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Krystyna GOLIŃSKA and Jacques BOHATIER

Action of Actinomycin D Upon Regenerative and Divisional Stomatogenesis in *Dileptus*[1]

Synopsis. Action of actinomycin D upon stomatogeneses of *Dileptus cygnus* and *Dileptus anser* was observed. It was found, that the regenerative stomatogenesis is resistant, while the divisional stomatogenesis is sensitive to AMD action. Resorption of divisional primordia is possible up to a very advanced stage, resorption of regenerative primordia was never observed. Possible explanations for this difference between regenerative and divisional oral primordia when affected by AMD, are discussed.

Introduction

The study presented here deals with two questions: the first comprises a comparison between regenerative stomatogenesis of fragments treated with actinomycin D and stomatogenesis of anucleate fragments. The second question concerns the differences between regenerative and divisional morphogenesis when both are affected by actinomycin D.

Among Ciliata the incapability of anucleate fragments to regenerate the oral apparatus is a common phenomenon (review Balamuth 1940). The only known exceptions are *Tracheloraphis* sp. (Torch 1962) and *Dileptus cygnus* (Golińska 1966). Torch (1964) observed in *Tracheloraphis* that regenerative stomatogenesis is resistant to actinomycin D treatment, with six successive regenerations of the oral apparatus possible in the presence of the inhibitor. It seems useful to examine whether in *Dileptus* also the independence of regenerative stomatogenesis from the nuclear apparatus is correlated with resistance to the action of actinomycin D.

The inhibiting action of AMD upon the development of oral primordia has been observed in many ciliates, and includes primordia forming in many developmental situations such as: division, regeneration, oral replacement and conjugation. The inhibition of divisional primordia development in *Paramecium* was described by Gill and Hanson (1964, 1968), Kaczanowska et al. (1974), in *Tetrahymena pyriformis* by Whitson and Padilla (1964), Frankel

(1965). Actinomycin D prevents the formation of divisional primordia in *Tetrahymena patula* (Gabe and de Bault 1973) and in *Stentor* (Ellwood and Cowden 1966, James 1967). Also, regenerative primordia are inhibited by AMD in *Stentor* (Whitson 1965, Ellwood and Cowden 1966, James 1967, Burchill 1968, Pelvat et al. 1973). Giese (1970) observed in *Blepharisma* the delay of development of the regenerative primordia after exposure to the drug. Oral replacement is inhibited in *Tetrahymena* (Frankel 1970). Sapra and Ammermann (1973, 1974), observed that during conjugation of *Stylonychia* the administration of AMD in sufficiently early stages of conjugation prevents the development of Ma-anlagen as well as formation of cortical primordia during reorganization.

The existence of differences in sensitivity to AMD between divisional and regenerative stomatogenesis is a new fact observed in this study. Fragments of *Dileptus* exposed to AMD form oral primordia several times as do fragments of *Tracheloraphis* (Torch 1964). Divisional primordia of *Dileptus*, however, are very sensitive to action of the drug. It is easy to prevent their formation, to arrest their development, and cause resorption. The possible causes of such a considerable difference in the properties of the oral primordia formed during division and regeneration are discussed after the presentation of results. It is supposed that the sensitivity to the AMD of different kind of oral primordia is related to the position of the primordia within the cell.

Material and Methods

The ciliates used in this investigation were two species of *Dileptus*: *D. cygnus* and *D. anser*. The culture methods were the same for both species. The culture medium is a boiled source water conveyed from the environs of Warsaw. *Colpidium colpoda*, cultivated separately on egg yolk suspension, was used as a food. The cultures of *Dileptus* were fed every other day.

The predividers and dividers were selected from culture dishes 24 h after feeding. Only so-called regular predividers and regular dividers were used in experiments, that is cells which could divide once without supplying food (Golińska and Jerka-Dziadosz 1973). Predividing individuals are considered cells of similar size as early dividers sampled from the culture dishes in the same time. After isolation a majority of the cells divide (Fig. 1 and 2, measurements in 0% of AMD).

Observations were performed on groups of individuals (usually containing 10 specimens) kept in single glass depressions in an amount of fluid not less than 0.5 ml. The operations were carried out by hand under the stereoscopic microscope PZO (100 ×), using a sharp microscalpel. Usually, transections in the middle of the cell were performed, or in the anterior 1/3 level. The formation of oral primordia was followed under the Ergaval (Zeiss) microscope. Anucleate fragments were cut off from the cells of *D. cygnus*, in which the position of nuclear apparatus is readily visible under the stereoscopic microscope. These operations were performed in culture dishes, the anucleate fragments were then transferred to single-depression slides containing 0.5 ml of culture medium.

A stock solution of 1000 μg/ml of Actinomycin D (Serva, Feinbiochemica, Heidelberg)

was prepared freshly every 3–4 days, using distilled water as a solvent. The experimental concentrations were prepared by adding culture medium to the stock solution. All experiments involved continuous exposure to AMD, without washing out the drug. Each experimental sample with AMD was matched with a control sample without the drug. The cells were usually observed for 24 h, but in several cases the time was prolonged to 48 h. The following concentrations of inhibitor were used: 10, 25, 100, 200, 500 and 750 µg/ml. The concentration of 1000 µg/ml was considered as lethal, because 100% of cells deteriorated during 24 h when exposed to it.

Results

Regenerative and divisional primordia are formed in the same manner and rate in both species of *Dileptus* (Golińska (1966 and this study). The posterior fragment completes all parts of the oral apparatus within 2 h after the operation. Three hours after the operation the mouth was observed to function. During division the oral primordium forms a complete ring in the equatorial region of the cell 2 h after the first sign of divisional primordium formation (Golińska 1972). Further development of the divisional primordium proceeds together with constriction of the division furrow, and lasts about 1 h from the stage of a full ring until the separation of the offspring.

Effects of AMD on the Regenerative Stomatogenesis

Preliminary experiments have revealed that AMD applied during regenerative stomatogenesis exerts no effect on the course of stomatogenesis. The inhibitor was thus introduced before the operation and cells were operated in the medium containing AMD. The results of observations on regenerative stomatogenesis are summarized in Table. 1. Fragments of *D. cygnus* were mainly used, since in this species the ability of anucleate fragments to regenerate the oral apparatus was previously described (Golińska 1966, Golińska and Grain 1969). Inhibition of stomatogenesis was observed only in fragments of cells which were exposed to 750 µg/ml of AMD for 19 h prior to the operation. Such cells showed considerable changes in body shape, were rounded up, had shortened proboscis and tails. Ten cells were operated — lost of a large amount of cytoplasm was observed during the operation. All fragments died during 3–4 h after the operation, showing no sign of regeneration. Fragments obtained from cells exposed for only 3 h to the same concentration of inhibitor were able to form oral primordia, but the regenerated proboscis was very short and thick and did not function, although the cytostome surrounded by the peristomial lip was readily visible (Pl. I 3). Ten of the regenerated fragments were left in the inhibitor for 24 h. The fragments degenerated and deteriorated by the end of this period, nonetheless the oral apparatuses were still visible and were not resorbed. A concentration of 750 µg/ml of AMD was also administered just

Table 1

Effects of Actinomycin D on Regenerative Stomatogenesis

Concentrations of AMD in µg/ml	Cell Number	Pretreatment Period Prior to Operation	Cell Shape	Proboscis		Mouth	
				long	short	functional	non functional
	Dileptus cygnus						
750	10	19 h	swollen		×		×
750	10	3 h	normal	×		×	
750	10	0 h	normal	×		×	
500	40	24 h	swollen		×		×
500	12	0 h	normal	×		×	
250	50	24 h	swollen		×		×
250	50	2–4 h	normal	×		×	
250	25	0 h	normal	×		×	
150	80	24 h	swollen		×		×
150	50	2–4 h	normal	×		×	
	Dileptus anser						
125	60	24 h	swollen		×		×
25	30	24 h	normal	×		×	

before the operation. In 10 observed cells regeneration proceeded normally and both the proboscis and cytostomal parts did not differ from those in control fragments (Pl. I 2). It seems that the inhibition of regenerative stomatogenesis obtained after 24 h exposure to 750 µg/ml of AMD could be attributed to an unspecific poisoning of the cell rather than to the action of AMD as a inhibitor of RNA syntheses.

The effects of lower concentrations of AMD on regenerative stomatogenesis were next studied. The following concentrations were used: 500, 250, and 150 µg/ml. The regeneration of oral parts was not affected by any of these concentrations. The cells survived in all concentrations for over 24 h. In 500 µg/ml they died before 48 h, while in lower concentrations they survived up to 48 h.

Cells were operated after 24 h exposure to the drug and regeneration was observed in 40 fragments at a concentration of 500 µg/ml, 50 fragments at 250 µg/ml, and 80 fragments at 150 µg/ml of AMD. All fragments of this series regenerated complete but non-functioning oral apparatuses. Killing and swallowing of the prey was never observed. The regenerated oral parts posessed a considerably shorter proboscis compared to controls. This shortening of the proboscis seems to be related to the duration of exposure rather than to the concentration of the inhibitor, and is connected with the state of the cell at the time of an operation. The regenerated proboscis is always very short when the cell was swollen before the opera-

tion and the extent of swelling was different in several samples exposed to the same concentration.

Regeneration after 2–4 h incubation in AMD was observed in 50 fragments at 250 μg/ml and 50 fragments at 150 μg/ml. All formed oral apparatuses, but only several functioned normally. In each sample of 10 cells only one or two cells posessed a normally functioning mouth. The rest were not able to kill and swallow prey. The shortening of the proboscis was observed only in some samples and was less conspicious than in the previous series.

Exposure to AMD just before the operation did not prevent the formation of a functional mouth with a proboscis of normal length, both at concentrations of 500 μg/ml (12 cells) and 250 μg/ml (25 cells).

Concentrations of AMD from 500 to 150 μg/ml thus do not cause an inhibition of regenerative stomatogenesis even after long-lasting exposure. A delay of regeneration or slowing down the rate of regeneration was never observed. The resorption of formed primordia was not found, even in fragments conspicuously affected by the drug. We did not observed an arrest of regeneration at any specific stage, unless one considers the time of functional maturation of the oral apparatus as such.

A similar effect of AMD upon the course of regenerative stomatogenesis was also observed in *Dileptus anser*. Sublethal concentrations of the inhibitor caused the formation of a shortened proboscis and lack of function of the oral apparatus. 60 specimens operated 24 h after exposure to 125 μg/ml regenerated a mouth that was unable to feed, with a short and thick proboscis. Only after the diminution of the concentration of the inhibitor to 25 μg/ml did the 24 h long exposure not prevent the formation of normal oral apparatus (observation made on 30 fragments).

Repetitive regeneration in the presence of AMD was observed in 50 fragments of *Dileptus cygnus*. The concentration of inhibitor was 125 μg/ml. The first operation was performed just after the administration of the drug, the second after 2 h, third − 5 h after adding of AMD. The first two stomatogeneses proceeded normally, while during the third one fragments formed shortened proboscis. Forty of control fragments formed normal oral parts after 5 succesive operations at 2–3 h intervals, till the fragments were too small to carry on the operation.

A short series of observations was carried out on anucleate fragments of *Dileptus cygnus*. In an earlier study (Golińska 1966) the fragments were observed in very small drops covered by fluid mineral oil. In this condition the lifetime of fragments was very short − a drop of the survival curve took place already after 2 h and the maximal length of life was 8 h. The observations on the lifetime of anucleate fragments were repeated in this study. The fragments were kept in depression slides containing 0.5 ml

of culture medium, in the same conditions as AMD-treated fragments. The anucleate fragments (36 fragments) survived in depression slides from 3 h (the shortest time) to 19 h (the longest time). That means that the fragments lacking the nuclear apparatus live for a considerably shorter time than the fragments maintained in sublethal concentrations of AMD. The lack of function of the regenerated mouth in anucleate fragments has been also confirmed.

Action of Actinomycin D on the Divisional Stomatogenesis

Oral primordia formed during division, unlike those formed during regeneration, are very sensitive to the action of AMD. The presence of the inhibitor can prevent the formation of primordia and also can arrest the development of primordia already present and cause their resorption and the cessation of the divisional process.

The specimens studied at a known stage of the division process were isolated into a known concentration of the inhibitor. The number of divisions achieved was calculated from the number of specimens in a given sample after a 24 h exposure to the drug.

The first series of experiments dealt with the difference in sensitivity to the drug between predividers and dividers. This series of experiments was performed using *D. anser*, for this species is less sensitive to handling and the predividers are easier to distinguish in mass culture than are predividers of *D. cygnus*. Results of experiments are presented in Fig. 1. The data
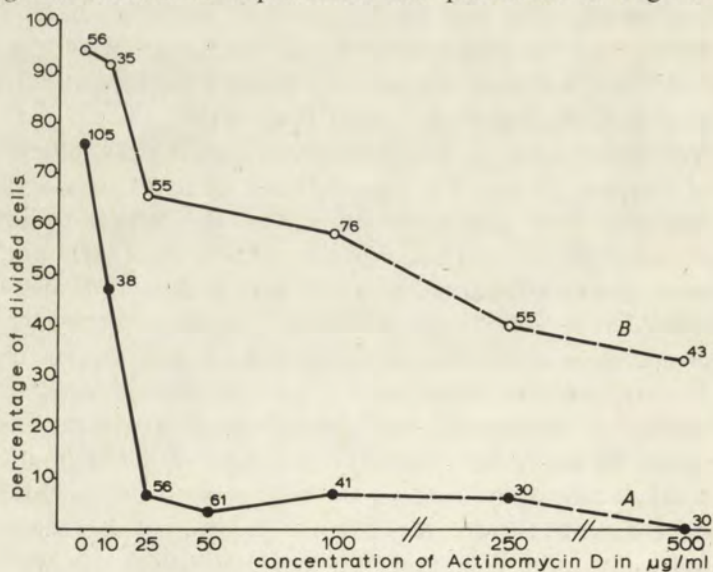

Fig. 1. The action of different concentrations of AMD upon predividers (*A*) and dividers (*B*) of *Dileptus anser*. The number adjoining each curve represent the number of animals treated in a given concentration

show that the predividing individuals are particularly sensitive to the inhibitor. Even relatively small concentrations (25 μg/ml) prevented division in over 90% of cells, and in 500 μg/ml division is arrested in 100%. For the majority of predividers the inhibiting effect of AMD is revealed by failure of formation of oral primordia. Some predividers could, however, form oral primordia during the first two hours of exposure to the inhibitor. Not all of these primordia develop further — resorption of primordia starts in the stage of early dorsal primordium.

The dividers of *D. anser* were isolated into the inhibitor at different stages of oral development, but all before the beginning of formation of the division furrow. In this series a concentration of 500 μg/ml prevents 65% of individuals from completing division (Fig. 1, *B*). In the others oral apparatus of the separated opisthe did not differ in size and length of the proboscis from the mouth of the control cells.

The aim of the next series of experiments was to study more closely the relationship between the extent of developmental advancement and the sensitivity of stomatogenesis to the presence of inhibitor. The experiments were performed using *D. cygnus* because this species posesses a moniliform macronucleus, which condenses into one rounded mass during division (Golińska 1965). The oral primordia begin to form before Ma condensation, and the circumferential primordial ring is fully formed when the Ma is condensed. The differentiation of primordia proceeds together with the development of divisional furrow and the elongation of Ma.

Two categories of dividing individuals were distinguished: early dividers with moniliform Ma, and late dividers with the Ma condensed. The results of observations on the effect of different concentrations of AMD on both kinds of dividers are presented on Fig. 2. There are conspicuous differences in the sensitivity of early and late dividers to the action of the inhibitor and of handling itself (Fig. 2, curve *A*, *B*, and the control in 0% of inhibitor). As the divisional processes advances the percentage of cells with inhibited division decreases. We did not find, however, a stage of development of primordia that is resistant to the action of AMD. Division was inhibited in 40% of late dividers treated with 500 μg/ml of AMD. The time between condensation of Ma until the separation of daugther cells is only one hour. If one presumes that there is certain amount of time necessary for the inhibitor to penetrate inside the cell then it can be inferred that very late stages of division can be inhibited. This situation changes rapidly after the separation of the offspring. The oral apparatus of the posterior daughter becomes immediately resistant to the action of inhibitor and does not undergo resorption. The size of oral apparatus and the length of proboscis of cells dividing in the presence of AMD does not differ from that of control cells.
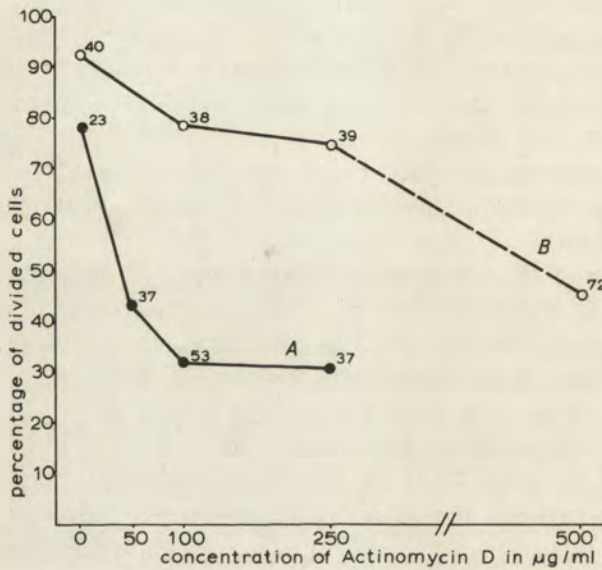
Fig. 2. The action of different concentrations of AMD on the early (*A*) and late (*B*) dividers of *Dileptus cygnus*. The number adjoining each curve represent the number of animals tested in a given concentration

The inhibition of divisional primordia is always expressed in the same manner: the primordia are arrested in development and after some time become resorbed. The arrest of development is possible in each stage of divisional stomatogenesis. The time lasting from the halting of development of primordia until their resorption was not measured, and it is not known to what extent this time is related to the advancement of primordium development at the moment of inhibition. In any case, even very advanced primordia are completely resorbed after 24 h. The inhibition of the development of divisional primordia is closely connected with the inhibition of development of the fission furrow. These two processes, cytokinesis and stomatogenesis, which in *Dileptus* seems to constitute an inseparable integrity in a morphological respect (Golińska 1972), cannot be separated by treatment with AMD. Inhibited dividers of *Dileptus* always returned to a single normal form.

In the next series of experiments we compared the action of AMD upon divisional and regenerative stomatogenesis when both processes take place in the same specimen. In a previous study (Golińska 1966) it was observed, that dividers of *D. cygnus* can regenerate new oral parts after removal of the proter mouth independently of the simultanously occurring formation of the opisthe oral primordia. The two stomatogeneses proceed each in its own rate — there is no synchronization of their development.

In this experiment, the operated dividers were exposed to different concentrations of AMD. The results are summarized on Fig. 3.
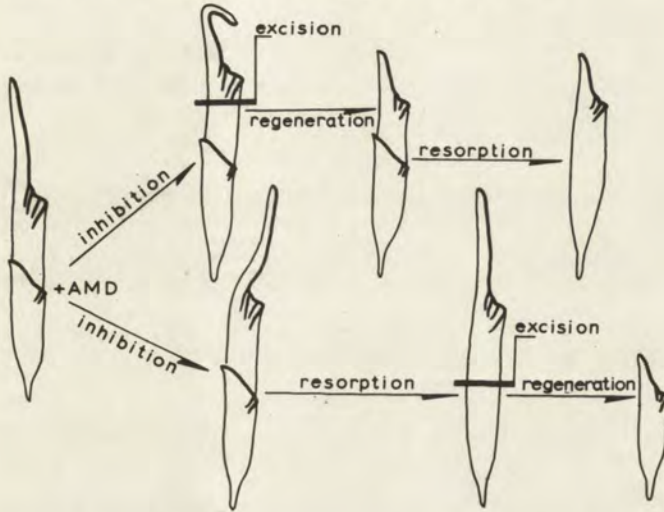

Fig. 3. Regeneration abilities of dividers inhibited by AMD. Upper row: the regeneration of the mouth in cells with arrested divisional primordia. Lower row: the regeneration of mouth in cells with resorbed divisional primordia

Dividers with visible primordia were isolated to the drug and transected in the middle of proter, so the whole oral apparatus was removed together with the nemadesms (Fig. 3, top). Eleven late dividers of *D. cygnus* were operated immediately after isolation into 200 μg/ml of AMD. All regenerated the proter's mouth in a time normal for regeneration, but only four completed division. Seven individuals showed an arrest of divisional stomatogenesis and the opisthe primordia were resorbed.

Sixteen dividers of *D. cygnus* were operated 3 h after incubation in 100 μg/ml of AMD. They probably were dividers with arrested development, with the primordia still readily visible. All operated cells regenerated the anterior mouth in normal time, but none have divided. It is difficult to determine whether development of anterior primordia and resorption of posterior primordia proceed simultanously, nonetheless posterior primordia were still visible after completion of the anterior mouth.

Twenty three dividers of *D. anser* were operated after 3 h exposure to 250 μg/ml of AMD. All regenerated the anterior mouth, and none divided during the next 24 h. The divisional primordia of all cells were resorbed after the formation of regeneration primordia.

In the last series of experiments the dividers with resorbed divisional primordia were transected in the equatorial region of the body, at the place where the primordia had been (Fig. 3, bottom). Fourteen specimens

of *D. anser* were operated after 20 h incubation in 250 µg/ml of AMD. All posterior fragments regenerated the oral parts in the normal time. In these regenerated cells the oral apparatus did not function and the proboscis was shorter than in the control.

Twenty two predividers of *D. anser* were isolated to 100 µg/ml of AMD. During two hours after isolation the divisional primordia were observed in 6 cells. After 18 h 2 cells divided, the rest did not show any sign of divisional primordia. Then all cells were transected in the middle of the body and the posterrior fragments all regenerated a new mouth in the normal time, although, as usually, after long action of the drug the newly formed proboscis was considerably shorter than in normal cells.

Discussion

Comparison of the lifetime of fragments of *Dileptus* cells treated with AMD with anucleate fragments shows that the anucleate fragments survived for a shorter time. It can be presumed that AMD in the concentration used does not completely block the nuclear syntheses necessary for the maintenance of metabolic processes in nucleate fragments, while in anucleate fragments these processes are naturally excluded. It should be stressed out, that the morphogenetical capabilities of fragments treated with AMD are very similar to those of anucleate fragments. Both kinds of fragments can form a complete oral apparatus, although the regenerated oral apparatus does not function when formed by anucleate fragment, and does not function after long pretreatment in AMD when formed by nucleate fragments.

The study on the ultrastructure of the oral apparatus formed by anucleate fragments (Golińska and Grain 1969) revealed that all basic structural elements, such as feeding cilia, nemadesms of both inner and outer baskets of the cytopharynx, the ventral fiber of proboscis etc. are present. Further ultrastructural study on possible deficiences in the oral structures regenerated by the fragments treated with AMD are planned, in order to determine the reasons for the lack of function of the mouth.

Torch (1964) postulated the existence, in *Tracheloraphis* sp. of a cyto-plasmic reserve of long-lasting RNA, that makes regeneration insensitive to AMD, and anucleate fragments able to regenerate. Morphogenetical abilities of anucleate fragments of *Dileptus* indicate an existence of cytoplasmic pool of oral proteins, over the whole lifetime. Utilization of this pool may be affected by the action of puromycin (Golińska 1974), but is not disturbed by actinomycin D. The idea, that utilization of oral protein pool simply does not lay in the path of AMD action, is consistent only with the fact that the regenerative primordia are insensitive to the inhibitor. The same

pool should have assured the formation and development of the divisional primordia as well as the regenerative ones. But a specimen, which is incapable of forming divisional primordium, or such which have resorbed its divisional primordium, is still able to form regenerative primordia — even in the same place where the divisional primordia should have been.

While regenerative stomatogenesis occurs despite the prolonged action of high concentrations of inhibitor, the divisional stomatogenesis is inhibited by low concentrations of AMD acting for a short period of time. The study on the action of different inhibitors upon the division of *Tetrahymena* (Frankel 1962, 1965) lead to distinction of transition point characterizing the stomatogenesis. Let us try to find this point in the course of the stomatogenesis of *Dileptus*. The term "transition point" will be used here as "The time at which 50% of the cells in the population have become insensitive" (Frankel 1962, p. 15). Transition point can be easily found in divisional stomatogenesis of *Dileptus* (Fig. 4). Its location in the course
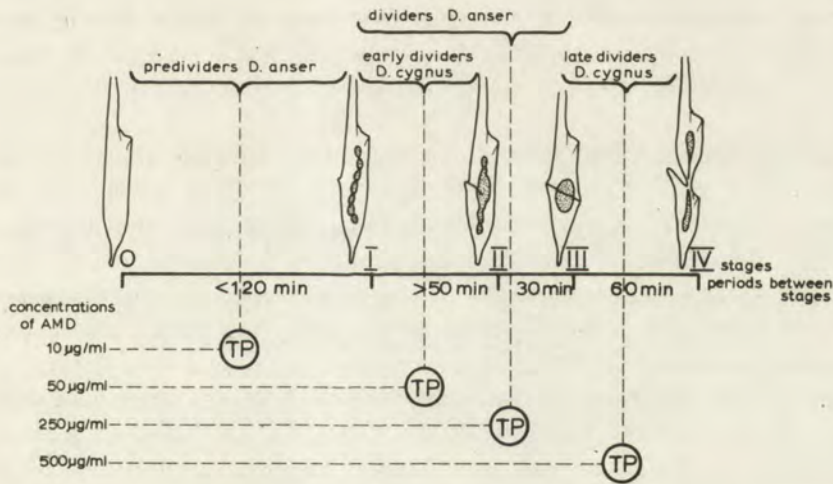

Fig. 4. Transition point (TP) in divisional stomatogenesis of *Dileptus* after treatment with different concentrations of AMD

of stomatogenesis depends upon the concentration of AMD. On the contrary, during regenerative stomatogenesis no transition point can be found. It seems, that in *Dileptus* transition points for AMD action concern some other divisional processes than the stomatogenesis. It should be recalled here, that regenerative stomatogenesis of other ciliates, like *Stentor*, being sensitive to the action of AMD (Whitson 1965, Elwood and Cowden 1966, James 1967, Burchill 1968, Pelvat et al. 1973) posess a transition point.

Another attribute found in the development of oral structures is that, that during stomatogenesis in single cells, when the primordium development

passes through a certain advanced stage it become insensitive to the action
of any drug, and can no longer be resorbed. This point in development,
called by Frankel 1962 "the stabilization point" exists not only in divisio-
nal stomatogenesis of *Tetrahymena*, but also was found to occur at the
same stage (stage 4) of development during divisional and regenerative
stomatogenesis in *Stentor* (de Terra 1960, Ellwood and Cowden 1966,
James 1967, Burchill 1968, Pelvat et al. 1973). In the divisional stoma-
togenesis of *Dileptus* we found no stabilization point. Development of oral
structures may be blocked in very advanced stages, in *D. cygnus* less than
1 h before separation of the offspring. If it exists before the separation of
the offspring, then it is so close to the moment of separation that the lack
of inhibition of primordia development could be attributed to the lack of
sufficient time for the inhibitor to penetrate inside the cell. In the regenerative
stomatogenesis of *Dileptus* primordia seem to be stabilized from the very
beginning of development.

Several possibilities were considered to explain the difference between
reaction of regenerative and divisional primordia to AMD. It was assumed,
that the same material, stored in the cytoplasm, that is utilized for regenera-
tive stomatogenesis, is also used in the course of formation of the oral struc-
tures during division.

The differences found in AMD action upon different stages of the cell
cycle (Child 1965, Pederson and Robbins 1972) or upon cells starved
and not starved (Frazier 1973) — also can not explain the differences in
reaction of regenerative and divisional primodia of *Dileptus* to the presence
of AMD, because the same cells at the same time can form regenerative
primordia, yet cannot form divisional primordia and often resorb existing
divisional primordia.

Another possibility exists that some divisional process other than stomato-
genesis is inhibited by AMD, while the primordia are resorbed secondarily.
This, however, implies a local resorption of divisional primordia. Otherwise,
if there exists a state of resorption of primordia overall the cell — then
the primordia of every kind should be resorbed — which is not true.
A possibility that divisional primordia are locally resorbed because fission
furrow development is inhibited by AMD cannot be ruled out, since the
divisional primordia and fission furrow are morphologically united (Go-
lińska 1972).

Another possible explanation considered is a difference in the ability of
cells to resorb regenerative and divisional primordia. Resorption of regenerative
primordia was never observed: neither in anucleate fragments, in fragments
treated with puromycin (Golińska 1974), in operated regenerants (Golińska
unpublished) nor in fragments treated with AMD. On the contrary, resorption
of divisional primordia was observed not only after treatment with actino-

mycin D, but also after microsurgical operations of dividers (Golińska and Jerka-Dziadosz 1973), and owing to mechanical stimulus caused by handling.

The lack of resorption of regenerative primordia could be a common phenomenon for all Ciliata with apically located oral apparatus, and the development of an apical mouth could be to a great extent independent of an immediate support by the nuclear apparatus. The divisional primordia of *Dileptus* are formed circumferentially in the equatorial region of the cell, that means — not apically. It is possible, that it is not the formation of structures themselves that is so easily disturbed in development of divisional primordia, but the process of localization and maintenance necessary just because the primordia are not apically located. This hypothesis is based on the supposition that apical location of primordia, being most primitive, does not to be assigned by the cell. After transection the anterior pole becomes the most natural place for the primordium to develop.

The positioning of primordia has been distinguished recently as a separate process in development of *Hypotricha* and other ciliates with primordia located ventrally (Frankel 1974, Jerka-Dziadosz 1974, Kaczanowska 1974). So far a very little is known as to the dependance of the positioning on the nuclear apparatus. It is well known that the development of oral primordia in *Tetrahymena* requires a material of nuclear origin untill the stabilization point (Frankel 1965, 1967 a, b). In *Stentor* the process of oral development is related to the state of morphogenetical activation (Tartar 1967) which can be achieved by the cell only in the presence of nuclear apparatus (Tartar 1968). In *Dileptus*, though, a formation of oral structures, together with the processes of differentiation and arrangement of the structures into the oral apparatus, can proceed on the apex of the cell in the absence of nuclear apparatus. It seems possible that the development of apically situated primordia (regenerative ones) do not require neither positioning nor active maintenance of development, and equatorially oriented primordia (divisional) require materials of nuclear origin for the positioning and for active maintenance of their development. This supposition will be tested in further studies.

RÉSUMÉ

Pour deux especes de *Dileptus*, *D. anser* et *D. cygnus*, les effets de l'Actinomycine D sur la formation du primordium de régénération chez des fragments posterieurs, et sur la formation du primordium chez des individus en prèdivision et en division, ont été ètudiés en exposant les cellules on maniere continue à des concentrations variables de l'antibiotique (25 à 750 μg/ml).

On demontre, que le primordium de régénération se developpe normalement, sans retard,

en preşence de l'inhibiteur. Le complexe buccal régénéré est complet. On n'observe jamais de resorption du primordium de régénération.

Par contre, chez des *D. anser* en prèdivision, l'AMD empeche la formation du primordium; chez des cellules en division (*D. anser* et *D. cygnus*), le developpement du primordium est arrété, et il y a resorption des èbauches. Les jeunes primordiums sont plus sensibles à l'antibiotique que les plus èvolués. Cependant, nous n'avons pas pu definir de stade resistant.

Les cellules dont la division a été inhibée, ou stoppée, sont capables de former un primordium de régénération, apres ablation de leur complexe buccal, même á l'emplacement où le primordium de division n'a pu se developper.

Il existe donc une difference quant à l'exigeances morphogenetiques parmi les primordium de régénération et primordium de division. Les primordiums de régénération, toujours situés en position apicale, ne peuvent pas être resorbés, et sont insensibles à l'action de l'AMD. Les primordiums de division, situés dans la region equatoriale de la cellule, sont sensibles à l'AMD et peuvent être resorbés. La position d'un primordium semble donc être particulierment importante en ce que concerne les facteurs qui règlent son developpment.

D'autres facteurs considérés comme importantes pour ce developpment particulière chez *Dileptus*, sont egalement discutés.

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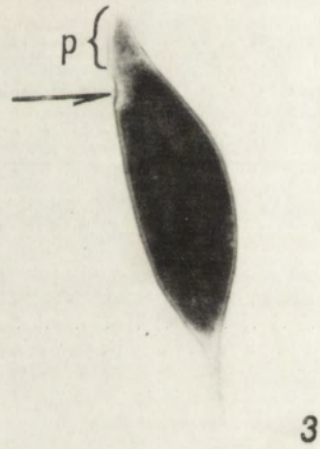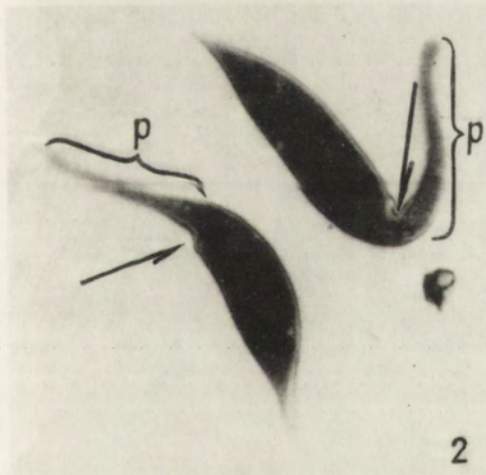Photographs taken from living cells. Magnification × 300
Arrows point to the cytostome, p − proboscis
1: Normal individual of *Dileptus anser*
2: Posterior fragments of *D. anser* 4 h after the operation
3: Posterior fragment of *D. anser* 4 h after the operation
Incubated for 24 h in 150 µg/ml of AMD before the operation

K. Golińska et J. Bohatier

auctores phot.

I. B. RAIKOV, Z. P. GERASSIMOVA-MATVEJEVA and P. DE PUYTORAC

Cytoplasmic Fine Structure of the Marine Psammobiotic Ciliate *Tracheloraphis dogieli* Raikov. I. Somatic Infraciliature and Cortical Organelles

Synopsis. Longitudinal somatic kineties of *Tracheloraphis dogieli* consist of paired, kinetosomes of which both are ciliated. A microfibrillar ribbon and 5–6 sheets of subkinetal microtubules lie beneath the kinetosome bases. Strong postcilliary ribbons of microtubules extend backwards along 12 to 24 kinetosome pairs and are stacked forming a thick laminated bundle. The kinetodesmal and the transverse fibrillar derivatives of a kinetosome pair are weakly developed. Strong myonemes, lying on the left of each kinety, are surrounded with perimyary vesicles which contact both the plasma membrane and the mitochondria. Each kinety is accompanied by dictyosomes. Cortical pigment granules and rod-like rhabdocysts are described.

Introduction

Electron microscopic studies of the cortex of various ciliates, steadily increasing in number, make more and more clear that large taxa of the ciliates have their own relatively stable patterns of organization of the ectoplasmic fibrillar system related to kinetosomes (Seravin and Matvejeva 1972). These patterns seem to be rather conservative and this might be used to clarify the phylogenetic relationships between various systematic groups of *Ciliata*.

The cortical ultrastructures of the lower ciliates — representatives of the order *Gymnostomatida* which occupies a key position in the entire class *Ciliata*, are still relatively little known. Of particular interest are the fibrillar systems of gymnostomatids belonging to the species-rich family *Trachelocercidae*: large, elongated or vermiform, strongly contractile marine ciliates which seem to be primitive in what concerns their nuclear apparatus consisting of numerous micronuclei and diploid macronuclei (for review see Raikov 1969). This family includes three genera, *Trachelocerca*, *Tracheloraphis*, and *Trachelonema*. Its members are highly peculiar to the biotope of marine mesopsammon (Dragesco 1960). Only fragmentary data (without published micrographs) exist in the literature for the cortical ultrastructures of *Tracheloraphis caudatus* (Raikov et Dragesco 1969) and *Tracheloraphis* sp. (Pitelka et al. 1971). The only detailed study of the ectoplasmic fine

structure of a Trachelocercid is available for *Trachelonema sulcata* (Ko-valeva 1974).

The present paper deals with *Tracheloraphis dogieli* Raikov, 1957, one of the largest forms among the *Trachelocercidae*, up to 2 mm in length. The morphology of this species has been described by Raikov (1957, 1962) and Dragesco (1963). Results of an electron microscopic study of its nuclear apparatus have been published elsewhere (Raikov 1972, 1973).

