivary glands of *Chironomus thummi* larvae by Stevens and Swift (1966).

In *Chilodonella cucullulus* the structures similar to ribosomes in the course of passage through the nuclear membrane were not observed, although some corpuscles, which would be preribosomal ones or so called nuclear ribosomes according to some authors, were present in the nucleus. Only slight possibility exists that these corpuscles would pass unchanged through the nuclear membrane. Some corpuscles situated in the pores of nuclear membrane, as observed in electron microscopy (Pl. V 13), are probably due to rays bending at the margin of the pore. Nevertheless it is obvious that the nucleolus material migrates to cytoplasm. It seems that the course of migration consists in transformation of the material, being in contact with the membrane, detachement of bearer (Bermann 1966 a, b) and then the formation of proper ribosomes and their accumulation in cytoplasm. Such course of this process seems to be most probable in *Chilodonella cucullulus* (Pl. VI 15).

Summary

The ultrastructure of nuclear apparatus in *Chilodonella cucullulus* is described as well as the changes in the nuclear apparatus in interphase concerning the number and size of nucleoli, their migration toward the nuclear membrane and passage of the nucleolus material to cytoplasm.

STRESZCZENIE

Praca niniejsza zawiera opis ultrastruktury aparatu jądrowego *Chilodonella cucullulus* oraz zmian jakie zachodzą podczas interfazy w aparacie jądrowym, zmian w ilości i wielkości jąderek, ich przesuwaniu się do błony jądrowej i migracji materiału jąderkowego do cytoplazmy.

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EXPLANATION OF PLATES I-VI

1, 2: Nuclear apparatus of *Chilodonella cucullulus* (O.F.M.) (Feulgen reaction)
3: Macronucleus (iron hematoxylin)
4: Macronucleus about 2 h after division (Feulgen test)
5: Macronucleus about 7-8 h after division (Feulgen test)
6: Macronucleus just before next division (Feulgen test)
7: Fragment of section through Ma and cytoplasm (2% glutaraldehyde, magnification 24,000 ×)
8: Fragment of section through Ma; the endosome with different structure of chromatin situated in the central part (2% glutaraldehyde, magnification 30,000 ×)
9: Fragment of longitudinal section through cytoplasm and Ma; the nucleolus with an apparent fibrillar structure inside (2% glutaraldehyde, magnification 24,000 ×)
10: Fragment of section through Ma, Mi, and cytoplasm; different structure of chromatin inside Mi (2% glutaraldehyde, magnification 30,000 ×)
11: Fragment of section through Ma; well visible nucleoli (2% glutaraldehyde, magnification 30,000 ×)
12: Fragment of section through cytoplasm and Ma; fibrillar structure inside the nucleolus in transversal section (2% glutaraldehyde, magnification 24,000 ×)
13: Fragment of section through cytoplasm; the pores visible in nuclear membrane (2% glutaraldehyde and 2% OsO₄, magnification 24,000 ×)
14: Fragment of section through Ma 2 h after division; small nucleoli set apart from the nuclear membrane (as baove, magnification 54,000 ×)
15: Fragment of section through Ma; passing out of the nucleolus material (as above)
Designations: Ma — macronucleus, Mi — micronucleus, c — cytoplasm, ch — chromatin, en — endosome, fv — food vacuole, m — mitochondria, nm — nuclear membrane, nu — nucleus, ort — orthomer, p — pores in the nuclear membrane, par — paramer, ri — ribosomes
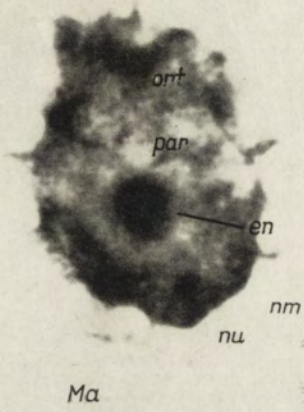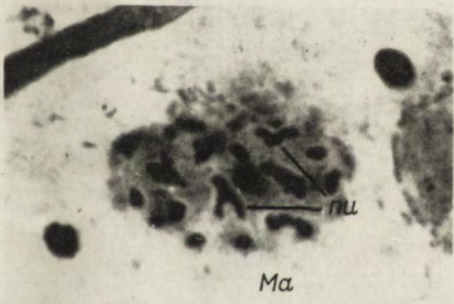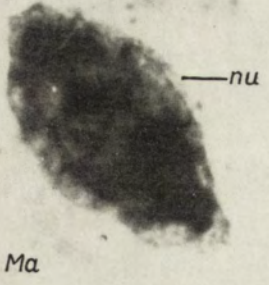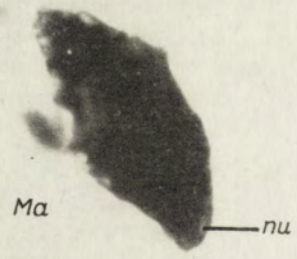
PLATE I

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PLATE II

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PLATE III

PLATE IV
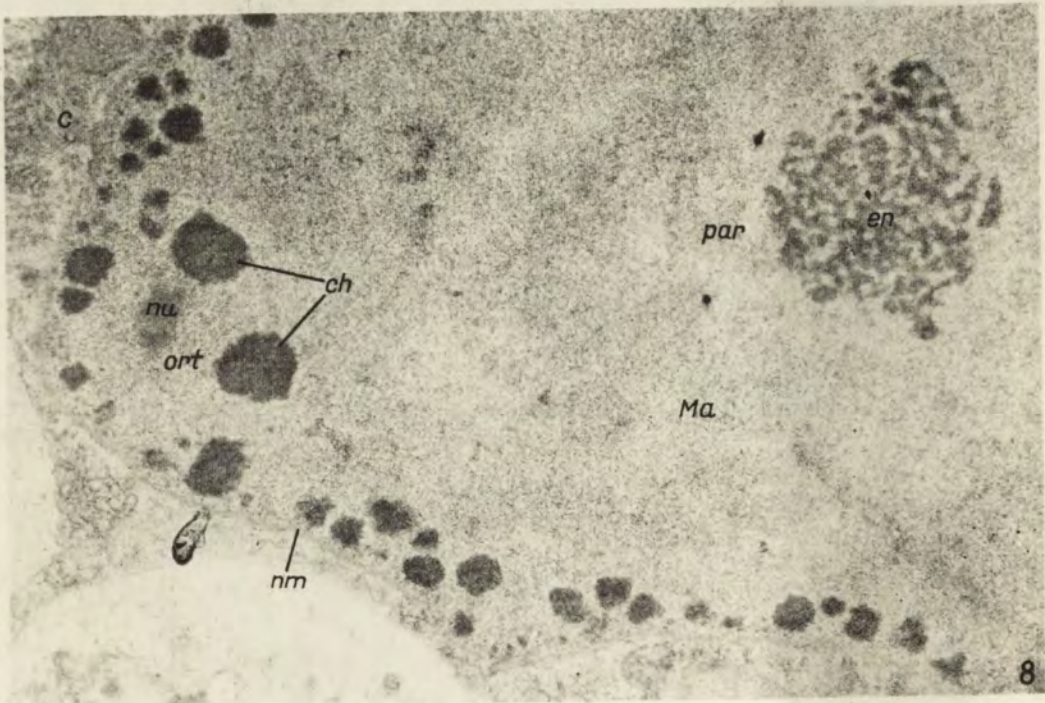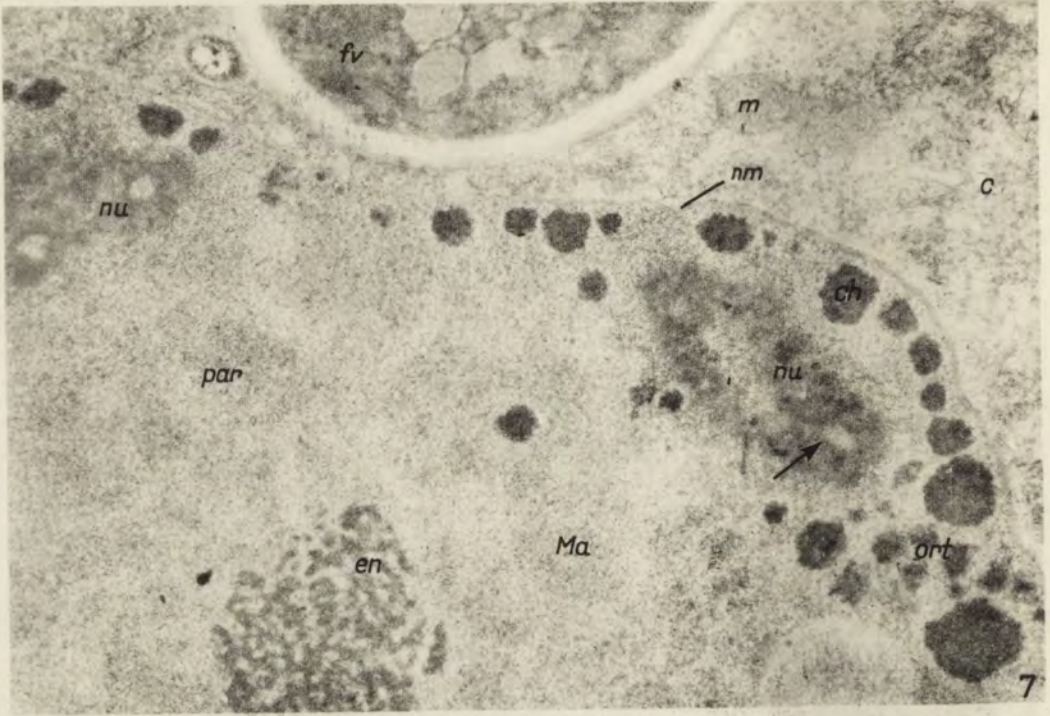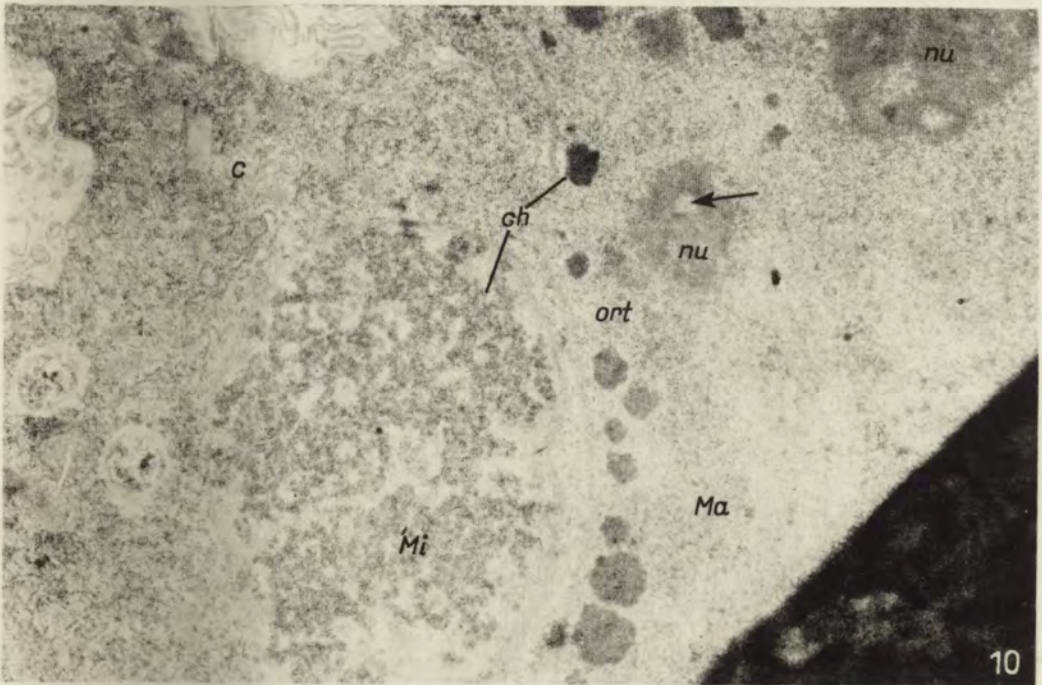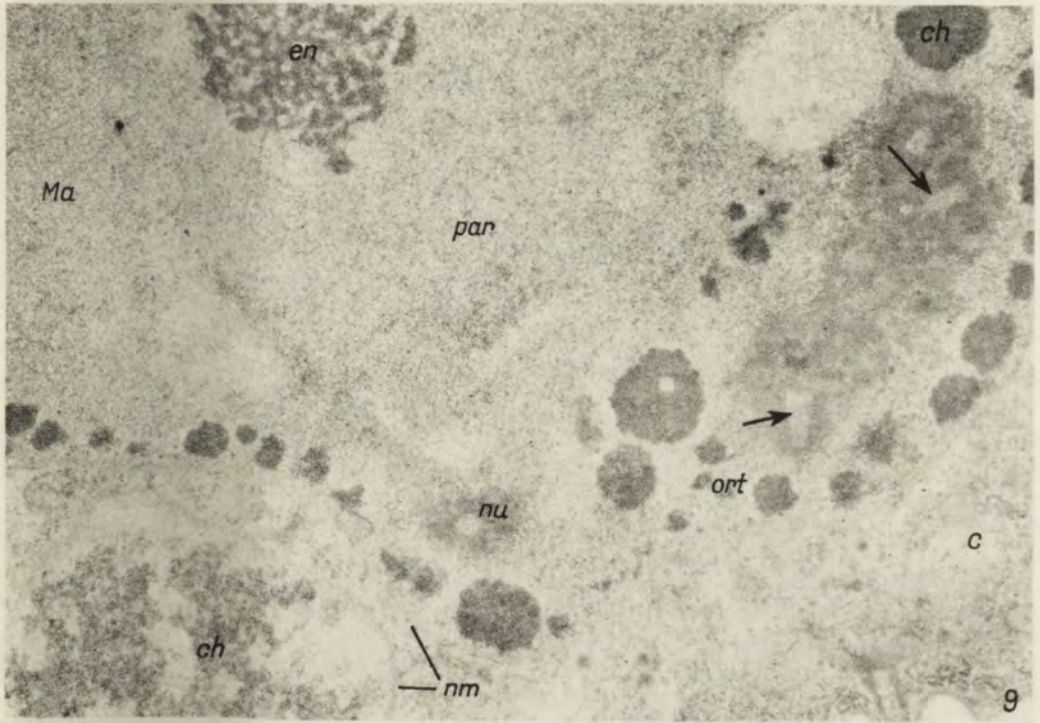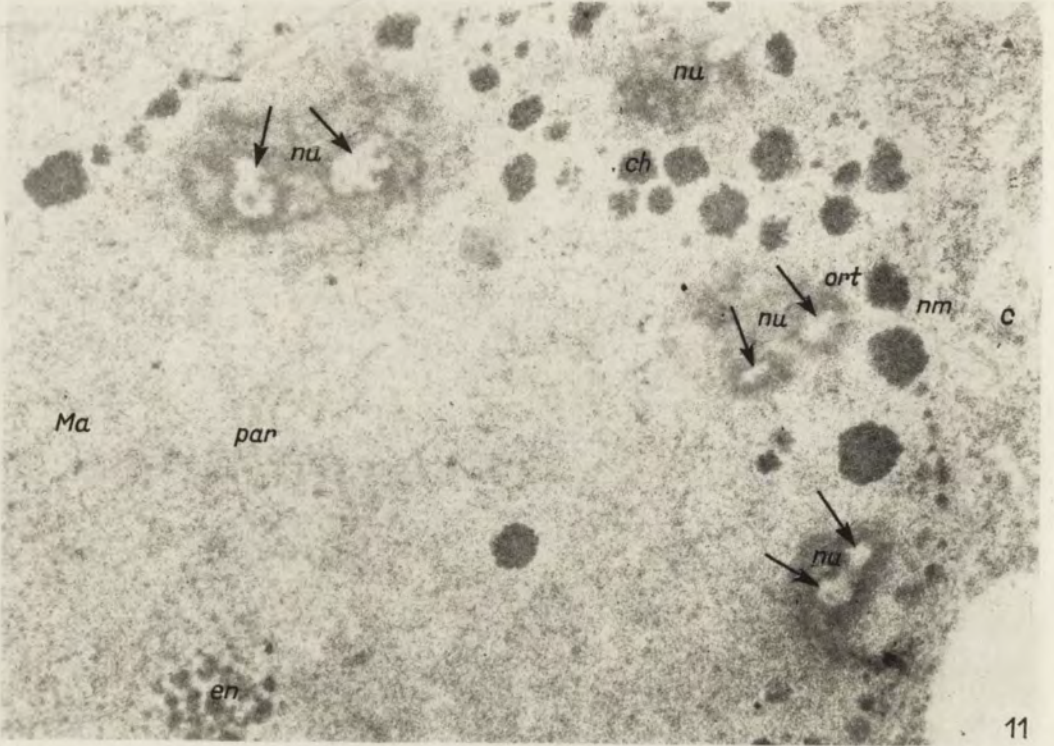
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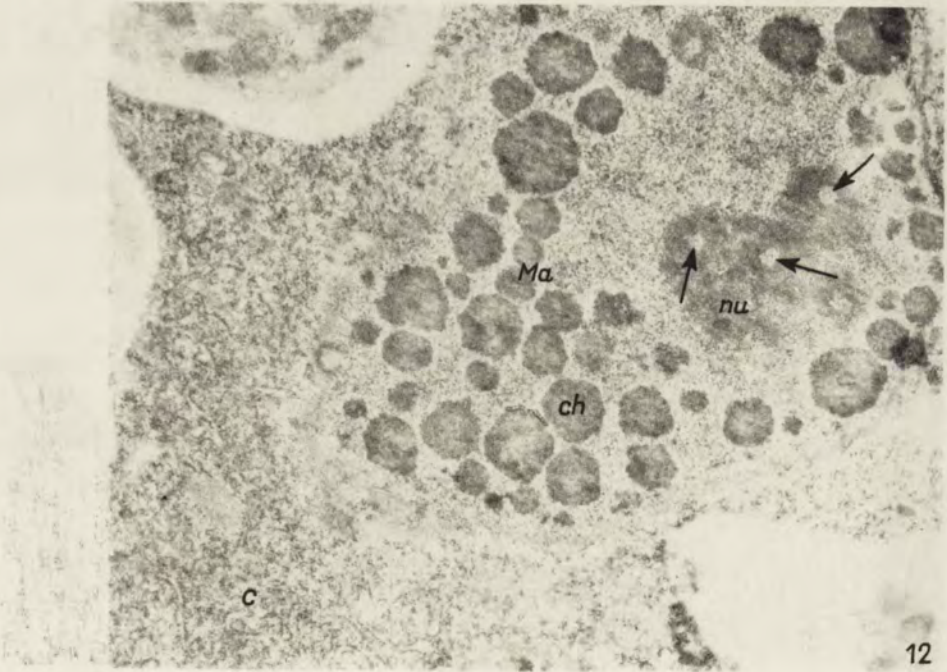
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PLATE V

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PLATE VI

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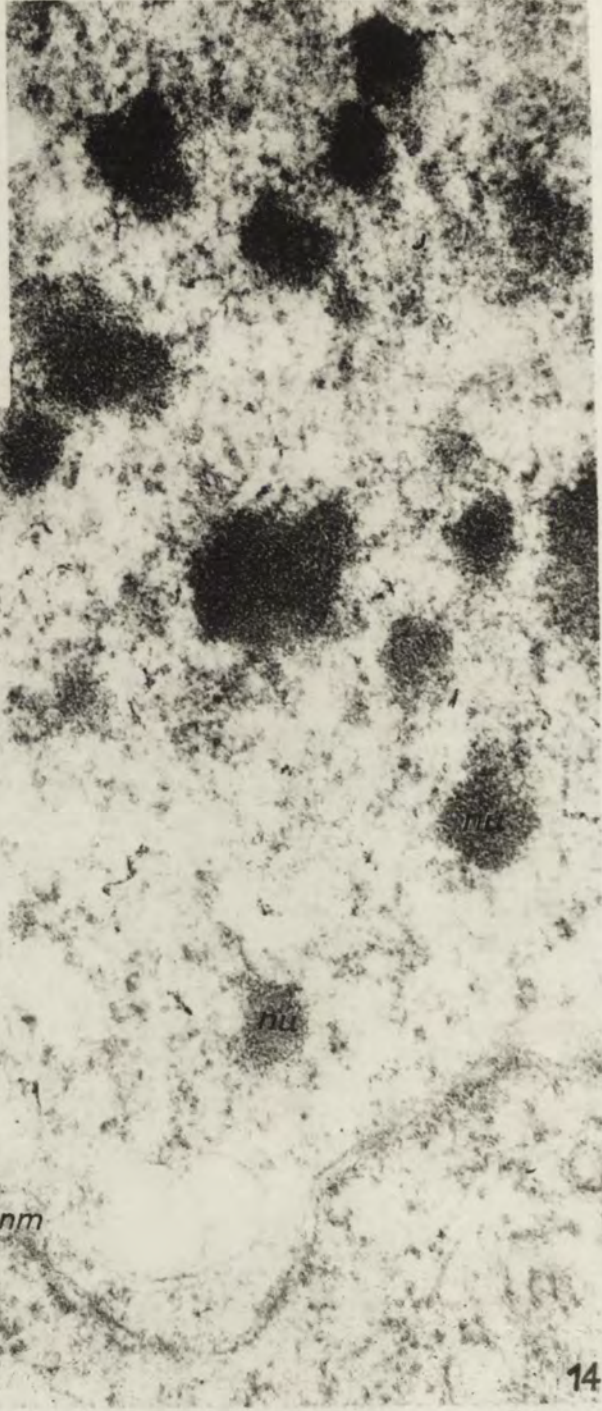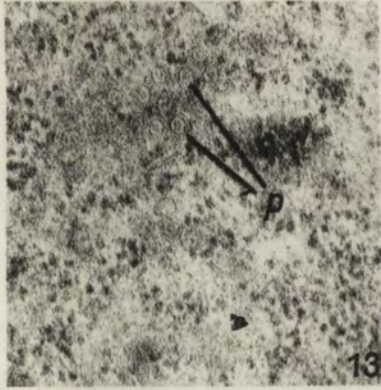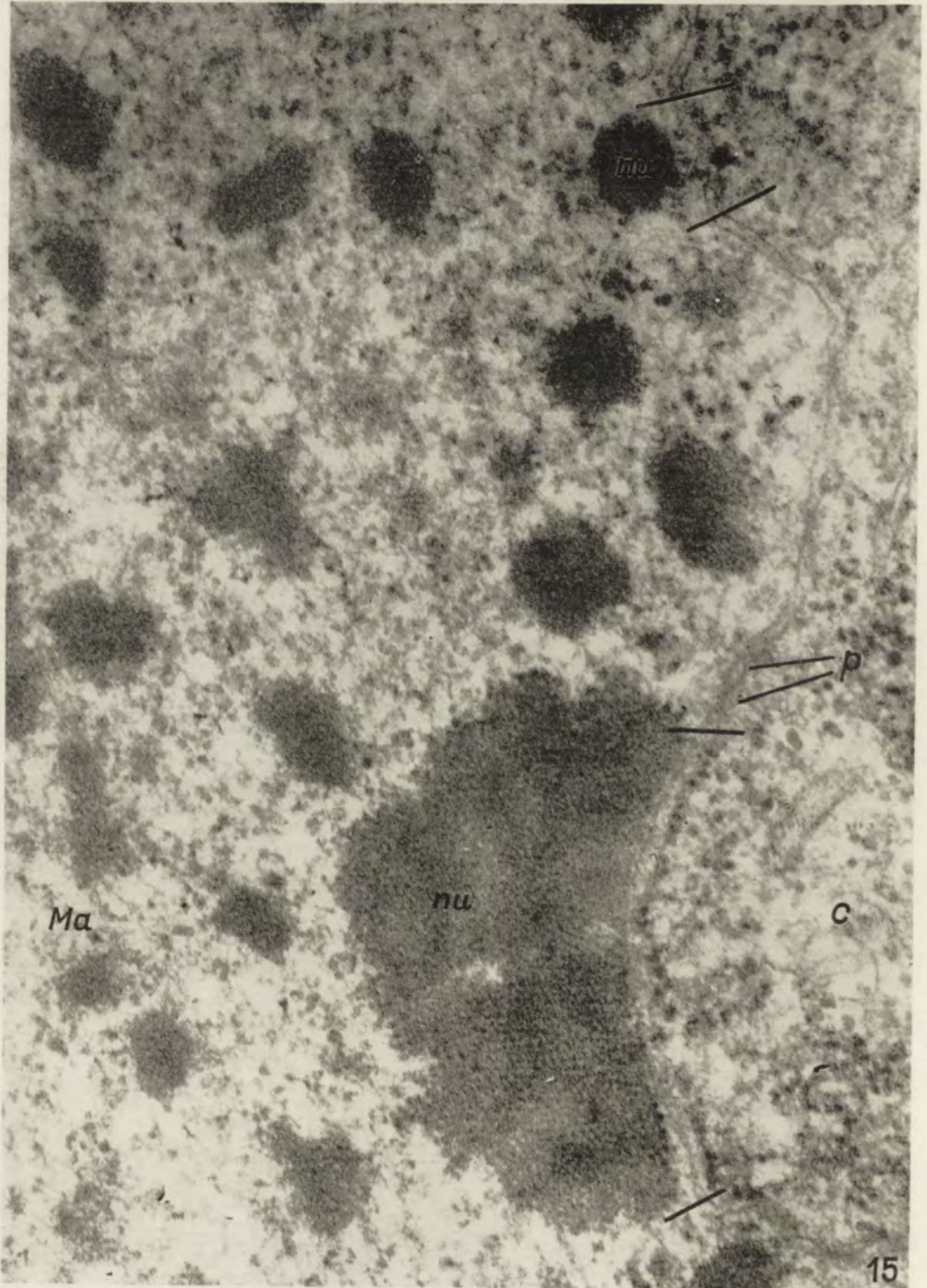
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Andrzej KACZANOWSKI

Morphological studies on Opalinids. II. Cortical patterns in *Opalina ranarum*

Badania morfologiczne nad Opalinata. II. Wzór kortykalny
u *Opalina ranarum*

The aim of the present study is formal analysis of cortical pattern in *Opalina*. Various shapes of opalinids even in the same population have fascinated a number of authors since M e t c a l f works 1909, 1923, 1940. A satisfactory description of these phenomena is very difficult because of lack of good marker points in the pattern of *Opalina*. The convinction that the cortical pattern of *Opalina* is different from other protozoans was the reason for dealing with this problem. Particularly the formal analysis of *Opalina* pattern is requested for a proper comparison with the cortical pattern in *Ciliata*.

It seems that the study of the cortical pattern in *Opalina* has some value for our knowledge about the types of general biological patterns. The most striking feature of this pattern is a monotonous arrangement of lineary organized microtubular strips and kineties which cover the two body surfaces in the very uniform manner. Therefore, the pattern can be imagined most simply, as a set of linear stripes able to grow by their active elongation. According to this assumption, some suggestions concerning the *Opalina* growth and body shape variability can be given on the basis of the pattern analysis only. This point was the most promoting for the presented study.

There are two main characters of pattern which can be analysed in the case of *Opalina*: directions (including eventual curvature of the stripes) and the length of the pattern stripes. The pattern differences between the left and the right body surface were referred in this way. These differences reflect the different unpacking up of the same material, though both body surfaces obey the same rule of the linear organization. This point brings to one's mind the problem of the growth regulation in two body surfaces. If one admits that the shape is a result of the pattern growth hence the growth of the different patterns on both

body surfaces has to be regulated in manner bringing the same regular body contour. In virtually all cases the flat *Opalina* represents different pattern, shape and sizes but the contour (outline) of its left and right body surfaces is identical.

It is not known, whether the variability of *Opalina* shape described in the paper has physiological or genetical character. Thus the present results cannot be directly applied to opalinids systematics.

Material and methods

Natural populations of *Opalina* from *Rana temporaria* were used for these studies. Frogs were collected in Warszawa countryside and they were sectioned during March and April, just before, or during seasonal spring palintomy. The Opalinids removed from the rectum were fixed in Bouin solution, embeded in gelatin and stained by protargol according to K a c z a n o w s k i 1970. In well stained specimens the pellicle (pellicular ribs) was heavily stained by protargol, while kineties and falx area were visible as bright lines and zones against the dark background.

All measurements and analysis of cortical pattern were performed on micro-photographs of whole stained specimens. There were taken photographs of all selected specimens under low microscopic magnification: objective 6,3 ×, eye-piece 10 ×, or 10 × followed additional magnification about 8 ×.

Results

A. General features of *Opalina* pattern.

A-1. The opalinid body is flat and asymmetrical, W e s s e n b e r g 1961, considers this flattening as a lateral. According to this point of view, there are distinguished: the shorter ventral body margin, the longer dorsal body margin and respectively two body surfaces, left and right (Fig. 1). Along the anterior body margin there extends a field of densely arranged kinetosomes called falx area. The structure and ultra-structure of this field were investigated by M o h r 1940, F e r n a n-d e z - G a l i a n o 1947, W e s s e n b e r g 1961, 1966 and K a c z a n o w-s k i 1970. The cell apex is strongly displaced toward the dorsal body margin.

A-2. The cortex of opalinids consists of longitudinal pellicular ribs and kineties (P i t e l k a 1956, B l a n c k a r t 1957, N o i r o t - T h i-m o t h é e 1958, 1959, W e s s e n b e r g 1966). In turn, each of pellicular ribs consists of a ribbon of microtubules. Pellicular ribs and kineties cover, in a uniform manner, both of *Opalina* body surfaces. Only some
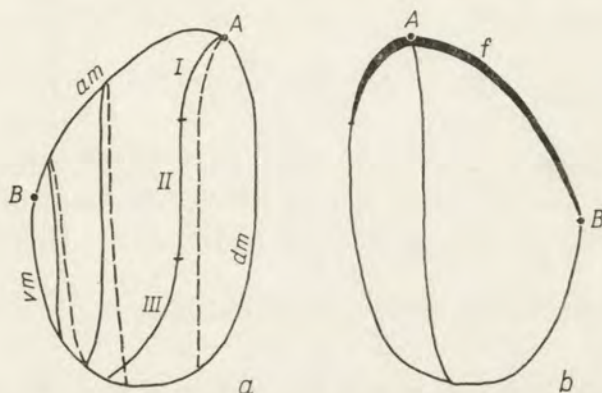
Fig. 1. The general scheme of orientation of the *Opalina* body shape and pattern, a — seen on the left body surface, b — seen on the right body surface. Solid lines represent left body surface kineties in Fig 1 a and right body surface kinety on the Fig. 1b, while dotted lines represent respectively right body surface kineties in Fig. 1 a. A — represents the cell apex. B — the ventral beginning of the falx. f — falx, *vm* — ventral margin, *dm* — dorsal margin, *am* — anterior margin. Note that in Fig. 1 a three segments of apical left kinety denoted as I, II, III are visible and the cell body axis is determined by the medium, nearly straight segment

of the kineties reach the posterior body margin, while others end at different distances from anterior and posterior body margins respectively.

This situation is caused by the intercalar ingrowth of new kineties arising from the falx area. All kineties are a little convergent backwards. And again the stripes of pellicular ribs limited by two adjacent kineties, narrows progressively backwards, because of the ingrowth of new pellicular ribs arising from falx between pre-existing ones (N o i-r o t - T h i m o t h é e 1959, W e s s e n b e r g 1966, K a c z a n o w s k i 1970).

A-3. The shape of the falx curvature the anterior body margin is similar to the shape of a logarithmic function curve (K a c z a n o w s k i 1969). The degree of the steepness of falx curvature decreases in comparison with the kineties along the falx — starting from its ventral beginning (Point *B* in Fig. 1).

A-4. The patterns of cortical lines (determined by kineties and pellicular ribs) on the left and right body surfaces are always different. However, in spite of the differences in the patterns of cortical lines the former assumptions A-1, A-2, A-3 are always respected by both left and right body surfaces. The incompatibility of pattern lines of the two surfaces can be observed at a glance under microscopic observations of one specimen in various focuses. However, it is obvious that kineties and pellicular ribs of each of the body surfaces realize the same contour.

Thus, the question arises what is the control of growth of pellicular ribs and kineties on both surfaces.

The lines determined by kinetom and pellicular ribs can be also considered as lines of cortical growth or as lines of cortical pattern.

A-5. Two kinds of divisions were found in *Opalina* — the longitudinal and the transversal (W e s s e n b e r g 1961). They will be analysed in details in subsequent papers of this series. For now it is important to point out that these divisions produce postdividers of different shape, while the body shape is always regulated by the growth without the whole pattern reorganizing. That is, the longitudinal division produces relatively narrow specimens while transversal one produces short specimens. General postdivisional regulation is therefore in the first case
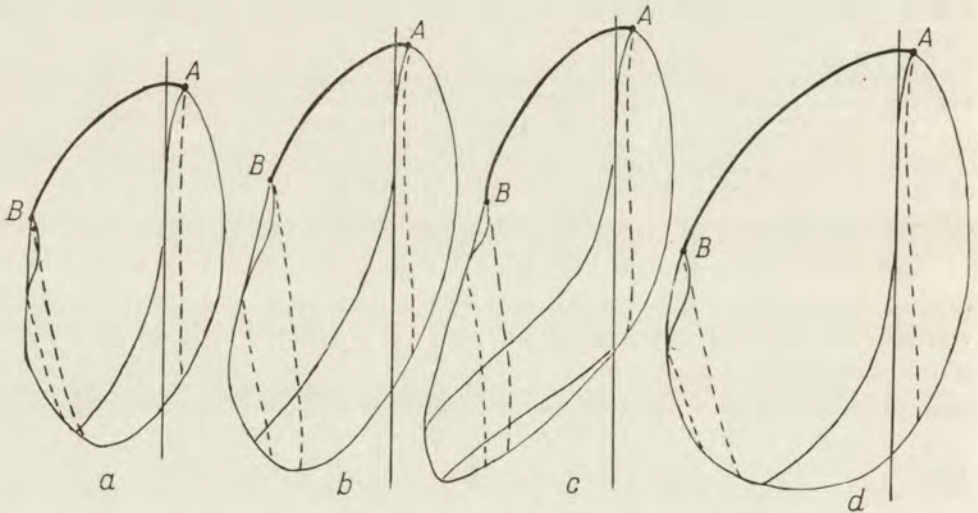
Fig. 2. The variability of large opalinids of form "A" in the same population. The Figs. 2 a, b and c represent elongation of the body shape compared with Fig. 5, while Fig. 2 d represents the wide specimen. Note that even in this specimen there is large deviation of the left apical kinety (above 1/2, however, its shape is similar to the shape of opalinids of form "B" in the Fig. 3 a). A — apex: solid lines kineties of the left body surface: apical one and kinety starting from the point B. B — ventral beginning of the falx, dotted lines corresponding with the kineties of the right body surface

expressed by growth in width and new development of falx curvature to the logarithmic like shape and in the other case by longitudinal growth. On the other hand the divisions of both kinds were observed many times in specimens of different sizes and shapes. In other words, no definite size and shape requested for the next division was observed. During the palintomy in spring season even the general shape regulation

mentioned above, does not proceed following divisions producing, as a result, subsequently smaller specimens. During palintomy, however, some large specimens do not usually enter in this process and keep their former character.