Material and Methods

This work has been performed using specimens of *T. dogieli* collected by one of us (I. B. Raikov) near the Roscoff Biological Station in Brittany (France) in November 1970, as well as those collected by Z. P. Gerassimova-Matvejeva and V. G. Kovaleva at the Barentz sea (near the Murman Marine Biological Institute) during summer 1971. The following fixation methods were used:

(1) Glutaraldehyde (6%) in 0.1 M cacodylate buffer (pH 7.4) containing 10% saccharose and 0.03% calcium chloride, 40 min on ice; a bath of cold buffer containing 30% saccharose; postfixation with 2% osmium tetroxyde in cacodylate buffer containing 27% saccharose (30 min on ice); four baths of buffer containing decreasing concentrations of saccharose. Used for material from Roscoff and partly for that from the Barentz sea.

(2) Osmium tetroxyde (2%) in 0.1 M cacodylate buffer (pH 7.4), containing 27% saccharose and 0.03% calcium chloride, 40 min on ice, followed by four baths of buffer with decreasing saccharose concentrations (part of the material from the Barentz sea).

(3) Osmium tetroxyde (2%) in 0.1 M phosphate buffer (pH 7.4), containing 14% NaCl (osmolarity of the fixative, 1090 milliosmoles), 20 min. on ice, followed by two baths of the same buffer but without NaCl (Barentz sea material only).

After fixation, the ciliates were pre-embedded into agar gel according to the method routinely used at the Zoology Department, Clermont-Ferrand University. For this, they were transferred with a minimum of water onto a gelatinized layer of agar on a slide. After withdrawing almost all water, the ciliates were moved on the agar surface to collect them into compact groups. A drop of melted agar was then carefully added onto each group. After gelatinization, small blocks, each containing a group of ciliates, were cut out of the agar "sandwich" and put into 70% alcohol. Thereafter, they were dehydrated and embedded into epon or araldite.

Sections, mounted on naked grids, were coloured 2–3 hours with saturated aqueous uranyl acetate and 5–15 min with Reynolds' lead citrate. After carbon coating, the sections were inspected with JEM-7 (at the Institute of Cytology in Leningrad), Elmiskop I (at the Zoology Department, Clermont-Ferrand University), and Hitachi HU 11 E (at the Leningrad University) electron microscopes.

Observations

Somatic ciliature

The body of *Tracheloraphis dogieli* is vermiform, somewhat flattened dorso-ventrally, strongly contractile, and has a characteristic brown pigmen-

tation. Its anterior end forms a "neck" bearing a terminal cytostome. The somatic ciliature consists of 32 to 60 longitudinal kineties, their number being higher in Barentz sea specimens (45–60, Raikov 1962) than in Roscoff specimens (32–46, Dragesco 1963). In the middle part of the body, the kineties are more numerous than near its ends, due to delineation of some kineties. A non-ciliated ("glabrous") stripe, some 8 kineties wide, extends along the median line of the dorsal body side (Fig. 1).
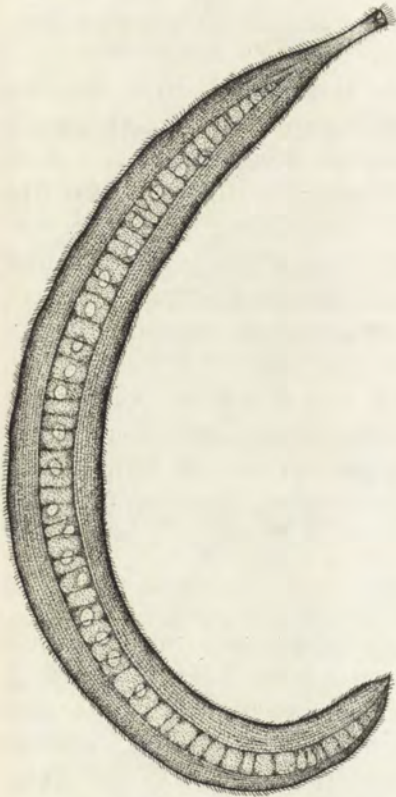
Fig. 1. General aspect of live *Tracheloraphis dogieli* showing the non-ciliated dorsal stripe. Approx. 130× (after Raikov 1962)

Figure 2 represents a three-dimensional diagram of organization of the ventral kineties, showing kinetosomes and their associated fibrils, based upon electron microscopical data. The ventral kineties are the most typical since they have a complete set of fibrils and are accompanied by well developed myonemes.

The kineties lie in deep furrows of the body surface, separated by interkinetal ridges (Fig. 2, Pl. I 1, II 2). In specimens contracted during fixation, the ridges are in their turn subdivided by transverse folds into separate knobs (Pl. I 1). *In vivo* observations show, however, that the transverse folds smooth out in extended animals.

In all kineties, the kinetosomes are paired, the anterior one lying somewhat to the left (if observed from its proximal end), and the posterior one, to the right of the kinety axis (Fig. 2, Pl. I 1, III 4, IV 7, 8, 9, 11, A and P). In other words, the axis of each kinetosome pair forms an angle of 30° to 45° to the axis of the kinety. In most kineties, both kinetosomes of a pair are ciliated (Fig. 2; Pl. I 1, III 4, IV 8, 9, Ci). However, in some kineties only the anterior kinetosome of a pair bears a cilium, the latter thus being implanted one by one (Pl. III 5). Tangential sections show that kineties with single cilia are sometimes adjacent to kineties with paired cilia.

In dorsal kineties lying to the right of the non-ciliated stripe, the cilia seem to be always solitary (i.e., the posterior kinetosome of each pair is non-ciliated, Pl. VI 13 at arrow). To the left of the non-ciliated stripe, both kinetosomes of each pair are, however, ciliated (Pl. II 3, V 12). The posterior kinetosome of a pair seems to be usually non-ciliated also near the anterior and the posterior body ends where the intervals between parallel kineties become very narrow. There are thus regional differences between various zones of the body surface of *T. dogieli* as to whether both or only one kinetosome of a pair are ciliated.

Free non-ciliated kinetosomes lying inside the cytoplasm occur along both edges of the glabrous dorsal stripe. These kinetosomes are not necessarily oriented perpendicular to the body surface and do not seem to form regular rows (Pl. V 12, VI 13, FKS). Their role is not clear. They have no well defined fibrillar derivatives.

Kinetosomes and their derivatives

Each row of kinetosomes is underlain by a ribbon-like basal plate, wide only enough to place a pair of kinetosomes on it (Fig. 2, Pl. I 1, II 2, 3, III 6, IV 7–10, BP). The plate is not uniformly structured: under a pair of kinetosomes it is thicker and appears transversely striated, whereas between kinetosome pairs it is less prominent (Pl. III 6, IV 10). Thus, the basal plate closes each kinetosome at its proximal end (Pl. II 2, 3, BP).

Tangential sections of the ciliate's body surface show that the basal plate is a layer of longitudinal microfilaments cemented with an amorphous substance which forms dark transverse or oblique bands of condensation (Pl. IV 7–10).

The basal plate is often underlain, at its cytoplasmic side, by several flat ribbons each formed by 6 to 8 microtubules, which resemble subkinetal (or basal) fibres (Pl. II 2, 3, V 12, SK). Each ribbon seems to start at the basal plate beneath a kinetosome pair and to extend along 5 or 6 other kinetosome pairs. Respectively, transverse section show 5 or 6 superposed ribbons of subkinetal microtubules at a given place (Fig. 2, Pl. II 3, SK).
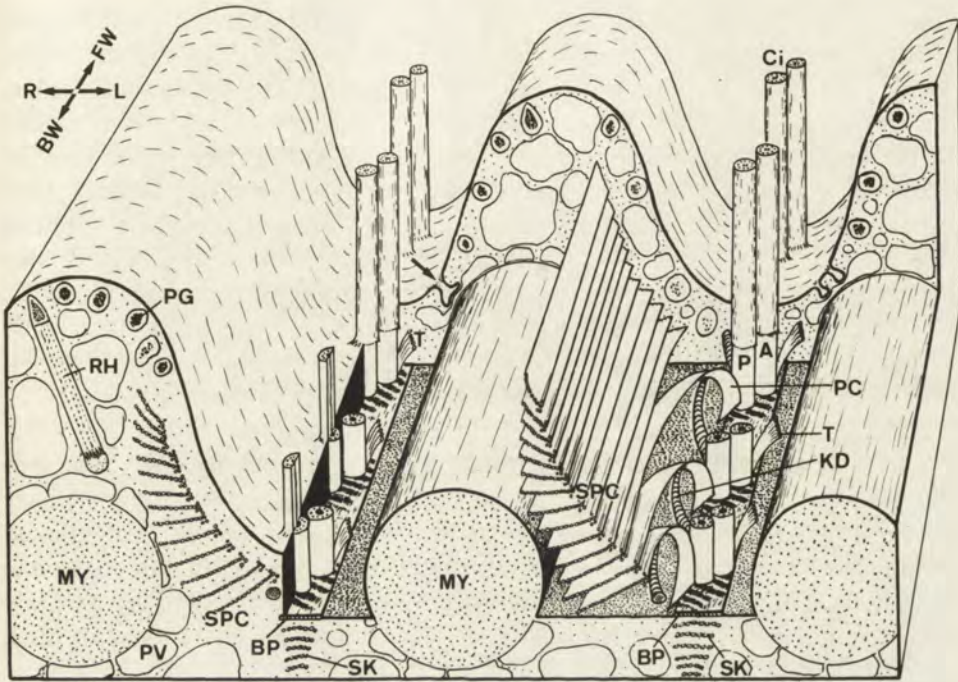
Fig. 2. Three-dimensional diagram of organization of the ventral kineties of *Tracheloraphis dogieli*. Right and left interchanged since the ventral surface is turned upwards. Abbreviations the same as in the Plates (see p. 41, an invagination of the body surface towards a myoneme at arrow

However, not all kineties of *T. dogieli* possess subkinetal ribbons, and, when present, they may be very differently developed. They seem to be more prominent under dorsal kineties, especially those to the left of the non-ciliated stripe (Pl. V 12), than at the ventral body side. They vary also from one individual to another, and the reason of this is unknown.

The kinetosomes of *T. dogieli* have typical structure (review: Grain 1969). They are closed at the distal end by the terminal plate, adjacent to which is a cup-like septum and the axial granule of the cilium (Pl. II 2, III 6). Non-ciliated kinetosomes retain their terminal plate but lack both the septum and the axial granule. The kinetosomes are united into pairs by their common basal plate and by two curved strands of dense material (Pl. III 4, 6, IV 7, 11, DM).

Each pair of kinetosomes has a common set of classical fibrillar derivatives: a postciliary ribbon of microtubules, a transverse fibre, and a kinetodesmal filament (Fig. 2, PC, T, KD). The most prominent of them is the postciliary ribbon consisting of 18 to 30 parallel microtubules lying in the same plane.

The postciliary ribbon of microtubules originates in the posterior right

segment of the rear kinetosome of a pair. The ribbon is at first parallel to the kinetosome (Fig. 2, PC). Then, the ribbon bends backwards, twists so that its edge is now turned to the surface of the interkinetal ridge, and proceeds posteriorly, accompanying the kinety on its right. It is nearer to the kinety than similar postciliary ribbons originating from more anterior kinetosome pairs of the same kinety. The stack of postciliary ribbons thus formed (Fig. 2, SPC) counts 12 to 24 individual microtubule ribbons (Pl. I 1, II 2, 3, V 12, VI 13, SPC). This means that postciliary ribbons are rather long and proceed backwards, in contracted animals, along 12 to 24 kinetosome pairs. The number of ribbons in the stack is usually higher in ventral kineties (18–24, Pl. I 1) than in dorsal ones (12–16, Pl. V 12, VI 13), suggesting their greater length in the former.

The first kinety at the left side of the non-ciliated dorsal stripe (Pl. V 12, asterisk) has a well developed stack of postciliary ribbons, only the free intra-cytoplasmic kinetosomes at the very edge of the stripe lacking postciliary fibres (Pl. V 12, FKS). However, at the right side of the non-ciliated stripe, not only the free kinetosomes (Pl. VI 13, FKS) but also the kinetosomes of the first kinety (asterisk) have no postciliary derivatives. Only the second kinety (two asterisks) is here accompanied by a typical stack of postciliary ribbons.

Both sides of the initial portion of each postciliary ribbon, not yet incorporated into the stack (Pl. II 2, 3, IV 7, 9, 11, PC), are covered by layers of dense material protruding from the rear kinetosome of a pair (Pl. I 1, II 2, 3, III 4, IV 7, 8, 9, 11, DL). The right one of these layers, strongly developed also in *Loxodes*, is often called a retrodesmal fibre (De Puytorac et Njiné 1970).

In stacked postciliary ribbons, the microtubules show a characteristic disposition seen in transverse sections (Pl. II 2, 3). At the ribbon edge which is nearest to the pellicle, each ribbon begins with three microtubules lying in a plane parallel to the pellicle and perpendicular to the rest of the ribbon (Fig. 2, Pl. II 2, 3, thin arrows). The main part of the ribbon is adjacent to the middle one of these three microtubules. There is also a single microtubule lying outside the main ribbon, behind the first (nearest to the kinetosome) microtubule of the subpellicular triad (Pl. II 2, 3, thick arrow).

The transverse fibre of the somatic kinetosome pair of *T. dogieli* is very short. It begins in the left anterior segment of only the anterior kinetosome as a ribbon of 6 microtubules, at first parallel to the kinetosome and directed towards the body surface (Fig. 2, Pl. IV 7, T). Then it gradually bends forward and to the left, becomes subpellicular, intercalates between the pellicle and the myoneme which accompanies the kinety on its left (Fig. 2, Pl. V 12, T), and here terminates.

The kinetodesmal filament is also short and poorly developed, consisting of dense material with a vague transverse banding (Fig. 2, Pl. III 4, IV 7, 11, KD). Starting in the right anterior segment of the rear kinetosome of a pair, it proceeds forward and to the right until it reaches the postciliary ribbon of the preceding kinetosome pair, and terminates joining the layer of dense material accompanying this ribbon (Pl. IV 11, arrows).

Thus, neither transverse nor kinetodesmal fibres leave in *T. dogieli* the limits of their own kinetosomal territory (Pitelka 1969 a, b), belonging in this case to a pair of kinetosomes. Only the strong postciliary fibres and, eventually, the less prominent subkinetal microtubules extend beyond this territory.

Myonemes

The myonemes of *Tracheloraphis dogieli* are very prominent, which corresponds to the strong contractility of the animals. These are longitudinal cylindrical structures accompanying each kinety on its left (Fig. 2, Pl. III 4, MY). They are especially thick at the ventral body side (up to 2 μm in diameter) and here they occupy almost the entire width of the interkinetal ridge base (Fig. 2, Pl. I 1, MY). The myoneme is adjacent at one side to its own kinetosome row (and possibly contacts its transverse fibres), and at the other side, to the stack of postciliary ribbons of the kinety next to the left (Fig. 2, Pl. I 1).

At the body sides and the dorsal surface, the myonemes are less developed, and the thinner the nearer to the non-ciliated stripe (Pl. II 2, 3, V 12, VI 13). The first kinety at the left side of the non-ciliated stripe has a very thin myoneme (Pl. V 12, MY_1). The first kinety at the right side of the stripe (Pl. VI 13, asterisk) has no myoneme at all, only the second kinety showing one (MY). No myonemes exist on the non-ciliated stripe and in the zone of free kinetosomes along its both edges (Pl. V 12, VI 13, FKS).

A myoneme has no membrane of its own. However, its surface is always lined with a layer of more or less flattened cytoplasmic vacuoles, the so-called perimyary vesicles (Fig. 2, Pl. II 2, 3, VII 16, VIII 17, PV), the proximal membranes of which delimit the surface of the myoneme as an almost continuous line (Pl. III 4, VI 15, VII 16, VIII 17). The perimyary vesicles communicate with other cisternae and vacuoles of the smooth endoplasmic reticulum which is highly developed in *T. dogieli*.

A row of tightly packed mitochondria accompanies each myoneme at its cytoplasmic side (Pl. I 1, VII 16, CH). These mitochondria lie in islets and trabeculae of the ground cytoplasm which immediately contact the perimyary vesicles (Pl. VII 16, PV). Moreover, most islets containing mitochondria show special mushroom-like protrusions filled with a finely granular material and directed towards the myoneme (Pl. VII 16, thin arrows). Some of them seem

to separate and to get inside the perimyary vesicle (thick arrows).

Deep invaginations of the outer surface of the ciliate, located in a row left to each kinety (Fig. 2, Pl. I 1, arrows), contact the outer surface of the myoneme. Pl. VII 16 shows that the plasma membrane of the ciliate (bent arrow) gets here into close apposition with the outer membrane of the perimyary vesicle (PV).

The myoneme itself is microfilamentous. Its component filaments, about 40 Å thick, form sometimes an irregular network (Pl. VIII 17) but more often are preferentially oriented along the myoneme (Pl. VII 16). Some myonemes, strongly contracted during fixation, show areas where the microfilaments are clearly longitudinal and display transverse bands of condensation which consist of thicker filaments (Pl. VI 14, 15). Such cross-banded portions of the myoneme pass without sharp transition into areas where the microfilaments are thinner and less organized (Pl. VI 15). Banded myonemes can be seen usually after glutaraldehyde fixation but not after osmium fixation.

Pellicular and cytoplasmic membranes

The pellicle of *T. dogieli* is usually formed by two closely adjacent unit membranes. The outer one is the plasma membrane which passes also onto the cilia (Pl. VII 16, VIII 18, 19, PM). The inner one is the distal membrane of voluminous subpellicular vacuoles (V) occupying most part of the volume inside the interkinetal ridges. The proximal membrane of these vacuoles is very sinuous and continuous with other cytoplasmic membranes, including those of the perimyary vesicles. Thus, the subpellicular vacuoles in *T. dogieli* are polymorphous and represent a part of the general, strongly developed lacunar system of the ciliate's cytoplasm.

No subpellicular vacuoles exist at the bottom of the ciliary furrows; the pellicle consists here of only the plasma membrane (Pl. II 2, 3, III 6, PM). Here, especially to the left of the kinety, there are numerous invaginations of the plasma membrane forming vesicles and convoluted channels resembling pynocytotic ones (Pl. II 2, 3, IV 8, PY). They are, however, irregularly spaced, thus differing from parasomal sacs.

The lacunar system of the cytoplasm is especially strongly developed in the region beneath the dorsal non-ciliated stripe. The body contents here consists almost exclusively of enormous vacuoles separated from each other by pairs of closely apposed membranes, virtually without layers of ground cytoplasm between them (Pl. IX 20, V). The outermost of these vacuoles (V_1) contacts, in many places, the plasma membrane. The ground cytoplasm is reduced to small islets suspended on membranes and intercalating between the vacuoles. These islets contain mitochondria, lipid droplets, etc. Similar units of ground cytoplasm are suspended also on the inner side of the plasma

membrane (Pl. IX 21, X 23, PM); some of these units are rich in ribosomes (Pl. IX 21, RS), others contain inclusions of very high electron density (Pl. IX 22, X 23, PG). These units hang down into the lumen of the large subpellicular vacuole (Pl. IX 21, 22, X 23, V_1). In areas where such cytoplasmic units are lacking, the plasma membrane and the distal membrane of the subpellicular vacuole are parallel and form a typical two-membraned pellicle (Pl. IX 21, arrows).

Some of the cytoplasmic vacuoles, occurring under the non-ciliated stripe, contain peripheral, electron dense, finely granular material. These vacuoles are usually more or less flattened (Pl. IX 22). The granular material may represent the precursor of the electron dense subpellicular inclusions present in the same sections.

The cortex of *T. dogieli* includes no epiplasmic layer peculiar to many other ciliates. It lacks also a typical layer of microfilaments at the boundary between ectoplasm and endoplasm, and the cytoplasm of this species is consequently not differentiated into these two areas.

Pigment granules and rhabdocysts

The subpellicular cytoplasm of the interkinetal ridges of *T. dogieli* contains many small (0.2μ m) vesicles with electron dense inclusions inside each one (Fig. 2, Pl. I 1, II 2, V 12, VIII 19, PG). They seem to correspond to the granules of brown pigment visible under the light microscope. At the same time they can be tentatively identified as protrichocysts (mucocysts) since we observed fusion of the vesicle membrane with the plasma membrane and extrusion of the electron dense inclusion from the ciliate's body (Pl. VIII 18, PG). All the vesicles appear empty after some fixations, which indicates simultaneous extrusion of all dense inclusions during fixation. The possible identity of pigment granules and protrichocysts (mucocysts) in *Trachelocercidae* has been discussed elsewhere (K o v a l e v a and R a i k o v 1972). The electron dense inclusions under the pellicle of the non-ciliated stripe (Pl. IX 22, X 23, PG) seem to be of similar nature, though larger and more poly-morphous.

Subpellicular organelles of another type occur in *T. dogieli* only in interkinetal ridges. These are rod-like bodies surrounded by a membrane, with a conical electron dense "head" at the end directed towards the pellicle (Fig. 2, Pl. VIII 18, 19, RH). The structure of these organelles, which occur also in *Kentrophoros latum*, has been considered elsewhere and they have been termed "rhabdocysts" (R a i k o v 1974). In *T. dogieli*, a rhabdocyst is about 2.5 μm long. It consists of a dense, concentrically lamellated head, a longitudinally striated shaft and an amorphous basal part (Pl. VIII 19). The rhabdocysts are formed in the endoplasm (this process will be described in a separate paper). Later, they migrate into the interkinetal ridges, where

they take position with their heads turned towards the pellicle (the rhabdocyst in Pl. VIII 19 has not yet assumed its final position). The head of a mature rhabdocyst often gets inside a pellicular protrusion directed outwards and covered by only the plasma membrane (Pl. VIII 18).

No discharge of the rhabdocysts has been observed in *T. dogieli*. The frequent images of rhabdocyst swelling *in situ*, without extrusion from the ciliate's body (Pl. I 1, RH) are likely to be fixation artifacts and not stages of a normal process of discharge.

Golgi elements

Tracheloraphis dogieli has numerous well differentiated dictyosomes localized mainly in the cortical zone of the cytoplasm, and lacking only under the dorsal non-ciliated stripe. The dictyosomes are especially abundant under the cineties and the myonemes (Pl. I 1, D). They are relatively small (0.5–1μ m), consist of 6–12 agranular cisternae (Pl. X 24, 25). The dictyosomes are usually cupuliform, their convex side corresponding to the side where new cisternae are formed. This side is often parallel to an agranular membrane of a cytoplasmic vacuole or lacuna (Pl. X 24, arrow). Numerous vesicles with an amorphous content of low electron density separate from the concave side of a dictyosome (Pl. X 24, VS). Ring-like dictyosomes, which are often met with (Pl. X 25) are likely to be transverse sections of cup-like organoids. The cytoplasm around the dictyosomes usually contains many free ribosomes (Pl. X 25, RS).

Discussion

Subpellicular fibrillar systems

The ectoplasmic fibrillar system of *T. dogieli* clearly belongs to the so-called "postciliary" type (Seravin and Matvejeva 1972), characterized by predominant development of postciliary kinetosome derivatives. This type is most peculiar to the *Heterotrichida* (Grain 1968, Bannister and Tatchell 1968, Huang and Pitelka 1973, etc.) and long seemed to be restricted to this order. However, recent studies demonstrated that many representatives of the order *Gymnostomatida* also belong to this type which shows here more morphological diversity than in heterotrichs.

Among the gymnostomatids, there are species with solitary kinetosomes and species with paired kinetosomes in somatic kineties. The former group includes *Helicoprorodon gigas* (Seravin and Matvejeva 1972), *Lacrymaria olor* (Bohatier 1970, Matvejeva 1973), *Chaenea vorax* (Fauré-Fremiet et Ganier 1969), *Lagynophrya fusidens* (Grain 1970), *Didinium nasutum* (Rieder 1971), *Dileptus cygnus* (Grain et Golińska 1969), *D. visscheri*

(Kink 1973), *Litonotus quadrinucleatus* (Bohatier et Njiné 1973), *Chilo-donella cucullulus* (Sołtyńska 1971), *Nassula* sp. (Tucker 1971), *Brooklynella hostilis* (Lom and Corliss 1971). Paired kinetosomes exist in all investigated representatives of the family *Trachelocercidae*, i.e. *Tracheloraphis caudatus* (Raikov and Dragesco 1969), *Tracheloraphis* sp. (Pitelka et al. 1971), *Trachelonema sulcata* (Kovaleva 1974), and *Tracheloraphis dogieli*. Paired kinetosomes are also peculiar of the family *Loxodidae* (*Loxodes magnus* — De Puytorac et Njiné 1970, *Kentrophoros latum* — Raikov 1971–72) and of the family *Geleiidae* (*Geleia nigriceps* — De Puytorac et al. 1973)[1].

In all gymnostomatids having paired kinetosomes, both kinetosomes of a pair are ciliated, except in *Tracheloraphis* sp. (Pitelka et al. 1971) and *Geleia nigriceps* (De Puytorac et al. 1973) where only the anterior kinetosome bears a cilium. However, in *Kentrophoros latum* and *Geleia nigriceps*, variations in the number of cilia per kinetosome pair exist. This character is instable also in *T. dogieli*, where the ciliation of one or both kinetosomes depends on the body region. It seems that in *Gymnostomatida* this character is less constant than in *Heterotrichida* where only the anterior kinetosome is usually ciliated (for review see Grain 1969). Though, among the *Heterotrichida* there are also species with both kinetosomes ciliated, e.g., *Nyctotherus cordiformis* (Paulin 1967, De Puytorac et Oktem 1967) and *Sicuophora xenopi* (De Puytorac et Grain 1968).

The morphology and topography of the gymnostomatid postciliary fibres is also variable. These are usually flat ribbons of microtubules, each originating from a kinetosome or a kinetosome pair. The ribbons are directed backwards and pass on the right of the respective kinety.

In most species, the ribbons are subpellicular and lie one beside the other, forming together a single sheet of microtubules which covers almost all the surface between two cineties (Fig. 3, A). This subpellicular type of postciliary fibres has so far been found only among species with solitary kinetosomes: *Helicoprorodon gigas* (Seravin and Matvejeva 1972), *Chaenea vorax* (Fauré-Fremiet et Ganier 1969), *Lagynophrya fusidens* (Grain 1970), *Didinium nasutum* (Rieder 1971), *Dileptus cygnus* (Grain et Golińska 1969), *D. visscheri* (Kink 1973), *Litonotus quadrinucleatus* (Bohatier et Njiné 1973), and *Lacrymaria olor* (Bohatier 1970, Matvejeva 1973). This type has not been discovered among species with paired kinetosomes (Table 1).

On the other hand, in forms with paired kinetosomes the topography

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[1] The taxonomic position of the *Loxodidae* and the *Geleiidae* it not clear, they have been considered Gymnostomatids, Trichostomatids and even Hymenostomatids. Recent electron microscopical and stomatogenesis studies seem to indicate that both either form a special branch inside the *Gymnostomatida*, or constitute a new taxon derived from this order and distinct from all other known orders of *Holotricha*. In a preliminary manner, we consider here both families as belonging to *Gymnostomatida* (see also De Puytorac et al. 1973).

of the postciliary ribbons (Fig. 3, C, D) resembles that in *Heterotrichida* (Fig. 3, E). The ribbons become superposed, turned to the pellicle with their edges, and thus form a laminated bundle which accompanies the kinety on its right. This pattern, clearly seen in *Tracheloraphis dogieli* (Fig. 3, D), exists also in other *Trachelocercidae* (Raikov et Dragesco 1969, Pitelka et al. 1971, Kovaleva 1974). In both *Trachelocercidae* and *Heterotrichida*, the postciliary ribbons consist of many microtubules per ribbon: 18 to 30
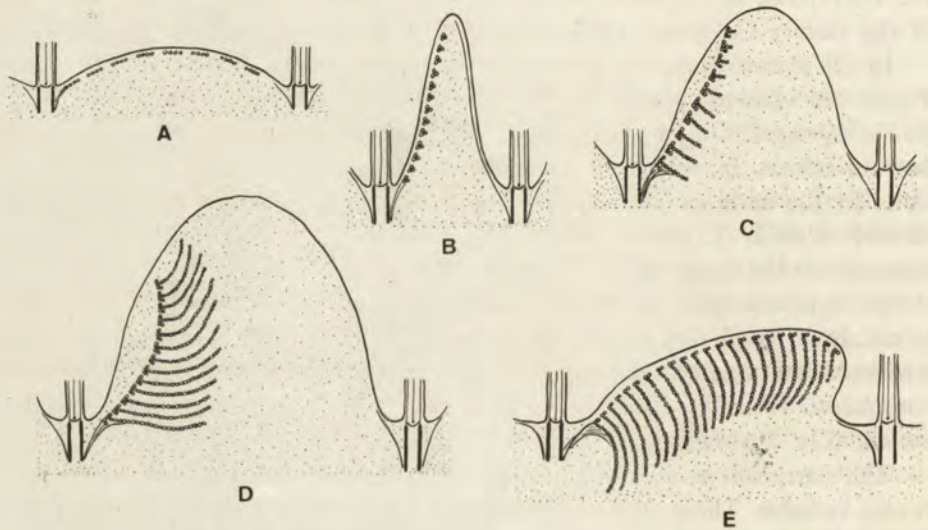
Fig. 3. Diagrams showing arrangement of postciliary ribbons in one interkinetal interval as they would be seen in an idealized transverse section. A — subpellicular arrangement (*Lacrymaria*), B — intermediate type with "triads" of microtubules (*Brooklynella*), C–E — laminated postciliary bundles (C — in *Kentrophoros latum*, D — in *Tracheloraphis dogieli*, E — in *Stentor coeruleus*). Compiled using data of Matvejeva 1973 (for A), Lom and Corliss 1971 (for B), Raikov 1971–72 (for C), and Huang and Pitelka 1973 (for E)

in *T. dogieli*, about 16 in *T. caudatus* (Raikov et Dragesco 1969), 21 in *Stentor coeruleus* (Bannister and Tatchell 1968, Huang and Pitelka 1973, Fig. 3, E), 19 in *S. igneus* (Grain 1968), up to 30 in *Condylostoma magnum* (Tartar and Pitelka 1969), about 16 in *Blepharisma undulans* (Kennedy 1965). In *Loxodidae*, the postciliary bundles are also laminated but the number of microtubules per individual ribbon is much smaller: 7–10 in *Kentrophoros latum* (Raikov 1971–72, Fig. 3, C), 4–6 in *Loxodes magnus* (De Puytorac et Njiné 1970).

A positive correlation possibly exists between the number of microtubules per postciliary ribbon and the degree of contractility of the ciliate. In strongly contractile forms (*Trachelocercidae, Stentor*) this number tends to be higher than in less contractile ones (*Kentrophoros*) and non-contractile ones (*Loxodes*). Such a correlation would be understandable because it has been shown in *Stentor* (Bannister and Tatchell 1968, Huang and Pitelka

1973) that postciliary fibres serve as functional antagonists of the myonemes. During the contraction phase, the ribbons passively slide onto each other and become more superposed, whereas re-extension of the body is assured by active sliding of the ribbons onto each other. This correlation is, however, incomplete since non-contractile (*Blepharisma*) and weakly contractile *Hetero-trichida* (*Condylostoma*) have nevertheless high numbers of microtubules per postciliary ribbon.

A third group of gymnostomatids has postciliary fibres which are morphologically intermediate between "subpellicular" and "laminated" ones. These are representatives of the families *Chlamydodontidae* and *Dysteriidae* which have solitary kinetosomes in somatic kineties. In *Chilodonella cucullulus* (Sołtyńska 1971), 5 postciliary microtubules start from each kinetosome and become packed into a "pentad" consisting of three microtubules in one row and two in another row. In *Brooklynella hostilis* (Lom and Corliss 1971) the number of microtubules is only 3, and they form a group which is triangular in cross section, a "triad" (Fig. 3, B). Such pentads or triads of microtubuli show subpellicular localization and cover approximately a half of the surface of the interkinetal ridge. In each group two or, respectively, one microtubule lies, however, in the second range from the pellicle and can be considered a primordium of a ribbon oriented with its edge towards the pellicle, like those in laminated postciliary bundles.

It must be noted that in *Kentrophoros* and *Loxodes*, where the ribbons are better developed, the first three microtubules of each ribbon remain nevertheless in a plane parallel to the pellicle and perpendicular to the plane of the rest of the ribbon (Fig. 3, C). Even in highly elaborate laminated postciliary bundles, e.g., in *Tracheloraphis dogieli* (Fig. 3, D), the first three microtubules of each ribbon are still subpellicular, and there exists also one microtubule which lies in the second row from the pellicle but outside the main ribbon (Pl. II 2, 3). This last observation is also true for *Kentrophoros latum* (see Fig. 5 in Raikov 1971–72) and probably for *Loxodes*.

A comparable but different configuration of the microtubules exists in *Stentor* (Bannister and Tatchell 1968, Huang and Pitelka 1973), with the difference that here only the first two microtubules of each ribbon are subpellicular and there is no single microtubule outside the main ribbon.

Thus, the triad-forming postciliary microtubules, resembling those of *Brooklynella*, may be considered intermediate between the "subpellicular" and the "laminated" types of the postciliary fibres.

Finally, some gymnostomatid species have subkinetal (or basal) sheets of longitudinal microtubules under the kinetosome bases, others not. These sheets start from the base of each kinetosome and, being almost parallel to the body surface, become stacked under the kinetosome row. Their occurrence seems to be rather irregular. Subkinetal microtubules are sporadi-

cally met with in both the group with paired kinetosomes and that with solitary kinetosomes (Table 1, asterisks). Among the *Trachelocercidae*, they have been recorded in *Tracheloraphis* sp. (Pitelka et al. 1971) but not in *Trachelonema sulcata* (Kovaleva 1974). In *T. dogieli*, the subkinetal fibres are especially prominent under dorsal kineties.

The subkinetal microtubules are highly developed in *Chlamydodontidae* (*Chilodonella*) and *Dysteriidae* (*Brooklynella*). However, even here they can have different directions, which seems to indicate their independent origin: in *Chilodonella*, they proceed backwards (Sołtyńska 1971), whereas in *Brooklynella*, forwards (Lom and Corliss 1971). The direction of the subkinetal microtubules in *T. dogieli* has not been determined.

Outside of the *Gymnostomatida*, subkinetal microtubules are known to be peculiar of the *Thigmotrichida* (Khan 1969, Lom and Kozloff 1969, Antipa 1971, etc.) and of suctorian swarmers (Bardele 1970, etc.). They also occur among the *Pleuronematina* (*Scuticociliatida*?), e.g., in *Cyclidium* (Beams and Kessel 1973). Subkinetal microtubules thus seem to have multiple and independent origin in various ciliate taxa, being produced by the "morphogenetic material" (Grain 1969) which occludes the proximal ends of the kinetosomes and is represented in *T. dogieli* by the cross-striated "basal plate". It looks as if subkinetal fibres were homologous to the ciliary roots or nemadesmata which become strongly inclined either forward or backward and transformed into a single sheet of microtubules. This homology appears substantiated in *Dileptus* (Grain et Golińska 1969, Kink 1973) where subkinetal fibrils dive steeper into the cytoplasm and thus somewhat resemble nemadesmata.

We attempted to classify the gymnostomatids of which the cortical ultra-structure is sufficiently known according to kinetosome pairing (if any) and type of postciliary derivatives (Table 1). This shows a clear correlation which exists between pairing of somatic kinetosomes and development of laminated postciliary bundles.

In all gymnostomatids studied, the kinetodesmal filaments are rather short and do not leave their own kinetosomal territories. They characteristically contact either the postciliary ribbon of the preceding kinetosome or pair of kinetosomes, as in *Nassula* (Tucker 1971), *Loxodes* (De Puytorac et Njiné 1970), *Kentrophoros latum* (Raikov 1971–72), or *Trachelonema sulcata* (Kovaleva 1974), or many groups of postciliary microtubules at once, as in *Brooklynella* (Lom and Corliss 1971). In both cases a structural connection between kinetosomes of the same kinety is assured.

The transverse fibres are also short in all gymnostomatids. They usually terminate close to the kinetosome where they started, and only in *Chilodonella* (Sołtyńska 1971) they are longer and reach the kinety next to the left. Sometimes they seem to contact the postciliary fibres of this neighbouring

Table 1

Classification of Gymnostomatid Species Studied with the Electron Microscope According to Various Features of their Cortex (Asterisks Mark Species where Subkinetal Microtubules Exist)

Kineto-somes / Postci-liary fibres	Solitary	Paired	
		with two cilia	with one cilium
Subpellicular	*Helicoprorodon gigas* *Lacrymaria olor* *Chaenea vorax* *Lagynophrya fusidens* *Didinium nasutum* * *Dileptus cygnus* * *Dileptus visscheri* *Litonotus quadrinucleatus*	—	—
Intermediate (with triads)	* *Chilodonella cucullulus* * *Brooklynella hostilis*	—	—
Laminated	—	*Tracheloraphis caudatus* * *Tracheloraphis dogieli* *Trachelonema sulcata* *Loxodes magnus* *Kentrophoros latum*	* *Tracheloraphis* sp. *Geleia nigriceps*

kinety, especially when these belong to the subpellicular type — e.g., in *Lacrymaria* (Matvejeva 1973).