In this complicated situation the question arises how to characterize in the best way any given *Opalina* population. It can be proposed primarily that not average specimens but the largest and those which have most developed falx curvature characterize the given *Opalina* population
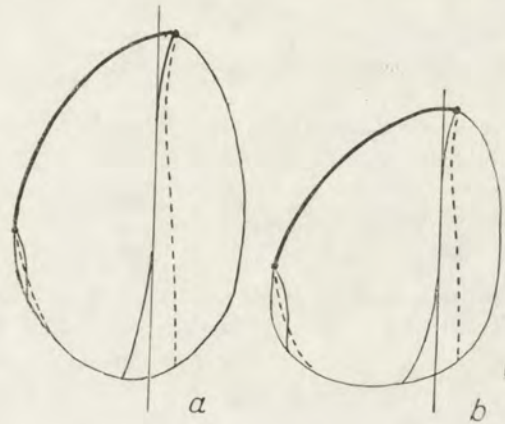
Fig. 3. The variability of medium sized opalinids of form "B" in the same population. All explanations and magnification as in Fig. 2

in the best way. In other words, they are specimens which realize in a most fully way the growth potention within this population. It is worth mentioning that on this basis different *Opalina* population can be practically distinguished even during palintomy period independently from subsequent diminishing of sizes of majority specimens. This stand-
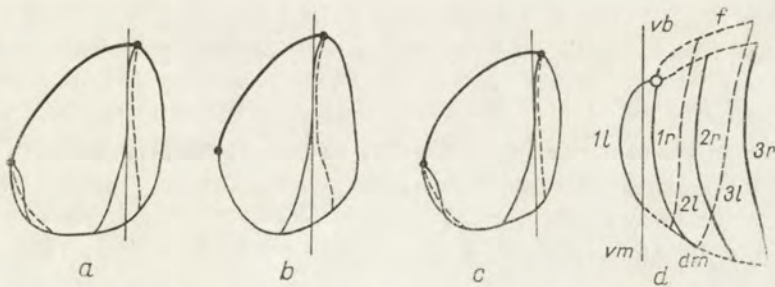
Fig. 4 a, b, c — The variability of little sized opalinids of form "B" in the same population. Explanations and magnification as in Fig. 2, d — the ventral portion of little trophont of form "B" if the 1st left kinety is a little displaced. 1*l*, 2*l*, 3*l* are the first, second and third left kineties, while 1*r*, 2*r*, and 3*r* — the first and next right kineties; *vb* — ventral beginning of the falx, *f* — falx, *vm* — ventral body margin, *pm* — posterior margin. Note that in ventral portion of the body right kineties are longer than left ones

point, however, may be opposed. One should keep in mind that in
Ciliata for example, the definite "maturity" of interdivisional stage can
be established on the basis of completing oral and other morphogenesis,
while in *Hypermastigina* — final defined number of kineties prior and
during cell division can be determined. But there is no possibility to
define in the similar way the mature interdivisional stage of *Opalina*
because of lacking of internal reorganization inside the former cortical
pattern.

B. Pattern of the left body surface ·

The left body surface kinety, arising from the apex, which can be
called also an apical kinety is sigmoid like. Three different segments of
this kinety can be distinguished. The medium segment (II) is nearly
straight while the short anterior segment (I) is directed toward the apex
and the posterior segment (III) is directed in the opposite direction,
towards ventral body margin (Fig. 1 a).

Distinguishing of the medium straight segment of apical kinety per-
mits to orientate all opalina shape figures in the similar comparable way
assuming that this segment is parallel to diagonal axis of the diagram
(see Fig. 1, 2, 3). Such orientation will be convenient and useful for
further considerations (section D).

It is worth notifying that other ways of comparable orientation of
different opalinid figures were not found because of the absence of good
marker points on the body surface, and a great variability of curvature
of all body margins including anterior falcular curvature.

Subsequent kineties from apical kinety toward ventral body margin
became gradually less and less sigmoid, while their first curvated
segments are reduced. Therefore, kineties arising in the central falx
region have curvated segments I and they run straight to their posterior
curvature (Fig. 1 and 2). In vicinity of the ventral body margin they are
almost arch-like. Wessenberg (1966) has observed, however, that
all kineties of the left surface arise obliquely from the falx and that
the I segment of the kinety always exists, though it can be strongly
reduced. In this case I segment is invisible, or weakly visible on
the anterior body margin in protargol slides, while falx is a little dis-
placed on the right body surface (Wessenberg 1961, 1966, Mohr
1940).

C. Pattern of the right body surface

Kineties of the right body surface run nearly straight. In most cases
their little curvature may be neglected. Only sometimes these kineties
are curvated slightly dorsally.

D. Variability of *Opalina*

Variability of *Opalina* can be related to their; sizes, shapes of the body, its proportion and cortical pattern, i.e., pattern of lines determined by kineties and pellicular ribs.

D-1. Variability of sizes of trophonts

Trophonts in the same population can differ in respect to their sizes. According to the previous assumption the largest sizes (not average) are reported as the best realization of growth potention within the given population. According to this standpoint opalinids were divided into 3 groups:

    (1) large opalinids up to 300 μm wide and 350-400 μm long,
    (2) middle sized opalinids up to 200 μm wide and 250-280 μm long,
    (3) little opalinids up to 159 μm wide and 160 μm long.

D-2. Variability of cortical pattern in *Opalina* and its classification

Analysis of cortical pattern of *Opalina* indicates that different populations of *Opalina* can differ in this respect. On this basis two different principal froms called "A" and "B" of *Opalina* were distinguished as primary proposition for classifying *Opalina* variability.

Form "A". There are large or sometimes middle size opalinids which are distinguished by a relatively long and more curvated segment III of kineties of the left body surface (apical and subsequent kineties). Thus the segments are directed relatively far towards ventral body margin (Fig. 2 a, Pl. I, 1 and 2). The first kineties and stripes of pellicular ribs starting from ventral beginning of the falx on the left body surface turn over the ventral body margin on the right body surface where they join the right surface pattern. Thus the whole length of the left body surface and the first kinety of the right body surface is similar, starting from the ventral beginning of the falx and ending in the same posterior region of the right body surface. Among large opalinids there are usually some relatively narrow and elongated specimens (Fig. 2 a, Pl. II 3 and 4).

Form "B". There are medium sized (Fig. 3, Pl. III 5 and 6) and small opalinids (Fig. 4, Pl. III 7 and 8), which distinguish themselves by rather small curvature of kineties (lines of cortical pattern) on the left body surface in contrast to form "A". The posterior segment III of an apical kinety is a short one and is only a little directed towards the ventral body margin. Marginal kineties and pellicullar ribs of the left body surface do not go over the ventral body margin reaching or nearly reaching its posterior margin (see Fig. 3, 4 and particularly 4 d). On the other hand the first kineties of the right body surface are much longer than the first

kineties of the left surface because they are going obliquely or are curvated toward the dorsal margin (Fig. 4 d). The ventral body margin in form "B" can be shortened and then the ventral beginning of the falx lies low or very low (Fig. 3 a and 4 a). It may be concluded that forms "A" and "B" differ by the shortening of posterior part of their body.

D-3. Theoretical interpretation of variability of *Opalina* body form

According to the description presented here the variability of *Opalina* body shape and pattern is related to the developing of curvature of the left body surface kineties. This curvature concerns the Ist and the IIIrd segment of the left surface kineties. Fig. 5 presents an idealized model of suggested transformation as referred to the same set of lines of cortical pattern of the left body surface. Two first profiles can be considered as form "B" and next 3 as form "A" according to the previous section C. It is clear that for each point of the curve of the left apical kinety lying between the first and the last profile, intermediary profiles can be easily found. So the whole transformation is continuous and is contained in change of growth proportion of the pattern. This growth on the cell surface is realized in the way of the ingrowth of new pellicular ribs and kineties starting from the falx and in the way of elongation of pre-existing elements.
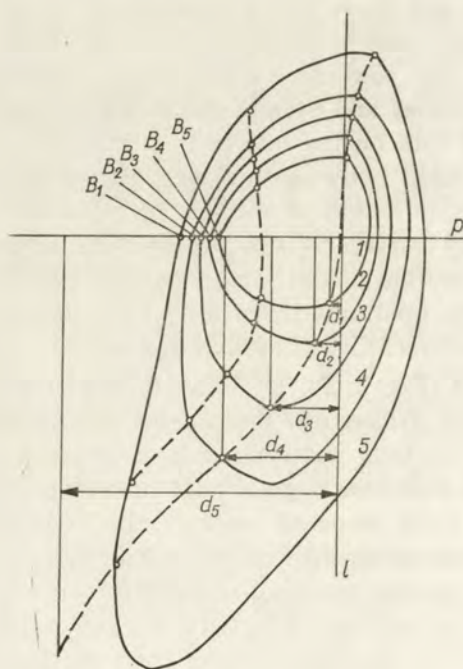
Fig. 5. The theoretical model of *Opalina* shape and pattern variability based upon the hypothesis about mutual different growth proportions. Points d_1, d_2, d_3 ..... represent different positions of posterior end of the apical kinety in the subsequent profiles while B_1, B_2, B_3 ..... positions of the ventral beginning of the falx. Deviations of apical left kinety are related to the p body axis perpendicular to the l, while l is determined by the medium straight II segment of this kinety

It means that the growth of the cell surface and its rate is a function of two different processes:

(1) formation of new initiative points for new pellicular ribs along the falx,

(2) elongation of pellicular ribs and kineties (longitudinal growth of the whole pattern).

Let us assume that the proportion of longitudinal growth of the pattern (pellicular ribs and kineties) in relation to the formation of new initiative points is different. If this longitudinal growth is higher, than curvature of the left body surface kineties is larger and form "A" is produced (Fig. 2 a). On the other hand, the relatively slow longitudinal growth of the whole pattern produces form "B" with low lying ventral beginning of the falx (Fig. 3 and 4).

It is not excluded and can be even postulated that the order and rate of formation of new initiation points along falx is similar in all cases during the active surface growth while the transformation of the pattern depends only on the different rate of its longitudinal growth. In this case the model would be the simplest and would need little information.

Then the question arises how the described variability of the pattern can be measured. It can be proposed to measure deviation of the left body kineties. This deviation is a distance d of the posterior end of the left apical kinety from the axis l which is determined by the middle straight segment of this kinety (Fig. 5).

For our purpose the deviation of the left body kineties should be referred to the body shape. In the model on Fig. 5 there is shown that deviations of the left body apical kineties d_1, d_2, d_3 ..... in subsequent profiles grow more, than the distance OB. This distance is measured along the axis p perpendicular to l while point B is the ventral beginning of falx and 0 is the intersection point of both l and p axes.

D-4. Application of the theoretical model of *Opalina* variability to really existing opalinid populations

In specimens of form "B", both in population of little opalinids and in populations of medium sized opalinids, the ratio d/OB is much smaller than in form "A".

There are, however, some limitations of this method: All the post-dividers should be recognized and rejected in the measurement; if the distance OB is reduced approximately by half during longitudinal division the ratio d/OB is changed. For this reason measuring of the postdividers particularly in form "B", should be omitted. Postdividers can be often

distinguished as relatively narrow cells without well developed falx curvature. Furthermore, many palintomic stages of little opalinids become more twisted and do not regulate their previous forms. Therefore, the estimation of ratio d/OB of given population can be applied only to rather wide specimens with well developed falx and its low lying ventral beginning. On the other hand, the transversal division can reduce deviation of left kineties. In practice, however, cells being just after this division were easily recognized by different character of posterior body margin and they were never numerous.

In spite of all these limitations different populations of *Opalina* can be rather easily distinguished by ratio d/OB.

In the population of middle-sized opalinids of form "B" this ratio was in the limits $1/7$-$1/4$. Two different populations of form "B", but little-sized opalinids, were investigated in this way. In the first population, the ratio d/OB, was in limits $1/4$-$1/5$. In this population there were many specimens just starting longitudinal division but only few postdividers. In the second population of little opalinids, there were numerous stages of palintomy but in wide cells with good developed falx, the ratio d/OB was similar to those in the previous case.

On the other hand, in three different populations of form "A" (large trophonts) the ratio d/OB was starting from $1/3$, while majority of the widest specimens in this population were characterized by the ratio nearly $1/2$ (see for example Fig. 3 a). Some elongated specimens of form "A" resemble *Opalina obtrigonoidea* and *O. virguloidea*, i.e., *O. agustae* Metcalf (see Fig. 2 c) Pl. II 3 and 4 corresponding to the last profile in the Fig. 3. According to the well developed falx curvature they were recognized as not "early" postdividers after longitudinal division. It means that they overpassed the shaping process following the previous division, however, it cannot be firmly concluded if their shape is definite, i.e., if they do not change more their bodies proportions in the way of further growth up to the next cell division.

The elongated specimens of form "A" belong to the longest in the observed populations; they are up to 400 μm long and about 150 μm wide and this fact suggests also that their shape is really the result of more longitudinal growth of whole pattern according to Fig. 5. In these specimens even the apical kinety goes over ventral body margin on the right surface and whole pattern is therefore twisted in the right direction. Thus the distance OB can be measured by the prolongation ("untwisting") of the apical kinety on the drawing and ratio d/OB in this case is larger then 1. It is worth notifying also that in the same way the ratio d/OB could be applied to *Opalina obtrigonoidea* and *O. virguloidea*

and therefore could be happened the morphological gap between *O. lata* Metcalf (*O. ranarum*) on the one hand, and *O. augustae* Metcalf (*O. virguloidea* and *O. obtrigonoidea*) on the other.

D-5. Divergency of the left and right body surface kineties

Two kineties starting from the same point of falx: one on the right body surface and another on the left surface run divergently each other see section B-3 (Figs. 1, 2, 3 and 4). This divergency determines, the divergency of longitudinal division plan on left and right body surfaces (it will be analysed in a separate paper). The divergency of the left and right kineties can be measured between the posterior ends of left and right body kineties against the cell body length. It is obvious that this divergency depends mainly on the left apical kinety deviation and a little upon the angle between the body axis and the right apical kinety. So the divergency of the left and right body kineties is larger in opalinids of form "A" than in those of form "B". Scheme on Fig. 6.
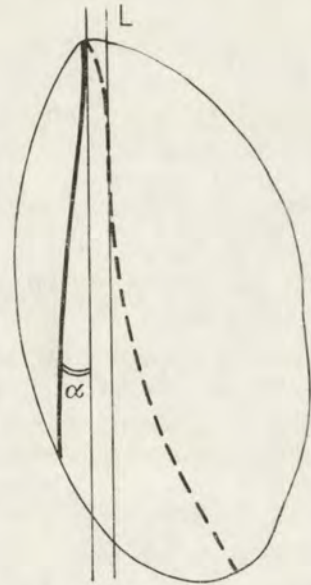
Fig. 6. The scheme of divergency of the apical left and right body kineties. The angle α is an angle between apical right kinety and *l* body axis

It is interesting, however, that either among forms "A" or among forms "B" particular populations can differ in respect to divergency of their kineties. It was recognized that these additional differences depend on some differences in the right body surface pattern expressed as variability of angle α, e.g., variability of kineties among two chosen populations of form "A" is presented in Fig. 7 a. The divergency of apical kineties (the left one, and the right one) is given against body
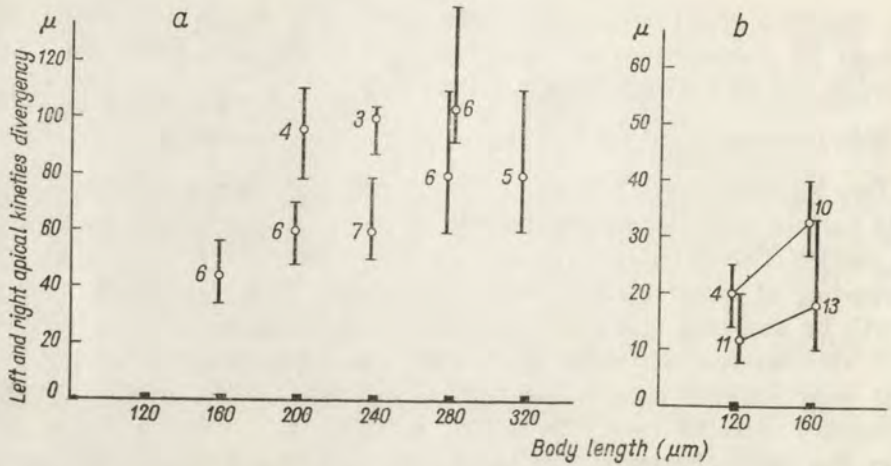
Fig. 7. Diagrams of left and right kineties divergency, a — for two populations of form "A" of large size with were different in this respect, b — for two populations of form "B". Average results limits of their variability and numbers of measurements in each class are given

length of specimens. In the second population of form "A" tested in this respect, the angle was larger, i.e., the right body kineties run more obliquely toward dorsal body margin than in first population, while the deviation of left body kineties and the ratio d/OB was similar. Similar differences of kinety divergency among two chosen populations of opalinids of form "B" are presented in diagram 7 b. The angle α in the second population is larger than in the first and it is rather similar to those in the first population in diagram 5. In the first population of the diagram, owing to the nearly 0 or minus value of the angle α, the divergency of the left and right kineties is so small that it can be neglected. The deviation of the left body surface kineties in both populations of form "B" was, similar just as among populations of form "A"; see diagram on Fig. 7 b.

E. Changes of kineties length along the falx

The shape of the falx curvature is similar to the shape of the logarithmic curve because the degree of the steepness of this curve in relation to the kineties fell down along the falx. Therefore the question arises if real differences of pattern lines length have the same character as it was suggested previously by K a c z a n o w s k i 1969.

For this purpose the kineties length was measured periodically along the falx according to Fig. 8. In this Figure the measured kineties keep the same distance of 25 μm plotted perpendicularly to the pattern lines from respective points of the falx. If the density of pellicular ribs is constant,
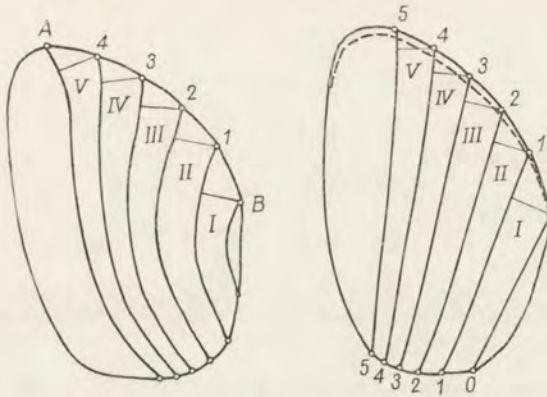
Fig. 8. Determination of pattern lines which keep constant perpendicular distance of 25 μm measured from respective falx points. They are the pattern lines equally distant, in respect to the number of rib-to-rib subdistances on the falx level. These pattern lines are given in arabics while subsequent falx sectors contained between them as I, II, III . . . . .

this distance (or any other distance plotted in the same way) represents in each case similar number of rib-to rib subdistances. Thus the diagram, prepared on this base, represents changes of the kineties (pattern lines) length plotted against the number of distances representing formation sites along the falx even if the exact number is not known. For the investigation 10 specimens of form "B" (little trophonts) and 10 specimens of form "A" were chosen at random. For each of them the diagram of kineties length on the left and right body surface was separately prepared according to the principles mentioned above.

The results were summarized in diagrams on Fig. 9 and 10 in which the real proportions of kineties length and distance of 25 μm were preserved. It was found that in form "B" the received curves have always logarithmic-like character. The diagram of the left surface kineties length is more steep than the diagram of the right body surface kineties length.

The same is true for opalinids of form "A" with only one limitation. Namely, the curve corresponding to the diagram of the length of the left body surface kineties is broken in the characteristic way in the point D in some distance from its begining. It was resulted by measuring the whole length of these kineties which in form "A" turn over by the ventral body margin on its right surface and join the right pattern (Section B-2). Therefore, the distances of these kineties which belong to the right body surface were added. Then the length of the first kinety of the right body surface and left body surface in contrast to the form "B" were similar, starting from the ventral beginning of the falx and
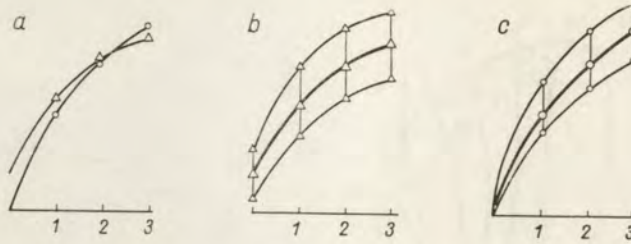
Fig. 9. The diagram of the change of kineties length along the falx in the popula-
tion of little opalinids of form B. Units on the low axis of the diagram represent
distances of 25 μm being kept. More steep curve (a) with dotted circles on it
represents diagram of the left surface kineties and less steep with triangles on it
for the right surface, b — presents the surface sector occupied by the bundle of
the length, the left surface kineties in 10 examined specimens, c — the respective
sector for the bundle of the length curves of the right body surface of the same
specimens
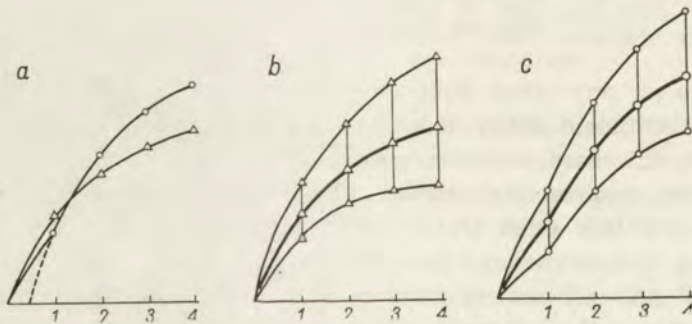
Fig. 10. The diagram of the change of kineties length along the falx in the
population of large trophonts of form "A". All designation as in Fig. 7. It is worth
noticing break point D of the left surface curve followed by their regular course

ending in the same region on the right body surface (see section D-2).
Beyond the point D further character of the curve representing diagram
of the left body surface kineties length in form "A" is again logarithmic-
like.

The different mode of kineties and pellicular ribs unpacking on the
left and right cell surfaces nevertheless realizes the same contour of
both body surfaces. It is obvious, that sigmoid kineties and pellicular
ribs of the left body surface are longer than the corresponding struc-
tures of the right body surface of the same specimen.

In other words, the left body surface contains smaller number of
kineties and pellicular ribs in comparison to the right body surface.
The larger average length of pattern lines on the left body surface is
compensated by the more pellicular ribs and kineties arising from the
falx to the right.

This difference between the left and right body surfaces is more accentuated in form "A" than in form "B". In form "A" the difference in numbers of plotted distances of 25 μm can be expressed, see in entire Pl. II photo 3 against 4, or Pl. III photo 5 against 6. Therefore, it is clear that the intersection point on the diagrams on Figs. 9 and 10 does not represent the true point on the falx, where both starting kineties left and right have the same length. In fact, this point is displaced more towards the apex relation to the intersection points of both curves on Figs. 9 and 10.

F. The density of kineties ingrowth

The density of kineties growth was calculated in the falx sectors determined by the distance of 25 μm plotted perpendicularly to the kineties respective points of the falx as it is presented on Fig. 8. In other words, number of kineties starting from the falx in relation to the respective number of pellicular ribs were counted along the falx according to the assumption given in the previous section of this paper. The falx sectors in which the density of kineties ingrowth were counted, were numbered I, II, III and so on. Results of these measurements are summarized in diagrams on Fig. 11 a and Fig. 11 b for large trophonts
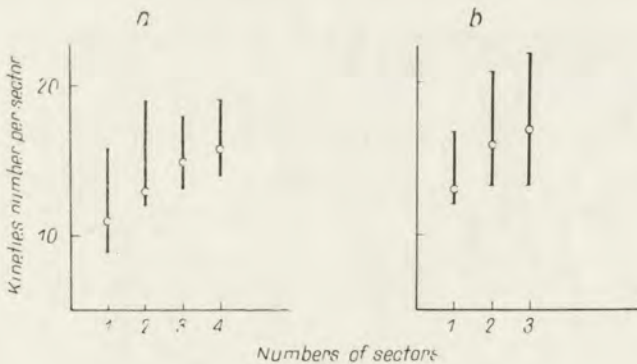
Fig. 11 a — The diagram of density of ingrowing kineties in subsequent sectors in form "A" which are determined by sectors of 25 μm plotted perpendicularly to the pattern lines starting from the respective falx points according to Fig. 6, b — the diagram of density of ingrowing kineties in subsequent sectors in form "B". These diagrams represent average numbers of kineties per sector and limits of their variability

of form "A" and little trophonts of form "B" respectively. Medium numbers and limits of their variability are plotted on these diagrams. The calculating of standard statistical deviation was omitted because of small number of measurements. Average numbers received for sub-

sequent sectors grew up and this growth was largest between the Ist
and IInd sector. Sometimes, however, the kineties number was equal in
the Ist and IInd sector growing up in the IIIrd one and in one case in
the IVth. But there was found no case of falling down of kineties
number from the Ist to the IInd sector. So in each case the number of
kineties in the first sector was the smallest number of kineties per
sector in the given specimen and no exception to this rule was found.
The comparison of the kineties ingrowth density in the left and right
body surface indicates that left and right body surfaces do not differ
in this respect.

To illustrate this, the results obtained for five chosen specimens of
little trophonts of form B are presented in the Table 1. They are given

Table 1

Ratio of the number of kineties of the right
and left body surface in the given sectors

Number of specimen	Sectors			
	I	II	III	IV
1	16/17	19/21	21/22	—
2	14/12	17/14	15/17	17/17
3	12/12	13/14	14/16	14/16
4	13/14	17/15	17/15	17/15
5	14/13	16/15	16/16	—

as a ratio where the numerator corresponds to the number of the right
body surfaces kineties and the denominator to the number of the left
body surface kineties of given corresponding sectors. It is also clear that
differences between the numbers of kineties per sector received for left
and right body surface in these same specimens are small and random.

General discussion

A. The concept of *Opalina* shape variability as a function of the level
of available molecular precursores for cortical microtubules formation

The formation of *Opalina* body shape was previously considered as
a result of two separate processess:

(1) formation of new initiative sites for new pellicular ribs and
kineties,

(2) elongation of pellicular ribs and kineties.

The investigation of number of kineties ingrowing from falx in different surface sectors indicates that this number is smaller in the first sector, i.e., in the vicinity of the ventral body margin.

According to the presented standpoint these differences of kineties numbers in different surface sectors reflect some asynchrony in formation of initiative sites for new pellicular ribs on the one hand and for new kineties on the other. The body shape regulation after longitudinal division requests particularly intensive surface in growth in the vicinity of the ventral body margin. Thus the smaller number of kineties in this sector can be considered as an effect o f t h e d e l a y o f n e w k i n e t i e s i n i t i a t i o n p r o c e s s i n r e l a t i o n t o t h e i n g r o w t h o f n e w p e l l i c u l a r r i b s.

This point is essential for further considerations.

On this base one can conclude that the surface growth is realized mainly by growth and ingrowth of new pellicular ribs while kineties occupy relatively small surface area and their ingrowth is delayed in comparison to the pellicular ribs. Therefore pellicular ribs play promoting role in surface growth and the problem of this growth can be outlined by the pellicular ribs system, neglecting kineties.

Since each pellicular rib consists of a ribbon of microtubules then the whole problem can be reduced to the rate of growth of microtubules ribbons within monotonous pattern. The growth of this system can be considered as a function of microtubules elongation and determination of new initiative sites. These processes would depend upon the level of available molecular precursors for microtubules formation. If the rate and order of determination of new initiative sites for pellicular ribs would be similar in different forms of *Opalina*, as it was proposed previously, than the *Opalina* body shape and form would be produced as a function of precursor (or precursors) level treated for microtubules formation in mathematical sense.

In the case of a higher level of these precursors, form "A" would be produced while in the case of smaller level of these precursors form "B" would be produced respectively.

The formation of new pellicular microtubules initiative sites is related to the falx kinetosomes multiplication and therefore to the expanding of the whole falx area. According to the suggestion of P i-t e l k a (1963) and W e s s e n b e r g's (1966) data, these points are determined by marginal falx kinetosomes. According to the presented concept, the falx expanding would be in some limits autonomous in comparison with the variable pattern elongation and its character would be similar among different *Opalina* forms.

The proposed concept is based on the assumption that the growth of microtubule bundles depends on the amount of molecular subunits, i.e., that this growth is unlimited in the mathematical sense.

Since some general properties of all microtubules are most probably similar it is worth notifying that the models of microtubules based mainly on cilia and flagella studies are built up of longitudinal columns combined with helicoidal pattern. Thus the ability to unlimited growth are contained within microtubules themselves (see K l u g 1967 for example). This pure theoretical point was supported experimentally by T u c k e r 1971 on the model of formation and elongation of the oral basket in the ciliate *Nassula*. This basket consists of rods; each of them represents an assembly of logitudinal microtubules. Their elongation can be arrested, by using protein synthesis antimetabolites (cycloheximide).

On the other hand the number and the position of rods in these experiments most likely did not depend on the tested factors being strictly related to the number of kinetosomes forming a new preoral kinety (T u c k e r 1970). Therefore the *Nassula* basket formation model is similar to the model of *Opalina* also in another respect: the determination of sites for microtubule synthesis and microtubules bundles elongation in both models are independent processes.

B. Comparison of the cortical pattern in *Opalina* with the pattern in ciliates

At the first glance *Opalina* is a ciliate: its body is covered by cilia which are aligned in longitudinal kineties. The same is true when investigating *Opalina* as a model of ciliary movement physiology and related phenomena. It is worth remarking that this point is not so formal and so superficial as the former one. But further analysis of the cortical pattern leads to unavoidable conclusion that cortical pattern in ciliates and in opalinids is contrary in many respects.

This conclusion can be considered most generally in terms of general and local pattern uniformity in *Opalina* on one hand and in terms of general and local non-uniformity pattern in *Ciliata* on the other hand respectively.

General pattern uniformity of *Opalina* is expressed by lacking of differentiated structures and territories within the surface of *Opalina*. The falx is a structure outside of the pattern. On the other hand in ciliates there are always some differentiated regions within the pattern; they are: oral apparatus, CVP-s, cytoproct and others. The importance of presented distinction is expressed when one considers the shape and pattern regulation problems. The body shape in opalinids is regulated when growing, which is realized through ingrowing new kineties and

pellicular ribs into the uniform pattern. There is lack of pattern regulation and reorganization of the whole pattern considered as an entity.

In contrast to *Opalina*, morphogenesis in ciliates is always connected with regulation of pattern as entity when new positions of differentiated structures are determined by entire cell geometry (T a r t a r 1962, S o n n e b o r n 1963, 1964, N a n n e y 1966, K a c z a n o w s k a 1971). The local uniformity of *Opalina* pattern can be expressed in the following way: s t r u c t u r e o f e a c h i n t e r k i n e t a l p o r t i o n o f c o r t e x i s r e p e t i t i v e. The local non-unformity in *Ciliata* is expressed by different positions of fiber ribbons in relation to kinetosomes and kineties in terms of cilliary unit territories. The asymmetry of kinetosomes is followed by asymmetry of unit territory organized by them (E h r e t and P o w e r s 1959, E h r e t 1960, D i p p e l 1965, 1968, P i t e l k a 1969).

It is worth to note, however, that the elementary structures building up cortex in ciliates and opalinids are similar. They are: kinetosomes, microtubular boundles and periodical fibers (kinetodesma in ciliates, kinetosome to kinetosome periodic fibers in *Opalina*). The main difference, however, is related to the uniform system of microtubules which are in *Opalina* independent in relation to the somatic kinetosomes; while in ciliates, they create many different systems organized by kinetosomes (postciliary and transversal fibers) or asymmetrically relate to the kinety (longitudinal fibers in *Tetrahymena* group). In the extensively studied model of ciliate, the surface growth is proceeded by the new kinetosomes formation which, in turn, organize new unit territories (E h r e t and de H a l l e r 1963, D i p p e l 1965, 1968, E h r e t 1967, G i l l i e s and H a n s o n 1968, S o n n e b o r n 1970, K a n e d a and H a n s o n 1972.

Since cortical microtubules within pellicular ribs do not keep any contact with somatic kinetosomes and since growth of new kineties is delayed in relation to the pellicular ribs, i.e., in relation to the cortical microtubules, it is clear that the whole concept of ciliary unit territories cannot be applied to *Opalina*. In other words somatic kinetosomes do not play the role of organizers of neighbouring territory systems. Lack of asymmetry aspect within cortical microtubules system is a consequence of described situation, while the asymmetry within ciliary unit territory in *Ciliata* is a result of its own kinetosome asymmetry. The fact that somatic kinetosome do not play the organizing part in *Opalina* in the described sense is related to the problem of cortical growth map. This map in ciliates is a map representing new and old kinetosomes in the given moment with theirs surrounding unit territories. The map of ciliates cortical growth is based on the fact that new kinetosomes are

formed anteriorly to pre-existing ones (E h r e t and de H a l l e r 1963, D i p p e l 1965, 1968, G i l l i e s and H a n s o n 1968). This process is going simultaneously on the given topographic level of pattern as measured to the divisional furrow and this process is followed by arising of the new unit territories. If any given line of ciliary unit territories encirling the whole body surface, termed by E h r e t 1967 "the paratene" represents the sites of one twice kinetosomes' proliferation in each of the meridional kineties, then the whole map of the cortical growth can be diagrammed as a set heavy and light stripes representing respectively old and new ciliary units, i.e., old and new paratenes.

If, however, the number of new units produced on the ventral and dorsal body surface is different (S o n n e b o r n 1970), the map of surface growth should be given separately for each of the body surface. Nevertheless, the whole concept of the map of growth in both cases is similar. It is a map of lines binding the pattern structures formed in the same time (i.e., old and new generations) related to the divisional furrow, or map of distinct regions covered by such structures which proliferate) in the case if the pattern of ciliary unit replication is more irregular and composed than their directly linear arrangement).

In *Opalina*, however, in contrast to ciliates, there is no possibility to determine any lines grouping the pattern points and structures formed at the same time. Even more, it is impossible to dinstinguish regions covered by such structures, if more than the fate of one single kinety is considered. The ingrowth of new kineties and new pellicular ribs between pre-existing ones causes that the new and old units lie side by side. The structurally uniform pattern is not uniform, but mosaique in the respect to the time of formation of its units, considering either the whole pattern or its local fragments only.

This conclusion does not depend upon the question where new kinetosomes are formed within the ingrowing kinety in *Opalina*, which up to now is unknown. It may be admitted only that two different possibilities could be taken in account:

(1) new kinetosomes are formed within the falx area nad they are added to the anterior end of kinety,

(2) marginal falx kinetosomes induce the synthesis of the first kinetosomes in kinety. In turn, these kinetosomes induce the formation of the next ones. It is a model of posteriad kinetal growth among the pre-existing pellicular stripes.

The first possibility seems, however, unlikely because it requests pushing and gliding of an entire kinety structure when the new kinetosomes are opposited anteriorly. As it was shown, the concomitant growth of the kineties and surrounding pellicular ribs in the falx area in pro-

portional way is excluded since there is delay of the kinety formation in ventral region in comparison to the pellicular ribs.

On the other hand the kinety is a solid structure even after isolation (K a c z a n o w s k i 1970) because of occurrence of kinetosome to kinetosome fibers.

S u m m a r y

The cortical pattern of *Opalina* consists of longitudinal ribs and kineties. The shape and pattern is considered as a result of growth of ribs and kineties.

Two processes involved in this growth could be distinguished:

(1) Determination of new initiative points along the margins of densely packed with kinetosomes field called the falx and extending of it on the anterior body margin.

(2) Elongation of pre-existing ribs and kineties.

On this basis, according to the hypothesis, differences in *Opalina* shape and cortical pattern are thought of as a result of different relations of the rate of determination of new initiative points and elongation of pre-existing elements. The relatively different elongation could depend upon the level of available molecular precursor for aggregation of microtubules within pellicular ribs. The cortical patterns of *Opalina* and *Ciliata* are compared in terms of the pattern uniformity in the case of *Opalina* and its nonuniformity in the case of *Ciliata*.