In more general terms, an inverse relationship seems to exist between various fibrillar derivatives of the somatic kinetosomes in ciliates: never more than one of them becomes strongly developed, the others being accordingly more or less suppressed. It looks as if the "morphogenetic potential" of a kinetosome or of a pair of kinetosomes was limited. Moreover, the more the kinetosomal triplets giving rise to respective fibrillar derivatives are close to each other, the more these derivatives seem to interfere ("rule of adjacent triplets"). This inverse relationship is, for example, especially obvious between the development of the kinetodesmal filament and that of the postciliary microtubular ribbon, whose bases are near each other (triplets 7 and 9, respectively). The transverse fibre, based upon triplets 4–5 which are relatively distant from both aforementioned ones even in the case of solitary kinetosomes and shifted to another (anterior) kinetosome in the case of paired kinetosomes, shows less interference with both the kinetodesmal and the postciliary fibres. In gymnostomatids and heterotrichs the postciliary

derivatives dominate, whereas in hymenostomatids and apostomatids, the kinetodesmal filament becomes preferentially developed (Pitelka 1969 a, b, Grain 1969, Didier 1970).

In this connection, the profound similarity of the subpellicular fibrillar system of some *Gymnostomatida* (*Trachelocercidae, Loxodidae, Geleiidae*) and that of *Heterotrichida*, already pointed out by De Puytorac et Njiné (1970), deserves special attention. This similarity could scarcely be explained by mere convergence. The paths of evolution of the ciliates being now profoundly revised (remember, e.g., the case of *Entodiniomorphida*), a suggestion might perhaps be allowed that *Heterotrichida* could descend not from the *Tetrahymenina* with which they share practically no common features in the fibrillar system, but directly from some lower gymnostomatids which already had paired kinetosomes and laminated postciliary fibres. The *Trachelocercidae — Loxodidae — Geleiidae* branch could then represent another line of evolution coming essentially from the same source.

Myonemes

The system of myonemes in *Trachelocercidae* seems to be homologous to the so-called "boundary fibrillar system", a continuous microfilamentous layer separating the ectoplasm from the endoplasm (Seravin and Matvejeva 1972). Such a layer exists, among the *Gymnostomatida*, in *Helicoprorodon, Dileptus, Lagynophrya*, etc. (De Puytorac et Kattar 1969, Seravin and Matvejeva 1972, Grain et Golińska 1969, Grain 1970) and seems to play the role of a contractile "muscle bag". Among the *Heterotrichida*, a similar contractile bag exists in *Spirostomum* (Yagiu et Shigenaka 1963 a, b, Finley et al. 1964, Grain 1968). In most contractile ciliates, the continuous boundary layer of microfilaments is, however, replaced by separate longitudinal myonemes, e.g., in *Lacrymaria* (Matvejeva 1973), *Trachelocercidae* (Raikov et Dragesco 1969, Kovaleva 1974), *Stentor* (Fauré-Fremiet et Rouiller 1958, Randall and Jackson 1958, Bannister and Tatchell 1968, Grain 1968, etc.). More complex systems of microfilaments, consisting of a layer of longitudinal and a layer of transverse (circular) strands, differentiate in some ciliates, even non-contractile ones, e.g., in *Astomatids* (De Puytorac 1961 a, b, 1963 a, b). Finally, in *Geleia nigriceps* (De Puytorac et al., 1973) each kinety is accompanied by two myonemes (one on each side of it), which are in their turn interconnected by transverse microfilamentous connections into a complex grid.

It is presently known that contraction of microfilamentous myonemes of ciliates is brought about by an increase of calcium ion concentration (Vivier et al. 1969, Legrand 1970, Huang and Pitelka 1973). The calcium ions enter the myoneme coming from the surrounding vacuoles of the smooth endoplasmic reticulum, the so-called perimyary vesicles. These

are invariably present in all contractile ciliates and either underlie the continuous "muscle bag" on its endoplasmic side (*Helicoprorodon, Dileptus, Spirostomum*) or surround individual myonemes, as in *Tracheloraphis dogieli* and also in *Kentrophoros latum* (Raikov 1971–72), *Geleia nigriceps* (De Puytorac et al. 1973), *Stentor* (Grain 1968, Bannister and Tatchell 1968), etc. Re-binding of calcium ions, bringing about relaxation of the myoneme, is an ATP-dependent process (Vivier et al. 1969, Legrand 1970, Huang and Pitelka 1973). An observation made in *Tracheloraphis dogieli* may be interesting in this respect: the mitochondria here are not only adjacent to myonemes but the cytoplasmic islets containing them also send special protrusions towards the myoneme (Pl. VII 16). Perhaps this visualizes the transport of ATP from mitochondria to the myoneme.

The myonemes of *T. dogieli* have no internal channels of endoplasmic reticulum (endomyary vesicles), like those of other *Holotricha* and *Spirotricha* and contrary to the myonemes of *Peritricha* which have such vesicles (Favard et Carasso 1965, Carasso et Favard 1966, Allen 1973 a, b).

The points of contact between perimyary vesicles and invaginations of the cell surface, existing in *T. dogieli*, are likely to transmit excitation from the plasma membrane to the membrane of perimyary vesicles. Similar contacts have been observed between the ends of endomyary vesicles of the spasmoneme and the plasma membrane in some *Peritricha* (Carasso et Favard 1966) and considered likely to transmit excitation to the former, bringing about release of calcium ions in them. In *Vorticella*, special "linkage complexes" have been described at the boundary between myonemes and perimyary vesicles, which here also contact the plasma membrane; these complexes are supposed to regulate calcium ion fluxes between the perimyary vesicles and the myoneme (Allen 1973 a, b).

Cross banding is not typical of the ciliate myonemes and in any case the striations are not regular sarcomeres. Banding has been recorded in *Lacrymaria* (Bohatier 1970, Matvejeva 1973). In the myonemes of *Tracheloraphis dogieli*, the dark bands seem to be temporary structures representing zones of strong local contraction which appear after some fixations.

The problem of whether myonemes depend on kinetosomes or are morphologically connected with them deserves further investigation. In ciliates having a continuous "muscle bag", the kinetosome bases are often implanted into the microfilamentous layer (Grain et Golińska 1969, Grain 1970, Seravin and Matvejeva 1972, Kink 1973, etc.). Connections between individual myonemes and kinetosomes (by means of microfilamentous tracts) have been observed in *Lacrymaria* (Matvejeva 1973) and, less clearly, in *Stentor* (Bannister and Tatchell 1968). The absence of myonemes on non-ciliated parts of the body, as the glabrous dorsal stripe of *Tracheloraphis dogieli*,

the dorsal body side of *Trachelonema sulcata* (Kovaleva 1974), or the left side of the body of *Kentrophoros latum* (Raikov 1971–72), also argues for some kind of morphogenetic dependence of the myonemes on kinetosomes.

Superficial and cytoplasmic membranes

The pellicle of *Tracheloraphis dogieli* is rather simple, its only permanent component being the plasma (or cell) membrane. The alveolar layer, underlying the plasma membrane in most ciliates, is here poorly differentiated from miscellaneous cytoplasmic vacuoles more or less randomly juxtapposing the plasma membrane. No uniform, specialized peripheral vesicles exist in *T. dogieli*.

The other *Trachelocercid* so far studied in this respect, *Trachelonema sulcata*, has a cortex consisting of only the plasma membrane and lacks completely the alveolar layer (Kovaleva 1974, Kovaleva et Raikov 1972).

This type of organization of the cortex, including only the plasma membrane, is frequent among the *Gymnostomatida*, especially in lower genera. It has been recorded in *Pseudoprorodon lieberkühni*, *P. niveus*, *Spathidium*, *Homalozoon*, *Trachelius* (Fauré-Fremiet et André 1968), *Chaenea* (Fauré-Fremiet et Ganier 1969), *Lagynophrya* (Grain 1970), *Helicoprorodon gigas* (Seravin and Matvejeva 1972), *Dileptus* (Grain et Golińska 1969, Kink 1973), *Litonotus* (Bohatier et Njiné 1973). Other species, sometimes belonging to the same genera (*Pseudoprorodon arenicola* — Kattar 1972, *Helicoprorodon multinucleatum* — De Puytorac et Kattar 1969) already have special flattened alveoli under the plasma membrane, but these are not yet arranged into a continuous layer and occupy no constant position in respect to kinetosomes. This is also the case of *Loxodes* (De Puytorac et Njiné 1970), *Kentrophoros latum* (Raikov 1971–72), *Geleia nigriceps* (De Puytorac et al. 1973), *Lacrymaria* (Bohatier 1970, Matvejeva 1973).

Most gymnostomatids, above all the *Cyrtophorina*, as well as virtually all trichostomatids and hymenostomatids, have, however, a highly differentiated alveolar layer, consisting of vesicles (or alveoli, or peribasal spaces) which form a regular mosaic, definite points of which are occupied by cilia and, eventually, trichocysts. Such cortex constantly includes three membranes, the plasma membrane, the distal and the proximal membranes of the alveoli. The first two membranes, usually parallel to each other, form the pellicle. Among the gymnostomatids, *Plagiocampa*, *Prorodon*, *Nassula*, *Paranassula*, *Chilodonella*, *Brooklynella* and other forms belong to this group (Fauré-Fremiet 1962, De Puytorac 1964, Fauré-Fremiet et André 1965, 1968, Sołtyńska 1971, Lom and Corliss 1971).

Remarkably, a very simple cortex appears again in *Heterotrichida*. In *Blepharisma* (Kennedy 1965), *Stentor igneus* (Grain 1968), *Condylostoma*

(Tartar and Pitelka 1969) it consists of only the plasma membrane. In other species (*Stentor coeruleus*) flattened alveoli exist under the plasma membrane but they are sparse and poorly differentiated (Bannister and Tatchell 1968, Huang and Pitelka 1973). If we consider that *Tetrahymenina* and other *Hymenostomatida* always have a highly organized cortex with regularly arranged subpellicular alveoli (Fauré-Fremiet and André 1968, Didier 1971), we are again incited to suppose that *Heterotrichida* might have descended not from *Hymenostomatida* but directly from some lower gymnostomatids.

Rhabdocysts and pigment granules

Recent studies showed that extrusive organelles of the ciliates, the "extrusomes" (Hausmann 1972 a), are far more diverse than previously supposed. Three categories of these organelles have usually been recognized, the mucocysts (or protrichocysts), the spindle trichocysts (or akontobolocysts), and the toxicysts (or "Nesselkapseltrichocysten") (Krüger 1936, Hovasse 1965), later supplemented by a fourth category, the microtoxicysts, including the suctorian haptocysts (Hovasse 1969). It has recently been demonstrated that extrusion of the mucocysts and that of the spindle trichocysts follows the same molecular principle, that of conformational "unfolding" of preformed but tightly packed protein filaments into a three-dimensional network (Hausmann 1972 b, c, 1973, Hausmann et al. 1972 a, b, etc.). The difference between mucocysts and spindle trichocysts is only that, in the former, the unfolding proceeds equally in all directions ("swelling"), whereas in the latter, it proceeds in only one direction ("stretching"). As to the toxicysts, their extrusion mechanism is entirely different: turning inside out of a long preformed internal tube (Hausmann und Wohlfarth-Bottermann 1973, and others).

It is important to note that true mucocysts must, according to Hausmann's definition, have a preformed protein structure usually visible as a paracrystalline periodicity of the "resting" mucocyst. If so, the pigment granules of some *Gymnostomatida*, such as *Trachelonema sulcata* (Kovaleva and Raikov 1972), *Loxodes* (Mashansky et al. 1963), *Tracheloraphis dogieli* (this paper), *Geleia nigriceps* (De Puytorac et al. 1973), also capable of "swelling" and extrusion and therefore considered to be mucocysts (Kovaleva et Raikov 1972) but showing no paracrystalline structure, might perhaps be separated from the true mucocysts into an independent category of "extrusible pigment granules", characterized by another molecular mechanism of "swelling". The same applies also to the pigment granules of *Heterotrichida* (for references see Kovaleva et Raikov 1972), which can also be extruded, and this is one more point in common between the *Heterotrichida* and the *Trachelocercidae* — *Loxodidae* — *Geleiidae* group.

New categories of "extrusomes" recently described in ciliates include the haptocysts, or phialocysts, of *Suctoria* (review: Hovasse 1969) and the somewhat similar "microtoxicysts" of *Chilodonella* (Pyne 1971) and *Tintinnidae* (Laval 1972), the "ampullocysts" of *Kentrophoros latum* (Raikov 1971-72, 1974), the cnidocysts of *Remanella multinucleata* (Raikov 1974), the "crystallocysts" of *Conchophthirius* (Antipa 1971), etc. In *Kentrophoros latum*, Raikov (1971-72) described, first under the name of "trichocysts", peculiar rod-like organelles which later have been re-named "rhabdocysts" (Raikov 1974) because their extrusion mechanism has nothing in common with that of true trichocysts, as defined by Hausmann.

The rod-like subpellicular organelles of *Tracheloraphis dogieli* are very similar to the rhabdocysts of *K. latum*. They have essentially the same form and dimensions, and show the characteristic differentiation into the same three portions, the head, the shaft, and the basal part. These organelles of *T. dogieli* must also be called rhabdocysts.

Extrusion of rhabdocysts has been followed in *Kentrophoros latum* but not in *T. dogieli*. It begins with swelling of their basal parts. Under the action of the pressure developed, the shaft with the head is entirely extruded from the ciliate's body, without, however, any substantial elongation of the shaft or conformational transformation of its internal structure. The rhabdocysts are thus clearly different from the mucocysts and the spindle trichocysts. They differ also from the toxicysts, having no concentric tubes likely to devaginate during extrusion. The rhabdocysts thus seem to be an independent category of "extrusomes". The number of these categories, in ciliates only, appears now to reach six (Raikov 1974) or even seven: the mucocysts (including or not the ampullocysts and the extrusible pigment granules), the spindle trichocysts, the rhabdocysts, the microtoxicysts (including the haptocysts), the toxicysts, and the cnidocysts.

Conclusions

The somatic kineties of *Tracheloraphis dogieli* consist of paired kinetosomes of which usually both are ciliated, but in some body regions, only the anterior kinetosome bears a cilium. Free non-ciliated intra-cytoplasmic

kinetosomes exist at the edges of the dorsal non-ciliated stripe running along the body.

Each row of kinetosomes is based upon a microfibrillar, transversely striated ribbon, usually doubled at its cytoplasmic side by 5–6 sheets of subkinetal microtubules (especially at the dorsal body side).

The posterior kinetosome of each pair gives rise to a prominent ribbon of 18–30 postciliary microtubules which extend backwards along 12 to 24 kinetosome pairs, being stacked at the right side of the kinety with similar ribbons of other kinetosome pairs. The outer three microtubules of each ribbon lie in a plane parallel to the pellicle, while the rest of them, in a plane perpendicular to it. The kinetodesmal filament is short, it starts in the anterior right sector of the rear kinetosome and terminates against the postciliary ribbon of the preceding kinetosome pair. Only the anterior kinetosome of a pair has 6 short transverse microtubules.

Each kinety is accompanied at its left by a strong myoneme which is microfilamentous, sometimes with dense bands of local contraction, and surrounded by perimyary vesicles. These contact both the plasma membrane and numerous mitochondria. The cortex consists of a single plasma membrane with irregular cytoplasmic vacuoles locally adjacent to it. The peripheral cytoplasm contains many dictyosomes. The interkinetal ridges contain subpellicular pigment inclusions probably homologous to mucocysts and rod-like organoids differentiated into a head, a shaft, and a basal portion, the so-called rhabdocysts.

The ectoplasmic fibrillar systems of *Gymnostomatida* belong to several modifications of the "postciliary" type of organization. One of these modifications, that of *Trachelocercidae* and *Loxodidae*, strongly resembles the ectoplasmic fibrillar system of the *Heterotrichida*.

РЕЗIOME

Соматические ресничные ряды *Tracheloraphis dogieli* состоят из парных кинетосом. Обычно обе кинетосомы пары несут реснички, но в некоторых зонах тела — только передняя. По краям безресничной продольной полоски на дорзальной стороне тела расположены свободные, безресничные, погруженные в цитоплазму кинетосомы.

Основания кинетосом располагаются на микрофибриллярной поперечно-исчерченной пластинке, проходящей вдоль ряда. Под ней, особенно на дорзальной стороне тела, часто располагается 5–6 плоских лент субкинетальных микротрубочек.

Задняя кинетосома каждой пары дает начало мощной ленте из 18–30 постцилиарных микротрубочек, которые, накладываясь друг на друга справа от ресничного ряда, проходят назад вдоль 12–24 пар кинетосом. В каждой ленте первые три микротрубочки расположены в плоскости пелликулы, а остальные — перпендикулярно ей. Кинетодесмальный филамент короткий, отходит от задней кинетосомы в ее переднем правом секторе и заканчивается, упираясь в постцилиарную ленту предыдущей пары кинетосом. Трансверсальные микротрубочки, в числе 6, очень короткие, имеются только у передней кинетосомы пары.

Слева от каждого ресничного ряда проходит мощная микрофибриллярная мионема, иногда обнаруживающая поперечные полосы сильного локального сокращения. Мионема окружена перимиарными пузырьками, которые тесно контактируют с плазматической мембраной и с митохондриями. Кортекс состоит из одной клеточной мембраны, местами подостланной малоспециализированными цитоплазматическими вакуолями. В периферической цитоплазме много диктиосом. В межресничных гребнях имеются субпелликулярные пигментные включения, вероятно, соответствующие мукоцистам, а также палочковидные органоиды, дифференцированные на головку, стержень и основание — рабдоцисты (Райков 1974).

Эктоплазматические фибриллярные системы *Gymnostomatida* относятся к разным вариантам "постцилиарного" типа организации. Один из этих вариантов, характерный для *Trachelocercidae* и *Loxodidae* — с парными кинетосомами и пластинчатыми постцилиарными тяжами — весьма сходен с эктоплазматической фибриллярной системой *Heterotrichida*.

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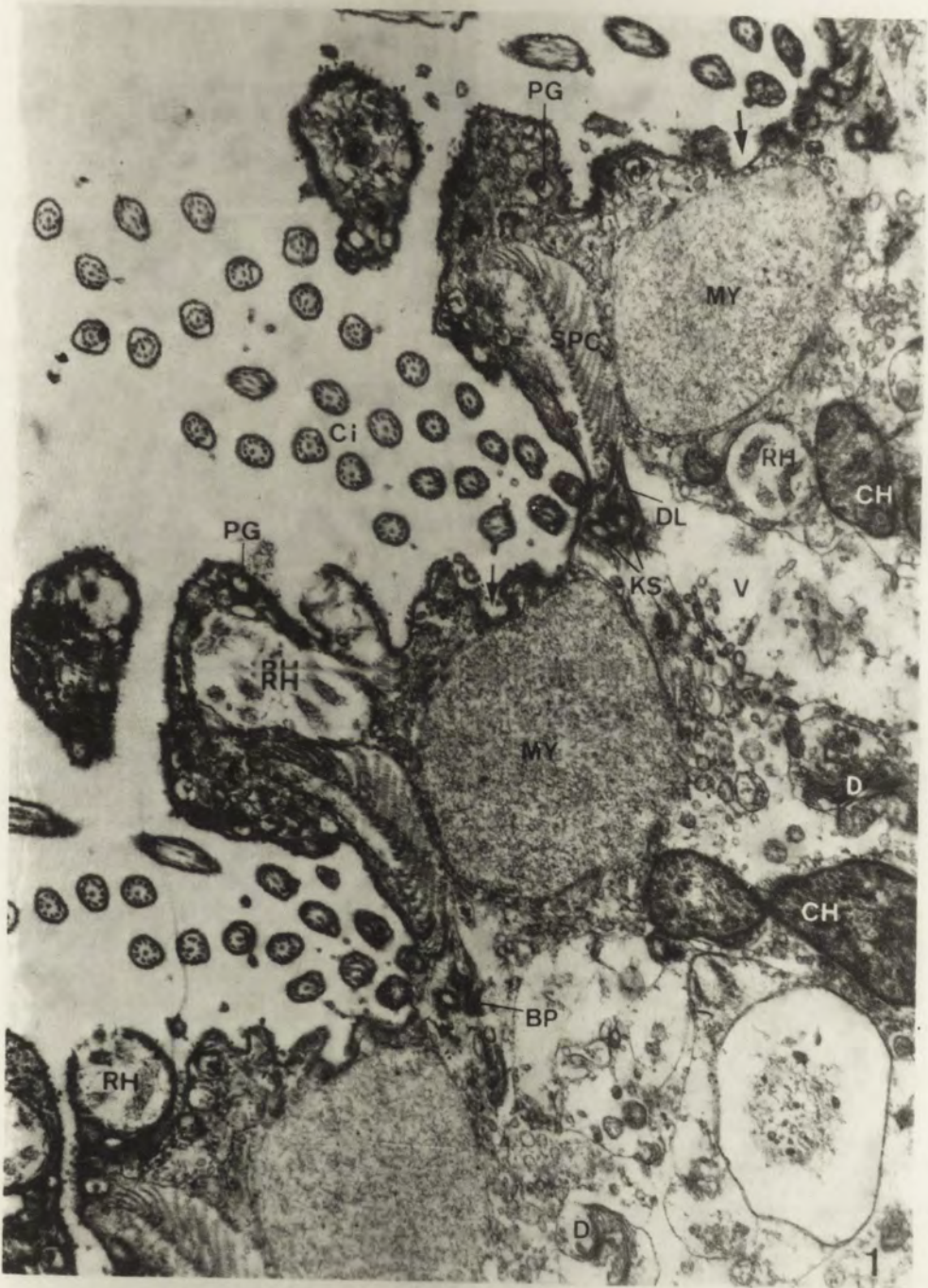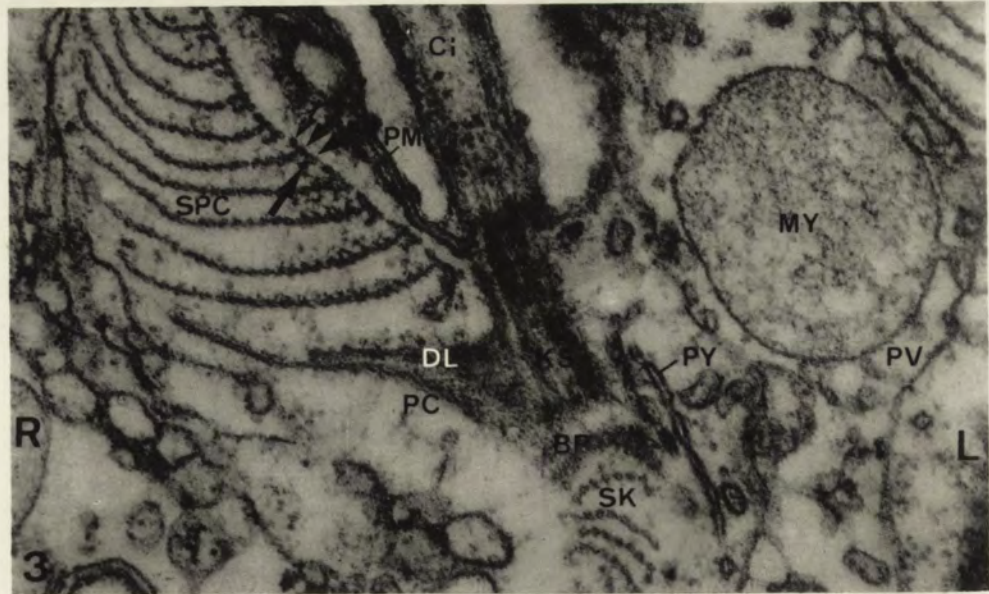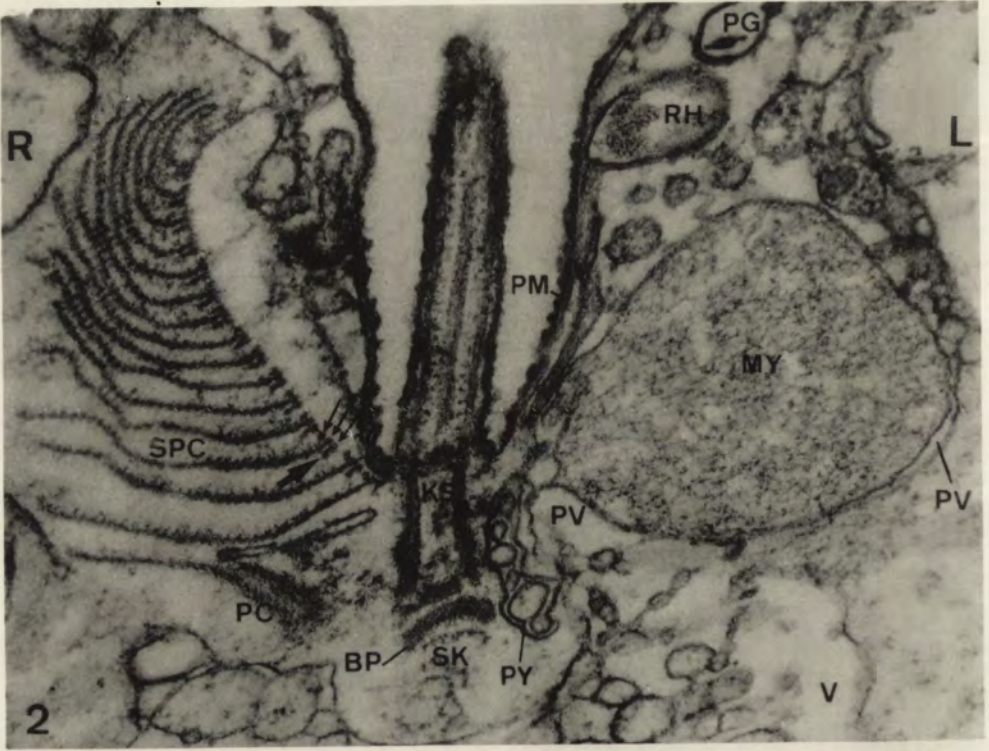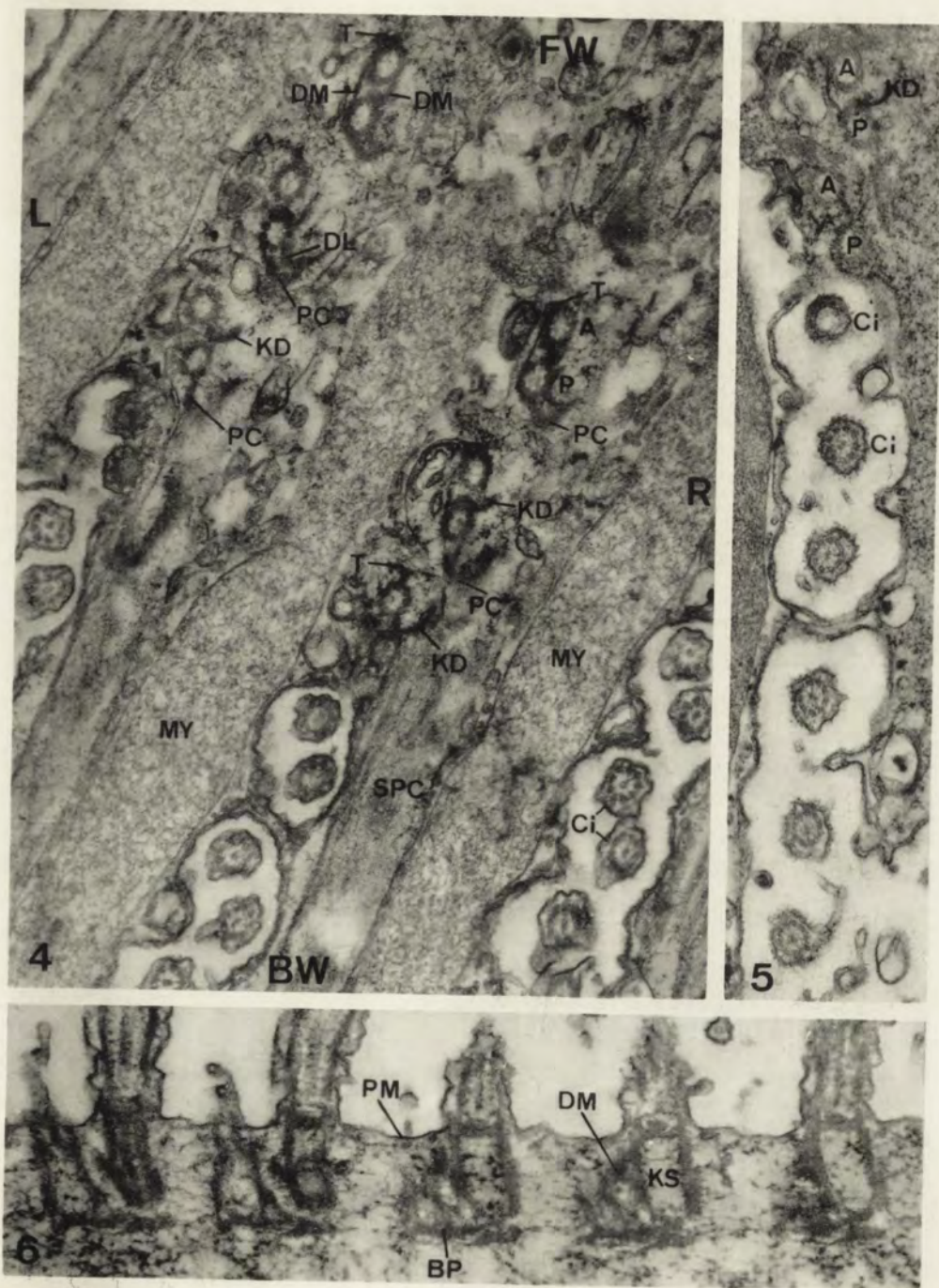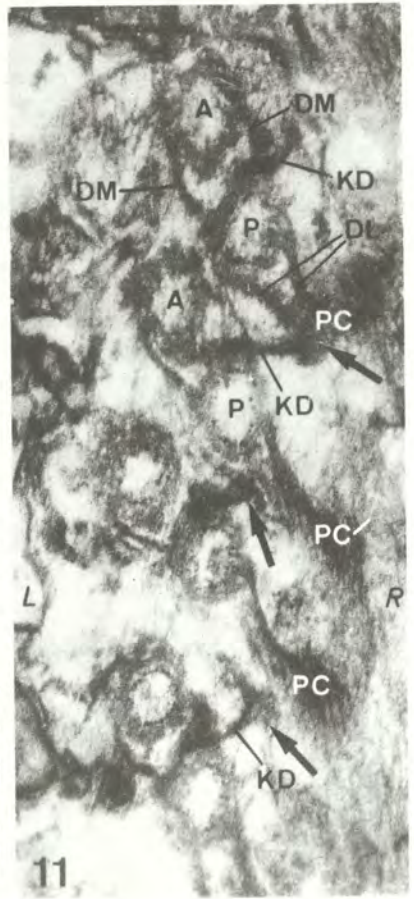
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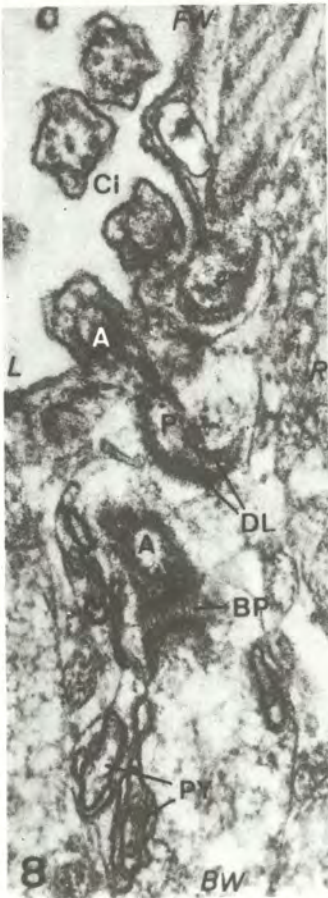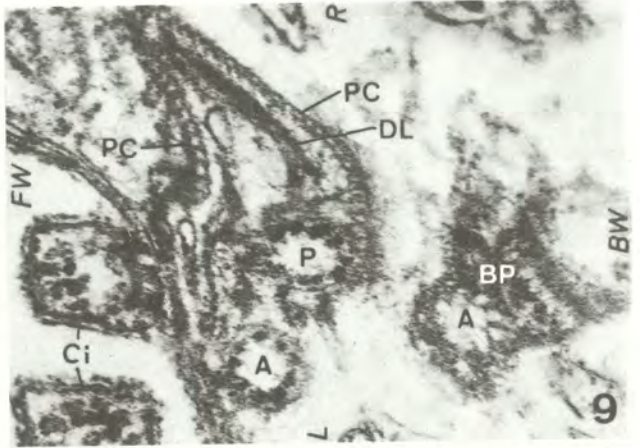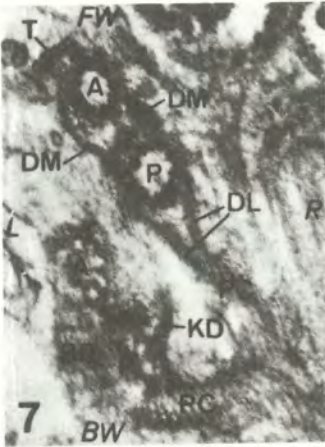