STRESZCZENIE

W skład wzoru kortykalnego *Opalina* wchodzą podłużne żeberka pellikularne i kinety.

Forma ciała i jego wzór są rozważane jako rezultat wzrostu kinet i żeberek pellikularnych. Wzrost ten jest funkcją dwóch oddzielnych procesów:

(1) Tworzenie się nowych punktów inicjacji dla nowych żeberek pellikularnych i kinet na brzegach pola gęsto ułożonych kinetosomów, które rozciąga się wzdłuż przedniej krawędzi ciała i zwane jest falksem.

(2) Wydłużanie się preegzystujących kinet i żeberek.

Na tej podstawie zaproponowano hipotezę, która rozważa zmienność formy i wzoru kortykalnego *Opalina* jako rezultat różnego stosunku tempa powstawania nowych miejsc inicjacji wzdłuż falksu i wydłużania się preegzystujących żeberek i kinet. Wówczas stopień ich wydłużania mógłby zależeć od poziomu dostępnego prekursora molekularnego potrzebnego do agregacji mikrotubul zawartych wewnątrz żeberek pellikularnych.

W dyskusji porównywany jest wzór kortykalny *Opalina* i *Ciliata* w terminach jednorodności wzoru u *Opalina* i jego niejednorodności u *Ciliata*.

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EXPLANATION OF PLATES I-III

1 and 2: The specimen of large *Opalina ranarum* of form "A" seen from the left (1) and right (2) body surface
3 and 4: The strongly elongated specimen of large *Opalina* of form "A" from the left (3) and right (4) body surface
5 and 6: The specimen of medium sized *Opalina* of form "B" seen from the left (5) and right (6) body surface
7 and 8: The specimen of little sized *Opalina* seen from the left (7) and right (8) body surface
All photographs are of the same magnification × 270
Dotted lines on photographs represent apical left and right kineties respectively. On photographs of the left surfaces the axis "1" is also plotted according to Fig. 2, 3, 4 and 5.

1

2

auctor phot.

PLATE II

3

4
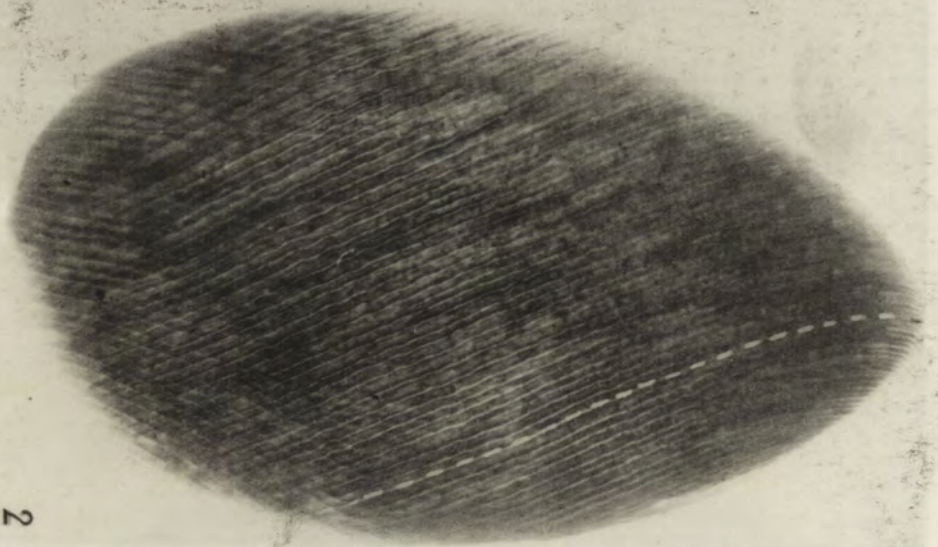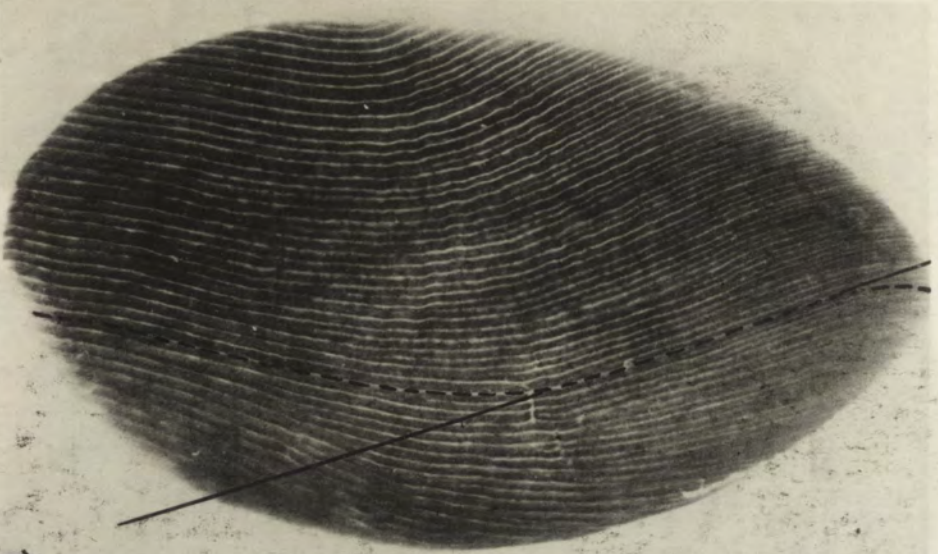
A. Kaczanowski

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PLATE III
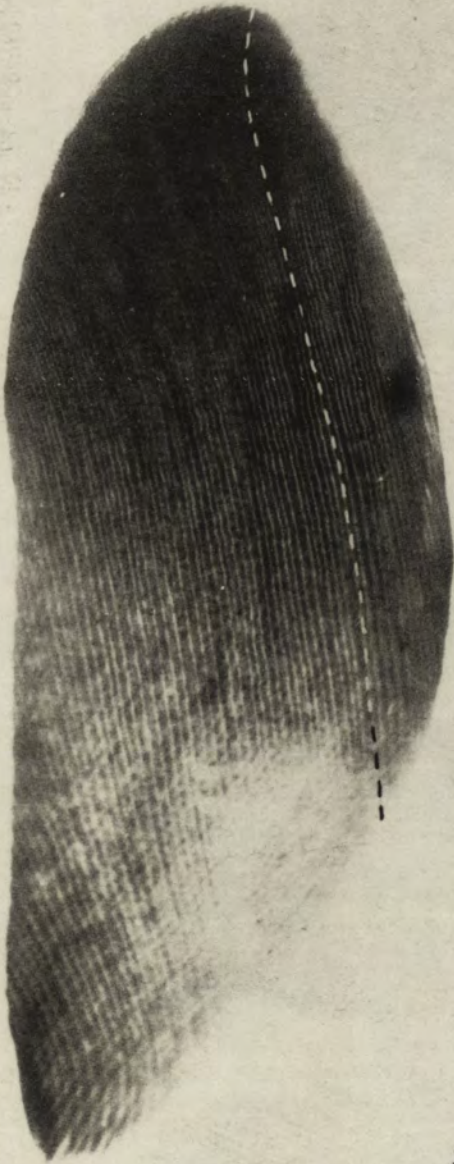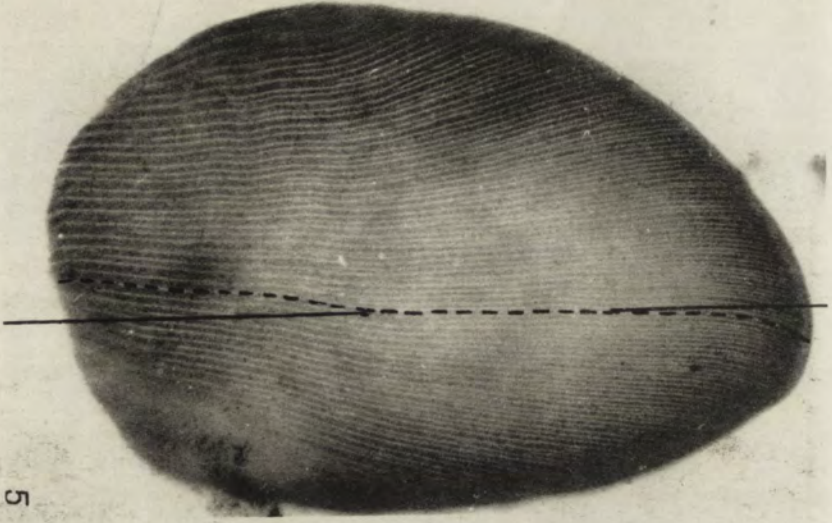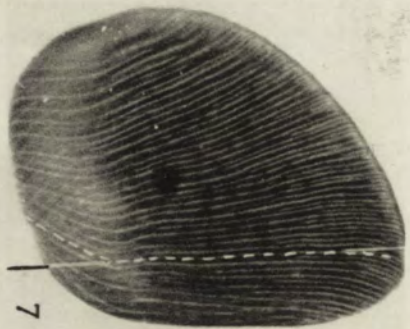
Irena TOTWEN-NOWAKOWSKA

Conditions of stability of the double system in the post-traumatic regeneration of doublets of *Stylonychia mytilus*

Warunki zachowania układu podwójnego podczas regeneracji dubletów
Stylonychia mytilus

The stability of doublets which retain their morphogenetic model pattern (Doroszewski and Raabe 1966) for a number of generations, has been explained by a solid junction between both components, as well as by their bilateral symmetry (Tartar 1961 a) Fauré-Fremiet (1948) considers the equilibrium between both components as the overlapping of the structural constraint upon the labile transformation of the return to the single form. According to Tchang and Pang (1965) only those double forms are steady in which the peristomes are in opposite position.

Doublet clones may perish: (1) by a mass dying out when the double system is preserved till the end of life (this is ascribed to the senescene of clone — Dawson 1920, Calkins 1925), (2) by return to the single form either by a gradual longitudinal separation of the doublet (e.g., in *Euplotes* — Kimball 1941, *Urostyla* — Fauré-Fremiet 1945, *Oxytricha* — Dawson 1920, *Chilodonella* — MacDougall 1929) and (3) by the resorption of one component (*Paramecium* — Sonneborn 1963, *Stentor* — Tartar 1961 a, *Leucophrys* — Fauré-Fremiet 1948), in similar way as in so-called "transitory doublets" reported in *Urostyla* by Jerka-Dziadosz 1967 and in *Stentor* by Johnson 1893.

A certain number of *Stylonychia* doublets returned to the single form by separation along the connection line from forward to backward. In prosperous cultures, however, this phenomenon was decreased to minimum. The doublet clones could be maintained for several months giving rise to several hundreds of generations (Totwen-Nowa-

kowska 1969). The death of single doublet clones might occur at different "age" and was mostly caused by the culture conditions, simultaneously with the death of the normal single forms serving as a control. This indicated that there was no reason to consider the mass death of doublets as consequence of the cell metabolism disturbances connected with the senescene of the culture.

The regular doublets of *Stylonychia* subjected to a prolonged starvation (approximately two weeks) underwent a repeated reorganization, similarly as the normal *Stylonychia* individuals (D e m b o w s k a 1938), reducing their volume nearly eight times, keeping, however, unchanged their doublet form; just a short time prior to death one of the components became resorbed (Pl. I 1) (T o t w e n - N o w a k o w s k a unpublished).

It has been also stated that the regular doublets of *Stylonychia* became complemented after the operations in which the fragments of one or of both components have been cut off (T o t w e n - N o w a k o w s k a 1969).

The aim of the present study was to follow the influence of different amputations, carried out on regular doublets of *Stylonychia mytilus*, on the preservation of their double system or on their return to the single form. This study does not concern the detailed course of the regeneration and reorganization morphogenesis itself since the early stages of development were not analysed. Such analysis would be certainly desired, as yet, however, it could not be carried out because of the difficulties connected with the vital observations and staining due to the great thickness of the doublet body and occurrence of numerous granulations in its cytoplasm. A short (up to 24 h) starvation prior to operation gave no satisfactory results and a more prolonged keeping of the doublets in the starvation conditions could involve the starvation reorganization. Therefore the earliest observable images of primordia in both components appeared at the stage III (according to the classification of F r i c k 1968, J e r k a - D z i a d o s z and F r a n k e l 1969), when the primordia of F-V-T cirri were formed of distinct kineties rows and in the AZM-s the membranelles had been differentiated.

In the present paper the attention was paid to possible relations of morphogenetic processes in the operated and in uninjured components of *Stylonychia mytilus* doublet. The following questions were put forward:

(1) Does the operation of one component involve similar regeneration processes in both parts of doublet?

(2) Is there synchrony or a sequence of morphogenetic changes in both components during induced post-traumatic regeneration?

(3) What is the role of proportion in size between two components

and what is the minimum size of injured component indispensable for the restitution of the double system?

(4) What is the role of the extent of union between components on the induction of reorganization morphogenesis in the uninjured component?

Material and methods

The exit material has been the ciliate *Stylonchia mytilus* collected from a pool at Królikarnia — Warszawa. The doublets used for experiments originated from clones which appeared spontaneously after conjugation (T o t w e n - N o w a k o w - s k a 1964), or were induced experimentally by application of the modified method of thermic shocks (Z e u t h e n and S c h e r b a u m 1954, F r a n k e l 1962, 1964, T o - t w e n - N o w a k o w s k a 1965). Doublets — similarly as the normal single *Stylo- nychia* individuals — were cultivated on *Tetrahymena pyriformis* culture in the Pringsheim solution.

Operations were performed manually under binocular microscope using steel needles or thin glass capillary needles following the method of D e m b o w s k a (1924, 1925). The experimental material placed on a slide a drop a Pringsheim solution was immobilized by removing partly the fluid. After operation, the fluid was supplemented and the operated doublets were isolated into microchambers made on slides (for vital observation) or in small glass containers (for subsequent fixation).

Material was stained with the protargol method of D r a g e s c o (1962) and of T u f f r a u (1964, 1967), as well as with the Feulgen reagent.

Microphotograms were executed using the Exacta-Varex apparatus and the Lumipan-Zeiss microscope which was also used for the vital observations applying the phase contrast and filters.

The structure of regular doublet

The regular doublet of *Stylonychia mytilus* consists of two equal components, each of them corresponding in its shape and proportions to the normal cell of *Stylonychia* (Fig. 1). So the single component has 18 cirri on its ventral side, in which 3 groups may be distinguished: 3 frontal, 10 ventral and 5 caudal cirri, according to the terminology of M a c h e m e r (1965) and single rows of right and left marginal cirri. On the dorsal side 5 rows of dorsal bristles are present and on the body end 3 long terminal cirri protrude. AZM containing long strong membranelles, begins in the median part of the cell and turns then towards the left margin and embraces along an arc the anterior part of the body. On silver impregnated material, an undulating membrane (UM) may be observed running from the centre forwards along the longitudinal body axis; it is constructed of two delicate membranelles. At the resting stage the nuclear apparatus consists of 2 macronuclei and 2-4 micronuclei.

The union region which joins both doublet components as a narrow

Fig. 1. Regular doublet of *Stylonychia mytilus* (after
D r y l and T o t w e n - N o w a k o w s k a 1972)

comb, runs between the 3rd and 4th row of dorsal bristles. A doublet
resting on the bottom adheres usually to it with ventral surface of one
of the components the other one being directed upwards. In the course of
separation along the union, the distance between the anterior parts of
the doublet increases, until both components become attached with
their ventral surfaces on the substrate (Pl. I 4-6). In this position
occurs the final separation of doublet into two single components, owing
to their active movement in two opposite directions (Pl I 4-6).

The division processes occur in both components according to the
pattern typical for *Stylonychia mytilus* as described by W a l l e n g r e n
(1901).

The formation of the surface structures shows a distinct synchroniza-
tion (Pl. II 7 a, b), whereas the processes occurring in the nuclear
apparatus at later stages may be not fully concurrent (Pl. II 9). Similar
disturbances in the synchronization of division processes had been
observed in proter and opisthor of *Stylonychia* (S a p r a and D a s s
1970). In the dividing doublets of *Uroleptus mobilis* (C a l k i n s 1925)
and *Chilodonella uncinata* (M a c D o u g a l l 1929) as well as in the
doublets of *Stylonychia mytilus* occur the cases of union of the neigh-
bouring macronuclei of both components throughout their connection
area (Pl. II 9, 10). In the doublets of *Stylonychia* a similar phenomenon
occurs also during starvation (Pl. II 11, 12).

Description of experiments

The doublets presenting the experimental material had been segregat-
ed into three groups according to the extent of union between both
components (Fig. 2).

Fig. 3. Results of different types of operation on single groups of doublets of *Stylonychia*

Explanations: (++) — regeneration of fragment and reorganization of induced component, (— +) — reorganization of induced component and resorption of fragment, (+) — regeneration of single individual, (—S) absence of the uninjured component induction — regeneration of fragment, (D) — single individual formed after the division of induced component, (+D) — regeneration of fragment and division of induced component, (—D) — division of induced component and resorption of fragment. The roman numerals determine the type of operation. (a — posterior fragment of the doublet, b — anterior fragment of the doublet. Arabic numerals in the right lower corners present the number of operated doublets and the achieved results

Group A. Doublets with tightly connected components. The union reaches the level of anterior macronuclei.

Group B. Doublets separating along the union plane which reaches the level between the anterior and posterior macronuclei (Fig. 4).

Group C. Doublets in the late phase of longitudinal separation; union reaches the level of posterior macronuclei or backwards.
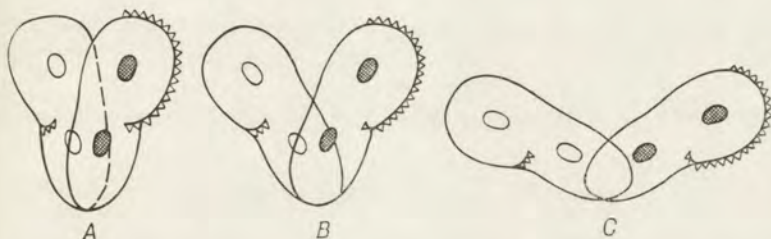
Fig. 2. Successive stages of return of *Stylonychia* doublet to single form by gradual separation from forward backwards along the connection plane. A — doublet with tightly connected components (see also Pl. I 4). B — separating doublet. Components in union along half of body length (see also Pl. I 5). C — doublets at late phase of separation. Union embrases only the posterior part of body to the level of posterior macronuclei (see also Pl. I 6)

The above mentioned material was applied for the following operations:

(I) Injury of the anterior part of one doublet component amputation of the anterior AZM fragment and of 2-3 frontal cirri.

(II) Amputation of approx. 1/2 of one component (nearly full removal of one AZM and of all frontal cirri, 4-5 ventral cirri and anterior macronucleus.

(III) Amputation of over 2/3 of one component (AZM is cut off as well as all frontal cirri, 5-7 ventral cirri and the anterior macronucleus).

(IV) Amputation of over 3/4 of one component with both macronuclei (removal of AZM, of all the frontal cirri, 7-8 ventral cirri and 1-2 caudal cirri.

(V) Cutting the doublet along the union plane. Injury of the dorsal bristles rows, the remaining cortical structures of both isolated components remain unimpaired.

(VI) Injury of the anterior part of both AZM-s.

(VII) Transversal section of doublet at the level between the anterior and posterior macronuclei. In this case:

(a) the posterior doublet fragment has both posterior macronuclei, 5 ventral cirri in each, all the caudal and terminal cirri from both components.

(b) anterior fragment of doublet has 2 anterior macronuclei, all frontal cirri, 5 ventral cirri from both components, and 2 AZM-s.

(VIII) Amputation of the posterior part of doublet below the posterior macronuclei brings about the deprivation of 3-4 caudal cirri and of all terminal ones (originating from both components) in the operated doublet.

Besides the above experiments, 7 operations were perfomed in which both macronuclei were removed from one component.

In the group A in which the union was fight the operation was impossible at the normal position of one component on the substrate, adhering to it by its ventral surface, whereas the other one was directed upwards.

Results of experiments

The results of operations in each group of doublets, and the results of experiments have been presented on the summarizing table (Fig. 3).

The morphogenetic processes in operated doublet components were similar to those in *Stylonychia mytilus* as described by D e m b o w s k a (1924, 1925) and F r i c k (1967, 1968). During the regeneration of the injured component a single area of 18 cirri primordia arose on the ventral side, near the anterior macronucleus (if the fragment preserved both of them) or near the preserved one. The primordium of the new AZM was formed below the old one, or — in a fragment deprived of AZM — in the region of the posterior macronucleus. The primordia of the dorsal bristles, similarly as the marginal cirri, arose near the old rows in the equatorial stripe of the doublet cell (Pl. III 13 b, c) similarly as in division processes in *Stylonychia* (T c h a n g et al. 1965, F r a n-k e l unpublished). The morphognetic processes occurring in the induced component could either develop similarly as in regeneration with formation of single morphogenetic area or they formed two of them like in cell division. The variety of morphogenetic processes appearing as well on the operated as on induced component might give different results even within one type of operation.

Usually the operation of one component evoked its activation and induction of the other non-operated component (Fig. 4, a).

When the injured component produced a regeneration area, and on the induced one a similar single reorganization (b) area arose, then a doublet was reproducted (b') as result of synchronic morphogenetic processes (Pl. III).

When the injured component produced one regeneration area while on the induced component two morphogenetic areas (c) arose (this
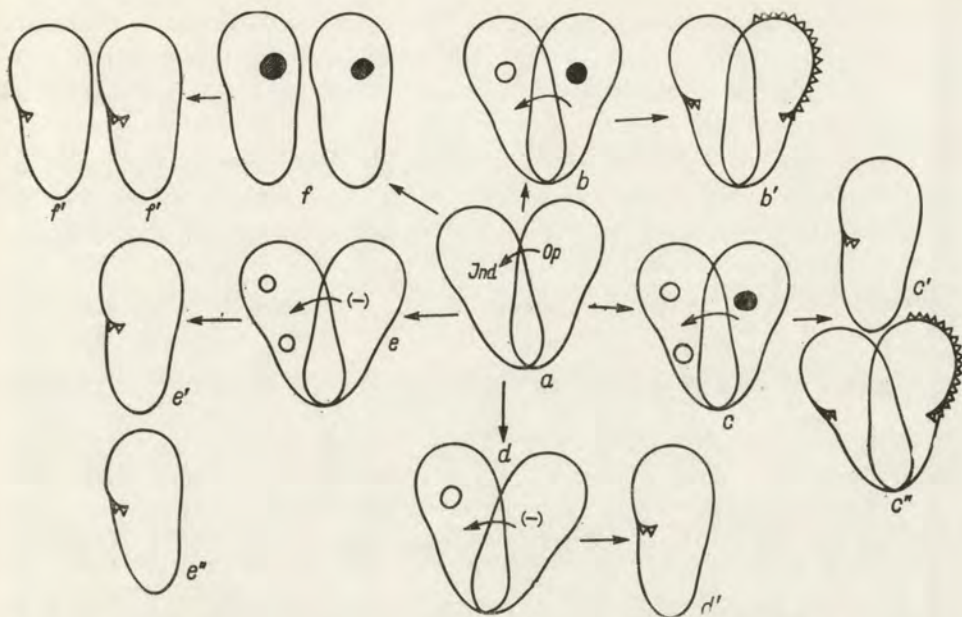
Fig. 4. Course of morphogenetic processes evoked by operations of *Stylonychia* doublets (detailed explanations in the text)

Abbreviations: op — operated, ind — induced (the arrow indicates the direction of morphogenetic induction of the operated component to the uninjured one), dashed area — regeneration area in the operated component, single clear field — area of reorganization primordia (in the non-operated component), clear double field — division primordia (in the unoparated component), (—) — absence of regeneration primordia

occurred in groups B and C, in the cases when the operated fragment was by half smaller than the induced one) — a single form (c′) arose from the proter of induced component, and a doublet (c″) — from its opisthor and the operated fragment.

When the injured component failed to form a regeneration area (—) whereas on the induced component a single regeneration area (d) arose in the course of morphogenetic processes occurring on the induced component, then the fragment of the second component was resorbed and a single individual (d′) was formed (Pl. IV 15-17).

If the injured component failed to form a regeneration area whereas in the induced one two areas of primordia (e) appeared then the injured fragment was resorbed during the division processes occurring in the induced component. As result, two single individuals arose from proter (e′) and opisthor (e″) of the non-operated component. Resorption concerned as well the anucleated fragments as those with one (mostly posterior) macronucleus. The subsequent fate of the resorbed nucleus has not been studied as yet. The absence (basing on the present results) of additional

macronucleus in the single individuals after division, allows to postulate that in the division following the regeneration and reorganization processes, all the macronuclei fuse together and a new segregation (?) of the nuclear material follows.

When both components were isolated by operation or separated in the course of morphogenetic processes (f), the subsequent course of morphogenesis occurred independently in each component, and 2 single forms arose (f') as result.

The induction processes occurred in both components up till a certain minimum of extent of union; below this limit a morphogenetic isolation followed. This took place usually when the union was reduced to a more or less thin cytoplasmic thread. Among 449 operations carried out in the present research only in one case the lack of primordia was stated on the non-operated component in doublet of group B. In this doublet the nuclear apparatus of both components was in similar stage which was characteristic for division, not for regeneration (the junction of macronuclei within each component (Pl. IV 18).

In the case of slight injury of one component (operation type I) when the proportions between the operated and uninjured component are not too distinct, similarly as after an equal injury of both components, the processes of regeneration and reorganization appeared and the doublet regenerated. The morphogenetic processes evoked often a stronger union of both components than it was in the doublet prior to operation.

After a removal of at least of half of one component, leaving the other unimpaired, the morphogenetic processes might have a different course even after a similar type of operation. If the fragment of operated component regenerated simultaneously with the reorganization of the induced component, then the dimensions of the cortical structures (the size of cirri, length of organelles) were the same for the whole, however, their proportions were nearer the initial doublet or the unimpaired component. If, however, instead of reorganization process, the induced component produced division areas, the structure proportions in the fragment were proportionally smaller — similarly as after the operation of type VII when the doublet was cut by half transversally. The division processes appeared in the induced component within this group of doublets (B, C).

In the cases when the proportion in size between injured and uninjured component was not higher than 1:3, the operated fragment failed to form primordia itself but induced morphogenetic process in the other component in which either a single reorganization area or two division primordia appeared. In the course of those processes, the fragment was

resorbed, independently of the presence of its nuclear apparatus. A fragment deprived of nuclear apparatus was always resorbed independently of the presence and dimensions of cortical structures. Seven operations of removing both macronuclei from one doublet component were performed. In all those cases, the resorption of the component occurred and a single form arose.

After a transverse section of doublet, different fragments arose depending on the extent of union: in the group A — two double fragments arose which regenerated as doublets being by half smaller than prior to operation. However, when the union reached only the half of the doublet (groups B and C) — two single fragments were formed from the anterior part as result of a transverse section regenerating subsequently as single forms as well as a posterior double fragment which regenerated as a doublet.

After injury of the posterior part of doublet belonging to group A (Operation type VIII) the double system was regenerated while in the group B and C the same type of operation caused separation of components just before or during the regeneration process.

Independently of the operation type, the primordia of cirri arose within $2^{1}/_{2}$-3 h after the treatment, as well in the cases of regeneration-reorganization processes or in cases of reorganization in the induced component without regeneration of the operated fragment.

The injury of the dorsal cirri in the course of section of the doublet into two components involved a total regeneration of both components, similarly as an injury of the other cortical structures.

In similar way as during division and starvation also in the course of regeneration the cases of union of neighbouring macronuclei of both components through the connection area were noticed (Pl. IV, 19, 20).

Discussion

In search for conditions deciding about the course of the doublet system, the following could be distinguished:

(1) The role of nuclear apparatus.

(2) The role of proportion in size between two components and the minimum size of injured component indispensable for restitution of the doublet system.

(3) The role of the extent of union between components on the induction of reorganization morphogenesis in the injured component.

(4) The occurrence of morphogenetic induction.

Role of the nuclear apparatus

The macronucleus of the doublets may be common for the whole as, e.g., in *Paramecium aurelia* (H a n s o n 1962, S o n n e b o r n 1963) or separate for each component like in *Euplotes* (K i m b a l l 1941), *Oxytricha* (D a w s o n 1920), *Uroleptus* (C a l k i n s 1925), *Chilodonella* (M a c D o u g a l l 1929). The presence of separate nuclear apparatus in each component of *Stylonychia mytilus* doublet might suggest the presence of morphogenetic autonomy of components. However, the cases of fusion of macronuclei in both components by adhesion during division, regeneration and starvation speak in favour of considering the doublet as a morphogenetic entity.

In certain cases the union of a *Stentor* cell containing nucleus with anuclear one led to formation of two AZM primordia (T a r t a r 1966), S u z u k i (1957) described a doublet of *Blepharisma japonicum* in which the nuclear apparatus was concentrated in one component. In contrast to this, removal of macronuclei in one component of *Stylonychia* doublet evoked always the resorption of this fragment although its cortical structures and AZM remained nearly unimpaired. D e m b o w s k a (1924, 1925) and J a n i s c h (1959) stressed the role of nuclear apparatus in regeneration of *Stylonychia*. Before the onset of morphogenetic processes, a regulation of the fragment shape takes place as well as a certain shift of the nuclear apparatus which becomes subsequently an orienting spot for the areas of arising primordia. Consequently the presence of at least one macronucleus in the impaired part of doublet is indispensable for the formation of regeneration primordia within it. This, however, is not a decisive factor since the fragments with one nucleus may undergo resorption as well.

The role of proportion in size between two components and the minimum size of injured component indispensable for restitution of the doublet system

The dimensions of cortical structures in regenerated *Stylonychia* doublet (AZM membranelles and cirri) are proportional to the size of whole animal. In the case of by-half section of the doublet (between the anterior and posterior mocronuclei), two by-half smaller doublets arise with correspondingly smaller cortical structures, similarly as after division (F r i c k 1968, De T e r r a 1969). The dimensions of both parts are different (e.g., 1:2) while proportions of the cortical structures are related to the larger doublet component. The morphogenetic field arises on the smaller injured fragment are related to the larger doublet com-

ponent up to a certain proportion of two components. When this pro-
portion is below 1:3, the smaller fragment is presumably too small to
produce primordia and becomes resorbed. The essential role of size pro-
portion is suggested by the fact that the doublets of similar dimensions
of both injured components, regenerated as a doublet even if each of its
parts was three times smaller than the operated doublet. Similarly
during starvation the doublet may reduce its dimensions even eight
times, still preserving its double system.

In the experiments of T a r t a r (1941, 1961 a), after the union of
two *Stentor* cells, a big and a small one, a doublet arose with a distinct
difference of dimensions of its both parts, however, the width of stripes
and the length of AZM membranelles were the same on the whole and
corresponded to the bigger part.

The isolation of the anterior part of the *Stentor* doublet (T a r t a r
1961 a) involved a proportional reduction of AZM-s and the resorption
of one of them.

The role of the extent of union between components on the induction of reorganization morphogenesis in the uninjured component

In regular doublet of *Stylonychia*, the connection line runs on the
dorsal side, between the 3rd and 4th rows of dorsal bristles. Considering
the possibility of union of the two doublet component macronuclei, the
absence of any internal barrier dividing both parts of the doublet should
be presumed. The return to a single form occurs by a gradual separation
of the doublet from anterior to the posterior part of the body, till the
moment when only a thin solid thread remains which bursts owing to
the violent movements of both components in two opposite directions
(T o t w e n - N o w a k o w s k a 1964, D r y l and T o t w e n - N o w a-
k o w s k a 1972). The separation of doublet may recess as a result of
regeneration and starvation reorganization, and the regenerated doublet
shows stronger union between components than prior to operation.

The process of separation of doublet components was not investigated
in detail so far. However, it was stated, that the deterioration of the
physiological condition of the doublet evokes weakening of the union
and pushing of both components, aside from one another. When the
anterior part of the doublet has been already deprived of union, the
induction in the unimpaired larger part may lead to formation of two
morphogenetic (division) areas: the anterior and the posterior one. This
occurs when the fragment of the second component has only one posterior
macronucleus, being by half smaller than the non-operated component.

As result of morphogenetic processes, a single individual is formed of proter of unimpaired component, and a doublet from its opisthor and remaining part of the operated component. The replacement of the regeneration morphogenesis by the divisional had been described in the normal forms of *Stylonychia* (D e m b o w s k a 1924, 1925, F r i c k 1968).

Occurrence of the morphogenetic induction

If we postulate that traumatic injury of *Stylonychia* cortex is a threshold stimulus for evoking a total morphogenetic reaction, then a question arises what would be the reaction of the unimpaired doublet component to this stimulus. Will be morphogenetic activization induce some definite changes in the second component? T a r t a r (1954, 1956, 1961 a, b, 1966) studied experimentally this problem on the double *Stentor* individuals formed after grafting, and ascertained that both components present a system of mutually inducing parts. Synchronization of processes in both components occurred in different ways. It had been stated beyond any doubt (448 positive for one negative result) by the experiments on *Stylonychia* doublets that the injury of one doublet component involves morphogenesis of the uninjured component which proves the occurrence of induction. Especially striking were the cases of removal of nearly whole one component from which only fragment of cytoplasm was left with an insignificant number of cortical structures. Even in this case, the morphogenesis of division or reorganization type was observed in the unimpaired component. The remaining fragments of the injured component were resorbed. Consequently the morphogenetic induction does not require either the presence of definite cortical structures or the nuclear apparatus in the injured fragment.

Resorption occurring in some cases of regeneration of *Stylonychia* doublet has no homologue in the divisional cells of this ciliate. In contrast to the influence of thermic shocks on *Tetrahymena pyriformis* (F r a n k e l 1962), and to the operations executed during division in *Spirostomum* (E b e r h a r d t 1962) or in *Oxytricha* (H a s h i m o t o 1961), the initiated division processes reach always their conclusion in *Stylonychia*, and the injured cell starts its regeneration only after the conclusion of division processes (D e m b o w s k a 1924, 1925). The thermic shocks applied to the dividing cells of *Stylonychia* (T o t w e n - N o-w a k o w s k a 1965) evoked some disturbances in formation of the division furrow resulting in the induction of various doublet types;

however, the morphogenetic processes attained their conclusion and only after their completion occurred the reorganization (occurring once or many times) in which one of the components became resorbed (T o t- w e n - N o w a k o w s k a unpublished). Similar phenomenon has been reported as a result of operation on dividing *Stylonychia* T c h a n g and P a n g 1965 and on dividing *Urostyla weissei, U. grandis* and *U. cristata* (J e r k a - D z i a d o s z 1967).

The cases of removal of the minor part of the component, or the operation of both components, permitted to compare the rate and synchronization of the morphogenetic processes. At least in the advanced stages, a full synchronization of processes in both doublet components was observed.

The operations consisting in the transection of the doublet along the connection plane permitted to ascertain that the injury of dorsal bristles similarly as of other cortical structures involves a complete regeneration of the *Stylonychia* cell.

The results concerning the morphogenetic induction in the *Stylonychia* doublet after injury of one component, are exceptionally clear. This is possibly connected with very low threshold of sensitivity to injury in *Stylonychia*. As a rule each injury of cortical structures evokes the activization of morphogenetic processes as a response of the whole cell or of the whole doublet. Those results are therefore different when compared with some experiments carried out on doublets of other ciliates e.g., *Blepharisma japonicum* (S u z u k i 1957), *Stentor* (T a r t a r 1961 a, 1966), *Paramecium* (H a n s o n 1962). The differences in results gained on those objects are connected with a different character of morphogenesis and with more determined localization of formation of primordia.