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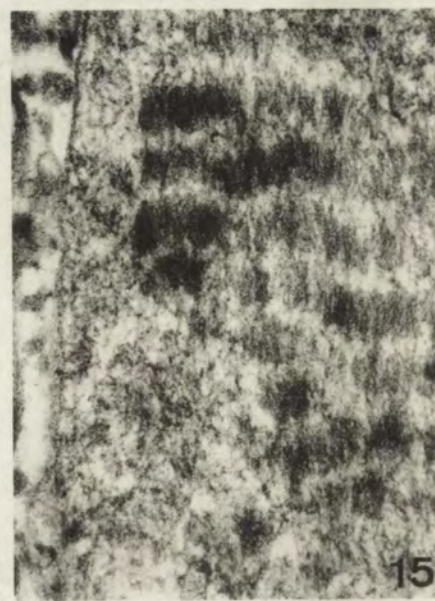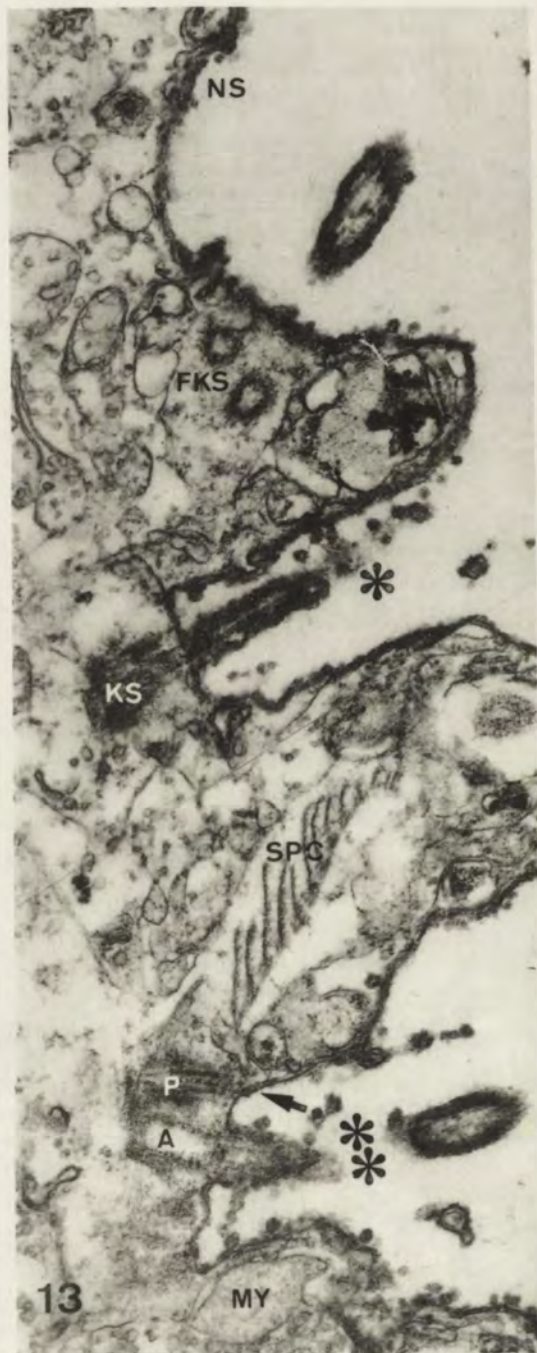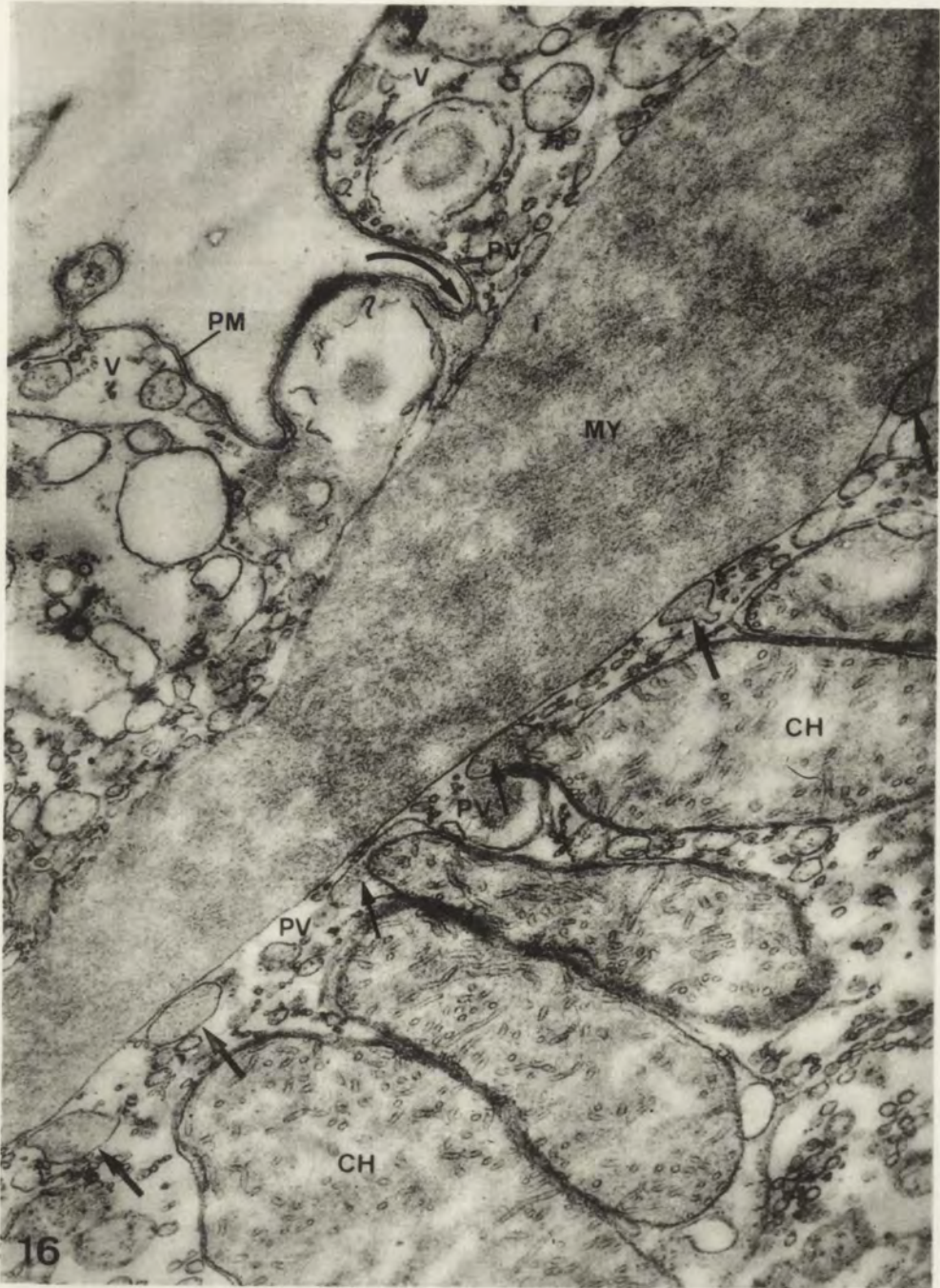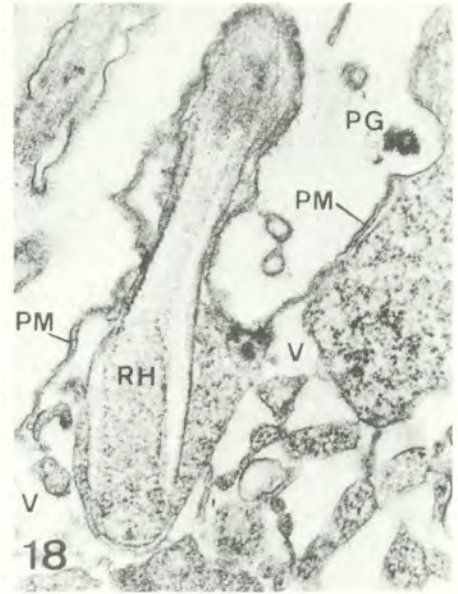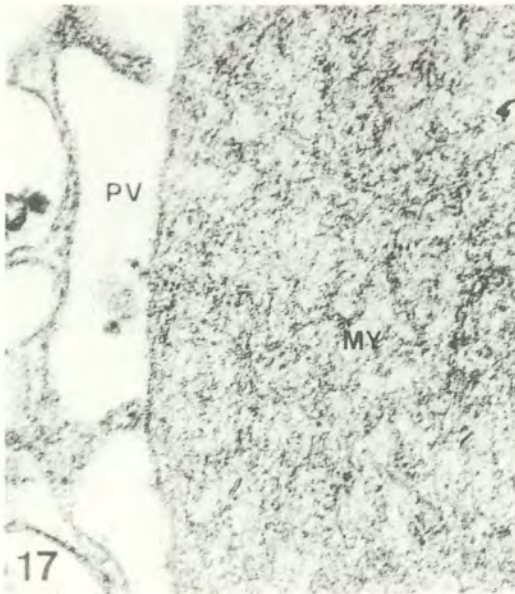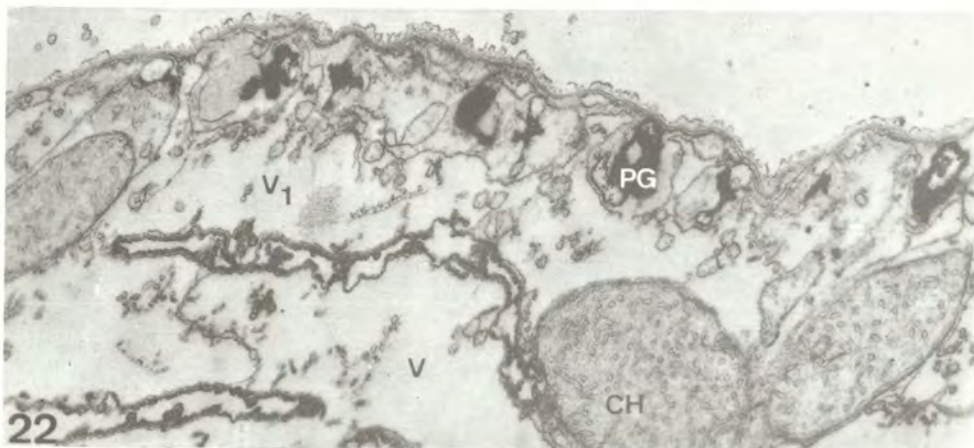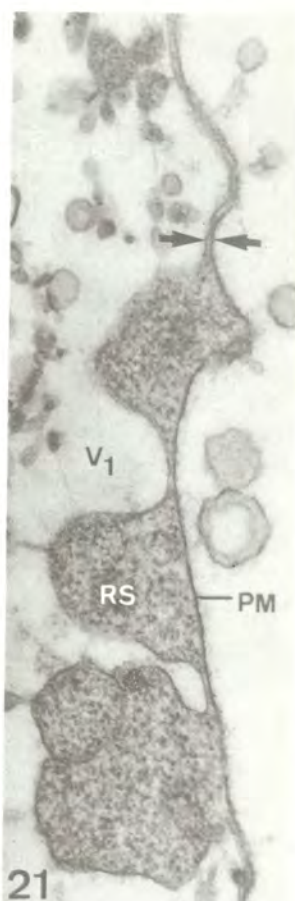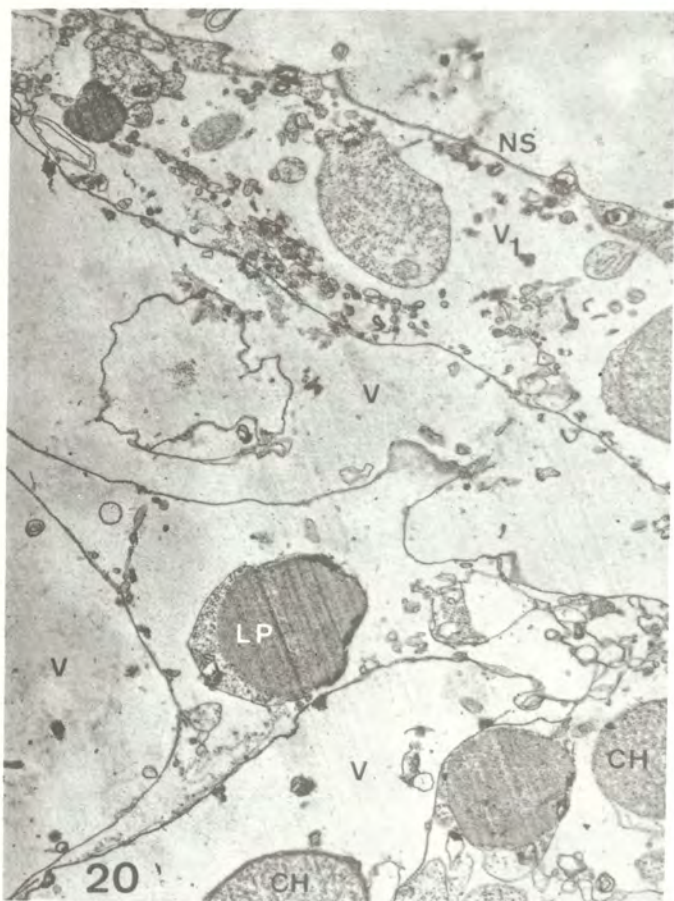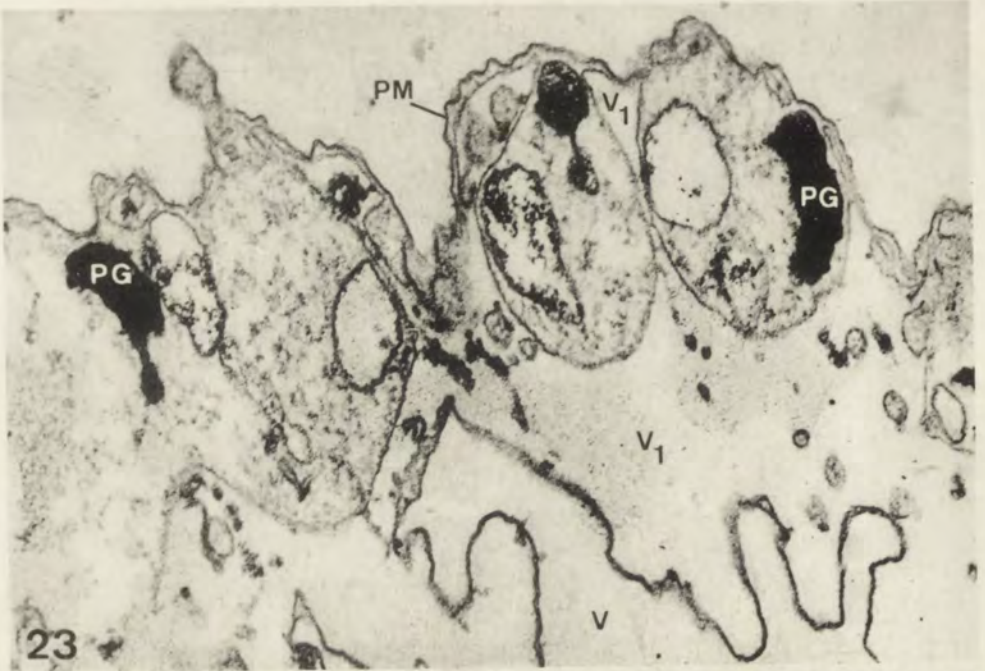
PLATE III

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PLATE IV

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PLATE VI

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PLATE VII

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PLATE VIII

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PLATE X

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Yagiu R. and Shigenaka Y. 1963 b: An electron microscope study of the fibrillar system of two heterotrichous ciliates, *Condylostoma spatiosum* and *Spirostomum ambiguum*. In: Progress in Protozoology, Proc. First int. Congr. Protozool., Prague 1961, 411—413.

EXPLANATION OF PLATES I–X

Cortical ultrastructure of *Tracheloraphis dogieli*

1: Oblique section of the ventral surface of the body showing interkinetal ridges with strong myonemes at their bases, stacks of postciliary ribbons, invaginations of the body surface towards the myonemes (at arrows). Os./Ph. — JEM, 14,000 ×

2: Transverse section of a dorso-lateral kinety at the level of the anterior kinetosome of a pair. Note the basal plate, subkinetal fibres, the subpellicular position of the first three microtubules in each postciliary ribbon (thin arrows), and the presence of a single micro-tubule outside the ribbon (thick arrow). Os./Ph. — JEM, 48,500 ×

3: Transverse section of the third kinety to the left of the non-ciliated dorsal stripe at the level of the posterior kinetosome of the pair, showing the beginning of the postciliary

ribbon and the characteristic topography of the microtubules in the ribbons (arrows the same as in Fig. 2). Well developed subkinetal microtubules are seen: the myoneme is very thin. Os./Ph. — JEM, 62,000 ×

4: Tangential section of the ventral body surface showing kinetosome pairs (both members ciliated) and their fibrillar derivatives. Os./Cac. — JEM, 25,000 ×

5: Tangential section of a kinety with paired kinetosomes but solitary cilia. Gl.-Os./Cac. — EL, 22,000 ×

6: Longitudinal section of a kinety showing the basal plate under the kinetosome pairs. Os./Cac. — JEM, 47,500 ×

7: Tangential section of the body surface showing a kinetosome pair, the basal plate with bases of another two kinetosomes, and the postciliary ribbon. Os./Ph. — JEM, 56,000 ×

8: The same, demonstrating pinocytotic vesicles and channels and the transverse striation of the basal plate. Os./Cac. — JEM, 46,500 ×

9: The same, showing the postciliary ribbon and the accompanying layer of dense material. Os./Ph. — JEM, 62,500 ×

10: Tangential section of the body surface at the level of the basal plate of a kinety. Os./Ph. — HU, 50,000 ×

11: Tangential section of the body surface at the level of kinetosomes. Note contact of the kinetodesmal filament with the postciliary ribbon of the preceding pair of kinetosomes (at arrows). Os./Ph. — HU, 60,000 ×

12: Transverse section of the dorsal kineties immediately to the left of the non-ciliated stripe (NS): the first (one asterisk) and the second (two asterisks). Free intra-cytoplasmic kinetosomes seen. Os./Ph. — JEM, 31,000 ×

13: Transverse section of the first (one asterisk) and the second (two asterisks) dorsal kineties immediately to the right of the non-ciliated stripe (NS). Free intra-cytoplasmic kinetosomes seen; the first kinety has neither postciliary fibre nor myoneme. The posterior kinetosome of a pair is non-ciliated (at arrow). Os./Ph. — JEM, 29,000 ×

14, 15: Regions of myonemes showing bands of local contraction. Gl.-Os./Cac. — EL, 14 — 26,500 ×, 15 — 40,500 ×

16: Longitudinal section of a myoneme. Cytoplasmic islets containing mitochondria show protrusions towards the myoneme (thin arrows), some of which seem to detach (thick arrows). Note invagination of the body surface towards the myoneme (bent arrow). Os./Ph. — HU, 30,000 ×

17: Part of a myoneme with perimyary vesicles. Gl.-Os./Cac. — JEM, 63,500 ×

18: Part of an interkinetal ridge containing a rhabdocyst and showing extrusion of a pigment granule. Gl.-Os./Cac. — EL, 28,000 ×

19: Mature rhabdocyst inside the interkinetal ridge. Gl.-Os./Cac. — EL, 39,000 ×

20: Vacuolized cytoplasm beneath the surface (at top) of the non-ciliated dorsal stripe. Gl.-Os./Cac. — EL, 12,000 ×

21: Surface of the non-ciliated stripe. Note the bi-membraneous pellicle in regions where the plasma membrane and the membrane of the cytoplasmic vacuole are adjacent (arrows). Gl.-Os./Cac. — JEM, 40,000 ×

22: Surface of the non-ciliated stripe showing large pigment granules and cytoplasmic vesicles with granulated electron dense material. Os./Ph. — HU, 22,000 ×

23: The same at higher magnification. Os./Ph. — HU, 50,000 ×

24: Dictyosome. Note the membrane of a lacuna of the smooth endoplasmic reticulum. Os./Ph. — HU, 50,000 ×

25: "Concentric" dictyosome in a cytoplasmic islet containing many ribosomes. Gl.-Os./Cac. — EL, 58,000 ×

Abbreviations used:

In legends: Gl. — glutaraldehyde, Os. — osmium tetroxyde, Cac. — cacodylate buffer, Ph. — phosphate buffer; microscopes: JEM — JEM-7, EL — Elmiskop I, HU — Hitachi HU 11 E.

On micrographs: A — anterior kinetosome of a pair, BP — basal plate, BW — backward direction, CH — mitochondrion, Ci — cilium, D — dictyosome, DL — dense lamella (retrodesmal fibre), DM — dense material connecting the kinetosomes into a pair, FKS — free kinetosomes, FW — forward direction, KD — kinetodesmal filament, KS — kinetosome, L — direction to the ciliate's left, LP — lipid droplets, MY — myoneme, NS — non-ciliated dorsal stripe, P — posterior kinetosome of a pair, PC — postciliary ribbon, PG — pigment granules, PM — plasma membrane, PV — perimyary vesicles, PY — pinocytotic vesicles, R — direction to the ciliate's right, RH — rhabdocyst, RS — ribosomes, SK — subkinetal microtubules, SPC — stack of postciliary ribbons, T — transverse fibre, V — cytoplasmic vacuoles, VS — vesicles separating from a dictyosome.

D. V. OSSIPOV

The Specificity of Localization of Omega Particles, the Intranuclear Symbiotic Bacteria in *Paramecium caudatum*

Synopsis. The specificity of the intranuclear localization of omega particles in *Paramecium caudatum* MI-48 has been studied for a further elaboration of the original "symbiotic" method of analysis of structure and function in the ciliate nuclei. With the homogenate of paramecia MI-48 omega, the cells of other 127 clones have been infected experimentally. The infected clones showed different peculiarities of their nuclear apparatus organization. In the experiments 84 micronuclear clones and 43 amicronuclear ones were used. No one of the 43 amicronuclear clones became infected with the *omega* particles. The results gained on micronuclear clones indicate that the presence of micronuclei in cells is the decisive condition of infection in paramecia. The capacity of clones to be infected with *omega* particles does not depend on the morphological type of micronuclei nor on the number of generative nuclei in the cell. So, the *omega* particles posses a rigorous specificity as to the localization in the nuclear apparatus of the ciliate *P. caudatum*.

Introduction

In the studies of structure and function of eucaryotic nuclei, much attention has been attracted by the unicellular organisms – especially ciliates – which show a specific organization of their nuclear apparatus.

In our former communications, an original method has been presented for the study of mechanism of the nuclear differentiation in ciliates. It is based on the analysis of the nucleo-cytoplasmic relation in the case of infection of the cell with symbiotic bacteria (Ossipov and Ivakhnyuk 1972, Ossipov 1973, Ossipov et al. 1973). In paramecia, numerous cases are known of revealing different symbiotic bacteria as well as organisms related to them (Wichterman 1953, Sonneborn 1959, Ball 1969, Beale et al. 1969). The great majority of the symbiont species are found in the cytoplasm of ciliates and only a few of them are intranuclear. Up till now, the majority of investigators have avoided – not without reason – to give them species names and do not attempt to determine their taxonomic position (Sonneborn 1959, Beale et al. 1969).

In the micronucleus of *Paramecium caudatum* clone MI-48 symbiotic Gram negative bacteria have been found by us. They were determined as *omega* particles. The cytoplasm and macronuclei of the infected cells proved to be free of them (Ossipov and Iyakhnyuk 1972). The cells of the MI-48 *omega* clone fail to evoke the killer effect after having been mixed with the cultures of *P. caudatum* and *P. aurelia*. Paramecia of the clone MI-48 *omega* keep for a long time their capacity to many cellular divisions, similarly as the normal cells.

The micronuclei of the "pure" clones of *P. caudatum* were successfully infected with the homogenate of MI-48 *omega* cells whereas 10 amicronuclear clones taken at random — were never infected with those bacteria (Ossipov 1973). We studied in special experiments the capacity of *omega* particles of the clone MI-48 *omega* to be maintained in the cells of some other protozoa species. The experiments were carried out on the cultures of 24 species of free living protozoa. As result a high species specificity of infection has been established since the *omega* particles could be kept in the *P. caudatum* cultures only.

By means of the electronmicroscopic study the characteristic morphological and ultramicroscopic changes could be revealed in the infected micronuclei of MI-48 *omega* cells. This allowed to postulate the redifferentiation of those nuclei into a functionally high active condition which is unusual for the generative nuclei of ciliates (Ossipov 1973). As yet the problem is not resolved concerning the difference of the functionally active state of the infected micronucleus and the usual condition of the normal macronucleus. Evidently they differ essentially in some way, since after the infection of micronucleus with *omega* particles, and after the alteration of its ultrastructure, the symbionts remain in it and continue to persist for many cellular generations. Further investigations in this line may help to elucidate the nature of the ciliate macronucleus, especially in connection with one of the recent hypotheses that not all the genes are present in it, when compared with the micronucleus (Ammermann 1970, 1971, Bostock and Prescott 1972, Soldo and Godoy 1972, Prescott et al. 1973). The changes of the infected micronuclei are highly specific and not accompanied by an observable rise of macronuclear activity nor by a disturbance of the ultrastructural organization of the other organoids of paramecium.

The peculiarities of the infection of paramecium with *omega* particles reveal great possibilities for an essentially new approach to the study of the mechanism of nuclear differentiation. Besides, the analysis of mutual relation of the endonuclear symbiont to the nucleus may prove to be one of the effective methods of studying *in vivo* the peculiarities of the functional activity of nuclei.

In the present study attempts have been made to gain some new experimen-

tal data, indispensable for the solution of the following questions: (1) will the localization of symbionts occur only in the micronucleus in all the cases of infection? (2) are the differences between the micro- and macronucleus connected with the character of differentiation, "perceptible" to the *omega* particles? In this way, the aim of the present study consists in determining the level of localization specificity of *omega* particles in the nuclear apparatus of ciliates, i.e., are the *omega* particles able to change their intracellular localization in the case of absence of micronucleus in the cell.

We stated in the previous experiments that no one of the 10 amicronuclear clones separated from 3 natural populations, became infected with *omega* particles in the experimental conditions securing a high effectiveness of infection in the normal cells (Ossipov 1973).

Out of 166 clones with a normal nuclear apparatus (1 macronucleus and 1 micronucleus), 36 resistant ("not infective") were stated by us. This permits to postulate that the inability of amicronuclear clones to infection with *omega* particles is evoked not by the absence of micronucleus in the cell but is connected with some features of paramecia genotype similarly as it has been stated for *kappa*, µm and other particles of *P. aurelia* (Sonneborn 1959, Beale et al. 1969). The conclusive answer to this question might be gained only after special experiments with the infection of pairs identical genetically, differing from one another only by the presence or absence of micronuclei. On the other hand, the more amicronuclear clones are used in experiments, clones gained independently of one another being, of different genotypes — the more reliable answer may be expected in the results.

The second approach to the analysis of factors which determine the specificity of the intracellular localization of *omega* particles is based on the polymorphism of micronuclei described before in *P. caudatum* (Chen 1940, Borchsenius et al. 1968, Borchsenius and Ossipov 1971 a, b, Ossipov and Borchsenius 1973). Within the species *P. caudatum*, clones have been revealed with micronuclei distinctly differing from one another by a number of morphological properties as: form, dimensions, structure, symmetry as related to the longitudinal and transverse axes of nucleus, content of DNA in the nucleus. The morphological type of interphase micronuclei of each clone is a hereditary feature since it is steadily repeated in a number of agamic generations.

The most characteristic morphological types of interphase nuclei of clones used in the present study as compared with the micronuclei of the "wild" type of *P. caudatum*, are presented in the Pl. I 1–11. By means of the cytofluorometric method, the content of DNA in nuclei was determined. Nearly 13-fold differences were revealed in the most remote morphological

types. The role of polyploidization of genomes has been ascertained as one of the factors leading to the polymorphism of micronuclei as described before in ciliates, especially in *P. caudatum* (Borchsenius and Ossipov 1971 a, b). Evidently the degree of polyploidy of nuclei of the marked polymorphic range of micronuclei varies largely within the limits from 2 n to 32 n. Despite such evident differences of the DNA content, sharp morphological differences as well as some others − the nuclei of all· the types of the polymorphic range are capable to the sexual reorganization, and to the normal mitotic divisions in the case of the cell reproduction (Borchsenius et al. 1968, Borchsenius and Ossipov 1971 a) i.e., they posses the fundamental properties of micronuclei.

It should be stressed that a direct correlation of the DNA amount in micro- and macronucleus does not exist in the *P. caudatum* clones used in this study. The maximal difference in the DNA amount in the clones of the polymorphic range in question does not exceed the double quantity (Borchsenius 1971). If we postulated that the essential factor determining the possibility of infection of nuclei with *omega* particles is connected with the functional properties of micronuclei and not with the level of polyploidy, nor with the morphological properties, then the equal effectivness of infection in different morphological types of micronuclei should be expected after an experimental infection with the *omega* particles. The question proved to be resolved most clearly owing to the application of the bi-hetero-micronuclear clones of *P. caudatum* (Skoblo 1968) in the experiments of infection with *omega* particles. Their nuclear apparatus consists of one macronucleus and two micronuclei which are different morphologically but represent the same genotype (Pl. II 16).

Author expressed his most vivid gratitude to L. N. Karvanen and M. S. Rautian for their help in the experiments and to I. I. Skoblo for his kind supply of some microphotograms (Pl. II 13–16).

Material and Methods

As the initial culture, the MI-48 omega *Paramecium caudatum* clone, infected with the *omega* particles (Ossipov and Ivakhnyuk 1972) has been used. In the nuclei of all the *Paramecium* specimens of this clone, several hundreds of symbionts are present. The method of Preer 1969 was followed in preparation of the MI-48 *omega* cells homogenate destined for infecting the "pure" cultures with the *omega* particles as well as for determination of the infection capacity of symbionts. Since the method of infection with the *omega* particles, as applied by us, allowed to gain an intense infection of pure clones, so the homogenate of MI-48 *omega* cells was exclusively used in all experiments for this purpose. No attempts were made to isolate a pure fraction of *omega* particles. No quantitative determination of *omega* particles concentration in the homogenate was carried out. All the experiments, repeated twice, were carried out on one kind of MI-48 *omega* homogenate prepared by the standard method.

The cytological pattern of infection and the intracellular localization of *omega* particles was determined by the light microscope in the dark field and in phase contrast, on unfixed intact as well as on squashed cells. After Feulgen or acetocarmine with Fast green after Dippel, the peculiarities of the nuclear apparatus organization in ciliates were observed. Every paramecium clone was infected no less than twice, some of them even 3–5 times.

The fundamental methods of culture and of manipulation with the paramecia cultures and with the living cells, were applied according to the generally accepted methods (Sonneborn 1950, 1970). The *P. caudatum* cultures were kept at $25° \pm 0.2°C$ in a buffered (pH 6.9) standard lettuce medium, previously infected with *Aerobacter aerogenes*. All the cultures used in the experiments with the infection by *omega* particles, were examined before the experiment as to the absence of symbionts in their nuclear apparatus. In no one case, except the clone MI-48 *omega*, they were detected.

20 or 30 days after addition of homogenate of paramecia MI-48 *omega* into the "pure" cultures, a sample of several hundreds of cells was taken from each clone, and the cytological observations were carried out. On each preparation, up to 100 individuals of paramecia were examined. A clone was determined as infected if at least in several cells the *omega* particles were found. However, in the established conditions of experiment the majority of cells of the culture proved to contain symbionts as a rule if the infection took place. Besides, each infected micronucleus increased in size considerably (up to $25–30\mu$ m after infection, instead of $7–8\mu$m for non-infected nucleus), and contained up to several hundreds of tightly packed *omega* particles (Pl. II 18). For proving that the clone remained "pure" (non-infected) after the experiment, 2–3 additional samples were examined, each of them containing several hundreds of paramecia. The spiral stages of symbionts, reaching up to 12μm of dimensions, are easily and reliably shown by means of the contrast phase microscope on squashed preparations.

In all the most important cases (series of clones with the micronucleus polymorphism and the series of multi-micronuclear clones), 35 days after addition of homogenate, a special cloning was carried out of 40 cells from some cultures which appeared as infected. 30 days after beginning of cloning, the presence of *omega* particles in the cells was determined as before. As result, from the cultures containing as well pure as infected cells, the subclones were isolated in which all the cells contained *omega* particles.

A short description will follow with presentation of the essential characters of *P. caudatum* cultures used in the present study for infection with *omega* particles (Table 1).

Series I contained 33 clones, selected out of 3 different natural populations. Their mating types are related to 3 not identified syngens, the nuclear apparatus is normal: 1 macro- and 1 micronucleus of "wild" type. In the former experiments (Ossipov 1973) they all were characterized by the capacity of an intense infection with *omega* particles. In this way, those cultures served in the experiments as a sort of control for evaluation of the infection capacity of *omega* particles in the prepared homogenate of MI-48 *omega* cells. The homogenate MI-48 *omega* applied in the experiments secured an intense infection of all the control cultures of *P. caudatum*.

Series II: 26 clones of two mating types (m. t.) of one syngen (m. t. M-13 and m. t. M-17) which have been initiated from exconjugants and exautogamonts (Borchsenius et al. 1968, Ossipov and Skoblo 1968) are characterized by micronuclei of different morphological types (Pl. I 1–11, Table 1). Despite the morphological differences in the interphase nuclei of those clones — as shown in the Pl. I — it is known that the ratio of DNA content in the micronucleus of type ODSI-22: MG-8aI-12: M-17-6-2-28-14: MS-47a-I-12-7 forms a range 1 : 2 : 8 : 16 if the DNA quantity in the micronuclei of the clone ODSI-22 has been accepted as a unit.

Series III of two bi-hetero-micronuclear clones: MM17-14a-14 and NG-14a4-41, each cell

contains two micronuclei of different morphological types, however, of an identical genotype (Pl. II 16).

Series IV consists of twenty multi-micronuclear clones containing as a rule from 2 up to 4 micronuclei of smaller dimensions than the micronuclei of the "wild" type (Skoblo 1968):

Table 1

Results of Experiments with Artificial Infection of *Paramecium caudatum* Clones with MI-48 *omega* Particles from the Homogenate. Designation: + clone proved to be infected, − clone remained "pure", clones with identical morphological types of micronuclei joined with bracket, cultures clonized after infection with *omega* particles are marked by asterisk

Series of Experiment and Indexes of Clones	Peculiarities of Organization of the Nuclear Apparatus of Clones	Capacity of Clones to Infection with *omega* Particles
1	2	3
I Series:		
M-8	normal nuclear apparatus	+
M-10	,,	+
M-15	,,	+
M-17-6	,,	+
8M-10	,,	+
8M-13	,,	+
8M-16	,,	+
8M-19	,,	+
8M-20	,,	+
8M-22	,,	+
MI-28	,,	+
MI-34	,,	+
MII-1	,,	+
MII-2	,,	+
MII-5	,,	+
MII-6	,,	+
MII-8	,,	+
MII-9	,,	+
MII-13*	,,	+
MII-14	,,	+
MII-15*	,,	+
MII-18	,,	+
MII-19*	,,	+
MII-20	,,	+
GtIII-11	,,	+
GtIII-1	,,	+
GtIII-2	,,	+
GtIII-7	,,	+
GtIII-10	,,	+
Čr-4	,,	+
Čr-5	,,	+
Čr-11	,,	+
Čr-15	,,	+

II Series:
MS-47a1-12-17	mutated micronucleus	+
MS-47a1-1	,,	+
MS-47a1-2	,,	+
MS-47a1-3	,,	+
MS-47a1-4*	,,	+
D-31a-37*	,,	+
D-66a-19	,,	+
M-17-6-2-28-14*	,,	+
KG-2a3	,,	+
KG-12a3	,,	+
MG-11a1	,,	+
MG-23a3	,,	+
MG-34a1	normal micronucleus	+
MG-38a1	,,	+
MG-38a3	,,	+
MG-55a3	,,	+
D-91a*	mutated micronucleus	+
D-199v1k-31	,,	+
D-199v1k-28*	,,	+
D-199v1k-15	,,	+
D-199v1k-32	,,	−
MG-8a1-12*	,,	+
DS-6a-20	,,	+
DS-3a-9*	,,	+
DS-1a-5*	,,	+
ODSI-22	,,	+

III Series:
MM17-14a-14*	bi-hetero-micronuclear	+
NG-14a4-41*	,,	+

IV Series:
D-II6a-2-19	bi-micronuclear	+
D-116a-28	,,	+
D-121a-23	,,	+
D-121a-28	,,	+
D-121a-7	,,	+
DM-6a	,,	+
MM55-31v	,,	+
MM17-150a	,,	+
NG-53a2	,,	+
3D-4a-8	,,	+
66-D-2-1	,,	+
DM128-91a*	tri-micronuclear	+
DM128-91a-2	,,	+
DM128-91a-3	,,	+
DM128-91a-7	,,	+
DM128-113a	,,	+
DM128-113a-36	,,	+
MM55-71v	,,	+
DM128-91a-52*	tetra-micronuclear	+
MM55-71v-18	,,	+

V Series:		
1st group		
8M-15	amicronuclear	—
8M-23	"	—
MII-4	"	—
MII-7	"	—
MII-10	"	—
MII-11	"	—
MII-12	"	—
GtIII-4	"	—
2nd group		
KG-11a3	normal micronucleus	+
KG-11a2	amicronuclear	—
M-17-6	normal micronucleus	+
M-17-6-10	amicronuclear	—
3rd group		
MG-40a1-1	amicronuclear	—
MG-40a1-2	"	—
MG-43a1	"	—
MG-43a2	"	—
MG-32a3	"	—
MG-51a1	"	—
MG-51a2	"	—
2D-10v	"	—
00-1	"	—
NG-16a1	"	—
MM17-39a	"	—
MM17-101a	"	—
8M-23	"	—
MII-7	"	—
MII-10	"	—
M-17-6-10	"	—
ODSI-3	"	—
ODSI-6	"	—
ODSI-7	"	—
ODSI-8	"	—
ODSI-11	"	—
ODSI-12	"	—
ODSI-14	"	—
ODSI-15	"	—
00-2	"	—
NG-1a2	"	—
NG-1a3	"	—
NG-1a4	"	—
NG-2a4	"	—
NG-12a1	"	—
NG-12a2	"	—
MM17-61a	"	—
NG-2a3	"	—

12 clones with 2 micronuclei. 7 clones with 3 macronuclei and 2 clones with 4 micronuclei (Pl. II 13–15).

Series V contained 40 amicronuclear clones of different origin, represented by 3 groups (Pl. II 17):

1st group of 8 clones gained out of 3 natural populations, the mating types belonging to two unidentified syngens.

2nd group of two pairs of clones: KG-IIa2 and KG-IIa3(Mi⁻): M-17-6 and M-17-6-10(Mi⁻). The amicronuclear mutants arose presumably as result of a spontaneous impairment of the normal distribution of telophase nuclei to the daughter cells (Ossipov and Skoblo 1968, Skoblo 1968).

3rd group of 33 clones of the same syngen as the clones of the 2nd group and the entire 2nd series. Macronuclei of those clones arose as a result of an unusual for *P. caudatum* process of nuclear reorganization: regeneration of macronucleus (Ossipov and Skoblo 1968, 1973, Skoblo 1968, 1969). The principal peculiarity of this process is connected with the fact that in the cell, after completion of the sexual process, the new macronucleus is developing, not as usually of the division products of zygotic nucleus, i.e., of synkaryon, but of separate fragments of the old desintegrated macronucleus (Sonneborn 1947). In contrast to *P. aurelia*, in which regeneration leads to formation of heterokaryotic cells (Sonneborn 1947), the new macronucleus being of the old genotype and micronucleus of hybrid one), according to our results, in *P. caudatum* the above process evokes the formation of exclusively amicronuclear cells. It should be, however, taken into account that without application of genetic markers, the possibility cannot be absolutely excluded that some of the amicronuclear clones of the 3rd group arose as result of a spontaneous loss of micronuclei at the most early stages of the life cycle after a normal nuclear reorganization. Of course we took care that this possibility would not influence the evaluation of our results and our conclusions.

Results and Discussion

In connection with the arising problems, the aim of our experiments was limited to an analysis of the character of correlation between the peculiarities of the nuclear apparatus of clones and their receptivity to infection with *omega* particles. The principal results of our experiments with artificial infection with *omega* particles from the homogenate of cells MI-48 *omega* clones with various peculiarities of their nuclear apparatus are presented in the Table 1 and may be formulated in the following way.

The MI-48 *omega* cell homogenate applied in the experiment, secured a high effectivness of the infection of clones which before showed the capacity of infection with the *omega* particles (Ossipov 1973). In the control experiment (series I), all the 33 clones with the normal nuclear apparatus, proved to be infected.

In these experiments especially striking appear the fully controversive results with the 43 amicronuclear clones (series V). No one of them was able to be infected and to support the *omega* particles. The only common property of those 43 clones is the absence of micronuclei in cells. In the combination of other properties as: origin of clone, the way of loss of

micronucleus, type of mating and syngen — a great variability could be expected. Consequently — the presence of micronuclei in cells is the obligatory condition of infecting paramecia with *omega* particles.

The direct correlation of the micronucleus absence and the loss of possibility of infection with symbionts stands out of the results in the 2nd group, of the V series of experiments, shown on the two pairs of clones: KG-IIa2 and KG-IIa3(Mi⁻), M-17-6 and M-17-6-10(Mi⁻). The clones of a pair differ only by the presence or absence of micronucleus, being fully similar genotypically. Both amicronuclear clones proved to be incapable to infection whereas both normal became infected by *omega* particles.

In contrast to the full absence of infected clones among the studied 43 amicronuclear ones, a striking factor appears in the high frequency (97.6% — 82 out of 84 clones) of infected clones among those containing micronuclei. As yet, the factor involving the lack of infection capacity in some clones with a normal nuclear apparatus (2.4% — 2 out of 84 clones) remains unknown. It may be postulated that this is connected with a certain genotypic character of those two exceptional clones. In this way, the presence of micronuclei in the cell appears to be not the only factor indispensable for infection at favourable conditions.

As shown by the results of the II, III and IV series of experiments, the capacity of clone to infection is independent of the morphological type of micronuclei nor of their number in the cell. The clones of all the types of micronucleus are able to be infected despite the considerable differences in their nuclear morphology and in the DNA content in single nuclei. It should be emphasize on the other hand, that the micronuclei of all the morphological types always increase in size (up to 25–30 μm, Pl. II 18) after being infected with the *omega* particles. This is characteristic not only for the "wild" and bigger morphological types but also for some small comet-shaped forms with a very low DNA content. In the infected paramecia of bi-hetero-micronuclear clones (the III series), a prefered infection of one of the types of micronucleus is not observed since both nuclei always contain symbionts.

Consequently such factors as the degree of polyploidy and morphology of nucleus fail to control the capacity of nucleus to the infection with symbionts. It should also be taken into consideration that the generative polyploidy of ciliates posesses a number of peculiar features which make it sharply different from the polyploidy of the majority of other organisms and from the polyploidy of macronuclei. It evidently fails to evoke the rise of functional activity of nuclei (Ossipov and Borchsenius 1973). The polyploidy of micronuclei does not appear phenotypically what is connected with the morpho-functional differentiation of nuclei. This secures a genetic inertness of micronuclei in the vegetative cells of ciliates. The capacity of

nuclei to infection is also independent of the number of micronuclei in the cell. Out of 20 multi-micronuclear clones (IV series), 19 were capable to infection with *omega* particles. The cytological analysis of infected multi-micronuclear paramecia proved that all the micronuclei become infected in the cells. After a prolonged keep of paramecium cultures (up to 2 months) "pure" micronuclei together with the infected ones have never been observed.

In conformity with the results of our former study concerning the mating type of clones of the II, III and IV series (Ossipov and Skoblo 1968, 1973, Skoblo and Ossipov 1968, Skoblo 1968, 1969) and our special experiments on the identification of the mating types in the majority of clones of the I series, it may be assumed that the cultures used in the present study should be related to 3 independent syngens (Sonneborn 1957, Ossipov 1963). We did not as yet succeed, however, to identify the clones of our 3 syngens with the cultures for which the generally accepted determination of mating types and syngens was established (Sonneborn 1957). Nevertheless the fact of revealing in our experiments a very high frequency of infection (97.6%) among the micronuclear clones of all the mating types studied, permits to state with a high probability that the genetic system controlling the mating type in *P. caudatum*, fails to exert influence on the capacity of micronuclei of infection with the *omega* particles. This fact makes the *omega* particles of *P. caudatum* sharply different from the other intranuclear symbionts, e.g., *alpha* particles revealed in the macronucleus of *P. aurelia* (Preer 1969). Out of 44 lines of *P. aurelia* only 6, corresponding to the syngen 2 proved to be capable of infection with *alpha* particles. A clear adaptation to some syngens only has been well established for some cytoplasmic symbionts of *P. aurelia* (Sonneborn 1959, Beale et al. 1969).

Out of experimentally infected cultures 16 were selected for cloning (in the Table 1 marked by asterisk) while 13 of them produced subclones contained *omega* particles in all cells. However, subclones could not be gained of 3 cultures although more than 200 cells instead of 40, as usually, were selected for every cloning. Three exceptional clones: ODSI-22 (220 cells were clonized), DS-3a-9 (225 cells), MG-8aI-12 (240 cells) could be kept in the test tube cultures only, whereas the cultures on plates of organic glass never gave origin to the cellular lines. Among all the remaining clones used for experiments, death of 100% of cells was observed, namely in these 3 above mentioned clones and in the case when cells were taken out of control cultures, not treated with MI-48 *omega* homogenate. Consequently the loss of cloning capacity in the 3 cultures mentioned is not connected with the infection of their micronuclei with the intranuclear symbionts. The process similar to that described above for 3 clones, is usually evoked by the progressive senescence of cultures (Wichterman 1953, Miyake 1957, Sonneborn 1957, 1960, Sonneborn and Dippell 1960).

Subsequently such cultures completely loose their reproduction capacity even in the test tubes, and die.

The experimental data of our study indicate with a high reliability that the *omega* particles are strictly specific as to their localization in the nuclear apparatus of *P. caudatum*. They are capable of infecting and persisting for a long time only in the micronuclei, being never found in macronuclei. This feature of *omega* particles permits to use the phenomenon of endonucleosymbiosis as an original method for analysis of the mechanisms of nuclear differentiation in ciliates.

The data concerning the symbionts of ciliates, reported in the literature permit to hope that the possibilities of the described method for the study of structure and function of the cell nucleus may prove to be very extensive. Recently symbiotic bacteria of a specific localization have been detected, independent of *omega* particles of *P. caudatum*. In the macronucleus of *P. aurelia alpha* particles of a specific localization have been studied in details by Preer (1969). In contrast to the *omega* particles, the symbionts of the *P. aurelia* nucleus do not evoke hypertrophic changes of dimensions of any structure of the nuclear apparatus. By means of electronmicroscopy, in *P. multimicronucleatum* only in the hypertrophic perinuclear space of the nuclear envelope of macro- and micronuclei, *epsilon* particles — rikketsia-like microorganisms — have been detected (Jenkins 1970). One electronograms of *Neobursaridium gigas*, in the macronuclei just of one of clones, numerous bacteria have been detected: 2000 particles in one nucleus, each symbiont being separated from the nucleoplasm by a separate membrane (Nilsson 1969). In this case endosymbiosis indicates characteristic changes in the ultrastructure of macronuclei. The nucleoplasm of macronucleus at the place of accumulation of symbiotic bacteria proved to be deprived of the typical chromatin granules (0.1 μm) as usually present in this type of nucleus, but is interlaced with a thin net of granule chains (300 Å in diameter) of an unknown nature. At last, in *P. caudatum*, besides the *omega* particles the *iota* particles have been found, being localized exclusively in macronuclei (Ossipov et al. 1973).

РЕЗЮМЕ

С целью дальнейшей разработки предложенного ранее оригинального "симбиотического" метода анализа структуры и функции ядер инфузорий (Ossipov and Ivakhnyuk 1972) изучена специфичность внутриядерной локализации омега-частиц *Paramecium caudatum* MI-48. Гомогенатом парамеций MI-48 омега искусственно заражали клетки других 127 клонов, обладающих разными особенностями организации ядерного аппарата. В работе использовали 84 микронуклеарных клона, из них: 28 — составляют широкий полиморфный ряд микронуклеусов, 2 — ди-гетеро-микронуклеарных и 21 — мульти-микронуклеарных — и 43 амикронуклеарных клона.

Ни один из 43 амикронуклеарных клонов не удалось инфицировать омега-частицами. Результаты, полученные с 84 микронуклеарными клонами, показывают, что наличие в клетках, микронуклеусов — обязательное условие инфекции парамеций. Способность клона к инфекции омега-частицами не зависит от морфологического типа микронуклеусов и числа генеративных ядер в клетке. Таким образом, омега-частицы обладают строгой специфичностью в отношении локализации в ядерном аппарате инфузорий *P. caudatum*.

Есть основания надеяться, что возможности "симбиотического" метода в изучении структуры и функции клеточного ядра окажутся весьма широкими, поскольку за последнее время в литературе появились сообщения (Nilsson 1969, Preer 1969, Jenkins 1970, Ossipov and Ivakhnyuk 1972, Ossipov et al. 1973, a,b). о различных симбиотических бактериях инфузорий, которые проявляют строго специфическую для каждого типа частиц локализацию в ядерном аппарате.

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EXPLANATION OF PLATES I–II

Light microphotograms of *Paramecium caudatum* with the peculiarities of the nuclear apparatus organization of some clones used in the experiments with the infection with *omega* particles MI-48. Whole mount preparations. Feulgen.

Plate I 1–11: Interphase micronuclei of the principal morphological types 400 × (after

1 — clone D-31a
2 — clone D-66a
3 — clone M-17
4 — clone D-91a
5 — clone ODSI-5
6 — clone D-199v-1k
7 — clone MG-8aI
8 — clone D-199v-5
9 — clone DS-3a

PLATE I

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D. V. Ossipov

auctor phot.
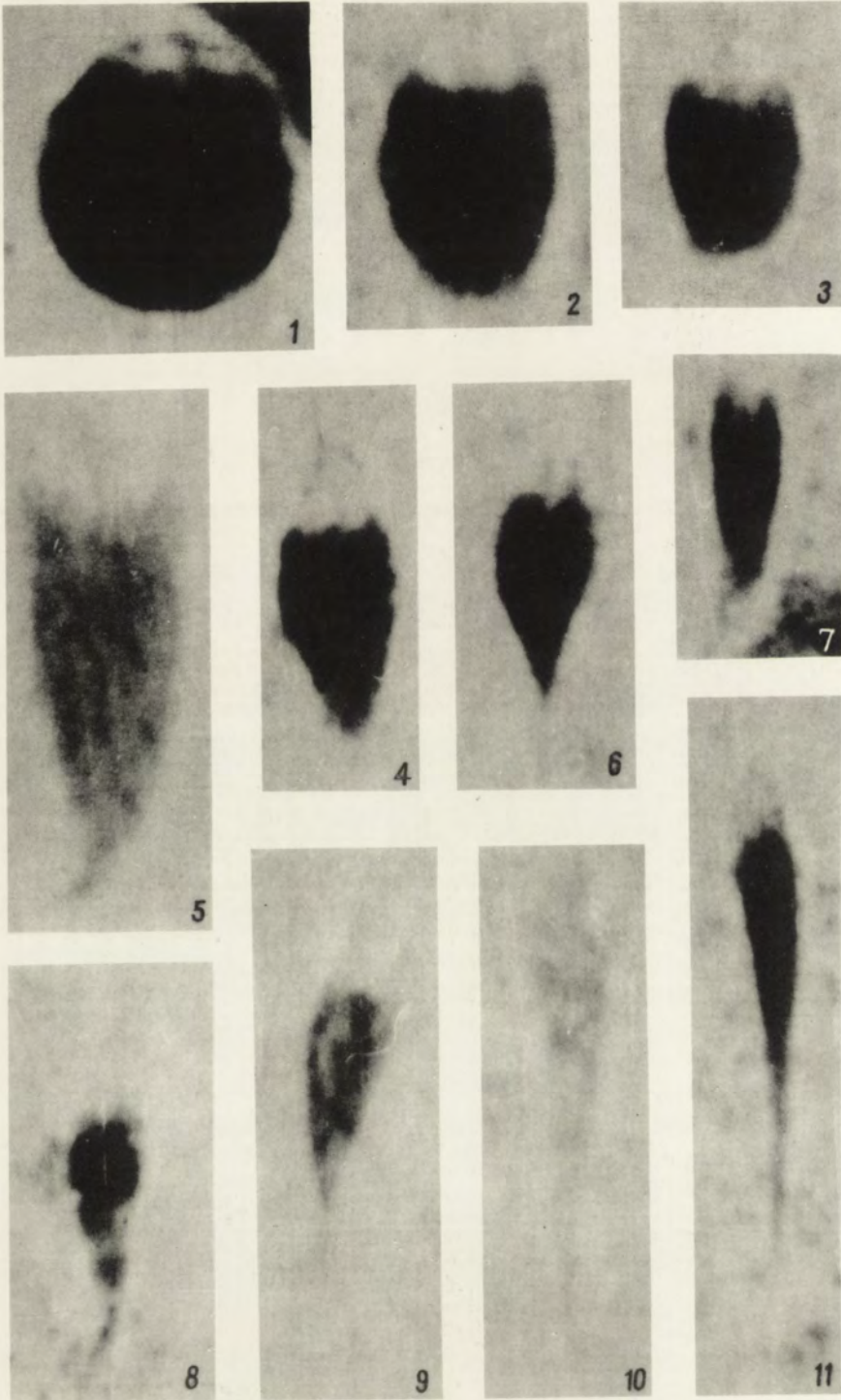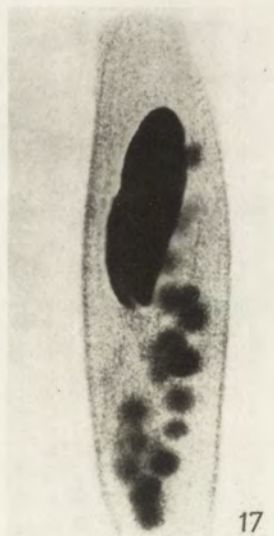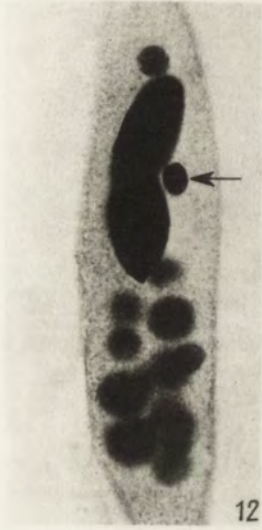
10 — clone DS-6a
11 — clone DS-1a

Plate II 12—18: Cells of clones with some organization peculiarities of the nuclear apparatus. Micronuclei are marked by arrows.
Numerous slightly stained bodies in the paramecia cytoplasm — food vacuoles.
12 — cell with normal nuclear apparatus (1 macro- and 1 micronuclei) 350 ×
13 — cell of bi-micronuclear clone 350 ×
14 — cell of tri-micronuclear clone 350 ×
15 — cell of tetra-micronuclear clone 350 ×
16 — dividing cell of bi-hetero-micronuclear clone 350 ×
17 — cell of amicronuclear clone 350 ×
18 — cell infected with *omega* particles 580 ×

F. DINI, P. BRACCHI and P. LUPORINI

Cellular Cycle in Two Ciliates Hypotrichs [1]

Synopsis. Macronuclear S phase occupies 1/3–1/4 and 1/2–1/3 of the cellular cycle of *Diophrys scutum* and *Oxytricha bifaria* respectively. Except for a short G_2, not exceeding 1 h, G_1 phase takes up the remaining period of the cell cycle in both species. The micronuclear S phase, which lasts from 30 to 60 min, occurs immediately before mitosis in both *Diophrys* and *Oxytricha*; a G_2 phase is thus lacking. Micronuclear S phase precedes the macronuclear one in *Diophrys*, while the opposite occurs in *Oxytricha*.

Introduction

The protozoan ciliates, characterized by the presence of two types of nuclei generally of different ploidy level, show a considerable heterogeneity in their cellular cycle. The chromatin material of S phase may involve both the macro- and the micronucleus (i) at different moments of the interdivision period, not only orthodoxally in the middle of the cycle, but also immediately before or just after nuclear division. This fact causes such a variation in the extension of both the G_1 and the G_2 phases, that, in the extreme cases, the presence of one phase exludes the other.

Moreover, the beginning of the synthesis phase does not coincide nor is it of equal duration in the two types of nuclei. Many examples are reported in Raikov's monography (1969) in which the author points out that a difference in stocks and in the experimental conditions can cause further variability at species and clonal levels.

Dealing with this problem other works have appeared in the meantime: Kudrjavtsev (1966) on *Paramecium putrinum*, Rao and Prescott (1967) on *Paramecium caudatum*, Minutoli and Hirshfield (1968) on *Blepharisma americanum* and *B. intermedium*, Radzikowski (1969) on *Chilodonella cucullulus*, Nilova (1969) on *Bursaria truncatella*, Jerka-Dziadosz and Frankel (1970) on *Urostyla weissei*, Ammerman (1970) on *Stylonychia mytilus*, Kitaoka (1971) on *Paramecium multimicronucleatum*, Takahashi (1972) on *Hemicycliostyla sphagni*, Ruthman (1972) on *Keronopsis rubra*, Luporini and Bracchi (1973) on *Euplotes crassus*.

This type of research was extended to two other genera of ciliates, *Diophrys* and *Oxytricha*, using ³H thymidine as nuclear marker. The study was carried out in order to increase our knowledge of the different types of cellular cycle in hypotrichs with morphologically rather similar nuclear apparatuses, and as a prerequisite for the study of the macro- and micronuclear cycle in sexual reproduction (P. Luporini and F. Dini, in preparation).

Material and Methods

A strain of *Diophrys scutum* collected at the Meloria sand-banks (Leghorn) and a strain of *Oxytricha bifaria* collected in the fresh water canals in San Rossore (Pisa) were used in the present research.

Both were grown at a temperature of 22–23°C. *Diophrys* was fed with the diatom *Phaeodactylum tricornutum*; *Oxytricha* was grown in lettuce medium inoculated with *Aerobacter* 24 h before use. The nuclear apparatus of both species in unlabelled experiments was stained by Feulgen reaction.

Labelling experiments were carried out using ³H thymidine (Radiochemical Centre Amersham England, specific activity of 5 Ci/mM) diluted in salt or fresh water respectively with a final activity of about 20 μci/ml for *Diophrys* and 10 μci/ml for *Oxytricha*.

Labelled animals were prepared for the autoradiographic examination as described by Luporini and Bracchi (1973). The ³H thymidine uptake was calculated by subjecting the cells to "pulses" of different duration (15–30–45–60 min). Macronuclear replication bands appeared to be labelled after 45–60 min in *Diophrys*, and after only 30 min in *Oxytricha*.

Results

The nuclear apparatus of *Diophrys scutum* consists of a macronucleus divided in two stick-like pieces of an average length of 70 μm and a variable number of micronuclei (0–14) that are 2–3 μm in diameter. The cellular cycle of well fed animals lasts approximately 15 h at 22–23°C.

Replication bands are usually present in 27.7% of animals and average number of micronuclei is 4.1 in unsynchronized vegetative cells examined three times in a month. This initial observation allows us to evaluate the duration of the macronuclear S phase as approximately 1/3–1/4 of the whole cycle on the basis of Monesi's (1969), and Prescott and Stone's (1967) assumptions. The duration of the S phase in homogeneous populations in which all cells divide, may, in fact, be deduced from the product of the generation time per the percentage of labelled cells, which in our case is equal to the percentage of animals with replication bands.

In a period of 30 min approximately 400 dividing cells were collected; after cell separation, daughter cells of equal age were Feulgen stained at 2 h intervals. The results are reported in Fig. 1. The average number of micronuclei which varies very little up to 10 h after cell division increases immediately thereafter being doubled at the time of cytokinesis. After 12 h,
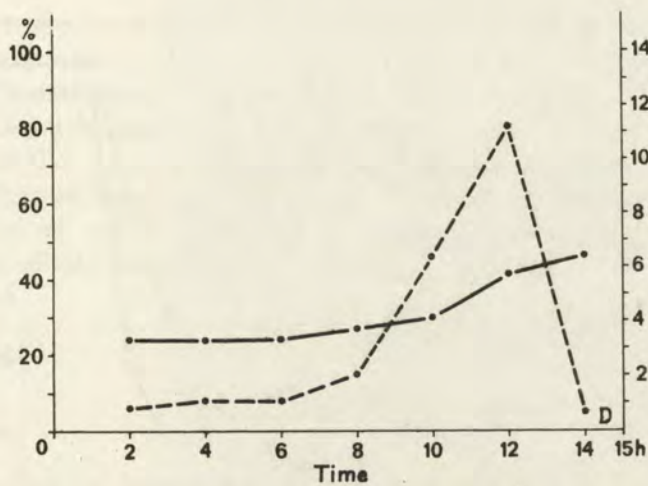
Fig. 1. The occurrence of macronuclear replication bands and number of micronuclei during cell life cycle in *Diophrys scutum*. The abscissa refers to the generation time. The left ordinate refers to the cell percentage with macronuclear replication bands (dotted line) and to mean number of micronuclei per cell the right ordinate (continuous line)

80% of the cells show macronuclear replication bands, which are present in only approximately 15% of the 8 h old cells. The 100% synchronization has never been reached under these laboratory conditions, even for a single cell cycle. As the percentage of cells with replication bands markedly increases after 9 h and rapidly decreases after 13 h since fission, one may conclude that the synthesis of macronuclear DNA requires approximately 4 h. Therefore, also these data confirm that the macronuclear S phase occupies 1/3–1/4 of the cellular cycle in *Diophrys*. This phase precedes a short G_2 of about 30 min corresponding to the fusion of the two macronuclear pieces occurring just before its amitotic division. The micronuclear mitoses precede the beginning of cytokinesis as suggested by the doubling of the average number of micronuclei occurring just before the appearance of the fission furrow.

At this point ^{3}H thymidine incorporation was used as marker for a more accurate study of the nuclear cell cycle timing. [3]H thymidine was added to a large group of cells two hours since their origin; from this culture, 60–80 cells at time were taken out and prepared for autoradiography every hour starting from the 6th up to the 13th hour of their interdivision period. Parallely, from a culture of asynchronous vegetative animals placed in ^{3}H thymidine solution every hour all dividing cells were singled out and examined.

The macronuclear cycle phases that result from these experiments (see Fig. 2) coincide with those deduced from the data reported in Fig. 1. In Graph A of Fig. 2 (first experiment) one sees that in the majority of
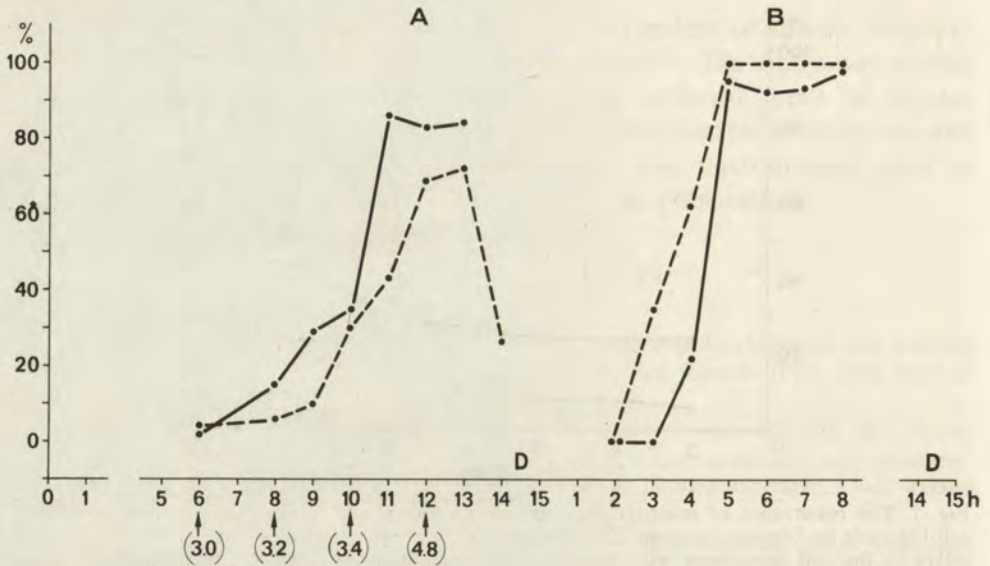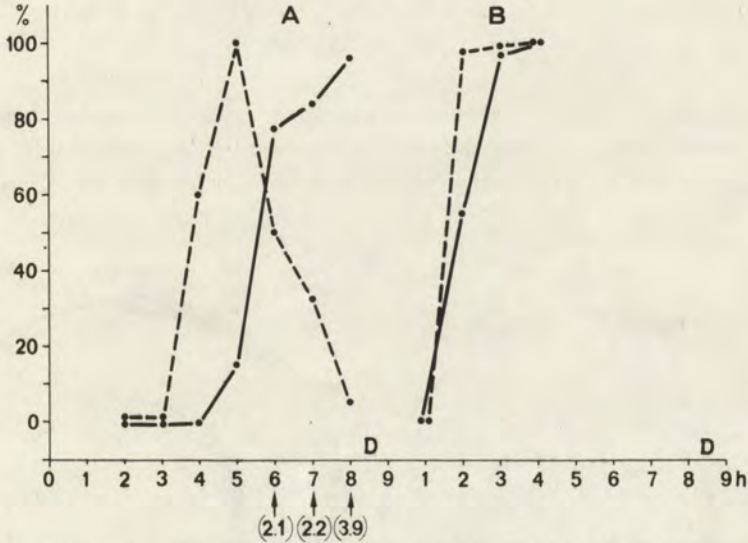
Fig. 2. Reorganization of nuclear apparatus during cell life cycle of *Diophrys scutum* in two series of Experiments (A and B) with ³H thymidine incorporation. The abscissa refers to the generation time. Micronuclear mean numbers per cell at different hours are reported in parenthesis. The ordinate refers to the percentage of cells with macronuclear replication bands (dotted line) and to the percentage of cells with at least one labelled micronucleus (continuous line)

10–14 h old cells (70–80%) there is labelling at various levels of the macronucleus. From Graph B Fig. 2 (second experiment) it appears that in the animals singled out in division, the macronucleus is still replicating three hours before fission. The slightly delayed cell cycle noticeable in the second set of experiments (comp. Fig. 1 and Fig. 2) may be ascribed to the delaying effect of ³H thymidine treatment. The micronuclear DNA replication which generally involves, in slight asynchrony, all micronuclei of a cell may begin (about 30% of animals) before the 10th h of interfission interval, whereas the majority of cells show labelled micronuclei just after this time (Graph A Fig. 2). The micronuclear S phase immediately precedes mitosis as the micronuclei mean number is already doubled at the 12th hour when the S phase is completed (see the data reported in parentheses on abscissa in Graph A of Fig. 2 and on the ordinate in Fig. 1). Moreover, (Graph B Fig. 2) labelling doesn't appear over micronuclei of cells kept in ³H thymidine for the last 3 h of cell cycle. This fact excludes the occurrence of S phase after mitosis. The data of Graph A also indicate that a G_2 phase is either lacking or very short. In fact, there is a two hour span between the beginning of S phase and the doubling of micronuclei: this time must be portioned between S phase and mitosis.

The complete cellular cycle of *Diophrys scutum* is shown in Fig. 4.

The other species of Hypotrich that was examined is *Oxytricha bifaria* which has a nuclear apparatus consisting of a macronucleus of two oval pieces, each approximatelly 25 μm in length, and two micronuclei, sometimes three, of 2–3 μm in diameter. The generation time of this species is about 9 h at 22–23°C.

The replication bands appear in Feulgen stained preparations about 4 h after the end of cellular fission and, in general, one does not observe micronuclear mitosis until the bands have extended over 3/4 of each macronuclear piece.

Fig. 3. Reorganization of nuclear apparatus during cell life cycle of *Oxytricha bifaria* in two series of Experiments (A and B) with ³H thymidine incorporation. Explanation see Fig. 2

In order to study the various phases of the cell cycle, the same type of experiments as in *Diophrys* was performed. Dividing cells were placed in a ³H thymidine solution, from which they were then removed in groups at intervals of 1 h. At the 5th h after division, the whole cell population present labelled macronuclei with ³H thymidine incorporation starting in the 3–4th h. At about the 7th h after division 70–80% of the cells show the two macronuclear pieces completely labelled (Graph A Fig. 3).

Vegetative cells were put into ³H thymidine solution, and as they presented the fission furrow were singled out and prepared for autoradiography at one hour intervals. Labelling granules do not appear over the macronucleus of cells maintained in ³H thymidine solution for only the last hour (Graph B Fig. 3) of the cell cycle.

Thus the macronuclear S phase of *Oxytricha* lasts approximately 3.5 h, preceded by a G_1 of equal duration and followed by a short G_2 as in *Diophrys*.

The beginning of synthetic micronuclear activity is asynchronous with respect to that of the macronuclei; labelling appears over the two micronuclei around the 5–6th h after fission (Graph A Fig. 3).

The micronuclear S phase of *Oxytricha*, which doesn't exceed 1 h, as the results reported in Graph. A Figure 3 suggest, seems to occur just before mitosis as in *Diophrys*. This is confirmed by the data of Graph B Fig. 3 which show the lack of labelling over micronucleus in the last hour before fission, when the micronuclear number has already doubled.

Conclusions

The cellular cycles of *Diophrys* and *Oxytricha* are similar and may be compared to those of *Urostyla* and *Stylonychia* among hypotrichs previously described by Jerka-Dziadosz and Frankel (1970) and by Ammermann (1970) respectively. In fact the macronuclear S phase occupies 1/3–1/4 and
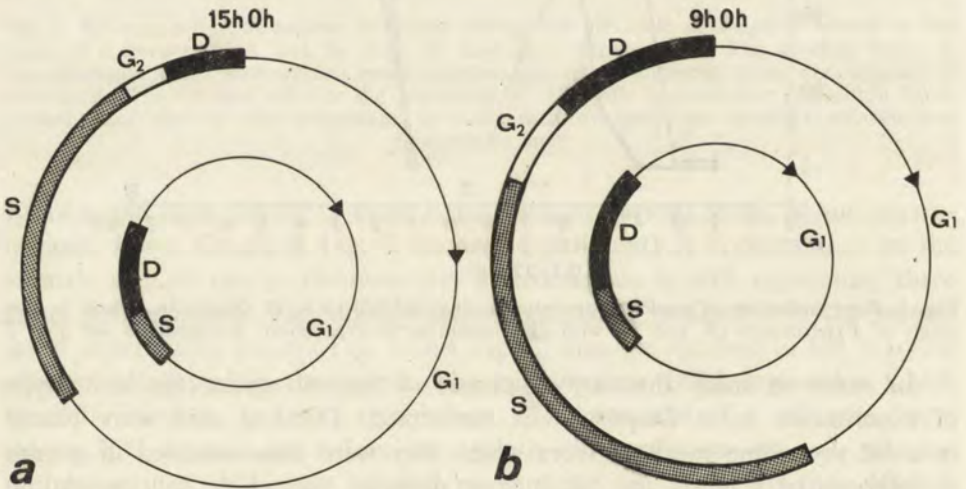
Fig. 4. Diagrams of the life cycle of *Diophrys scutum* (a) and *Oxytricha bifaria* (b). The cycle proceeds clockwise. The outer circle refers to macronuclear, the inner one to micronuclear cycle

1/2–1/3 of the interdivision period respectively in *Diophrys* and *Oxytricha*, whereas the G_1 phase takes up almost the whole remaining period in both species (Fig. 4).

Moreover, micronuclear DNA synthesis, as far as we were able to deduce, lasts approximately 30–60 min in both species and occupies only a small part of the cell cycle during which micronuclei are in G_1.

By and large, the few discrepancies observed, which do not invalidate the general pattern described above, are the following: in *Diophrys* the

micronuclei S phase precedes the macronuclear one, whereas in *Oxytricha* it is clearly the contrary. In addition, probably due to their high number, the micronuclei of *Diophrys* sometimes replicate slightly asynchronously, a phenomenon that never occurs in *Oxytricha*.

The most evident peculiarities of the cell cycle of these four Hypotrichs may be identified, at macronuclear level, by the presence of long and well defined G_1 and S phases, and at micronuclear level by the absence of the G_2 period, since synthesis of the micronuclear chromatin material immediately precedes mitosis. The uniformity of the number of phases that take place in the cellular cycle and their comparable duration, likely reflects a similar structure at least at the morphological level (fragmented macronuclei and more than one micronucleus).

The absence of the macronuclear G_2 in *Stylonychia*, as revealed by Ammermann (1970), may be regarded as not affecting the homogeneity in the cell cycle of this group of Hypotrichs. In fact, even when this phase is present, (*Diophrys*, *Oxytricha*, *Urostyla*) not only it is short, but it almost corresponds to the fusion process of the macronuclear pieces and the subsequent rearrangement of the chromatin material in resulting nucleus. We may regard these phenomena as part of the amitotical division.

This behaviour contrasts with what is known in *Euplotes* characterized by a single C-shape macronucleus and one micronucleus. The peculiar cell cycle of *Euplotes* consists of a long macronuclear S phase, that lasts more than half of the generation time, and above all by the lack of the micronuclear G_1 phase (Prescott et al. 1962).

In any case, it should be pointed out that there are remarkable differences in the cellular cycle among Ciliates, which are not matched by the remaining Eucariotes. In the Metazoan cells a constancy of both S and G_2 phases, with few exceptions, exists not only in the same cell population itself, although subjected to different physiological and environmental conditions, but also in different cellular types (see Monesi 1969). It is known that different metabolic activities are linked to the various cellular phases (Monesi 1969) or to the macronuclear phases in Ciliates (Prescott and Stone 1967). It may thus be supposed that triggering of sexual processes in Ciliates are associated with well defined phases of their cellular cycle.

RÉSUMÉ

La phase S du macronoyeau représente 1/3–1/4 du cycle céllulaire chez *Diophrys scutum* et 1/2–1/3 chez *Oxytricha bifaria*, en dehors d'une brève phase G_2 qui ne dépasse pas 1 h, tout le reste du temps du cycle cellulaire chez le deux espèces est couvert par la phase G_1.

La phase S du micronoyeau qui dure 30 à 60 min intervient chez *Diophrys* comme chez *Oxytricha* juste avant la mitose, il manque donc de la phase G_2.

La phase S du micronoyeau précéde celle du macronoyeau chez *Diophrys*, tandis que c'est le contraire chez *Oxytricha*.

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Ulrich BUITKAMP

The Morphogenesis of *Oxytricha agilis* Engelmann (*Ciliatea, Hypotrichida*)

Synopsis. Infraciliature and morphogenesis of *Oxytricha agilis* Engelmann were investigated by means of a modified Protargol technique. The binary fission begins with the appearance of an oral primordium between the 3 postoral ventral cirri and the left marginal row. In this *O. agilis* differs from other species of *Oxytricha*, where an oral primordium appears near the first transverse cirrus. The caudal cirri develop from the 1st, 2nd and 3rd dorsal kinety. The 4th dorsal ciliary row is a product of the marginal row. It takes no part in the formation of any cirri.

Introduction

According to Borror (1972) the following species of ciliates which were named by Kahl (1932) are synonymous, *Uroleptus agilis* Engelmann, *Oxytricha* (*Urosoma*) *macrostyla* Wrześniowski, *Opisthotricha emarginata* Stokes and *Oxytricha* (*Urosoma*) *gigantea* Horváth. He therefore suggests to rename this species *Oxytricha agilis* Engelmann. It is the purpose of this work to describe the morphology and morphogenesis of this relatively unknown hypotriche ciliate.

Materials and Method

The ciliate was found in the upper 0–3 cm of the soil from a pasture near Bonn. For investigation *O. agilis* was cultivated in petri-dishes. Culture medium was diluted soil-extract. *O. agilis* fed on algae and bacteria which developed on a grain of polished rice. The division stages were stained by a modification of the Protargol technique of Tuffrau (1967) (Modification by Wilbert, in press).