The stability of *Stylonychia* doublets which persists for a number of generations — as well as the results of experiments described in this paper — indicate a considerable biological integration of both doublet components. This result is in conformity with the data of G r i m e s (unpublished) concerning the excystation of the *Oxytricha* doublet even after the resorption of kinetosomes in the cyst.

Summary

In the operated component of *Stylonychia mytilus* doublet containing nuclear apparatus, the regeneration primordia appeared if the proportion in size between injured and uninjured component was not higher than 1 : 3. When both components were operated and their proportion in size was 1 : 1, the doublet could be reproduced even if the remaining part of the body was two times smaller than before operation. After removal of the half of one component in the stage of advances separation of union in the anterior region of body, the cell division process could be induced instead of regeneration. The morphogenetic processes could be evoked by injury of dorsal bristles in similar way as by amputation of other cortical structures.

STRESZCZENIE

Związki regeneracyjne tworzą się w obrębie operowanego, posiadającego aparat jądrowy komponentu dubletu *Stylonychia mytilus*, o ile proporcje wymiarów między operowanym i nieoperowanym komponentem nie będą większe niż 1 : 3. Forma podwójna może zregenerować z części dubletu o połowę mniejszej niż dublet operowany, o ile zostaną zachowane proporcje wielkości obu uszkodzonych komponentów 1 : 1. W dublecie, którego przednia część nie jest połączona, odcięcie ok. połowy jednego komponentu może spowodować w obrębie komponentu nieoperowanego wystąpienia procesów podziałowych zamiast regeneracji. Podobnie, jak amputacja innych struktur powierzchniowych, również uszkodzenie szczecinek grzbietowych może wywołać całkowitą regenerację komponentu dubletu.

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EXPLANATION OF PLATES I-IV

1: Final starvation stage of *Stylonychia* regular doublets. One of the components has been resorbed. Additional macronucleus is seen in the posterior part of the cell
2: Side view of the anterior part of the doublet union with rows of dorsal bristles
3: Side view of the union in the posterior part of doublet with rows of dorsal bristles
4-6: Successive stages of separation along the connection plane between components (after D r y l and T o t w e n - N o w a k o w s k a movie: "Morphology and movement of *Stylonychia mytilus* doublets")
7: Dividing regular doublet of *Stylonychia*. Agglomeration of macronuclei in each component. General view
7 a: Same as 7, higher magnification, dividing component, seen from the ventral side
7 b: Same as 7. Second component in division, seen from the dorsal side
8: Later division stage. Disturbances in synchronization of macronuclei division in both components
9-10: Cases of union of macronuclei of both components in the course of division, 9 — union in the former division doublet, 10 — union of the former macronuclei originating of both components in the doublet formed during division (anterior individual)
11-12: Cases of union of macronuclei of both components during starvation. 11 — union after 4 days of starvation, 12 — union after 12 days of starvation
13: Regeneration of doublet with tightly connected components (group A) after a slight injury of component (operation I). Primordia of FVC cirri and AZM are seen. General view
13 a: The same doublet (as in 13) photographed at higher magnification. Primordia of dorsal bristles are seen in equatorial band of doublet as well as the row of primordia of the right marginal cirri of one of the components
13 b: Equatorial zone of the doublet. Primordia of dorsal bristles (magnification as in 13 a)
14: Regeneration of a doublet of group B. A half of one component is cut off. General view
14 a: (operation III). The same (as in 14) at higher magnification. The induced morphogenesis in uninjured component
14 b: The same (as in 14) the view of regenerating fragment
15: The induced reorganization in uninjured component and resorption of the fragment with nucleus (macronucleus is seen in the lower part of the photogram)
16: Similar case as in 15, at later stage of induced reorganization of uninjured component
17: Induction of morphogenesis in uninjured component and resorption of anucleated part of operated component
18: Disturbances in synchronization of morphogenetic processes in operated doublet. General view
18 a: The same doublet (as in 18), higher magnification, regeneration primordia are seen in operated component
18 b: The same doublet (as in 18), absence of primordia in the uninjured component
19-20: Cases of union of macronuclei of both doublet components throughout the connection plane in the course of regeneration

PLATE I

I. Totwen-Nowakowska   S. Dryl, K. Golińska et I. Totwen-Nowakowska phot.
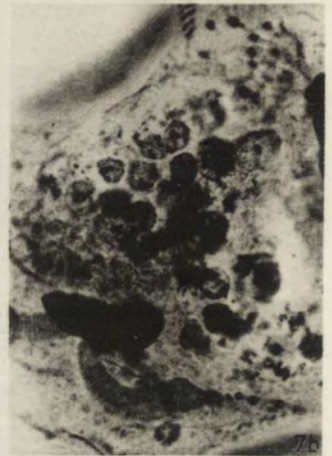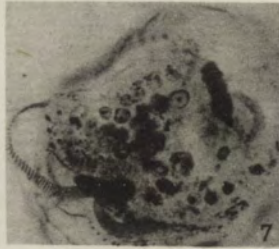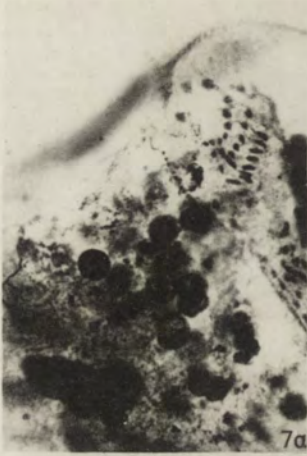
PLATE II

7a

7

7b

8

9

10

11

12

I. Totwen-Nowakowska

K. Golińska et I. Totwen-Nowakowska phot.

PLATE III

13

13a

13b

14

14a

14b

I. Totwen-Nowakowska

K. Golińska et I. Totwen-Nowakowska phot.

PLATE IV

15

16

17

18

18a

18b

19

20
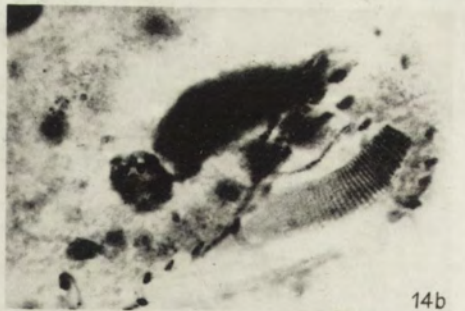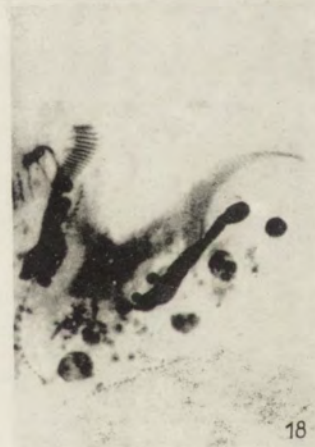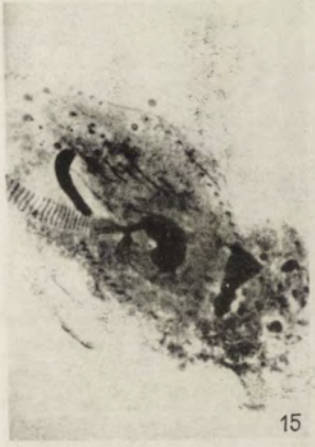
I. Totwen-Nowakowska

K. Golińska et I. Totwen-Nowakowska phot.

N. HÜLSMANN and M. HABEREY

Phenomena of amoeboid movement. Behavior of the cell surface in *Hyalodiscus simplex* Wohlfarth-Bottermann

Phänomene der amöboiden Bewegung. Das Bewegungsverhalten der Zelloberfläche von *Hyalodiscus simplex* Wohlfarth-Bottermann

In free living protists the cell membrane has a special importance because it fulfills a protective function as a barrier against the surrounding outer medium. The role, however, that this membrane plays in the locomotion of amoeboid cells is not yet fully understood.

Today it appears no longer tenable to consider the plasmalemma as the structure which generates motive force as was formulated in the surface tension theory (Berthold 1886, Bütschli 1892, Rhumbler 1898). In view of recent findings contraction of filamentous structures in the groundplasm, similar to actomyosin, seems to be responsible for amoeboid locomotion (see Komnick et al. 1972). The contraction is possibly controlled by bioelectric phenomena at the cell membrane which serves as a continuous and permanent conductor of regulating impulses (Bingley and Thompson 1962, Bingley et al. 1962).

Exact knowledge of turnover rates and dynamic behavior of the cell membrane is necessary to investigate the participation of the plasmalemma in locomotion of amoebae. For *Amoeba proteus* Goldacre 1952 postulated a new formation of the whole cell membrane in a time interval equivalent to that necessary for the cell to move a distance corresponding to its own length. According to this model the membrane would be disintegrated at the posterior end into vesicles or molecules which then could be used to rebuild the membrane at the anterior tip of the cell.

Contradictory results to this model have been obtained, however, by labelling the membrane with different marker techniques. According to these experiments the membrane is a permanent structure which can follow the movement of the amoebae without disintegration. A number of authors have attempted to explain the behavior of the membrane

during locomotion by postulating a rolling movement of the plasmalemma (J e n n i n g s 1904, R h u m b l e r 1905, S c h a e f f e r 1920, M a s t 1926, G r i f f i n and A l l e n 1960, A b é 1962, S e r a v i n 1964). Others have explained the movement of the cell membrane by alternating folding and unfolding of either the whole surface area (C z a r s k a and G r ę b e c k i 1966) or of special regions of the plasmalemma (H a b e r e y et al. 1969, H a b e r e y 1970).

In this work *Hyalodiscus simplex* was investigated to test these models. This amoeba of the Limax type differs from *Proteus* amoebae mainly in respect to size and the permanent differentiation into hyaline groundplasm and endoplasm which contains the granular and vesicular inclusions.

Since endocytotic activity has been shown (W o h l f a r t h - B o t - t e r m a n n 1960, H a u s m a n n and S t o c k e m 1972) and quanti- tatively measured (H a u s m a n n 1971, H a u s m a n n et al. 1972), investigations about the dynamic behaviour of the cell membrane during normal locomotion seemed to be of great interest for the elucidation of the locomotive mechanism in *Hyalodiscus simplex*.

Material and methods

Hyalodiscus simplex was repeatedly found and collected in the ponds of the "Botanischer Garten der Universität Bonn" as well as in the outdoor basins of the "Institut für Cytologie und Mikromorphologie". The amoebae were cultured according to the method of W o h l f a r t h - B o t t e r m a n n 1960 in 0.1⁰/o Knop's solution and simultaneously on 1⁰/o Agar prepared with water or Knop's solution. Material was pipetted with a micropipette from the agar surface or from the bottom of the culture dish into the object chambers. Closed chambers (Fig. 1 a) and chambers which allowed continuous change of solutions were used to allow sufficient oxygen supply, protection from evaporation, and good objective lens clearance even with oil-immersion objectives. Profile observations were carried out in modified microbasins (after D e l l i n g e r 1906) (Fig. 1 b).

Motion picture films were taken only during the first 48 h even though the protists usually lived for 14 days or longer before encystation or death occurred in the observation chambers; in this way abnormal behavior could be eliminated. Suspensions of Latex particles (Dow-Latex, Serva, Heidelberg) with constant dia- meters (1.099 μm and 0.500 μm) were added to the observation chambers in minimal concentrations to avoid too heavy labelling. Incidentally-adhering bacteria or other objects were used also for analysis of movement behavior of the membrane.

For microcinematographical registration a Zeiss "Mikro-Kino-Kamera" was used. The microscopical equipment (Zeiss-Standard WL) allowed the use of bright field, phase contrast, and differential-interference-contrast (N o m a r s k i 1955). Profile views (see D e l l i n g e r 1906) were recorded by a combination of a Zeiss "Standard WL" microscope and a motion picture camera (Beaulieu). Some pictures were taken
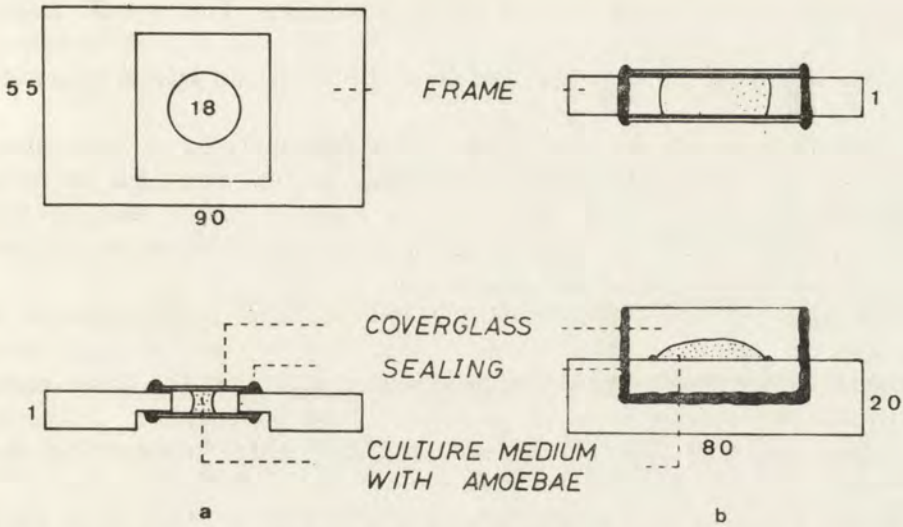
Fig. 1. Drawing of the closed observation chamber for normal observation (a) and open microbasin for profile view (modified after D e l l i n g e r), (b). All numbers are measurements in mm

on the Zeiss "Photomikroskop" or Zeiss "Planktonmikroskop". Recording speed was either 2 fps or 1 fps. Analysis of the final motion picture films was carried out on a Lytax-Analyser (Lytax GmbH). Evaluation of movement phenomena could be achieved by projection of single frames and graphic reproduction on transparent drawing paper.

Results

The suitability of Latex particles for labelling the cell surface was shown by S t o c k e m 1966 and H a b e r e y et al. 1969. In electron micrographs the particles can be seen adhering to the mucous layer of *Amoeba proteus* as well as *Hyalodiscus simplex* (H a u s m a n n, personal communication).

That *Hyalodiscus simplex* is distinctly polarized can be seen both in vertical (Pl. I and Fig. 2-3) and in side views. The advancing tip is always characterized by a lobopodial hyaloplasmic layer (W o h l f a r t h- B o t t e r m a n n 1960, H a u s m a n n and S t o c k e m 1972), which is normally 2 μm thick (measured in side view) but can rise up to 8 μm. The endoplasm (granular plasm) is exclusively situated in the posterior cell region and forms a more or less spherical "hump". This hump extends up to 20-25 μm above the underlying ground and contains the cell organelles. Nucleus, nucleolus, food vacuoles, and pulsating vacuole are clearly visible in the light microscope, whereas mitochondria can

only be identified under optimal optical conditions. The overall length of the amoeba varies between 30 and 80 μm, the lateral extension between 40 and 80 μm. Normal ratio of longitudinal and lateral extension is 40 : 50 μm.

Movement phenomena of the cell surface was analysed with amoebae, which in the course of normal locomotion, moved over one or more Latex particles and carried them over a distance longer than several times the cell length. In these cases a firm contact between marker particle and mucous layer was guaranteed.

The amoeba moves over particles without any visible change of direction. In most cases, however, a connection between mucous layer and marker is made visible by the movement behavior of the Latex bead. Sometimes the marker material is taken up by phagocytotic processes, but usually the particles move over the cell body, representing the movement behavior of the membrane.

In Pl. I 4-6, an amoeba is shown moving with constant speed onto and over a marker particle (see arrow). The particle is then found between the substrate and the lower surface of the cell. There is apparently no influence on the rate of locomotion or a change of contours, and the Latex bead remains in the same position during the process (Fig. 2). Small positional changes, however, show that contact to the
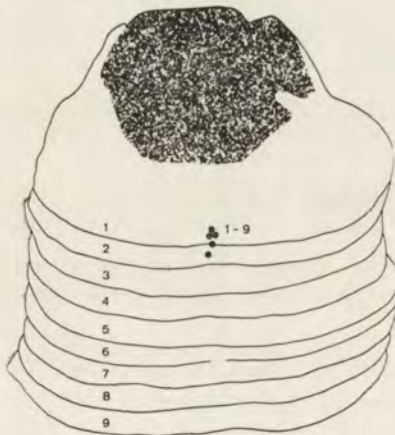
Fig. 2. Stationary particle behavior on the lower surface of *Hyalodiscus simplex* (positions 1-9). Small positional changes demonstrate the connection between marker and mucous layer of the amoeba. Locomotion is shown by successive outlines of the frontal border (lines 1-9). Microcinematographic single frame analysis, interval 10 sec

mucous layer exists. In this figure the particle remains in a nearly constant position during 80 sec. The lack of distinct particle movement can be explained only by stationary behavior of the plasmalemma. Even amoebae with discontinuous or abnormal movement exhibit this phenomenon. This stationary behavior of the lower cell surface seems to be a typical feature of movement in *Hyalodiscus simplex*.
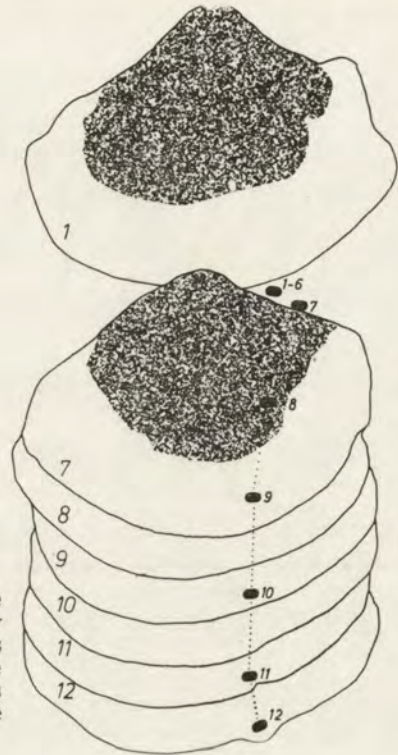
Fig. 3. Membrane movement on the upper side of *Hyalodiscus simplex*. The primarily stationary particle (1-6) moves upwards (6-8) and forwards (9-11). In position 12 the particle reaches the lower side again (compare position 1) and stops moving. Microcinematographic single frame analysis, interval 10 sec

By the forward movement of the amoeba the stationary particle consequently will be situated in the uroid region (Fig. 3, 1-6). The connection with the cell surface is demonstrated clearly in state 7. Within states 6-8 the cell membrane glides along the posterial contour towards the dorsal surface and begins to move forward away from the posterior end of the cell. With nearly constant velocity this area advances towards the anterior contour (state 7-11) where it moves downward again to the lower side of the hyaline layer (12). During the following phase the amoeba continues the forward movement while the particle is stationary on the lower surface until it reaches the posterior region after approximately 1 min. The same process can be seen again in the cinematographic sequence in Pl. I 1-3.