List of Abbreviations

AZM	= adoral zone of membranelles	MCL	= left marginal cirri
CC	= caudal cirri	MCLP	= left marginal cirri primordium
CCP	= caudal cirri primordium	MCR	= right marginal cirri
CF	= cirral field	MCRP	= right marginal cirri primordium
CV	= contractile vacuole	OP	= oral primordium

DC   = dorsal cilia
(4.)DCP = (fourth row of) dorsal ciliary pri-
                mordium
FC   = frontal cirri
MA, MI = macro-, micronucleus

RB   = reorganization band
TC   = transverse cirri
UM   = undulating membrane
VC   = ventral cirri

Results

Oxytricha agilis is about 130 μm long and lancet-shaped. It has two macronuclei, whereas the number of micronuclei varies from 2 to 5. The contractile vacuole lies on the left side in the middle of the body. It empties every 10 to 12 sec in the culture dishes. For infraciliature of the vegetative form see Fig. 1.

Fig. 1. *Oxytricha agilis*, infraciliature of the ventral surface
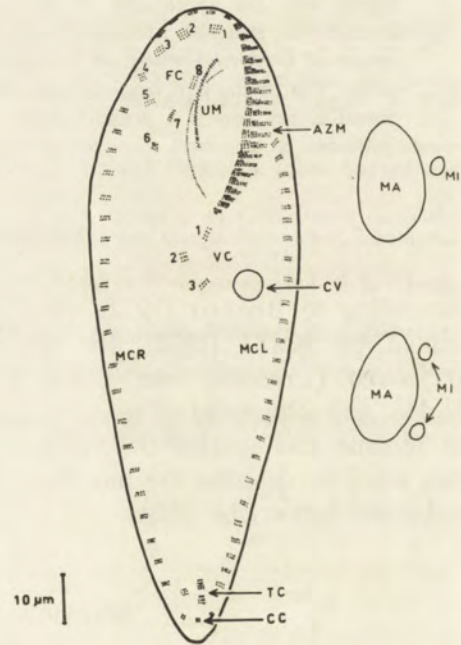
The adoral zone of membranelles (AZM) comprises one fourths of the whole length of the ciliate and consists of 25 membranelles. In the frontal field eight cirri (FC) are arranged in the manner typical of *Oxytricha*. The only ventral cirri (VC) are situated postorally in a group of 3. Only the 3 transverse cirri (TC) of about 15 μm in length insert close to the caudal pole. The marginal rows which are built of 24–26 cirri on the left (MCL), and of 20–22 on the right (MCR), all about 12 μm in lengths, are not confluent posteriorly. The 15 μm long caudal cirri (CC) have moved further to the dorsal surface. The dorsal cilia (DC) are 3–4 μm long and arranged in 4 rows.

The morphogenetic stages of *O. agilis* described in the following passages are shown in Fig. 2–8.

An oral primordium (OP) appears between the postoral ventral cirri and the left marginal row (Fig. 2 and Pl. I 1, 2). A proliferation of new kinetosomes starts from here and proceeds towards the caudal areas of the ciliate. At that time the reorganization bands (RB) have already traversed one half of the length of the macronuclei.

The OP increases in length. The frontal cirri 6, 7 and 8 as well as the ventral cirri begin to break up (Fig. 3 and Pl. I 3). The rest of the frontal cirri will be resorbed after the binary fission. The basal bodies of the former ventral cirri participate in building up the new undulating membranes (UM') and the frontal, the ventral and the transverse primordium (FVT) of the opisthe. The reorganization bands have then traversed about 70% of the length of the macronuclei.

In AZM' new membranelles start to develop from front to back. In the proter the kinetosomes of the former frontal cirri 6, 7 and 8 form six parallel ciliary rows (cirral field, CF, Pl. I 4). Several kinetosomes building up the later UM' can be recognized (Fig. 4). The rest of the basal bodies in this area also form six rows of cilia (CF'). The anlagen of the dorsal ciliary primordia (DCP) 1, 2 and 3 are visible (Fig. 9a–d and Pl. III 9, 10). The 2nd and 3rd cirrus of the right marginal row begin to stretch and build new kinetosomes, while the 1rst remains intact until the binary fission takes place. Apart from this the 10th, 11th and 12th cirrus of this same marginal row begins to stretch, while at that time the left marginal cirri still remain unchanged. The reorganization bands have nearly traversed the whole length of the macronuclei.

The six newly developed ciliary rows (CF, CF') can be clearly recognized as double rows of kinetosomes (Fig. 5). In the left marginal row changes take place as well. The first 3 cirri and the 10th, 11th and 12th cirrus begin to stretch. The reorganization bands have almost disappeared.

AZM', UM and UM' have finished their development (Fig. 6 and Pl. II 5). The double ciliary rows of the ciliary fields are reorganized into single cirri. It is now that the 4th row of the dorsal ciliary primordium is built up out of the foremost parts of the right marginal cirral primordium (MCRP) (see Pl. II 6, 7). The marginal cirral primordia show three rows of kinetosomes. The parts of the MA fuse, the mitosis of the MI begins. A narrowing of the cell becomes visible.

From front to back marginal cirri start to develop (Fig. 7). The transverse cirri primordia begin to move in caudal direction out of the complex of the new FVT – cirri (Pl. II 8). The MA stretches, the mitosis of the MI proceeds.

In the new marginal rows all cirri are clearly visible (Fig. 8). The new
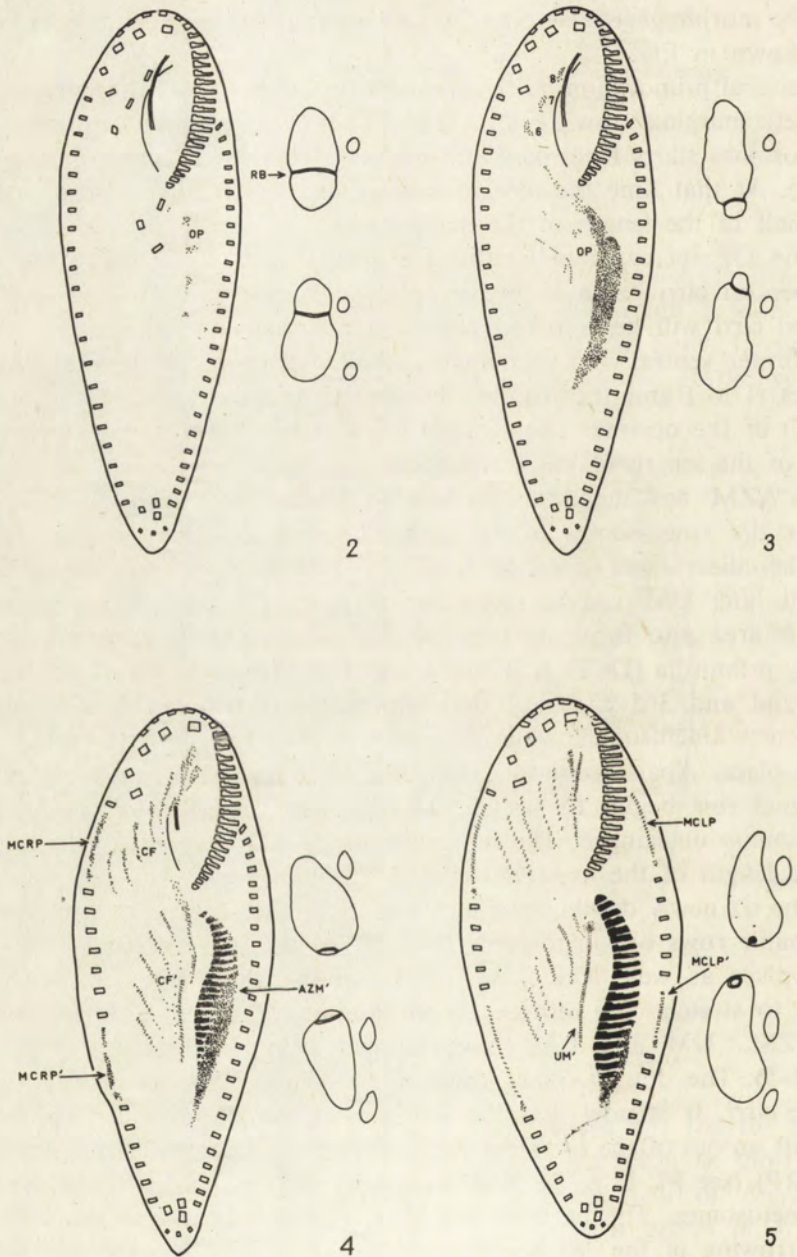
transverse cirri have almost moved to the caudal pole-of each half of the
cell. The fronto-ventral cirri separate and arrange in a typical manner.
In both, the proter and the opisthe, the MA divides into two. The mitosis

6

7

8
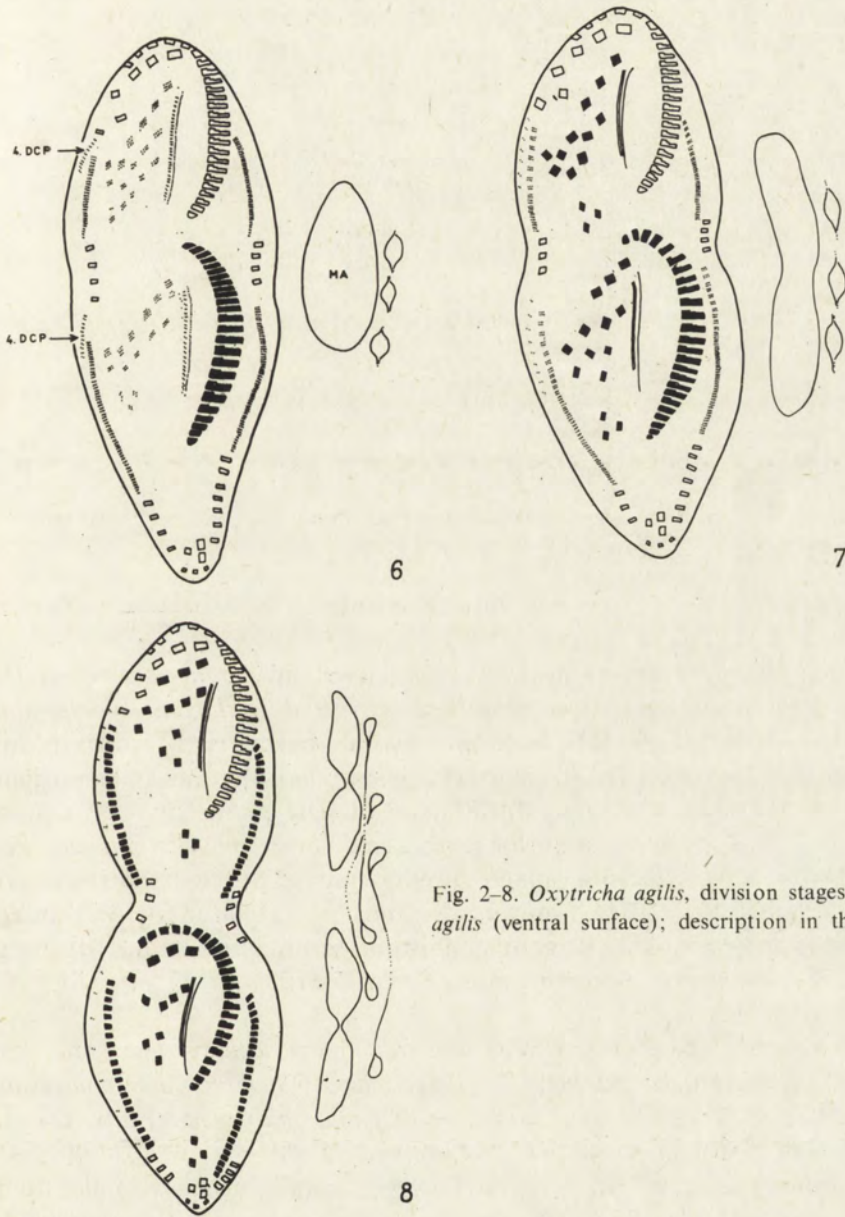
Fig. 2–8. *Oxytricha agilis*, division stages of *O. agilis* (ventral surface); description in the text

of the MI has almost come to an end. The binary fission of the cell is almost completed. After the fission the old cirri except for the AZM are resorbed.
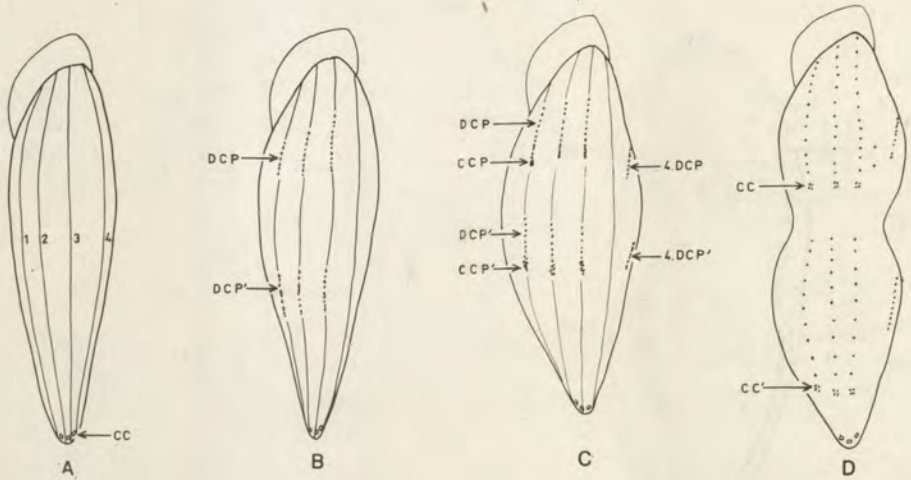
Fig. 9. *Oxytricha agilis*, development of the dorsal cilia and the caudal cirri

Discussion

According to observations by Tuffrau 1969 and Jerka-Dziadosz (1972) the oral primordium of lower hypotrichs (*Urostyla, Kahliella, Hypotrichidium*) develops close to the left postoral row of ventral cirri, while in higher hypotrichs (*Opisthotricha, Gastrostyla, Stylonychia*) the oral anlage originates close to the transverse cirrus furthest on the left (= the first TC). It seems that these observations cannot be generalized. In *Stylonychia putrina* Grolière (1969) found that the oral anlage develops out of the first transverse cirrus. Sapra and Dass (1970), however, described the origin of the oral anlage in *Stylonychia notophora* to lie between postoral ventral cirri and the left marginal row. *S. notophora* is synonymous for *S. pustulata* (O. F. M.) Ehrb. (Borror 1972).

A differing place of origin of the oral anlage among one genus can be found in *Oxytricha* as well. In *Oxytricha (Opisthotricha) monspessulana* Chatton et Séguéla and *Oxytricha (Steinia) platystoma* Ehrb. Grolière (1969 and 1970) observed the development of the oral anlage out of the first transverse cirrus. In *O. agilis*, however, conditions are similar to those in *Stylonychia pustulata* (O. F. M.) Ehrb. No kinetosomes or any structures which are responsible for the origin of the oral primordium could be found (About the problem of preexisting structures in cortical development see Jerka-Dziadosz and Janus 1972). It seems that in higher hypotrichs the development of the oral anlage out of a transverse cirrus is a new achievement, while in certain species the "old" type of development is maintained.

In *O. agilis* the formation of the new dorsal cilia is remarkable. The first 3 dorsal kineties of the proter and opisthe develop out of the preexisting dorsal cilia by kinetosomal proliferation. The 4th dorsal row is a product of the right marginal row. The caudal cirri emerge out of the dorsal ciliary rows 1–3 (Fig. 9 a–d and Pl. III 10). The old caudal cirri are resorbed later. This means that conditions are rather similar to those in *Urostyla weissei* Stein (Jerka-Dziadosz and Frankel 1969), *U. grandis* Ehrb., *U. cristata* Jerka-Dziadosz, *Stylonychia mytilus* O. F. M. (Jerka-Dziadosz 1972) and *Parastrombidium* (*Urosoma*) *planctonicum* Horváth (Wilbert, personal information).

ACKNOWLEDGMENTS

ZUSAMMENFASSUNG

Die Infraciliatur und die Morphogenese von *Oxytricha agilis* Engelmann wurde mittels einer modifizierten Protargolmethode untersucht. Die Teilung beginnt mit dem Erscheinen einer Oralanlage zwischen den 3 postoralen Ventralcirren und der linken Marginalreihe. Hierin unterscheidet sich *O. agilis* von anderen *Oxytricha* – Arten, bei denen die Oralanlage nahe dem ersten Transversalcirrus entsteht. Die Caudalcirren der Tochterzellen entwickeln sich aus den ersten drei Dorsalreihen der Mutterzelle, während die 4. Dorsalkinete ein Produkt der rechten Marginalreihe ist und an einer Cirrenausbildung nicht beteiligt ist.

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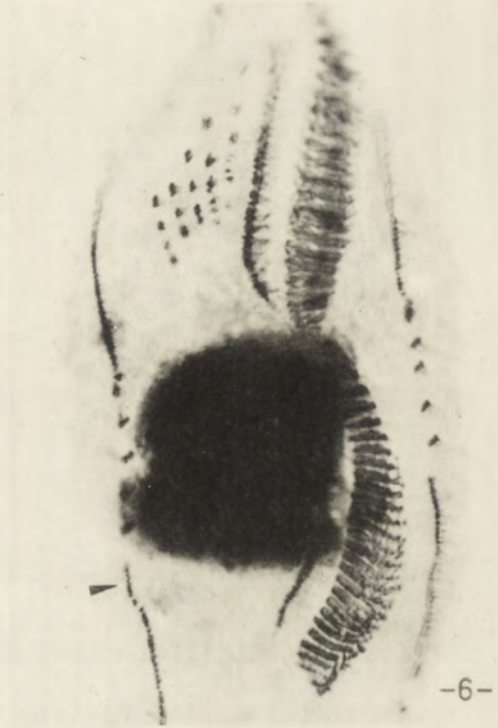Wilbert N. 1974: Eine verbesserte Methode der Protargolimprägnation für Ciliaten. Mikrokosmos (in press).

EXPLANATION OF PLATES I–III

Oxytricha agilis, morphogenesis
1: Place of origin of the oral primordium
2: Proliferation of kinetosomes building up the OP
3: Frontal cirri 6, 7 and 8 breaking up
4: Six double rows of kinetosomes each forming CF and CF′
5: The fusion of the two parts of the MA, AZM′ and UM′ is completed
6, 7: Development of the 4th dorsal ciliary primordia (arrows) emerging from the right marginal cirral primordia
8: The 4th dorsal ciliary primordia stretches (arrow); the new marginal cirri and the FVT — cirri are visible; MA — division
9: Development of the other three dorsal ciliary rows
10: The dorsal cilia with the CCP of the proter

PLATE I

U. Buitkamp

auctor phot.

PLATE II

U. Buitkamp

auctor phot.

-9-

-10-

CCP

U. Buitkamp

auctor phot.
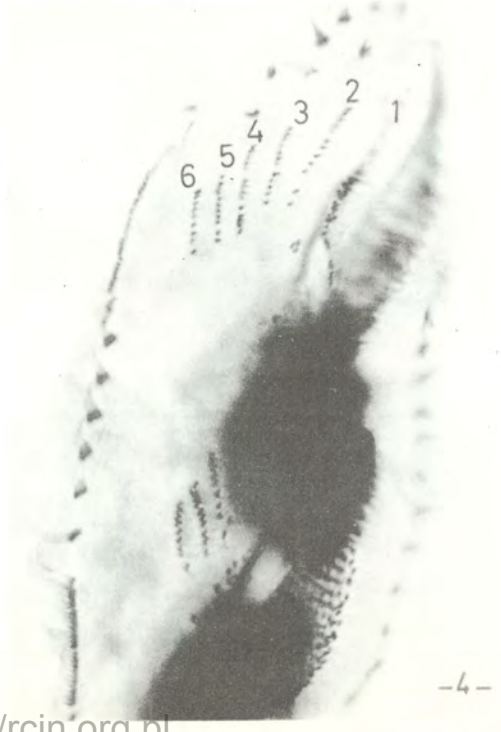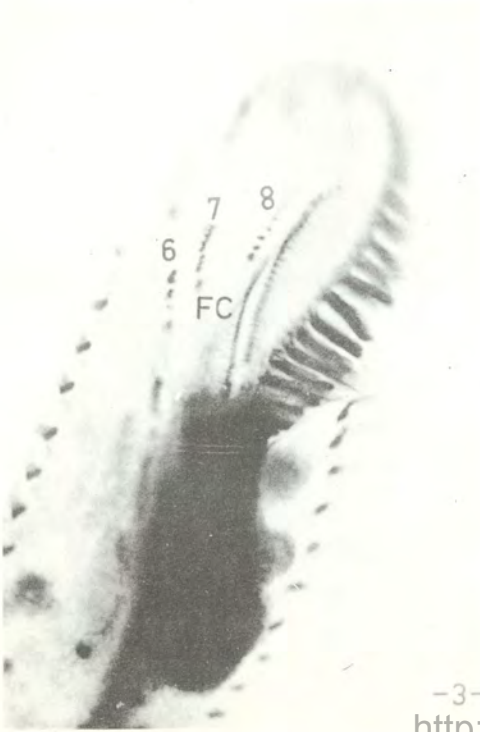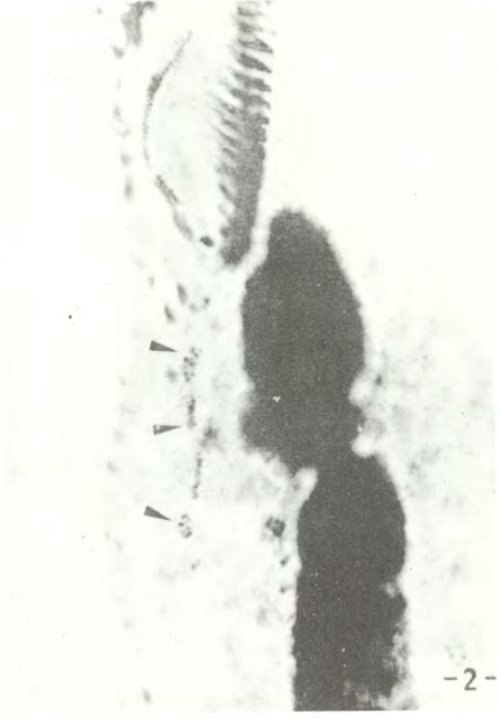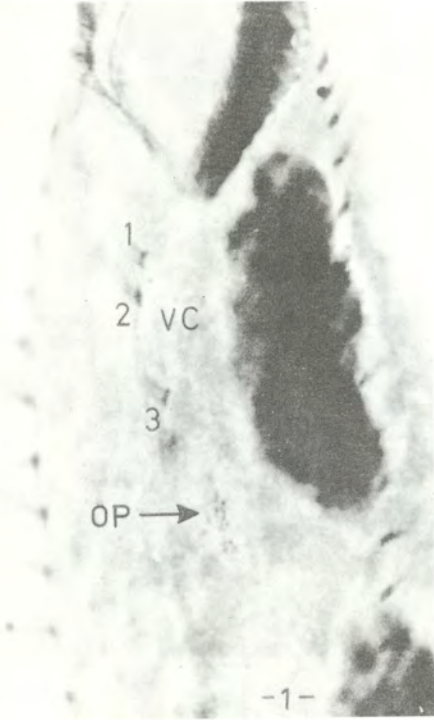
Stanisław DRYL and Irena TOTWEN-NOWAKOWSKA

Motor Response of Double Animals of *Stylonychia mytilus*. I. Response to Potassium Ions in External Medium

Synopsis. Unlike single forms, the doublets of *Stylonychia mytilus* in the early stage of potassium-induced continuous ciliary reversal react by detachment from the substrate and then are swimming backwards along spirals; this response might be the result of strong excitation of two AZMs which play an essential role during swimming. After some time of adaptation to high concentrations of KCl in external medium, the normalization of movement occurs in both components of doublets in the following order: caudal cirri, left marginal cirri, AZMs and then remaining groups of cirri. In analogy to its morphological pattern, the right component of the "flat" doublet is a functional mirror immage of the left one, i.e., the right marginal cirri of this form correspond in function to the left ones of the single form of *Stylonychia*.

Introduction

Double forms of *Stylonychia mytilus* are particularly suitable for investigations on the morphological and functional differentiation of various groups of locomotive organelles, of which the number and location within the cell are characteristic of a given cell form (Dryl and Totwen-Nowakowska 1972).

Direct observations show that "spherical" doublets of *Stylonychia* — where the components are joined by the dorsal sides are swimming and moving forms, having a decidedly limited contact with the substrate, while "flat" doublets are forms of poor mobility, moving slowly with a marked thigmotactic tendency, manifested in an almost constant contact with the substrate and very rare periods of swimming, i.e., of moving in the liquid medium by spiral motion.

The authors suppose that the great mobility and swimming tendency of "spherical" doublets is due to the occurrence of a double set of locomotor organelles (cirri and Adoral Zone of Membranelles (AZM) taking part in the movement) located outside in relation to the central axis of the body running along the back concrescence of both components. While moving on the substrate the "spherical" doublet touches it with the locomotor

organelles of only one component while AZM and cirri of the other have contact only with the liquid medium. Photographically registered (Dryl 1958) tracks of movement of "spherical" doublets on solid substrate and under the liquid surface resemble tracks of movement of single forms of *Stylonychia* (compare Pl. I a^1a^2 and Pl. II a).

In "flat" doublets the connection of the components by their lateral zones causes a nearly double increase of the ventral surface and an increase of the number of FVC cirri (frontal, ventral, caudal) as well as a lack of right marginal cirri occurring in single forms of *Stylonychia*. The result of these changes is a slower motion and a stronger tigmotaxis than in single forms (Pl. III a).

The subject of the present work is a study of responses of both forms of *Stylonychia* doublets exposed to various concentrations of potassium ions, which — similarly as in *Paramecium* and some other ciliates — cause in Hypotricha a depolarization of cell membrane (Machemer 1970, De Payer 1973) accompanied by the reversal of the direction of the effective beat of AZM and cirri. An attempt was made in the present paper of determining the threshold of excitability and the possible differences of response in various forms of *Stylonychia* and in particular groups of locomotor organelles to chemical treatment.

Material and Methods

Investigations concerned double forms: "spherical" and "flat" doublets of *Stylonychia mytilus*, obtained by the previously described method of ca. 15 min temperature shocks (34–35°C), used during cell division of a single form of *Stylonychia* (Totwen-Nowakowska 1965). Observation of single forms was also carried out for comparison and control.

Preliminary observation has shown that *Stylonychia* tolerates very badly to be transferred into media containing the investigated substances in specially prepared buffer solutions with definite pH (e.g., solutions of 1 mM Tris-HCl + 1mM $CaCl_2$ or phosphate-citrate solutions etc.) 15–30 min after transfer into the new medium there occurred undesirable disturbance of movement such as slower onward movement, numerous spontaneous reversals of effective beat of AZM and cirri, weaker response to mechanical stimuli etc. These symptoms were accompanied by lesions or even the loss of the AZM. That is why investigations were carried out only in the Pringsheim Liquid medium (pH 7.3) in which the Ca level was supplemented to 1 mM concentration. Protozoa placed in this medium did not display any behavioral or morphological changes. For direct observation a binocular microscope was used. The duration of the reversed movement of AZM or cirri was measured with a stopper in 20 animals and the observed response was considered as finished when less than 50% of ciliates behaved in a typical way.

The movement of the animals was registered by means of photomacrographic technique in the dark field after Dryl's method (1958).

Discussion of Results

Depending on the KCl concentration used the investigated forms of *Stylonychia* displayed highly different motor responses. Preliminary series of

experiments have shown that 1 mM and 2 mM concentrations of KCl did not affect visibly the motile behaviour of the doublets, while in 4 mM concentration only an increase in frequency of spontaneous reversed movement of AZM and cirri could be observed; however, its quantitative evaluation was difficult because the intensity of this response changed greatly as the experiment proceded. That is why it was arbitrarily assumed that the threshold response was the occurrence of reversed movement reversal of AZM and cirri lasting at least ca. 20 sec and displayed in single individuals as backward movement along an arc of ca. 2 mm of diameter (Pl. I b[1]), and in doublets — as swimming backward along a spiral line (Pl. II b[1] and Pl. III b[1]). The reason of the above mentioned response differences of doublets is probably a strong excitation and an increased activity of two AZM in the first fast phase of reversal, which results in the detachment of the doublets from the substrate and their relatively fast backward swimming. In a single individual the activity of one AZM may be insufficient to detach the ciliate from the substrate. Such an interpretation seems to be in full accordance with the previously formulated opinions of the authors (Dryl and Totwen-Nowakowska 1966, 1972) on the decisive part of AZM in the swimming action.

Microscope observation has shown that, at the beginning, the movement reversal comprised all groups of motoric organelles, i.e., marginal, frontal, ventral and caudal cirri (classification of cirri groups according to Machemer 1965) as well as AZM. During short, threshold reversal responses the action of the particular groups of organelles soon was back to normal. It is interesting, however, that in threshold concentrations (6–8 mM KCl), after the disappearance of continuous ciliary reversal, there occurred a long-lasting increase of frequency of spontaneous reversal responses, which is in accordance with the so called Periodic Ciliary Reversal (PCR) described in *Stylonychia* (Dryl 1965) and *Paramecium* (Dryl 1961) and induced by the action of barium ions. In both forms of doublets this response was manifested by short, violent backward movement after which the animals were detached from the substrate and performed several turns in the liquid medium, then they dropped again onto the substrate. After a short moment of immobility the backward movement and the successive phases of periodic reversal were repeated (Pl. I b[4], Pl. II b[4], Pl. III b[4]). This type of response subsisted during the whole experiment (over 15 min).

In 12 mM and 16 mM concentrations of KCl, similarly as in *Paramecium* (Dryl and Grębecki 1966) two phases of reversed movement could be observed: (a) the phase of continuous reversal (CCR) comprising all motor organelles and (b) the phase of partial cilliary reversal (PaCR) when the movement of AZM causes back to the normal pattern while somatic cilia are still in reversion. The detailed microscopic observation

and investigations carried out on fragments of cells containing particular groups of motor organelles (S. Dryl and I. Totwen-Nowakowska, not published) have shown that within the components of a "spherical" doublet,
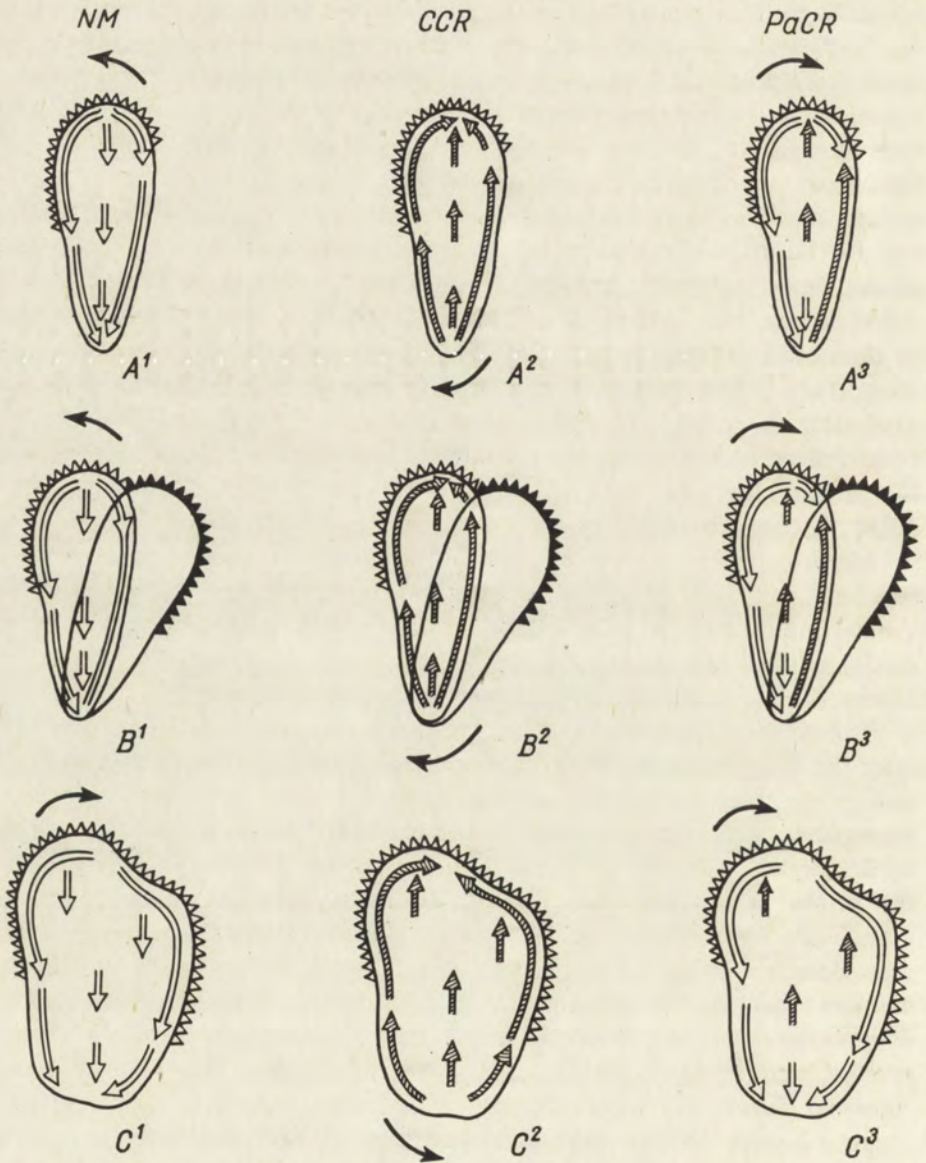
Fig. 1. Analysis of movement of three forms of *Stylonychia*: NM — normal movement, CCR — continuous ciliary reversal, PaCR — partial ciliary reversal, PCR — periodic ciliary reversal. Empty arrows — normal direction of beat of cirri and AZM membranelles. Hatched arrows — reversal of direction of beat of cirri and AZM membranelles. Black arrows — direction of movement of the whole organism, A^1, A^2, A^3 — single form of *Stylonychia*, B^1, B^2, B^3 — "spherical doublet, C^1, C^2, C^3 — "flat" doublet

just like in a single *Stylonychia* cell (D r y l and D e P e y e r 1970), the left marginal cirri and caudal cirri are coming back to normal pattern of movement during the same time or even little sooner than AZM while frontal, ventral and right-marginal cirri show reversed beat for a long time. Owing to the gradual renormalization of movement in the particular groups of locomotor organelles the movement of the swimming doublets became slower, the covered paths shorter and the animals dropped onto the substrate (Pl. II b²). Since the "spherical" doublet touched the substrate with the motor organelles of only one component just as during normal movement, the motor response in this phase proved similar as in single individuals, i.e., the animal moved backwards along a left-turned arc (compare Pl. I b² and Pl. II b², Fig. 1 A², B²). The movement renormalization of some motor organelles on the left side of the body (AZM the left-marginal and caudal cirri), and the still lasting reversal of movement of right-marginal frontal and ventral cirri causes the animal swim along smaller and smaller clockwise running circles until it is turning around within one spot (Pl. I b³, Pl. II b³ and Fig. 1 A³, B³).

The gradual renormalization of movement of AZMs and marginal cirri

Table 1

Duration of Particular Phase of Motor Response Occurring in the Three Investigated Forms of *Stylonychia mytilus* in Various KCl Concentration

Form of *Stylonychia*	Concentration of KCl mM	Duration of Induced Motor Response in sec		
		CCR	PaCR	PCR
Single form	4	–	–	20 ± 5.0
	6	20 ± 8.8	15 ± 5.0	+
	8	35 ± 7.8	100 ± 4.3	+
	12	50 ± 8.2	+	–
	16	55 ± 26.3	+	–
"Spherical" doublet	4	–	–	20 ± 5.0
	6	20 ± 3.5	–	+
	8	45 ± 7.7	90 ± 3.5	+
	12	60 ± 11.1	+	–
	16	65 ± 8.8	+	–
"Flat" doublet	4	–	–	20 ± 5.0
	6	25 ± 7.7	–	+
	8	45 ± 10.9	110 ± 5.0	+
	12	70 ± 25.7	+	–
	16	75 ± 20.7	+	–

Data obtained from 10 experimental series carried out with 20 specimens used in each experiment.
Explanations: CCR – continous ciliary reversal, PaCR – partial ciliary reversal, PCR – periodic ciliary reversal.
(–) – no response (+) – response lasting longer than 15 min.

in "flat" doublets caused slowing down of movement of the swimming animals and their dropping onto the substrate on which they moved backwards at first, along counter-clockwise directed arcs (Pl. III b[2], Fig. 1 C[2]). Owing to final renormalization of movement of both AZMs caudal cirri and both groups of marginal cirri with reversed movement in the groups of frontal and ventral cirri, and due to the domination of motor activity in the left component, the "flat" doublet — after a short time of immobility — changed its turns to clockwise direction (Pl. III b[3] and Fig. 1 C[3]).

The discordance of direction of beat of AZM membranelles in both components caused the right-hand turns to occur at slower rate than in single individuals and the movement was interrupted by frequent stopping. The duration of particular phases of motor disturbance induced by potassium ions in various forms of *Stylonychia* has been presented in Table 1. The threshold of sensitiveness to external stimuli was the same in all three forms, and this may suggest that both the single and the double forms of *Stylonychia* possess the cell membrane characterized by similar receptor properties. In response to stimulation with potassium ions the components of "spherical" doublet, as well as single form of *Stylonychia* (Dryl and De Peyer 1970), show transversal gradient of normalization of movement running from left to right while in a "flat" doublet this gradient runs from two sides of the body towards its central part. Considering the similarity of duration of reversal in both rows of marginal cirri in the "flat" doublet, it is suggested that the right component is a functional image of the left one what has been already pointed out in the morphological description of this form (Tchang, Shi and Pang 1964, Totwen-Nowakowska 1965).

After prolonged adaptation in 12 mM and 16 mM KCl concentrations both "spherical" and "flat" doublets were loosing the spontaneous ciliary reversal responses which proved to be typical for single and double forms of *Stylonychia* exposed to medium devoid of potassium (Machemer 1965).

RÉSUMÉ

Les formes doubles de *Stylonychia* pendant la prémière phase du rebroussement ciliaire continu (CCR) provoqué par les ions K^{+} quittent le substrat et nagent en spiralisant à reculons, ce qui ne se produit pas chez les individus normaux. Cette réaction peut être une conséquence d'une forte agitation de deux AZM dont le rôle pendant la nage est essentiel.

Dans des concentrations élévés du KCl, après une période d'adaptation, la normalisation du mouvement apparait chez les deux composants de la forme double, en ordre suivant: le cirres anales, les cirres marginales gauches, les deux AZM, et enfin les autres groupes des cirres.

Conformément au plan structurel d'une forme double "plate" sa composante droite est symétrique par rapport à la composante gauche comme son empreinte, donc le rang droit de ses cirres marginales correspond en ses fonction au rang marginal gauche d'une *Stylonychia* normale.

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EXPLANATION OF PLATES I–III

Photomacrographic registration of movement in single form of *Stylonychia mytilus*

a^1 — normal forward movement on the substrate
a^2 — normal forward movement on the substrate and under the liquid surface of medium (arrow)
b^1 — CCR
b^2 — transition from CCR to PaCR
b^3 — PaCR
b^4 — PCR

Photomacrographic registration of movement in "spherical" doublets of *Stylonychia mytilus*

a^1 — normal forward movement on the substrate and during swimming in liquid medium
b^1 — CCR (continuous ciliary reversal)
b^2 — transition from CCR to PaCR
b^3 — PaCR (Partial ciliary reversal)
b^4 — PCR (Periodic ciliary reversal)

Photomacrographic registration of movement in "flat" doublets of *Stylonychia mytilus*

a^1 — normal forward movement
b^1 — CCR
b^2 — transition from CCR to PaCR
b^3 — PaCR
b^4 — PCR

PLATE I
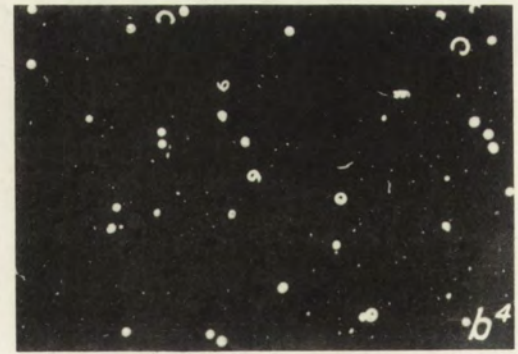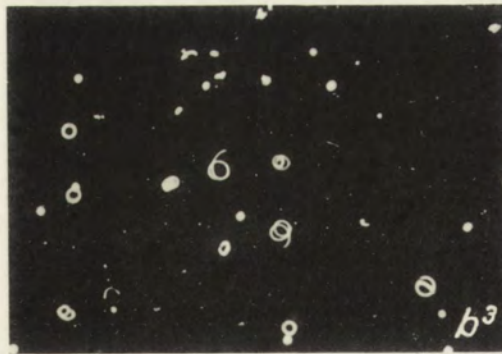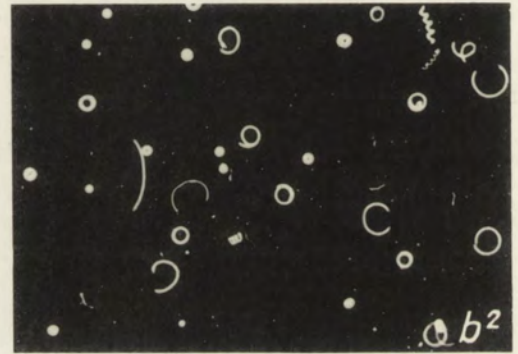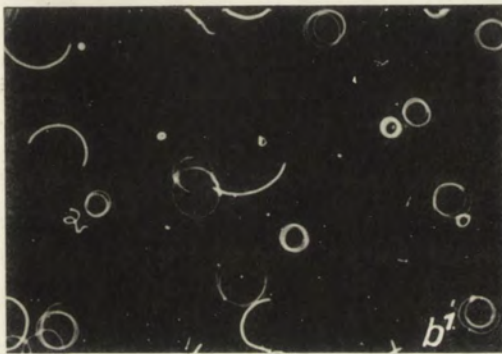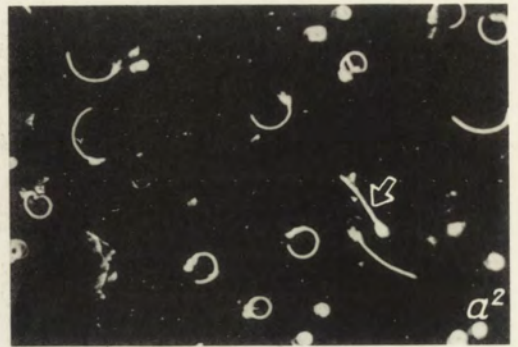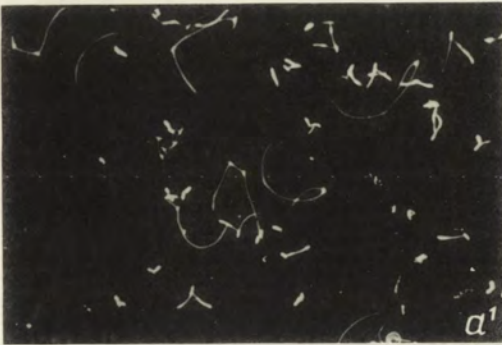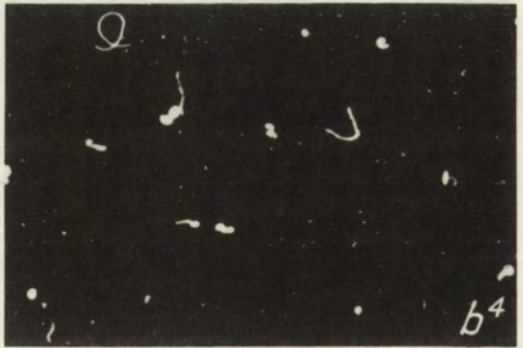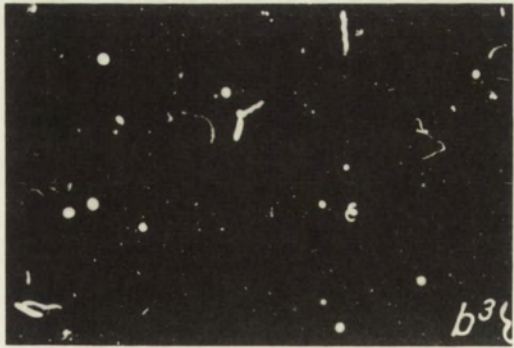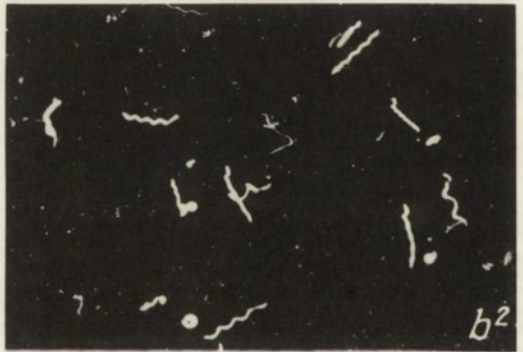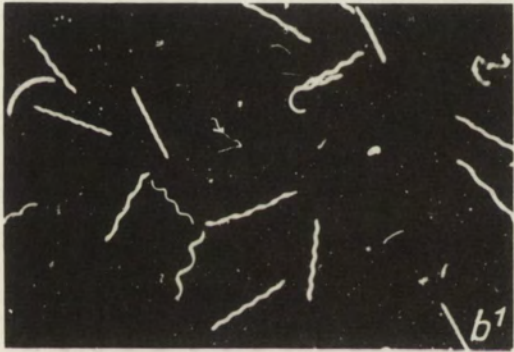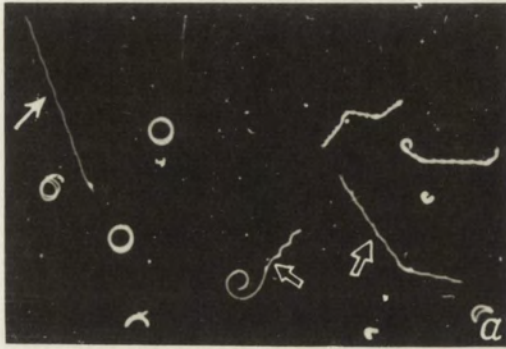
S. Dryl et I. Totwen-Nowakowska

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PLATE III
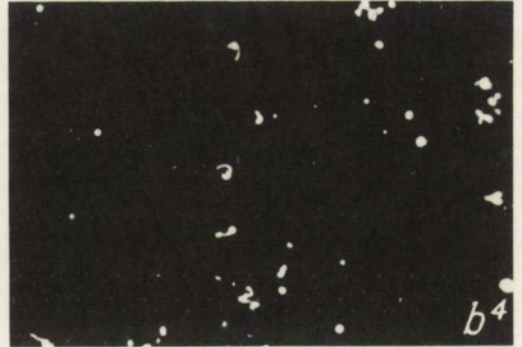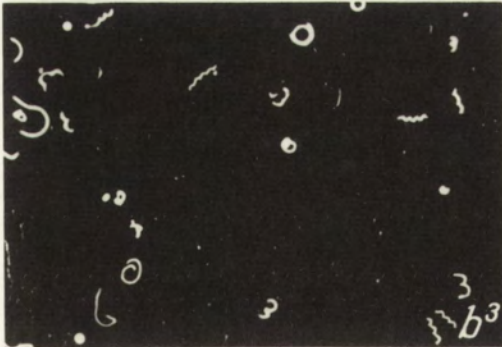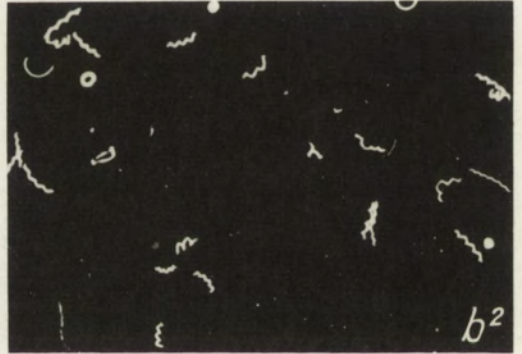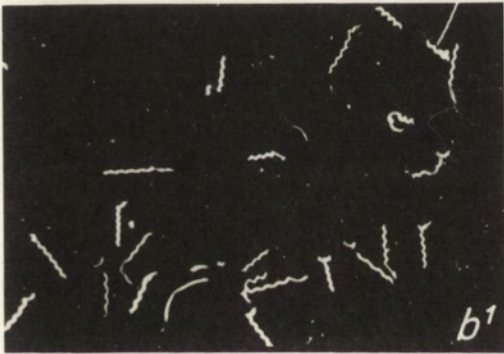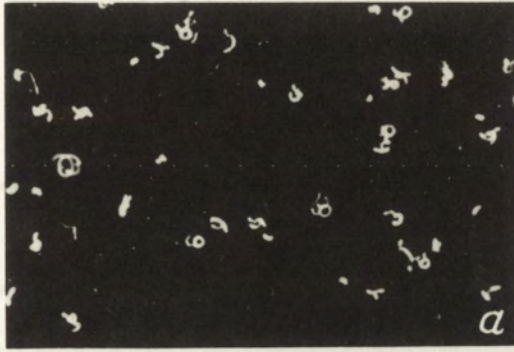
S. Dryl et I. Totwen-Nowakowska

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Ryszard PADO

The Effect of White Light on Kinesis in the Protozoans *Paramecium bursaria*

Synopsis. The object of the present investigation was to study and record changes in the movement of single *Paramecium bursaria* cells in successive stages of the light reaction.

Photokinesis proceeding in *P. bursaria* in white light was found to be of a markedly twophase character. Moreover, certain types of movement characteristic of the respective phases of the light response were identified. For the first 0–20 sec the protozoans swam very quickly towards the illuminated area, following the paths in the form of long broad spiral. But if the ciliates were in the illuminated zone at the moment of switching on of light, they swam immediately towards the source of light — paths were short and tightly coiled helix.

Introduction

The object of the present study is the protozoan *Paramecium bursaria* which is characterized by an intense green colour. This colour comes from green endosymbionts — algae of the genus *Chlorella* — present therein. The number of endosymbiotic algae in one protozoan is not a constant value but may vary considerably depending on the light intensity applied during culturring (Pado 1965).

The investigations carried out by Siegel (1960), Karakashian (1963), and Pado (1965, 1967, 1969) have shown that endosymbionts manifest a fairrly close relation with the protozoans. Owing to the existence of such a closse symbiosis between the animal organism and green algae, this organism may be self-sufficient regarding its nutrient conditions. Under favourable light conditions, the photosynthetic production of vegetal endosymbionts meetss in full the demand for nutrient of both the algae and the animal, as haas been ascertained by Pado (1967).

Unlike the colourless Paramecia, *Paramecium bursaria* exhibits distinct locommotive reactions dependent on illumination conditions, which phenomenoon was associated by Wichterman (1948) with the presence of algae in thae described species.

In more recent investigations (Pado 1972), the problem of phototactic reactions was studied in more detail for the whole population of the protozoans *Paramecium bursaria*. In this study threshold doses of white light required for the induction of a positive light reaction as well as the dose at which the protozoan reacts by escape, were determined.

Besides, the population of the protozoan *Paramecium bursaria* was examined for its response to chromatic light. The reaction was found to vary depending on the spectrum range of the operating light. In blue light (with its maximum sensitivity of ca. 450 nm), the reaction of the protozoans manifested by their agglomeration in or escape from illuminated places was almost instant and reached its optimum in as early as 7 to 10 min. On the other hand, this process proceeded much more slowly in red light (ca. 680 nm), where a positive light reaction was recorded only after 90 min of exposure, and the phototactic optimum was attained after about 3 h and a half. On this basis two photosystems controlling the light reaction in the symbiotic complex of *Paramecium bursaria* were distinguished:

(1) early-appearing, quick-operating system, in which riboflavin or carotenoids may function as a photoreceptor.

(2) long-time, "photosynthetic" system which occurs much later.

The method which was used for the population of the protozoans *P. bursaria* cannot be taken as a basis for answering the question on the behaviour of individual cells in the course of experiment.

The aim of the present investigation was to study and record the movement of individual specimens of *P. bursaria* in successive stages of the light reaction.

To this end a method was applied which permitted a photographic registration, in a suspension, of the paths of particular cells of the microorganisms. Fergusson's technique (1957) in Dryl's modification (1958) has found wide application in many similar experiments, contributing largely to the advancement of the studies on motor reactions of the ciliates. The main advantage of this method lies in a simultaneous registration of a large number of paths followed by the studied organisms. Moreover, this is a "permanent" record on the photographic material, which makes possible the determination of several parameters of the locomotive reaction of the organism in question, such as, e.g., the direction of movement, its speed, a kind of spiralling, the angle of swimming in relation to the incident light beam, and the like.

Material

The protozoans *Paramecium bursaria* used for the above experiments were caught in the field (Municipal Park at Leszno Wielkopolskie). The experimental clone was derived from one specimen.

Mass-breeding was carried out under white light of fluorescent tubes with the intensity of ca. 2000 lux, in light thermostats at the temperature of 26–27°C. The basic nutrient medium was an extract made from the lettuce (*Lactuca sativa*) according to Sonneborn (1950), at the proportion of 1.5 g dry lettuce leaves per 1 l distilled water, and contained no calcium in the form of $CaCO_3$. After the medium had been filtered on Schott and paper filtres, its pH was established in the range from 6.5 to 6.7 by means of 1 N NaOH, and next the medium was tyndallized three times in the Koch apparatus at 24 h intervals. 24 hours prior to inoculation with the protozoans, the nutrient was mixed with distilled water at the ratio 1 : 1. Every 7 to 10 days the exhausted nutrient was replaced by a fresh one, this being conditioned by changes in pH of the medium. A decrease of the pH values below 6.1 or their rise above 7.0 signalled the necessity to replace the medium. The pH values were checked at a few days' intervals by means of an LBS-63 A pH-meter ("Eureka" — Warsaw).

The breeding of the protozoans was carried out in cuboidal glass vessels 100 mm high with 250 ml capacity, under continuous illumination. For each experimental series the protozoans were cultured so as to prevent their excessive condensation in the medium. The average number of specimens was ca. 1200/1 ml medium. At higher values, the nutrient conditions for the protozoans deteriorated.

Methods of Measurement and Registration of Photokinesis

The experimental series of the protozoans *Paramecium bursaria* were obtained with mass cultures by condensation of the material in graduated flasks of 100 ml capacity, with 1 mM of "Tris" buffer of pH = 7.1. The protozoans suspended in this buffer tended to accumulate in the neck of the flask from where they were transferred, by means of a pipette, to special measuring cuvettes with fresh medium mixed with 1 mM "Tris" buffer at the ratio 3 : 1. The cuvette was then covered with a piece of cut glass and exposed to weak light (ca. 300 lux) for the period of 18–24 h in order to adapt the material to new environmental conditions. Such an adaptation period had a positive effect on the protozoans as it enhanced their sensitivity to light. For each experimental series the principle was observed to maintain a constant protozoan content, as converted to a unit of capacity, amounting to about ± 2500 cells/1 ml medium. This value was set by means of the stereoscopic microscope.

A recorder similar in outline to the one described in Pado's previous study (1972) was the basic apparatus employed in these experiments. The essential modification consisted in the introduction of a camera and a revolving measuring cuvette. This recorder is shown in Fig. 1. The cuvette (A) with a suspension of protozoans was protected by a light-tight cover of black printing-paper. A slot 1.5 mm wide was left in the cover along the entire periphery of the cuvette, precisely on the axis of the stream of light. Moreover, the cuvette with its revolving base (R) was placed in a larger light-tight casing (B). This casing was blackened inside with soot and it embraced the lens of the camera (F), thus protecting the whole set against

penetration of diffuse light from outside. The front wall of the casing (B) was provided with a round hole (0) lying on the axis of the light beam. Besides, this wall was fitted with a holder (C) to install and change neutral net filters.

A light beam directed by a set of lenses (H) penetrates through the hole (0) and illuminates the cuvette in the place of the slot; thereby the illuminated zone of definite thickness (1.5 mm) is obtained. A Narva 110
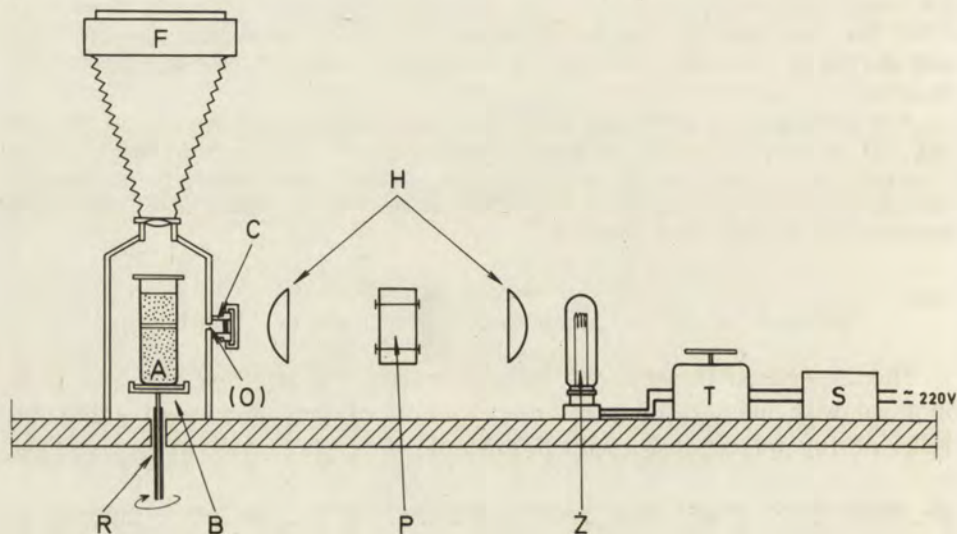

Fig. 1. Scheme of apparatus used for illumination and for taking readings of photokinesis, (A) – cylindrical measuring cuvette covered with mask, (B) – light-tight casing, (C) – holder for replacing neutral filters, (F) – photographic camera, (H) – system of lenses, (P) – infra-red filter with water, (R) – rotating base, (S) – stabilizer, (T) – transformer, (Ż) – 750 W filament bulb, (O) – hole in light-tight casing

V 750 W projection filament bulb (Ż) was the source of light for both tests and measurements. It was set in a vertical position and connected to a voltage control (T) stabilized by an equalizer (S) of great power.

The above set yielded the intensity of white light ranging from 0 to 35 000 lux.

A water filter for infra-red (P) filled with distilled water without $CuSO_4$ was placed between the lenses on the axis of the light beam. The camera (F) was positioned on a special stand over the cuvette. The photographed area covered the entire diameter of the cuvette. During the experiment the film was exposed for 5 sec. At this time of exposure most of the paths lay within the photographed area on their entire length.

Measurements of photokinesis of the proztozoans *Paramecium bursaria* were taken in the following way. The cuvette with a standard number of the ciliata subjected to prior 24 h adaptation in weak light, was placed in

the measuring apparatus on a revolving base (R). It was next set in rotation at the speed of 24 rev/min for the period of 3 min, in order to mix thoroughly the cuvette content. After switching off of the engine, the cuvette remained at rest for another 5 min.

In this period the protozoans were "segregated" into those which settled at the bottom and on the walls of the vessel and the others which swam freely in the medium. The latter ciliata were subsequently registered photographically according to the method adopted in the present study. Both the mixing of the ciliates and the period of rest proceeded in complete darkness.

In these experiments kinesis of *Paramecium bursaria* was registered at a constant light intensity of 2600 lux – this is the white light intensity which can still induce a positive light reaction. The paths of the swimming individual *P. bursaria* cells were registered on the photographic film. This was possible because a certain amount of light was diffused on particular cells swimming in the illuminated zone, which produced the effect of the "luminosity" of the cells. When the cell swam at the open shutter of the camera, its path was finally registered.

The photographs obtained with this technique formed the basis for the quantitative analysis of the process. The lengths of the paths were reduced from large magnifications by means of a curvimeter. On this basis the speeds of the swimming individual *P. bursaria* cells were calculated. Only the paths that had come out on the film equally light and sharply outlined were subjected to analysis.

Results

The present results refer to single series which by several repetitions were found to be most representative for this process. These findings permit the statement that at the applied white light intensity (2600 lux) photokinesis in the protozoans *Paramecium bursaria* is markedly of a two-stage character.

While surveying the yielded photographic documentation (Pl. I–III), the following distinct traits of the recorded light reaction are discernible:

(a) a variety of types of the paths followed by particular cells of *P. bursaria*,

(b) the direction of the swimming ciliate towards the source of light is always accompanied by increased spiralling,

(c) a correlation between the direction of movement and a type of its path.

On the basis of this documentation an attempt was made to distinguish certain types of movement characteristic for this organism in respective phases of light reaction. The results are shown in Table 1. In the successive vertical columns 5 types of movement (paths) are distinguished which differ

Table 1

Numerical ratio of particular kinds of swimming (type of paths) of *Paramacium bursaria* depending on duration of light reaction

Duration of light reaction	Particular kinds of swimming				
	1	2	3	4	5
5 sec	27	0	0	2	0
10 sec	49	3	0	19	0
15 sec	36	5	0	5	0
20 sec	19	4	0	1	12
30 sec	8	0	0	3	31
40 sec	3	1	0	2	35
50 sec	3	21	1	3	14
1 min	4	47	0	5	4
2 min	5	52	1	9	6
5 min	9	38	4	5	5
10 min	7	42	8	2	2
15 min	3	8	15	2	3
20 min	1	1	20	1	1
30 min	6	2	16	1	2

Explanations:

1 Broad spiral — long
2 loosely coiled spiral (a high — pitched one)
3 tightly coiled spiral (small — pitched) — or straight line — "direction" line.
4 spiral according to item 2 or 3 upward or inward movement of infusorian.
5 broad spiral with directional orientation.

essentially from one another and can be ascribed to the first or second phase of the light reaction. In addition, Table 1 gives the numerical values of the participation of each kind of path in different periods of this reaction. It can be seen from this comparison that in the first stage of the experiment (0 to ca. 20 sec) the protozoans swim very quickly towards the illuminated area. At this period they follow elongated paths (see Table 1 *1*) which assume the form of long broad loops or of an almost straight line. Such a mode of swimming enables the protozoans to cover the distance by the shortest way (Fig. 2, Plate I a, b, c). In the initial phase of the reaction the above-described paths lie at various angles to the direction of the incident light beam.

On the other hand, the response of the infusorians which at the turning on of the light were in the illuminated area is quite different. The direction
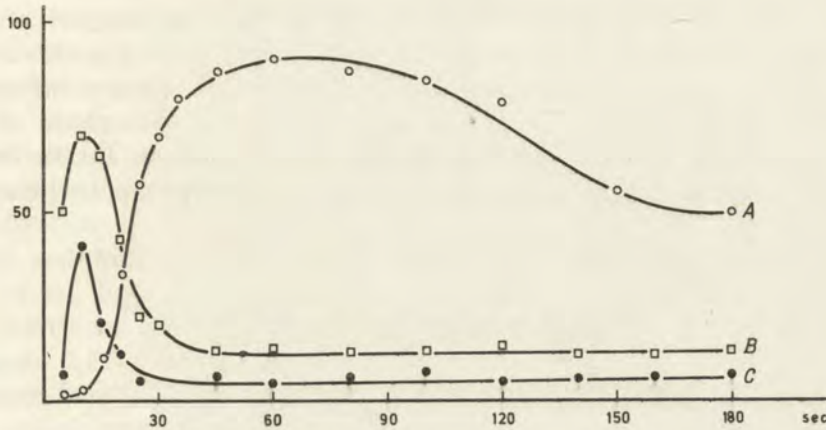
Fig. 2. Numerical ratio of individual kinds of swimming paths during light reaction, depending on duration of exposure to white light, x — abscissa, light reaction time in sec, y — ordinate, A — short spiral paths directed toward light source, B — long broad paths in the light plane, C — protozoans swimming upwards perpendicularly in a spiral (toward the light area)

of swimming of these cells coincides from the start with that of the light beam — they swim immediately towards the light source. This oriented swimming is accompanied by a change in spiralling. The infusorians follow now the paths in the form of very short and tightly coiled spirals. Now and then the proximate coils of these spirals lie so close to one another as to overlap, this appearing on the photograph as a thickened segment of a straight line. Plate IV presents the successive intermediate phases which lead to the afore-mentioned type of movement.

In all probability, the swimming of the above-described type (Table 1 *3*) is specific for the species *Paramecium bursaria*. The present author has not found any mention in the literature about a similar mode of swimming of other ciliata of the genus *Paramecium*.

A comparison of the paths of the infusorians which at the moment of light operation were in the dark area with those which at that moment were in the illuminated zone reveals fairly significant divergences. As has already been observed, the former protozoans *P. bursaria* tried to reach the illuminated areas as quickly as possible, therefore they followed the paths forming broad loops. The question arises what makes these organisms distinguish infallibly the illuminated from dark areas. Apparently, despite a strict demarcation of the illuminated zone there occurs some diffusion of light producing the light gradient on the borderline. It may be assumed that in this situation the chaotically swimming protozoans reach by chance the places where diffuse light penetrates. But even this scarce amount of light is sufficient for the protozoan to react to it. In consequence the protozoan swims towards the increasing light gradient reaching finally the

illuminated area. The infusorians swimming in this way do not at first respond to the direction of the incident light beam. This is confirmed by a variety of angles to this direction, at which individual cells are swimming.

The speed of movement of the infusorians in the first phase of the reaction is higher than in the later period (Figs. 3 and 4). In the former phase of "quick swimming" this organism can move at the speed of ca 1 mm/sec,
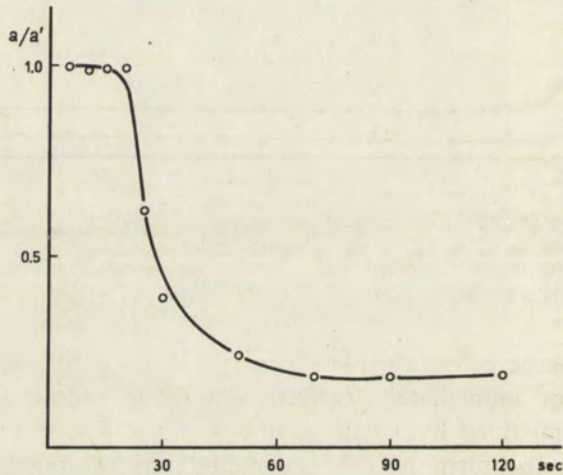

Fig. 3. The swimming of *Paramecium bursaria* in white light (2600 lux) expressed by the ratio of covered route to the distance covered at the same period of time, a' — distance covered, a — covered route, × — abscissa, reaction time in sec, y — ordinate, ratio a/a'
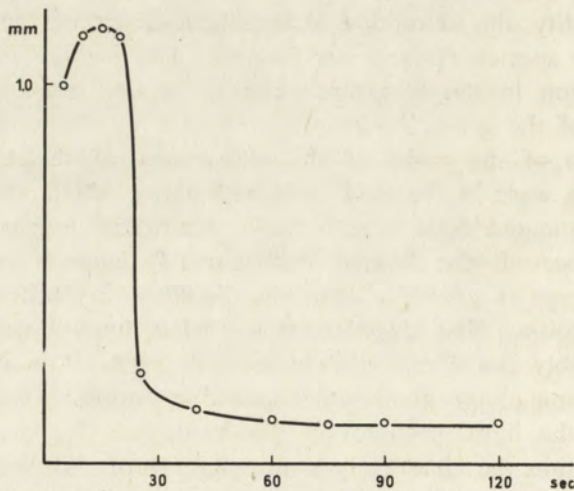
Fig. 4. Ability of covering distances in mm/1 sec by a single individual of *Paramecium bursaria* depending on total duration of light reaction, × — abscissa, light reaction time in sec, y — ordinate, distance covered during 1 sec in mm

thus covering the distance ten times longer than its own length. In several series the cases were recorded in which the speed attained the value of even 2 mm/sec.

In the second phase of the light reaction, i.e., when the protozoans have already reached the illuminated area in the above-described way, a marked directional orientation is noticeable in them. The observed change of spiralling causes that the protozoan begins to describe a spiral of a small pitch and with time swims just like the specimens which after the turning on of the light (in 0 sec) have already been in the light zone (this type of movement has been described in the present paper). It is worth adding though that the *P. bursaria* cells already found in the illuminated area slacken their movement. At this time the reaction acquires the characteristics of typical orthokinesis (i.e., deceleration under the effect of a stimulus). The distances covered at such a mode of swimming range between 0.2 and 0.3 mm/sec.

The behaviour of the protozoans *P. bursaria* exposed to white light for over 40 sec is presented on Pl. II a, b, c and III a, b, c. Side by side with a still large number of long paths (Pl. II a) which relate to the organisms swimming successively into the illuminated area, there begin to appear numerous short straight paths. They lie parallelly to the direction of the incident test white light beam. This tendency is even better seen in the following figures (Pl. II b, c and III a, b) which show the increased participation of directionally oriented paths.

On the ground of these results an attempt was also made to study more extensively the quantitative changes in directionally oriented swimming during the light reaction. This relation was determined by means of the angle between the direction of the incident test beam and that of the swimming of individual *P. bursaria* cells. The thus obtained data are shown graphically in Fig. 5. Taking this as a basis, it can be stated that in the first phase of the process (from 0 to 20 sec) the infusorians swim at various angles to the direction of the light beam. In the next phase, from the 40th second onwards, they demonstrate a marked tendency to directionally oriented swimming. This increases the per cent participation of the paths running parallel to the direction of the operating light. Such an arrangement of the paths is particularly well visible in Fig. 5 — for one and two minutes of exposure to light. At this time about 80% cells swim at an angle ranging from 0 to $\pm 5°$ to the direction of the incident light beam. The prolongation of the light reaction over 30 min does not alter its character. With the increasing total time of exposure the experimental material exhibits a final establishment of the directional orientation of swimming.

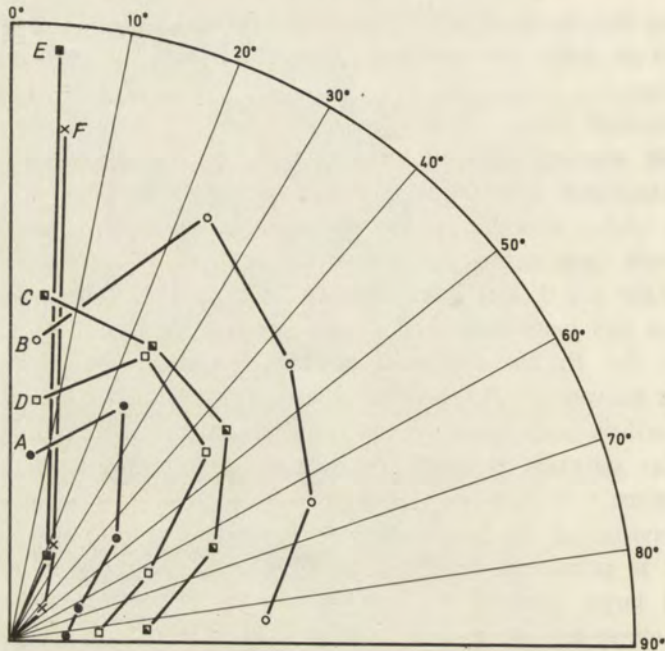In the experimental series where the measuring cuvettes were intentionally

Fig. 5. Numerical ratio of ciliates in population of *Paramecium bursaria* swimming in specific direction in relation to the light source at various times of light reaction (in the picture the swimming angles in relation to the light source are marked with figures from 10° to 90°, A – time 0 sec, B – time 5 sec, C – time 20 sec, D – time 40 sec, E – time 1 min, F – time 2 min

filled with larger than normal quantities of the protozoans, the direction of the swimming of particular *P. bursaria* cells towards the light source is so regular that it resembles very closely the effects attained by Dryl (1963) for *Paramecium caudatum* during a normal cathodal galvanotaxis (Pl. III c).

Discussion

The locomotive reactions of the photosynthetizing organisms may depend on light in various ways. Taking the final effect as a criterion, Nultsch (1970) distinguishes two basic kinds of reaction. If exposure to light brings about the local aggregation of the organisms in the illuminated spot, this process is defined by him as phototaxis. If, on the other hand, the organism reacts to light by a change in the speed of its movement, this phenomenon is called photokinesis.

All the motor reactions of the organism associated with the light effect have been termed photomotion by Wolken and Shin (1958). On the

basis of the above nomenclature, Nultsch (1970) distinguished the following types of the organism's response to light:

(1) positive photokinesis (+), i.e., acceleration or induction of movement by light,

(2) negative photokinesis (−), i.e., slackening of movement or, finally, immobility,

(3) positive phototopotaxis (+) − movement towards the light source,

(4) negative phototopotaxis (−) − escape from the light source

(5) positive photophobotaxis (+), i.e., reversal of the direction of movement evoked by a sudden decline in light intensity or by illumination to a steep gradient, which results ultimately in agglomeration of the organisms in the light field,

(6) negative photophobotaxis (−) induced by a sudden increase of light intensity which causes that lighter areas become empty.

As mentioned by Nultsch (1970), the photokinetic phenomena were described a long time ago, in 1878, by Strasburger, and the term "photokinesis" was used for the first time by Engelmann in 1882. Since then these processes have been investigated in many organisms. However, the obtained results indicate clearly that the response of the organisms sensitive to light is not uniform and may take a different course even in similar forms. This is illustrated, among others, by the below given examples.

The green zoospores *Ulotrix zonata* as well as colourless ones in the fungus *Chytridium vorax* exposed to light become motionless in a short time. In darkness, on the other hand, their motility is maintained for several days. This may be regarded as a classical example of negative photokinesis. Other organisms respond to this factor in quite a different way. Thus, e.g., certain species of purple bacteria lose their locomotive ability in darkness. However, short illumination is sufficient for them to recover their motility. The movement of these bacteria accelerates with the increasing light intensity, being therefore the example of positive photokinesis. Nevertheless, prolonged illumination, especially at high light intensity, may induce the occurrence in the purple bacteria of negative photokinesis − immobilization.

Bolte (1920) (cit. after Nultsch, 1970) has found the motility of many organisms exposed to light to depend on the presence of CO_2 in the environment, concluding on this basis that there exists a correlation between positive photokinesis and photosynthesis.

The object of the present study − the endosymbiotic protozoan *Paramecium bursaria* − is a rather specific organism as it consists of two essentially different and separate organisms: the animal infusorian and vegetal endosymbionts − the green algae *Chlorella*. In view of this, on account of, e.g., its locomotive capacity, this protozoan may be comparable with the green zoospores of other organisms. On the other hand, a comparison with *Euglena*

which has been studied extensively for its phototactic responses (Diehn 1969, 1970; Diehn and Kint 1970), is difficult owing to a very complex structure of its photoreceptive apparatus which has no equivalent in the presently examined symbiotic complex.

Nor can the protozoans *P. bursaria* be compared directly with the purple bacteria or *Cyanophyceae*, since such a comparison would concern different structural classes.

In the case of other ciliates of the genus *Paramecium* which are typical animal forms (colourless), light does not exert any effect on their movement. As a rule they follow long paths assuming the form of broad spirals. Under normal conditions, *P. caudatum* exibits left spiralling. By means of several factors diverse ciliary reversals may be evoked in this infusorian, which leads to changes in the character of its movement (Dryl and Grębecki, 1966). Likewise diverse changes of paths ensue from the process of inversion in this organism (Grębecki et al. 1967).

The author failed to find any description of the type of paths observed in *P. bursaria* during the present experiments in the available literature pertaining to the mechanism of movement in the genus *Paramecium*. As it was already mentioned the ciliates which in the dark follow long broad spirals, change essentially the character of their movement as soon as they find themselves in the light area. The *P. bursaria* cells which at the moment of switching on of the light were in the illuminated zone, swim almost instantly towards the incident light. A similar behaviour is observed in the ciliates which entered this zone in later stages of the experiment. In the cases of oriented movement towards the source of light the protozoans describe a compact spiral with a small pitch. Sometimes neighbouring coils of such a spiral lie so close to one another as to appear on the photographs as a thicker segment of a straight line. The below-shown type of path illustrates the directional orientation of the swimming ciliate towards the source of light and probably ought to be regarded as typical of this organism. Perhaps this is related with the presence in this protozoan of green endosymbionts and with the appropriate localization of the photoreceptor. If the existence of such a photoreceptor in this organism is assumed, as has already been postulated by Pado (1972), it may be noted that it functions rapidly and efficiently.

This coincides with Pado's earlier experiments (1972) in which the light response of the whole population of *P. bursaria* was examined. Two photosystems participating in the light reaction of these organisms were then distinguished:

Ist. Early-appearing photo-receptor system operating in the range of 375–475 nm, with its maximum at about 450 nm, which indicates that riboflavin or carotenoids may function as a photoreceptor in it. This is

a fast-operating and probably very sensitive system. It can be expected that its task is to bring quickly the protozoan into the illuminated area, i.e., where green endosymbionts can start their photosynthetic production.

IInd. Later-acting photo-receptor system called photosynthetic, whose operation becomes revealed after some time and with action spectrum characteristic for the absorption spectrum of chlorophyll.

In photokinesis of *P. bursaria* the operation of photosystem I seems to be quite certain due to a very short time of reaction. Further data concerning this process should be yielded by investigations on the role of monochromatic light in photokinesis of *P. bursaria*. These investigations are now in progress.

The present results demonstrate that the kinetics of this process varies depending on the time at which the process is recorded. It appears that for the first few seconds of the action of the stimulant the infusorians swim energetically, or, strictly speaking, cover the distance more effectively. Consequently they migrate very quickly from the dark into the illuminated area, or in the inverse direction. The character of movement is determined by the direction of swimming in relation to that of the incident light. As soon as *P. bursaria* finds this direction, its path is seen to assume a different form of spiralling – short and compact. As mentioned in the Results, this is accompanied by slowing down of the movement – orthokinesis, or, according to Nultsch's nomenclature (1970), such a behaviour of the organism may be regarded as a sign of negative photokinesis.

Unfortunately, no data on the photoreception in other animal organisms living in symbiosis with algae are available in the relevant literature. Nevertheless, the flavin system has been found to steer locomotive reactions in the cases of plant phototropism and to control the chloroplast migration (Haupt and Schoenfeld 1962, Zurzycki 1962, 1967). It seems highly probable that riboflavin or carotenoids play a part as a photoreceptor in photokinesis of the endosymbiotic protozoans *P. bursaria*, especially in the initial stages of the reaction.

Another interesting question arising in the present study are the causes of such a change of the character of movement in *P. bursaria* exposed to white light. This is presumably associated with the mechanism conducting the very stimulus that induces the motor reaction.

The mode of movement of the ciliate in light was observed to change during a few or several seconds. In the dark and for the first seconds following the switching on of the light (Figs. 3 and 4) the protozoans followed the elongated paths, whereas in the next few seconds they slowed down the movement displaying simultaneously an enhanced spiralling.

In all likelihood, exposure to light induces the local and increasing depolarization of the membrane of the protozoan *P. bursaria* manifested by a slackened

forward movement and enhanced spiralling. Such a possibility of the light effect on the cell membrane has been testified by researches carried out by Okumura (1964), who proved that the decreased resistance of the cell membrane as well as the ciliary reversal had occurred as a result of irritation of the colourless ciliate *Paramecium caudatum* with a light stimulus, this reminds of results reported by Kinosita, Dryl and Naitoh (1964 a, b, c) who brought evidence that Balca-induced depolarizing spikes are accompanied by short-lasting reversal of ciliary beat in *Paramecium caudatum*.

ACKNOWLEDGMENT

RÉSUMÉ

On a obsérvé et enrégistré les particularités du mouvement de *P. bursaria* dans les phases consécutives de leur réponse à la lumière. On a utilisé la technique d'enregistrement photographique des trajectoires individuelles.

La photocinèse du *P. bursaria* dans la lumière blanche comporte deux phases. On a identifié les particularités du mouvement typiques de ces phases. Pendant les 20 premiéres secondes de l'exposition à la lumière blanche de l'intensité de 2600 lux les ciliés se déplacent vite vers la zone illuminée en décrivant leurs trajets en forme des spirales allongées. Par contre si un cilié se trouve dans la zone illuminée dès le début, il se met à nager dans la direction de la source lumineuse, en suivant une trajectoire très différente, en forme d'une spirale courte et très serrée. Les ciliés qui en nageant vite en spirales allongées entrent dans la zone illuminée, changent également le caractère de leur mouvement pour les trajectoires formant les spirales courtes et serrées.

La nage orientée vers la source lumineuse est donc accompagnée de l'orthocinèse (ralentissement du mouvement) ou de la photocinèse négative selon la terminologie de Nultsch 1970.

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Kinosita H., Dryl S. and Naitoh Y. 1964 c: Spontaneous change in membrane potential of *Paramecium caudatum* induced by barium and calcium ione. Bull. Acad. Pol. Sci., Cl. II, Ser. Sci. biol., 459–461.

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Pictures of light reaction of *Paramecium bursaria* registered by photomacrographic technique at various times (the arrow in the pictures indicates the direction of light beam stimulating the reaction):
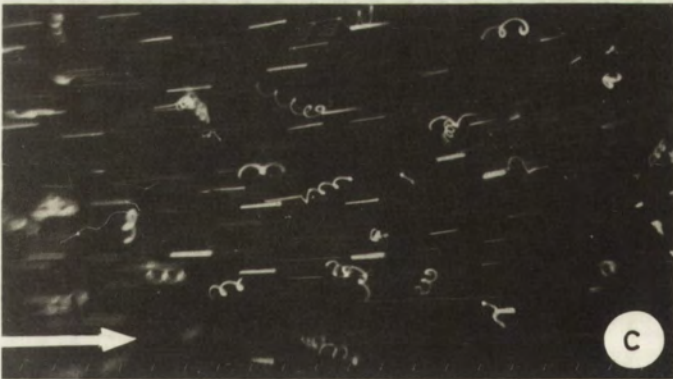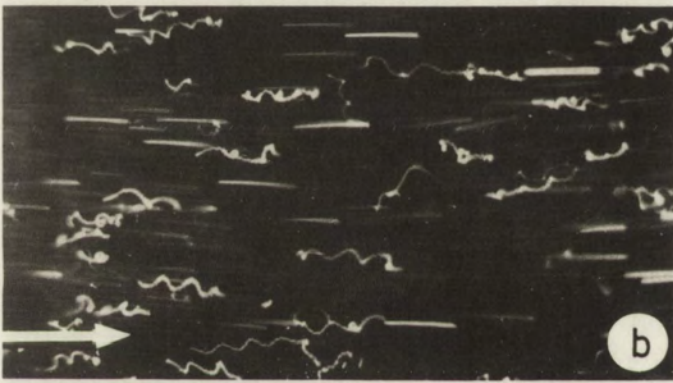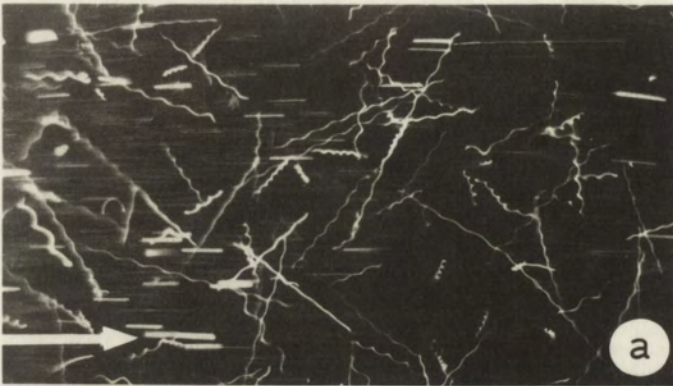
Pl. I a − 0 sec, Pl. I b − 5 sec, Pl. I c − 20 sec, Pl. II a − 40 sec, Pl. II b − 1 min, Pl. II c − 2 min, Pl. III a − 5 min, Pl. III b − 10 min, Pl. III c − 10 min

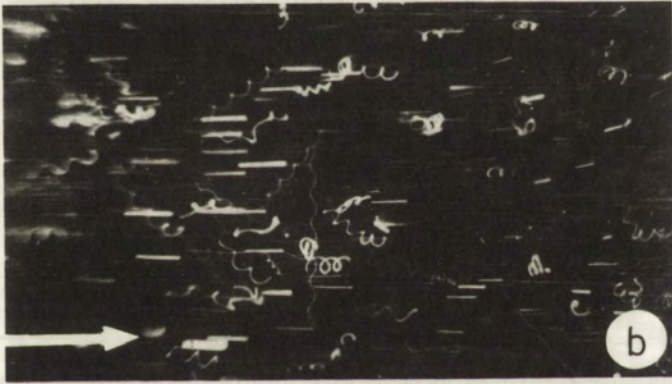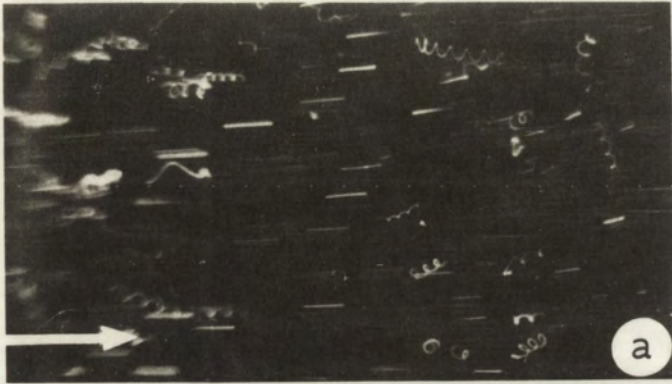Pl. IV − Successive transitional phases bringing about short tight path with specific direction (magnified 20 ×)
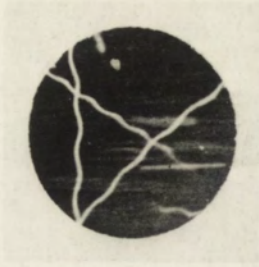
PLATE I

R. Pado

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PLATE II

R. Pado

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PLATE III
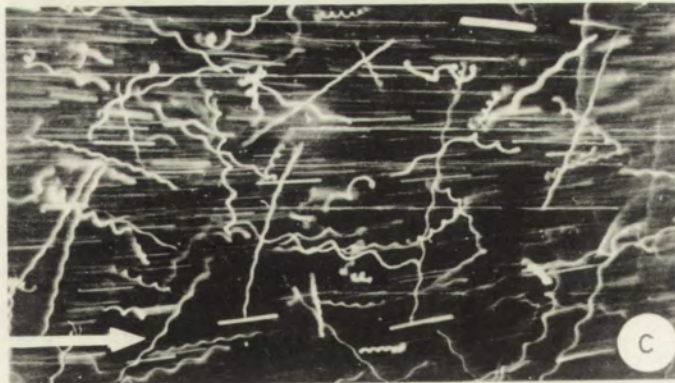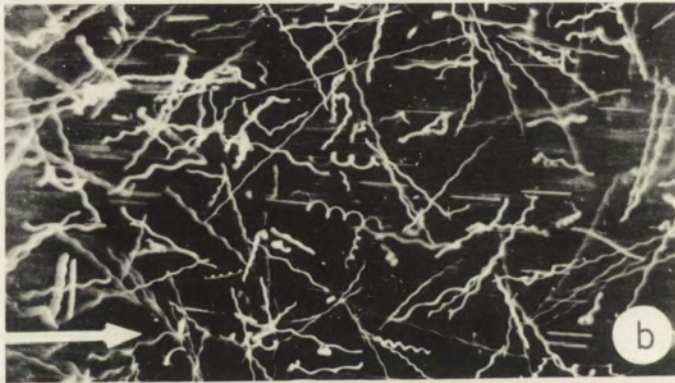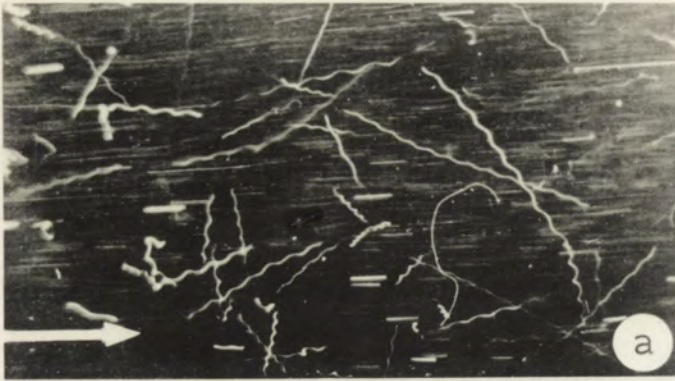
R. Pado

auctor phot.

R. Pado   auctor phot.

Anthony T. W. CHEUNG

Reactivation of Glycerinated Cilia from *Opalina*

Synopsis. Cilia were extracted from *Opalina obtrigonoidea* by glycerol, and were reactivated by ATP. Under Nomarski interference contrast and phase contrast optics, the cilia were observed to beat and locomote in a helical propagatory manner. The majority of the cilia, when reactivated, showed vigorous bending patterns. At the time when such bending waves were traveling progressively along the entire length of the cilia at a slow rate of about 1 wave per second, these isolated cilia rotated about their long axis simultaneously, spiralling through the medium at a speed of about 2 µm per sec. Such simultaneous movements constituted a continuous traveling helix. It had been revealed (and also documented) previously that the cilia of a freely swimming *Opalina* beat with a continuous traveling helical wave. The demonstration of the existence of a similar helical pattern of ciliary movement in the reactivation of isolated *Opalina* cilia could help to further confirm the existence of a continuous traveling helical beat for the cilia of freely swimming *Opalina*.

Introduction

High speed cinemicrographs revealed that the cilia of freely swimming *Opalina* beat with continuous traveling helical waves, propagating from base to tip along the entire length of the cilia, differing totally from the classical discontinuous forward and return stroke pattern popularly believed. It is predicted that if the continuous helical pattern of ciliary movement of *Opalina* is authentic, then the isolated cilia of *Opalina*, when reactivated by ATP under ideal physiological conditions, should demonstrate this helical pattern of movement.

Several methods of cilia isolation have been described in the literature (Child 1959, Watson et. al. 1961). Although they are very sophisticated in technique and excellent for many purposes, such procedures cannot provide isolated *Opalina* cilia capable of being reactivated.

This report describes a method for the extraction and isolation of the cilia of *Opalina* by glycerol, and reactivation by ATP. This experimental procedure is based on the Hoffman-Berling technique (1955) as modified by Gibbons (1965) and Preston (1972) to reactivate the cilia of *Tetrahymena pyriformis* and also utilized by Brokaw and Gibbons (1973) for sperm tail

reactivations. The preparations of isolated cilia obtained are relatively pure and free from cellular debris contaminations. Under ideal physiological conditions, such cilia can normally be reactivated when treated with ATP. Under Nomarski interference contrast and phase contrast optics, the reactivated cilia of *Opalina* can be seen to beat and locomote with a continuous helical pattern as predicted.

Materials and Methods

Biological Material

Opalina obtrigonoidea were obtained from the rectum of common grass frogs purchased from biological supply firms in Southern California. They were suspended in Naitoh's *Opalina* physiological medium (1964), and then washed thoroughly to remove all rectal contents with the same medium. The organisms were obtained by centrifugation, washed at room temperature with a hypertonic solution containing 0.2% NaCl and 0.2 M sucrose, and then resuspended in 15–20 ml of fresh wash solution. This pure suspension of *Opalina* cells could be concentrated by centrifugation, and the above procedures could be repeated until a reasonable amount of cells was obtained.

Extraction Medium
Medium (1) 70% glycerol (v/v), 50 mM KCl and 2.5 mM $MgSO_4$ buffered at pH 7.5 at 0°C.
Medium (2) 70% glycerol (v/v) in Naitoh's *Opalina* physiological medium.

Reactivation medium
0.2 mM ATP, 50 mM KCl and 2.5 mM $MgSO_4$ buffered at pH 7.5 at room temperature (20°C). An alternate medium could be made with 0.2 mM ATP and 2.5 mM $MgSO_4$ in Naitoh's *Opalina* physiological medium buffered at pH 7.5 at 20°C.

Procedure
The concentrated cell suspension was cooled to 0°C, and then mixed with the extraction medium, with 5 ml of concentrated cells to every 25 ml of reagent. Immediately after thorough mixing, the mixture of cells in glycerol was cooled to −20°C, and maintained at this temperature. Agitation of this suspension on a Vortex mixer for about 30–45 sec would cause the majority of the cilia to become detached without breaking up the cell bodies. The cell bodies were subsequently removed by centrifugation at 12000 g for 10 min. The supernatant, which consisted of a suspension of relatively pure isolated cilia, was removed and used immediately. Occasionally, they were stored at −20°C for future use. However, it had been demonstrated that the potential for motility in reactivation deteriorated in course of time.

The best reactivation was obtained by mixing a very small volume of the cilia suspension with 3 vol. of the reactivation medium. A few drops of this mixture were immediately transferred to a clean glass slide for observation under a microscope, equipped with Nomarski interference contrast and phase contrast optics.

Stored stock cilia were sometimes used, but the results were not as encouraging as that of freshly extracted ones.

Observations (Results)

The isolated cilia of *Opalina*, when reactivated with ATP under ideal physiological conditions, swim and locomote in a manner similar to the basic movement patterns of Spirochaetes, though in a much slower forward speed

of locomotion. The same type of helical propagation pattern had been suggested by Gibbons (1965) and later confirmed by Preston (1972) for *Tetrahymena pyriformis*.

Reactivated ciliary movement started immediately after the application of the reactivation medium, if at all, and could last for a maximum of 2 to 3 min. The majority of the cilia, when reactivated, showed a vigorous bending movement. At the time when the bending waves were propagating along the entire length of the cilia at a rate of about 1 wave per second, these isolated cilia rotated about their long axis simultaneously, propagating through the medium at a slow speed of about $2-3\,\mu$m per second.

A quick glance at the movement of the reactivated cilia showed undulatory waves propagating along the entire length of the cilia. Detailed and careful analysis revealed that the undulatory waves were actually propagating in a circular manner and that such waves were three-dimensional and helical in pattern — a fact that was previously suggested by Gibbons (1965), and later confirmed by Preston (1972) in *Tetrahymena pyriformis* and by Cheung (1973 a) in *Opalina*, in the same laboratory at the University of California at Los Angeles.

The average length of the cilia of *Opalina* was $12\pm2\,\mu$m and the cilia could be seen locomoting with the original base of the cilia moving foremost, suggesting that the waves were moving along the entire length of the cilia from base to tip; a fact that was established and documented by high speed cinemicrographs in my previous work in determining the ciliary beat pattern of freely swimming *Opalina* (Cheung 1973 a, b, c).

Discussion

Since the publication of Gray's work (1928, 1930), the planar discontinuous pattern of ciliary beat had been considered the basic pattern of ciliary movement, and not much work had been done on this field until the perfection of the rapid fixation technique by Párducz (1967) and later modified by Grębecki (1964) and also by Tamm and Horridge (1969, 1970). The updated pattern of ciliary beat was considered to be discontinuous but three-dimensional; with the rapid forward stroke going uni-directionally in one plane, and the slower recovery stroke returning in a path similar to that of the curved arc of the letter 'D' in an angularly deviated manner.

The continuous traveling helical ciliary pattern of freely swimming *Opalina*, as determined by high speed cinematography (Cheung 1973 b) differs completely from the classical discontinuous planar concept of Gray (1928, 1930) and Sleigh (1962), and it also differs remarkably from the discontinuous three-dimensional idea as proposed by Párducz (1967) and Tamm and Horridge (1969, 1970).

At a glance, the cilia appear to undulate in a manner similar to that of sea urchin sperm tail (Gray 1955), bull sperm tail (Gray 1962, Rickmenspoel 1965) or the posterior flagellum of *Ceratium* (Jahn et. al. 1963) in a planar propagatory manner. Detailed analysis and careful observations revealed that the ciliary beat of *Opalina* is actually three-dimensional. The ciliary beat is continuous and not separated into the popularly believed forward and recovery strokes. This three-dimensional traveling helical wave of *Opalina* it distally directed, and it exerts a locomotory force from base to tip along the entire length of the cilia in a continuous manner; thus propelling the organism to move in a direction opposite to the movement of the propagatory waves. Such observations are confirmed by careful frame-by-frame analysis of the high speed movies.

The helical propagatory pattern as demonstrated by the reactivation of glycerinated cilia of *Opalina* further confirms the authenticity of the continuous helical ciliary pattern of *Opalina*. However, it should be pointed out that NOT all ciliates locomote in this helical pattern. It had been demonstrated that *Spirostomum* locomote in a manner similar to the popularly believed pattern in that the forward stroke is planar and that the recovery stroke is angularly deviated (Cheung and Winet 1974). It should also be pointed out that *Paramecium multimicronucleatum* (Kuźnicki et. al. 1970), *Tetrahymena pyriformis* (Preston 1972) and *Colpidium* (Wilson et. al. 1974) beat with this continuous helical pattern.

This isolation of cilia from *Opalina* by treatment with glycerol is analogous to the preparation of glycerol-extracted muscle fibres. In most cases the movement obtained is some sort of a wave bending, with slow progression through the medium. The speed of forward movement and the rate of propagation of the waves are very slow compared with the actual motion of the cilia of a freely swimming *Opalina*. Theoretically, isolated cilia can be reactivated by ATP to give a wave propagation along the cilia at a rate close to that of the normal pattern under normal freely swimming conditions. The slowing down of the wave propagation and the sluggishness in forward movement suggested very strongly the fact that the extraction process by glycerol had not broken down completely the physiological membrane "barrier" for ATP action. This results in a slowing down of the ciliary activity. Preliminary trial attempts with Triton-X-100 as an extraction reagent had provided better results in that the rate of wave propagation was about 2 to 3 times faster than that of glycerinated models, though still much slower than the normal ciliary beat rate.

Variation of the concentration of ATP in the reactivation process affected the movement and propagation of the cilia in a manner similar to that described for *Polytoma uvella* by Brokaw (1961, 1963). ATP is the essential and the only available energy source of the reactivation process, and no

ciliary movement is observed in its absence or at low concentrations.

Reactivations had occasionally been obtained at pH values of from 6.8 to 7.8. However, the best results were obtained at pH 7.5. From the experimental data obtained, the pH value is not a very significant reactivation factor. As long as the pH values are within the 6.8 to 7.8 limit, reactivation can take place.

Free calcium ions did not appear to be essential for reactivation. The first reactivation medium used was Ca^{2+} free, while the alternative medium contained 0.1 mM $CaCl_2$ (Naitoh's *Opalina* physiological medium). In both cases, reactivation of ciliary activity was obtained. Chelating agents had been added to the first medium in control measures to remove any possible trace amount of Ca^{2+}. In all such attempts, reactivation was achieved, with or without free Ca^{2+} ions, illustrating that Ca^{2+} ions were not required for reactivation.

Complications can easily arise if the *Opalina* cells are lysed in the extraction process, as the lysed cells can contaminate the intact cells to form clumps in the glycerol solution. Such clumps can cause serious contaminations in the final cilia preparation and will have a drastic effect on reactivation attempts. For ciliary reactivation, a pure cilia sample is absolutely necessary. This cell lysis can be prevented by the utilization of a strong hypertonic NaCl-sucrose solution as a washing (and at times even as a suspension) medium. With this precautionary measure, the final suspension of cilia is very pure; thus enhancing the potential of reactivation, and avoiding other possible experimental complications.

It should be stressed that such a reactivation technique of *Opalina* cilia has yet to be perfected, as not all the attempts were successful. Nearly 50% of the attempts were in vain as the reactivation succeeded only in causing the cilia to lash about or to wriggle about for a few seconds, without any forward movement and without demonstrating any definite pattern of propagation. However, the ones that were reactivated showed an obvious helical pattern of movement as predicted.

RÉSUMÉ

Les cils de l'*Opalina obtrigonoidea*, après leur extraction avec du glycérol, étaient réactivés par l'ATP. En utilisant le contraste d'interférence de Nomarski et le contraste

de phase, on a constaté que les cils battent et se déplacent de façon hélicoïdale. Ils montrent en majorité, après la réactivation, des courbatures très prononcées. Les courbatures forment des ondes qui suivent toute la longueur du cil en progressant lentement avec une fréquence d'une onde par sec environ, et en même temps le cil isolé tourne autour de son axe longitudinal, dont résulte un mouvement spiral à travers le milieu d'une vélocité de 2 μm par sec en moyenne. Tous ces mouvements simultanés forment un hélix de progression continue. Il a été démontré et prouvé précedement que les cils d'une *Opalina* en nage libre battent en formant une onde hélicoïdale qui progresse de façon continue. La démonstration que chez les cils isolés et réactivés de l'*Opalina* existe un pareil caractère hélicoïdal du mouvement aide à reconfirmer l'existence du battement ciliaire en forme des ondes hélicoïdales continues chez les *Opalina* en nage libre.

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The Infectivity of Culture Forms of Trypanosomes to Tsetse Flies

Synopsis. Bloodstream and culture forms of *Trypanosoma brucei* and *T. congolense* were fed through chicken skin membrane to *Glossina morsitans*. The experimental procedure was controlled in such a way that flies fed on different forms of each species of trypanosomes were exposed to similar concentration in the infective feed and they were treated similarly before and after infective feed. The culture of *T. brucei* harvested early produced similar infection rate in tsetse flies as the blood forms, while the culture harvested late had its infectivity to flies significantly reduced. Both early and late cultures of *T. congolense* produced significantly higher rate of infection than the blood forms. The differences in the infection rates are attributed to the morphological and physiological differences between the blood and culture forms and between the culture forms of different ages.

Introduction

When tsetse flies were fed on culture forms of trypanosomes, the midgut infection rate was observed to be higher than when they were fed on blood forms; the rate of mature infections, however was similar in both cases (Thomson and Sinton 1912, Reichenow 1939, Gordon and Miller 1961). The experiments from which these workers made their observation were not controlled. Flies were fed on culture forms under conditions different from those that prevailed when they were fed on blood forms. In this investigation, the experiments were controlled and the experimental flies were treated similarly before and after ingestion of both forms of trypanosomes.

Materials and Methods

Bloodstream and culture forms of derivatives of *T. brucei* Treu 667 and *T. congolense* Treu 692 were used. The bloodstream forms of these species were made into stabilates and preserved in liquid Nitrogen ($-196°C$). Before preservation, the number of trypanosomes per ml blood was estimated with the aid of a Neubauer haemocytometer (Table 1 and 2).

The species of tsetse fly used was *G. morsitans*. In control experiments stabilates of each species of trypanosomes were diluted 1 : 100 with defibrinated ox blood and fed to the flies with the aid of the artificial membrane feeding technique (Dipeolu 1972). Those that fed on *T. brucei* were divided into two batches. One batch of flies was dissected 3 days and the other 30 days post infection. Flies that fed on *T. congolense* were treated similarly and the two batches were dissected 3 and 21 days post infection.

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The stabilated trypanosomes were inoculated into 3 mice. After the mice had become parasitaemic, culture forms were prepared by introducing the blood stream forms into hanging drop cultures of complete alimentary tract of *G. morsitans* pupae, which were older than 21 days. Subcultures were prepared every 4 days. This method of culturing trypanosomes has been described (Cunningham 1971). Cultures of *T. brucei* were harvested 3 and 11 days after initiation then the parasites were counted, mixed with defibrinated ox blood to the concentration comparable with that of the control experiment, and finally fed to the flies through the membrane. Cultures of *T. congolense* were harvested 3 and 9 days after initiation, then treated as described above. Flies which fed on the culture forms were dissected at the same intervals as those that fed on their corresponding blood forms.

All experimental flies which fed on bloodstream and culture forms were treated similarly before and after infective feed. They were maintained at 26°C and relative humidity of 65–70%. The first feed of the flies was the infective feed given within 36 h of eclosion. Those flies kept under observation for 21 and 30 days were maintained on mice, one mouse being allocated to each fly. Flies in which trypanosomes were found during dissection in the midgut 3 days after infection were classified as "infected" while those which had infected the mice on which they had fed during their observation for 21 or 30 days were recorded as having "mature" infections. Results were compared by calculating X^2 by means of a 2×2 contingency table. Where the total number for comparison was less than 200, Yates correction was applied (Fisher 1941).

Results

The *G. morsitans* which had fed on the bloodstream and culture forms of *T. brucei* and dissected 3 days later possessed similar infection rates (Table 1). Prolongation of the culture period (11th day culture) had, however, reduced the infectivity of the trypanosomes to the fly. In case of

Table 1

Results of Feeding Flies on Bloodstream and Culture Forms of *Trypanosoma brucei*

Designation	*Trypanosoma* concentration per ml blood	Total flies fed	Day dissected after feed	Total infected	Total mature	Average prepatent period in mice	Average length of development cycle
Bloodstream forms	5.3×10^6	67 100	3 30	34	 3	6 days	25 days
3rd day culture forms	5.0×10^6	25 75	3 30	11	 1	6 days	25 days
11th day culture forms	5.0×10^6	25 75	3 30	4 *p 0.01	0	—	—

* Value of x^2 was obtained by comparing the infection rates of the bloodstream forms and the 11th day culture forms 3 days after infective feed

T. congolense, the infection rates among flies which fed on the 3rd and 9th day culture forms and dissected 3 days later were similar but were significantly higher than the infection rate among the flies that fed on blood forms; however, the proportion of mature infections were similar for both forms.

The period of the development cycle was prolonged and the prepatent period in mice became longer in flies which fed on culture forms of *T. congolense* (Table 2).

Table 2

Results of Feeding Flies on Bloodstream and Culture Forms of *Trypanosoma congolense*

Designation	Trypanosoma concentration per ml blood	Total flies fed	Day dissected after feed	Total infected	Total mature	Average prepatent period in mice	Average length of development cycle
Bloodstream forms	4.4×10^6	77	3	47			
		115	21		10	8 days	9 days
3rd day culture forms	4.4×10^6	25	3	25			
		50	21	*p 0.01	3	12 days	19 days
9th day culture forms	4.4×10^6	45	3	41			
		75	21		3	12 days	19 days

* Value of X^2 was obtained by comparing the infection rates of the bloodstream forms and the 3rd day culture forms 3 days after the infective feed

Discussion

The results of this investigation confirm the observations of the previous workers that the culture forms of trypanosomes are capable of undergoing complete development cycle in *Glossina* and that the number of mature infections, when compared with that obtained by feeding the flies on blood forms, is not influenced. However, the observation that the culture forms produced an initial higher rate of midgut infections was only confirmed for *T. congolense*. With *T. brucei* it appears that the age at which the culture is harvested may be one of the determining factors affecting the infectivity to tsetse flies. The 3rd day culture forms of both species of trypanosomes were observed to be morphologically similar to the blood forms while the 9th and 11th day culture forms had assumed the broad and long shape very similar to the initial "typical" midgut form described by Dipeolu (1972). It could therefore be explained that the results of the

3rd day dissections of flies that fed on bloodstream and culture forms of *T. brucei* are similar because the flies ingested trypanosomes of identical morphology and concentration in the infective feed. It is however, not known why such similar results were not manifested in flies which fed on the bloodstream and 3rd day culture forms of *T. congolense* and dissected on the 3rd day post infection.

Brown and Evans (1971) found that, apart from the morphological changes, two distinct physiological forms of trypanosomes can be obtained when bloodstream forms of *T. brucei* are placed in a suitable culture medium. These forms they name Type A, which is found immediately after transfer from blood to culture medium, and Type B, which develops later. They found some biochemical differences between these two types such as the difference in sensitivity of their succinoxidase to cyanide. It is probable that most of the physiological forms of the 3rd day culture trypanosomes belong to Type A while of those of the 11th day culture trypanosomes belong to Type B. The difference in the susceptibility of these types to tsetse flies may be the results of their different biochemical reactions to the midgut environment. This explanation cannot yet be extended to *T. congolense*, since very little culturing of this species had been reported, and it is not known which, if any, physiological forms are present in its culture.

Some authors, basing their observations on morphology (Thomson and Sinton 1912), ultrastructure, (Vickerman 1969) and biology (Reichenow 1939) had assumed that the culture and the fly midgut forms of trypanosomes are identical. Dar (1971), however, concluded that the similarities are not as rigid as assumed. The results of this investigation appear to agree with Dar's conclusion. If both forms were rigidly similar, it should be expected that the culture forms would be "pre-adapted" to development in the fly and that the duration of the development cycle would be shortened. It should also be expected that the midgut infection rate would be high. The latter expectation was fulfilled in *T. congolense* but not in *T. brucei*. The former expectation was not fulfilled at all and in fact, the duration of development cycle of culture forms of *T. congolense* was prolonged.

ZUSSAMENFASSUNG

Die mit Rinderblut in Verhältnis 1 : 100 vermischte Blut- und Kulturformen der *Trypanosoma brucei* und *T. congolense* wurden durch Huhnhautmembran von *Glossina morsitans* gesaugt. Das Experiment wurde kontrolliert, damit die Tsetsefliegen, die die Blutformen, und diejenigen, die die Kulturformen gesaugt haben, derselbe konzentration von Trypanosomen ausgesetzt waren. Das Infektionsrate in Tsetsefliegen, die die früh gewonnenen Kulturformen von *T. brucei* gesaugt haben, war nicht von bedeutsamer Abweichung von dem der Fliegen, die die Blut-formen gesaugt haben. Das Infektionsrate der die spät gewonnenen Kulturformen von *T. brucei* gesaugten Fliegen war sehr niedrig und von bedeutsamer Abweichung von dem

der Blutformen oder der frühgewonnenen Kulturformen gesaugten Fliegen. Die früh und spät gewonnenen Kulturformen von *T. congolense* produzierten bedeutsame hohere Infektionsraten in Tsetsefliegen als die Blutformen. Die Ungleichheiten in Infektionsraten sind auf die morphologischen und physiologischen Verschiedenheiten zwischen den Blut- und Kulfurformen einerseits und zwischen den früh und spät gewonnen Kulforformen zuruchgeführt.

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