Further insight into the movement pattern of the membrane can be obtained with amoebae marked simultaneously by several particles. Comparative measurements of membrane velocity and velocity of the forward moving contour are shown in the following analysis (Fig. 4).

Latex particles moving over the upper surface of the cell all show the same velocity: A ratio of 2 : 1 against the overall forward movement of the cell as measured by the movement of the advancing contour at
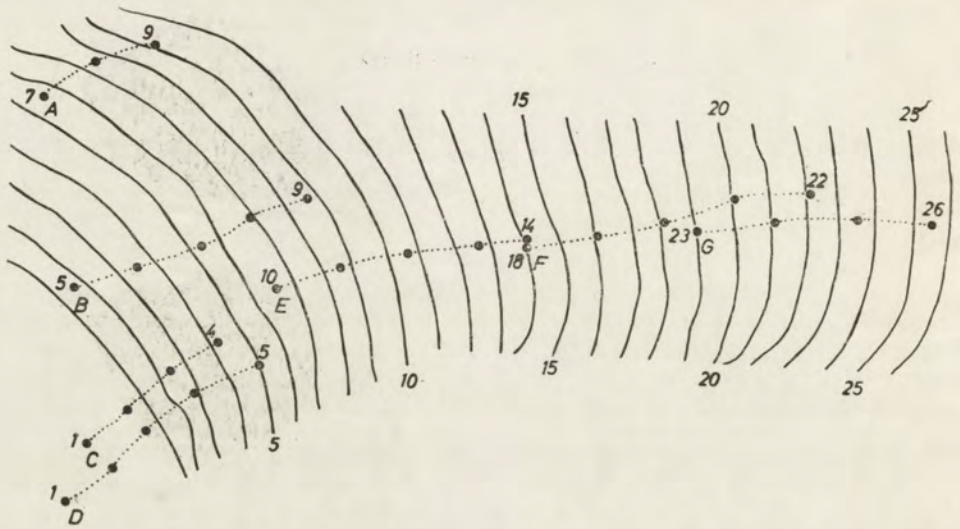
Fig. 4. Particle movement in relation to the movement velocity of the frontal hyaloplasmic border in *Hyalodiscus simplex*. Particles A-G represent the gliding of the plasmalemma on the upper side of the frontal lobe. Comparison of velocities is shown in Table 1 (for further explanations see text). Microcinematographic single frame analysis, interval 10 sec

the tip. The dorsal cell membrane advances twice as fast as the cell itself (see Table 1).

These results could be confirmed in all analysed sequences and were independent from the marker material. The velocity of particles in the central region of the dorsal surface of a single amoeba is identical, independent from the individual position of the markers (Fig. 4, particles A 7-9 and B 7-9).

Table 1

Ratio of overall forward movement of the cell against particle velocity on the upper surface of the lobopodium (analysis of the same sequence as in Fig. 4)

Particle A, 7-9	1 : 2.00
Particle B, 5-9	1 : 1.81
Particle C, 1-4	1 : 1.90
Particle D, 1-5	1 : 1.96
Particle E, 10-14	1 : 1.99
Particle F, 18-22	1 : 1.96
Particle G, 23-26	1 : 2.13
average	1 : 1.96

To demonstrate the movement in lateral areas of the cell locomotion of an amoeba was recorded which carried a particle for a comparatively long period of more than 190 sec (Fig. 5). The first centripedal path (1-4) then changes into a centrifugal direction (5-7) while the marker moves forward over the lateral periphery. During this time (phase I) the cell moves a distance equivalent to the cell length.

In 7 the particle assumes a position symmetrical to the starting point (1) in relation to an axis through the lateral tips. In phase II (7-13) the Latex bead moves backward along the lower surface of the cell and reaches a position similar to state 1. The following sequence (13-20) is a repetition of phase I.

In relation to the cell body the particle moves in an ellipsoid path, moving in a forward direction when on the dorsal surface of the amoeba, and backwards, when on the lower surface of the cell.

The visible complex movement of the particle has two components: (a) a rotating movement around the lateral tips and (b) the forward movement of the amoeba. The sum of both movements results in a periodical velocity distribution reaching a maximum in phase I (Fig. 5,
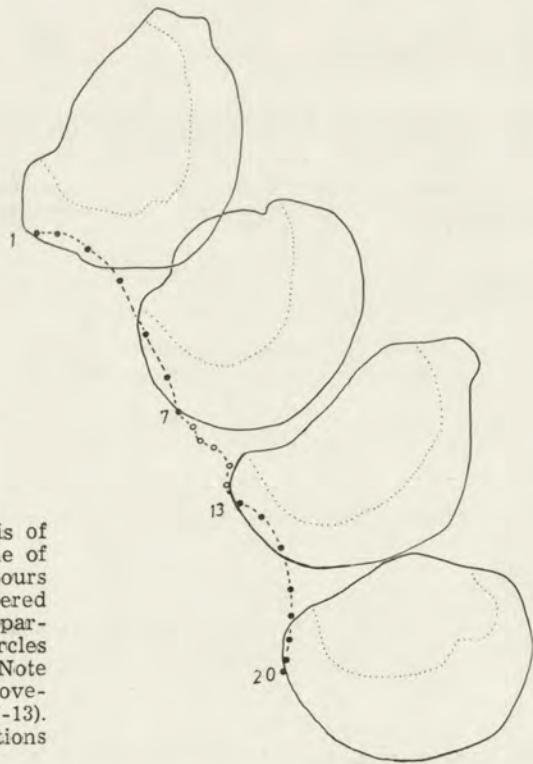
Fig. 5. Microcinematograhic analysis of marker behavior in the lateral zone of *Hyalodiscus simplex*. The contours shown correspond to the numbered particle positions. Dots represent particle position on the upper side, circles are positions on the lower surface. Note that there is a distinct particle movement on the ventral surface (7-13). Interval between particle positions 10 sec
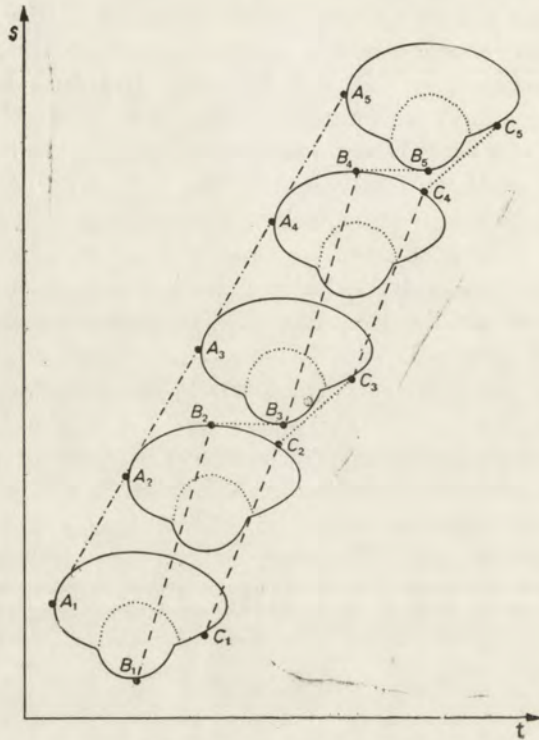
Fig. 6. Diagram of movement and plasmalemma behavior in *Hyalodiscus simplex*. While particle A on the lateral periphery moves with a constant velocity equivalent to the movement of the cell, particle B alternatively moved twice as fast on the upper surface (broken line) or is stationary on the lower surface (dotted line). Particle C shows periodic velocity changes according to its position on upper (broken line) or lower surface (dotted line); velocity is greater on the upper surface and less on the lower surface than that of the cell itself

1-7) and a minimum in phase II (Fig. 5, 7-13). Phase III is the repetition of phase I. Particles moving in the central region (Fig. 6 B) or on the surface of the lateral wings (Fig. 6 C) continuously revolve around an imaginary axis that would penetrate the surface on the lateral tips. Particles situated on these tips, therefore, exhibit no or very little movement relative to the amoeba (Fig. 6 A). In view of the unhindered movement of the lower surface in lateral areas the cell apparently has no ground contact in this region.

Discussion

In recent years cytological and biochemical investigations have dominated research involving the phenomenon of amoeboid movement. Important details, for example, the participation of plasmalemma in

cell motility, could not be elucidated by these techniques and, therefore, played a more subordinate role. In many cases this problem was subjected to various unproven theories.

Recent investigations on endocytotic activities demonstrated a connection between amoeboid movement and vesiculation mechanisms (Goldacre and Lorch 1950, Wohlfarth-Bottermann 1959 and 1960, Wolpert and O'Neill 1962, Marshall and Nachmias 1965, Wohlfarth-Bottermann and Stockem 1966, Stockem 1969). Permanent ingestion of cell membrane in the uroid region could be shown in small amoebae (*Hyalodiscus simplex*, Wohlfarth-Bottermann 1959 and 1960, Hausmann and Stockem 1972) as well as in amoebae of the *Proteus* type. Reversible processes must be postulated to balance the resulting loss of surface membrane (Stockem 1969).

Continuous uptake of plasmalemma by permanent endocytosis in *Hyalodiscus simplex* is responsible for a turnover of 100% in 7-8 h (Hausmann 1971, Hausmann et al. 1972). This turnover rate is not sufficient to cause the high renewal rate that would be necessary for Goldacre and Lorch's model.

Therefore, membrane vesiculation and turnover rate cannot be considered to play a decisive role in locomotion of *Hyalodiscus simplex* or *Amoeba proteus* (Czarska and Grębecki 1966, Haberey et al. 1969, Haberey 1970, Stockem 1972).

Marker particle behavior as shown by *Hyalodiscus simplex* in our observations disqualifies a theory implying extensive new building of the membrane. The plasmalemma is apparently a largely permanent structure and follows the movements and shape transformation of the cell. Exocytosis and endocytosis are quantitatively immeasurable within the microcinematographically recorded periods.

Even the small relative changes of marker positions on advancing pseudopodia of *Hyalodiscus* cannot be explained by rapid surface extension due to exocytosis nor by elasticity of the membrane. Those particle movements can best be explained by surface folding phenomena.

The total membrane area necessary to cover the surface of new, expanding regions of *Hyalodiscus simplex* is apparently provided by the gliding of dorsal plasmalemma in a forward direction. While this phenomenon is comparable to the gliding behavior of membrane in *Amoeba proteus* (Haberey et al. 1969, Stockem et al. 1969, Haberey 1970), differences are given by the velocity distribution. In *Hyalodiscus simplex* gliding velocity on the dorsal side is twice as high as the overall locomotion of the cell, whereas the ventral cell membrane is stationary to the ground. Intensive adhesion to the underlying substrate could be

responsible for the stationary behavior of the ventral cell membrane and would force the plasmalemma on the upper side of the cell to glide faster. Interactions between ventral cell membrane and substrate, therefore, are probably more important to the movement pattern of plasmalemma in amoeboid locomotion than cell topographical localisation of motive force generation.

Lateral lobopodial zones show no or little adhesion to the ground (compare Fig. 5). The rolling pattern of membrane movement in this region must be interpreted differently than in the central region where intensive ground contact could be demonstrated. The forces necessary to cause a gliding of membrane in lateral regions could, however, be transmitted along the membrane from central regions where the described ground-adhesion leads to a rolling movement. This passive gliding movement would provide a mechanism by which extensive deformation or shearing of the membrane could be avoided. Further preliminary results indicate that temporary changes of cell shape and alterations in the gliding velocity can be used by the cell to avoid membrane shearing. In some cases folding of the membrane can be observed, mainly in posterior regions. In accordance with results obtained in *Amoeba proteus* the folded regions can represent a membrane reservoir. In *Hyalodiscus simplex* these regions are integrated into the rolling movement but folding and unfolding as in *Amoeba proteus* could not be observed and does not appear to be necessary for the locomotion of this amoeba.

In addition regulation of surface area is brought about by periodic changes of shape in the posterior cell region in connection with a rounding up of the protoplasmic "hump".

The problem of cell topographic localisation of motive force generation and possible causal connections to the behavior of the cell membrane, endoplasmic movements, and membrane folding is still unsolved. It appears necessary first to understand most of the movement phenomena in the locomotion of *Hyalodiscus simplex* before further information about coordination and control of movement in this species can be expected.

Summary

The movement of the cell membrane during normal locomotion of *Hyalodiscus simplex* was demonstrated by marker techniques and investigated by microcinematographic single frame analysis. The cell membrane follows the forward movement of the cell by a rolling motion thereby retaining relative surface permanency. The results were compared with conditions found in *Proteus* amoebae.

ZUSAMMENFASSUNG

Das Bewegungsverhalten der Zellmembran von *Hyalodiscus simplex* während der normalen Lokomotion wurde mit Hilfe von Partikelmarkierungen mikrokinematographisch untersucht. Durch Einzelbildanalyse konnte eine weitgehende Permanenz der Zellmembran nachgewiesen werden. Das Plasmalemm folgt der Vorbewegung der Zelle nach Art einer Rollbewegung. Diese Ergebnisse wurden mit den bei Proteus-Amöben gefundenen Verhältnissen verglichen.

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EXPLANATION OF PLATE I

Motion picture sequences of normal movement by *Hyalodiscus simplex*
1-3: Particle moving on dorsal surface, 3 — turning to lower surface,
4-6: stationary behavior between substrate and lower surface
eh — endoplasmic hump, hl — hyaloplasmic lobopodium, n — nucleus, pv — pulsating vacuole, ○ — stationary bacterium as reference marker on the substrate, ＞ — plasmalemma marker particle. Interval 8 sec, magn. 950×

PLATE I
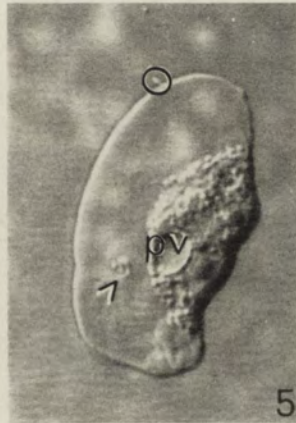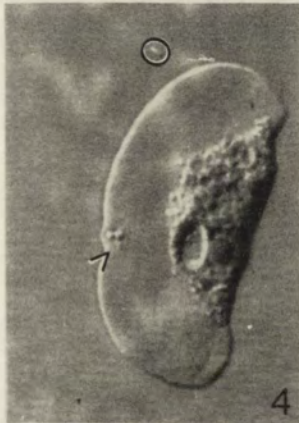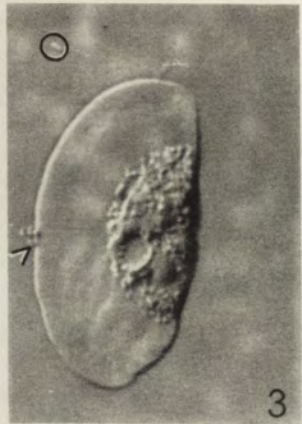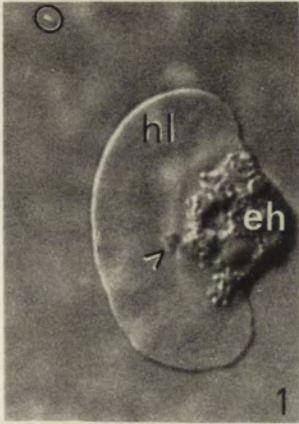
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G. I. P o l j a n s k y and I. S. I r l i n a: On the variability in the norm of reaction of *Paramecium caudatum* to various cultivation temperature. [Об изменчивости] нормы реакции *Paramecium caudatum* на разные температуры культивирования J. J. P a u l i n and S. K r a s c h e n n i n i k o w: An electron microscopic study of *Balantidium caviae* [L'etude au microscope electronique de *Balantidium caviae*] — S. R a d z i k o w s k i: Elimination of chromatin from the micronucleus of *Chilodonella cucullulus* (O.F.M.) during the 1st meiotic division [Eliminacja chromatyny podczas pierwszego podziału mejotycznego z mikronukleusa *Chilodonella cucullulus* (O.F.M.)] — K. K. M i s r a and R. F. N i g r e l l i: On *Babesiosoma*; *Haemosporidia* of cold-blooded vertebrates. [Über *Babesiosoma*; *Haemosporidia* kaltblüting wirbeltiere] — V. S. K a l n e y, B. N. K u d r y a v t s e v and M. V. K u d r y a v t s e v a: Relation between radiosensitivity and DNA content in nuclei of various *Amoeba proteus* strains [Соотношение между радиочувствительностью и содержанием ДНК в ядре у разных штаммов *Amoeba proteus*] — M. S h a d a k s h a r a s - w a m y and P. S. J o y t h y: Effect of ph on *Blepharisma* intermedium 1. Changes in fission rate and oxygen consumption. [Der Einfluss des pH auf *Blepharisma* intermedium 1. Die Änderungen im Teilungstempo und der Suerstoffverbrauch] — T. R. I s q u i t h and A. B o b r o w: The effect of high intensity magnetic field on the entrance of various molecules into *Paramecium caudatum*. [Der Einfluss eines magnetischen Feldes hoher Intensität auf den Eintritt verschiedner Moleküle in das *Paramecium caudatum*]

Fasciculi: