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Body Contraction and Ultrastructure of *Euglena*

Synopsis. On the basis own physiological and ultrastructure studies of *Euglena gracilis, Euglena ehrenbergii* and critical review of literature concerning *Euglenoidina* authors suggest that: (1) Longitudinal and lateral changes in the euglenas cell shape are evoked by contraction of the fibrillar continuous structure (fibrillar sac), lying immediately under the strips. (2) Contraction occur as a effect of a reorientation of the locomotory flagellum. Flagellum taking the phobic position causes deformation of the resorvoir shape in turn stimulate to contraction the fibrillar sac. (3) Fibrillar structures within the strips and forming the periplast together with microtubules, are subjected to stress during contraction, which would bring the cell back to its cigar-like shape. These stresses would also play an essential role in peristaltic movements. (4) The relationship between the flagellum-reservoir system and the contractile system occurring in *Euglena* have served as basis for the development of receptor → effector relationship in multicellular organisms.

Locomotion of eukaryotic cells and change of their direction of movement occur either by means of specialised organelles or else by a change in the cell shape. There are organisms among protozoans which exhibit both these types of motor activity. To this group belong also a number of species classified as *Euglenoidina*. Body shape changes of these organisms have been described up to the middle of the bygone century as "metaboly" or "euglenoid movement" (Fig. 1) and they are still the least known kind of cellular movements, at any rate their principle and character have so far not been elucidated. It is hoped that the results of investigations presented in this paper, conducted mostly on *Euglena gracilis* (Fig. 2) will contribute to the explanation of some
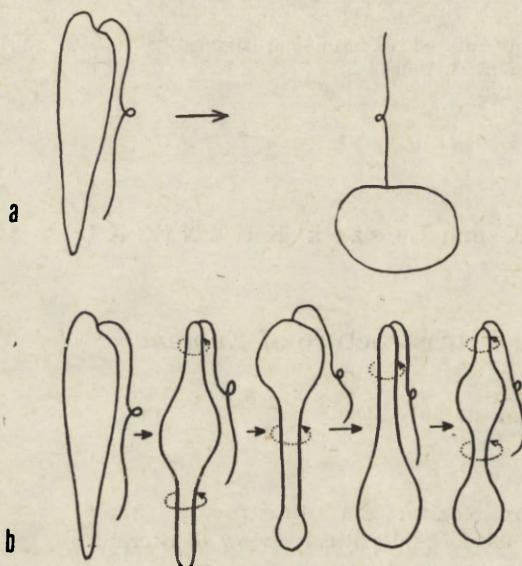
Fig. 1. Scheme of the euglenoid movements of *Euglena gracilis*. a — logitudinal contraction; b — peristaltic wave. Arrows mark the contracted zone

aspects of these movements. Another aim in view was the demonstration of a relationship between movements of the flagellum and change of cell body shape.

Euglenoidina are an interesting group also from the evolutionary aspect (L e-e d a l e 1967, J o h n s o n 1968). According to classical views, *Euglenoidina* were supposed to belong to green algae because of the presence of chloroplasts. It is known at present that *Euglenae*, on account of the structure of their nucleus and the course of mitosis, differ esentially from all green algae thus far known. They actually have many more common traits with heterotrophic flagellates than with green algae.

Chloroplasts present in green algae seem to have arisen from endosymbionts of prokaryotic algae, while the chloroplasts of *Euglena* are transformed cells of eukaryotic algae (S t e w a r t and M a t t o x 1975, G i b b s 1978). All these facts make *Euglena* a particularly interesting object for studies on evolution.

The opinion prevails at present that flagellates are the oldest group among *Protozoa* and that *Metazoa* are derived from colonial flagellates. It is also doubtless that colonial flagellates and the group *Euglenoidina* exhibit a number of common traits. The genealogical relationship between flagellates with euglenoid structure and *Metazoa* is greatly strengthened by considerations concerning the evolution of receptor cells in animals (V i n n i k o v 1946, 1971, 1974, 1979). If we consider chloroplasts as organelles of symbiotic origin, a cell with *Euglena gracilis* structure may be treated as the precursor structure of receptor cells of invertebrates and vertebrates (Fig. 3). The parts of the Figure surrounded by a circle, are shown on Pl. Ill 19–21, Pl. IV 22, 23. This supposition may prompt a discussion on the role which contraction processes played in evolution, making euglenoid movements possible. This problem actually boils down to the consideration of euglenoid move-

ments as primary forms of cell body contraction, from which the contractions occurring in other protozoans are derived.

A further problem here is to what extent the primary contraction systems of flagellates could have undergone evolution towards specialised cells and muscle tissues.
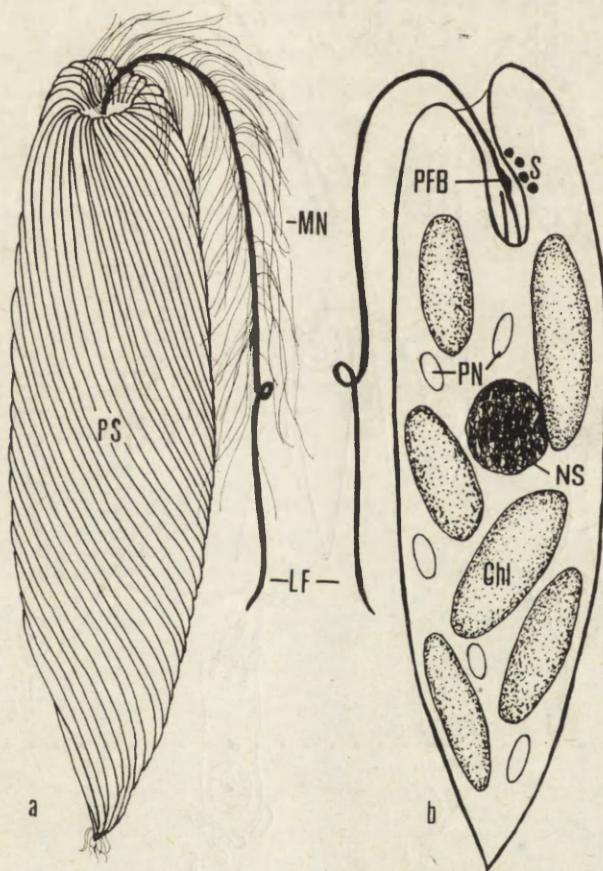
Fig. 2. Exterior view (a) and interior structure (b) of *Euglena gracilis*. PS — pellicular strips, LF — locomotor flagellum, MN — mastigonemes, S — stigma, PFB — paraflagellar body, Chl — chloroplast, PN — paramylon, NS — nucleus

An exhaustive answer to this question is not possible at the present state of knowledge. The consideration of these problems is, however, useful since it shows the gaps which should be filled in the future.

Different Forms of Cell Body Movements

When investigating the behaviour of euglenoid flagellates, a diversity was observed of the changes in their body shape. In *Euglena gra-*

cilis, there may be either longitudinal (anterior–posterior) contractions (Fig. 1 a, Pl. I 12, 13) or peristaltic waves (Fig. 1 b, Pl. I 1–6). The latter appear as a ring of contraction advancing slowly from the posterior to the anterior of the cell (C h a d e f a u d and P r o v a s o l i 1939,
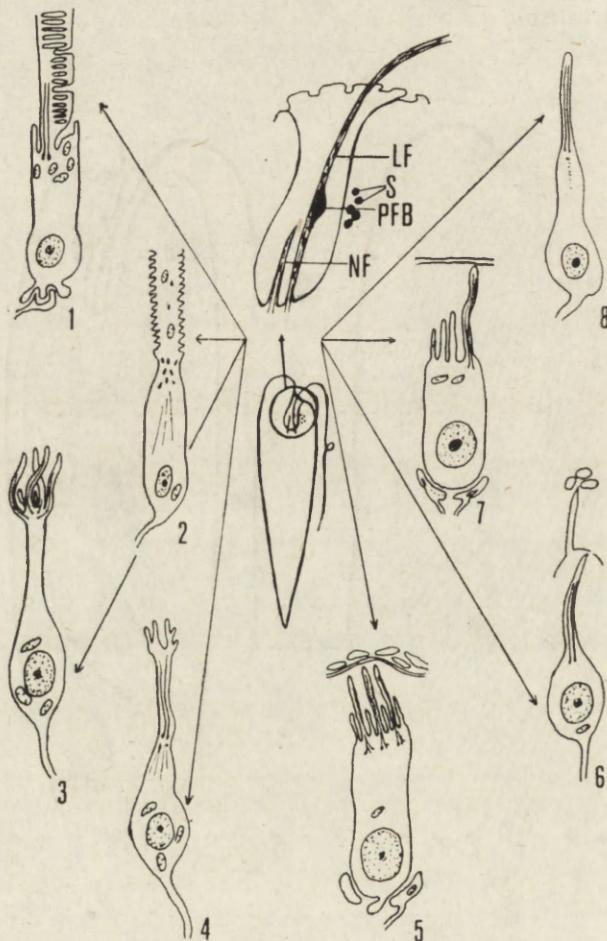
Fig. 3. *Euglena* — the starting point for other sensory cells. 1 — photoreceptor of vertebrates, 2 — photoreceptor of molluscas, 3 — olfactory cells of *Chordata*, 4 — olfactory cells of insects, 5 — receptor of gravity in cephalopods, 6 — receptor of gravity of arthropods, 7 — auditory cells of vertebrates, 8 — auditory cells of insects, NF — nonmergent flagellum, LF — locomotor flagellum, PFB — paraflagellar body, S — stigma

M a c k i n n o n and H a w e s 1961, M i k o ł a j c z y k 1972). Peristaltic waves also appear, for instance in *E. mutabilis* (H o l l a n d e 1942), *Astasia captiva* (B e a u c h a m p 1911), *Peranema trichophorum* (C h e n 1950). Euglenoid movements may take the form of lateral bendings which are commonly observed in *E. ehrenbergii* (Pl. II 15) (H a m b u r-

ger 1911, Pringsheim 1956, Heimpel 1972, Mikołajczyk 1975), *E. terricola* (Günther 1927), *E. limosa* (Conrad 1940), *E. granulata* (Arnott and Walne 1966) and others. Moreover, such euglenoid movements have been referred to as surface fluctuation (Arnott and Walne 1966), or oscillatory movements (Günther 1927). The particular type of euglenoid movements may occur in sequence, for instance in *E. gracilis* anterior–posterior contractions may be followed by peristaltic waves.

Various species of *Euglenoidina* were found to be characterised by a different ability of changing their shape. From this aspect *Euglenoidina* have been classified into four groups (Pringsheim 1948, 1956, Mignot 1965, 1966): (1) with strong euglenoid movements (e.g., *E. granulata, E. mutabilis*), (2) moderately rigid ones (*E. agilis, E. gracilis*), (3) almost rigid ones (*E. acus, E. tripteris*) and (4) completely rigid ones (e.g., species of the genus *Phacus, Menoidium* and *Rhabdomonas*). It was found that organisms with strongly marked strips, the periplast of which is thick and forms teeth, have a very limited range of euglenoid movements. The strips seem to stiffen and mechanically limit the cell movements, thus determining the range of euglenoid movements performed (Wager 1900, Günther 1927, Chu 1946, Jahn 1946, Gojdics 1953). However, it was also observed for instance that *E. acus* which, as regards the structure of the pellicular complex (highly developed periplast forming "teeth" under the strip), belongs to the group of almost rigid organisms, contracts strongly when the environmental conditions (e.g., pH) change (Deflandre 1924, Le Fèvre 1931, Szabados 1936). Similarly *E. ehrenbergii* contracts when it loses its locomotor flagellum, although in view of its structure it should be classified as an almost rigid *Euglena* (Pl. II 4–18) (Chu 1946, Conrad and Van Meel 1952, Heimpel 1972, Mikołajczyk 1975). Chu (1946) advanced the hypothesis that the ability of changing the body shape in *Euglena* does not depend on the structure of the strips, but on the periplast content. The more compact it is, the more limited are the euglenoid movements. Therefore, *E. ehrenbergii*, the periplast of which is not very compact, in spite of the structure of its pellicular complex and particularly of its very thick periplast (in the dorsal zone of the strips ca. 1 μm), is capable of strong contractions. Pringsheim (1948, 1956) and Mignot (1965, 1966) believe, however, that neither the pronounced strips nor their size are closely connected with the ability of body shape change in *Euglena*.

Distinct striation is for instance characteristic both of "rigid" *Euglena* (e.g., *E. tripteris*, the genus *Phacus, Rhabdomonas*) as well as of the highly contractile ones (*E. granulata, Distigma proteus*). Delicate, little

pronounced striation has been noted both in contractile *Euglena,* such as *E. mutabilis* and in the almost "rigid" *E. acus.* On the other hand, changes in shape and size of the pellicular strips were observed in contracted and uncontracted body parts of *E. viridis* (G u t t m a n and Z i e g l e r 1974, F o i s s n e r 1977). The strips of the contracted part of the cell are higher and more depressed in the ridge part of the strip, whereas in the uncontracted or only slightly contracted part they are wide and flat. Even neighbouring strips may differ widely. According to F o i s s n e r (1977) this seems to indicate that the particular strips may change their shapes independently. These observation confirm earlier observations of M i g n o t (1966) who suggested that metaboly is a resultant of deformations occurring at the level of the particular strips.

In the opinion of the present authors, all *Euglena* species — the swimming and the creeping ones — are capable of changing their body shape and their forms may be extremely diversified. The pattern of euglenoid movement of *E. gracilis* leads to the supposition that these movements are evoked by contractions of the structure surrounding the whole cell interior and lying immediately under the strips.

Factors Stimulating and Immobilising Euglenoid Cell Movements

It has long been noted that various factors of chemical and physical nature evoke euglenoid movements, and this has been contemporarily confirmed (D i s k u s 1956, M i k o ł a j c z y k 1973, D o u g h t y and D i e h n 1979, M i k o ł a j c z y k and D i e h n 1978, Y o u q u e et al. 1979). *E. terricola* for instance is so sensitive that it is sufficient to transfer it to fresh culture medium to produce long lasting euglenoid movements (G ü n t h e r 1927, H a l l 1933). Strong body contractions appear at pH changes both from alkaline to acids and vice versa (A l ex a n d e r 1931, H a l l 1933, S z a b a d o s 1936, C o n r a d 1940, K a m i y a 1939, 1953). B o v e e and A c u ñ a (1970) demonstrated relations between the presence of ATP in the medium and the appearance of body contractions during the photophobic reaction of *Euglenae.* While in an ATP-free medium *Euglena* emerge and leave an illuminated region without body contractions and without strong photophobic reaction, in the presence of ATP this photophobic reaction is associated with euglenoid movements.

Body contraction may be caused by temperature changes (D a n-

geard 1902, Youque et al. 1979), X-rays (Wichterman 1955), changes in light intensity (Lozina-Lozinsky 1963, Bovee and Acuña 1970, Mikołajczyk and Diehn 1976), electric current evoking body contraction of the *Euglena* on the anode side (Bancroft 1915) and mechanical factors (Jahn and Bovee 1968, Nickols and Rikmenspoel 1977, Mikołajczyk and Diehn 1979).

Thus, any factor causing a shock reaction of the organism manifested by a change in the position of the flagellum, or producing its autotomy, may at the same time evoke euglenoid movements. This relation has long been noted and confirmed many times (Dangeard 1902, Jennings 1906, Bancroft 1915, Hall 1931, Le Fèvre 1931, Fritsch 1935, Hilmbauer 1954, Wichterman 1955, Mackinnon and Hawes 1961, Bovee and Acuña 1970, Mikołajczyk and Diehn 1976, Youque et al. 1979) (for the other references see Leedale 1976, and Jahn and Bovee 1968). An exception from this regularity are the peristaltic waves in *E. gracilis* which may appear without simultaneous noticeable changes in the motion of the flagellum (Mikołajczyk 1973).

A known factor causing immobilisation of the cilia with simultaneous preservation of the ability of reorientation is $NiCl_2$ (Kuźnicki 1963, Naitoh 1966, Grębecki and Mikołajczyk 1968). As regards *E. gracilis* $NiCl_2$ causes immediate immobilisation of the cells (Doughty and Diehn 1979). After immobilisation the flagellum stimulated with light of intensity producing in swimming individuals a photophobic reaction did not change its position. According to Doughty and Diehn (1979), immobilisation of swimming occurs much more slowly under the action of nickel ions in the presence of Ca^{2+} and Mg^{2+} ions. Our preliminary observations of the behaviour under these conditions of nonmotile *Euglenae* allowed to establish the following chain of motor reactions of *E. gracilis*: the first step — when the flagellum is still active and light stimulation causes its maximum reorientation. If the stimulus lasts sufficiently long an anterior–posterior body contraction occurs. In the second step the flagellum is completely immobilised in normal position (along the body). The light stimulus, however, produces its maximal reorientation and an anterior–posterior body contraction. It would seem, therefore, that for body contraction flagellar beating is not indispensable, only a change of the flagellum position to the phobic maximum (full reorientation).

In contrast to the stimulating factors, a reversible immobilisation of euglenoid movements may be only evoked by some physical and chemical factors.

Kitching (1957), Byrne and Marsland (1965) and Gross (1965) investigated the influence of hydrostatic pressure on *Euglena*. Byrne and Marsland established that flagella moved in *Euglena* subjected to hydrostatic pressure of 15 000 psi, whereas the euglenoid movements were inhibited at a pressure as low as 10 000 psi, both in rounded and in elongated cells. The authors ascribe the rounding of cells to changes which may occur in the structure of the gel (periplast) in the pellicular complex. At the moment of decompression *Euglena* exhibited a post-decompression contraction like many other protozoans. Inside a hyaline layer was visible surrounding the contracted dense granular mass with the nucleus and chloroplasts in the centre of the cell. In the course of 3 min nearly all individuals started to swim normally.

Diskus (1956) investigated *in vivo* the influence of dyes on euglenoid movements. He demonstrated that cation dyes are absorbed in the periplast of *E. halophila,* causing at first strong contractions which are later inhibited.

The lack of a carbon source in the medium causes a gradual diminution of the cell dimensions, disability to swim, shedding of the flagellum and after 30 days a loss of ability to change the body shape (Leedale and Buetow 1976).

Silwerman and Hikida (1976) while studying the influence of a colchicine solution on the course of flagellum regeneration in *E. gracilis* noted that deflagellation occurring owing to 'drastic pH changes from acid to alkaline, with simultaneous shaking, caused a rounding of the cells and disappearance of the euglenoid movements. The latter reappeared after about 10 min, whereas in cells treated with colchicine this occurred much later. It should be stressed, however, that regeneration of the flagella in the presence of this drug is also delayed. Thus, it is not clear whether the delayed appearance of euglenoid movements is connected with the retarded regeneration of the flagella or with the direct action of colchicine on the mechanism responsible for the euglenoid movements.

Dinitrophenol (DNP) is a factor inhibiting immediately but reversibly body contraction in all individuals (Mikołajczyk 1973). This compound also produces autotomy of flagella in general. After transfer to a medium free of DNP the ability of euglenoid movements returns before full regeneration of the flagellum (before its appearance outside the reservoir). Immediate immobilisation of euglenoid movements by DNP supports the supposition that the structures responsible for these movements lie in the pellicular complex or in its close vicinity.

Relationships Between the Flagellar Responses and Cell Body Contraction

All studies to date have demonstrated that in *E. gracilis* the maximum flagellar reorientation is often followed by anterior–posterior cell contractions. During the shock reaction under the influence of stimuli (e.g., light) there occurs in the swimming individual a reorientation of the locomotor flagellum. From its normal position (along the body) the flagellum passes to the minimal phobic position (Fig. 4 2) perpen-

Fig. 4. Scheme of relationship between flagellar responses and longitudinal contraction of *Euglena gracilis*
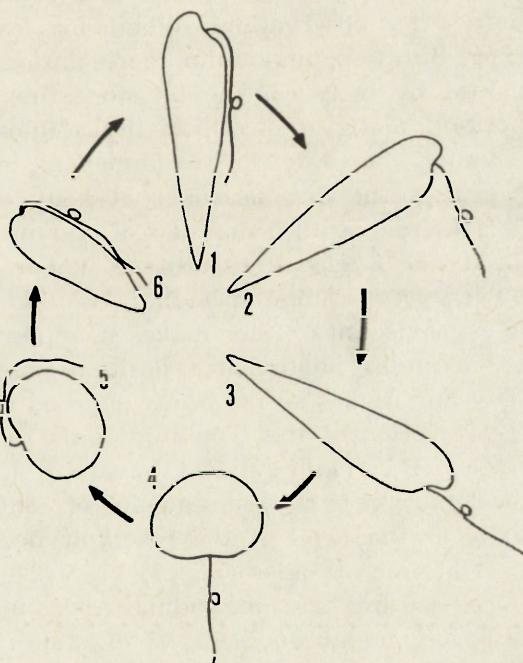
dicular to the long body axis. This results in the arrest of forward swimming and a starting of tumbling. Body contractions are not associated under these conditions (with such a position of the locomotor flagellum) with the phobic reaction of the cell (M i k o- ł a j c z y k and D i e h n 1976).

In a medium with raised viscosity the light impulse of the same intensity as before produces in 100% of the individuals maximal phobic reorientation of the flagellum and after about a subsequent body contraction (M i k o ł a j c z y k and D i e h n 1976). The same reaction can be elicited by immobilising and *Euglena* by way of sucking its posterior end into a capillary. Under these conditions light stimulation also evokes maximal reorientation of the flagellum and body contraction (M i k o- ł a j c z y k and D i e h n 1979).

If the stimulus acts sufficiently long, a strong body contraction causes the *Euglena* to assume a disc shape (M i k o ł a j c z y k 1973). In this form the *Euglena* may rotate for as long as several minutes (Fig. 4 4). Gradually, in spite of the presence of the stimulating factor, body contraction relaxes after the return of the flagellum to the normal position (along the body) (Fig. 4 6).

The time between the appearance of the contraction and light stimulation depends on the length of the flagellum. This was shown by the experiments carried out on *Euglena* without the locomotor flagellum outside the reservoir (M i k o ł a j c z y k — unpublished). The more the regeneration of the flagellum is advanced, the shorter is this time (60 to $\approx$ 1 s). These observations become understandable if we assume that the flagellum taking the maximal phobic position deforms the reservoir to an extent dependent on its length. Changes in the shape of the reservoir, in turn, stimulate the occurrence of anterior–posterior body contractions. This explanation would be in agreement with other observations. When, for instance, the acting stimulus is of short duration, maximum reorientation of the flagellum is not accompanied by body contraction since the flagellum rapidly returns to its position along the cell. Methylcellulose at viscosities above 4000 cp causing, when *Euglena* are immersed, immediate autotomy of the flagella, raises the disappearance of body contraction due to light stimuli. A decrease of the viscosity of the medium ($>$ 4000 cp) restores the ability of *Euglenae* to contract under the influence of light (M i k o ł a j c z y k — unpublished). It would seem, therefore, that a medium of high viscosity either makes it impossible for the flagellum to assume a maximum photophobic position or it prevents deformation of the reservoir. It has so far been supposed (P i c c i n i and O m o d e o 1975) that erection of the flagellum is the result of changes in the reservoir shape. P i c c i n i and O m o d e o (1975) suggested that flagellum reorientation is the consequence of contraction of the "fibrillar matrix" lying at the level of the reservoir neck between the longitudinal and circular microtubules (Fig. 7, Pl. V 24, 25) or of the interaction of the fibrils joining the longitudinal microtubules to one another and to the reservoir membrane (Pl. V 26, 27) (M i k o ł a j c z y k and D i e h n 1979). The sequence of reactions between introduction of the light stimulus and body contraction of the *Euglena* would be, according to the above quoted authors, as follows: (1) reception of the stimulus by PFB (Fig. 7, Pl. IV 22, 23), (2) transduction of the impulse further to the effector — fibrillar matrix or fibrils joining the longitudinal fibrils with the reservoir membrane, (3) contraction of the fibrillar matrix or interaction of the fibrils with the longitudinal microtubules of the reservoir, (4) change of reservoir shape, (5) reorientation of the flagellum, (6) body contraction.

On the basis of our observations we suggest a different sequence (Fig. 5 *1*): (1) the light stimulus reaches PFB (Fig. 5 *2*), (2) it switches on the contraction system within the flagellum, this causing maximal erection (Fig. 5 *2*), (3) subsistence of the flagellum in erect position

causes deformation of the reservoir which when it reaches a certain extent produces anterior–posterior body contractions (Fig. 5 3), (4) transduction of the reservoir shape changes to the contractile system of the cell body may occur either directly as the results of mechanical stimulation of the layer under the pellicular strips or by means of the reser-
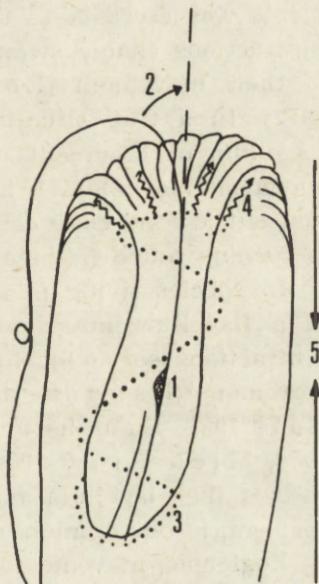
Fig. 5. Hypothetical correlation between the flagellar reorientation and longitudinal contraction of *Euglena gracilis*. The chain of the cell responses from the impulse reception to body contraction. 1 — reception of the light impulse by PFB, 2 — maximal flagellar reorientation (broken line), 3 — changes of the reservoir shape (dotted line), 4 — stimulation of the contractile structure (fibrillar sac) within pellicular complexes of the cell body (zig-zag arrows), 5 — longitudinal cell contraction. Arrows show the direction of contraction

voir membrane as previously suggested by M i k o ł a j c z y k and D i e h n (1979), (5) anterior–posterior contraction (Fig. 5 5). B o u c k et · al. (1978), M i l l e r and M i l l e r (1978), M i k o ł a j c z y k and D i e h n (1978), and R o g u l s k i and B o u c k (1978) demonstrated that in *Euglena gracilis* the membrane covering the flagellum, reservoir and canal differs in structure and properties from the cortical membrane. The latter is of crystalline nature, whereas the reservoir membrane is of fluid type and resembles other biological membranes taking part in the uptake, transport and metabolic processes occurring in the cell. The reservoir membrane is connected, moreover, with the surrounding microtubules by the fibrils (Pl. V 26, 27). These data suggest that this membrane may, therefore, perform-mechanosensory functions (M i k o-ł a j c z y k and D i e h n 1979).

The process of flagellum erection produces also anterior–posterior body contractions — two cooperating contractile systems. The interaction of these systems explains the behaviour of *Euglena* under natural conditions, which is modified by the adaptation of the particular species to their life conditions.

Euglena, in view of the character of their movements, may be classified as: (a) freely swimming by means of one or more locomotor flagella, (b) creeping — living freely and (c) parasitic with a very short flagellum or without any outside the cell.

In freely swimming species the main role in movement is played by the locomotor flagellum, while body contractions are auxiliary. Such a role was ascribed to them by D a n g e a r d as early as 1902. Body contractions enable swimming *Euglena* to rapidly change the direction of their movement (L o w n d e s 1936, C h e n 1950, M i k o ł a j c z y k 1972). In a trap situation, for instance in a capillary the locomotor flagellum of *E. gracilis* turns the cell around with the cooperation of euglenoid movement (Pl. I 7–11). An *Euglena* without a visible flagellum outside the body can only contract at both ends, but it is incapable of turning round (M i k o ł a j c z y k 1972).

In species living in silt and in parasitic ones, where the presence of a flagellum outside the body is functionally not necessary, body contractions serve for locomotion and for changes of the direction of movement (D a n g e a r d 1902, B e a u c h a m p 1911, G ü n t h e r 1927, H a l l 1931, B r a c h e r 1938, C h e n 1950, H e i n 1953, H i l m b a u e r 1954, M i c h a j ł o w 1956, M a c k i n n o n and H a w e s 1961). In these species the flagellum in the reservoir and other reservoir structures perform, in our opinion, sensory functions exclusively.

Euglenoid movements in *Astasia cyclopis* counteract the peristaltic movements of the host intestine and consequently prevent evacuation of the parasite with faeces (M i c h a j ł o w 1956).

In parasitic individuals the flagellum is fully regenerated after their release from the host body. Regeneration of the flagellum in *E. leucops* for instance, after emergence from the host and reaching 1/2 of its normal length, causes a disappearance of euglenoid movements (B e a u c h a m p 1911), possibly since the flagellum takes over the main role in cell movements.

Both contractile systems may substitute for each other dependent on the environmental conditions.

In contrast to the anterior–posterior contraction (Fig. 1 a, Pl. I 12, 13) the "peristaltic" movements (Fig. 16) are independent of the behaviour of the flagellum and may occur during swimming forward. These movements were studied in detail in *E. gracilis* (M i k o ł a j c z y k 1972). The contraction ring appears in the posterior part of the body and progresses to the front (Pl. I 1–6).

H o f m a n n and B o u c k (1976) suggested the existence of separate contractile structure responsible for changes in the cell shape in longitudinal and transverse direction. The disappearance of the peris-

taltic wave transmission when the cell is constricted by means of the electronic fluid "Fluorinert" (M i k o ł a j c z y k and D i e h n 1979) — whereas the anterior–posterior contractions are not canceled by this fluid — seems to confirm the interpretation suggested by K u ź n i c k i and M i k o ł a j c z y k (1973).

Peristaltic movements are not controled by the same mechanisms which are responsible for longitudinal or lateral cell shape changes. Rhythmic peristaltic waves appear only in those individuals which previously underwent anterior–posterior contraction and did not return to their initial cylindrical shape on their whole length. We believe that the peristaltic wave would, therefore, be a manifestation of local return of the elastic structure of the pellicular complex to normal. The constriction advancing from the posterior to the anterior end of the euglena's body produces a shift of endoplasm and an increase of the body thickness in other parts of the cell. This determines the long duration and rhythmic character of the peristaltic movements. In brief, peristaltic waves would be the result of straining of elastic structures without full relaxation of the contractile system. On this basis we advance the opinion that in *Euglena gracilis* there exists only one contractile system integrated with the flagellum. The return of the cell to its normal elongated shape is the consequence of relaxation of the sac-like contractile system with simultaneous return to the initial position of the previously (during contraction) stretched elastic structures included in the pellicular complex.

Structures Involved in Contraction and Relaxation
of the Cell Body

P e r t y (1852) was the first to speculate on the mechanism of euglenoid movements and the localisation of cell structures. D a n g e a r d (1902), G ü n t h e r (1927), P r i n g s h e i m and H o v a s s e (1950) and D i s k u s (1956) believed the contractile structures to be present within the periplast (diagram of strip structure shown in Fig. 6

Fig. 6. Scheme of the fibrillar system within the pellicular strip of *Euglena gracilis*. A, B, C — groups of microtubules. Broken lines — fibrills connecting: 1 — microtubule A with periplast (P); 2, 5 — microtubule A with microtubules of group B; 3 — microtubule A with fibrillar continuous structure (fibrillar sac); 4 — microtubule of group B with periplast (P). Arrows show the fibrillar continuous structure (fibrillar sac)
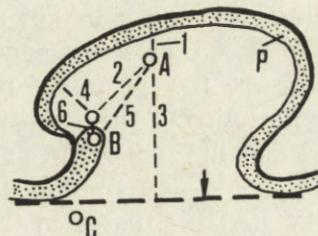
and Pl. VI 30). H i l m b a u e r (1954), P i t e l k a (1963) and L e e d a l e (1966), however, claimed that these structures should be searched for in the endoplasm. At present all authors agree that changes in the shape of *Euglena* are caused by processes occurring in the pellicular complex of the cell. The endoplasm and the structures within it are passively shifted. The pellicular complex includes: periplast, microtubules, and fibrillar structures which are taken into account when discussing the mechanisms of contraction. The knowledge of their structure and distribution became possible owing to electron microscopy.

Various contradictory hypotheses have been advanced concerning the participation of the particular pellicular complex structures in euglenoid movements. Microtubules are present in this complex in all *Euglena* examined so far. In *E. gracilis* elongated microtubules appear at the basis of the reservoir (Fig. 7, Pl. III 19–21), they run along it
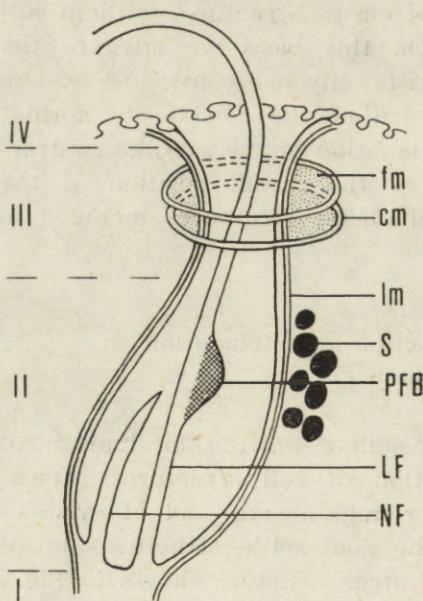
Fig. 7. Diagrammatic representation of the reservoir of *Euglena gracilis*. I — bottom, II — reservoir bulb, III — neck, IV — outlet. LF — locomotory flagellum, NF — nonemergent flagellum, PFB — paraflagellar body, S — stigma, lm — longitudinal microtubules, cm — circular microtubules, fm — fibrillar matrix

as if forming a basket around it. They further become components of the pellicular strips appearing at the outlet of the reservoir (Pl. VI 28, 29). At the reservoir neck level circular microtubules may be seen lying outside the longitudinal ones (Pl. V 24, 25) (W o l k e n and P a - l a d e 1953, S o m m e r 1965, L e e d a l e 1967, B u e t o w 1968, P i - t e l k a 1968). In the strips the microtubules form three groups. The first (A) is situated in the ridge of the strip, the second (B) at the level

of the notch on the strip (where the latter is deprived of periplast) and the third (C) beneath the groove of the strip (Fig. 6, Pl. VI 30).

The number of microtubules is variable and depends not only on the *Euglena* species, but also on their location within the given cell. For instance *E. acus* and *E. gracilis* have in the dorsal part one or two microtubules, whereas *Distigma proteus* has 4–9 (M i g n o t 1965, 1966). The number of microtubules in the anterior or posterior part of the *Euglena viridis* cells is larger than in the middle part (F o i s s n e r 1977). This may be connected with the reduction of the pellicular strips closer to the body end and the entrance into the reservoir depression (Fig. 2 a).

Both an active and a passive role is ascribed to microtubules in euglenoid movements. M i g n o t (1965, 1966), L e e d a l e et al. (1965), L e e d a l e (1966, 1967) and G u t t m a n and Z i e g l e r (1974) advanced the supposition ' that microtubules maintain and restore the normal shape of *Euglena* after contraction, but they have no effect on body contractions. In *E. acus* — a rigid organism — there is only one micro-tubules in group A (ridge), whereas in the extremely contractile *Distigma proteus* there are 4–9.

Recently H o f m a n n and B o u c k (1976) put in doubt the view that microtubules may play a cytoskeletal role. "Ghosts" of *E. gracilis* cells, in spite of the absence of microtubules, maintain their striated shape. The cell shape and that of the individual strips are stabilised by protein components of the periplast. This supposition was confirmed lately by L e f o r t - T r a n et al. (1980). These authors consider that in *E. gracilis* the periplast has a fibrillar structure. This 100–120 Å electron dense layer underlying the cell membrane consists of twisted fibres of 10 nm diameter arranged periodically at 20 nm intervals.

Dasgupta (1964), Arnott and Walne (1967), Arnott and Smith (1969), Hofmann and Bouck (1976) ascribed to microtubules an active role in the changes of boody shape of *Euglena*. In our opinion the latter paper is the most clear-sighted contemporary analysis of the mechanism of euglenoid movement. H o f m a n n and B o u c k (1976) suggested the hypothesis of the existence in *E. gracilis* cells of two independent systems of contractile mechanisms. One would be responsible for lon-gitudinal contraction and the other for peristaltic waves. According to these authors, anterior–posterior body contractions are due to fibrils arranged perpendicularly to the microtubules (cross bridges, Fig. 6 6). The result of their interaction is a sliding of the microtubules B in relation to one another, giving as effect a shortening of the long body axis. This system would also be responsible for relaxation of the cells.

Submembraneous (periplast) and traversing fibres, according to the

same authors, are responsible for the peristaltic waves and expansion
of the pellicle strips (Fig. 6, Pl. VII 31–33).

The supposition of H o f m a n n and B o u c k (1976) concerning
the interaction of periplast in the side contractions (peristaltic move-
ments) of *Euglena* has been lately confirmed by Lefort-Tran et al.
(1980). According to the latter authors, it not only takes part in eugle-
noid movements (cell curling) but it could play a role in retaining the
ridge after disruption.

The ultrastructure of the reservoir is different from that of the
strips. Within the reservoir, at the level of the neck a fibrillar matrix
was noted lying between the longitudinal and circular microtubules
(S o m m e r 1965, S o m m e r and B l u m 1965, B u e t o w 1968) (Pl.
V 24, 25). P i c c i n i and O m o d e o (1975) attribute to this matrix
contractile properties changing the shape of the reservoir.

M i k o ł a j c z y k and D i e h n (1979), on the other hand, are of
the opinion that the change of the reservoir shape is the result of
interaction between the longitudinal microtubules and the fibrils joining
them to one another and to the resorvoir membrane (Pl. V 25, 26). In
the present paper we advance our view which differs from those sug-
gested to date concerning the structural basis of euglenoid movements.
We believe that anterior–posterior changes of body shape in *E. gracilis*
are due to the contraction of the fibrillar layer lying immediately under
the pellicular strips. (This layer in Fig. 6 and Pl. I 31–33 is denoted
by arrows). There is no doubt that this structure forms a continuous
layer (sac) separating the pellicular complex from the cell interior. It
is not known whether it also lines the reservoir. This layer corresponds
to what is called traversing fibres by H o f m a n n and B o u c k
(1976).

Observations of the behaviour of living *Euglena* and analysis of the
cross sections of the strips were the basis for the conclusion that the
anterior–posterior contraction is produced by this continuous fibrillar
layer. During the anterior–posterior contraction the strips slide passi-
vely on this fibrillar sac. The range of contraction undergone by the
cell from elongated form to disc shape would cause, if there existed
contractile structures within the strips, deformation of the latter
(changes of the cross sections) which would be impossible in view of
the parallel close arrangement of the strips in elongated protozoans.
The endoplasmic reticulum lies in the endoplasm under the fibrillar
sac in its nearest neighbourhood, whereas it is absent in all membra-
neous structures of this type within the strips.

Fibrillar structures within the strips (Fig. 6 *1–6*), together with the
fibrillar structures forming the periplast and microtubules, are sub-

jected to stress during contraction, which would bring the cell back to its cigar-like shape. These stresses would also play an essential role in peristaltic movements, according to the interpretation of their character and mechanism given in the preceding chapter.

In this connection it would seem not very probable that there would be an interaction between the microtubules and the interconnecting fibres (Fig. 6 *1–6*) — analogously as in the dynein-microtubular systems, as postulated by other authors and most recently H o f m a n n and B o u c k (1976).

Crucial Problems

Our hypothesis of the principle and mechanisms of euglenoid movements like all those previously advanced is of speculative character, above all because of the lack of immediate evidence that the fibrillar structures are capable of contraction. We do not even know whether in this group of organisms body contraction is caused by the interaction of actin and myosin or some other molecular mechanism.

Initial biochemical studies of the pellicular layer consisted in the analysis of their chemical composition from the aspect of their percentual content of proteins, lipids and sugars (B a r r a s and S t o n e 1965, K e m p n e r and M i l l e r 1965).

It is only lately that H o f m a n n and B o u c k (1976) and G a l l o et al. (1978) started a series of biochemical and immunological investigations in order to check the existence of an actino-myosin system in *Euglena*.

Analysis of the protein components of *E. gracilis* cell "ghosts" on acrylamide SDS gel performed by H o f m a n n and B o u c k (1976) demonstrated the presence of a protein with molecular weight 75 000 which proved to be the main immunogenic component of the pellicle. Moreover, the presence of 25 000, 40 000 and 80 000 proteins (main strips) and from 25 000 to 200 000 (weaker strips) was noted. It remains an open question whether the detected proteins or the products of their degradation are actually molecules involved in the contraction phenomena.

J i r o v e c (1929) and K l e i n (1930) ascertained the presence in flagellates of the *Euglenoidina* group of a silverline system very similar in its architecture to that described previously in *Ciliata*. C h a d e f a u d (1937, 1938), C h a d e f a u d and A r l e t (1944) considered that the argentophilic system of *Euglena* is not of fibrillar nature nor is it an

element of the cell pellicular complex. It consists simply of impregnated pellicular strips. Comparative studies on the argentophilic system of *Euglena* with the use of light and electron microscopy prompted some authors to the conclusion that the argentophilic system cannot be considered artefacts (P o c h m a n 1953, M i g n o t 1965, 1966, S c h a n t z et al. 1975, F o i s s n e r 1977). The latter author combined investigation of silver-impregnated preparations with physiological observations. He established relationships between euglenoid movements and the argentophilic system. He also demonstrated that cytochalasin B (CCP) destroys this system. In all *E. viridis* cells with impaired argentophilic system the euglenoid movements were inhibited.

In our opinion the described argentophilic system is identical with some elements of the interconnecting fibres (Fig. 6 *1–6*). The fibrillar structures (fibrillar sac), however, which, according to our belief, control body contraction cannot be impregnated by this method. This impregnation method reveals only certain characteristic traits of the structure of some fibrillar systems which are, however, strikingly similar in *Euglena* and in *Ciliata*. This resemblance may, therefore, be considered indirect evidence that the fibrillar systems of these ciliates are derived from those occurring in *Euglena*.

Euglenoid movements are probably one of the most primitive forms of contraction phenomena, giving as effect a change of the body shape of eukaryotic cells. They appeared in the primary flagellates and have subsisted in but little changed form in some *Euglena* species. In its primary form this was a contraction with a dual function: locomotor and making an avoidance reaction possible. The avoidance reaction and the change of swimming direction connected with it are also evoked in flagellates by specific contractile phenomena occurring in the flagellum. In the contemporary forms known to date which have flagella and exhibit euglenoid movements these two contraction processes are closely related. The flagellum is the first to react to an external stimulus, notwithstanding stimulus character or receptor — the body shape changes later. On the basis of up-to-date knowledge it cannot be decided which of the motor systems was the primary one and which secondary. The above described sequences seem to speak in favour of the flagella movement as primary. The parallel development of both systems cannot, however, be ruled out. It seems highly probable, however, that the primary contractile system in the cytoplasm developed directly under the membrane (as in the case with the development of sarcomeres in mammal embryos). Simultaneously the primary flagellates had a restricted possibility of shifting their plasma since it was limited by the cortical layer. This means that the primary flagellate cell

resembled a sac with contractile properties in various directions, preserving, however, always the same volume. In the group from which *Euglenoidina* are derived the tendency to a rigid cortical layer prevailed and probably none of the contemporary forms exhibits the "flexibility" which the primary forms possessed.

Considerations on the relation between euglenoid movements and muscle contraction systems are difficult, owing to the lack of knowledge of contractile and regulator proteins conditioning body contraction in *Euglenoidina*. At any rate the ultrastructure of the fibrillary sac and of other fibrils of the pellicular complex differs essentially from those so far described in muscle and nonmuscle actino-myosin systems. Even if the contraction processes conditioning euglenoid movements are based on a specific molecular principle in the process of evolution the relationship of the flagellum-reservoir system and the fibrillar systems occurring in *Euglena* could have served as basis for the development of receptor → effector systems in multicellular organisms. K u ž n i c k i et al. (1979) demonstrated the presence of calmodulin in the cells of *E. gracilis,* it is, therefore, quite probable that the calmodulin-Ca^{2+} complex regulates not only the contraction mechanisms of both systems (flagellar and fibrillar), but also the interaction between them.

C h e n (1950) advanced the supposition that in *Euglenoidina* the locomotor flagellum and reservoir have a coordinating function, preserving the elongated body shape during swimming, creeping and spiralisation. This suggestion has been confirmed by our microsurgical experiments with *Peranema trichiphorum* (Pl. VIII and IX). The cutting off of even a small fragment of the anterior part of the cell makes any coordinated movement of the remaining fragment impossible, whereas the anterior fragment of *Peranema* with intact reservoir behaves like an intact organism.

It would seem, therefore, that one of the evolutional tendencies within *Euglenoidina,* if they preserved their ability of changing the cell shape, was the perfecting of the interaction between the flagellum and the receptor (sensory) system connected with it, and the contractile system of the body.

V i n n i k o v (1946, 1971, 1974, 1979) considered, on the basis of comparative studies, *Euglena* as cellular structures being the starting point in the evolution of receptor structures of *Metazoa*. The information and arguments compiled in this paper lead to a further-reaching conclusion. Flagellates with a structure similar to that of *Euglena* possessed a functional and structural basis for the simultaneous development of two system — the receptor and the effector one. The former arose by transformation of the flagellum and reservoir structures with

the microtubular junctions, the latter from fibrillar structures separating the pellicular complex from the endoplasm.

To conclude, we believe that the primeval *Flagellata* from which the contemporary *Euglena* are derived both as regards ultrastructure and functions, may have constituted the original form in the evolution of *Metazoa* organisms.

REFERENCES

A l e x a n d e r G. 1931: The significance of hydrogen ion concentration in the biology of *Euglena gracilis* Klebs. Biol. Bull., 165–184.
A r n o t t H. J. and W a l n e P. L. 1966: Metaboly in *Euglena granulata*. J. Phycol., (suppl) 2, (Abstr.).
A r n o t t H. J. and W a l n e P. L. 1967: Observations on the fine structure of the pellicle pores of *Euglena granulata*. Protoplasma, 64, 330–344.
A r n o t t H. J. and S m i t h H. E. 1969: Analysis of microtubular structure in *Euglena granulata*. J. Phycol., 5, 68–75.
B a n c r o f t F. W. 1915: Heliotropism, differential sensibility, and galvanotropism in *Euglena*. J. Exp. Zool., 15, 383–428.
B a r r a s D. R. and S t o n e B. A. 1965: The chemical composition of the pellicle of *Euglena gracilis*. Biochem. J., 97, 14P–15P.
B e a u c h a m p de P. 1911: *Astasia captiva* n. sp. Euglénien parasite de *Catenula lemnae*. Ant. Dug. Arch. de Zool. Exp. et Gen., ser. 5, T 6, 53–58.
B o u c k G. B., R o g a l s k i A. and V a l a i t i s A. 1978: Surface organization and composition of *Euglena*. II. Flagellar mastigonemes. J. Cell Biol., 77, 805–826.
B o v e e E. C. and A c u ñ a M. L. 1970: Effects of adenosine triphosphate (ATP) on flagellar movements of *Euglena gracilis* in photophobotaxis. J. Protozool., 17, Suppl., 14.
B r a c h e r R. 1938: The light relations of *Euglena limosa* Gard. J. Lennean Soc. (London) Botany, 51, 23–43.
B u e t o w D. E. 1968: Morphology and ultrastructure of *Euglena*. In: The Biology of *Euglena*. (ed. D. E. B u e t o w), Acad. Press N. Y. and London, 1, 110–184.
B y r n e J. and M a r s l a n d D. 1965: Pressure — temperature effects on the form — stability and movements of *Euglena gracilis* var. Z. J. Cell. Comp. Physiol., 65, 277–284.
C h a d e f a u d M. 1937: Anatomic comparèe des Eugléniens. Botaniste, 28, 85–185.
C h a d e f a u d M. 1938: Nouvelles recherches sur l anatomic comparèe des Eugléniens: les Peranèmines. Rev. Algol., 11, 189–220.
C h a d e f a u d M. and P r o v a s o l i L. 1939: Une nouvelle Euglène graciloide: *Euglena gracilis* Klebs var. *urophora* n. var. Arch. Zool. exp. gèn., 80, 55–60.
C h a d e f a u d H. and A r l e t J. 1944: Sur la cuticule des Eugléniens et son appareil argyrophile. C. R. A. S. (Paris), 219–222.
C h e n Y. T. 1950: Investigations of the biology of *Peranema trichophorum* (*Eugleninae*). Quart. J. Microscop. Sci., 91, 279–308.
C h u S. P. 1946: Contribution to our knowledge of the genus *Euglena*. Sinensia, 17, 75–134.
C o n r a d W. 1940: Sur une Euglène du psammon de l'Escant. Bull. Museè Roy. Hist. Nat. Belg., 16, 1–12.
C o n r a d W. and V a n M e e l L. 1952: Matèriaux pour une monographie de *Trachelomonas* Ehrenberg C. 1834, *Strombomonas* Deflandre G. 1930, et *Euglena* Ehrenberg C. 1832, genres d'euglenacées. Mem. Inst. Roy. Sci. Nat. Belg. 124.
D a n g e a r d M. P. A. 1902: Recherches sur les Eugléniens. Botaniste, 8, 97–357.
D a s g u p t a J. 1964: Submicroscopis Morphology of *Euglena gracilis* Klebs. Ann. Sci. Natur. Zool. Paris, 6, 215–227.

Deflandre G. 1924: A propos de l'*Euglena acus* Ehrbg. Revue Algologique, 3.

Diskus A. 1956: Färbestudien an den Scheimkörperchen und Schleim anscheidungen einiger Euglenen. Protoplasma 45, 460–477.

Doughty M. J. and Diehn B. 1979: Photosensory transduction in the flagellated alga, *Euglena gracilis*. I. Action of different cations, Ca^{2+} antagonistics and Ca^{2+} ionophore on motility and photobehavior. B. B. A., 588, 148–168.

Foissner W. 1977: Comparative light and electron microscopical studies on the argyrophilic structures of *Euglena viridis*. Acta biol. Acad. Sci. hung., 28, 157–174.

Fritsch F. E. 1935: The structure and reproduction of the Algae. Cambridge Univ. Press, 791.

Gallo J. M., Goldstein S. F., Philippe M. and Schrevel J. 1978: Les mouvements de métabolie chez *Distigma proteus*. J. Protozool. 25, 54A.

Gibbs S. P. 1978: The chloroplasts of *Euglena* may have evolved from symbiotic green algae. Can. J. Bot., 56, 2883–2889.

Gojdics M. 1953: The Genus *Euglena*. Univ. Wisconsin Press, Madison, 268.

Grębecki A. and Mikołajczyk E. 1968: Ciliary reversal and re-normalization in *Paramecium caudatum* immobilized by Ni ions. Acta Protozool., 5, 297–304.

Gross J. A. 1965: Pressure — induced color mutation of *Euglena gracilis*. Science, 147, 741–742.

Günther F. 1927: Über den Bau und die Lebensweise der Euglenen, besonders der Arten *E. terricola, geniculata, proxima, sanguinea* und lucens nov. spec. Arch. Protistenk., 3, 511–590.

Güttman H. N. and Ziegler H. 1974: Clarification of structures related to function in *Euglena gracilis*. Cytobiologie, 9, 10–22.

Hall S. R. 1931: Observations on *Euglena leucops*, sp. nov., a parasite of *Stenostomum*, with special reference to nuclear division. Biol. Bull., 60, 327–344.

Hall R. P. 1933: On the relation of hydrogen — ion concentration to the growth of *Euglena anabaena* var. *minor* and *Euglena deses*. Arch. Protistenk, 79, 239–248.

Hamburger C. 1911: Studien über Ehrenbergii, insbesondere über die Körperhülle. Carl Winters Universitätsbuchhandlung, 1–22.

Heimpel H. 1972: Ein Verwandlungskünstler unter den Euglenen *Euglena ehrenbergii*. Mikrokosmos 61, 100–101.

Hein G. 1953: Über *Euglena mutabilis* und ihr verhalten zu sauren Medien. Arch. Hydrobiol., 47, 516–525.

Hilmbauer K. 1954: Zellphysiologische Studien an Euglenaceen, besonders an Trachelomonas. Protoplasma, 43, 192–226.

Hofmann C. and Bouck G. B. 1976: Immunological and structural evidence for patterned intussusceptive surface growth in a unicellular organism. A postulated role for submembranous proteins and microtubules. J. Cell Biol., 69, 693–715.

Hollande A. 1942: Etude cytologique et biologique de quelques flagellés libres. Arch. Zool. Exptl. Gen., 83, 1–268.

Jahn T. L. 1946: The euglenoid flagellates. Quart. Rev. Biol., 21, 246–274.

Jahn T. L. and Bovee E. C. 1968: Locomotive and motile responses in *Euglena*. In: The Biology of *Euglena*. (ed. D. E. Buetow), Academic Press N. Y. and London, 1, 45–108.

Jennings H. S. 1906: The Behavior of Lower Organisms. Indiana Univ. Press, Bloomington, Indiana, 366.

Jirovec O. 1929: Die Silverlinien bei einigen Flagellaten. Arch. Protistenk., 68, 209–214.

Johnson L. P. 1968: The taxonomy, phylogeny, and evolution of the genus Euglena. In: The Biology of *Euglena*. (ed. D. E. Buetow), Academic Press. N. Y. and London, 1, 1–25.

Kamiya N. 1939: Die Rhythmik des metabolischen Formwechsels der Euglenen. Ber. dt. bot. Ges., 57, 231–240.

Kempner E. S. and Miller J. H. 1965: The molecular biology of *Euglena gracilis*. I. Growth conditions and cellular composition. BBA, 104, 11–17.

K i t c h i n g J. A. 1957: Effects of high hydrostatic pressures on the activity of flagellates and ciliates. J. Exp. Biol., 34, 494–510.

K l e i n B. M. 1930: Über das Silverliniensystem einiger Flagellaten. Arch. Protistenk., 72, 404–419.

K u ź n i c k i L. 1963: Reversible immobilization of *Paramecium caudatum* evoked by nickel ions. Acta Protozool., 6, 301–312.

K u ź n i c k i L. and M i k o ł a j c z y k E. 1973: Contraction and elasticity involved body movements of *Euglena gracilis*. Progress in Protozoology, Abstr. Fourth Int. Congr. Protozool., Clermont-Ferrand, 1973, 237.

L e e d a l e G. F. 1966: *Euglena*: A new look with the electron microscope. Advan. Sci., 23, 22–37.

L e e d a l e G. F. 1967: The Euglenoid Flagellates. Englewood Cliffs: Prentice-Hall, Inc., New Jersey, 242.

L e e d a l e G. F., M e e u s e B. J. D. and P r i n g s h e i m E. G. 1965: Structure and physiology of *Euglena spirogyra*. Arch. Mikrobiol., 50, 68–102.

L e e d a l e G. F. and B u e t o w D. E. 1976: Observations on cytolysome formation and other cytological phenomena in carbon — starved *Euglena gracilis*. J. Micr. Biol. Cell., 25, 149–154.

L e F é v r e M. 1931: De la valeur des caractères spécifiques chez quelques Eugléniens. Trav. crypt. dédies a L. Mangin, Paris, 343–354.

L e f o r t - T r a n M., B r e M. H., R a n c k J. L. and P o u h i l e M. 1980: Euglena plasma membrane during normal and vitamin B_{12} starvation growth. J. Cell Sci., 41, 245–261.

L o w n d e s A. G. 1936: Flagella movement. Nature, 138, 210–211.

L o z i n a - L o z i n s k y L. K. and Z a a r E. I. 1963: Metabolija i immobilizacija kletok *Euglena gracilis* v zavisimosti ot tipa pitanija i osvešennosti. Citologija, 5, 263–272.

M a c k i n n o n D. L. and H a w e s R. S. J. 1961: An introduction to the study of *Protozoa*. Oxford Univ. Press, 69–87.

M i c h a j ł o w W. 1956: Developmental cycle of the flagellate *Astasia cyclopis* n. sp. Acta Parasitologica Polonica, 4, 1–26.

M i g n o t J. P. 1965: Ultrastructure des Eugléniens. I. Comparaison de la cuticule chez différent espèces. Protistologica, 1, 5–17.

M i g n o t J. P. 1966: Structure et ultrastructure de quelques Euglénomonadines. Protistologica, 2, 51–117.

M i k o ł a j c z y k E. 1972: Patterns of body movements of *Euglena gracilis*. Acta Protozool., 11, 317–324.

M i k o ł a j c z y k E. 1973: Effect of some chemical factors on the euglenoid movement in *Euglena gracilis*. Acta Protozool., 12, 133–142.

M i k o ł a j c z y k E. 1975: The Biology of *Euglena ehrenbergii* Klebs. I. Fine structure of pellicular complex and its relation to euglenoid movements. Acta Protozool., 14, 233–240.

M i k o ł a j c z y k E. and D i e h n B. 1976: Light — induced body movement of *Euglena gracilis* coupled to flagellar photophobic responses by mechanical stimulation. J. Protozool., 23, 144–147.

M i k o ł a j c z y k E. and D i e h n B. 1978: Morphological alterations in *Euglena gracilis* induced by treatment with CTAB (Cetyltrimethyl ammonium bromide) and Triton X-100: correlations with effects on photophobic behavioral responses. J. Protozool. 25, 461–470.

M i k o ł a j c z y k E. and D i e h n B. 1979: Mechanosensory responses and mechanoreception in *Euglena gracilis*. Acta Protozool., 18, 591–602.

M i l l e r K. R. and M i l l e r G. J. 1978: Organization of the cell membrane in *Euglena*. Protoplasma, 95, 11–24.

N a i t o h Y. 1966: Reversal response elicited in nonbeating cilia of *Paramecium* by membrane depolarization. Science, 154, 660–662.

N i c h o l s K. M. and R i k m e n s p o e l R. 1977: Mg^{2+}-dependent electrical control of flagellar activity in *Euglena*. J. Cell Sci., 23, 211–225.

P e r t y M. 1852: Zur Kenntnis kleinster Lebensformen. Bern.

P i c c i n i E. and O m o d e o P. 1975: Photoreceptors and phototactic programs in protista. Boll. Zool., 42, 57–79.

P i t e l k a D. R. 1963: Electron — microscopic structure of *Protozoa*. Pergamon Press, New York.

P i t e l k a D. R. 1968: Fibrillar systems in *Protozoa*. In: Research in Protozoology, (ed. T z e-T u a n C h e n). Pergamon Press, Oxford and New York, 3, 280–388.

P i t e l k a D. R. and S c h o o l e y C. N. 1957: The pellicuar fine structure of *Euglena gracilis*. J. Protozool., 4, (Suppl.), 10.

P o c h m a n A. 1953: Structur, Wachstum und Teilung der Körperhülle bei den Eugleninen. Planta, 42, 478–548.

P r i n g s h e i m E. G. 1948: Taxonomic problems in the *Euglenineae*. Biol. Rev., 23, 46–61.

P r i n g s h e i m E. G. 1956: Contributions toward a monograph of the genus *Euglena* Nova Acta Leopoldina, 18, 1–168.

P r i n g s h e i m E. G. and H o v a s s e R. 1950: Les relations de parentè entre Astasiacees et Euglenacees. Arch. Zool. Exptl. Gen., 86, 499–549.

R o g a l s k i A. and B o u c k G. B. 1978: A flagellar membrane glycoprotein does not extend over the cell surface in *Euglena*. J. Cell Biol., 79, 281a.

S i l v e r m a n H. and H i k i d a R. S. 1976: Pellicle complex of *Euglena gracilis*: characterization by disruptive treatments. Protoplasma, 87, 237–252.

S o m m e r J. R. 1965: The ultrastructura of the pellicle complex of *Euglena gracilis*. J. Cell Biol., 24, 253–257.

S o m m e r J. R. and B l u m J. J. 1965: Cell division in *Astasia longa*. Exp. Cell Res., 39, 504–527.

S c h a n t z M. L., R e i c h l e r Y. and O h a d I. 1975: The ultrastructure of the pellicle of *Euglena gracilis* Z as revealed by the freeze-etching technique. Israel J. Med. Sci., 11, 407.

S t e w a r t K. D. and M a t t o x K. R. 1975: Comparative cytology, evolution and classification of the green algae with some consideration to the origin of other organisms with chlorophylls A and B. Bot. Rev., 41, 104–135.

S z a b a d o s M. 1936: *Euglena* Untersuchungen. Acta Biol. Szeged, 4, 49–95.

V i n n i k o v Y. A. 1946: Opyt filontogenetičeskoj klassifikacii receptorov (organov čustv) pozvonočnyh. Žurn. Obst. biol., 7, 345–368.

V i n n i k o v Y. A. 1971: Citologičeskie i molekuljarnye osnovy recepcii. L., 1971.

V i n n i k o v Y. A. 1974: Sensory reception. Cytology, molecular mechanisms and evolution. Heidelberg–New York.

V i n n i k o v Y. A. 1979: Evoljucija receptorov. Citologičeskij, membrannyj i molekuljarnyj urovni. Nauka, 138.

W a g e r H. 1900: On the eye-spot and flagellum in *Euglena viridis*. J. Linnean Soc. Zool. London, 27, 463–481.

W i c h t e r m a n R. 1955: Survival and other effects following X-irradiation of the flagellate, *Euglena gracilis*. Biol. Bull., 109, 371.

W o l k e n J. J. and P a l a d e G. E. 1953: An electron microscope study of two flagellates. Chloroplast structure and variation. Ann. N. Y. Acad. Sci., 56, 873–889.

Y o u q u e W. H. Jr., B e r r e n t B. L. and C a i r n s J. Jr. 1979: Survival of *Euglena gracilis* exposed to sublethal temperature and hexavalent chromium. J. Protozool., 26, 122–125.

EXPLANATION OF PLATES I–IX

Pl. I. 1–6: Consecutive stages of the peristaltic wave of *Euglena gracilis*. Arrows mark contracted zone. (according to M i k o ł a j c z y k 1972)

7–11: Turning around of *Euglena gracilis* inside a narrow capillary. (Cinematographs). Anterior end (a), posterior end (p) of the cell; arrows show the direction of turning

12–13: Longitudinal contradiction of *Euglena gracilis*. Arrows mark the direction of contraction. (Cinematograph)

Pl. II. 14–17: Light microscopic pictures of different forms of euglenoid movement of *Euglena ehrenbergii*. (according to M i k o ł a j c z y k 1975)

18: Electronmicrograph of the cross section of the pellicular complex of *Euglena ehrenbergii*. Nontreated cell. (according to M i k o ł a j c z y k 1975). × 40 000

Pl. III. 19–21: Electronmicrographs of the proximal part of the flagella of *Euglena gracilis*
19: Longitudinal section through the locomotor flagellum with paraflagellar body. Cell treated with 80 µl ethanol/5 ml cell suspension. $\times$ 30 000
20: Longitudinal section through the locomotor flagellum with kinetosome. Cell treated with 70% glycerol. $\times$ 30 000
Pl. IV. 21: Cross section through the kinetosomes. Nontreated cell. $\times$ 45 000
22: Paraflagellar body visible in the light microscope. Cell fixed with glutaraldehyde and osmium tetroxide
23: Electronmicrograph of the locomotor flagellum with paraflagellar body. Nontreated cell. $\times$ 75 000
Pl. V. 24–27: Electronmicrographs of the reservoir bulb and reservoir neck of *Euglena gracilis*
24: Longitudinal section through the reservoir neck with visible circular microtubules (cm); longitudinal microtubules (lm) and fibrillar matrix between them (fm). Cell treated with ionophore — 40 µM A 23187. $\times$ 37 500
25: Cross section through the reservoir neck, with circular (cm) and longitudinal (lm) microtubules and fibrillar matrix (fm). $\times$ 60 000
26: Cross section through the middle part of the reservoir. Arrows mark fibrils connecting longitudinal microtubules with reservoir membrane. *Euglena* treated with 15 mM Triton X-100. $\times$ 45 000
27: Cross section through the middle part of the reservoir with visible fibrils connecting longitudinal microtubules (arrows). Cell treated with 70% glycerol. $\times$ 75 000
Pl. VI. 28–30: Electronmicrographs of the reservoir outlet of *Euglena gracilis* with pellicular strips apparent in this region.
28: Oblique section. *Euglena* treated with 80 µl ethanol/5 ml cell suspension. $\times$ 22 500
29: Cross section with very well marked pellicular strips (PS). Nontreated cell. $\times$ 22 500
30: Cross section through pellicular strips with three groups of microtubules: A — arranged under the pellicular ridge. B — on the level of pellicular notch (N), C — under the pellicular groove. Nontreated cell. $\times$ 75 000
Pl. VII. 31–33: Electronmicrographs of fibrillar structures of the pellicular complex of *Euglena gracilis*.
31: Section of the cell with continuous fibrillar structure separating cytoplasm and pellicular complex (arrows). Cell treated with 1 mM CTAB. $\times$ 6000
32: Cross section of the pellicular strips with visible continuous fibrillar structures (arrows). Periplast (P). Cell treated with 1 mM CTAB. $\times$ 45 000
33: Fibrillar system within the pellicular strips. Fibrils connecting: 1 — microtubule A with periplast (P), 2, 5 — microtubule A with microtubules of group B; 3 — microtubule A with continuous fibrillar structures (arrows); 4 — microtubule of group B with periplast (P); 6 — microtubules of group B among themselves. CTAB treated cell. $\times$ 75 000
Pl. VIII. 34–39: Uncoordinated body movements of a posterior fragment without flagellum and reservoir of *Peranema trichophorum*. a — anterior fragment (non-motile)
Pl. IX. 40–49: Coordinated body movements of an anterior fragment of *Peranema trichophorum*

Abbreviations used:
LF — locomotor flagellum, NF — nonemergent flagellum, S — stigma, PFB — paraflagellar body, T — tooth, N — notch, PS — pellicular strip, PL — plasmalemma, P — periplast, R — reservoir, m — microtubules, cm — circular microtubules, lm — longitudinal microtubules, f — fibrils, fm — fibrillar matrix.
For electronmicroscopic procedure see — M i k o ł a j c z y k and D i e h n (1976).
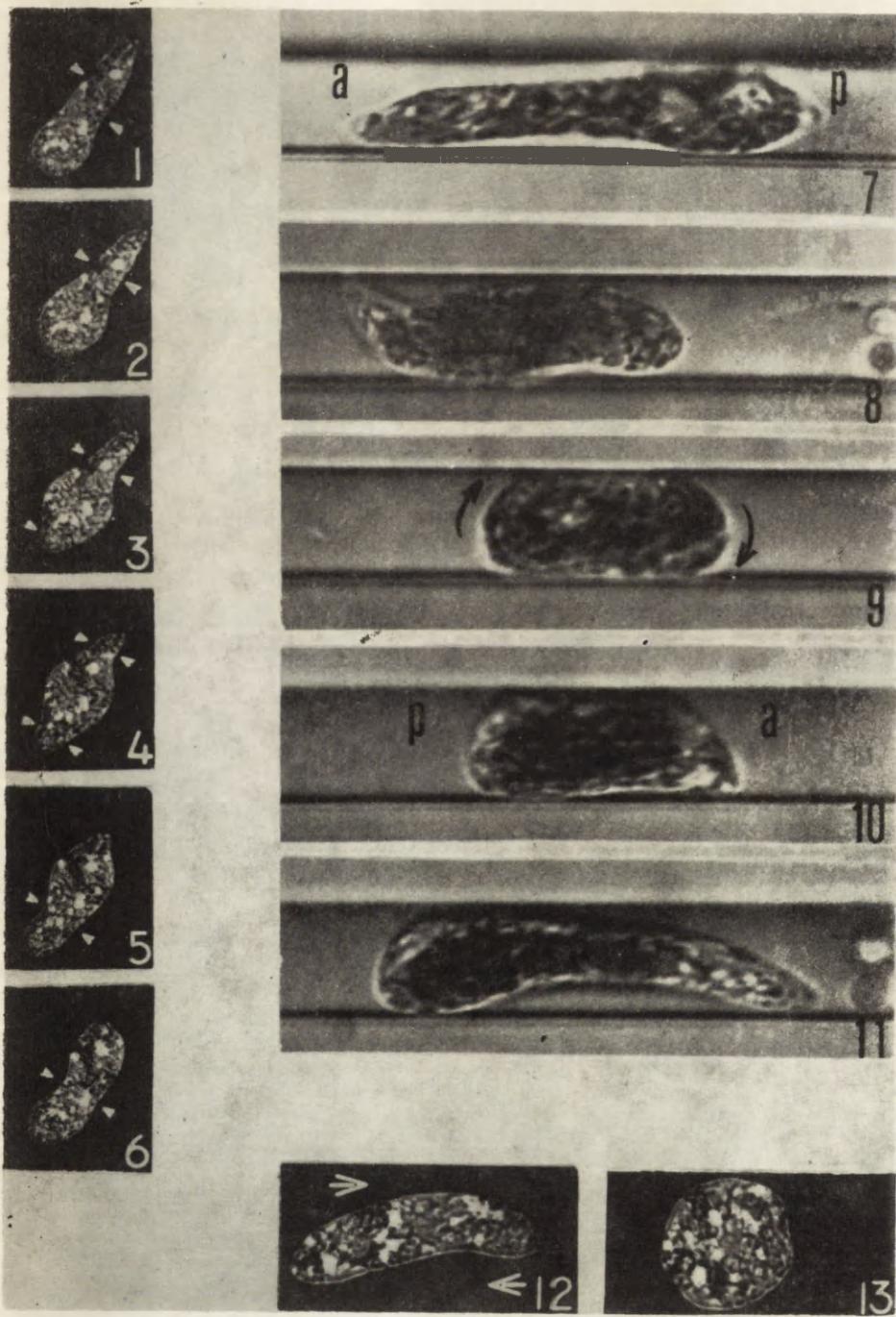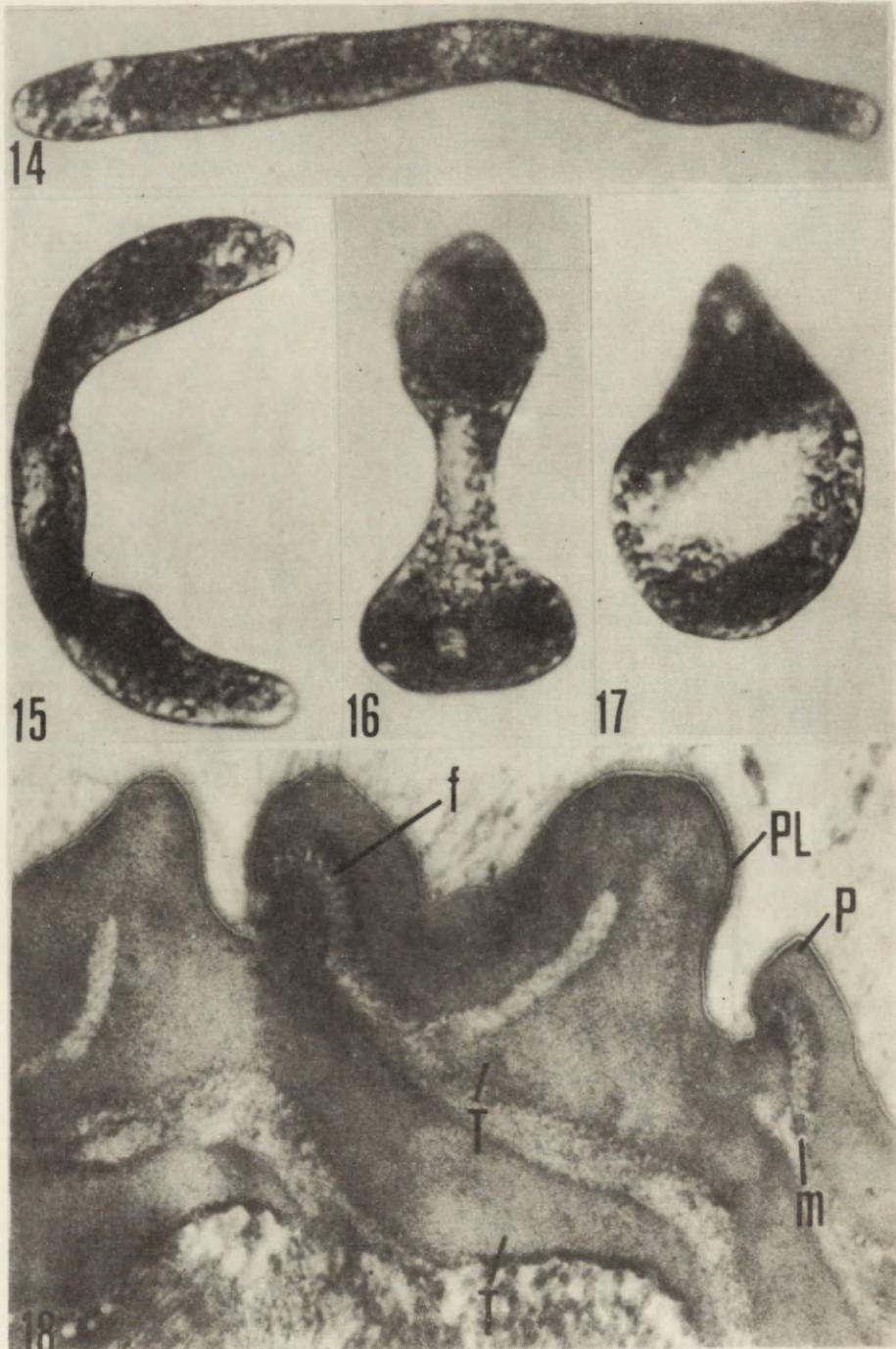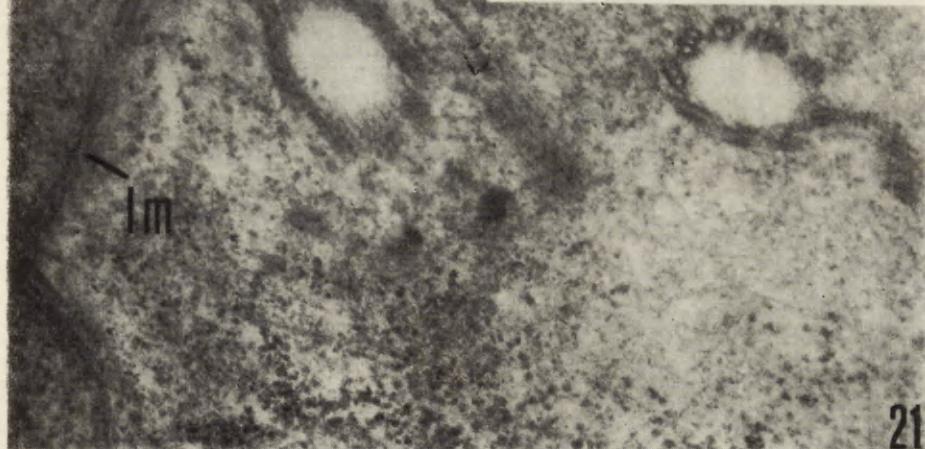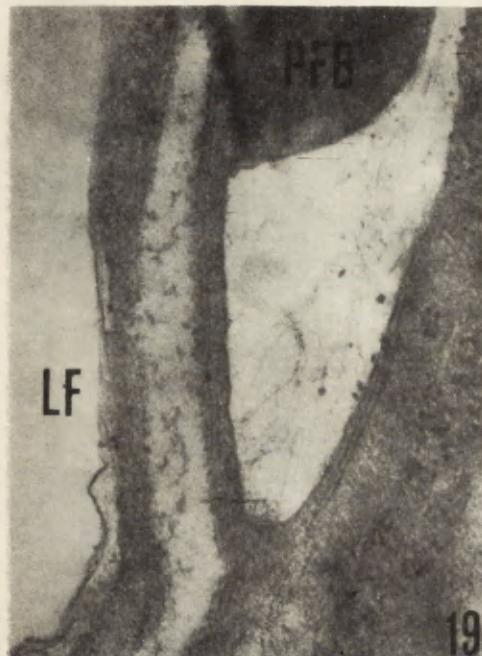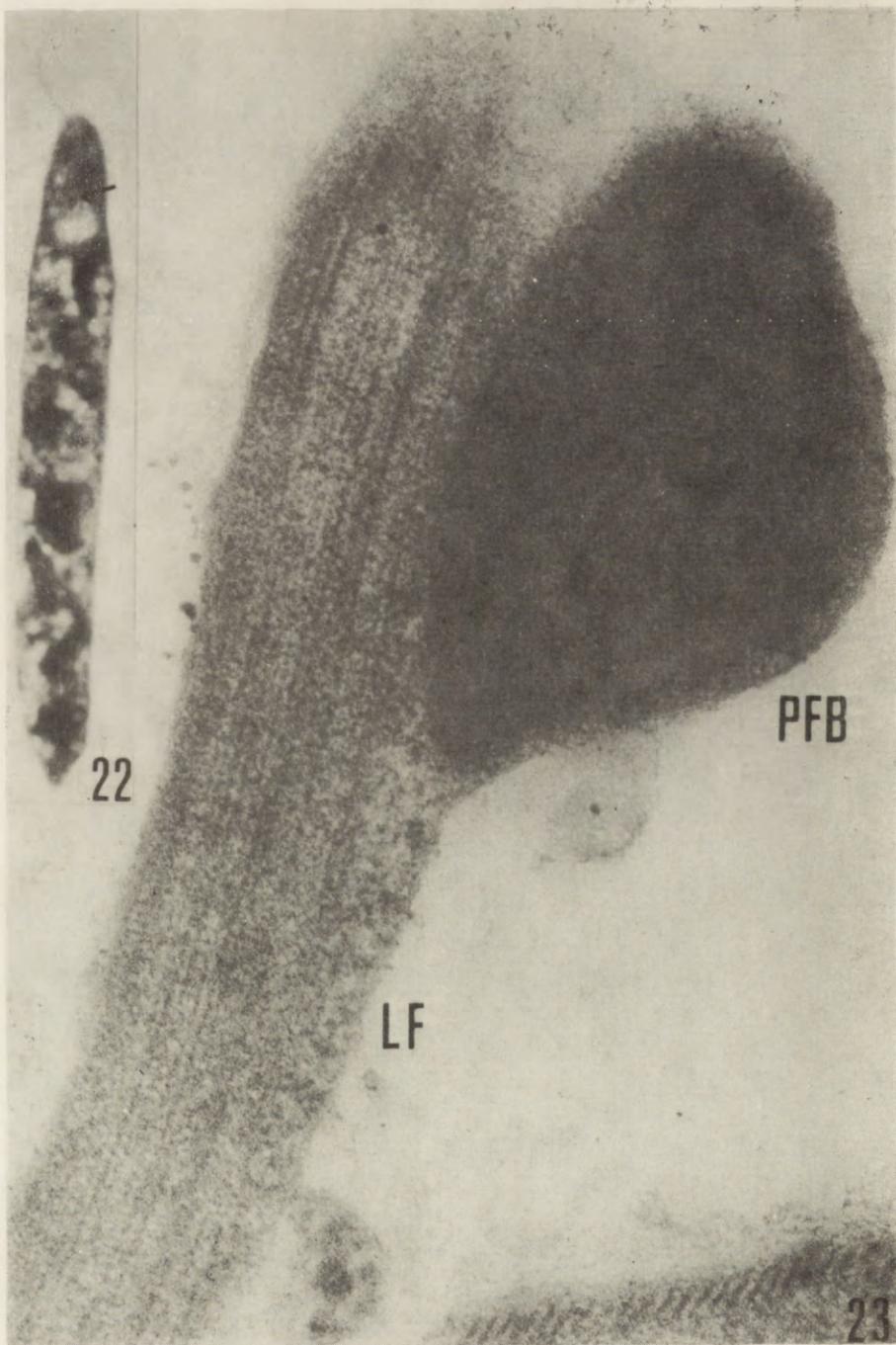
PLATE I

E. Mikolajczyk et L. Kuźnicki

PLATE II

E. Mikolajczyk et L. Kuźnicki

auctores phot.

PLATE III


E. Mikołajczyk et L. Kuźnicki

auctores phot.

E. Mikołajczyk et L. Kuźnicki   auctores phot.

PLATE V

E. Mikołajczyk et L. Kuźnicki

auctores phot.

E. Mikolajczyk et L. Kuźnicki   auctores phot.

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PLATE VIII

E. Mikołajczyk et L. Kuźnicki

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PLATE IX
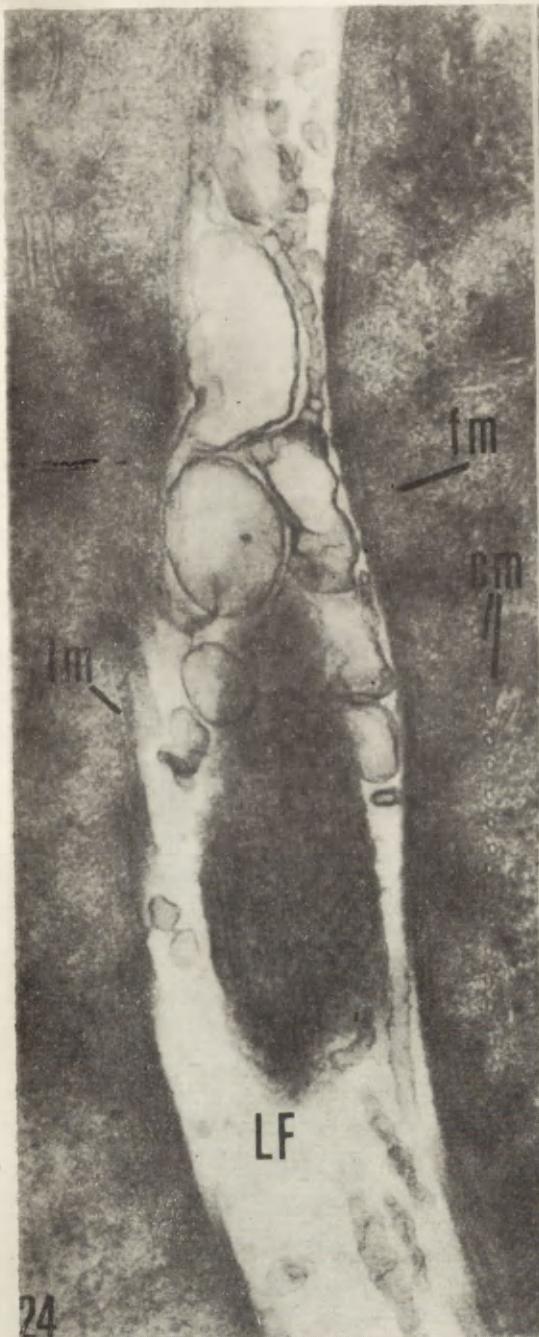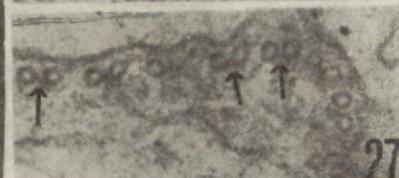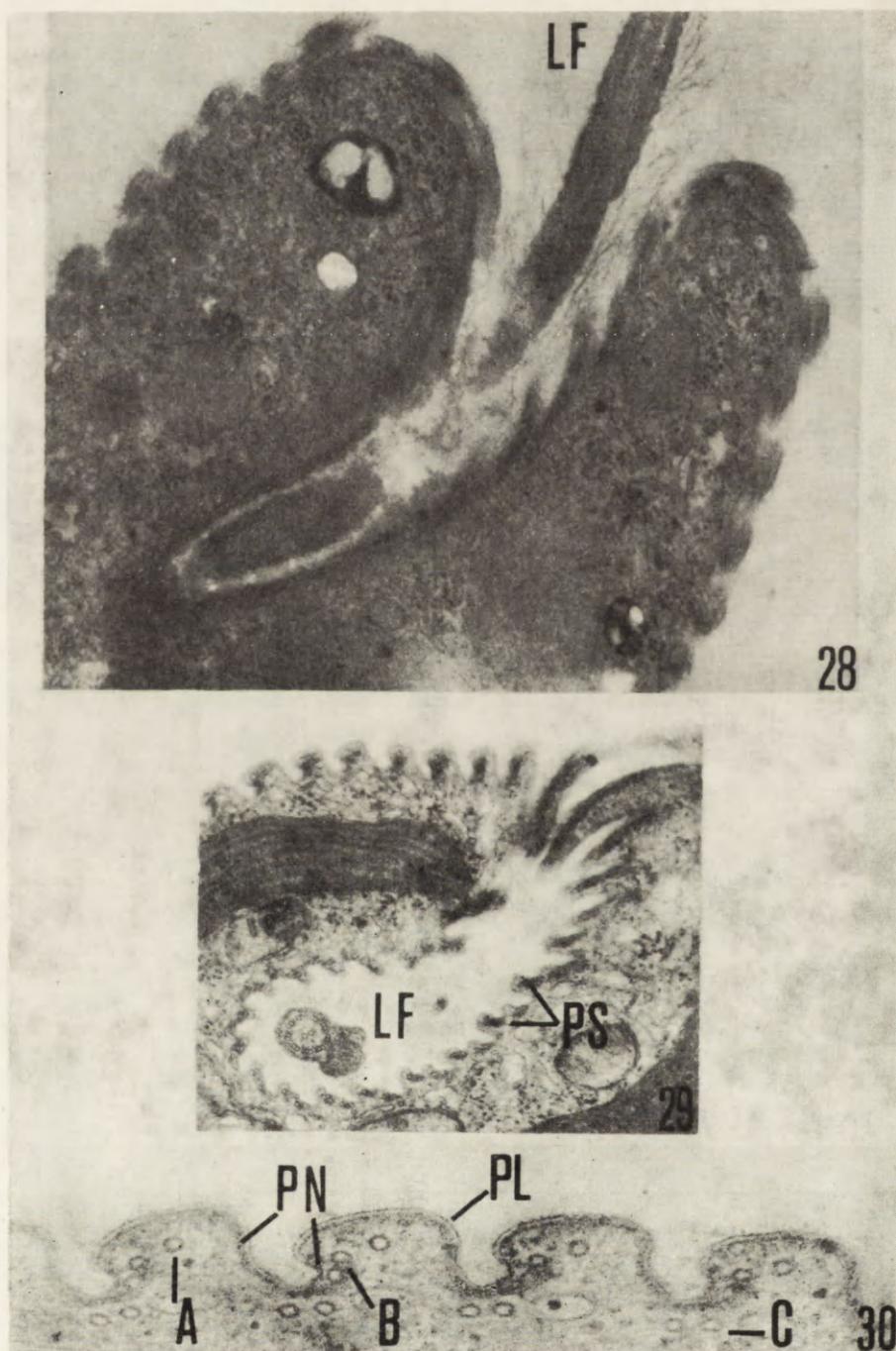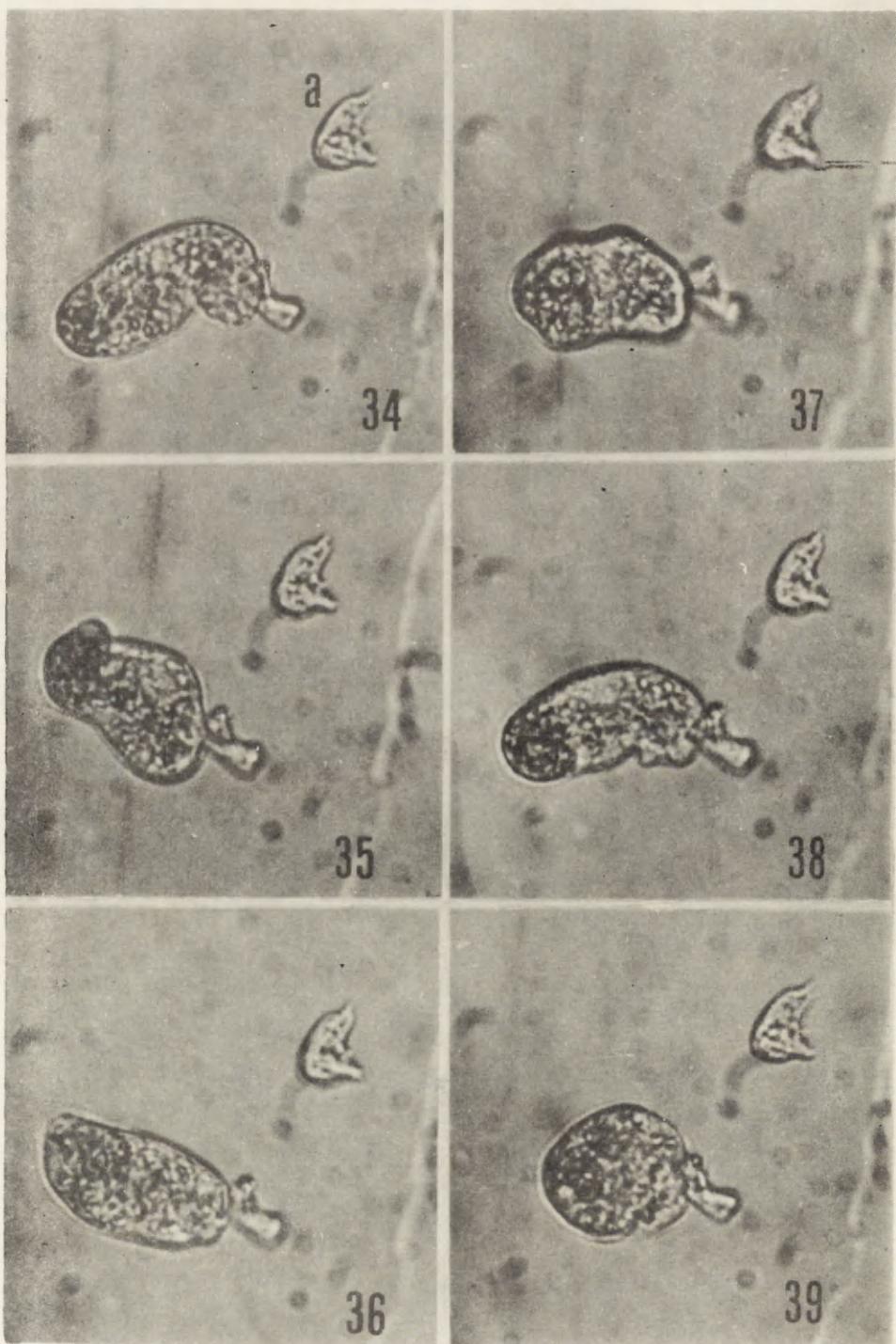
E. Mikołajczyk et L. Kuźnicki

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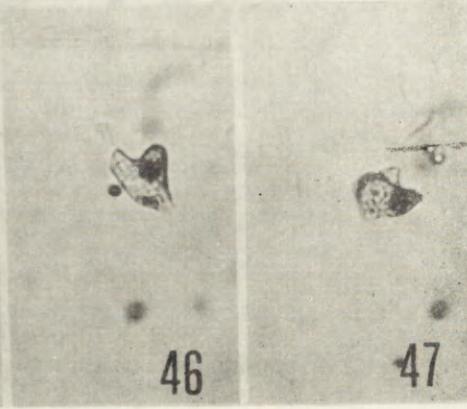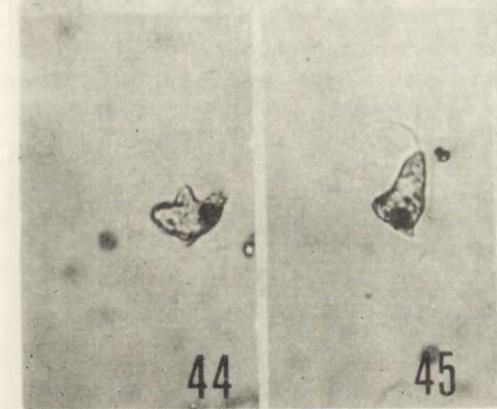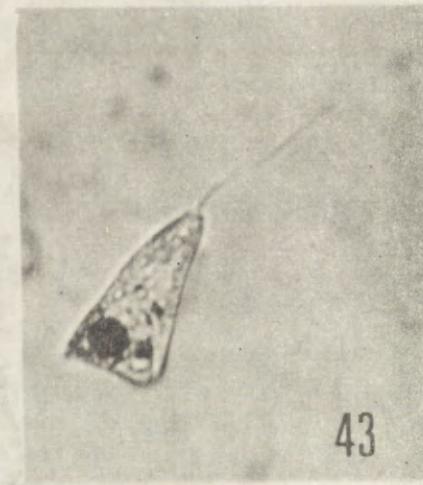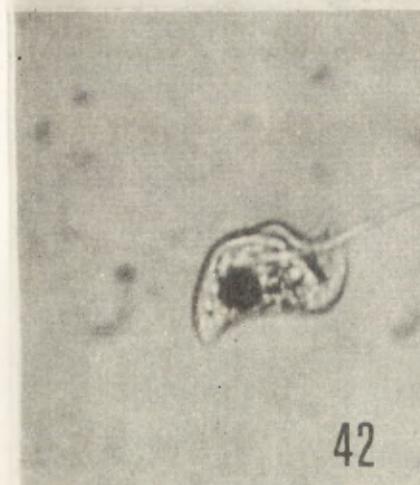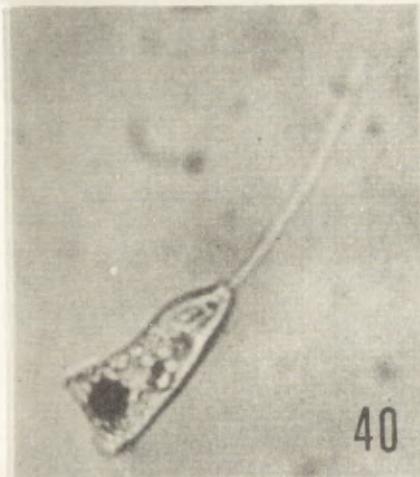
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Julita BĄKOWSKA

The Ultrastructural Analysis of the Regulation of Frontal Cirri in *Paraurostyla weissei*

Synopsis. A size-dependent regulation of frontal cirri (FC) of *P. weissei* has been shown. Cells of normal and reduced size possessing 6 FC disposed in two rows have been morphometrically analysed. The total number of kinetosomes (Nks) composing each cirrus and the number of transverse kinetosomal rows (KsR) in FC is reduced together with the cell dimensions. The ratio NKs/KsR is size dependent for all except the last posterior cirrus. The results are discussed in relation the morphogenesis of frontal cirri and to regulation of other ciliary structures.

The ultrastructural studies on regulation of cortical pattern in *Paraurostyla weissei* have been undertaken paralelly on different complex ciliary structure. The analysis of alterations within the oral ciliature caused by cell size reduction (Bąkowska and Jerka-Dziadosz 1980) revealed that the elements of ciliary pattern existing as a single structure (preoral membranelles) are not eliminated when the cell dimensions decreases. The number of serially repeated structures such as frontal and ventral adoral membranelles is proportionally reduced. The size of ventral membranelles decreases together with the dimensions of cell. The results of studies on regulation of left marginal cirri (Bąkowska 1980) indicate that both the number of adoral membranelles and left marginal cirri is size dependent, the proportion between them is characteristic for a given cell size. The number of kinetosomes composing left marginal cirri and the kinetosome distribution within these cirri changes together with the organism's size. The size reduced cells possess less numerous and smaller left marginal cirri.

The present paper is the third part of the study on changes in ultrastructure of ciliary organelles caused by cell size reduction. The

paper concerns the regulation of size of individual elements being the anterior fragments of FVT primordial streaks (J e r k a - D z i a d o s z and F r a n k e l 1969). The morphometric analysis indicates that alterations of the cell size lead to the reduction of the total number of kinetosomes composing each cirrus. Both the length and the width of cirri are regulated in a definite and unproportional manner. The results are discussed in relation to the morphogenesis of these structure and the previous results concerning ciliary pattern regulation in *P. weissei*.

Material and Methods

The line Z-6 of *Paraurostyla weissei* is used in this investigations. The line has been isolated from a single exconjugant after total conjugation (J e r k a - - D z i a d o s z and J a n u s 1975). The cells were maintained in sterile Prings-heim solution and fed on green algae *Chlorogonium* after H e c k m a n n (1963). The observations were preformed on single cells of normal and reduced size. The size-reduced cells were obtained as previously described (B ą k o w s k a and J e r k a - D z i a d o s z 1980). The cells analysed under light microscope Ortholux Leitz Wetzlar were stained with protargol after (J e r k a - D z i a d o s z and F r a n k e l 1969). For electron microscopy, cells were prepared using methods described in previous publications (B ą k o w s k a and J e r k a - D z i a d o s z 1978, 1980). Kinetosomes in cirri were scored on ultrathin sections examined under an electron microscope JEM 100B.

The number of kinetosomes composing frontal cirri and the number of transverse kinetosomal rows in which kinetosomes are disposed within cirri were statistically analysed using: multiple linear regression analysis and two tests for proportionality described in previous publication (B ą k o w s k a and J e r k a - D z i a d o s z 1980). The description of these routine techniques can be found in S o c a l and R o h l f (1969). Preparation of the statistic programs and computations were performed by the Computing Center of the Polish Academy of Sciences.

Results

Each cell of *P. weissei* possesses six or eight frontal cirri (FC). The cirri are disposed into two transverse rows — anterior (A) and posterior (P). The cirral rows are located immediately posterior to the frontal part of adoral zone of membranelles (fAZM). The frontal cirri in each row are enumerated from left to right (Pl. I 1). The anterior frontal cirri (FC_A) are slightly larger than the posterior ones (FC_P). The kinetosomes with in each cirrus are disposed in longitudinal and transverse rows. (Pl. I 2). The longitudinal rows can be considered as kineties according to de P u y t o r a c et al. (1976). Kinetosomes can be disposed within
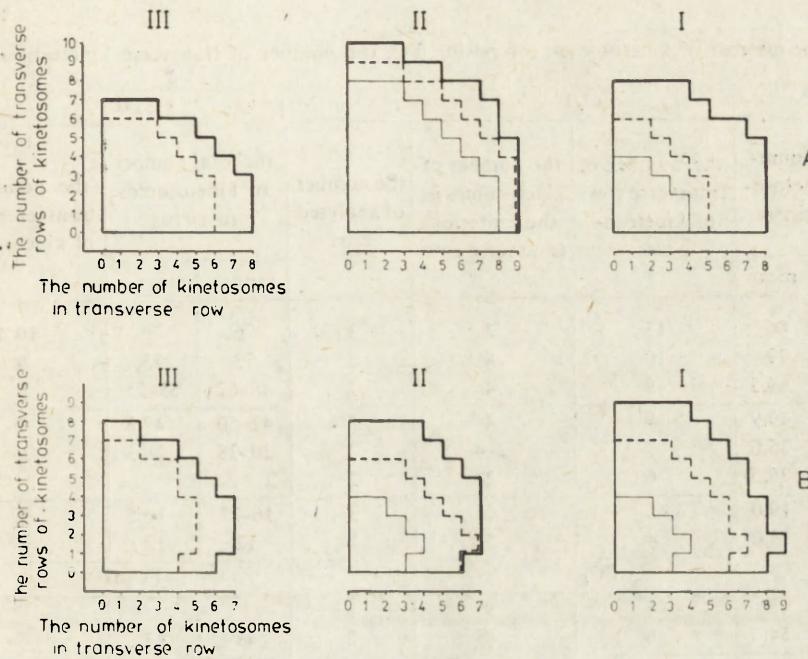
Fig. 1. The first (A) and the second (B) pattern of kinetosome disposition within anterior frontal cirri in normal and size-reduced cells. I, II and III — the enumeration of frontal cirri
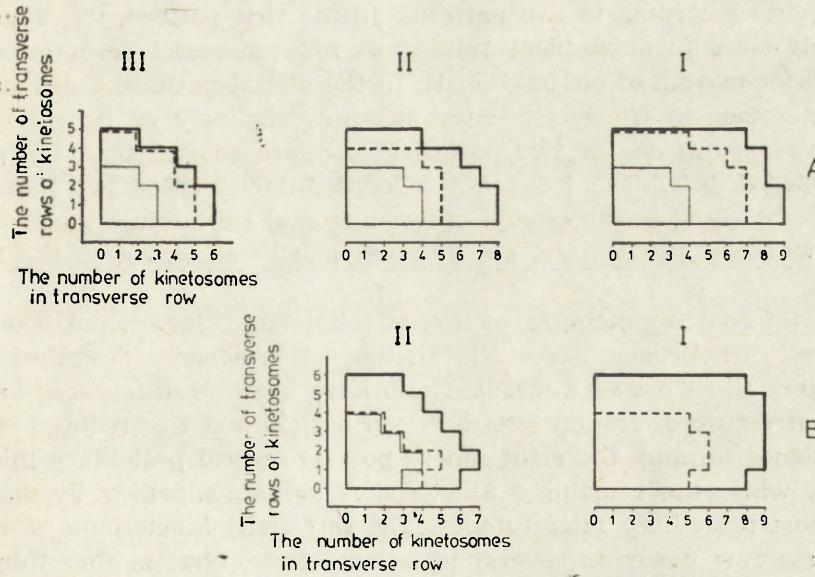

Fig. 2. The first A and the second B pattern of kinetosome disposition within posterior frontal cirri in normal and size-reduced cells. I, II and III — the enumeration of frontal cirri

The number of kinetosomes composing FC, the number of transverse kinetosomal rows

FCₐI					FCₐII		
the total number of kinetosomes in cirrus		the number of transverse rows of kinetosomes	the number of kinetosomes in the anterior transverse row	the number of analysed cirri	the total number of kinetosomes in cirrus		the number of transverse rows of kinetosomes
range	mean				range	mean	
86	86	13	3 ·	1	76	76	10
79	79	10	4	1	73	73	9
62–67	64.5	9	4	2	48–62	55–25	8
46–56	49.9	8	4	5	42–50	47.3	7
32–44	36.0	7	2–4	3	20–38	28.3	6
24–29	26.5	6	2	2			
16–23	19.0	5	2	3	16–21	18.5	5
13	13.0	4	2	1	12	12	4
FCₚI					FCₚII		
63	63	7	9	1			
52–57	54.5	6	8	2	43	43	6
31–54	43.6	5	4–9	5	25–42	34.6	5
17–33	22.0	4	4–5	6	13–19	16.25	4
11	11	3	2	1	11	11	3

frontal cirri according to two patterns. In the first pattern, the number of kinetosomes in subsequent transverse rows increases from anterior to posterior margin of cirrus (Fig. 1). In the second pattern, the number of kinetosomes in transverse rows increases similarly as it is in the first pattern, but one or two posterior rows are shorter than the previous ones (Fig. 2). The frequency of occurrence of these two patterns seems to be equal in the case of anterior frontal cirri, while the kinetosomes within posterior cirri are disposed mostly according to the first pattern.

The internal architecture of frontal cirri (the kinetosomal connectives and the fibrous basket) is similar as previously described for other cirri (Bąkowska 1980). There have been no differences found in ultrastructure of frontal cirri between normal and size reduced cells. Kinetosomes forming the right kinety possess several postciliary microtubules, while the remaining kinetosomes are accompanied by single, very short postciliary microtubules. The last (left) kinetosome of each transverse row possesses several transverse microtubules. Microtubular fibers connected with frontal cirri are very prominent. The anterior frontal cirri do not possess a separate anterior longitudinal fiber (AL)

in FC and the number of kinetosomes in the anterior transverse row in frontal cirri

FCAII		FCAIII					
the number of kinetosomes in the anterior transverse row	the number of analysed cirri	the total number of kinetosomes in cirrus		the number of transverse rows of kinetosomes	the number of kinetosomes in the anterior transverse row	the number of analysed cirri	The size of cells
		range	mean				
4	1						
4	1						normal
3	8	44–57	50.67	8	3	3	
3	3	38–45	42.67	7	3	3	
2–3	6	22–31	27.81	6	2–3	5	meedium
?	2						tiny
2	1						
FCpII		**FCpIII**					
							normal
5	1	29	29	6	2–4	2	
4-6	5	17–34	23.17	5	2–3	6	medium
2-4	4	16–20	18.00	4	2–3	2	
3	1	8	8	3	2	1	tiny

as do the remaining cirri of *P. weissei*. Instead, numerous microtubules attached to the anterior and left margin of cirral basket together with transverse microtubules form several bundles directed anteriorly. These bundles marge with microtubules forming the submembranellar fiber (SMF) connected with frontal membranelles (B ą k o w s k a and J e r-k a - D z i a d o s z 1978). A similar connection of microtubules running from frontal cirri with microtubular system accompanying frontal membranelles has been described in *P. hymenophora* (G r i m e s and L'H e r n a u l t 1978) and *Stylonychia* (T u f f r a u et al. 1968, de P u y-t o r a c et al. 1976). The small subectoplasmic rootlet (SSR) connected with ventral and marginal cirri is absent. Microtubules originating in the posterior margin of cirrus join the postciliary microtubules connected with kinetosomes of the right kinety and together they form the posterior longitudinal fiber (PL) directed posteriorly. The posterior frontal cirri possess the microtubular system less prominent than characterizing anterior frontal cirri. The microtubules originating in anterior and left cirral margin form a single fiber. The first posterior frontal cirrus (FCpI) possesses microtubular connections with microtubular system of preoral membranelles.

In order to analyse the alterations of size of frontal cirri the number of kinetosomes and the number of kinetosomal transverse rows were counted in each cirrus. The results concerning the kinetosomal composition of frontal cirri are groupped according to the cirral enumeration and cell sizes. The data are listed in Table 1. The reduction of cell size is connected with decrease of both the total number of kinetosomes composing cirri and the number of transverse kinetosomal rows in which kinetosomes are disposed. Comparison of the subsequent groups of cirri shows that kinetosomes of anterior cirri are disposed in more numerous transverse rows than the similar number of kinetosomes composing posterior cirri. In anterior frontal cirri the number of kinetosomes composing the first transverse row changes from 4 to 2. In the first posterior frontal cirri the number of kinetosomes composing this row changes from 9 to 2 and in the second posterior cirrus — from five to two. The results presented in Table 1 also show that the cirri possessing the same number of transverse kinetosomal rows may differ in the total number of kinetosomes composing them. This indicates that the kinetosome number range rather than their particular number is connected with a given number of transverse kinetosomal rows.

The statistical analysis of the relation between the number of transverse kinetosomal rows in a cirrus (KsR) and the total number of kinetosomes (NKs) composing it has been performed separately for each group of cirri of the same designation. The results are listed in Table 2. The regression analysis shows that the values of coefficients a and b are similar in the case of all except FC III_P, cirri. In order to find out whether the differences are significant they were compared using analysis of multiple regression. The analysis revealed that both regression coefficients are significantly different (a — $F = 17.097$, $F_{0.01} = 3.25$, $DF = 5/81$, b — $F = 3.5606$, $F_{0.01} = 3.26$, $DF = 5/76$). The further analysis revealed that excluding the $FC_A I$ and $FC_P III$ the remaining frontal cirri do not differ significantly in respect to the values of coefficient b at $P = 0.05$. Thus, these coefficients can be replaced by the one, common coefficient $b = 12.781$. These data indicate that the elimination of one transverse row of kinetosomes is connected with reduction of the total number of kinetosomes composing each of these four cirri by about 13 kinetosomes. The comparison of coefficients a calculated for these cirri show a statistically significant differences between them. That means, the lines illustrating the relation between the total number of kinetosomes and the number of transverse kinetosomal rows for each of the four cirri characterized by the same slopes but differ in respect to their elevation. Significant differences between the elevations indicate that the size (the total number of kinetosomes) of these

Table 2

The relation between the total number of kinetosomes composing frontal cirri and the number of transverse kinetosomal rows in which they are disposed

| Cirral row	The number of cirrus	The number of analysed cirri	Regression coefficient a	Regression coefficient b	95% confidence interval includes the value $x=0$	The total number of kinetosomes changes with the number of kinetosome rows	The ratio NKs/KsR changes with NKs	Regression coefficients common regression a	common regression b	general regression a	general regression b
anterior cirri	I	18	−28.078	9.9196	no	yes P = 0.001	yes P = 0.001			−32.254 DF = 2/47 F = 3.005 $F_{0.05}$ = 3.21	10.510 DF = 2/45 F = 3.115 $F_{0.05}$ = 3.21
	II	22	−42.000	12.1820	no	yes P = 0.001	yes P = 0.001	−38 613	12.781		
	III	11	−41.536	11.679	no	yes P = 0.001	yes P = 0.001	DF = 3/54 F = 28.?8 $F_{0.05}$ = 2.79	DF = 3/51 F = 0.8652 $F_{0.05}$ = 2.79		
posterior cirri	I	15	−35.174	14.924	no	yes P = 0.001	yes P = 0.010				
	II	11	−33.405	13.189	no	yes P = 0.001	yes P = 0.010				
	III	11	− 9.523	6.523	yes	yes P = 0.010	no P = 0.100				
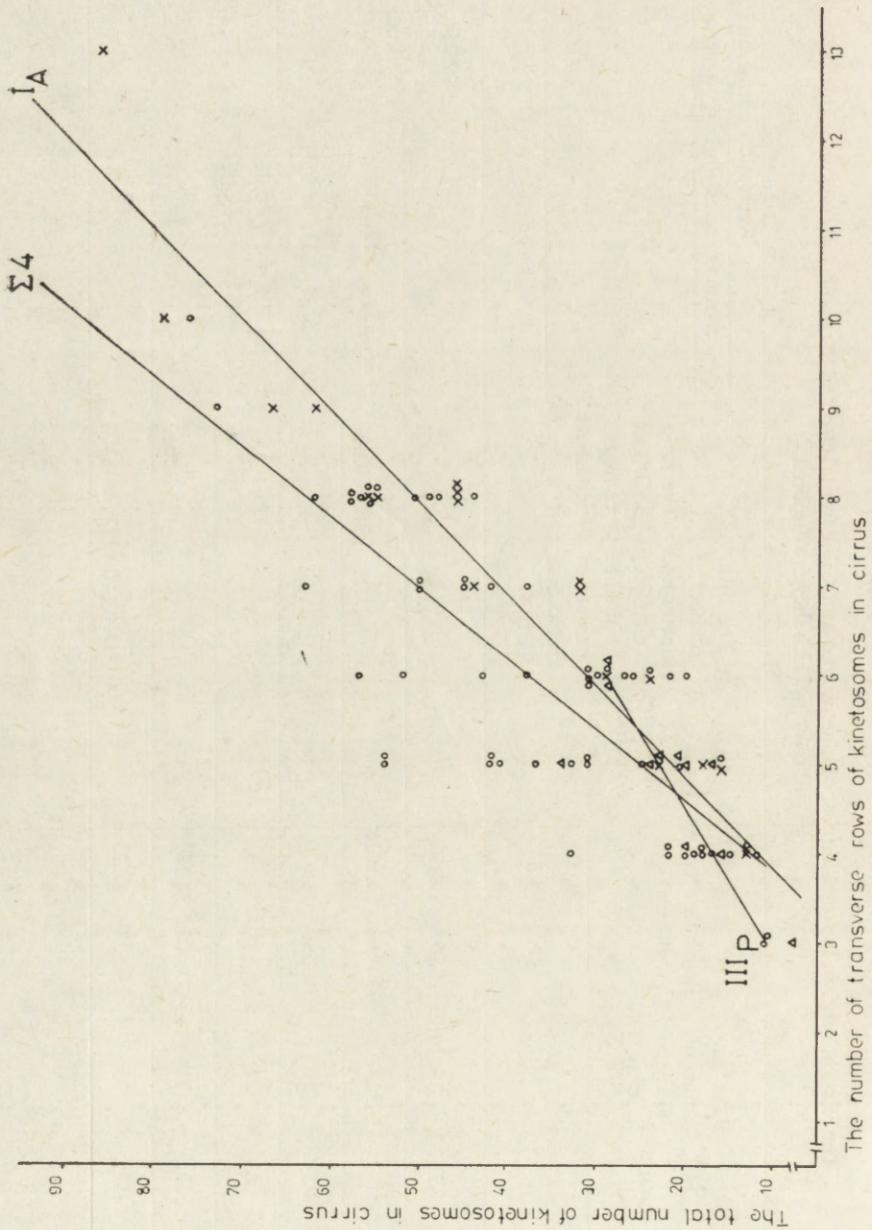
Fig. 3. The relation between the total number of kinetosomes composing frontal cirri and the number of transverse rows in which the kinetosomes are disposed. 4 — the common regression line for frontal cirri No. II, III of the anterior row and No. I and II of the posterior row, I_A — The first frontal cirrus of the anterior row, III_P — The last frontal cirrus of the posterior row

cirri are not similar. The relation between the total number of kinetosomes and the number of transverse rows in which they are disposed in the case of the remaining two cirri (FC_AI and FC_PIII) is described by the separate equations (Table 2, Fig. 3). The elimination of one transverse kinetosomal row is connected with the reduction of the total number of kinetosomes composing FC_AI by about ten kinetosomes and FC_PIII by about 6.5 kinetosome. The further elimination of groups of cirri from statistical analysis shows that the relation between the NKs and KsR is similar when only anterior frontal cirri are considered. In this case, both the coefficients a and b do not differ significantly. Therefore, these cirri are of similar size and elimination of one transverse row of kinetosomes in each of them is connected with the reduction of the total number of kinetosomes composing a given cirrus by about 10.5 kinetosome. These data indicate that the differences in regulation of posterior cirri are statistically significant and greater than the ones characteristic for anterior cirri.

In the equations describing the linear relation between the total number of kinetosomes composing cirri and the number of transverse rows in which kinetosomes of a cirrus are disposed, the confidence interval of Y-intercept does not include the value $X = 0$ in the case of all but one (FC_PIII) of frontal cirri. The second test for proportionality reveals that the ratio NKs/KsR depends on the size of all cirri except the last posterior cirrus. Thus, FC_PIII differs from the other in size, in the relation between the total number of kinetosomes composing it and in the number of transverse kinetosomal rows. These characteristic features can be connected with the morphogenesis of this cirrus (J e r k a - D z i a d o s z and F r a n k e l 1969).

Discussion

The question of how the developing system is regulated become especially acute in situation where both the size of elements and their number can change together with the organism's dimensions. This type of pattern regulation is exemplified by ciliary system of *Paraurostyla weissei*.

The somatic ventral ciliature of *P. weissei* is formed in a following way: (J e r k a - D z i a d o s z 1980a):

(1) Formation of kinetosomal pairs (so called — the first round of proliferation) and their ordering into longitudinal paired row (double kinety) paralell to the antero-posterior cell axis.

(2) Addition of new kinetosome to the pre-existing pairs of kineto-

somes (so called — the second round of proliferation). New kineto-
somes are added along the left margin of a double kinety.

(3) Transverse fragmentation of the primordium begins after the
initiation but not before the termination of the second round of proli-
feration.

(4) The final differentiation of the "cut off" segments of primordial
streaks leads to the formation of mature cirri.

(5) The process of new ciliary elements formation is accompanied
by the resorption of the old set of ciliary structures.

The second streak of   The first streak c
FVT primordium   FVT primordium

III$_A$ II$_A$

II$_P$ I$_P$

III$_P$
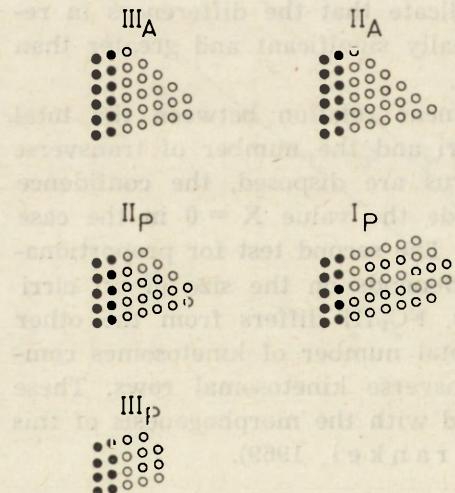
Fig. 4. The two first anterior parts of
streaks of fronto-ventro-transverse cir-
rial primordium. The first segments of
the two left primordial streaks which
differentiate into frontal cirri. I–III —
the enumeration of frontal cirri, A —
segments forming cirri of the anter-
ior row, P — segments forming front-
al cirri of the posterior row, ● — ki-
netosomal pairs formed in the course
of the first round of proliferation, o —
kinetosomes formed during the second
round of proliferation

The frontal cirri (except the frontal cirrus I of the anterior cirral
row) are formed in the above described way. Frontal cirri are the
anterior segments of the first two (left) fronto-ventro- transverse streaks
(FVT) of ciliary primordia (Fig. 4). The first (left) frontal cirrus of the
anterior cirrial row (FC I$_A$) is formed partially of the kinetosomes of
the primordium of preoral membranelles. After the lost of continuity
with this primordium FC I$_A$ develops similarly to the remaining frontal
cirri i.e., new kinetosomes are added to the preexisting ones in the
course of the second round of proliferation (Jerka-Dziadosz
1981b). In all (except FC I) frontal cirri the first two kineties are the
products of the first round of proliferation.

The results of present investigations show that the length of anterior
segments "cut off" from the FVT streaks undergoes regulation and

decreases together with the cell size. It has been shown for marginal cirri (B ą k o w s k a 1980) that the length of primordial streak is regulated according to the cell size. Thus, it can be expected that the length of segments in the case of FC depends also upon the total length of primordium streaks. The number of kinetosomes composing a given frontal cirrus is directly related to the number of transverse kinetosomal rows in which they are disposed. The number of transverse kinetosomal rows in cirri is equal to the number of kinetosomal pairs in a "cut off" segment. Thus, the results indicate that the intensity of both rounds of proliferation are interrelated and together determine the size of each ciliary structures — adoral and preoral membranelles (B ą k o w s k a and J e r k a - D z i a d o s z 1980) left marginal cirri (B ą k o w s k a 1980) as well as frontal cirri.

The maximal number of kinetosomes in the anterior transverse row in each frontal cirri of the anterior cirral row is four. It means that during the second round of proliferation two kinetosomes have been added to the first kinetosomal pair of each primordial streaks. In cells of medium size only one kinetosome has been formed next to this kinetosomal pair. In tiny cells the first transverse row of these cirri consists of only two kinetosomes. This indicates that there has been no addition of kinetosomes to the existing kinetosomal pairs during the second round of proliferation. These facts suggest that the limits of the zone of the second round of proliferation are set up in different manner than these controlling the size of the zone of kinetosomal pairs formation. The analysis of LMC regulation (B ą k o w s k a 1980) revealed that in cells of normal size the posterior cirri are composed of two transverse rows with 4–5 kinetosomes in each of them, while in tiny cells kinetosomal rows in posterior cirri are composed only of two kinetosomes. This indicates that in posterior part of cells the similar relationship between the zones of both round of proliferation can occurs as described in this paper for the anterior part of FVT streaks.

There is little question that microtubule organizing centers (MTOCs) in cells play a major role in governing the temporal and spatial ordering of microtubular structure assembly (T u c k e r 1979, F u l t o n and S i m p s o n 1979). Cells could position the nucleating elements for the first round of proliferation according to the species-specific pattern and adjust their number in respect to organisms size. The alternative possibility is that cells can activate in a needed quantity the preexisting nucleating elements disposed over cell surface according to the specific pattern. The nucleating elements for the secound round of proliferation seem to be connected with preexisting pairs of kinetosomes. The length of paired kinety defines the maximal number of potential sites

for new kinetosome formation. In normal cells the extent of new kineto-
somal addition seems to be limited by the number of nucleating ele-
ments which is equal to the number of pre-existing kinetosomal pairs.
The remaining material (except the nucleating elements for kinetosomal
pair formation) is disposed over cell surface in such a manner that it
decreases both to anterior and posterior poles of the cell. In normal
cells the potential for kinetosomal formation lengthens beyond
the paired kinety formed within LMC primordium and FVT primordial
streaks.

This relation between the extent of both round of proliferation is
shown on the Fig. 5 A. That could explain why several kinetosomes are
added to the first kinetosomal pairs of FVT streaks and to the anterior
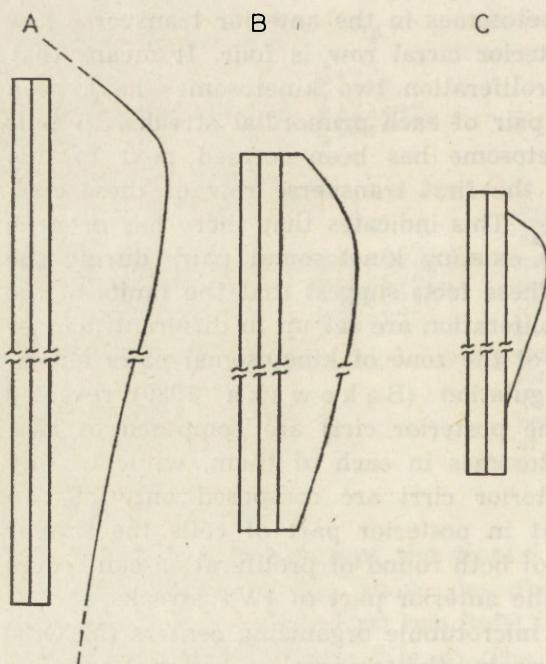
Fig. 5. The schematic represen-
tation of the supposed relation
between the extents of two ro-
unds of proliferation in normal
(A), medium-size (B) and tiny
cells (C). The length of paired
kiney is marked by the two pa-
rallell lines. The curved continu-
ous line indicates the extent of
the second round of proliferation.
The dashed lines mark the hy-
potetical potentiality of kineto-
somal formation during the se-
cound round of proliferation

and posterior kinetosomal pairs of LMC primordium. In cells of medium
size, the length of double kinety formed within primordia of somatic
ventral ciliature during the first round of proliferation seems to be
equal to the extent of potential possibilities of kinetosomal formation
during the second round of proliferation. This situation where only
one kinetosome is added to the first kinetosomal pair of the streak is
shown on Fig. 5 B. In tiny cells, the sites of new kinetosome addition
are delimited not by the lack of nucleating elements connected with

pre-existing kinetosomal pairs but rather by the extent of available material for kinetosomal formation. That could explain why there is no kinetosomal addition to the first kinetosomal pair of primordial streaks. This relation is ilustrated in the Fig. 5 C.

The newly formed kinetosomes along the left margin of paired kinety aquire the function of the kinetosomal pairs in nucleating new kinetosomal formation. However, the subsequently formed kineties become progressively shorter. The number and the length of repetitively formed kineties next to the first paired kinety could be controlled in either of the two ways: (1) by the factor arresting the formation or activation of nucleating elements, (2) by exhausting of the precursors for kinetosome formation other than MTOCs.

The same frontal cirri of different cells being equally long segments of the primordial streak can differ in the number of kinetosomes composing them. This could suggest that either the intensity of the second round of proliferation is not exactly controlled in respect to the length of structure (length of segment) or the pattern of primordium segmentation is to some degree random. The results obtained for the regulation of adoral membranelles (B ą k o w s k a and J e r k a - D z i a d o s z 1980) indicated that the intensity of both rounds of proliferation are precisely adjusted. On the other hand, the investigations on the regulation of left marginal cirri (B ą k o w s k a 1980) revealed that the pattern of primordial streaks of similar length can differ. Therefore, it seems that the difference in primordium segmentation could be responsible for observed irregularities in formation of frontal cirri.

Summarizing above considerations it can be stated that the size of frontal cirri is regulated according to the cell dimensions. It seems that different factors are involved in regulation of both rounds of proliferation. Thus the final relation between the total number of kinetosomes composing each frontal cirrus and the number of transverse rows in which these kinetosomes are disposed within this cirrus could be the results of harmonious action of different controling factors.

REFERENCES

B ą k o w s k a J. 1980: Size dependent regulation of serially repeated structures of a protozoan *Paraurostyla weissei*. Acta Protozool., 19, 297–313.

B ą k o w s k a J. and J e r k a - D z i a d o s z M. 1978: Ultrastructural analysis of the infraciliature of the oral apparatus in *Paraurostyla weissei* (*Hypotricha*). Acta Protozool., 17, 285–301.

B ą k o w s k a J. and J e r k a - D z i a d o s z M. 1980: Ultrastructural aspect of size dependent regulation of surface pattern of complex ciliary organelle in a protozoan ciliate. J. Embryol. Exp. Morphol., 39, 355–375.

F u l t o n C. and S i m p s o n P. A. 1979: Tubuline pools, synthesis and utilization. In: Microtubules. (ed. R o b e r t s K. and H y a m s J. S.) Acad. Press, London, 117–174.

G r i m e s G. W. and L'H e r n a u l t S. W. 1978: The structure and morphogenesis of the ventral ciliature in *Paraurostyla hymenophora*. J. Protozool., 25, 65–74.

H e c k m a n n K. 1963: Paarungssystem genabhängige Paarungstypdifferenzierung bei dem hypotrichen Ciliaten *Euplotes vannus* O. F. Müller. Arch. Protistenk., 106, 393–421.

J e r k a - D z i a d o s z M. 1980 a: Ultrastructural study on development of the hypotrich ciliate *Paraurostyla weissei*. I. Formation and morphogenetic movements of ventral ciliary primordia. Protistologica, XVII (in press).

J e r k a - D z i a d o s z M. 1980 b: Ultrastructural study on development of the hypotrich ciliate *Paraurostyla weissei*. II. Formation of the adoral zone of membranelles and its bearing on problems of ciliate morphogenesis. Protistologica, (submitted).

J e r k a - D z i a d o s z M. and F r a n k e l J. 1969: An analysis of the formation of ciliary primordia in the hypotrichous ciliate *Urostyla weissei*. J. Protozool., 16, 612–637.

J e r k a - D z i a d o s z M. and J a n u s I. 1972: Localization of primordia during cortical development in *Keronopsis rubra* (Ehrbg., 1838), (*Hypotrichida*). Acta Protozool., 10, 249–262.

de P u y t o r a c P., G r a i n J. and R o d r i g u e s de Santa Rosa M. 1976: A propos de l'ultrastructure corticale du cilié hypotriche *Stylonychia mytilus* Ehrbg., 1838: les caracteristiques du cortex buccal adoral et paroral des *Polyhymenophora* Jankowski, 1967. Trans. Am. Microsc. Soc., 95, 327–345.

S o c a l R. R. and R o h l f F. J. 1969: Biometry. The Principles and Practice of Statistics in Biological Research. (ed. W. H. F r e e m a n), San Francisco.

T u c k e r J. B. 1977: Shape and pattern specification during microtubule bundle assembly. Nature, 266, 22–26.

T u c k e r J. B. 1979: Spatial organization of microtubules. In: Microtubules. (ed. R o b e r t s K. and H y a m s J. S.) Acad. Press, London, 315–357.

T u f f r a u M., P y n e C. K. and de H a l l e r G. 1968: Organisation de l'infraciliature chez quelques cilies hypotriches. Protistologica, 4, 289–301.

EXPLANATION OF PLATE 1

1: The anterior part of the size reduced cell in *Paraurostyla weissei*. I, II and III — the enumeration of frontal cirri, A — anterior row of frontal cirri, P — posterior row of frontal cirri, OPM — preoral outer membranelle, AZM — adoral zone of membranelle. 8160 ×

2: The first frontal cirrus of the posterior row (FCₚI) of the cell of normal size

PLATE I

J. Bąkowska

auctor phot.

Janina KACZANOWSKA and Jerzy MORACZEWSKI

Short-range Positioning of Contractile Vacuole System in a Ciliate
Chilodonella steini

Synopsis. The unicellular ciliate organism *Chilodonella steini* has well defined ventral field. During divisional morphogenesis, two sets of new contractile vacuole pore (CVP) primordia appear on this ventral surface, while the old set of CVPs still persists and is resorbed later. The number and distribution of the CVP primordia manifest some variability and indeterminacy of large-scale pattern of CVPs in *Chilodonella steini*. New CVP primordia are always generated in the vicinity of the left-anterior sites of the ciliary basal bodies. During maturation of the CVP primordia, they move into a central position between ciliary meridians, and a union of CVP structures with well differentiated subcortical spongiome takes place. This organellogenesis reveals some local autonomy, since CVP primordia near one another may differ in their rate of development. It follows from the above that the large-scale mechanism of positioning manifests some indeterminacy, while short-range fine positioning has local and strictly spatially oriented character.

Ciliates are single celled organisms with an ordered pattern of distribution of their organelles over the cell cortex. This spatial pattern is controlled by a single nuclear apparatus operating in an unpartitioned cytoplasmic continuum. New pattern formation results from genetical control but is influenced by the preexisting organization of the cell (Sonneborn 1963, Frankel 1974, 1979). These is evidence that the large-scale mechanism of positioning of organelles is encoded in the ciliate genome (Heckmann and Frankel 1968, Jerka-Dziadosz and Frankel 1979). Qualitatively different nearest-neighbour, or short-range positional control can be considered as a "supramolecular scaffold" (Frankel 1979) of developmental program of pattern formation (Jerka-Dziadosz 1980 a).

Chilodonella steini is a ciliate species that reveals a striking variability of the spatial patterns of its excretory organelles among sister cells of similar size. The contractile vacuole pores (CVPs) easily impregnated with silver are markers of the underlying contractile vacuole system. In early dividing cells a large number of CVP primordia appear, exclusively on the well delimited ciliated ventral field. At this stage of morphogenesis the parental CVPs are still preserved. Their distribution marks the final parental pattern of the dividing cells. During further development some of the CVP primordia develop, while others are resorbed with the total resorption of the parental CVPs.

By studying CVP organellogenesis of early dividing cells of *Chilodonella steini* one may explore two different questions:

(1) Does polymorphism reflect some indeterminacy of the large-scale mechanism of positioning or result from an indeterminacy induced by the preexisting pattern and expressed at the ultrastructural level?

In some mutants of *Tetrahymena* N g and F r a n k e l (1977) and N g (1977) found that the local rotation of ciliary meridians in an area of expected occurrence of the CVP yielded the proper general placement of this organellum. However, the fine positioning of the latter organellum was observed on the opposite side of an inverted ciliary meridian, as compared to the stable positioning of CVPs to the left of a nonrotated ciliary meridian. Thus the two sides of the CVP meridian have different morphogenetic properties, and this difference is determinative in the asymmetrical fine-positioning of the CVP. Recognition of two different aspects of CVP formation, large-scale cellular organization and fine-positioning has been also confirmed in further studies on *Tetrahymena* (J e r k a - D z i a d o s z and F r a n k e l 1979, N g 1979). Studies on CVP differentiation in *Chilodonella steini* throw some light on the role of basal body in positioning of this organellum.

(2) How is the actual CVP organellogenesis within cortical layer associated with differentiation of the specialized region of excretion in the cytoplasmic continuum?

CVPs of ciliates are highly elaborated cortical structures which discharge the fluid content from the underlying contractile vacuoles (CVs) (E l l i o t t and B a k 1964, F a u r é - F r e m i e t and R o u i l - l e r 1959, M c K a n n a 1973, P a t t e r s o n and S l e i g h 1976, J e r - k a - D z i a d o s z, 1980 b). It is expected that the cortical organellogenesis of the CVP primordium is somehow integrated with the compartmentalization of the subcortical excretory system (H a u s m a n n and A l l e n 1977). Studies on the CVP and the spongiome differentiation in *Chilodonella steini* reveal the structural markers of this integration.

Material and methods

Chilodonella steini (*Ciliata, Kinetofragmophora*) R a d z i k o w s k i and G o-ł e m b i e w s k a (1977) non-selfing line 527/10 in the immaturity period (K a c z a-n o w s k i et al. 1980) has been isolated from one exconjugant. The general characteristic of this species and methods of culturing of cells followed these of R a d z i k o w s k i and G o ł e m b i e w s k a (1977). The mean generation time of cells varied from 12–19 h when maintained in normal daily rhythm of light and fed every second day.

Cells from two-day mass clonal culture were used either for silver impregnation (F r a n k e l and H e c k m a n n 1968) or were isolated for electron microscope fixation. In the latter case the early dividers were recognized by their modified shape and slight indentation in the right side of the equatorial zone. For EM studies the routine methods were used, with fixation with glutaraldehyde of 0.5% in 0.05 M cacodylate buffer (15′) followed by 2% osmium in the same buffer (2 h) and stained with acetate uranyle. Unoriented sections were made with a diamond knife on LKB ultramicrotome III. Sections were analyzed with a Tesla Electron Microscope.

Results

CVPs Differentiation as Observed at Cellular Level of Organization of Dividing *Chilodonella steini*

Morphology and divisional morphogenesis of *Ch. steini* conforms the general scheme described by R a d z i k o w s k i and G o ł e m-b i e w s k a (1977). Polymorphism of CVPs distribution and the level of variability of CVPs pattern have been recently described (K a c z a-n o w s k a in preparation).

CVPs are distributed only over the ventral surface, but they never appear near the oral apparatus or at the margin of the ventral surface. In silvered specimens, CVPs appear as round black circles in the middle of certain pairs of ciliary meridians. In early dividing cells CVP primordia appear as the small dots or slits a c r o s s the intermeridional spaces. The CVP primordia are positioned exclusively at the left side of the selected ciliary rows preferentially in three longitudinal sectors (K a c z a n o w s k a in preparation). In early dividers new CVP primordia are readily distinguished from parental CVPs both by their shape and localization in the intermeridional space. One may discern some variability of the dimension of both parental CVPs and of CVP primordia. Parental CVPs in the postoral portion of the ventral surface are about 1.4 μm of diameter, while in the preoral part they are only about 0.7 μm. of diameter. Very small CVP primordia occur in the preoral part of the cell, and in postoral part in the vicinity of larger

perpendicular slits. During further divisional morphogenesis some new CVP primordia change into long perpendicular slits across the whole intermeridional space, while others remain with no transformation as dots at the left side of ciliary meridians. During early cytokinesis some CVP primordia (slits) are transformed into final round orifice in the middle of the intermeridional spaces, while the parental set of CVPs and some underdeveloped new CVP primordia (dots) completely disappear. Different fates of the CVP primordia might be based either on their different position as seen at the cellular level of organization of the CVP pattern, or on the different local microenvironment around a given CVP primordium. Therefore the question arises whether the local development of the individual CVP primordium is strictly spatially determined in respect to the cortical structures, or not.

Cortical and Subcortical Markers of Spatial Orientation of Cortical Components

The structure of the somatic cortex of *Ch. steini* conforms to that described for other ciliates of the same group (G r a i n 1969, S o l- t y ń s k a 1971, L o m and C o r l i s s 1971). The cell membrane covers the entire cell including the cilia, contractile vacuole pores, parasomal sacs, membranous sacs (P a t t e r s o n 1978) and cytostome. Alveoli are located below the cell membrane and consist of an outer alveolar membrane, an inner alveolar membrane and an alveolar space. The alveolar space is compartmentalized by septa formed by the union of inner and outer alveolar membranes of adjacent alveoles. The height of alveoles varies from 1.4 um in intermeridional spaces to about 0.2 um in grooves occupied by the ciliary meridians.

The entire cytoplasmic surface of the inner alveolar membrane is covered with an electron-dense fibrillar epiplasm, but is pierced by parasomal sacs, membranous sacs and a diaphragm at the bottom of the CVP lumen (Fig. 1, Pl. I 2, III 12).

In the subcortical cytoplasm free ribosomes, endoplamic reticulum, mitochondria, smooth electron empty vesicles, food vacuoles and dense granules are observed (Pl. I 2). In this subcortical layer in the vicinity of the oral apparatus an abundance of wavy ribbons of puzzling "tubule complexes" (P y n e and T u f f r a u 1970) are observed (Pl. I 3). The character of the subcortical cytoplasm dramatically changes in the region below the CVP (see below).

Kinetosomes are spaced along the somatic meridians in cortical grooves. Each kinetosome is associated with spatially oriented systems of fibrillar and membranous structures (Fig. 1, Pl. I 3). At least four

different fibrillar structures are specifically oriented around the ciliated somatic kinetosome.

The striated kinetodesmal fiber (KD) originates in the wicinity of triplet 6 (orientation and enumeration of triplets according to G r a i n's 1969). This fiber extends toward the surface perpendicular to the main axis of the meridian. It forms a blade tapered at the end in the vicinity of the next meridian to the right (Fig. 1, Pl. I 3).
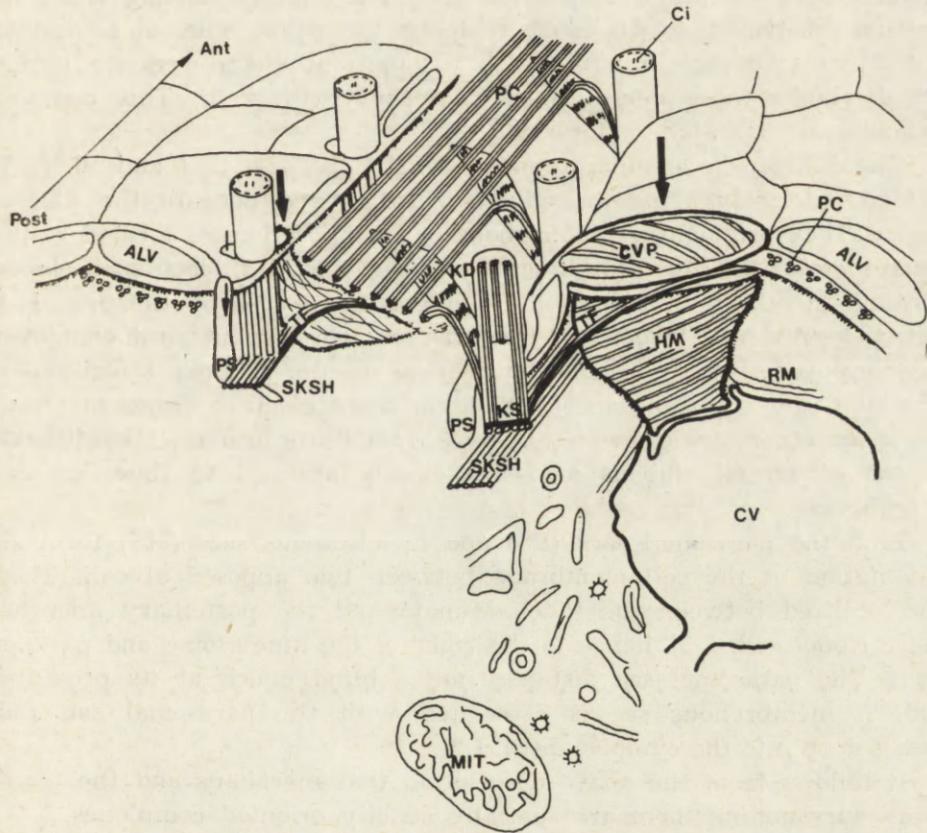
Fig. 1. A three-dimensional schematic representation of the various cortical and subcortical components of *Chilodonella steini* with two sequential stages of development of the contractile vacuole system. Abbreviations: Ci — cilium, ALV — alveole, PC — postciliary ribbon of microtubules, TF — transverse complex (transverse microtubules and transverse spur), KD — kinetodesmal fiber, SKSH — submeridional microtubular sheet, KS — kinetosome, PS — parasomal sac, CVP — contractile vacuole pore, HM — helical microtubules, RM — radiating microtubules, MIT — mitochondrium. The heavy arrows mark an entrance into CVP lumen

The postciliary ribbon (PC) consists of three microtubules that originate at the kinetosome base in the position of triplet 9. The postciliary microtubules extend steeply at a right angle toward the surface

just below the epiplasm of the intermeridional ridge and then sharply bend posteriorly to become parallel to bundles of triple postciliary ribbons extending from more anterior kinetosomes (Fig. 1, Pl. I 3). The length of any triple postciliary ribbon extends for a distance of about 10 kinetodesmal territories. These ribbons form a roof about kineto-desmata.

A traverse microtubular complex consists of two transverse micro-tubules (TF) extending to the left at a right angle starting from the position of triplet 4. An electron dense transverse spur is associated with two transverse microtubules through an electron dense bridge which flanks these microtubules posteriorly (Pl. I 3). This complex extends just below the cytoplasm.

The submeridional microtubules (SKSH) (Pl. I 2, 3, 4 and Pl. II 7) extend anteriorly along the ciliary meridian and beneath the kineto-somes. They originate as flat ribbons of six microtubules formed at the basal plate of the preceding kinetosome (Fig. 1 Pl. I 3); each such ribbon continues to the next kinetosome where it is covered by the preceding newly formed flat ribbon. In this way a very distinct cord composed of many rows of six microtubules joins the meridional kinetosomes. The two upper ribbons are included in more electron dense material, while the others are connected in rows by delicate bridges. Microtubules of the sequential ribbons are each closely apposed to those of the neighbours.

Both the parasomal sacs (PS) and membranous sacs (MS) form an indentation of the cell membrane between two apposed alveoli. They are localized between the kinetodesmata and the postciliary microtu-bular ribbon (Pl. I 3), hence to the right of the kinetosome and parallel to it. The parasomal sac distends into a blind pouch at its proximal end. A membranous sac is associated with the parasomal sac and passes deep into the cytoplasm (Pl. I 2).

It follows from the above description that meridians and the terri-tories surrounding them are spatially strictly oriented complexes.

CVP Organellogenesis

The new CVP primordium begins as an invagination of the cell membrane between septa of alveoli adhering to the kinetosome (arrow at Pl. I 4). In all cases this invagination begins at the anterior side of kinetosome at the level of its terminal plate and is located a n t e r i o r to the transverse complex (TF). This invagination forms a bottle-shape pouch (Pl. II 5) extending from the upper anterior part of the kineto-some toward the cytoplasm parallel to the transverse fibrillar complex.

From the beginning this pouch is lined with an electron dense material similar to that of the epiplasm with at least some associated microtubules partly embedded in this electron dense material. Microtubules are sparse and they lie loosely parallel to the axis of the pouch (Pl. II 5). Their proximal ends sometimes may be traced even beyond the pouch. The origin of these microtubules remains unknown. The primordial pouch spreads across the intermeridional space and is associated with a trail of delicate fibrillar material at its proximal end.

Even at a very early stage of CVP differentiation there is observed a clear differentiation of the spongiome beneath this primordium (Pl. I 4). In this subcortical territory there is no endoplasmic reticulum but there are many smooth membrane vesicles and canaliculi reminiscent of the tubular system of the spongiome. In some sections structures resembling "decorated fluid segregation tubules" (M c K a n n a 1976) (FST? at Pl. I 4) and sporadic "tubule complexes" (P y n e and T u f- f r a u 1970) are clearly distinguished.

In the second stage of CVP primordium development an invagination of the pouch extends perpendicularly across the whole intermeridional space. The small round opening observed at Pl. II 5 transforms into an elliptical slit (Pl. II 6, 7). At this stage microtubules are occasionally found in sections of the deep part of the pouch (Pl. II 7). At this stage some canals are dilated and some form regular connecting canals.

In the third and final stage of CVP development (Pl. II 8, III 12) a microtubular sheet surrounding of the CVP lumen is differentiated. The orifice, initially in the form of a slit, becomes round and entirely separated from the adjacent kinetosome through interposition of one or two newly formed alveoli. In this stage the diaphragm and the bottom of CVP tube is formed and radiating microtubules are arranged into regular bundles.

In transverse sections (Pl. II 8, III 9) microtubules are regularly displayed around the wall of the CVP lumen and they are tightly spiralized since they are differently slanted in the same picture. It is not known whether the numerous microtubular profiles seen in the sections result from new assembly, or from the tight twisting and precise ordering of previously assembled microtubules. Following calculations of M c K a n n a (1973) for the number of microtubules around the CVP tube in *Paramecium,* it is expected that their number in the CVP tube in *Chilodonella* may be fewer than observed in a given section.

Newly formed alveoli arise between the adjacent kinetosome and the adhering rim of the CVP orifice (Pl. III 10 ALV). These alveoli definitely separate the kinetosome from the rounding CVP orifice. The shorter axis of the orifice becomes greater, while the long axis of the

slit becomes shorter. The final diameter of such a CVP orifices varies from 0.7 µm to about 1.2 µm. In some sections the CVP may occupy the space of two interkinetosomal distances along the long axis, while in others the CVP is framed by two sequential transverse fibers which touch the CVP wall on both its anterior and posterior sides.

Microtubules are regularly displayed around the CVP orifice (Pl. II 8, III 9, 10) at a constant distance of 25 nm from the outside membrane. From each of the microtubules, a thin fibrillar thread 12–15 nm long extends radially and touch an electron dense ring outside the ring of microtubules (Pl. Ill 9). Thus this thread is much shorter than the cross bridges connecting the hexagonally arranged microtubules of ne-madesmata of *Ch. steini,* which are about 25–30 nm long. In a transverse section of the CVP orifice, whose orientation may be confirmed by transverse section of nearby cilia, a right hand spiralization of microtubules is discerned (Pl. Ill 9) by an analysis similar to that of M c K a n n a (1973) in *Paramecium.*

The rounding of the CVP orifice and its displacement into the middle of intermeridional space is accompanied by a transformation of the pouch of the CVP primordium into a vase-shaped tube (Pl. III 11, 12), whose bottom is devoid of electron dense material and of microtubules. There is a very close contact of the rounding contractile vacuole with the bottom of the CVP tube. Thus the diaphragm (D) (Pl. III 12) is formed at the bottom of the CVP tube while maintaining strict continuity of the CVP membrane with the cell membrane. Physical compression exerted by the rigid cortical structures of the CVP tube upon the subcortical contractile vacuole is suggested by the deformation of the shape of the latter by the proximal end of the CVP (Pl. III 12).

Another set of structures might be involved in the final shaping of the CVP tube, microtubular spiralization and the functional connection between the CVP and the spongiome. These are radiating microtubules (RM) which are sparsely at the slit stage of morphogenesis. At more advanced stages (Pl. II 8, III 11, 12) as it is described in *Paramecium* (H a u s m a n n and A l l e n 1977) they consist of bundles of microtubules which spread from the wall of the CVP obliquely deep into the cytoplasm over the upper hemisphere of contractile vacuole.

While in sections some primordia of the CVP system are very advanced in their differentiation, others represent a very early stage of organellogenesis. Thus an asynchrony of the CVP organellogenesis over very short distance is discerned.

An integrity of a whole contractile vacuole functional unit is manifested by the attachment of the helical microtubules to an outer rim, by the cross bridges of the set of helical microtubules themselves, by

the close opposition of the membrane of contractile vacuole and of the bottom of the CVP tube with an aid of the radiating bundles of microtubules.

This functional unit is inserted in the middle of the intermeridional space: it may be flanked anteriorly and posteriorly by the transverse complexes, and is separated from the adjacent kinetosome by new alveoli.

Discussion

Chilodonella steini manifests a reproducible variability of patterns of CVPs distribution within the ventral field of sister cells of the same or similar size. This polymorphism is observed during differentiation of sets of CVP primordia in early dividing cells and is maintained after resorption of some of the CVP primordia.

Observations made on silvered specimens, and at the ultrastructural level reveal an asynchronous development of individual CVP primordia, even if they are near to each other. Some of these may be arrested and later resorbed (K a c z a n o w s k a in preparation and this paper). CVP morphogenesis is not restricted to any particular meridians nor to the age of the particular area of the cortex, and is autonomous with respect to the fate of the other primordia.

At the level of fine positioning the cortical events involved in CVP formation are spatially oriented and regularly restricted by the nature and polarity of the structures within the ciliary territory. The selected kinetosome serve as a nucleating center for CVP organellogenesis at the l e f t side of ciliary meridian. This statement conforms data on *Tetrahymena* (N g and F r a n k e l 1977, N g 1979). As far, selected kinetosomes seems to be not structurally involved in CVP organellogenesis. Thus it is suggested that the kinetosomal territory form a framework for the appearance of the CVP pouch in a region of the cell membrane deprived of alveoli and subcortical fibers. The asymmetry of the kinetosome and the "empty" area on its anterior left side may account for the difference between fine positioning anterior to the kinetosome in *Chilodonella* and posterior in *Tetrahymena* (N g 1979).

Small CVPs occur in the anterior preoral part of the ventral field, while double-sized CVPs predominate in the equatorial part of this field. In the anterior part of the ventral field the kinetosomes possibly do not proliferate (K a c z a n o w s k a 1975). These old kinetosomes may be equipped with transverse fibers that limit the size of a CVP primordium. On the other hand, on the postoral part of the ventral

field kinetosome proliferation occurs before divisional morphogenesis. The new CVP primordia may develop within enlarged areas, since distances between kinetosomes increase just before their proliferation: new kinetosomes may fail to develop transverse fibers if a CVP develops nearby.

It may be concluded that strictly spatially oriented autonomous and local organellogenesis of the individual CVP primordium is very different from the indeterminacy of assesment of territories with CVP primordia and of the number and fate of CVP primordia observed at the cellular level of organization.

Description of contractile vacuole unit corresponds to the type A of morphological organization as defined by P a t t e r s o n (1980). At least CVPs structures are lost during divisional morphogenesis and all pores develop de novo. The CVP once formed moves relative to other structures. The ultimate position of the CVP is regulated by an appearance of new alveoli. A striking phenomenon in the cytodifferentiation of CVPs and of the subcortical spongiome is the e x a c t f i t of the areas of the spongiome with the ultimate position of the CVP tubes in the middle of intermeridional space. At first the CVP primordium develops at the left anterior side of an adjacent kinetosome, and its central localization in the intermeridional space is reached at the stage when the spongiome is well differentiated. The later formation of the diaphragm and the spreading of the radiating microtubules mark the phenomenon of i n t e g r a t i o n of these two separate parts of the elaborated secretory system of *Ch. steini*.

ACKNOWLEDGEMENTS

REFERENCES

E l l i o t A. M. and B a k I. J. 1964: The contractile vacuole and related structures in *Tetrahymena pyriformis*. J. Protozool., 11, 250–261.
F a u r é - F r e m i e t E. and R o u i l l e r C. 1959: Le cortex de la vacuole contractile et son ultrastructure chez les ciliés. J. Protozool., 6, 29–37.
F r a n k e l J. 1974: Positional information in unicellular organisms. J. Theor. Biol., 47, 439–481.
F r a n k e l J. 1979: An analysis of cell-surface patterning in *Tetrahymena*. In:

Determinants of Spatial Organization. (eds. S u b t e l n y S. and K o n i g s-
b e r g I. R.), Academic Press, 215–246.

F r a n k e l J. and H e c k m a n n K. 1968: A simplified Chatton-Lwoff silver
impregnation procedure for use in experimental studies with ciliates. Trans.
Am. Microsc. Soc., 87, 317–321.

G r a i n J. 1969: Le cinétosome et se dérivés chez les ciliés. Ann. Biol., 8, 53–97.

H a u s m a n n K. and A l l e n R. D. 1977: Membranes and microtubules of the
excretory apparatus of *Paramecium caudatum*. Cytologie, 15, 303–320.

H e c k m a n n K. and F r a n k e l J. 1968: Genic control of cortical pattern in
Euplotes. J. Exp. Zool., 168, 11–38.

J e r k a - D z i a d o s z M. 1980 a: Control of pattern formation in ciliated *Pro-
tozoa*. In: Cell Compartmentation and Metabolic Channeling, (eds. N o v e r
L, L y n n e n F. and M o t h e s K.), Veb. G. Fischer Verlag, Jena, 495–502.

J e r k a - D z i a d o s z M. 1980 b: Ultrastructural studies of cortical pattern in
janus mutant of *Tetrahymena thermophila* with an inverted morphogenetic
field. Acta Protozool., (in press).

J e r k a - D z i a d o s z M. and F r a n k e l J. 1979: A mutant of *Tetrahymena
thermophila* with partial mirror image duplication of cell surface pattern.
I. Analysis of phenotype. J. Embryol. Exp. Morph., 49, 167–202.

K a c z a n o w s k a J. 1975: Shape and pattern regulation in regenerants of
Chilodonella cucullus (O.F.M.). Acta Protozool., 13, 343–360.

K a c z a n o w s k i A., R a d z i k o w s k i S., M a l e j c z y k J. and P o l a k o w-
s k i I. 1980: Study on intraclonal pairing in *Chilodonella steini*. Evidence
of abortive conjugation. J. Exp. Zool., (in press).

L o m J. and C o r l i s s J. O. 1971: Morphogenesis and cortical ultrastructure
of *Brooklynella hostilis*, a dysteriid ciliate ectoparasitic on marine fishes.
J. Protozool., 18, 261–281.

M c K a n n a J. A. 1976: Fine structure of fluid segregation organelles of *Para-
mecium*. J. Protozool., 20, 631–638.

M c K a n n a J. A. 1976: Fine strucure of fluid segregation organelles of *Para-
mecium* contractile vacuoles. J. Ultrastruct. Res., 54, 1–10.

N g S. F. 1977: Analysis of contractile vacuole pore morphogenesis in *Tetra-
hymena pyriformis* by 180° rotation of ciliary meridians. J. Cell Sci., 25,
235–246.

N g S. F. 1979: The precise site of origin of the contractile vacuole pore in
Tetrahymena and its morphogenetic implications. Acta Protozool., 18, 305–
312.

N g S. F. and F r a n k e l J. 1977: 180°-rotation of ciliary rows and its morpho-
genetical implications in *Tetrahymena pyriformis*. Proc. natn. Acad. Sci.
USA, 74, 1115–1119.

P a t t e r s o n D. J. 1978: Membranous sacs associated with cilia of *Paramecium*.
Cytobiologie, 17, 107–113.

P a t t e r s o n D. J. 1980: Contractile vacuoles and associated structures: their
organization and function. Biol. Rev., 55, 1–46.

P a t t e r s o n D. J. and S l e i g h N. A. 1976: Behavior of the contractile vacuole
of *Tetrahymena pyriformis*: A redescription with comments on the termi-
nology. J. Protozool., 23, 410–417.

P y n e C. K. and T u f f r a u N. 1970: Structure et ultrastructure de l'appareil
cytopharyngien et des tubules complexes en relation celui-ci chez le cilié
gymnostome *Chilodonella uncinata* Ehrbg. J. Microsc., 9, 505–518.

R a d z i k o w s k i S. and G o ł e m b i e w s k a M. 1977: *Chilodonella steini*. Mor-
phology and culture method. Protistologica, 13, 381–389.

S o ł t y ń s k a M. S. 1971: Morphology and fine structure of *Chilodonella cucul-
lulus* (O.F.M.) cortex and cytopharyngeal apparatus. Acta Protozool., 9,
49–82.

S o n n e b o r n T. M. 1963: Does preformed cell structure play an essential role
in cell heredity? In: The Nature of Biological Diversity. McGraw-Hill, New
York, 165–221.

EXPLANATIONS OF PLATES I–III

2: A longitudinal section of the somatic kinetosome and adjacent cortex of *Ch. steini*. Postciliary microtubules (PC), submeridional microtubules (SKSH), parasomal sac (PS) and underlying membranous sac (MS) indicate the right side of the meridian and its direction. Other abbreviations as in Fig. 1. Magn. 48 000

3: A tangential section through the cortex with kinetosomes (KS) seen at the different levels. The upper edge of the figure corresponds to the anterior part of the meridian. The origin of the kinetodesm (KD) and postciliary microtubules (PC) and their course may be traced in the vicinity of the upper kinetosome. The left transverse microtubular complex (TF) is associated with a transverse electron-dense spur and is seen in the lower sections of the level of cilia. Magn. 48 000

4: A very early stage of CVP primordium formation. A slight indentation of alveoles adhering to a kinetosome (arrow) marks formation of the CVP orifice. In the subcortical layer structures reminiscent of decorated fluid segregating tubules (FST?), with some transverse sections across forming canals (CC) are seen. Other abbreviations as before. Magn. 48 000

5: Grazing sections through a very early stage of the CVP primordium. The small anterior orifice of the pouch is localized in close proximity of adjacent kinetosome. The sparse fibrils (arrow) are seen embedded in electron-dense material and some filamentous trails (MF) lie below the epiplasm. Magn. 60 000

6: A tangential and slightly oblique section of an early primordium of the CVP, still localized in the vicinity of the meridian. Very small alveole (ALV) is seen. Numerous microtubules are seen anchored at the rim of the CVP orifice. Magn. 36 000

7: An oblique section of a CVP primordium at the proximal level of the pouch. Some scanty microtubules are embedded in the electron-dense material (arrow). The lumen of the pouch is still in the neighbourhood of a kinetosome, but it extends across the intermeridional space. Magn. 27 000

8: A transverse section through a rounding young CVP. Insertion of helical microtubules (HM) and bundles of radiating microtubules (RM) are observed. Magn. 36 000

9: A transverse section through the CVP at the level of its orifice. Tight spiralization of helical microtubules (HM) and their insertions on the outer membrane of the CVP lumen are seen (arrow). An outer rim of electron-dense material separates the CVP structures from the adhering alveoles. Cross section of adjacent cilia reveals direction of dynein arms marking the orientation of the section. Magn. 48 000

10: A longitudinal section through a young CVP. Small alveole (arrow ALV) separate the CVP structure from the nearest kinetosome. The oblique angle of section through the helical microtubules marks the tight spiralization of the microtubules along the tube. Beneath the CVP tube there is formed a contractile vacuole (CV). Magn. 36 000

11: Section through the diaphragm (D) separating the lumen of the CVP tube from the contractile vacuole sac. Magn. 36 000

12: Another section through the diaphragm (D) embedded into the contractile vacuole. Radiating microtubules (RM) spread from the CVP wall. Magn. 36 000

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PLATE III

. Kaczanowska et J. Moraczewski
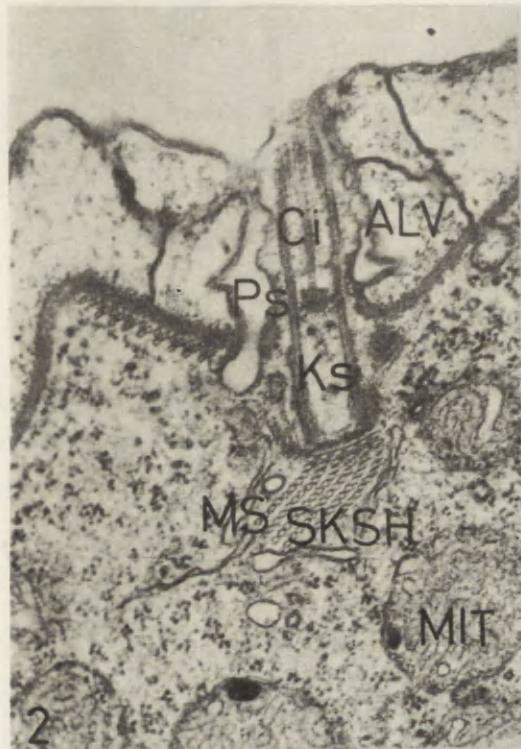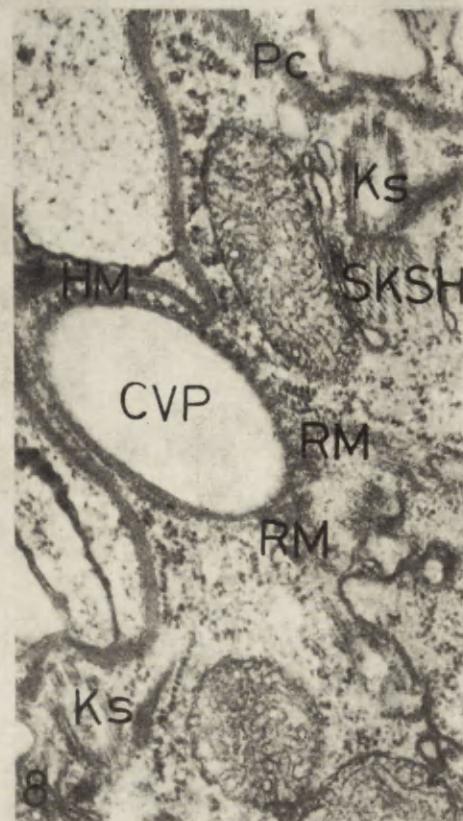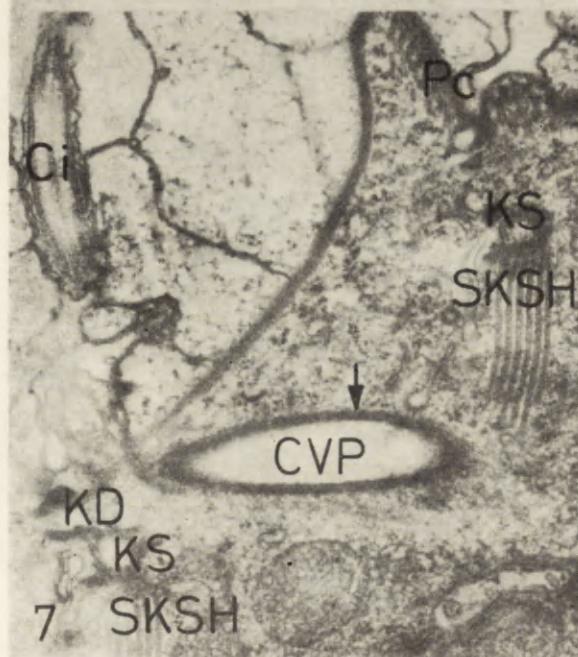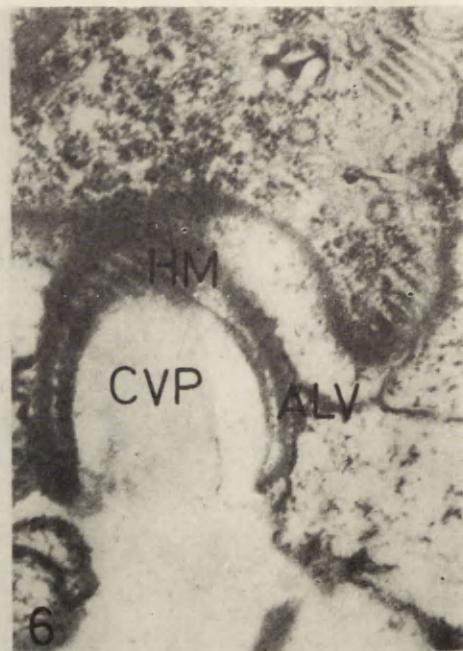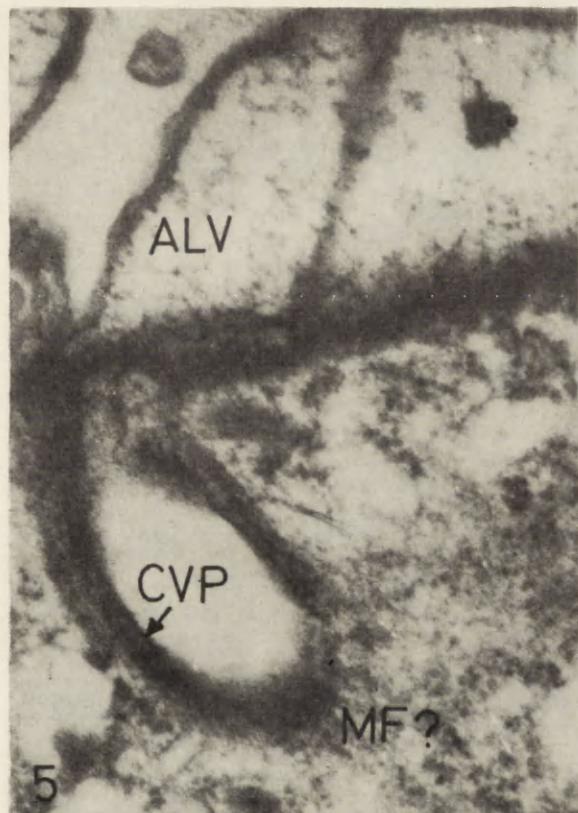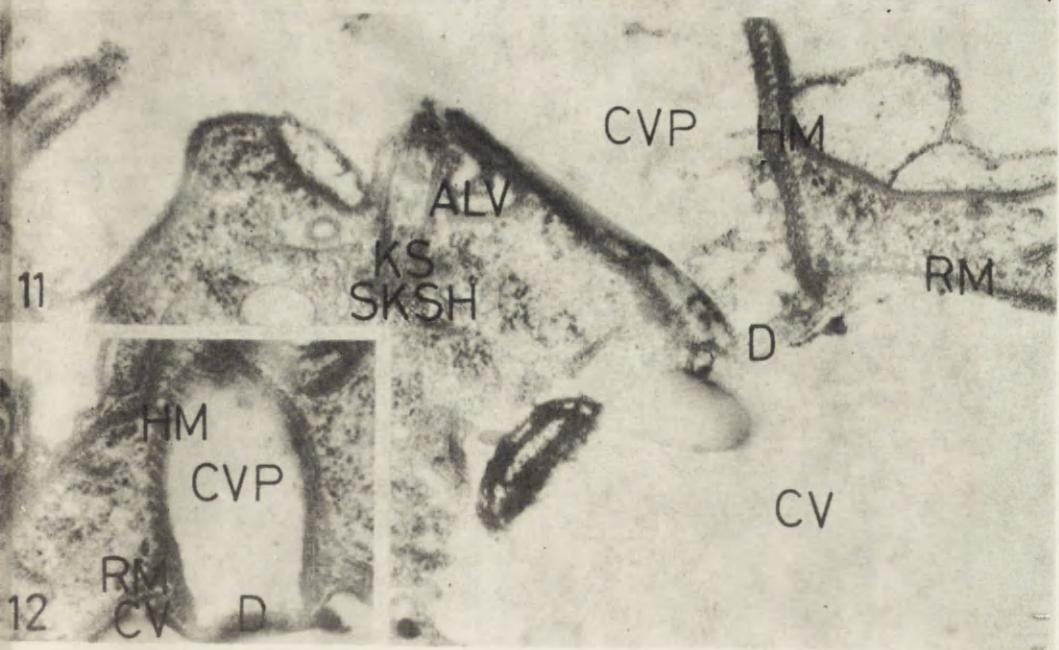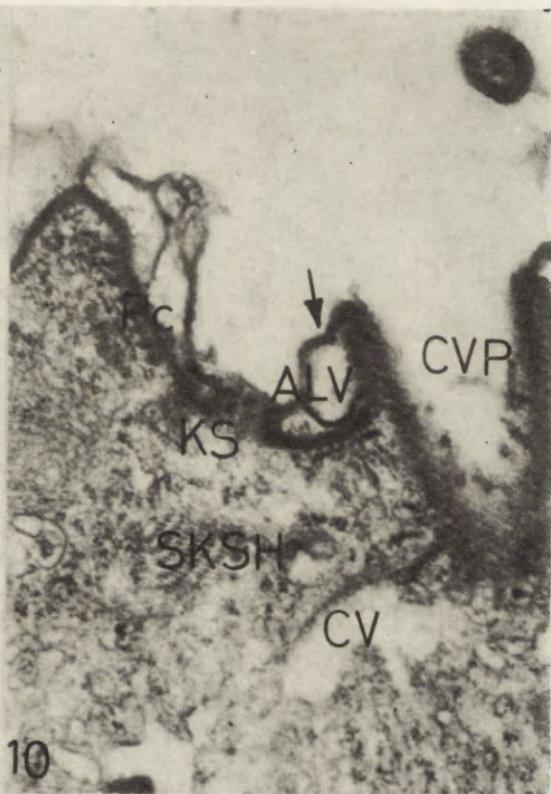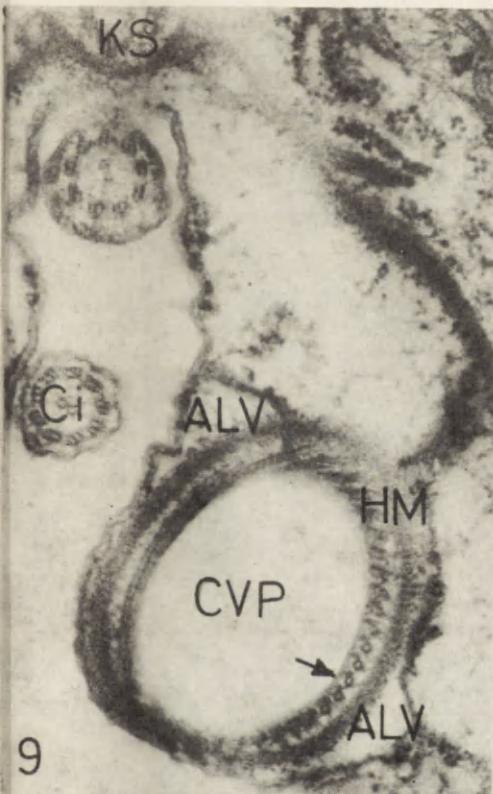
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Generative Nucleus Control over Cell Vegetative Functions in *Paramecium*

Synopsis. An investigation has been carried on clones of *Paramecium caudatum, P. bursaria, P. putrinum, P. woodruffi* and *P. jenningsi* in which the micronuclei were removed by UV microbeam. It has been shown that beside the activity of the macronucleus, in the normal vital activity of ciliates during the vegetative phase of life the micronucleus plays also an important role. On the ground of these investigations the authors set up a hypothesis on the role of functional activity of generative nuclei in ciliates. It is stated that execution of a complete range of vegetative functions controlled by the nuclear apparatus involves not only the transcription activity of MA but also some activity of MI genome. Various aspects of this hypothesis are discussed with literature data.

Ciliates have a fairly peculiar cell organization which makes them the focus of studies for biologists of different specialities. Nuclear duality is one of such peculiarities. It consists in the presence in the cell of nuclei that display morphological and functional differences. These differences appeared within *Ciliophora* and underwent a number of considerable phylogenetic changes (R a i k o v 1967, 1978). The cell of higher ciliates features a polygenomatic somatic nucleus, macronucleus (MA), whose DNA is markedly capable of transcription, and a diploid or low poliploid generative nucleus, micronucleus (MI), which is unable to synthesize RNA in any significant quantity during the vegetative period of the organism's life.

Up to now, investigations on nuclear duality have given preference to the study of MA, its structure, functions and evolutionary changes (R a i k o v 1967, 1968, 1976, 1978; R a i k o v and A m m e r m a n n 1976). MI has been neglected. It is a widespread belief that the nuclei

of higher ciliates display a highly differentiated control over somatic
and generative functions. The macronucleus ensures the cell life func-
tions due to an extremely high transcription activity during the ve-
getative period of life cycle, whereas the micronucleus is responsible
for the nuclear apparatus reorganization during the sexual process
(G r e l l 1973, R a i k o v 1978). In other words, the ciliate MI is com-
monly believed to be a nucleus completely functionally inert in the
vegetative life of the cell. It is also stated that the MI genome may
be used as a promising model of an inactive nucleus, though the re-
sults of some investigations suggest that such conclusions are prema-
ture (B o r c h s e n i u s 1975, S a l v a n o 1975).

Full understanding is extremely important here, for some ciliates are
used in increasing frequency as model objects for resolving a number
of karyological and cytogenetic problems. Thus, it is obvious that all
evidence concerning the MI functional activity becomes a matter of
particular interest to scientists.

Specific Methods of Revealing the Functional Activity of Nuclei in the Cells Having a Heteromorphic Nuclei

Apparatus

Experimental studies on the MI genome activity make use of a great
variety of methods. These are methods of quantitative cytochemi-
stry, biochemistry and autoradiography which make it possible to de-
termine the intensity of RNA synthesis in MI (G o r o v s k y 1973), com-
parative electron microscopy of MA and MI ultrastructure (R a i k o v
1978), a genetic method involving the formation of cell lines-heteroka-
ryons of ciliates in which MA and MI are distinguished by particular
genes and the phenotypic effect of alleles can be intimately controlled
(S o n n e b o r n 1966, 1974 a, b, A l l e n and G i b s o n 1973), purpo-
seful generation of amicronuclear (MI⁻) ciliates owing to a general or
local action of chemical and physical factors on the cell to be followed
by analysis of cell function changes associated with the loss of MI
(T a y l o r and F a r b e r 1924, K i m b a l l 1941, O s s i p o v 1966,
F r i c k 1967, A m m e r m a n n 1970).

A critical study of each of these methods as regards their reliabi-
lity allows us to state (though we cannot adduce here all our arguments
for the sake of brevity and lack of space) that many of them, in fact,
either have an insufficient resolution or fail to provide an unequivocal
answer to the problem of MI functional activity. It should be noted
that the evaluation of nuclear activity in uninuclear *Protozoa* and in

metazoan cells is fairly simple. But ciliates with their nuclear duality require a technique of high s e l e c t i v i t y and precise l o c a l i z a- t i o n of its action.

One of the most promising methods of nuclear function analysis involves a special kind of their blocking, namely inactivation (A s t a- u r o v 1974). In its most general form, the method consists in eliminating the nuclei from usual participation in the control and genetic deter- mination of cell functions. Among the techniques of cell inactivation noted for precision of localization and selectivity of their action, we would single out the method of ultraviolet (UV) micro-irradiation (A m m e r m a n n 1970, F o k i n and O s s i p o v 1975), micrurgy (T a y l o r and F a r b e r 1924, T a r t a r 1964, F u j i s h i m a and H i- w a t a s h i 1978) and the symbiotic method (O s s i p o v et al. 1976, S k o b l o et al. 1978). Alongside these methods it is by all means ex- pedient to use autoradiography for determining the presence of RNA synthesis in MI. But the conclusions concerning the MI activity based on autoradiography should be considered with great caution. This re- serve is necessitated, first of all, by the possibility of transport of some RNA forms (e.g., shuttle RNA) from one nucleus of the cell to the other as has been proved for heterokaryons of amoebae and hybrids of somatic cells in multicellular organisms (H a r r i s 1970, G o l d s t e i n 1974, 1975, Y u d i n 1977). Though no such data have been obtained for ciliates, one cannot rule out *a priori* the possibility of transport of some RNA forms between the MA and the MI. The mere fact of the presence of RNA in MI is not yet a proof of RNA synthesis *in situ* indicative of a transcription activity in the generative nucleus genome.

An argument often resorted to in discussing the distribution of func- tions between MA and MI is based on a well documented absence of nucleoli or any similar structures in MI during the vegetative period (R a i k o v and A m m e r m a n n 1976, R a i k o v 1978). But this fact shows merely that there is no intense synthesis of ribosomal RNA in MI leaving fully open the question whether other forms of RNA can be synthesized in MI.

The above considerations allow us to choose what we believe to be the most efficient approach to the study of MI significance in the cell vegetative life. It consists in obtaining some MI⁻ ciliates from normal strains by means of local selective actions to be followed by analysing the cell function changes due to the loss of generative nucleus. Using this approach we may hope to evaluate the role of MI in the life of cell at its phenotypic level. Information concerning the influence of the generative nucleus loss on the vitality of ciliates has been available

since long ago (C a l k i n s 1911, 1912, D a w s o n 1920, W o o d r u f f 1920, 1921, M o o r e 1922, 1924, R e y n o l d s 1932, B e e r s 1946).

The results of studies on the viability of MI⁻ cells as compared with the initially normal clones of some ciliates suggested a certain significance of MI during the vegetative period of life cycle. Thus, T a y l o r and F a r b e r (1924) studied the effect of MI micrurgical removal on the viability of *Euplotes patella*. The removal of generative nucleus resulted in the animal's death five days after the operation, while the removal of a small part of cytoplasm or a fragment of MA had no effect on the viability of the specimens operated. It is interesting that, once the removed MI was re-transplanted into the cell cytoplasm, the specimen produced viable progeny. The same pattern was confirmed by K i m b a l l (1941) in his studies on the viability of MI⁻ *E. patella* where he applied merotomy to initially normal and doublet cells.

Different experimental methods of MI removal from vegetative cells of *Paramecium caudatum* used by various authors show that the MI⁻ clones appear invariably less viable and die out after a limited number of cell divisions (L e w i n 1910, S c h w a r t z 1934, M i y a k e 1956, T a r t a r 1964, O s s i p o v 1966). A lower division rate associated with the spontaneous loss of MI has only been recorded in *P. bursaria* (S c h w a r t z 1947, G o l i k o v a 1978), *P. multimicronucleatum* (W i c h t e r m a n 1959), *P. aurelia* (Ruiz et al. 1976).

Attesting to the significance of MI in the ciliate vegetative life is the evidence of the cell viability after the induced loss of MI in *Stylonychia mytilus*. No matter what method of denucleation, micrurgy or β-irradiation, all the operated cells that had lost their MI inevitably died (F r i c k 1967).

Very interesting results were obtained by W e l l s (1961) who investigated the viability of *Tetrahymena pyriformis* clones following a general γ-irradiation of the cell. It appeared that in spite of frequent appearance of MI⁻ cells in the irradiated cultures, the formation of MI⁻ clones was extremely rare and all of them featured no vitality. According to the author, such a short life of MI⁻ ciliates is due to the exhaustion of the stock of potential metabolic products synthesized prior to irradiation.

A m m e r m a n n (1970) observed anomalies in cell morphogenesis among the MI⁻ *S. mytilus* and the death of anomalous cells. The loss of MI after the irradiation resulted in the death of most ciliates; however, part of the MI clones proved to be viable and according to the author this should be accounted for by the compensation of MI loss due to the appearance of small fragments of the MA Feulgen-positive structures functionally resembling MI. Such bodies, called pseudomicro-

nuclei (pseudo-MI), greatly exceeded the usual number of generative nuclei in the cell. Their appearance was associated with the restoration of a normal division rate in the progeny of irradiated ciliates. The disappearance of pseudo-MI as a result of a repeated irradiation was associated with a sharp decrease in the division rate and a prompt death of ciliates. Thus, A m m e r m a n n's results are also in favour of the significance of MI in the vegetative life of *Stylonychia*. The very fact of the pseudo-MI appearance after the loss of generative nucleus may suggest the necessity of some kind of the MI functional activity during the vegetative period of ciliate life cycle. It is of interest that the appearance in the MI^- cell cytoplasm of such structures as resemble a defective MI was also reported for *P. caudatum* and *P. bursaria* (L e D a n t e c 1897, S c h w a r t z 1958, G o l i k o v a 1978).

Many ciliates, however, are known to have, both in nature and in the laboratory conditions, viable MI^- lines. A possible explanation of this phenomenon in the light of the data on the MI activity will be given in the end of this article.

Method of MI Selective Inactivation by Means of Local UV Microirradiation

The data discussed in the previous section outline the problems of MI functional activity in the cell vegetative life still to be solved and reveal some of the causes of the existing contradictions. Up to now, studies in this field have been executed on ciliates fairly far apart systematically, with the methods being not always adequate to the task. This accounts for the inability, to get any definite solution concerning the significance of MI. That is why we believed it extremely expedient to conduct a comparative study of the generative nucleus role during the cell vegetative life in systematically related ciliates with the use of a uniform method adequate to the task. The UV-irradiation was chosen for the purpose (F o k i n and O s s i p o v 1975). The present paper sums up the results of the first stage of investigations on the MI functional significance by means of selective inactivation of MI in *Paramecium*.

T h e m e t h o d o f U V i r r a d i a t i o n makes it possible, in contrast with numerous other techniques, not only to damage (inactivate) particulae organelles and living cell elements but also to observe the consequence of the operation on the same cell or on its remote progeny. In our investigations, the UV light-waves within range 240–280 nm (with a maximum of 260 nm) were used to irradiate individual elements of *Paramecium* nuclear apparatus on a Carl Zeiss Jena device based on a Nf microscope. The basic scheme of the experiments (Fig. 1) together

with all the manipulations on the cells after irradiation was described earlier (F o k i n and O s s i p o v 1975). The irradiance actinometrically measured (C a l v e r t and R e c h e n 1952) was 17 ± 5 erg/mm² s. The time of illumination in the basic experiments, ranged from 180 to 300 s. The UV probe ares was 100 μm. Control experiments involved illumination with a similar probe of a fragment of MA or cytoplasm having no cytopharynx or contractile vacuoles. To understand the influence of photoproducts that appear due to a UV microbeam irradiation on the viability of operated cells and on the morphology of their nuclear apparatus, we also illuminated the part of cytoplasm immediately adjacent to MI. Cells of non-synchronized cultures were used.
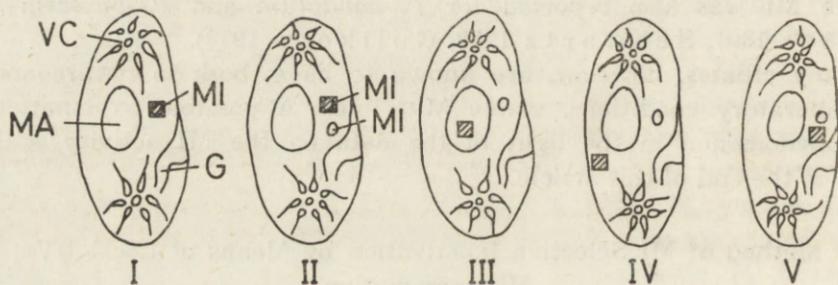

Fig. 1. Basic variants of experiments on local microbeam UV irradiation of cell components in *Paramecium*

Legend: I — irradiation of the only MI in the cell; II — irradiation of one of the two MI in the cell; III — irradiation of an MA fragment of the same area; IV — irradiation of a cytoplasm fragment of the same area; V — irradiation of a cytoplasm fragment immediately adjoining MI. G — cytopharynx; MA — macronucleus; MI — micronucleus; VC — contractile vacuole. The cross-hatched square marks the UV beam area, the size of which has been enlarged for the sake of clarity

The operated specimens of *Paramecium* and their progeny were studied for the effect of MI loss on the viability, division rate and morphology of nuclear apparatus. Such cells were considered viable which produced subclones that would live intensively for many months to come. Regarded as inviable were both the cells that died right after the irradiation and those in which the subclones displayed a reduced division rate and eventually died as well. Those cultures did not die all the same time.

For the nuclear apparatus study the cells were fixed with Bouin's and Carnouat's fluid. Staining required for DNA detection was executed after a revised Feulgen technique suggested by De Lamateur (S o n n e-b o r n 1950). In a few cases, some preparations were at the same time stained with rivanol-SO₂ (H a c h a t u r o v and S m i r n o v a 1966) and with azure after Romanowski.

A Comparative Study of the Effect of MI Removal
in Five Species of *Paramecium*

The choice of the subjects was determined, on one hand, by a good knowledge of the genus *Paramecium* as far as its morphological and functional features are concerned (W i c h t e r m a n 1953, B o m f o r d 1965, J a n k o w s k i 1972, S o n n e b o r n 1974 a, V a n W a g t e n d o n k 1974). On the other hand, this choice was suggested by the fact that, being a fairly distinct group of species, *Paramecium* varies in its MI organization at the light microscopic level. In our investigations we were trying to use specimens from all the three subgenera (J a n k o w s k i 1969): *Helianter* — *P. putrinum*, *P. bursaria*; *Cypreostoma* — *P. woodruffi*; *Paramecium* — *P. caudatum*, *P. jenningsi*.

(1) *P. caudatum*. We have studied the effect of MI on the viability and cell division rate in two unimicronuclear clones, Be-1-4 and Be-5 (B o r c h s e n i u s and O s s i p o v 1978). A comparative study has also been carried out with a view of understanding the effect of MI irradiation on the cell viability in stable uni- and bimicronuclear clones, M-505-46 and M-505-19, that have genotypically identical MA (F o k i n and B o r c h s e n i u s 1978). In the course of experiments on the MI inactivation in the Be-1-4 and Be-5 cells, 217 specimens were irradiated (Table 1), with a line started from each operated cell. However, a viable progeny could be obtained from no more than 27 cells all of which started lines containing MI. These often differed morphologically from the MI of a "wild" type. The progeny of all the other irradiated cells usually died out several days after a sharp reduction in their division rate. A comparison between the cloning of the operated cells' progeny and the MI distribution in the progeny of the first and second post-irradiation cell division showed that all the MI$^-$ cells thus produced are inviable (Table 2).

The experiments on a local UV irradiation of generative nucleus in the cells of M-505-46 and M-505-19 yielded quite different results. Irradiation of MI (n = 56) in the unimicronuclear cells of the clone M-505-46 resulted in the following (Table 1). The operated cells produced 53 UV subclones, 45 (80 per cent) of which appeared to be inviable and MI$^-$. The generative nuclei of *Paramecium* of these lines underwent morphological changes possibly due to the loss of part of DNA (cf. O s s i p o v and B o r c h s e n i u s 1973). Figure 2 (curve 1 a) shows the relationship between the percentage of the dead MI$^-$ UV subclones and the time that passed after the irradiation of parental cells.

Different consequences were reported for irradiating one of the two MI's in the cells of the clone M-505-19. The operated *Paramecium* (35)

Table 1

The Results of Local Microbeam UV Irradiation of Generative Nucleus in Vegetative Cells of Five Species of the Genus *Paramecium*

Species	Clone	Number of irradiated cells	Dose erg/mm²	Number of UV subclones obtained				Authors
				Total	Unimicronuclear	Amicronuclear inviable (percentage of irradiated subclones)	Amicronuclear viable (percentage of irradiated subclones)	
P. caudatum	Be-1-4	59	1020	59	7	52 (88%)	—	Borchsenius and Ossipov 1978
	Be-1-4	111	5100	111	4	107 (96%)	—	
	Be-5	21	1020	21	10	11 (52%)	—	
	Be-5	26	5100	26	6	20 (77%)	—	
	M-505-46	56	5100	53	8	45 (80%)	—	Fokin and Borchsenius 1978
	M-505-19*	35	5100	33	25	8 (23%)	—	
P. jenningsi	K-72-UV-3	37	3060	34	5	29 (78%)	—	Data unpublished earlier
	K-72*	35	3060	32	30	—	—	
P. putrinum	S-6-1	60	3060	60	9	51 (85%)	—	Fokin 1978 a
	L-2-1	35	3060	33	3	30 (86%)	—	
	K-177	27	3060	26	8	11 (42%)	7 (27%)	
P. bursaria	T-2	184	3060	182	47	32 (17%)	103 (56%)	Fokin 1979 a
	T-4	52	3060	51	10	12 (23%)	29 (56%)	
P. woodruffi	OK-3-5UV-14	48	3060	48	3	45 (94%)	—	Data unpublished earlier
	OK-3-5*	46	3060	43	43	—	—	

* Note: The UV probe area is 100 um²; irradiation of a cytoplasm fragment of the same area involved no morphological changes of nuclear apparatus; * bimicronuclear clones

Table 2

The Nuclear Apparatus of Progeny of the First and Second Post-Irradiation
Divisions in Cells of *Paramecium caudatum*

Clone	Progeny of Division I			Progeny of Division II			Authors
	Presence of MI		Number of pairs	Presence of MI		Number of tetrads	
	MI⁻	MI⁺		MI⁻	MI⁺		
Be-1-4	0	2	0	0	4	1	Borchsenius
	1	1	12	1	3	1	and Ossipov
	2	0	2	2	2	6	1978
		Total	14	3	1	6	
				3	1 (2MI⁺)	2	
				4	0	0	
					Total	16	
M-505-46	0	2	0	0	4	0	Fokin and
	1	1	16	1	3	0	Borchsenius
	2	0	8	2	2	0	1978
		Total	24	3	1	7	
				4	0	8	
					Total	15	

The part of perish UV treated-subclones (%)
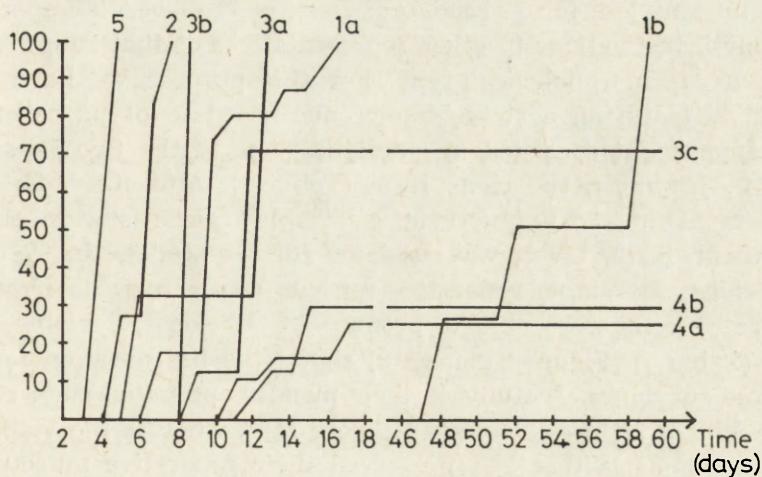
Fig. 2. The longevity of amicronuclear subclones obtained after the UV irradia-
tion of generative nucleus in the vegetative cells of five ciliate species of the
genus *Paramecium* (Fokin 1978 b)

Legend: 1 a, b — *P. caudatum*; 2 — *P. jenningsi*; 3 a, b, c — *P. putrinum*,
4 a, b — *P. bursaria*, 5 — *P. woodruffi*. Irradiation dose 5100 erg/mm² (1 a, b);
3060 erg/mm² (2–5)

produced 33 UV subclones (Table 1), only 8 (23 per cent) of which proved to be inviable. The cells of these eight subclones did not display a single generative nucleus. All cells of inviable cultures died out during 1.5 to 2 months after the parental cells' irradiation (Fig. 2, curve 1 b). An analysis of nuclear apparatus in the cells of 25 viable UV subclones allowed us to identify 23 of them as unimicronuclear.

Thus, the inviability caused by a UV microbeam is displayed differently in the cultures under study: the unimicronuclear cells die much earlier after the loss of MI than the bimicronuclear ones (Fig. 2). But in both cases the loss of MI results inevitably in the death of paramecia following a sharp decrease in the division rate. On the contrary, the cell division rate in the viable UV subclones is the same as in the control experiment.

Study of the nucleus apparatus in the progeny of the first two post-irradiation divisions of M-505-19 and M-505-46 cells showed that, unlike the clones Be-1-4 and Be-5, the irradiated nucleus mostly degraded and fully disappeared during that period (Table 2). The irradiation of part of MA (n = 10) or cytoplasm (n = 15) in an immediate proximity of MI affected neither the viability of the cells nor the morphology of their nuclear apparatus (Table 1).

(2) *P. jenningsi*. As it is known, the subgenus *Paramecium* embraces several ciliate species varying in the morphological type of MI (J a n-k o w s k i 1969). Therefore, having studied the consequence of the generative nucleus loss for *P. caudatum* cells, we believed it important to extend our investigations to other representatives of that subgenus with a different MI morphological type. For this purpose we have chosen *P. jenningsi* featuring a stable bimicronuclear state of its cells.

The experiment consisted in irradiating one of the two MI's in the cells of *P. jenningsi*, the clone K-72 (Table 1). Altogether, 35 ciliates were operated and 30 unimicronuclear viable subclones were obtained. One of them, K-72, UV-3, was used for further studies. In 37 cells of that subclone, the single generative nucleus was subject to irradiation. The operated cells were able to produce 34 subclones, 5 viable and 29 inviable (Table 1). Figure 2 (curve 2) shows the life duration for those 29 inviable subclones. A study of their nuclear apparatus state revealed that the cells of all inviable cultures were MI⁻, whereas the viable ones contained paramecia that had preserved their generative nucleus. Control irradiation of parts of MA (n = 10) or cytoplasm (n = 10) displayed neither any changes in the nuclear apparatus morphology nor a decrease in viability. The results obtained in the experiments on *P. jenningsi* reveal a distinct correlation between the inviability due to a UV

microbeam irradiation and the lack of the generative nucleus in the cell.

(3) *P. putrinum.* According to J a n k o w s k i (1969), this species is the most primitive among the paramecia of the subgenus *Helianter* featuring an unusual type of MI organization. In the course of the experiments on the generative nucleus inactivation we irradiated 146 cells of three clones *P. putrinum* (F o k i n 1978 a, b): 60 ciliates of the clone C-6-1, 35 of the clone L-2-1 and 51 of the clone K-177 (Table 1) [1]. The results show that the MI⁻ UV subclones thus derived usually appear inviable and die 9 to 13 days later (Fig. 2, curves 3 a, b, c). At the same time, the cells that preserve their MI after the irradiation divide normally and give rise to viable subclones. Their generative nuclei, however, are morphologically different from the MA of a "wild" type and respond less intensively to staining after Feulgen. We managed to show for three UV subclones with a modified MI of the clone K-177 that the irradiated generative nucleus may become defective and disappear some time later (after cca. 30 days).

It should be noted that seven MI⁻ subclones of *P. putrinum* obtained from the cells of the clone K-177 still proved to be viable. It is remarkable that the nuclear apparatus of those particular cultures displays a unique MA f r a g m e n t a t i o n and an aberrant division of the somatic nucleus. The MA fragmentation was not recorded until the macronuclei were irradiated; neither has it ever been observed in the cells of control series (irradiation of parts of MA or cytoplasm). These facts are suggestive of an interaction between the irradiated MI and intact MA (F o k i n 1979 a).

The second investigation (2 months later) of the state of the nuclear apparatus in the three subclones featuring the unusual MA fragmentation showed a complete termination of that process. It is interesting that the cytoplasm in the cells of these cultures (55 to 70 per cent) contained, besides MA, a great number (up to 20) of Feulgen-positive bodies. These are probably formations of pseudo-MI type (A m m e r-m a n n 1970), but we failed to prove their functional significance.

Thus, the data obtained are indicative of a dynamic interaction between MA and MI during the vegetative period of life of *P. putrinum.* The results of the viability studies conducted on the MI⁻ UV subclones of that species substantiate a conclusion as to the importance of generative nucleus in the cell vegetative life. The loss of MI involves either a lethal outcome or preservation of viability associated with some process of MA reorganization.

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[1] The Table contains only such cells as received 3060 erg/mm².

(4) *P. bursaria.* The experiments on irradiation of MA in the cells of another primitive *Paramecium, P. bursaria,* revealed, as compared with the species studies earlier, an unusually high viability of experimentally obtained MI⁻ subclones (F o k i n 1979 b). Submitted to irradiation were 236 cells of two clones: 184 cells of the clone T-2 and 52 cells of the clone T-4 (Table 1). The operated cells produced 176 MI⁻ subclones and 57 subclones with a modified MI. In contrast to the above three species of *Paramecium, P. bursaria* had no more than 44 inviable MI⁻ lines that died out some 12 to 17 days after the irradiation of parental cell (Fig. 2, curve 4 a, b). Most of the MI⁻ UV subclones, along with the lines with a modified generative nucleus, remained viable during almost 1.5 years of observation. It is significant that, among the MI⁻ UV subclones of *P. bursaria,* some cultures contained cells with a fragmented MA.

Thus, the results of our experiments show that the vegetative cells of *P. bursaria* display a slightly different response to the loss of their generative nuclei after irradiation than other species of *Paramecium.*

(5) *P. woodruffi.* The results of investigations as described above allowed us to establish the functional significance of MI in the vegetative cells of ciliates that belong to the subgenera *Helianter* and *Paramecium.* Similar investigations were conducted on *P. woodruffi,* a species of *Paramecium* from the subgenus *Cypreostoma.* The experiments involved a stable bimicronuclear clone OK-3-5. On irradiating one of the two MI's in the cells of that clone (n = 46) 43 viable unimicronuclear UV subclones were obtained (Table 1). Two subclones preserved both generative nuclei. Then the single MI of the cells in the subclone OK-3-5 UV-14 was exposed to irradiation: 48 operated cells produced subclones of which only three appeared viable. A study of the nuclear apparatus in the viable cultures revealed that their MI had been preserved. The remaining 45 out of 38 UV subclones were found to be MI⁻ and inviable. The operated cells went through no more than 4 to 5 divisions, while their progeny ceased dividing 4 or 5 days later and eventually died. The percentage of the dead cultures as a function of the time elapsed after irradiation is shown in Fig. 2 (curve 5). Thus, the MI-less cells may reproduce for a short time, but than suffer inevitable death. These facts are convincing evidence of the functional significance of generative nuclei in the vegetative life of *P. woodruffi* cells.

A Hypothesis of the MI Functional Activity

The results of experiments on the generative nucleus irradiation in the agamic cells of five species of *Paramecium* — with the use of UV microirradiation as a single method — allowed, for the first time, a com-

parative analysis of the MI functional role. It should be noted that the conditions of inactivation (removal) of MI were identical in all the ciliate species under study. The MI local irradiation was executed with a UV beam of a constant area (100 µm) in doses of the same order (3060 and 5100 erg/mm²). Local irradiation of the single MI in the cells of ciliates of the genus *Paramecium* enables us to establish a lethal effect of the generative nucleus loss on most species of *Paramecium*. This fact points to the functional significance of MI in the ciliate vegetative life.

However, different species varied in their response to the loss of MI depending on what forms they represented, higher or lower. Higher forms were remarkable for almost simultaneous death of most MI⁻ UV subclones. At the same time, irradiation of one of the two MI's in the cells of initially bimicronuclear clones of *P. caudatum, P. jenningsi* and *P. woodruffi* usually resulted in the appearance of viable unimicro-nuclear cultures. The share of such subclones as produced by the ope-rated cells was 71, 79 and 83% for *P. caudatum, P. jenningsi* and *P. wo-odruffi* respectively. The ciliates of the subgenera *Helianter, P. bursaria* in particular, displayed an opposite ability to form a considerable number of MI⁻ viable subclones. But this, as will be shown later, does not necessarily involve a lack in MI of vegetative functions.

From the universally accepted viewpoint of molecular biology (K o-r o c h k i n 1978, W a t s o n 1978), it should be assumed that the func-tional activity of MI is accounted for by transcription of some RNA forms. In this connection, a delay in the death of cells in the MI⁻ lines may be due to the presence in the ciliate cytoplasm of a cell of hypo-thetical products of the MI gene activity. The viability of *Paramecium* after the destruction of MI owing to UV irradiation depends on how soon this stock gets exhausted.

The data on the functional significance of generative nucleus obtain-ed in our experiments suggest that in the viable UV subclones with MI of an altered morphology and a reduced amount of DNA, these nuclei account for the same cell vegetative functions as the "wild" MI. Neither the division rate nor the viability of the cells of such cultures are dif-ferent from the control specimens. The ability of the morphologically transformed MI of *P. caudatum, P. bursaria* and *P. putrinum* to fulfil their functions is associated primarily with the polyploid nature of ge-nerative nuclei in those ciliate species (C h e n 1940, D i l l e r 1940, J a n-k o w s k i 1972, O s s i p o v and B o r c h s e n i u s 1973, C u l l i s 1973, G o l i k o v a 1974). It will be recalled that we do not know yet what particular elementary vegetative functions are controlled by the MI ge-nome. Therefore, our major emphasis is put on the fact that such func-tions exist altogether. It will be the task of further investigations to

identify those individual elementary functions that the MI genome controls.

On the basis of local irradiating the generative nuclei in the ciliates of the genus *Paramecium* having MI's of various types, one may suggest that different organization of generative nuclei was of no special consequence for their functional differences. The death at least part of MI⁻ subclones obtained due to UV irradiation was reported for all the species studied.

Morphological transformations of somatic nucleus in response to the MI loss were more distinct in primitive *P. bursaria* and *P. putrinum*. The fragmentation of MA after local irradiation of micronuclei together with the existence in those ciliates of seemingly unusual viable MI⁻ subclones could be explained on the strength of an assumption that the heteromorphic nuclear apparatus of ciliates is an s i n g l e i n t e g r a t-e d, f u n c t i o n a l s y s t e m. In fact, if the ciliate nuclear apparatus is to be regarded as a system of related elements, MA and MI, the MA response to the damage of nuclear apparatus will appear quite logical.

Indirect evidence brought by some authors (S c h w a r t z 1958, A m-m e r m a n n 1970, A l l e n and W e r e m i u k 1971, W e i n d r u c h and D o e r d e r 1975) is indicative of a possible existence of interaction between nuclei in the ciliates. This does not rule out a functional change in the spectrum of active genes. Thus, the MI aneuploidy in a number of clones of *T. pyriformis* is associated with a diminished size of MA genome as compared with the genome of somatic nuclei in the normal lines (A l l e n and G i b s o n 1972). The same material demonstrated that the MI-less GL strains had extrachromosome copies of r-DNA with a smaller molecular weight than those of a normal strain (K a r r e r and G a l l 1976). In some ciliate species, an experimental elimination of MI involves morphological and functional changes in MA. Their major expression is the loss of ability to normal divisions of the vegetative nucleus (D i l l e r 1965, F r i c k 1967, A m m e r m a n n 1970, R u i z et al. 1976), together with occassional MA fragmentation (G o l i k o v a 1978). Finally, some experimental data indicate that there are also interactions of a regulatory nature between the cell micronuclei in *E. crassus* (L u p o r i n i and B r a c c h i 1977), *Homalozoon vermiculare* (S a x e-n a and S a x e n a 1978) and *P. caudatum* (F u j i s h i m a and H i w a-t a s h i 1978).

One of the principal statements of our hypothesis holds that during the cell vegetative life the genetic control functions are shared by MA and MI, being of a dynamic nature. Given this peculiarity of ciliate nuclear apparatus, one can understand why the MI loss does not always

result in a lethal outcome. A prolonged viability of MI⁻ cell lines may be accounted for by the changes in the MA genome differential activity. In the cases when such a redistribution of genetic control is possible, the MI⁻'s form quite viable lines, in spite of the loss of MI.

The evidence brought by other authors and our own data suggest that the MA fragmentation as observed in some cases may be a mor- phological expression of such a redistribution of genetic control over the functions at the light microscopic level. That process accounts for the spatial isolation of part of MA so that in the end the MA fragments become capable of replacing the generative nucleus functionally.

As shown earlier, the loss of MI derivatives during the sexual reor- ganization of nuclear apparatus causes the formation with a high fre- quency up to 65% of fully viable exautogamont MI⁻ cell lines in *P. caudatum* (O s s i p o v and S k o b l o 1968). Similar phenomenon has been also observed in 89% of conjugating pairs of *P. caudatum* between MI⁻ and normal ones (O s s i p o v and S k o b l o 1973). It's worth men- tioning that in both cases the formation of MA in the gamonts of MI cultures was due to the MA regeneration. Here, a definitive MA deve- loped from an isolated fragment of the old MA and not from the syn- karyon derivative as is the case under the normal conditions. A com- parison with the results of MI removal from the vegetative cells by means of a UV beam permits the suggestion that after the loss of MI by the vegetative cells, a reorganization of the MA genome differential activity is either impeded or cannot be completed before the organism's death. On the contrary, in exautogamont and exconjugants that have lost their MI, the MA reorganization process is completed with a high frequency, being probably facilitated, by the development of MA from a fragment of the old one. Thus, we can see that d i f f e r e n t s t a g e s o f t h e l i f e c y c l e v a r y a s t o t h e p r o b a b i l i t y o f t h e MI l o s s c o m p e n s a t i o n. Consequently, the formation of viable MI⁻ cell lines in *P. caudatum* after the sexual process does not contradict our assumptions concerning the MI activity. There is evidence that a spontaneous or induced loss of MI is more often lethal for older clones than for younger ones in *S. mytilus* (F r i c k 1967), *T. pyriformis* (W e i n d r u c h and D o e r d e r 1975), *P. bursaria* (G o l i k o v a 1978), *P. putrinum* (F o k i n 1978 a).

The experimental data obtained by us together with some of the above evidence of other authors indicate that a certain activity of MI is essential for the normal functioning of the cell during its vegetative life. Again, there is no indication that the vegetative functions are ex- clusively the responsibility of MA. We suggest that with the appear- ance, in the course of evolution, of a heteromorphic nuclear apparatus

system, the ciliate MI genome was given a unique chance not to synthesize some forms of RNA such as rRNA or other non-informational types. We have already discussed such technical problems as arise when one tries to discover RNA synthesis in MI. It is due to the MI functional significance that the MI⁻ cells which appear spontaneously in the population have a reduced selective value through their low division rate or display a complete inviability. Thus, the biological sense of MI functional activity is to be found in the preservation of generative nucleus as an active component of the nuclear apparatus throughout the agamic period of life-cycle in ciliates. Of an increased selective value are only those cell lines which preserve their generative cells during the entire agamic period.

The ciliate evolution includes probably other mechanisms aimed at the preservation of MI over a long succession of agamic generations. Among such morphological and functional adaptations one can mention those well-known from ciliate karyology (R a i k o v 1967, 1968, 1978). This is, first of all, the formation in the course MI mitotic division of an extremely large spindle accounting for the daughter nuclei movement toward the opposite poles of a dividing ciliate. Secondly, it is a widespread phenomenon of multiple generative nuclei in ciliates having a fairly large cell size; this, of course, reduces the probability of the appearance of MI⁻ cells. And thirdly, the preservation of MI is certainly facilitated by morphological contacts between the MA and MI membranes (T u c k e r 1967, I n a b a and K u d o 1972, G o l d e r 1976). It is believed that the reliability of MI preservation in Paramecium is achieved through a combination of various factors. In *P. caudatum*, *P. putrinum*, *P. bursaria* it is undoubtedly associated with the large size of MI division spindles and with polyploidy of that nucleus. In *P. woodruffi* and *P. jenningsi* a greater reliability of MI preservation is accounted for by an increase in the number of generative nuclei up to 2–4.

The evidence available indicates that the ciliate MI is an active component of an integrated system of cell nuclear apparatus. This may explain the reasons why different patterns of MI loss in a number of ciliate species do not have the same effect on the viability of MI⁻ cells. It is quite evident that each pattern of denucleation disturbs in its own way the system of interactions between the cell nuclei.

Discussing this problem on a comparative basis, one should bear in mind that the appearance and development of heteromorphism in ciliate nuclei as a pattern of functioning nuclear apparatus has evolved within that type of *Protozoa* (R a i k o v 1967, 1968, 1976, 1978). In different

taxonomic groups of *Ciliophora* an interaction between the elements of nuclear system has obviously been developed independently. So, one can suggest that MI of different groups of *Ciliophora* controls a different complex of functions. This explains why it is so important for a comparative study of MI functional significance to use a single method of investigations, adequate to the purpose.

While evaluating the results of manipulations with MI it should be remembered that some ciliate species display a considerable difference in the molecular size of MA and MI genomes. Thus, in *S. mytilus* the genome of a definitive MA is no more than 1.6% of the MI genome, while in *T. pyriformis* it is as great as 80 to 90% (M u r t i 1973, A m m e r m a n n et al. 1974). But it was *S. mytilus* that featured the appearance of pseudo-MI from the MA derivatives after the loss of generative nucleus. There is indirect evidence of reduction of the MA genome also in *P. bursaria* (S c h w a r t z and M e i s t e r 1975). This allows to a suggest that the MI genes, active during the vegetative life, are not eliminated in the course of formation of the definitive MA in those ciliates, thus accounting for the compensation of MI loss and the viability of MI⁻ cells.

The widely known data on frequent occurrence of MI⁻ in the "laboratory" cultures of numerous ciliate species (E l l i o t t 1973, B o r c h- s e n i u s 1975, B o r c h s e n i u s and O s s i p o v 1978) and on the high share of fully viable MI⁻ cell lines in the natural populations of such species as *T. pyriformis* (M c C o y 1976, N a n n e y and M c C o y 1976, N a n n e y 1977), seem inconsistent with our reasoning. Though the mechanism of appearance of MI⁻ cells in reported cases has never been established, those facts are usually used as a basic argument to substantiate an absolute functional inertness of MI in ciliates (A l l e n and G i b s o n 1973). We believe that the major fault of such arguments is regarding the nuclear apparatus of cell as a simple aggregate of fully independent heteromorphic nuclei and not as an integrated system.

Thus, the well-established fact of the existence in some ciliates of MI⁻ cell lines, cannot be, in our opinion, a valid proof of MI being functionally inert. There is a similar situation when one tries to evaluate the functions of organelles in a eukaryotic cell on the sole basis of their physical loss. The possibility to obtain mutants with an altered structural organization and functions of plastids, mitochondria and kinetoplasts or, else, a complete loss of those organelles does not imply the initial lack of their specific and extremely important functions (S a - g e r 1972, Y o s t 1972, M a r g u l i s 1976, K a l l i n i k o v a 1977). In fact, the cells having such mutational damages are capable of compen-

sating the functional defects in organelles and even their loss by using different metabolic means and energy sources.

Thus, our experimental results together with the data published by other authors permit us to put forward a hypothesis concerning the functional activity of generative nuclei. Its major statements are given below. Execution of a complete range of vegetative functions controlled by the nuclear apparatus involves not only the transcription activity of MA but also some activity of MI genome. In terms of contemporary molecular biology the MI functional activity should consist basically in the synthesis of specific RNA forms. Therefore, one should consider such evidence as points to the process of RNA synthesis in the generative nucleus to be the best proof of its functional activity. But the cell duality in ciliates interferes with an unequivocal interpretation of the presence of RNA synthesis *in situ* in MI. However, it should be noted that some authors have demonstrated the inclusion of traced precursors of RNA in MI (P a s t e r n a k 1967, R a o and P r e s c o t t 1967, A m-m e r m a n n 1970, G o r o v s k y 1973). But does it mean that the MI transcription activity thus observed is identical to that associated with the MA genome of the same cell? From the viewpoint of our hypothesis one can suggest that the transcription in MI is of qualitative specificity as compared with MA. What happens in MI is, probably, the synthesis of some may be not numerous, but unique forms. So far, we know of only one short contribution substantiating the above suggestion (P u m o and G o l d s t e i n 1977). That paper, devoted to the results of electrophoresis in polyacryloamide gel compares low molecular stable RNA's (sn RNA) isolated from MA and MI. Though it is quite obscure what part that fraction of RNA plays, structural or functional, one fact deserves consideration. The banding pattern of sn RNA appears to be distinct in both types of nuclei: each nucleus displays at least two sn RNA bands missing in the other, while the concentration of the remaining fractions is different in MA and MI.

The data obtained with the help of experimental methods involving inactivation (destruction) of nuclei demonstrate convincingly the functional significance of MI in a number of ciliates. The generative nucleus activity is a guarantee that during the reproduction process in a species only those cell lines will be mainly preserved which have not lost their generative nucleus. The range of MI functions may vary from one taxonomic group in *Ciliophora* to another. Our hypothesis allows a new approach to the cytogenetic and molecular biological data on the ciliate nuclear apparatus, since functional inertness of MI seems to be doubtful.

SUMMARY

By applying the method of a UV microbeam irradiation, the micronucleus (MI) was removed from the cells of five species of *Paramecium* (*P. caudatum, P. bursaria, P. putrinum, P. woodruffi, P. jenningsi*). The data obtained show that the normal vital activity of ciliates during their vegetative life cannot be controlled by intense transcription of macronucleus genome (MA) alone; the MI functional activity is also essential. This implies that MI is an active component of an integrated system of cell nuclear apparatus where interactions between nuclei play a special role. During the cell vegetative life the genetic control functions are shown by MA and MI and have a dynamic nature.

It is still unknown what particular elementary functions are controlled by the MI genome. Numerous data concerning a fairly normal viability of specimens which have lost their MI (MI-) are to be explained by changes in the differential activity of MA genome.

Contradictory evidence on the effect of MI loss known from other publications is probably due to the fact that different methods of denucleation differently alter the system of interactions between the cell nuclei. The cell response to the MI loss varies considerably even within closely related species of *Paramecium*, representatives of higher and lower forms. In different groups of *Ciliophora* the micronucleus probably controls a different complex of functions. It has been demonstrated for *P. caudatum* that the probability of MI loss compensation varies at different stages of the life cycle (vegetative cells, gamonts). The MI functional activity may be regarded as a kind of mechanism which render possible the preservation of generative nucleus over many agamic cell generations until the next reproduction process. Our hypothesis concerning the MI functional activity provides a new approach to the cytogenetic and molecular biological data on the ciliate nuclear apparatus.

РЕЗЮМЕ

Используя метод УФ укола, произведено удаление микронуклеуса (МИ) из клеток парамеций пяти видов (*Paramecium caudatum, P. bursaria, P. putrinum, P. woodruffi, P. jenningsi*). Полученные данные доказывают, что для нормальной жизнедеятельности инфузорий в период вегетативной жизни, помимо интенсивной транскрипции генома макронуклеуса (МА), существенна функциональная активность МИ. Это означает, что МИ является активным компонентом единой системы ядерного аппарата клетки, в которой существенную роль играют межъядерные взаимодействия. Во время вегетативной жизни клеток функции генетического контроля распределены между МА и МИ и носят динамический характер. Пока не известно, какие именно элементарные функции вегетативной клетки контролирует геном МИ. Многочисленные данные о сравнительно нормальной жизнеспособности особей, утративших МИ (МИ-), мы объясняем изменением дифференциальной активности генома МА. Известные противоречия литературных данных о последствиях утраты МИ, по-видимому, основаны на том, что различные способы денуклеации по-разному нарушают в клетке систему межъядерных взаимодействий. Реакция клетки на потерю МИ существенно отличается даже у близких видов парамеций: представителей низших и высших форм. Вероятно в различных группах *Ciliophora* МИ контролирует неодинаковый набор функций. Установлено, что у *P. caudatum* вероятность компенсации утраты МИ различна на разных стадиях жизненного цикла (вегетативные клетки, гамонты). Функциональная активность МИ может рассматриваться

как своего рода механизм, гарантирующий сохранение генеративного ядра в течение многих агамных клеточных поколений до очередного полового процесса. Предложенная нами гипотеза о функциональной активности МИ позволяет под новым углом зрения рассматривать цитогенетические и молекулярно-биологические данные по ядерному аппарату инфузорий.

REFERENCES

A l l e n S. L. and G i b s o n I. 1972: Genome amplification and gene expression in the ciliate macronucleus. Biochem. Genet., 6, 293–313.

A l l e n S. L. and Gibson I. 1973: Genetics of Tetrahymena. In: Biology of Tetrahymena. Dowden, Hutchinson and Ross, Inc., Stroudsburg, 307–373.

A l l e n S. L. and W e r e m i u k S. L. 1971: Defective micronuclei and genomic exclusion in selected C subclones of Tetrahymena. J. Protozool., 509–515.

A m m e r m a n n D. 1970: The micronucleus of the ciliate Stylonychia mytilus: its nucleic acid synthesis and its function. Expl. Cell Res., 61, 6–12.

A m m e r m a n n D., S t e i n b r ü c k G., B e r g e r L. and H e n n i n g W. 1974: The development of the macronucleus in the ciliated protozoan Stylonychia mytilus. Chromosoma, 45, 273–280.

B e e r s C. D. 1946: Tilina magna: micronucleus number, encystment and vitality in diverse clones; capabilities of amicronucleate races. Biol. Bull., 9, 256–271.

B o m f o r d R. H. 1965: Genetic studies on Paramecium bursaria. Thesis Doct. Phil. University of Edinburg.

B o r c h s e n i u s O. N. 1975: K voprosu ob aktivnosti generativnogo jadra infuzorij. Vest. LGU, 21, 7–16.

B o r c h s e n i u s O. N. and O s s i p o v D. V. 1978: Vlijanie izbiratel'noj inaktivacii generativnych jader infuzorii Paramecium caudatum na vegetativnye funkcii kletok. Acta Protozool., 17, 331–345.

C a l k i n s G. N. 1911: Regeneration and cell division in Uronychia. J. Expl. Zool., 10, 95–116.

C a l k i n s G. N. 1912: The paedogamus conjugation of Blepharisma undulans. J. Morphol., 23, 667–687.

C a l v e r t J. G. and R e c h e n H. T. 1952: Precision actinometry at low light intensities with malachite green leucocyanide. J. Am. Chem. Soc., 74, 2101–2103.

C h e n T. T. 1940: Polyploidy and its origin in Paramecium. J. Heredity, 31, 175–184.

C u l l i s C. A. 1973: DNA amounts in the nuclei of Paramecium bursaria. Chromosoma, 40, 127–133.

D a w s o n J. A. 1920: An experimental study of amicronucleate Oxytricha. J. Expl. Zool., 30, 129–153.

D i l l e r W. F. 1965: The relation of amicronuclearity to stomatogenic activity during conjugation in several ciliates. J. Morphol., 116, 51–64.

E l l i o t t A. M. (ed.), 1973: Biology of Tetrahymena. Dowden, Hutchinson and Ross, Inc., Stroudsburg, 508.

F o k i n S. I. 1978 a: Vlijanie lokal'nogo UF obluchenija generativnogo jadra na vegetetivnye funkcii infuzorij Paramecium putrinum. Citologija, 20, 1426–1430.

F o k i n S. I. 1978 b: Funkcional'naja značimost' generativnogo jadra infuzorij roda Paramecium v vegetativnoj zizni kletki. Avtoref. kand. diss., Leningrad.

F o k i n S. I. 1979 a: O suščestvovanii mežjadernych vzaimodejstvij u infuzorij. Citologija, 21, 1348–1352.

F o k i n S. I. 1979 b: Vlijanie lokal'nogo UF obluchenija generativnogo jadra na vegetativnye funkcii kletok Paramecium caudatum i Paramecium bursaria. V: Sb. Fotobiologia zivotnoj kletki. Leningrad. „Nauka", 200–202.

F o k i n S. I. and B o r c h s e n i u s O. N. 1978: Vlijanie lokal'nogo UF obluchenija na vegetetivnye funkcii odno- i dvumikronuklearnych kletok Paramecium caudatum. Citologija, 20, 570–575.

F o k i n S. I. and O s s i p o v D. V. 1975: Vlijanie lokal'nogo UF mikroobluce-
  nija na jadernyj apparat i citoplazmu infuzorii *Paramecium caudatum*.
  Citologija, 17, 1073–1080.
F r i c k D. 1967: Organellbildung und kernverhaltnisse bei *Stylonychia mytilus*.
  Biol. Zbl., 86, 629–655.
F u j i s h i m a M. and H i w a t a s h i K. 1978: Transplantation of germ nucleus
  in *Paramecium caudatum*. I. Nuclei in the pre-meiotic S-phase can enter
  into mitotic cycle. Expl. Cell Res., 111, 468–471.
G o l d e r T. K. 1976: The macro-micronuclear complex of *Woodruffia meta-
  bolica*. J. Ultrastr. Res., 54, 169–175.
G o l d s t e i n L. 1974: Movement of molecules between nucleus and cytoplasm.
  In: The cell nucleus. Academic Press. New Jork, London, 1, 387–438.
G o l d s t e i n L. 1975: Proteins and RNA's in nucleocytoplasmic interactions.
  In: Biochemistry of the cell nucleus mechanism and regulation of gene
  expression. Akademiai Kiado, Budapest, 33, 189–204.
G o l i k o v a M. N. 1974: Citofluorimetriceskoe issledovanie kolicestva DNK v mi-
  kronukleuse *Paramecium bursaria*. Acta Protozool., 13, 109–120.
G o l i k o v a M. N. 1978: Vnutrijadernye paraziticeskie bakterii v mikro- i makro-
  nukleuse infuzorij *Paramecium bursaria*. Citologija, 20, 576–580.
G o r o v s k y M. A. 1973: Macro- and micronuclei of *Tetrahymena pyriformis*:
  A model system for studying the structure and function of eucaryotic nuclei.
  J. Protozool., 20, 19–25.
G o r o v s k y M. A. and W o o d a r d J. 1969: Studies on nuclear structure and
  function in *Tetrahymena pyriformis*. I. RNA synthesis in macro- and
  micronuclei. J. Cell Biol., 42, 673–681.
G r e l l K. G. 1973: Protozoology. Springer-Verlag, Berlin, Heidelberg, New York,
  554.
M a c h a t u r o v E. N. and S m i r n o v a B. A. 1966: Primenenie rivanola SO_4
  dlja citofluorimetrii DNK. Izv. AN SSSR, 6, 900–905.
H a r r i s H. 1970: Nucleus and cytoplasm. Clarendon Press, Oxford, 190.
I n a b a F. and K u d o N. 1972: Electron microscopy of the nuclear events during
  binary fission in *Paramecium multimicronucleatum*. J. Protozool., 19, 57–63.
J a n k o w s k i A. V. 1969: Predlagaemaja klassifikacija roda *Paramecium* Hill,
  1752 (*Ciliophora*). Zool. zurn., 48, 30–40.
J a n k o w s k i A. V. 1972: Poliploidija mikronukleusa u *Paramecium* kak vido-
  voj priznak. Genetika, 8, 78–84.
J u d i n A. L. 1977: Dvizenie makromolekul mezdu jadrom i citoplazmoj po dan-
  nym opytov s amjobami. V sb.: Problemy eksperimental'noj biologii. Nauka,
  Moscow, 144–165.
K a l l i n i k o v a B. D. 1977: Kletocnaja organella kinetoplast. Nauka, Leningrad,
  127.
K a r r e r K. M. and G a l l J. G. 1976: The macronuclear ribosomal DNA of
  Tetrahymena pyriformis is a palindrome. J. Mol. Biol., 104, 421–453.
K i m b a l l R. F. 1941: Double animals and amicronucleate animals in *Euplotes
  patella* with particular reference to their conjugation. J. Expl. Zool., 86,
  1–32.
K i m b a l l R. F. and P r e s c o t t D. M. 1964: RNA and protein synthesis in
  amacronucleate *Paramecium aurelia*. J. Cell Biol., 21, 496–497.
K o r o c h k i n L. I. 1977: Vzaimodejstvie genov v razvitii. Nauka, Moscow, 280.
L e D a n t e c F. 1897: La regeneration du micronuclei us chez quelques Infu-
  soires. Compt. Rend. Acad. Sci., 125, 51–52.
L e w i n K. R. 1910: Nuclear relations of *Paramecium caudatum* during the
  asexual period. Proc. Cambridge Philos. Soc., 16, 39–41.
L u p o r i n i P. and B r a c c h i P. 1977: On the determination of micronuclear
  mitosis in plurimicronucleate cells of *Euplotes crassus* (*Ciliata, Hypotrichi-
  da*). Arch. Protistenk., 119, 178–187.
M a r g u l i s L. 1976: A review. Genetic and evolutionary consequences of sym-
  biosis. Expl. Parasitol., 39, 277–349.
M c C o y J. W. 1976: Updating the tetrahymenids. II. Domestic and natural
  variation of amicronucleate species of the *Tetrahymena pyriformis* complex.
  Acta Protozool., 13, 235–243.

M i y a k e A. 1956: Artificially induced micronuclear variation in *Paramecium* caudatum. J. Inst. Polytech. Osaka City Univ., ser. D, biol., 7, 147–161.

M o o r e E. L. 1922: Regeneration in *Spathidium spathula* and *Blepharisma undulans.* Proc. Soc. Expl. Biol. Med., 19, 128–189.

M o o r e E. L. 1924: Regeneration at various phases in the life history of *Spathidium spathula* and *Blepharisma undulans.* J. Expl. Zool., 39, 249–316.

M u r t i K. G. 1973: Electron-microscopic observations on the macronuclear development of *Stylonychia mytilus* and *Tetrahymena pyriformis (Ciliophora, Protozoa).* J. Cell. Sci., 13, 479–509.

M u r t i K. G. and P r e s c o t t D. M. 1970: The micronuclear ribonucleic acid in *Tetrahymena pyriformis.* J. Cell Biol., 47, 460–467.

N a n n e y D. L. 1977: Molecules and morphologies: the perpetuation of pattern in the ciliated protozoa. J. Protozool., 24, 27–35.

N a n n e y D. L. and M c C o y J. W. 1976: Characterizations of the species of the *Tetrahymena pyriformis* complex. Trans. Am. Micros. Soc., 95, 664–682.

O s s i p o v D. V. 1966: Metody polučenija gomozigotnych klonov *Paramecium caudatum.* Citologija, 8, 108–110.

O s s i p o v D. V. and B o r c h s e n i u s O. N. 1973: Osobennosti poliploidii mikronukleusov u infuzorij. Citologija, 15, 243–253.

O s s i p o v D. V. and S k o b l o I. I. 1968: The autogamy during conjugation in *Paramecium caudatum* Ehrbg. II. The ex-autogamont stages of nuclear reorganization. Acta Protozool., 6, 33–48.

O s s i p o v D. V. and S k o b l o I. I. 1973: Morphogenetic activity of generative nucleus in sexual cell reorganization of ciliates. Acta Protozool., 12, 151–164.

O s s i p o v D. V., G r o m o v B. V., M a m k a e v a K. A., R a u t i a n M. S., S k o b l o I. I. and B o r c h s e n i u s O. N. 1976: Ispol'zovanie simbiotičeskich bakterij dlja analiza struktury i funkcii jadernogo apparata infuzorij. V sb.: Kariologija i genetika prostejših. Nauka, Leningrad, 101–139.

P a s t e r n a k J. 1967: Differential genic activity in *Paramecium aurelia.* J. Expl. Zool., 165, 395—418.

P u m o D. E. and G o l d s t e i n L. 1977: A comparison of small nuclear RNA species isolated from micronuclei and macronuclei of *Tetrahymena.* J. Cell Biol., 75, 125a.

R a i k o v I. B. 1967: Kariologija Prostejših. Nauka, Leningrad, 260.

R a i k o v I. B. 1968: Macronucleus of ciliates. In: Research in Protozoology. Pergamon Press. Oxford–New York, 3, 1–128.

R a i k o v I. B. 1976: Evolution of macronuclear organization. Ann. Rev. Genet., 10, 413–440.

R a i k o v I. B. 1978: Jadro Prostejših. Morfologija i Evoljucija. Nauka. Leningrad, 328.

R a i k o v I. B. and A m m e r m a n n D. 1976: Novye uspechi v izučenii makronukleusa infuzorij. V sb.: Kariologija i Genetika Prostejših. Nauka, Leningrad, 64–90.

R a o M. V. N. and P r e s c o t t D. M. 1967: Micronuclear RNA synthesis in *Paramecium caudatum.* J. Cell Biol., 33, 281–285.

R e y n o l d s M. E. 1932: Regeneration in an amicronucleate infusorian. J. Expl. Zool., 62, 327–361.

R u i z F., A d o u t t e A., R o s s i g n o l M. and B e i s s o n J. 1976: Genetic analysis of morphogenetic processes in *Paramecium.* I. A mutation affecting trichocyst formation and nuclear division. Genet. Res., 27, 109–122.

S a g e r R. 1972: Cytoplasmic genes and organelles. Academic Press, New York and London, 423.

S a l v a n o P. 1975: Sur les acides nucleiques des Cilies. Ann. Biol., 14, 1–28.

S a x e n a R. and S a x e n a D. M. 1978: Cytology and DNA synthesis of a gymnostome ciliate *Homalozoon vermiculare* (Stokes). Arch. Protistenk., 120, 128–137.

S c h w a r t z V. 1934: Versuche über Regeneration und Kerndimorphismus der Ciliaten. Nacher. ges. Wiss. Göttingen, Math-physik YI, N. F., 1, 143–155.

S c h w a r t z V. 1947: Über die Physiologie des kerndimorphismus bei *Paramecium bursaria.* Z. Naturforsch., 26, 369–381.

S c h w a r t z V. 1958: Chromosomen im Makronucleus von *Paramecium bursaria.* Biol. Zbl., 77, 347–364.

S c h w a r t z V. und M e i s t e r H. 1975: Die Extinktion der feulgengefarbten Makronucleusanlage von *Paramecium bursaria* in der DNS-armen Phase. Arch. Protistenk., 117, 60–64.

S k o b l o I. I., R a u t i a n M. S. and O s s i p o v D. V. 1978: Poterja generativnych jader u infuzorii *Paramecium caudatum* wyzyvaemaja na rannich stadijach infekcii ich simbioticeskimi bakterijami. Acta Protozool., 17, 321–330.

S o n n e b o r n T. M. 1950: Methods in the general biology and genetics of *Paramecium aurelia.* J. Expl. Zool., 113, 87–143.

S o n n e b o r n T. M. 1966: Genetika prostejsich i ejo otnosenie k obscej genetike. Genetika, 11, 31–41.

S o n n e b o r n T. M. 1974 a: *Paramecium aurelia.* In: Handbook of Genetics. Plants, Plant Viruses, and Protists. Plenum Press, New York, 2, 469–594.

S o n n e b o r n T. M. 1974 b: *Tetrahymena pyriformis.* In: Handbook of Genetics. Plants, Plant Viruses, and Protists. Plenum Press, New York, 2, 433–467.

T a r t a r V. 1964: Experimental technique with ciliates. In: Methods in Cell Physiology. Academic Press, New York, 1, 109–125.

T a y l o r C. V. and F a r b e r W. P. Fatal effects of the removal of the micronucleus in *Euplotes.* Univ. Cal. Publ. Zool., 26, 134–144.

T u c k e r J. B. 1967: Changes in nuclear structure during binary fission in the ciliate *Nassula.* J. Cell Sci., 2, 481–498.

V a n W a g t e n d o n k W. J. (ed.) 1974: *Paramecium.* A Current Survey. Elsevier. Amsterdam, London, New York, 499.

W a t s o n J. 1978: Molecular Biology of the Gene. W. B. Benjamin, Inc, 719.

W e i n d r u c h R. H. and D o e r d e r F. P. 1975: Age-dependent micronuclear deterioration in *Tetrahymena pyriformis,* syngen 1. In: Mechanisms of Ageing and Development. Elsevier Sequoia S. A. Netherland, 4, 263–279.

W e l l s C. 1961: Evidence for micronuclear function during vegetative growth and reproduction of the ciliate, *Tetrahymena pyriformis.* J. Protozool., 8, 284–290.

W i c h t e r m a n R. 1953: The biology of Paramecium. Blakiston Comp., Inc., New York, 527.

W i c h t e r m a n R. 1959: Mutation in the protozoan *Paramecium multimicronucleatum* as a result of x-irradiation. Science, 129, 207–208.

W o o d r u f f L. L. 1920: Amicronucleate infusoria. Proc. Soc. Exp. Biol. Med., 18, 28–29.

W o o d r u f f L. L. 1921: Micronucleate and amicronucleate races of infusoria. J. Expl. Zool., 34, 329–337.

Y o s t H. 1972: Cellular Physiology. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 864.

Е. Е. МАХЛИН

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Жизнеспособностъ ядерно-цитоплазматических „грибридов” межлу амебами различных штаммов *Amoeba proteus*

Viability of Interstrain Nucleocytoplasmic Hybrids in *Amoeba proteus*

Синопсис: Путем трансплантации ядер между разными штаммами (культурами) *Amoeba proteus* получали ядерно-цитоплазматических„гибридов”и определяли их жизнеспособность. Определяли также жизнеспособность внутрикультуральных ренуклеатов (ВР) разных культур. Для разных культур доля нежизнеспособных ВР варьировала от 5 до 64%. При исследовани „гибридов” четыре комбинации культур оказались полностью несовместимыми, а шесть совместимыми. Не обнаружено комбинации культур, характеризующихся частичной несовместимостью. Обнаружено так, что трансплантационные отношения амеб двух штаммов в ходе их длительного культивирования могут измениться. Высказывается предположение, что трансплантационная несовместимость амеб может быть обусловлена какими-то эпигенетическими различиями. Полученные в работе данные о жизнеспособности ядерно-цитоплазматических „гибридов” амеб сопоставляются с литературными данными о жизнеспособности гетерокарионов, полученных для тех же комбинаций штаммов.

Амебы *Amoeba proteus* относятся к небольшому кругу клеток, хорошо переносящих имплантацию ядра и цитоплазмы. Это означает, что если трансплантации произведены между амебами одной культуры[1], большинство амеб-реципиентов (гомотрансплантантов) оказываются жизнеспособными. Гибель некоторого количества гомотрансплантантов объясняется, по-видимому, как тем, что в культуре, из которой были взяты для операции амебы, могут присутствовать нежизнеспособные по тем или иным причинам клетки, так и тем. что некоторые клетки травмируются в результате операции необратимо.

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[1] Данной культурой амеб в настоящей статье называется совокупность амеб одного штамма, культивируемых в одном сосуде. Новая культура амеб данного штамма может быть получена из существующей путем изоляции и последующего культивирования нескольких амеб или одной амебы. В последнем случае новая культура будет представлять собой субклон по отношению к исходной культуре.

Если трансплантации производятся между амебами, взятыми из культур разных штаммов[2], результат может отличаться от описанного. При использовании некоторых комбинаций штаммов либо все, либо преобладающее большинство амеб-реципиентов (гетеротрансплантантов) оказываются нежизнеспособными. Такой результат можно рассматривать как проявление некой трансплантационной несовместимости амеб разных штаммов. Амебы разных штаммов не обязательно трансплантационно несовместимы; бывают трансплантационно совместимые штаммы амеб, т.е. в этом случае доля нежизнеспособных гетеротрансплантантов не превышает долю нежизнеспособных гомотрансплантантов для каждой из двух культур амеб.

Трансплантационная несовместимость амеб обнаруживается как при имплантации ядер, так и при инъекциях цитоплазмы амеб одного штамма в амеб другого. В первом случае получают либо гетерокарионов (если собственные ядра клеток-реципиентов не удаляются), либо ядерно-цитоплазматических "гибридов" (если ядра клеток-реципиентов предварительно удаляются; в дальнейшем просто гибридов). Хотя до сих пор лишь для трех комбинаций трансплантационно несовместимых штаммов амеб производились как трансплантации ядер, так и инъекции цитоплазмы (Lorch and Danielli 1953, Hawkins and Cole 1965, Lorch and Jeon 1969, Lorch 1969, Махлин и Юдин 1969), полученные данные позволяют считать, что гетерологичная цитоплазма менее „токсична", чем гетерологичные ядра, поскольку амебы с инъецированной гетерологичной цитоплазмой выживают чаще, чем с имплантированными ядрами. До сих пор ни разу не наблюдалась 100%-ная нежизнеспособность амеб, которым была инъецирована гетерологичная цитоплазма. Вместе с тем 100%-ная нежизнеспособность зарегистрирована для гетерокарионов и гибридов, полученных для ряда комбинаций штаммов.

Наряду с комбинациями трансплантационно несовместимых штаммов, для которых не было получено ни одного жизнеспособного гетерокариона или гибрида, известны комбинации, для которых наблюдалось редкое выживание гетеротрансплантантов этих типов. Если гетеротрансплантанты данного типа, полученные для разных комбинаций штаммов, различаются по частоте (вероятности) выживания, то частоту выживания можно было бы рассматривать как показатель степени трансплантационной несовместимости (совместимости) штаммов[3].

В предыдущих работах, выполненных в нашей лаборатории, транспланта-

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[2]Штаммом у амеб называют потомство одной амебы, взятой из природного водоема. Поскольку у амеб отсутствует половой процесс, понятие штамм для них является синонимом понятия клон.

[3]В качестве показателей степени трансплантационной несовместимости амеб можно было бы использовать, как очевидно, и другие особенности поведения гетеротрансплантантов, такие, например, как частота делений нежизнеспособных клеток, численность клонов, образуемых нежизнеспособными клетками, срок первого после операции деления жизнеспособных клеток, скорость размножения амеб в клонах, образуемых жизнеспособными гетеротрансплантантами, и т.д.

ционная совместимость амеб оценивалась путем определения жизнеспособности гетерокарионов (Махлин 1971, Юдин 1975). В этих работах ставилась задача квалифицировать штаммы амеб в попарных комбинациях как совместимые и несовместимые. Однако, разные комбинации штаммов, которые были квалифицированы как несовместимые, различались в отношении частоты выживания гетерокарионов: для одних комбинаций штаммов не было получено ни одного выжившего гетерокариона, для других наблюдалось редкое их выживание (до 20%). С другой стороны, гетерокарионы, полученные для разных комбинаций совместимых штаммов, также несколько различались по жизнеспособности (от 70 до 95% жизнеспособных). Последний факт также мог бы свидетельствовать о разной степени совместимости амеб разных штаммов, если бы при контрольных операциях, т.е. при получении дигомокарионов, амебы разных штаммов выживали бы с одинаково высокой частотой. Однако в рассматриваемых работах жизнеспособность дигомокарионов для каждого использованного штамма не определялась.

В связи со сказанным мы попытались в настоящей работе выяснить, различаются ли по степени трансплантационной совместимости (несовместимости) амебы ряда имевшихся в нашем распоряжении штаммов.

Для оценки трансплантационной совместимости амеб проще получать гетерокарионы, а не гибриды, поскольку в первом случае не нужно удалять ядра клеток-реципиентов. Однако, если иметь в виду регистрацию редких случаев выживания гетеротрансплантантов несовместимых штаммов, определение жизнеспособности гибридов кажется более надежным по следующей причине. При трансплантации ядер из одних амеб в другие бывают случаи, когда трансплантируемое ядро не прорывает плазматическую мембрану одной из клеток (донора или реципиента) и оказывается в клетке-реципиенте заключенным в "чехол" из мембраны (Lorch and Danielli 1953, Jeon 1970). Такое мнимо имплантированное ядро вскоре после операции выбрасывается из клетки-реципиента. При некотором опыте выполнения трансплантаций ядер у амеб обычно не представляет труда решить, была ли имплантация успешной или нет. В последнем случае мнимо оперированная клетка выбраковывается. Однако, нельзя исключить возможность того, что в единичных случаях неудачная имплантация ядра может быть принята за успешную. При получении гетерокарионов мнимо оперированная клетка будет представлять собой одноядерную амебу с собственным ядром. При определении жизнеспособности гетерокарионов, полученных для несовместимых штаммов, такая амеба выживет, что будет расценено, как редкое выживание гетерокариона. Если же целью операции является получение гибридов, то мнимо оперированная амеба окажется безъядерной и, естественно, погибнет. Имея в виду эти соображения, мы в настоящей работе характеризовали трансплантационные отношения амеб разных штаммов путем получения и определения жизнеспособности гибридов.

Таким образом, задача работы состояла в том, чтобы на основании определения жизнеспособности гибридов между амебами различных штаммов выяснить, различаются ли разные комбинации штаммов по степени трансплантационной несовместимости.

Материал и методика

Культуры амеб

Были использованы 9 культур амеб, по одной культуре штаммов A, B, C, L, T_1P и по две культуры штаммов Bk и Da, обозначенных далее как Bk1 и Bk2, Da1 и Da2. Сведения о происхождении в нашей коллекции штаммов A, B, Bk, C, Da и L даны в работе Юдина (1975). Амебы штамма T_1P, обозначаемые далее как амебы P и широко используемые в ряде лабораторий (см.: Jeon and Lorch 1973), были в 1975 г. любезно присланы в нашу лабораторию H. J. Ord (University of Southampton, Англия). Культуры Bk1 и Bk2 происходят от одной культуры Bk, которая ведется в нашей лаборатории с 1969 г. (см.: Юдин 1975). Культура Bk1 была основана в 1972 г. и велась в нашей лаборатории при 25°. Культура Bk2 была основана в 1969 г. и велась в Лаборатории зоологии беспозвоночных Петергофского научно-исследовательского биологического института при Ленинградском университете при 20°. Точно так же культуры Da1 и Da2 происходят от одной культуры амеб Da, основанной в нашей лаборатории в 1970 г. (см.: Юдин 1975). Обе культуры также велись в нашей лаборатории, культура Da1 основана в 1972 г. и велась постоянно при 25°, культура Da2 основана в 1970 г. и до того, как была использована в настоящей работе, велась при комнатной температуре, затем содержалась при 25°.

Амеб культивировали в солевой среде, используя в качестве корма инфузорий *Tetrahymena pyriformis* (Prescott and Carrier 1964). Массовые культуры амеб и клонируемые клетки содержались при 25°.

Трансплантация ядер и определение жизнеспособности амеб

Ядра амеб трансплантировали на агаре (Jeon and Lorch 1968) с помощью микроманипулятора MM-1; использовали объектив ОСФ-26.

Ренуклеированных амеб содержали поодиночке и определяли их судьбу. Известно, что при трансплантационной несовместимости амеб гибриды не обязательно погибают сами, но могут делиться, образуя клоны из нескольких клеток, которые однако погибают (Lorch and Danielli 1950, 1953, Jeon 1969, Lorch 1969). Максимальное число клеток, наблюдавшееся в клонах, образованных нежизнеспособными гибридами, равнялось 16 (Jeon 1969). В связи с этим в настоящей работе ренуклеированная клетка квалифицировалась как жизнеспособная, если число амеб в образованном ею клоне достигало[4] по крайней мере 16. При клонировании неоперированных амеб жизнеспособной считали клетку, давшую начало клону по крайней мере из 8 амеб.

В результате серии операций, произведенных в один день, получали гибридов между амебами какой-нибудь одной пары культур — по 20-25 гибридов каждого из двух реципрок-

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[4] В преобладающем большинстве клонов, возникших в результате деления ренуклеированных амеб, которые были квалифицированы как жизнеспособные, было зарегистрировано большее число клеток — от 20 до 40.

ных типов. В большинстве случаев в тот же день производили ренуклеации такого же числа амеб той и другой культуры ядрами клеток той же культуры (внутрикультуральные ренуклеаты — ВР). Одновременно с клонированием ренуклеированных амеб начинали клонировать по 25 неоперированных амеб той и другой культуры. Жизнеспособность гибридов, полученных для каждой комбинации культур, была протестирована от 3 до 5 раз, жизнеспособность ВР каждой культуры — от 4 до 7 раз. Жизнеспособность гибридов большинства типов, кроме гибридов между амебами Bk2 и Da2, определялась в период с февраля 1977 г. по июль 1978 г., гибридов между амебами двух указанных культур — с марта по июнь 1979 г

Обозначения

В настоящей работе используется общепринятый способ обозначенияо перированных амеб (см., Yudin 1979), например: $A_N A_C$ — амеба А, у которой собственное ядро заменено ядром другой амебы А; $A_N B_C$ — амеба В, у которой собственное ядро заменено ядром амебы А; $A_N B_{NC}$ — гетерокарион, полученный в результате имплантации ядра амебы А в амебу В.

Результаты

Жизнеспособность ВР и неоперированных амеб

Авторы, ранее определявшие жизнеспособность ВР у амеб, в каждом случае чаще всего имели дело с одной-двумя, реже — с большим числом культур амеб (Lorch and Danielli 1950, 1953, Ord and Danielli 1956, Jeon 1968, 1969, 1972, 1975, Ord 1968, Lorch and Jeon 1969, Hawkins and Willis 1969, Махлин и Юдин 1970, Ord and Bell 1970, Махлин 1974, 1977, Chatterjee and Rao 1974). Доля нежизнеспособных ВР, по данным цитируемых авторов, колеблется в пределах 10–30%. В настоящей работе в одних и тех же экспериментальных условиях была определена жизнеспособность ВР для 9 культур амеб. Поскольку жизнеспособность ВР, полученных для разных культур, оказалась различной, эти данные заслуживают специального рассмотрения.

Доля нежизнеспособных ВР от суммарного их количества, полученного для каждой культуры, варьирует от 5% для амеб Da2 до 64% для амеб Bk1 (табл. 1). В ряду культур, ранжированных по доле нежизнеспособных ВР, статистически значимым оказывается, например, различие между амебами Da2 и С (5 и 17% нежизнеспособных ВР соответственно), между амебами А и Bk1 (42 и 64%; Р<0,05, критерий χ^2). Отметим также, что достоверные различия долей нежизнеспособных ВР получены для разных культур как штамма Bk, так и штамма Da.

Крайние значения долей нежизнеспособных ВР, полученные для разных групп амеб одной культуры, ренуклеированных в разные дни, для некоторых культур различаются больше, чем следовало бы ожидать при биномиальном распределении выборочных долей. Если принять доверительную вероятность

Таблица 1

Жизнеспособность амеб, ренуклеированных ядрами клеток
той же культуры, и неоперированных амеб

Table 1

Viability of amoebae renucleated with nuclei of the same culture and of
unoperated amoebae

Культура амеб Culture of amoebae	Число ренук- леированных амеб No. of renucle- ated amoebae	Доля нежизне- способных, % % of nonviable ones	Число неопе- рированных амеб No. of unope- rated amoe- bae	Доля нежизне- способных, % % of nonviable ones	Доля амеб, по- гибших в резу- льтате ренукле- аций, %* % of amoebae dying as a result of renucleation*
Da2	75	5	75	0	—
C	163	17	175	11	—
Da1	99	20**	100	6	15
L	96	21	100	22	—
P	99	22**	100	4	19
Bk2	96	27***	100	14	15
A	149	42	150	32	—
B	168	54**	175	22	41
Bk1	162	64**	175	31	48

   * Рассчитана, исходя из правила сложения вероятностей двух независимых совместимых событий.
   * Calculated on the base of the rule of summation of probabilities of two independent compatible events.
  ** Достоверно отличается от доли нежизнеспособных неоперированных амеб, P<0.01.
  ** Significantly different from the % of nonviable unoperated amoebae, P < 0.01.
 *** Достоверно отличается от доли нежизнеспособных неоперированных амеб, P<0.05.
 *** Significantly different from the % of nonviable unoperated amoebae, P < 0.05.

0.05, то различие крайних значений выборочных долей нежизнеспособных ВР следует считать значимыми для 5 культур: A, B, Bk, C и L; при доверительной вероятности 0,01 — для 3 культур: A, B и L. Наибольшим рассматриваемое различие оказалось для амеб B: 5 и 20 нежизнеспособных клеток из 25 ренуклеированных. Если принять генеральную долю нежизнеспособных амеб B равной 54% (Табл. 1), то для выборок указанного объема при биномиальном распределении долей 95%-ный доверительный интервал соответствует 8–18 клеткам (расчет доверительного интервала с помощью φ-преобразования Фишера). Таким образом, для ряда культур те их свойства, которые определяют вероятность выживания ренуклеированных амеб, на протяжении выполнения работы не оставались неизменными.

Доля нежизнеспособных неоперированных амеб для разных культур также оказалась различной (Табл. 1), хотя она колеблется в меньших пределах, чем доля нежизнеспособных ВР: от 0 для амеб Da2 до 32% для амеб A. При

использованном для клонирования числа амеб различие в доле нежизнеспособных клеток оказывается статистически значимым, например, для культур C и B (11 и 22%, P<0.01), C и L (11 и 22%, 0.01<P<0.05). Значимым оказывается также различие в доле нежизнеспособных амеб Bk1 и Bk2 (31 и 14%, P<0.01).

В разных выборках неоперированных амеб из одной культуры, также как и в случае ВР, доля нежизнеспособных клеток для ряда культур, особенно для культур Bk1 и L, варьировала в больших пределах, чем следовало бы ожидать при биномиальном распределении признака. Так, максимальное число нежизнеспособных амеб Bk1 из 25 клонируемых было 13, минимальное — 2; при средней доле 31% (Табл. 1) 95%-ный доверительный интервал при биномиальном распределении соответствует 3–12 клеткам. При использованных объемах выборок (не более 25 амеб) невозможно установить, связаны ли статистически неслучайные колебания выборочных долей нежизнеспособных ВР данной культуры с колебаниями долей нежизнеспособных неоперированных амеб.

При сравнении суммарных долей нежизнеспособных неоперированных амеб и ВР для каждой культуры оказывается, что для 4 культур — A, C, Da2 и P — различия не значимы; для остальных 5 культур доля нежизнеспособных ВР значимо превышает долю нежизнеспособных неоперированных амеб (Табл. 1). Хотя смертность после операции наиболее значительно возросла для амеб B и Bk1, характеризующихся исходно плохой клонируемостью, в целом полученные данные не позволяют считать, что амебы тем хуже переносят ренуклеацию, чем больше нежизнеспособных клеток в культуре, поскольу ренуклеация обусловила гибель ощутимой доли амеб Da1 и P, характеризующихся относительно высокой эффективностью клонирования.

Таким образом, обнаружено, что разные культуры амеб, использованные в работе, на протяжении нескольких месяцев различались по доле присутствующих в них жизнеспособных клеток и что амебы разных культур с разной частотой необратимо повреждаются в результате ренуклеации. Оба эти фактора обуславливают разную жизнеспособность амеб разных культур после ренуклеации их ядрами клеток той же культуры.

Жизнеспособность гибридов

Гибриды были получены для 10 пар культур, для каждой пары — оба реципрокных типа гибридов. Жизнеспособность гибридов сравнивали с жизнеспособностью только тех ВР "родительских" культур, которые были ренуклеированы в те же дни, в какие были получены гибриды, или в ближайшие к ним дни. Поэтому данные о выживаемости ВР одной и той же культуры, представленные в Табл. 2 в качестве контроля для разных гибридов, могут

несколько различаться, также как они могут отличаться от суммарных данных о выживании ВР, представленных в Табл. 1.

Для гибридов разного типа доля нежизнеспособных клеток варьирует в широких пределах: от 7% для гибридов $Da1_N A_C$ до 100% для гибридов ряда типов (Табл. 2). Все гибриды обоих реципрокных типов между амебами Da1 и Р оказались нежизнеспособными. Для трех комбинаций культур — С и Da1, С и Р, Bk2 и Da2 — было получено по одной жизнеспособной гибридной амебе; гибриды реципрокного типа в каждом случае характеризуются 100%-ной нежизнеспособностью. При полученном числе гибридов различие между результатом, когда жизнеспособной оказалась только одна клетка, и результатом, когда ни один гибрид не выжил, не является статистически значимым. Таким образом, для четырех рассматриваемых комбинаций культур получен однотипный результат. Во-первых, амебы разных культур в каждой из четырех комбинаций оказались трансплантационно несовместимыми. Во-вторых, нет оснований считать, что разные комбинации культур различаются по степени несовместимости амеб; несовместимость для рассматриваемых пар культур следует считать максимально выраженной. В-третьих, можно допустить, что при максимально выраженной несовместимости амеб двух культур возможно редкое выживание гибридных амеб — по нашим данным, с частотой около 1%.

Среди гибридов всех остальных типов максимальной нежизнеспособностью — 62% — характеризуются гибриды $B_N C_C$ и $Bk1_N A_C$ (Табл. 2). Для того, чтобы решить, можно ли считать амеб какой-либо из 6 пар культур хотя бы частично несовместимыми, необходимо сравнить смертность гибридов со смертностью ВР обеих "родительских" культур. Для трех из шести рассматриваемых комбинаций культур — В и С, В и L, Bk1 и Ba1 — доля нежизнеспособных ВР для двух культур оказалась значимо различной (Р<0.01). Для четырех комбинаций культур — А и Bk1, А и Da1, В и С, Dk1 и Da1 — значимо различаются доли нежизнеспособных гибридов реципрокных типов (для двух первых комбинаций 0.01<Р<0.05, для двух последних Р<0.01). Если долю нежизнеспособных гибридов любого типа сравнить с долей нежизнеспособных ВР той "родительской" культуры, у которой она выше, то ни для одной пары культур жизнеспособность гибридов не оказывается значимо более низкой, чем жизнеспособность ВР. Следовательно, нет оснований считать амеб ни одной из шести рассматриваемых комбинаций культур хотя бы в слабой степени трансплантационно несовместимыми.

Для 9 из 12 типов гибридов между совместимыми культурами доля нежизнеспособных клеток в разных партиях гибридов одного типа, полученных в разное время, варьировала несколько больше, чем следовало бы ожидать при биномиальном распределении выборочных долей. Возможно, этот факт связан с тем, что смертность неоперированных амеб и ВР некоторых культур на протяжении периода тестирования также не была неизменной.

Для двух комбинаций совместимых культур — В и С, Bk1 и Da1 — оказа-

Таблица 2

Жизнеспособность ядерно-цитоплазматических
,,гибридов" амеб

Table 2

Viability of nucleocytoplasmic hybrids

Комбинация культур Pairs of cultures	Тип клеток Type of cells	Число клеток No. of cells	Доля нежизнеспособных, % % of nonviable amoebae
A–Bk1	$A_N Bk1_C$	116	47
	$Bk1_N A_C$	111	62
	$A_N A_C$	124	47
	$Bk1_N Bk1_C$	115	57
A–Da1	$A_N Da1_C$	71	21
	$Da1_N A_C$	68	7
	$A_N A_C$	74	28
	$Da1_N Da1_C$	75	17
Bk1–Da1	$Bk1_N Da1_C$	85	50
	$Da1_N Bk1_C$	86	27
	$Bk1_N Bk1_C$	97	68
	$Da1_N Da1_C$	99	20
B–C	$B_N C_C$	117	62
	$C_N B_C$	114	15
	$B_N B_C$	119	55
	$C_N C_C$	114	18
B–L	$B_N L_C$	96	56
	$L_N B_C$	99	42
	$B_N B_C$	99	51
	$L_N L_C$	96	21
C–L	$C_N L_C$	96	18
	$L_N C_C$	99	8
	$C_N C_C$	93	15
	$L_N L_C$	96	21
C–Da1	$C_N Da1_C$	91	99
	$Da1_N C_C$	91	100
	$C_N C_C$	92	16
	$Da1_N Da1_C$	99	19
C–P	$C_N P_C$	96	100
	$P_N C_C$	100	99
	$C_N C_C$	94	14
	$P_N P_C$	99	22
Da1–P	$Da1_N P_C$	69	100
	$P_N Da1_C$	70	100
	$Da1_N Da1_C$	99	19
	$P_N P_C$	99	22
Bk2–Da2	$Bk2_N Da2_C$	100	100
	$Da2_N Bk2_C$	97	99
	$Bk2_N Bk2_C$	96	27
	$Da2_N Da2_C$	75	5

лось, что значимо различаются как доли нежизнеспособных ВР двух культур, так и доли нежизнеспособных гибридов реципрокных типов. При этом более высокой жизнеспособностью характеризуются гибриды, содержащие ядра амеб той из двух культур, клетки которой чаще выживают после контрольных ренуклеаций. Хотя мы столкнулись всего с двумя такими ситуациями, можно предположить, что вероятность гибели амеб после ренуклеации (как ядрами клеток той же культуры, так и — при трансплантационной совместимости — гетерологичными ядрами) определяется главным образом свойствами имплантируемых ядер.

Заслуживает внимания тот факт, что для разных культур одной и той же комбинации штаммов амеб — Bk и Da — получен разный результат в отношении трансплантационной совместимости. Амебы Bk1 и Da1 оказались вполне совместимыми, амебы Bk2 и Da2 — совершенно несовместимыми.

Нежизнеспособные амебы разных типов (неоперированные, ВР или гибриды) либо погибали сами, либо делились и погибало их потомство. Оказалось, что нежизнеспособные гибриды между амебами несовместимых культур и нежизнеспособные гибриды между амебами совместимых культур разли-

Таблица 3

Частота делений нежизнеспособных амеб разных типов

Table 3

Division frequency of nonviable amoebae belonging to different types

Тип амеб Type of amoebae	Число нежизнеспособных амеб No. of nonviable amebae	Доля клонов с разным максимальным числом клеток, % % of clones with different maximal number of cells			
		1	2	3–4	4
Неоперированные Unoperated	207	51	24	16	9
Ренуклеированные ядрами клеток той же культуры Renucleated with the nuclei of the same culture	376	65	13	9	13
Гибриды между совместимыми культурами Hybrids between compatible cultures	420	68	9	10	13
Гибриды между несовместимыми культурами Hybrids between incompatible cultures	711	75	22	3	0

чаются по частоте различных форм нежизнеспособности. 25% от общего количества нежизнеспособных гибридов между несовместимыми амебами разделились (Табл. 3). В 3% клонов одна или обе дочерние клетки разделились в свою очередь, но ни один рассматриваемый гибрид не дал более 4 клеток-потомков. Нежизнеспособные гибриды между совместными амебами делились чаще и в 13% клонов число клеток, впоследствии погибших, превышало 4 (Табл. 3). В этом отношении проявление нежизнеспособности у гибридов между совместными амебами не отличалось от того, что наблюдалось для ВР и неоперированных амеб (Табл. 3). Таким образом, формы проявления нежизнеспособности у гибридов между несовместимыми амебами менее разнообразны, чем у других ренуклеированных или у неоперированных амеб. Этот факт позволяет предположить, что нежизнеспособность гибридов между несовместимыми амебами обусловлена если не одним, то по крайней мере меньшим числом факторов, чем нежизнеспособность амеб в других случаях.

Обсуждение

Нежизнеспособность неоперированных амеб

Тот факт, что в массовых культурах амеб могут присутствовать нежизнеспособные клетки, отмечался неоднократно (Ord and Danielli 1956, Юдин 1961, Кальний 1967, Парибок и др. 1968, Jeon 1968, Махлин и Юдин 1969, 1970, Махлин 1971, Sopina 1976). Ранее также отмечалось, что доля нежизнеспособных амеб в данной культуре не может быть очень стабильным показателем, поскольку она должна определяться многими факторами среды (Yudin and Sopina 1970).

Недостаточная стандартность условий культивирования объясняет, по-видимому, тот факт, что в настоящей работе для некоторых культур были получены достоверные различия долей нежизнеспособных амеб в разных выборках, взятых для клонирования в разное время. Это же обстоятельство может быть причиной того, что для одного и того же штамма амеб разные авторы получают различающиеся данные о доле нежизнеспособных клеток в культуре (об эффективности клонирования амеб). Так, по данным одних работ (Юдин 1961, Кальний 1967, Парибок и др. 1968, настоящая работа), в культурах амеб В присутствовало около 20% нежизнеспособных клеток, по данным других работ (Кальний 1969, Sopina 1976), доля таких клеток не превышала 1%. Среди амеб С, клонируемых при 25°, по данным Sopina (1976), количество нежизнеспособных клеток не превышало 2%, по данным настоящей работы — достигало 11%.

Следует, однако, отметить, что рассматриваемые различия данных разных

авторов в отношении эффективности клонирования амеб одного и того же штамма могут быть результатом, по крайней мере, еще двух причин: (1) различий в способах отбора партий амеб для клонирования и как следствие этого — неодинаковой репрезентативности выборочных данных в отношении всей культуры; (2) существования наследуемых различий каких-то свойств амеб разных культур одного штамма, проявляющихся в разной частоте утраты клетками жизнеспособности даже при сходных условиях культивирования.

Хотя высокая лабильность такого показателя как доля нежизнеспособных клеток в культуре амеб очевидна, тем не менее при сравнении разных культур амеб, культивируемых в одних и тех же условиях, в некоторых случаях обнаруживаются стойкие различия по этому показателю. Впервые различие культур амеб по частоте утраты клетками жизнеспособности описал Юдин (1961); такие же различия обнаружены в настоящей работе. В обоих случаях эти различия были характерны для сравниваемых культур на протяжении нескольких месяцев, поэтому можно предположить, что они обусловлены различиями каких-то наследуемых свойств амеб разных культур. Кроме того, по данным Юдина (1961), субклоны, полученные из сравниваемых культур, точно так же различались по частоте утраты амебами жизнеспособности, как и сами эти культуры, что также подтверждает наследственный характер рассматриваемого различия.

Отметим, однако, что данные разных авторов, сравнивавших эффективность клонирования амеб одних и тех же штаммов, могут существенно различаться. Так, Юдин (1961) наблюдал в культуре амеб B около 20% нежизнеспособных клеток, а в культуре амеб L — не более 8%; по данным Sopina (1976), при клонировании амеб этих штаммов при 25° нежизнеспособными оказались около 1% амеб каждого штамма, а при 17° — 1% амеб B и 14% амеб L; в настоящей работе амебы этих штаммов не различались по эффективности клонирования, причем обе культуры характеризовались значительной долей нежизнеспособных клеток (22%). По данным Кальний (1969) амебы B и C характеризовались почти 100%-ной эффективностью клонирования; при клонировании амеб этих штаммов при 25° Sopina (1976) также получила 99% клонов амеб B и 98% клонов амеб C; в настоящей же работе для амеб этих же двух штаммов получены более низкие и достоверно различные значения (78 и 89%).

Как и различия данных разных авторов в отношении эффективности клонирования амеб какого-то одного штамма, расхождение данных в отношении различий культур одной и той же пары штаммов может быть результатом разных причин. Во-первых, можно предположить, что не все авторы производили отбор партий амеб для клонирования случайно. Если сознательно или бессознательно отбирать по тем или иным показателям „хорошие", „здоровые" клетки, то можно не обнаружить различия в доле присутствующих в культурах нежизнеспособных амеб. Во-вторых, можно предположить, что если какие-то наследуемые свойства амеб определяют вероятность утраты ими жизнеспособ-

ности, различие разных культур по этим свойствам проявляется не при любых внешних условиях, а только на фоне условий культивирования, в какой-то мере отклоняющихся от оптимальных. В-третьих, можно предположить, что культуры амеб двух штаммов, с которыми работал один автор, различались в отношении наследственно обусловленной вероятности утраты клетками жизнеспособности, а культуры, использованные другим автором, не различались.

О последней возможности свидетельствует, с нашей точки зрения, обнаруженное в настоящей работе различие доли нежизнеспособных амеб в двух культурах одного штамма Bk. Хотя эффективность клонирования амеб этих культур определялась не одновременно, нет оснований подозревать, что обнаруженное различие может быть связано с различием процедуры отбора клеток для клонирования или с различием условий культивирования амеб Bk1 и Bk2. Поэтому мы считаем, что различие в эффективности клонирования амеб Bk1 и Bk2 свидетельствует о наследуемых различиях каких-то свойств амеб этих культур. При этом оказывается, что амебы Bk1 отличаются, например, от амеб C по частоте утраты жизнеспособности, а амебы Bk2 — не отличаются.

Стойкое повышение частоты утраты жизнеспособности обнаружено в культурах амеб, подвергавшихся рентгеновскому облучению (Бычковская и Очинская 1972) или воздействию повышенной температуры (Бычковская и Очинская 1977). Повышенная частота гибели клеток в таких культурах регистрируется на протяжении нескольких лет (Бычковская и Очинская 1973). Поскольку наследуемые изменения, обуславливающие повышение частоты гибели клеток, происходят у подвергнутых воздействию амеб с большой частотой, высказано мнение, что эти изменения относятся к классу эпигенетических наследуемых изменений (Bychkovskaya et al. 1980). Возможно, что закономерная утрата жизнеспособности некоторыми клетками в ряду их поколений, обнаруживаемая в культурах амеб, не подвергавшихся специально каким-либо воздействиям, обусловлена теми же генетическими механизмами, как и в случае экспериментально повышенной частоты гибели амеб в опытах Ъычковской и соавторов.

Коль скоро в культуре амеб присутствует некоторое количество нежизнеспособных клеток, нежизнеспособными должны оказаться как некоторые из амеб этой культуры, ренуклеированных ядрами клеток той же культуры, так и некоторые гибриды между амебами этой и какой-то другой культуры.

Нежизнеспособность внутрикультурных ренуклеатов

В настоящей работе впервые обнаружено, что амебы разных культур различаются в отношении способности переносить ренуклеацию. Ренуклеация амеб одних культур не приводит к гибели клеток, некоторое количество амеб

других культур в результате ренуклеации утрачивает жизнеспособность. Поскольку культуры различались в этом отношении на протяжении ряда месяцев, есть основания предполагать, что различие в способности переносить ренуклеацию связано с различием каких-то наследуемых свойств амеб.

Для трех из шести комбинаций совместных культур — B и C, B и L, Bk1 и Da1 — доля нежизнеспособных BP для двух культур оказалась статистически значимо различной. При этом для одной из этих комбинаций (B и L) доли нежизнеспособных гибридов реципрокных типов не различались, а для двух остальных — различались. В последних двух случаях чаще погибали те гибриды, которые имели ядра культуры, характеризующейся более частой нежизнеспособностью BP. Такой результат позволяет предполагать, что различие в жизнеспособности BP разных культур связано с различной повреждаемостью при операциях ядер, а не цитоплазмы амеб.

Трансплантация ядер у амеб производится с помощью стеклянной микроиглы. При электронно-микроскопическом исследовании трансплантированных амебных ядер обнаружено, что у многих ядер повреждается оболочка, вплоть до ее разрывов (Flickinger 1970). Показано также, что по крайней мере у некоторых ядер эти нарушения обратимы и что в восстановлении нормальной структуры оболочки принимает участие эндоплазматический ретикулум (Flickinger 1970, 1974, 1978). По-видимому, трансплантированное амебное ядро может быть повреждено также и необратимо. Различная частота утраты жизнеспособности амебами разных культур в результате ренуклеации может быть связана как с разной механической прочностью оболочки их ядер, так и разной эффективностью процессов репарации повреждений оболочки.

Если некоторые амебные клетки, ренуклеированные ядрами амеб той же культуры, утрачивают жизнеспособность, трудно ожидать, что гибриды между амебами этой и какой-то другой культуры будут всегда жизнеспособны даже при трансплантационной совместимости амеб этих культур.

Жизнеспособность гибридов

На основании определения жизнеспособности гибридов использованные комбинации культур амеб четко дифференцируются на две группы: совместимые и несовместимые. Подчеркнем, что также как и в предыдущих работах, выполненных в нашей лаборатории (Махлин 1971, Юдин 1975), продемонстрирована трансплантационная совместимость амеб некоторых культур (штаммов). Таким образом, вопреки высказываемому в литературе утверждению (Jeon and Lorch 1979) трансплантационная совместимость амеб разных штаммов — столь же обычное явление, как и трансплантационная несовместимость.

При трансплантационной несовместимости культур для каждой из четырех их комбинаций нежизнеспособными оказались гибриды того и другого реци-

прокных типов. Таким образом, также как и в большинстве других описанных случаев (см.: Yudin 1973, Yudin 1979, Jeon and Lorch 1973, 1979) мы имеем дело с такой формой трансплантационной несовместимости амеб, которая может быть названа симметричной. Для одной комбинации штаммов амеб описана несовместимость другой формы — несимметричная: большинство гибридов одного типа были нежизнеспособными, большинство гибридов реципрокного типа — жизнеспособными (Jeon 1972, 1975). В дальнейшем речь будет идти только о симметричной несовместимости у амеб.

В настоящей работе не обнаружено комбинаций культур, которые можно было бы квалифицировать как частично несовместимые. Ранее разными авторами были получены двоякого рода данные, либо определенно свидетельствующие о частичной несовместимости амеб, либо позволяющие предполагать,

Таблица 4

Редкое выживание ядерно-цигоплазматических „гибридов" и
гетерокарионов у амеб

Table 4

Rare survival of nucleocytoplasmic hybrids and heterokaryons in amoebae

Тип гетеротрансплантантов Type of heterotransfers	Число полученных No. of obtained cells	Число выживших No. of viable cells	Источник References
	Гибриды Hybrids		
$P_N D_C$	61	1	Lorch and Danielli 1950
$G_N P_C$	85	28	Lorch and Jeon 1969
$G_N D_C$	93	28	Lorch and Jeon 1969
$P_N I_C$	149	33	Chatterjee and Rao 1974
$I_N P_C$	242	8	Chatterjee and Rao 1974
	Гетерокарионы Heterokaryons		
$P_N D_{NC}$	114	2	Jeon and Lorch 1969
$D_N P_{NC}$	186	1	Jeon and Lorch 1969
$D_N S_{NC}$	76	8	Jeon and Lorch 1969
$D_N S_{NC}$	108	27	Lorch and Jeon 1969
$C_N P_{NC}$	54	1	Lorch and Jeon 1969
$A_N C_{NC}$	50	4	Махлин 1971
$C_N A_{NC}$	49	11	Махлин 1971
$Bk_N C_{NC}$	49	2	Махлин 1971
$C_N Bk_{NC}$	50	2	Махлин 1971
$C_N Dis_{NC}$	74	4	Махлин 1971
$Dis_N C_{NC}$	73	7	Махлин 1971
$Bk_N B_{NC}$	40	3	Юдин 1975
$Bk_N L_{NC}$	24	1	Юдин 1975

что такие ситуации существуют: (1) данные о разной частоте гибели гетеротранс-
плантантов, полученных при использовании совместимых штаммов; (2) данные
о редком выживании гетеротрансплантантов, полученных при использовании
несовместимых штаммов. Данные первого рода касаются разной частоты
гибели гетерокарионов, полученных для ряда пар совместимых штаммов амеб
(Махлин 1971, Юдин 1975). Однако в цитируемых работах не определялась
жизнеспособность дигомокарионов для каждого использованного штамма.
Между тем, как показывают результаты настоящей работы, жизнеспособность
гомотрансплантантов, полученных при использовании амеб разных культур,
варьирует в широких пределах. В связи с этим, обсуждаемые данные о разной
частоте гибели гетерокарионов, полученных для разных комбинаций совме-
стимых штаммов амеб, не могут свидетельствовать о частичной транспланта-
ционной несовместимости некоторых штаммов.

Данные второго рода получены как для гибридов, так и для гетерокарио-
нов (Табл. 4). Редкое выживание гибридов наблюдалось для четырех комби-
наций штаммов амеб. Из 61 гибрида $P_N D_C$ жизнеспособным оказался только
один. Если иметь в виду статистическую оценку значимости различий выбо-
рочных данных, такой результат не свидетельствует о меньшей несовмести-
мости амеб P и D по сравнению с несовместимостью амеб, для которых была
получена 100%-ная нежизнеспособность гибридов. Ощутимое количество жи-
знеспособных гибридов (около 30% гибридов одного из двух реципрокных
типов в каждом случае) было получено для двух комбинаций несовместимых
штаммов: G и P, G и D. Отметим, однако, что штамм G — это не природный
штамм амеб, а экспериментально полученный клон. Амебы этого клона
являются потомками единственного выжившего гетерокариона $D_N P_{NC}$ (см.
Табл. 4) и ядра их происходят от ядра амебы P (Jeon and Lorch 1969, Lorch
and Jeon 1969). Трансплантационные взаимоотношения амеб G с амебами
исходных штаммов P и D оказались весьма необычными: во-первых, в отличие
от рассматриваемых гибридов $G_N P_C$ и $G_N D_C$ ни один гибрид реципрокных
типов не выжил[5]; во-вторых, гетерокарионы $G_N P_{NC}$ и $G_N D_{NC}$ оказались
нежизнеспособными почти в 100% случаев (выжил только один гетерокарион
$G_N P_{NC}$ — Табл. 4). Подобные трансплантационные взаимоотношения не на-
блюдались до сих пор ни для какой другой комбинации несовместимых шта-
ммов. Возможно, своеобразие этих отношений как-то связано с тем, что амебы
G являются экспериментально наследственно измененными амебами P.

Ввиду выживания некоторых гибридов между амебами штаммов P и I
(Табл. 4) несовместимость амеб этих штаммов следует считать меньшей,
чем при 100%-ной нежизнеспособности гибридов. Это наиболее убедительный

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[5] Имея в виду такой результат, Jeon and Lorch (1979) квалифицируют несовместимость
штамма G со штаммами P и D как несимметричную; поскольку, однако, гибриды $G_N P_C$
и $G_N D_C$ были преимущественно нежизнеспособными, нам такая оценка представляется
весьма спорной.

из фигурирующих в литературе примеров неполной несовместимости штаммов, обнаруживаемой при определении жизнеспособности гибридов. Интересно отметить, что штаммы P и I рассматриваются Chatterjee and Rao (1974) как штаммы амеб разных видов: *Amoeba proteus* и *A. indica*.

Для ряда комбинаций несовместимых штаммов амеб было получено редкое выживание гетерокарионов (Табл. 4). В тех случаях, когда регистрировалось выживание одного–двух гетерокарионов данного типа, можно было бы предположить, что этот результат является артефактом, о котором шла речь в начале настоящей статьи. Однако подобное сомнение кажется неоправданным в отношении случаев более частого выживания гетерокарионов. Таким образом, на основании данных, представленных в Табл. 4, несовместимость, например, между штаммами A и C следовало бы считать меньшей, чем между штаммами, для которых не было получено ни одного жизнеспособного гетерокариона (см.: Махлин 1971, Юдин 1975). Допуская, что комбинации штаммов, для которых было получено редкое выживание гетерокарионов, характеризуются не максимальной несовместимостью, мы для трех таких комбинаций, представленных в Табл. 4 — A и C, Bk и C, Bk и L — получили по 20–25 гибридов того и другого реципрокных типов. Ни один полученный гибрид не выжил. Такой результат не позволял надеяться, что при определении жизнеспособности гибридов рассматриваемые комбинации будут характеризоваться не максимальной несовместимостью. Сопоставляя этот результат с данными о редком выживании гетерокарионов, полученных для тех же комбинаций штаммов (Табл. 4), можно высказать предположение, что если штаммы несовместимы, вероятность (редкого) выживания гетерокарионов выше, чем вероятность выживания гибридов.

В целом вопрос о существовании градации в степени трансплантационной несовместимости у амеб далек от ясности. Данные, представленные в Табл. 4, позволяют считать, что такая градация существует. Однако в настояшей работе при определении трансплантационных отношений амеб для 10 комбинаций культур различий в степени совместимости обнаружено не было. Можно предположить, что частичная несовместимость штаммов амеб встречается не часто. Можно также предположить, что различия в степени несовместимости штаммов проявляются в разной частоте выживания гетерокарионов и не проявляются в частоте выживания гибридов.

О соотношении жизнеспособности гибридов и гетерокарионов,
полученных для одних и тех же комбинаций штаммов амеб

В недавно опубликованном обзоре, посвященном явлениям трансплантационной несовместимости у амеб, Jeon and Lorch (1979) рассматривают нежизнеспособность гибридов и нежизнеспособность гетерокарионов как не-

связанные друг с другом явления (ядерно-цитоплазматическая несовместимость в первом случае и ядерно-ядерная — во втором). Однако, если бы оказалось, что для каждой из тех комбинаций штаммов, для которых нежизнеспособны гетеротрансплантанты одного типа, также нежизнеспособны и гетеротрансплантанты другого типа, то такое совпадение позволяло бы предполагать, что нежизнеспособность гетеротрансплантантов разного типа у амеб обусловлена общими причинами. Другими словами, вопрос может быть сформулирован следующим образом: всегда ли оценка трансплантационных взаимоотношений двух штаммов, полученная на основании определения жизнеспособности гибридов, совпадает с оценкой, полученной на основании определения жизнеспособности гетерокарионов.

Для 10 комбинаций штаммов, для которых в настоящей работе была определена жизнеспособность гибридов, ранее (Махлин 1971, Юдин 1975) определялась жизнеспособность гетерокарионов. Сопоставление тех и других данных (Табл. 5) показывает, что для 8 комбинаций штаммов оценки трансплантацион-

Таблица 5

Жизнеспособность гетерокарионов и ядерно-цитоплазматических „гибридов", полученных для одних и тех же комбинаций штаммов амеб

Table 5

Viability of heterokaryons and nucleocytoplasmic hybrids obtained for the same pairs of amoeba strains

Комбинация штаммов Pair of strains	Жизнеспособность гетерокарионов Viability of heterokaryons	Жизнеспособность гибридов Viability of hybrids
B–C	+	+
B–L	+	+
C–L	+	+
Bk–Da	+	+ или −
A–C	−	−
Bk–C	−	−
Bk–L	−	−
C–Da	−	−
A–Bk	−	+
A–Da	−	+

„+" — гетеротрансплантанты жизнеспособны, „−" — гетеротрансплантанты нежизнеспособны. Жизнеспособность гетерокарионов — по данным Махлина (1971) и Юдина (1975); жизнеспособность гибридов — по данным настоящей работы.

„+" — viable heterotransfers, „−" — nonviable heterotransfers. Viability of heterokaryons according to M a k h l i n (1971) and Y u d i n 1975); viability of hybrids — evidence of this investigation.

ных отношений совпадают[6]. Различия в жизнеспособности гетерокарионов и гибридов обнаруживаются для двух комбинаций штаммов: A и Bk, A и Da. Для того, чтобы выяснить, действительно ли для этих комбинаций штаммов жизнеспособность гибридов и гетерокарионов различна, мы получили небольшие партии гетерокарионов обоих реципрокных типов для использованных в нашей работе культур амеб A и Da1. Оказалось, что из 24 гетерокарионов $Da1_N A_{NC}$ нежизнеспособны были 6, а из 25 гетерокарионов $A_N Da1_{NC}$ — только 2. Таким образом, гетерокарионы, полученные для использованных нами культур штаммов A и Da, также как и гибриды оказались жизнеспособными. Поэтому расхождение в оценках трансплантационных отношений амеб A и Da, полученных при определени гетерокарионов (Юдин 1975) и при определени жизнеспособности гибридов (настоящая работа), обясняется не тем, что для этой пары штаммов гибриды жизнеспособны, а гетерокарионы нет, а тем, что культуры амеб этих штаммов, использованные Юдиным, были несовместимы, а культуры, использованные в настоящей работе — совместимы. То же самое, очевидно, верно и в отношении штаммов A и Bk.

Таким образом, полученные к настоящему времени данные позволяют считать, что для любой пары культур амеб, для которой нежизнеспособны гетеротрансплантанты одного типа (гибриды или гетерокарионы), нежизнеспособны также и гетеротрансплантанты другого типа[7]. Такое совпадение позволяет думать, что нежизнеспособность гетеротрансплантантов обоих типов обусловлена общими причинами и что трансплантационная несовместимость у амеб выражается в нежизнеспособности клеток, в которых совмещены гетерологичные клеточные компоненты, независимо от того, какова конкретно комбинация этих компонентов.

Нежизнеспособность гетерокарионов при трансплантационной несовместимости амеб есть основания объяснять нарушением функционирования амебных ядер в результате действия на них некого легального фактора, содержащегося в амебах любого штамма (Махлин и Юдин 1969, 1970, Jeon and Lorch 1969, 1970). Ранее были высказаны соображения, согласно которым нежизнеспособность гибридов у амеб также может быть объяснена нарушением

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[6] Для разных культур штаммов Bk и Da в настоящей работе получены разные результаты в отношении трансплантационной совместимости амеб этих штаммов; этот факт обсуждается далее. Поскольку гибриды между амебами Bk1 и Da1 оказались жизнеспособными — также как и гетерокарионы, полученные для этой комбинации штаммов Юдиным (1975) — нет оснований говорить, что для рассматриваемой комбинации штаммов жизнеспособность гибридов и гетерокарионов различна.

[7] Упомянутые взаимоотношения штамма G со штаммами P и D (Jeon and Lorch 1969, Lorch and Jeon 1969) не являются, как очевидно, исключением из этого правила. Поскольку для той и другой пары штаммов (G и P, G и D) все гибриды одного типа были нежизнеспособны, а среди гибридов реципрокных типов выжили только 30%, гибриды между штаммами в целом следует считать нежизнеспособными.

функционирования имплантированного ядра в результате действия на него летального фактора, содержащегося в цитоплазме амебы-реципиента (Махлин и Юдин 1970, Махлин 1977).

О стабильности трансплантационных взаимоотношений штаммов амеб

До сих пор исследователи, получая данные о трансплантационных отношениях амеб, взятых из культур разных штаммов, рассматривали эти результаты как характеристику взаимоотношений штаммов в целом. Полученные в настоящей работе данные показывают, что такая позиция не всегда правомочна.

Мы столкнулись с тремя случаями изменения трансплантационных взаимоотношений амеб разных штаммов. Во-первых, различными оказались трансплантационные отношения амеб разных культур штаммов Bk и Da. Во-вторых, трансплантационные отношения амеб штамма А с амебами штаммов Bk и Da оказались иными, чем в работе Юдина (1975). В каждой рассматриваемой комбинации штаммов изменились свойства амеб по крайней мере одного штамма. Что касается штаммов Bk и Da, мы не можем сказать, амебы какого именно штамма изменились[8]. В случае взаимоотношений амеб А с амебами Bk1 и Da1 можно предполагать, что изменились амебы А, поскольку взаимоотношения амеб Bk1 и Da1 оказались такими же, как и взаимоотношения амеб этих штаммов по данным Юдина (1975).

Ранее уже было описано несколько случаев спонтанного изменения признаков амебных культур в ходе их длительного поддержания — признаков, которые до изменения длительное время были стабильными (Jeon and Danielli 1971, Jeon and Lorch 1973, Goriunova and Kalinina 1978). Для понимания природы происходящих в таких случаях наследственных изменений амеб весьма важно знать, возникли ли изменения во многих клетках или первоначально изменилась только одна клетка, так что измененная культура является клоном, возникшим из измененной клетки. В отношении случаев изменения устойчивости амеб к действию некоторых веществ (Goriunova and Kalinina 1978) авторы считают, что изменения произошли во многих клетках культуры, в связи с чем они предполагают, что эти изменения имеют эпигенетическую природу. Накопленные к настоящему времени данные о характере различий амеб разных штаммов позволяют предполагать, что эти различия во многих случаях детерминированы на эпигенетическом уровне (Yudin and Sopina 1970). Подобная возможность должна приниматься во внимание и в отношении описанных

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[8] Культуры Bk1 и Da1 по независящим от экспериментатора причинам погибли до того, как были использованы культуры Bk2 и Da2. Поэтому мы не имели возможности выяснить, каковы трансплантационные отношения амеб разных культур одного штамма.

в настоящей работе изменений свойств амеб, проявляющихся в изменении их трансплантационных отношений с амебами другого штамма.

Многие штаммы амеб в настоящее время существуют в виде многих культур, содержащихся в разных лабораториях. Таковы, например, штаммы $_{T1}P$, Bk, Da, C. При определении трансплантационных отношений амеб из культур таких широко распространенных штаммов полученные результаты, как очевидно, не следует распространять на штаммы в целом. Это соображение очевидно справедливо и в отношении других возможных стойких различий между культурами амеб разных штаммов.

О закономерностях трансплантационных отношений в группе штаммов (культур) амеб

Юдин (1975), использовав 7 штаммов *A. proteus*, определил трансплантационные взаимоотношения для каждой из 21 возможной попарной комбинации разных штаммов. В одних комбинациях штаммы были совместимы, в других — несовместимы. При этом отношения между штамами во всей их группе характеризовались следующими закономерностями: (1) если данный штамм был несовместим с каким-либо другим, он был несовместим и со всеми штаммами, совместимыми с последним; (2) если каждый из двух штаммов был совместим с третьим, то они были совместимы друг с другом. Полученный Юдиным результат можно сформулировать еще следующим образом: не было найдено ни одной такой комбинации из трех разных штаммов (всего в обсуждаемой работе можно было составить 35 таких комбинаций), чтобы первый штамм был совместим со вторым и третьим, а второй и третий были несовместимы.

В настоящей работе были использованы 9 разных культур амеб. Амебы Bk2 и Da2 использованы только в одной комбинации. Трансплантационные отношения амеб Bk2 и Da2·с амебами других культур остались неизвестными. Трансплантационные отношения остальных 7 культур были определены в ряде попарных комбинаций. Помимо 9 комбинаций, рассмотренных в разделе „Результаты", как уже указано, были получены гибриды для комбинаций культур A–C, Bk1–C и Bk1–L. Во всех этих случаях амебы оказались несовместимыми. Было получено также по 24-25 гибридов того и другого реципрокных типов для еще двух комбинаций культур: A–P и Bk1–P; все эти гибриды также оказались нежизнеспособными. Таким образом, при использовании 7 культур были определены трансплантационные отношения всего для 14 пар разных культур. Полученные данные позволяют рассмотреть взаимоотношения амеб в 12 комбинациях из трех культур, для которых известны взаимоотношения для каждой из трех возможных пар культур. Как оказывается, эти взаимоотношения ни в одном случае не противоречат сформулированным выше правилам.

Использованные нами культуры B, C, L образуют одну группу совместимых культур. Вторая такая группа включает амеб A, Bk1 и Da1. В 4 испытанных (из 9 возможных) попарных комбинациях культур первой и второй групп амебы оказались несовместимыми. Амебы P во всех испытанных комбинациях с амебами других культур (4 из 6 возможных) оказались несовместимыми с последними. Отличие наших результатов от результатов Юдина (1975) заключается в том, что амебы A оказались совместимыми с амебами штаммов Bk и Da. Кроме того, впервые были определены трансплантационные взаимоотношения амеб штамма P с амебами некоторых других штаммов нашей коллекции.

Учитывая полученные в настоящей работе данные о возможности изменения трансплантационных взаимоотношений амеб двух штаммов, мы считаем возможным говорить о трансплантационных взаимоотношениях конкретных культур амеб, а не штаммов в целом.

О природе наследственных различий амеб, обусловливающих их трансплантационную несовместимость

На основании результатов определения трансплантационных взаимоотношений амеб разных штаммов одного вида (*A. proteus*) и амеб разных видов (Махлин и Юдин 1969, Махлин 1971, Юдин 1975) ранее было высказано предположение о том, что трансплантационная несовместимость у амеб есть следствие некого суммарного генотипического различия (Махлин и Юдин 1971, Юдин 1975). Некоторые полученные к настоящему времени данные, касающиеся трансплантационной несовместимости амеб, побуждают рассмотреть и другие возможности.

Сюда относятся, во-первых, данные о возможности изменения трансплантационных взаимоотношениях амеб двух штаммов в ходе их длительного культивирования, о чем свидетельствуют результаты настоящей работы. Во-вторых, в ряде случаев было установлено, что амебы, являющиеся потомками редких выживших гетеротрансплантантов, полученных при трансплантации ядер между амебами несовместимых штаммов, характеризуются иными трансплантационными отношениями с амебами „родительских" штаммов, чем следовало бы ожидать, если бы свойства ядер и цитоплазмы у потомков гетеротрансплантанта не изменились (Danielli 1958, Hawkins and Cole 1965, Jeon and Lorch 1969, Lorch and Jeon 1969, Jeon and Danielli 1971, Chatterjee and Rao 1974). Эти факты противоречат представлению о том, что трансплантационная несовместимость у амеб всегда обусловлена различиями по многим генетическим факторам. Как одну из возможностей следует иметь в виду возможность того, что в некоторых случаях трансплантационная несовместимость амеб обусловлена различием устойчивых состояний генетического

аппарата клетки, т.е. обусловлена эпигенетическими различиями клеток. Такая точка зрения не исключает, однако, представления, согласно которому на определенном этапе генотипической дивергенции штаммов амеб возникшая трансплантационная несовместимость становится необратимой.

РЕЗЮМЕ

Путем трансплантации ядер получали ядерно-цитоплазматических „гибридов" между амебами *Amoeba proteus*, взятыми из культур разных штаммов, и определяли их жизнеспособность. Выясняли, различаются ли разные комбинации штаммов (культур) в отношении частоты жизнеспособности „гибридов", обусловленной трансплантационной несовместимостью амеб. Если бы это было так, то частота нежизнеспособности „гибридов" могла бы служить показателем степени трансплантационной несовместимости амеб разных культур. Использованы 7 штаммов *A. proteus*: A, B, Bk, C, Da, L и T_1P (последний штамм в дальнейшем обозначается как P). В случае штаммов Bk и Da использованы по две культуры каждого штамма, обозначенных соответственно как Bk1 и Bk2, Da1 и Da2. Была определена жизнеспособность амеб каждой из 9 культур, ренуклеированных ядрами клеток той же культуры (внутри-культуральные ренуклеаты). Для разных культур доля нежизнеспособных внутрикультуральных ренуклеатов варьировала от 5 до 64%. Нежизнеспособность внутрикультуральных ренуклеатов обусловлена как постоянным присутствием некоторого количества нежизнеспособных амеб в культурах, из которых брались клетки для операций, так и необратимым повреждением некоторых клеток в результате операции. „Гибриды" были получены для 10 пар культур: A–Bk1, A–Da1, Bk1–Da1, Bk2–Da2, B–C, B–L, C–L, C–Da1, C–P, Da1–P. Для каждой пары культур получали „гибридов" обоих реципрокных типов. Число полученных „гибридов" одного типа варьировало от 68 до 117. Доля нежизнеспособных „гибридов" разного типа варьировала от 7 до 100%. Все „гибриды" между амебами Da и P оказались нежизнеспособными. Для трех комбинаций культур — C и Da1, C и P, Bk2 и Da2 — было получено по одному жизнеспособному „гибриду". При использованном количестве „гибридов" каждого типа (около 100) различие между результатом, когда жизнеспособным оказался только один „гибрид", и результатом, когда ни один „гибрид" не оказался жизнеспособным, не является статистически значимым. Таким образом, во всех четырех рассматриваемых комбинациях культур амебы оказались полностью несовместимыми; следует при этом считать, что при полной несовместимости амеб возможно редкое (с частотой около 1%) выживание „гибридов". Для „гибридов" остальных 12 типов („гибриды" между амебами A и Bk1, A и Da1, Bk1 и Da1, B и C, B и L, C и L) доля нежизнеспособных варьировала от 7 до 62%. При этом доля нежизнеспособных „гибридов" любого типа не отличалась статистически значимо от доли нежизнеспособных внутрикультуральных ренуклеатов для той из двух „родительских" культур, для которой она была выше. Следовательно, в каждой из шести рассматриваемых комбинаций культур амебы оказались полностью совместимыми. Таким образом, в использованных комбинациях культур амебы были либо полностью совместимыми, либо полностью несовместимыми; не обнаружено комбинаций культур, характеризующихся частичной несовместимостью. Трансплантационные отношения амеб разных культур одной и той же пары штаммов — Bk и Da — оказались различными: амебы Bk1 и Da1 оказались совместимыми, амебы Bk2 и Da2 — несовместимыми. Трансплантационные отношения использованных в работе культур штаммов A, Bk и Da оказались иными, чем отношения культур тех же штаммов, ранее использованных Юдиным (1975): по нашим данным, амебы A и Bk1, A и Da1

совместимы; по данным Юдина, в каждой из этих двух комбинациях штаммов амебы несовместимы. Таким образом, трансплантационные отношения амеб двух штаммов в ходе их длительного культивирования могут измениться. Высказывается предположение, что трансплантационная несовместимость амеб может быть обусловлена какими-то эпигенетическими различиями. Полученные в работе данные о жизнеспособности ядерно-цитоплазматических „гибридов" амеб сопоставляются с литературными данными о жизнеспособности гетерокарионов, полученных для тех же комбинаций штаммов. Сравнение показывает, что для данной комбинации штаммов жизнеспособность гетеротрансплантантов обоих типов одинакова.

SUMMARY

Nucleocytoplasmic hybrids between amoebae taken from the cultures of different strains were obtained by means of nuclear transplantation. The purpose was to elucidate whether the different pairs of cultures may be differentiated by a percent of nonviable hybrids. In this instance the portion of nonviable hybrids could be regardered as an indicator of the degree of transplantation incompatibility of amoebae. Seven amoebae strains were used in this investigation: A, B, Bk, C, Da, L and $_{Tl}P$ (the latter strain will be henceforth designated as P). In the case of Bk and Da strains two cultures of each strain were used. These cultures will be henceforth designated as Bk1 and Bk2, Da1 and Da2. The viability of amoebae in each of 9 cultures renucleated with the nuclei of the same culture (intracultural transfers) were determined. For different cultures the percent of nonviable intracultural transfers was found to vary from 5 to 64. The percent is due to the constant presence of nonviable amebae in the cultures and to irreversible damage of some amoebae as a result of operation. Hybrids were obtained for 10 pairs of the cultures: A–Bk1, A–Da1, Bk1–Da1, Bk2–Da2, B–C, B–L, C–L, C–Da1, C–P, Da1–P. Both reciprocal types of hybrids were obtained for each pair. The number of obtained hybrids of one type varied from 68 to 117. The portion of nonviable hybrids of one type varied from 7% to 100%. All the hybrids between amoebae Da1 and P proved nonviable. Only one viable hybrid was obtained for each pair of the cultures C and Da1, C and P, Bk2 and Da2. With the number of obtained hybrids (about 100 hybrids of each type) the case when only one cell is viable and the case when all cells are nonviable do not differ statistically. Thus amoebae of all the 4 culture pairs were found to be fully incompatible. It must be acknowledged that hybrids between quite incompatible amoebae may rarely survive — with the frequency of about 1%, according to our evidences. The proportion of nonviable hybrids of the remaining 12 types (the hybrids between amoebae A and Bk1, A and Da1, Bk1 and Da1, B and C, B and L, C and L) varied from 7% to 62%. The percent of nonviable hybrids of any type did not exceed significantly that of intracultural transfers in one of the two parental cultures, for which this portion was higher. Consequently amoebae of each of 6 pairs of cultures were completely compatible. Thus among the culture pairs used amoebae turned to be either quite compatible or quite incompatible. Not a single pair that is partially incompatible was detected. Transplantation relationships between different cultures of the same pair of strains Bk and Da proved to be different: amebae Bk1 and Da1 were compatible, amoebae Bk2 and Da1 incompatible. Transplantation relationships between

the used cultures of strains A, Bk and Da were different as compared to those between the cultures of the same strains which had been used earlier by Y u d i n (1975): according to our evidences amoebae A and Bk1, A and Da1 are compatible, according to Yudin, amoebae in each of the two pairs of strains are incompatible. Hence possible changing of transplantation relationships of two cultures in the course of their durable lifespan is established. It is suggested that transplantation incompatibility of amoebae may be determined by some epigenomic differences. The obtained results concerning the viability of nucleocytoplasmic hybrids are compared to the evidences about the viability of heterokaryons obtained earlier for the same pairs of strains. The comparison shows that for a given pair of strains the viability of heterotransfers of two types is the same.

ЛИТЕРАТУРА

Бычковская И. Б. и Очинская Г. Н. 1972: Зависимость динамики пострадиационной гибели амеб от дозы облучения. Цитология, 14, 137–140.

Бычковская И. Б. и Очинская Г. Н. 1973: О жизнеспособности птомков облученных клеток (опыты на *Amoeba proteus* и *Paramecium caudatum*). Радиобиология, 13, 211–215.

Бычковская И. Б. и Очинская Г. К. 1977: Стабильное наследуемое повышение уровня клеточной смертности (опыты на *Amoeba proteus*, подвергнутых термическому и лучевому воздействию). Генетика, 13, 1289—1293.

B y c h k o v s k a y a I. B., O c h i n s k a y a G. K. and Y u d i n A. L. 1980: An analysis of hereditary increase in frequency of cell mortality induced by external factors (experiments on nuclear transplantations in *Amoeba*). Arch. Protistenk., 123, 1–11.

C h a t t e r j e e S. and R a o M. V. N. 1974: Nuclear-cytoplasmic interaction in amoeba interspecific hybrids. Exp. Cell Res., 84, 235–238.

D a n i e l l i J. F. 1958: Studies of inheritance in amoebae by the technique of nuclear transfer. Proc. Roy. Soc., B148, 321–331.

F l i c k i n g e r C. J. 1970: The fine structure of the nuclear envelope in amoebae: alteration following nuclear transplantation. Exp. Cell Res., 60, 225–236.

F l i c k i n g e r C. J. 1974: The role of endoplasmic reticulum in the repair of amoeba nuclear envelopes damaged microsurgically. J. Cell Sci., 14, 421–437.

F l i c k i n g e r C. J. 1978: Interaction between endoplasmic reticulum and nuclear membranes of different types of cells during repair of damaged amoeba nuclei. Exp. Cell Res., 111, 427–436.

G o r j u n o v a L. B. and K a l i n i n a L. V. 1978: Changes of hereditary properties observed during prolonged cultivation of amoebas. Acta Protozool., 17, 77–88.

H a w k i n s S. E. and C o l e R. J. 1965: Studies on the basis of cytoplasmic inheritance in amoebae. Exp. Cell Res., 37, 26–38.

H a w k i n s S. E. and W i l l i s L. R. 1969: Nuclear transplantation studies of the action of dimidium bromide on amoebae. Nature, 221, 86–87.

J e o n K. W. 1968: Nuclear control of cell novement in amoebae. Nuclear transplantation study. Exp. Cell Res., 50, 467–471.

J e o n K. W. 1969: Nuclear-cytoplasmic relations in lethal amoeba hybrids. Exp. Cell Res., 55, 77–80.

J e o n K. W. 1970: Micromanipulation of amoeba nuclei. Methods in Cell Physiology (D. M. P r e s c o t t, ed). Academic Press, New York and London, 4, 179–194.

J e o n K. W. 1972: Development of cellular dependence on infective organisms: micrurgical studies in amoebas. Science, 176, 1122–1123.

J e o n K. W. 1975: Selective effects of enucleation and transfer of heterologous

nuclei on cytoplasmic organelles in *Amoeba proteus*. J. Protozool., 22, 402–405.

J e o n K. W. and D a n i e l l i J. E. 1971: Micrurgical studies with large free-living amoebas. Int. Rev. Cytol., 30, 49–89.

J e o n K. W. and L o r c h I. J. 1968: New simple methods of micrurgy on living cells. Nature, 217, 463.

J e o n K. W. and L o r c h I. J. 1969: Lethal effect of heterologous nuclei in amoeba heterokaryons. Exp. Cell Res., 56, 233–238.

J e o n K. W. and L o r c h I. J. 1970: Strain-specific mitotic inhibition in large mononucleate amoebae. J. Cell. Physiol., 75, 193–198.

J e o n K. W. and L o r c h I. J. 1973: Strain specificity in *Amoeba proteus*. In: The Biology of *Amoeba* (K. W. J e o n, ed). Academic Press, New York and London, 549–567.

J e o n K. W. and L o r c h I. J. 1979: Compatibility among cell components in large free-living amebas. Int. Rev. Cytol., Suppl. 9, 45–62.

Кальний В. С. 1967: Радиочувствительность амеб в зависимости от условий облучения и стадии клеточного цикла. Цитология, 9, 1535—1542.

Кальний В. С. 1969: Сравнительная радиочувствительность двух штаммов амеб. Цитология, 11, 768—771.

L o r c h I. J. 1949: The rate of attachment of amoebae to the substratum: a study of nuclear-cytoplasmic relationship. J. Cell. Physiol. 73, 171–177.

L o r c h I. J. and D a n i e l l i J. E. 1950: Transplantation of nuclei from cell to cell. Nature, 166, 329–330.

L o r c h I. J. and D a n i e l l i J. E. 1953: Nuclear transplantation in amoebae. II. The immediate results of transfer of nuclei between *Amoeba proteus* and *Amoeba discoides*. Quart. J. Micr. Sci., 94, 461–480.

L o r c h I. J. and J e o n K. W. 1969: Character changes induced by heterologous nuclei in amoeba heterokaryons. Exp. Cell Res., 57, 223–229.

Махлин Е. Е. 1971: Явления несовместимости при трансплантации ядер у амеб. III. Жизнеспособность гетерокарионов, полученных при трансплантации ядер между различными штаммами одноядерных амеб. Цитология, 13, 1020—1027.

Махлин Е. Е. 1974. О нарушении функциональной активности ядер амеб после введения летального фактора из амеб другого штамма. Цитология, 16, 1406—1410.

Махлин Е. Е. 1977: Предотвращение гибели амеб, в которых были введен летальный фактор из амеб другого штамма, путем имплантации в них дополнительных ядер из клеток „своего" штамма. Цитология, 19, 445—451.

Махлин Е. Е. и Юдин А. Л. 1969: Явления несовместимости при трансплантации ядер у амеб. I. Нежизнеспособность гетерокарионов, полученных при трансплантации ядра *Amoeba proteus* в клетку *Chaos chaos*. Цитология, 11, 744—759.

Махлин Е. Е. и Юдин А. Л. 1970: Явления несовместимости при трансплантации ядер у амеб. II. Нежизнеспособность *Amoeba proteus*, ядра которых побывали в *Chaos chaos*. Онтогенез, 1, 199—205.

O r d M. J. 1968: The viability of the anucleate cytoplasm of *Amoeba proteus*. J. Cell Sci., 3, 81–88.

O r d M. J. and B e l l L. E. 1970: Viability of isolated nuclei. Nature, 226, 854–855.

O r d M. J. and D a n i e l l i J. E. 1956: The site of damage in amoebae exposed to lethal concentrations of methyl-di-(β-chloroethyl)-amine (a "nitrogen mustard"). Quart. J. Micr. Sci., 97, 17–28.

Парибок В. П., Кальний В. С. и Иконникова Т. Б. 1968: Эффект мощности дозы при рентгеновском облучении амеб. Цитология, 10, 1479—1484.

P r e s c o t t D. M. and C a r r i e r R. F. 1964: Experimental procedures and cultural methods for *Euplotes eurystomus* and *Amoeba proteus*. In: Methods in Cell Physiology (D. M. Prescott, ed.) Academic Press, New York and London, 1, 85–95.

S o p i n a V. A. 1976: The multiplication rate of amoebae related to the cultivation temperature. J. Thermal. Biol., 1, 199–204.

Юдин А. Л. 1961: О роли ядра и цитоплазмы в наследовании некоторых признаков у амеб. Цитология, 3, 569–576.

Y u d i n A. L. 1973: Nuclear-nuclear interactions in heterokaryons. In: The Biology of *Amoebae* (K. W. J e o n, ed). Academic Press, New York and London, 505–524.

Юдин А. Л. 1975: Явления несовместимости при трансплантации ядер у амеб. IV. Транс-
     плантационная совместимость различных штаммов *Amoeba proteus*. Генетика, 11,
     122—128.
Yudin A. L. 1979: Nuclear transplantation studies in *Amoeba proteus*. Int. Rev.
     Cytol., Suppl. 9, 63–100.
Yudin A. L. and Sopina V. A. 1970: On the role of nucleus and cytoplasm
     in the inheritance of some characters in amoebae (nuclear transfer experi-
     ments). Acta Protozool., 8, 1–39.

TOKER A. H. 1974. Rozkład nierównomierny... ... ...
...antropogenicznych pokrywowe... ... ...
12—120.

... ...

... ...
in the inhabitants of some localities in ... ...
annual Pol. Pestearch, 8, 1—50.

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Morphological Variability of *Trichodina nigra* Lom (*Ciliata, Peritrichida*), a Parasite of *Lucioperca lucioperca* (L.) from Szczecin Gulf

Synopsis. The morphological variability and its sources has been studied in a ciliate *Trichodina nigra* Lom (*Peritrichida*), parasitizing perch-pike, *Lucioperca lucioperca* (L.) in Szczecin Gulf. Small differences noted in the body dimensions of trichodinas collected in cooler and warmer seasons of the year appeared to be statistically not significant. While the variation among populations, although not so much pronounced, was statistically significant. The present paper is the first one concerning interpopulational variation in trichodinas occurring on fish.

The studies on morphological variability in ciliates, carried from several years, have called our attention to interpopulational variation in these organisms (K a z u b s k i 1979, 1980). In this aspect, however, only the trichodinas parasitizing the urinary bladder of amphibians have been hitherto recognized. Thus, it seems to be interesting to recognize the range and sources of variation in this group of parasites occurring in other hosts and habitats, i.e., on the body surface and gills. The present paper is the first one dealing with interpopulational variability of trichodinas occurring on fish.

Material and Methods

The material used in the present paper has been collected by the junior author from perch-pike, *Lucioperca lucioperca* (L.) from Szczecin Gulf (Zatoka Szczecińska) during April–July 1977. More precise data concerning the methods of collecting trichodinas as well as the data on other protozoans found on these

fishes are included in the paper by P i l e c k a - R a p a c z (1981). In the present paper only 7 most numerous subpopulations of *Trichodina nigra* Lom have been used. Measurements of the body and of the adhesive disc were made on silver impregnated preparations after Klein. The trichodinas were collected from gills, only in the case of a subpopulation No. 8 the material originated both from gills and skin of a fish (both groups of ciliates were joined together as no statistically significant differences had been found between them).

From each subpopulation a number of ciliates, from several to some scores of individuals, were measured according to the principles adopted in former papers (K a z u b s k i 1979, 1980). The following features were measured: (1) body diameter, (2) diameter of the adhesive disc with border membrane, (3) diameter of the adhesive disc without border membrane, (4) diameter of the denticulate ring, (5) number of denticles, and (6) length of denticles. Moreover, for each subpopulation mean length of an arch of the denticulate ring corresponding to one denticle was counted according to the formula:

$$\frac{\text{denticulate ring diameter}}{\text{mean number of denticles}} \times \pi.$$

The variation was studied with the aid of statistical methods, mainly the analysis of variance was made using two level nested ANOVA with unequal sample sizes (S o k a l and R o h l f 1969, Box 10.4). This analysis was made for three features: (1) diameter of the adhesive disc without border membrane, (2) denticulate ring diameter, and (3) the number of denticles. Choice of these characters, regarded as representative for trichodinas, was argued by K a z u b s k i (1979).

The variation between particular subpopulations was analysed as well as the variation between trichodinas collected in cooler (April–May) and warmer (June–July) seasons of the year.

Results and Discussion

The metric and meristic values of particular features in seven examined subpopulations of *Trichodina nigra* are given in Table 1. In this Table mean values of trichodinas collected in cooler (April–May) and warmer (June–July) seasons of the year are given as well as mean values for the total material. These values, especially the summarized ones, do not differ essentially from the data given by various authors for *Trichodina nigra* (L o m 1961, B y k h o v s k y 1962, K a z u b s k i and M i g a ł a 1968, K u l e m i n a 1968). Slight differences between these data may be regarded as a result of occurrence of these trichodinas on different host species or due to geographical factors.

The material given in Table 1 has been collected from a proportionally small area of Szczecin Gulf and from only one host species, *L. lucioperca*. Thus, the host dependent variability and the geographical variability could not be taken into account.

In his material, however, some differences in mean values of trichodinas collected in April–May may be observed when compared with

Table 1

Mean values (M) and standard deviation (SD) of main characters in samples of 7 subpopulations and two season groups of *Trichodina nigra* from *Lucioperca lucioperca*

No. of fish	Month	Diameter (μm)											No. of denticles			Length of denticle (μm)			Width of denticle (μm)
		body			adhesive disc with border membrane			adhesive disc			denticulate ring									
		M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	M	SD	n	
8	April	65.89	6.58	9	50.37	3.56	16	41.58	2.57	19	27.92	2.60	24	22.30	1.06	23	13.80	0.76	25	3.93
9	,,	77.00	–	1	53.66	2.16	16	45.25	5.26	8	27.92	3.15	12	23.25	0.62	12	13.83	1.53	12	3.77
12	May	75.36	11.50	25	53.09	4.47	42	44.33	4.33	45	29.49	3.22	51	22.38	1.82	55	14.23	1.82	58	4.14
16	,,	60.67	4.85	9	50.33	5.38	12	41.67	4.68	12	26.58	3.32	12	21.83	1.27	12	13.54	1.39	13	3.83
26	June	63.00	6.68	8	50.80	4.26	10	42.50	3.47	10	27.46	3.33	13	23.08	2.19	12	13.15	1.07	13	3.74
27	,,	65.91	6.43	22	48.97	4.04	40	40.26	3.77	42	26.00	2.68	50	22.12	0.87	50	12.96	1.39	51	3.69
34	July	70.71	7.16	14	52.44	4.76	16	42.82	4.90	17	28.06	3.34	17	22.00	1.13	15	14.17	1.54	18	4.41
	April–May	70.45	11.21	44	52.13	4.46	76	43.42	4.31	84	28.57	3.21	99	22.40	1.54	102	13.87	2.05	108	
	June–July	66.91	7.14	44	50.09	4.44	66	41.22	4.15	69	26.67	3.06	80	22.24	1.29	77	13.25	1.45	82	
	Total	68.68	9.51	88	51.18	4.55	142	42.42	4.37	153	27.72	3.26	179	22.33	1.44	179	13.61	1.84	190	

Table 2

Two-level nested ANOVA table for three examined characters of *Trichodina nigra* from *Lucioperca lucioperca*

Source of variation	Degree of freedom	F_0 — value			Critical value	
		diameter of adhesive disc	diameter of denticulate ring	number of denticles	$F_{0.05}$	$F_{0.01}$
Among groups from various seasons	$2-1 = 1$	3.467 ns	4.672 ns	0.232 ns	6.608	—
Among particular subpopulations	$7-2 = 5$	3.147 s	3.712 s	2.294 s	2.290	3.173
Within subpopulations	$n^a - 7$					

[a] diameter of adhesive disc n = 153, diameter of denticulate ring n =179, and number of denticles n = 179.

those collected in June–July. Dimensions of the body, of the adhesive disc and of the denticulate ring in trichodinas collected during warmer months are slightly smaller. Only the mean number of denticles is almost the same in both groups of protozoans. However, the analysis of variance made for these three features (Table 2) has shown that the observed differences are statistically not significant at the 5% level of error. Nevertheless, it is worth of mention that the direction of changes — diminution of body dimensions in warmer months of the year, is the same as it has been observed in other species of protozoans (Kazubski 1975). Small differences observed in the present material are probably connected with proportionally small changes of water temperatures in such great reservoir as Szczecin Gulf. Additionally, the temperature preference of fish host (optimum temperature) ought to be also taken into account contributing to more stable thermic conditions of parasite habitat and, indirectly, to the morphology of parasites.

On the other hand the differences among subpopulations (Table 1) are statistically significant (Table 2) at the level of error slightly lower than 1% for the diameter of denticulate ring, slightly above 1% for the diameter of the adhesive disc and equal to 5% for the number of denticles. In general, it may be stated that the variation among subpopulations of *T. nigra* occurring on *L. lucioperca* is fairly small although statistically significant. In this respect this species of trichodina clearly differs from *T. vesicularum* and *T. ranae* parasitizing the urinary bladder of newts and frogs (Kazubski 1979, 1980). The last two species show high interpopulation variation — the value of F_0 oversteps some times the critical value at 1% level of error.

The reason for such difference between *T. nigra* and *T. vesicularum* and *T. ranae* probably lays upon the mode of life of these ciliates. *T. nigra* occurs on the surface of gills and skin of fish so subpopulations on particular host individuals are "open" and the exchange of ciliates between hosts is not so difficult. While subpopulations of *T. vesicularum* and *T. ranae,* occurring in inner organ (urinary bladder) of their hosts, are much more isolated what accounts to their greater differentiation (K a z u b s k i 1979, 1980).

REFERENCES

B y c h o w s k y B. E. 1962: Opredelitiel' parazitov presnovodnyh ryb SSSR. Izd. Akad. Nauk USSR. Moskva-Leningrad 1962, pp. 776.
K a z u b s k i S. L. 1975: On the variability of ciliates. J. Protozool., 22, (3), 64 A
K a z u b s k i S. L. 1979: Morphological variability of *Trichodina vesicularum* Faure-Fremiet and *T. faurefremieti* Kazubski, parasites of newts from Poland and France. Acta Protozool., 18, 385–400.
K a z u b s k i S. L. 1980: *Trichodina ranae* da Cunha, 1950 (*Ciliata, Peritrichida*), a parasite of *Rana esculenta* s. l. and its morphological variability. Acta Protozool., 19, 207–223.
K a z u b s k i S. L. and M i g a ł a K. 1968: *Urceolariidae* from breeding carp — *Cyprinus carpio* L. in Żabieniec and remarks on the seasonal variability of trichodinids. Acta Protozool., 6, 137–160.
K u l e m i n a I. V. 1968: Parazitičeskije infuzorii (*Peritricha, Urceolariidae*) s molodi nekotoryh ryb oz. Seliger. Acta Protozool., 6, 185–208.
L o m J. 1961: Ectoparasitic trichodinids from fresh water fish in Czechoslovakia. Vest. čsl. Spol. zool., 25, 215–228.
P i l e c k a - R a p a c z M. 1981: Pierwotniaki ryb z rodziny *Percidae* Zalewu Szczecińskiego. Zeszyty naukowe AR w Szczecinie, (in press).
S o k a l R. R. and R o h l f F. J. 1969: Biometry, W. H. Freeman and Co., San Francisco, 776 pp.

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Uptake and Metabolism of DDT by a Ciliate Protozoan, *Stylonychia notophora* (Stokes)

Synopsis. Stylonychia notophora, when exposed to one ppm p,p'-DDT accumulated it rapidly. Maximum concentration of total DDT residue was obtained within 1 h. Accumulation factor varied from 97 to 295. Different metabolic products of DDT obtained as a result of metabolism were DDMU, p,p'-DDE and Kelthane. DDMU was the major metabolite and its quantities were always higher than the parent compound. p,p'-DDD was not detected in this system.

The persistent pesticide DDT[1] has been recently shown to be decomposed extensively by microorganisms (J o h n s e n 1976, B u t l e r 1977, W i l l i a m s 1977). The common metabolic products of DDT particularly DDE and DDD are more persistent in the environments (K e n a g a 1972) and are equally toxic to certain biological systems (L i n c e r 1975). These metabolites are mainly generated by microbial activity (J u e n g s t and A l e x a n d e r 1976).

Protozoa, algae and bacteria form the broad base of aquatic food chain (B o r r o r 1963, F e n c h e l 1967, C o o l e y et al. 1972). Ciliate protozoans are most numerous organisms in the environment and are able to concentrate certain persistent pesticides (G r e g o r y et al. 1969). This may permit the movement of these chemicals through food chain and the effects of such toxicants could be exerted at higher trophic levels. However, not much information is available concerning the accumulation and metabolism of DDT by ciliates. In the present paper accumulation and metabolism of DDT in a fresh water hypotrichous ciliate, *Stylonychia notophora* is described.

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[1] DDT and its metabolites are identified in Table 1.

Materials and Methods

Specimens of *Stylonychia notophora* were isolated from water samples collected from fresh water ponds around Delhi in 1975. Subsequently they were acclimatized to a medium of sterilized hay infusion inoculated with bacteria, *Klebsiella aerogenes*. The medium was fortified with Horlick's malted milk (produced in India by Hindustan Milk Food Ltd., for Horlicks limited Brenford, Middlesex, U. K.) periodically to aid the rapid growth of bacteria. Cultures were maintained in 250 ml conical flasks in a B. O. D. Incubator at $22 \pm 1°C$. Old cultures were subcultured once in a week.

Stock solution of 1000 ppm p,p'-DDT was prepared in acetone. The purity of DDT was more than 99% as determined by gas liquid chromatography (GLC). Appropriate quantity of solution was added to have a desired concentration of DDT in the culture medium.

Ciliates from log phase cultures were transferred to Chalkley's medium. This medium contains (w/v) 0.01% sodium chloride, 0.004% potassium chloride and 0.0006% calcium chloride (R a n d a l l and J a c k s o n 1958). Organisms were kept in Chalkley's medium for 24 h before treatment. One ppm concentration of DDT was used to study the accumulation and metabolism of DDT. This concentration of DDT and the solvent (0.1% acetone) had no effect on the growth of *Stylonychia notophora* (R u p L a l and S a x e n a 1979).

In order to study the uptake and metabolism of DDT, ciliates from log phase cultures were centrifuged and repeatedly washed with sterilized Chalkley's medium. These animals were transferred to conical flasks containing 100 ml Chalkley's medium. Animals were allowed to grow for 24 h in this medium and then treated with one ppm DDT. Appropriate controls were run simultaneously. The treated cultures were shaken periodically to keep the cells in suspension. After regular intervals of time, cultures were centrifuged at 3000 rpm for 10 min. The supernatant consisting of medium was collected separately from the pellet. Subsequently the pellet was washed with toxicant free medium thrice and the washings were added to the medium which was already collected. DDT and its metabolites were then extracted from the pellet and analysed by GLC according to the method described alsewhere (R u p L a l et al. 1978).

DDT and its metabolites (Table 1) obtained in this way were further confirmed by using thin layer chromatography (TCL), a GLC column of different po-

Table 1

Chemical names of DDT and its metabolites

Common name	Chemical name
DDD	1,1'-bis (p-chlorophenyl) -2,2-dichloroethane
DDE	1,1'-bis (p-chlorophenyl) -2,2-dichloroethylene
DDMS	1,1'-bis (p-chlorophenyl) -2-chloroethane
DDMU	1-chloro-2,2-(p-chlorophenyl) ethylene
DDNU	1,1'-bis (p-chlorophenyl) ethylene
DDT	1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane
Kelthane	1,1-bis (p-chlorophenyl) 2,2,2-trichloroethanol

larity, chemical derivation techniques, and subsequent GLC of the different metabolites. All treatments were replicated three times. The recovery of the standards from spiked samples from the pellet was: p,p'-DDT 95%, p,p'-DDE 92%, DDMU 96% and Kelthane 93%. The concentrations of DDT and its metabolites were calculated on dry weight basis but not corrected for recovery.

Results

Pattern of uptake and metabolism of DDT is shown in Table 2. Total concentration of DDT in the organisms increased rapidly after the initiation of treatment and maximum uptake occurred within 1 h. However, this peak in accumulation was accompanied by increase or decrease in the amount of total DDT at different treatment periods but never reached the concentration obtained after 1 h. The concentration factor varied from 97 to 295 during the experimental period.

Table 2

Concentration of DDT and its metabolites in ppm at different time
intervals by *Stylonychia notophora* exposed to 1 ppm of p,p'-DDT for 10 days[1]

Exposure time in hours	Kelthane	DDMU	p,p'-DDE	p,p'-DDT	Total DDT/AF[2]
1	32.7±4.66 (0.11)	164.7±23.24 (0.55)	38.5±5.96 (0.13)	59.0±7.00 (0.20)	294.8
6	44.9±5.18 (0.17)	155.5±9.10 (0.58)	31.7±2.60 (0.12)	35.6±5.12 (0.13)	267.5
12	7.5±1.09 (0.07)	68.1±1.43 (0.70)	11.2±1.15 (0.11)	10.3±2.24 (0.10)	97.1
24	18.7±2.31 (0.08)	157.3±10.73 (0.70)	20.8±1.73 (0.09)	24.9±3.84 (0.11)	221.6
48	19.6±0.58 (0.14)	67.8±9.07 (0.50)	25.0±1.57 (0.19)	20.8±1.61 (0.15)	133.3
72	19.6±0.58 (0.10)	97.0±10.76 (0.50)	28.8±0.40 (0.14)	47.6±6.22 (0.25)	192.9
96	25.4±1.46 (0.10)	149.7±5.78 (0.59)	31.9±1.18 (0.12)	44.6±1.57 (0.17)	251.5
144	18.8±0.59 (0.08)	95.7±10.66 (0.47)	24.0±1.00 (0.11)	65.7±6.43 (0.32)	204.3
192	30.5±1.58 (0,14)	105.1± 1.73 (0.50)	30.8±0.74 (0.14)	43.2±0.44 (0.20)	209.6
240	33.6±3.03 (0.12)	130.3±18.4 (0.49)	26.1±0.22 (0.09)	71.7±6.17 (0.27)	261.6

[1] Values in parentheses represent quotient for each component.
[2] AF = Accumulation factor.

Studies relating to the metabolism of DDT revealed that this ciliate rapidly metabolized DDT into three metabolites viz., DDMU, p,p′-DDE and Kelthane. DDMU was found to be the major metabolite and the quotient (DDMU/total DDT) was as high as 0.70 and varied from 0.47 to 0.70. Maximum amount of DDMU (164.7 ppm) was obtained after 1 h of treatment. Kelthane and p,p′-DDE accounted for more or less similar concentrations. The maximum amount of Kelthane was observed during first 6 h. The quotient (Kelthane/total DDT) varied from 0.07 to 0.17 during the period of experiment. Maximum amount of p,p′-DDE was found after 1 h of treatment and a rapid decline in the amount was observed thereafter. The ratio of p,p′-DDE to DDT was maximum (0.19) in 48 h samples and minimum (0.09) on the 10th day of treatment.

Discussion

Rapid accumulation of DDT and other related persistent pesticides has been reported in some microorganisms such as *Aerobacter aerogenes* and *Bascillus subtilis* (J o h n s o n and K e n n e d y 1973), *Chlorella* sp. (S o d e r g r e n 1968), *Tetraselmis chuii* and *Ankistrodesmus amalloides* (R i c e and S i k k a 1973, N e n d o r f and K h a n 1975) and *Crethidia fasciculata* (F r e n c h 1976). A number of factors have been proposed to account for such a rapid accumulation in microorganisms. Some of these are water insolubility of DDT, its lipophilic nature and its properties to get absorbed quickly (K e i l and P r i e s t e r 1969, C o x 1970). In microorganisms such as algae, rapid accumulation of DDT has also been attributed to the phase partitioning of DDT between the medium and the lipid portion of the cells (C o x 1972).

The biological magnification of DDT in *Stylonychia notophora* ranged from 97–295 and is comparable with those reported for *Euglena gracilis* as 99 and *Paramecium bursaria* as 264 (G r e g o r y et al. 1969). However, *Paramecium micromultinucleatum* biomagnified DDT by 969 times (G r e g o r y et al. 1969).

The products most widely reported to result from DDT metabolism by microorganisms are DDD, DDE, DDMU, DDMS, DDNU and Kelthane (J o h n s e n 1976, W i l l i a m s 1977). *Stylonychia notophora* converted DDT to DDMU, Kelthane and DDE. DDMU a metabolic product of DDT is mainly generated by bacteria (J o h n s o n 1976, W i l l i a m s 1977). Since DDMU was the major metabolite in *Stylonychia*, the possibilities that ciliates also play a role in the conversion of DDT to DDMU in nature cannot be ruled out. DDD which is an inter-

mediate product of DDT metabolism during the formation of DDMU was not detected in this organism. This probably indicates divergent metabolic pathway for DDT metabolism or DDD in converted so quickly to DDMU that it is not being detected. The latter possibility seems to be more tenable because in another ciliate protozoan, *Tetrahymena pyriformis,* DDD when added separately was metabolized to DDMU (R u p L a l et al. 1978). Further enzymatic conversion of DDT to different products has been reported in many microorganisms (F r e n c h and H o o p i n g a r n e r 1970, J o h n s e n 1976) and a separate enzyme in each step rather than a single system has been suggested (W e d e-m e y e r 1967 a, b).If it is assumed that the conversion of DDT to DDMU is a two step reaction involving different enzymes, the second enzyme would appear to be more efficient and perhaps with a much lower KM value, whereby any DDD formed is quickly changed to DDMU. Thus DDD is not detected in the system.

REFERENCES

B o r r o r A. C. 1963: Morphology and ecology of the benthic ciliated protozoa of Alligator Harbor, Florida. Arch. Protistenk., 106, 465–534.
B u t l e r G. L. 1977: Algae and pesticides. Res. Rev., 66, 19–62.
C o o l e y N. R., K e l t h e r J. M. and F o r e s t e r J. 1972: Mirex and Arodor 1254. Effect and accumulation by *Tetrahymena pyriformis* Strain W. J. Protozool., 19, 636–638.
C o x J. L. 1970: Low ambient level uptake of ^{14}C-DDT by three species of marine phytoplankton. Bull. Environ. Contam. Toxicol., 5, 218–221.
C o x J. L. 1972: DDT residues in marine phytoplankton. Res. Rev., 44, 23–28.
F e n c h e l T. 1967: The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. Ophelia, 4, 121–137.
F r e n c h A. L. and H o o p i n g a r n e r E. 1970: Dechlorination of DDT by membranes isolated from *Escherichia coli.* J. Econ. Entomol., 63, 756–759.
F r e n c h J. E. 1976: The effects of the chlorinated polycyclic hydrocarbons, p,p'-DDT and polychlorinated biphenyls (PCBs) on the flagellated protozoan, *Crithidia fasciculata.* Diss. Abstr., Int., 13, 37, 96–97.
G r e g o r y W. W., R e e d J. R. and P r i e s t e r L. E. 1969: Accumulation of parathion and DDT by some algae and protozoa. J. Protozool., 16, 69–71.
J o h n s e n B. T. and K e n n e d y J. O. 1973: Biomagnification of p,p'-DDT and methoxychlor by bacteria. Appl. Microbiol., 26, 66–71.
J o h n s o n R. E. 1976: DDT metabolism in microbial system. Res. Rev. 61, 1–28.
J u e n g s t E. W. and A l e x a n d e r M. 1976: Conversion of 1.1-trichloro-2.2-bis (p-chlorophenyl ethane (DDT) to water soluble products by microorganisms. Agr. Food Chem., 29, 111–115.
K e i l J. E. and P r i e s t e r L. E. 1969: DDT uptake and metabolism of a marine diatom. Bull. Environ. Contam. Toxicol., 4, 169–173.
K e n a g a E. E. 1972: Guidelines for environment study of pesticides: determination of bioconcentration potential. Res. Rev., 44, 73–113.
L i n c e r J. L. 1975: DDE induced egg shell thining in American Kestrel. A comparison of the field situation and laboratory results. J. Appl. Ecol., 12, 781–793.
N e n d o r f S. and K h a n M. A. O. 1975: Pick up and metabolism of DDT,

dieldrin and photodieldrin by fresh water alga (*Ankistrodesmus amalloides*) and microcrustacean (*Daphinia pulex*), Bull. Environ. Contam. Toxicol., 13, 443–450.

R a n d a l l J. F. and J a c k s o n S. 1958: Fine structure in *Stentor polmorphus*. J. Biophys. Biochem. Cytol., 4, 807.

R i c e C. P. and S i k k a H. C. 1973: Uptake and metabolism of DDT by six species of algae. J. Agr. Food Chem., 21, 148–152.

R u p Lal, S a x e n a D. M. and A g a r w a l H. C. 1978: Biocentration of DDT and its metabolites in a ciliate protozoan. *Tetrahymena pyriformis* and its relationship with their octanol/hexane: Water partition coefficients. Proceedings of the All India Symposium on Pesticide in the Environment, Bangalore, India.

R u p Lal and S a x e n a D. M. 1979: Cytological and biochemical effects of pesticides on microorganisms. Res. Rev., 73, 49–86.

S o d e r g r a n A. 1968: Uptake and accumulation of ^{14}C-DDT by *Chlorella* sp. (*Chlorophyceae*). Oikos, 19, 126–138.

W e d e m e y e r G. 1967 a: Dechlorination of DDT by *Aerobacter aerogenes*. Metabolic products. Appl. Microbiol., 15, 569–574.

W e d e m e y e r G. B. 1967 b: Biodegradation of DDT intermediate in dichlorophenyl acetic acid metabolism by *Aerobacter aerogenes*. Appl. Microbiol., 15, 1494–1495.

W i l l i a m s P. P. 1977: Metabolism of synthetic organic pesticides by anaerobic microorganisms. Res. Rev., 66, 63–139.

Vassil GOLEMANSKY

Description de trois thécamoebiens (*Protozoa: Rhizopodea*) nouveaux des eaux souterraines littorales des mers

Three New Thecamoeba (*Protozoa, Rhizopodea*) from Psammon of Marine Littoral Zone

Synopsis. Three new taxons of testate amoebae are described: *Campascus interstitialis* sp. n., *Centropyxiella patystoma* sp. n. et *Cryptodifflugia paludosa* sp. n. The three species inhabit the underground supralittoral waters of the seas and are considered at psammobionts.

Campascus interstitialis sp. n. Fig. 1, a–d, Pl. I, 1–4

Description: La thèque est allongée, plagiostome, en forme de bouteille à goulot recourbé, incolore et transparente. La base de la thèque est arrondie, rarement ogivale. La section transversale est circulaire. Le pseudostome s'ouvre sur un col très court. Il est muni d'une collerette chitineuse très délicate. Le pseudostome est rond, rarement largement oval.

La thèque est chitinoide et formée d'une membrane sur laquelle sont incorporés des idiosomes et rarement de xénosomes plats, serrés les uns contre les autres. Parfois ils donnent l'aspect d'une réticulation fine de la thèque, semblable à celle d'une *Cyphoderia*.

Le corps cytoplasmique est clair et hyalin, avec un seul noyau. Les filopodes de nombre 2–3 sont longues et très fins.

Les dimensions observées: longueur de la thèque: 32–45 µm, largeur (diamètre) de la thèque: 15–23.5 µm. Pseudostome: 8–11 µm, collerette: 9–15 µm.

Discussion: Jusqu'à présent cinq espèces de genre *Campascus* sont connues de biotopes dulçaquicoles et muscicoles. Parmi elles la plus proche de la nouvelle espèce est *C. minutus* Penard. *C. intersti-*
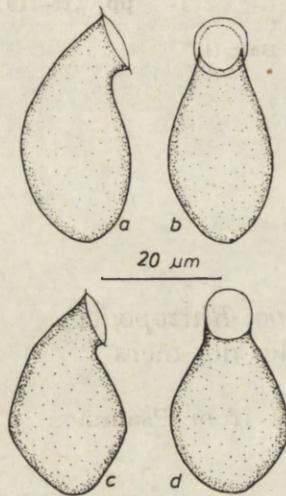
tialis sp. n. diffère de *C. minutus* Penard par ses dimensions plus petites, par sa section transversale triangulaire et surtout par son écologie. Rappelons que *C. minutus* Penard habite les lacs intercontinentaux et sa longueur varie de 50 à 60 µm (P e n a r d 1902, 1908, C h a r d e z 1965).

E c o l o g i e: *C. interstitialis* sp. n. habite les eaux souterraines des plages sableuses de la Mer Noire, Mer Baltique et l'Atlantique. C'est une espèce euryhaline observée chez une salinité de l'eau variant de 3.4‰ (Mer Baltique, Dziwnów, 29.VII.1969) à 22.85‰. (Atlantique, Santa Maria del Mar, 2.XII.1967). Il semble que c'est un psammobionte stricte de supralittoral sableux des mers.

Centropyxiella platystoma sp. n. Fig. 2 a–c, Pl. II, 1

D e s c r i p t i o n: La thèque est allongée, plagiostome, comprimée dorso-ventralement et arrondie en arrière. Elle est incolore et semitransparente. De vue ventrale le pseudostome est rond ou légérement oval et disposé au centre d'un élargissement de la thèque. De vue latérale cet
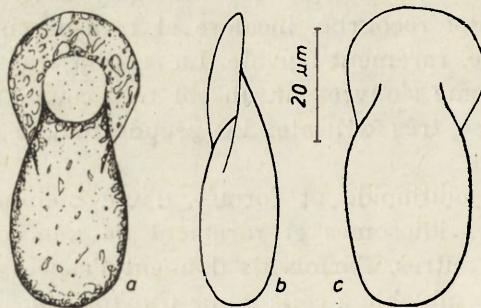
Fig. 2. *Centropyxiella platystoma* sp. n.
a — vue ventrale, b — profil, c — vue dorsale

élargissement à l'aspect d'une large visière, recouvrant le pseudostome. Les bords latéraux de cette visière commencent de la première tierce de la thèque. Chez tous les cas observés la largeur de la visière est plus grande que la largeur de la thèque. La partie ventrale de la thèque est plate, tandis que la partie dorsale est bombée.

La thèque est formée de xénosomes et d'idiosomes plats et poly-morphes, collés sur une base chitinoide. Parfois dans la région de la visière et du pseudostome on observe des xénosomes plus gros comme chez les autres espèces du genre *Centropyxiella* (V a l k a n o v 1970, G o l e m a n s k y 1971).

Les dimensions de la thèque varient en diapazon large, mais ne dé-passent pas 65 µm de longueur. Longueur de la thèque: 45–64 µm, largeur de la thèque: 22–31 µm, épaisseur de la thèque: 14–19 µm, pseudostome: 13–18 µm, largeur de la visière: 28–38 µm.

D i s c u s s i o n: Elles sont connues jusqu'à présent six espèces du genre *Centropyxiella,* habitant exclusivement les eaux souterraines litto-rales des mers. Chez toutes ces espèces le pseudostome est disposé au centre d'un élargissement de la thèque, qui en vue ventrale a l'aspect d'un disque entourant l'ouverture buccale. *C. platystoma* sp. n. diffère de tous les espèces connues du genre par la présence d'une large visière, dont les bords commencent de la partie ventrale de la thèque à côté du pseudostome.

E c o l o g i e: *C. platystoma* sp. n. est trouvée jusqu'à maintenant dans trois stations de plages bulgares de la Mer Noire (Potamjata, Arkoutino et Nessebar) et dans une station sur les côtes polonaises de la Mer Baltique (Świnoujście). Le sable dans les stations citées est assez fin et homogène (Mo = 0.31–0.8 mm). La salinité des eaux varie de 0.69‰ à 2.05‰. En comparaison des autres espèces du genre qui sont eury-halines et survivent une salinité jusqu'à 37‰ *C. platystoma* sp. n. préfère une salinité de l'eau souterraine beaucoup plus base. La présen-ce de la visière caractéristique à côté et devant le pseudostome nous donne la raison de considérer la nouvelle espèce comme un psammo-bionte stricte de la taxocénose thécamoebienne du supralittoral sableu des mers.

Cryptodifflugia paludosa sp. n. Fig. 3 a–c, Pl. II, 2

D e s c r i p t i o n: La thèque est allongée-ovoide, légérement retré-cie dans la région du pseudostome et arrondie en arrière. Elle est sou-vent colorée en jaune-pâle. La section transversale est ovale. Le pseudo-stome est largement oval, presque rond.
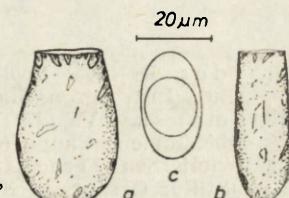
Fig. 3. *Cryptodifflugia paludosa* sp. n. a — vue latérale, b — profil, c — vue apicale

La thèque est chitinoïde et transparente. Souvent on observe des plaquettes chitinoïdes polymorphes collées sur la base ou xénosomes silicieux arrangés surtout dans la région du pseudostome.

Les dimensions observées: longueur de la thèque: 32–45 µm, largeur de la thèque: 20–32 µm, épaisseur de la thèque: 14–18 µm, ouverture du pseudostome: 10–15 × 8–13 µm.

Discussion: *C. paludosa* sp. n. est la troisième espèce du genre *Cryptodifflugia* trouvée dans les eaux souterraines littorales des plages sableuses des mers. Elle diffère de *C. lanceolata* Gol. par ses dimensions supérieures de la thèque et la base arrondie. Rappelons que *C. lanceolata* Gol. ne dépasse jamais 25–30 µm de longueur et sa base est ogivale ou lanceolée. La nouvelle espèce differe de *C. brevicolla* Gol. par ses dimensions près de deux fois plus grandes et surtout par la manque de la collerette caractéristique dans la région du pseudostome. *C. paludosa* sp. n. diffère et des autres espèces du genre, connues de biotopes dulçaquicoles et terricoles, par ses dimensions supérieures, par sa forme caractéristique, la structure de la theque et par sa écologie différente.

Ecologie: *C. paludosa* sp. n. est trouvée dans cinq stations sur les côtes bulgares de la Mer Noire (Achtopol, Mitchurin, Arkoutino, Pomorie et Nessebar), dont les sables sont de dimensions moyennes et relativement homogènes (Mo = 0.31–1.0 mm). La salinité de l'eau souterraines varie de 0.69‰ à 16.26‰. La profondeur maximale dans le sable chez laquelle a été trouvée la nouvelle espèce est 120 cm. Il semble que *C. paludosa* sp. n. est aussi un psammobionte stricte de supralittoral marin.

REMERCIEMENTS

BIBLIOGRAPHIE

Chardez D. 1965: Ecologie générale des Thécamoebiens (*Rhizopoda Testacea*). Bull. Inst. Agron. Stat. Rech. de Gembloux, 33, 3, 307–341.
Golemansky V. 1971: Taxonomische und zoogeographische Notizen über die thekamoebe Fauna (*Rhizopoda, Testacea*) der Küsten grundgewässer der Sowietischen Fernostküste (Japonisches Meer) und der Westküste Kanadas (Stiller Ozean). Arch. Protistenk., 113, 235–249.

P e n a r d E. 1902: Faune rhizopodique du bassin du Léman. Genève, (édit. K ü n-
d i g), 714 pp.
P e n a r d E. 1908: Catalogue des invertébrés de la Suisse. I. Sarcodinés. Genève,
(édit. G e o r g) 165 pp.
V a l k a n o v A. 1970: Beitrag zur Kenntnis der Protozoen des Schwarzen Me-
eres. Zool. Anzeiger, 184, 3/4, 241–290.

EXPLICATIONS DE PLANCHES I–II

Planche I

1, 2: *Campascus interstitialis* sp. n., vue latérale (1 — $\times$ 2000, 2 — $\times$ 2500)
3: Id., vue du pseudostome ($\times$ 2000)
4: Id., grossissement de la collerette ($\times$ 5000)

Planche II

1: *Centropyxiella platystoma* sp. n. ($\times$ 2000)
2: *Cryptodifflugia paludosa* sp. n. ($\times$ 1000)
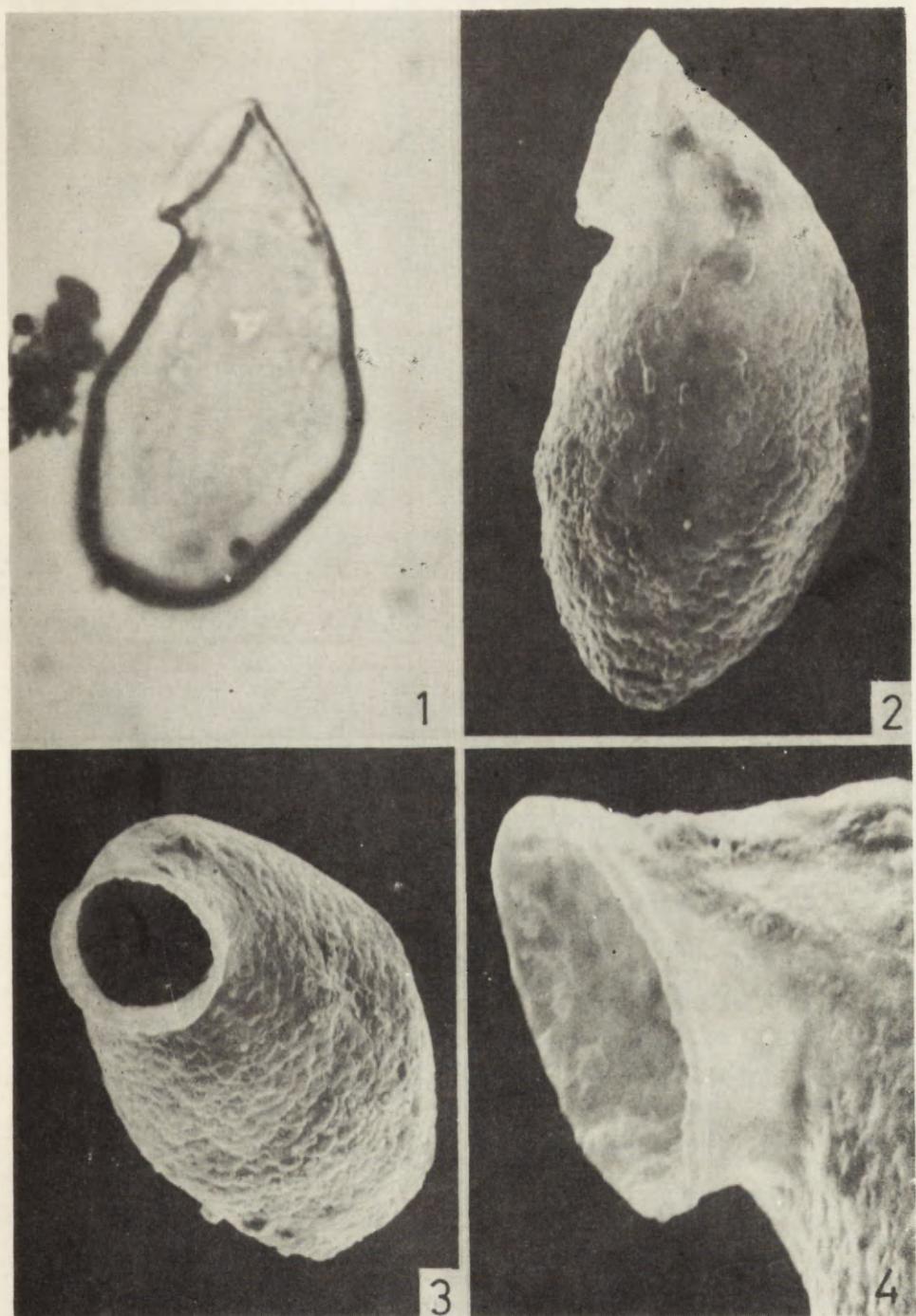
V. Golemansky   auctor phot.

V. Golemansky

auctor phot.

N. K. SARKAR[1] and D. P. HALDAR

Cephaline Gregarine *Actinocephalus ellipsoidus* sp. n. Parasite of an Odonate *Ischnura delicata* Hagen from India

Synopsis. A new species of cephaline gregarine (*Protozoa: Sporozoa*) *Actinocephalus ellipsoidus* sp. n. is described from the midgut of an odonate *Ischnura delicata* Hagen. The gregarine has the ratios of LP : : TL = 1 : 7.6 and WP : WD = 1 : 1.3. It is compared with the other two species of this genus described from odonate insects to establish its distinctiveness.

Stein (1848) created the genus *Actinocephalus* to accommodate a cephaline gregarine *Actinocephalus lucani* from *Lucanus* sp. Frantzius (1848) transferred *Gregarina conica* Dufour, 1837 to the new genus *Actinocephalus* Stein and named it *A. conicus* (Dufour). Watson (1916) observed that *A. lucani* Stein and *A. conicus* (Dufour) Frantzius were the same species and redescribed them as *A. conicus* (Dufour) Frantzius, 1848: Dufour (1837) having described the species first and Frantzius (1848) having given its proper taxonomic status. Since then Schneider (1875), Léger (1892, 1899), Crawley (1903), Wellmer (1911), Ellis (1913, a, b), Triffit (1927), Théodoridès (1955), Théodoridès and Jolivet (1959), Tuzet and Tarroux (1959), Stein (1960), Rodgi and Ball (1961), Desportes (1963), Théodoridès and Desportes (1966, 1967), Ormières (1967), Geus (1969), and Sarkar and Chakravarty (1969) described many new species of cephaline gregarines under the genus *Actinocephalus* Stein from various arthropod hosts. Of these, only two species have been recorded from odonate insects. The first, one, *Actinocephalus sieboldii* (Kölliker)

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from the larvae of several species of *Agrion* sp., was first described as *Gregarina sieboldii* by Kölliker in 1848 and later transferred to this genus by F r a n t z i u s (1848). This revision was also accepted by K a m m (1922) and G e u s (1969). The second species, *A. ceriagrionae* was described by S a r k a r and C h a k r a v a r t y (1969) from *Ceriagrion coromandelianum* (Fabr.).

While examining the various arthropods for their protozoan parasites, a cephaline gregarine has been obtained from the midgut of the odonate *Ischnura delicata* Hagen from West Bengal, India. It is described hereunder as new species for several of its special features.

Materials and Methods

The insects were brought alive to the laboratory and their alimentary canals were examined in 0.5% saline solution under a dissecting binocular microscope. Smears of infected midgut contents were made on grease-free slides. The methods employed have been elaborated by us elsewhere (H a l d a r and S a r k a r 1979). The figures have been drawn with the aid of camera lucida. The ratios used are the ratio of length of protomerite to total length (LP : TL) and the ratio of width of protomerite to width of deutomerite (WP : WD).

Observations

Structure of the Trophozoite and Sporadin

The earliest stage obtained from the migdut contents is a trophozoite having epimerite, protomerite and deutomerite (Fig. 1 *1*). The epimerite is globular, placed on a short cylindrical neck. There is a stellate plate on the dorsal surface of the epimerite, bearing rudiments of 7–9 radiating processes. The protomerite is ovoidal, followed by a fusiform deutomerite which bears the nucleus. When fully grown, the trophozoites become elongated, vermiform bodies (Fig. 1 *2*) measuring 307.7 µm in length and 50.0 µm in width in the average. The epimerite now becomes disc-like with nine slightly backwardly directed pointed processes (Fig. 1 *3*). The epimerite is placed on a short neck. The conical protomerite is separated from the neck by a septum. The deutomerite is elongated and tapers posteriorly to a pointed end. The ovoidal nucleus, containing a few fine granules, is placed anteriorly in this segment.

The sporadins are elongated and solitary having a conical protomerite and a fusiform deutomerite (Fig. 1 *4*). In the average the sporadins are 736.0 µm in length and 81.9 µm in width. The ovoidal nucleus in the deutomerite is situated immediately below the septum. The nucleus measures 37.5 µm × 25.8 µm in the average.
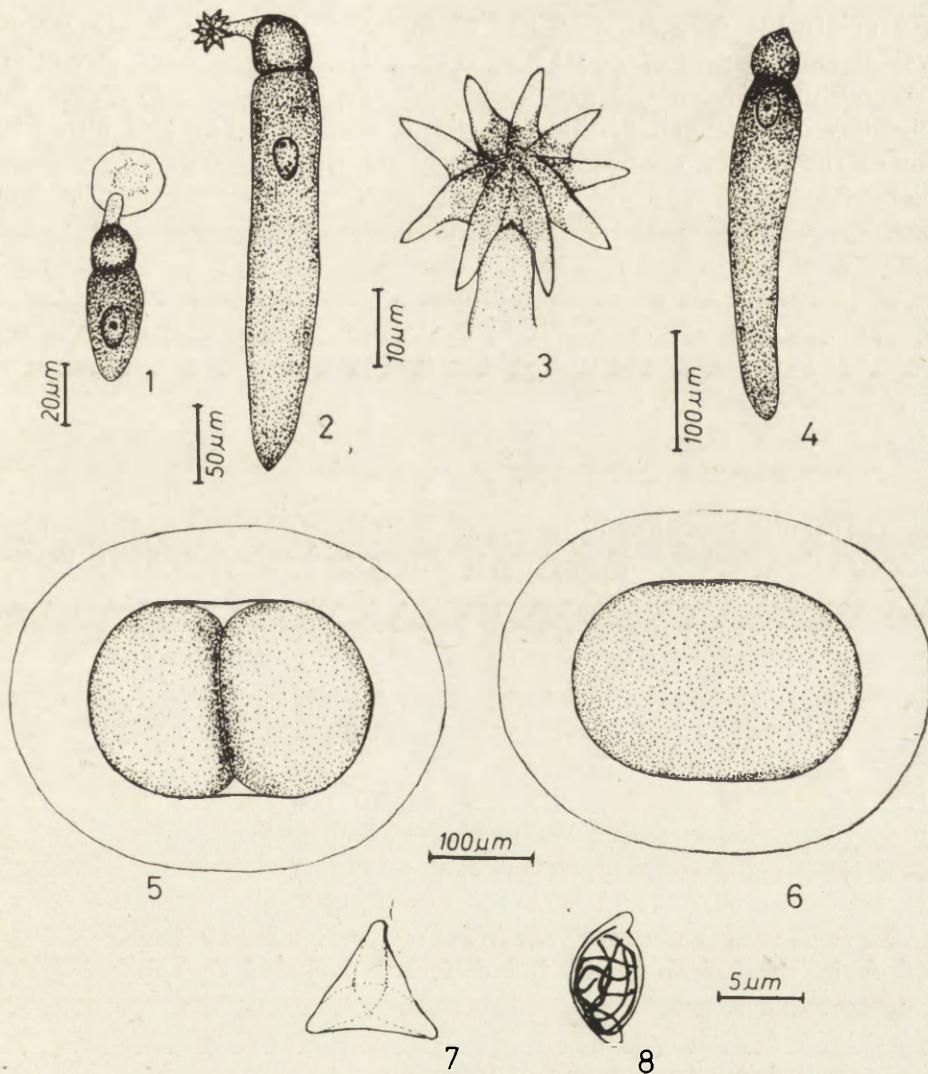
Fig. 1 *1–8*. Camera lucida drawings of *Actinocephalus ellipsoidus* sp. n. *1* — Trophozoite — early stage, *2* — *Trophozoite* — fully grown, *3* — Epimerite — enlarged, *4* — Sporadin — fully grown, *5* — Gametocyst — early stage, *6* — Gametocyst — mature, *7* — Spores, in the form of triangle, *8* — A spore, with filiform sporozoites inside

Gametocyst and Spore

The gametocysts are rarely seen and only one such cyst was found from the midgut of an infected odonate in association with large number of trophozoites and sporadins. It is milky white, ellipsoidal, covered by a thin cyst wall. It measures 276.0 µm × 190.0 µm in dimension. There

is a gelatinous ectocyst, 85.0 µm in thickness. The cyst encloses two ovoidal gametocytes of equal size (Fig. 1 5). After 24 h of formation, the septum between the gametocytes disappears (Fig. 1 6). After the third day of development, the ectocyst disintegrates. The cyst bursts by simple rupture of the cyst wall after the fifth day. The spores are released mostly in clusters of three, in the form of a triangle (Fig. 1 7). Each spore is biconical, and measures 8.0 µm $\times$ 4.5 µm bent in the middle with eight filiform sporozoites inside it (Fig. 1 8).

The examination of numerous serial sections of the heavily infected midguts of the hosts has failed to locate any intracellular stage of the gregarine. This indicates that the early development of the gregarine is probably extracellular.

Measurements in µm

The summary of measurements of the different parts of the trophozoites and sporadins with the mean within parenthesis is given below:

Trophozoites
TL = 67.8 — 458.3 (307.7); LNc = 4.2 — 41.7 (29.2);
DE = 10.6 — 25.0 (18.8);
LP = 33.3 — 41.7 (36.4); WP = 33.3 (33.3);
LD = 200.0 — 386.7 (275.0); WD = 33.3 — 50.0 (40.6).

Sporadins
TL = 133.6 — 916.8 (736.0);
LP = 25.0 — 133.3 (57.7); WP = 16.7 — 200.0 (64.8);
LD = 108.6 — 783.5 (345.0); WD = 33.4 — 241.7 (81.9);
LN = 12.5 — 50.0 37.5); WN = 11.6 — 50.0 (25.8);
    LP: TL = 1; 4.4—13.7 (7.6); WP: WD = 1; 0.9—2.0 (1.3).
Details of measurements of the different parts of 25 specimens are given in Table 1.

Seasonal Intensity and Site of Infection

The insects were collected during post-rainy season of the year and 20.0% of them were found to be infected in their midguts.

Material

Holotype — on slide No. Od6k-3, prepared from the midgut content of *Ischnura delicata* Hagen, collected at Kalyani, West Bengal, India on 28 October, 1978 and deposited at the Department of Zoology, University of Kalyani. Paratype — many, on above numbered slide and on other slides.

Table 1

Showing details of measurements (in μm) of the different parts of 25 specimen
of *Actinocephalus ellipsoidus* sp. n.

Serial No.	TL	DE	LP	LD	LNc	LN	WP	WD	WN	LP:TL	WP:WD
1	383.4	20.8	37.5	233.4	41.7	33.3	33.3	50.0	12.5	1 : 10.2	1 : 1.5
2	275.0	16.7	33.3	200.0	25.0	25.0	33.3	41.7	—	1 : 8.2	1 : 1.6
3	354.2	20.8	41.7	250.0	41.7	—	33.3	33.3	—	1 : 8.5	1 : 1.0
4	458.3	25.0	33.3	366.7	33.3	33.3	33.3	37.5	12.5	1 : 13.7	1 : 1.2
5	67.8	10.6	12.7	40.3	4.2	12.7	16.9	25.4	11.6	1 : 5.3	1 : 1.5
6	354.2	—	37.5	316.7	—	37.5	37.5	41.7	16.7	1 : 9.4	1 : 1.1
7	166.6	—	33.3	133.3	—	25.0	33.3	50.0	16.7	1 : 5.0	1 : 1.5
8	204.2	—	37.5	166.7	—	33.3	37.5	41.7	20.8	1 : 5.4	1 : 1.1
9	208.3	—	25.0	183.3	—	—	33.3	50.0	—	1 : 8.3	1 : 1.5
10	333.4	—	37.5	295.9	—	—	37.5	54.2	—	1 : 8.9	1 : 1.4
11	416.7	—	54.2	362.5	—	—	50.0	66.7	—	1 : 7.7	1 : 1.3
12	366.7	—	33.3	333.4	—	—	41.7	37.5	—	1 : 11.0	1 : 0.9
13	316.7	—	33.3	283.4	—	—	37.5	41.7	—	1 : 9.5	1 : 1.1
14	334.0	—	41.7	292.3	—	—	37.5	50.0	—	1 : 8.0	1 : 1.3
15	283.9	—	33.4	250.5	—	—	37.5	50.0	—	1 : 8.5	1 : 1.3
16	217.1	—	33.4	183.7	—	—	37.5	54.3	—	1 : 6.5	1 : 1.4
17	133.6	—	25.0	108.6	—	—	16.7	33.4	—	1 : 5.3	1 : 2.0
18	308.9	—	41.7	267.2	—	—	41.7	41.7	—	1 : 7.4	1 : 1.0
19	326.5	—	38.1	288.4	—	—	42.4	50.9	—	1 : 8.5	1 : 1.2
20	433.4	—	83.3	350.1	—	41.6	66.7	100.0	20.0	1 : 5.2	1 : 1.5
21	366.7	—	83.3	283.4	—	41.6	66.7	91.6	33.3	1 : 4.4	1 : 1.4
22	600.1	—	83.3	516.8	—	41.6	75.0	83.3	33.3	1 : 7.2	1 : 1.1
23	916.8	—	133.3	783.5	—	50.0	200.0	225.0	50.0	1 : 6.9	1 : 1.1
24	850.1	—	133.3	716.8	—	50.0	175.0	241.7	50.0	1 : 6.4	1 : 1.4
25	916.8	—	133.3	783.5	—	50.0	191.7	233.4	33.3	1 : 6.9	1 : 1.2

Discussion

Stein (1848) emphasized the following characters for the genus *Actinocephalus*:

(1) epimerite sessile or with short neck;

(2) eight to ten short spines or simple digitiform processes in the epimerite;

(3) smooth, biconical spores.

The gragarine from *Ischnura delicata* Hagen has almost similar features and thus belongs to this genus. However, a comparison of the present gregarine with the other two species of this genus reported from odonate insects viz. *Actinocephalus sieboldii* (Kölliker) Frantzius, 1848 and *A. ceriagrionae* Sarkar and Chakravarty, 1969, given in Table 2

Table 2

A comparison of the two species of the genus *Actinocephalus* Stein, 1848, described from the odonate insects, with *Actinocephalus ellipsoidus* sp. n.

Characters	Parasites		
Body	*A. sieboldii* (Kolliker) Sporadin solitary, obese, "0.162 to 0.188" (Kolliker) in length	*A. ceriagrionae* trophozoite elongated; sporadin large, elongated	*A. ellipsoidus* sp. n. trophozoite elongated, vermiform; sporadin elongated, 736.0 μm in length (average)
Epimerite	Four to six sharp recurved hooks, set upon a long cylindrical neck	Globular crown with sixteen or more short stout lateral digitiform processes, set upon a short cylindrical retractile neck	Disc-like with nine slightly bent processes, set upon a short neck
Nucleus	Spherical with one or two large karyosomes	Ellipsoidal	Ovoidal with a few fine granules
LP : TL	1 : 4–5	1 : 5.5	1 : 4.4–13.7
WP : WD	1.3 : 1	—	1 : 1.3
Gametocyst	—	Spherical, white; dehisces by simple rupture after 90–116 h	Ellipsoidal, milky white, ectocyst thick and uniform; dehisces by simple rupture after 120 h
Spore	—	Biconical; 2.84 μm × 4.28 μm	Biconical, bent in the middle, released in the cluster of three forming a triangle; 8.0 μm × 4.5 μm
Development	—	—	Extracellular
Host	Several species of *Agrion* sp.	*Ceriagrion coromandelianum* (Fabr.)	*Ischnura delicata* Hagen
References	Frantzius (1848), Kamm (1922), Geus (1969)	Sarkar and Chakravarty (1969)	Present study

clearly indicates its distinctiveness.

The distinctive features of the gregarine are summarized below:

(1) discoidal epimerite with nine finger-like processes;

(2) ellipsoidal gametocyst;

(3) difference in measurements of various body parts and of spores; and

(4) separate host.

The gregarine is, therefore, considered to be a new species for which the name *Actinocephalus ellipsoidus* sp. n. is given. The specific trivial name is derived from the characteristic ellipsoidal shape of its gametocyst.

REFERENCES

C r a w l e y H. 1903: The polycystid gregarines of the United States. Proc. Acad. Nat. Sci. Philad. 55, 632–644.

D e s p o r t e s I. 1963: Quelques Gregarines parasites d'insects aquatiques de France. Ann. Parasit. Hum. Comp., 38, 341–377.

D u f o u r L. 1837: Recherches sur quelques entozoaires et larves parasite des insects Orthopteres et Hymenopteres. Ann. Sci. Nat., 7, 5–20.

E l l i s M. M. 1913 a: A descriptive list of the cephaline gregarines of the New World. Trans. Amper. Microsc. Soc. 322, 259–296.

E l l i s M. M. 1913 b: Three gregarines from Louisiana. Zool. Anz., 42, 200–202.

F r a n t z i u s A. 1848: Einige nachtragliche Bemer-Kugen über Gregarinen. Arch. Naturgesch., 14, 188–196.

G e u s A. 1969: Sporentierchen, *Sporozoa* Die Gregarinida. Die Tierwelt Deutschlands. 57 Teil.: 608 p.

H a l d a r D. P. and S a r k a r N. K. 1979: Cephaline gregarine *Leidyana linguacta* sp. n. parasite of a gryllid *Pteronemobius concolor* Walker from India. Acta Protozool., 18, 435–440.

K a m m M. W. 1922: Studies on Gregarines. II. Synopsis of the polycystid gregarines of the world excluding those from the *Myriapoda, Orthoptera* and *Coleoptera.* Ill. Biol. Monogr., 7, 1–104.

L é g e r L. 1892: Recherches sur les gregarines. Tabl. Zool., 31, 1–182.

L é g e r L. 1899: Sur les Gregarines des Dipteres et description d'une espece nouvelle. Ann. Soc. Sci. ent. France 68, 526–533.

O r m i è r e s R. 1967: Apropos de quelques gregarines de *Blaps* de Grece. Vie et Millieu, 18, 247–250.

R o d g i S. S. and B a l l G. H. 1961: New species of gregarines from millipedes of Mysore state, India. J. Protozool., 8, 162–179.

K ö l l i k e r A. 1848: Beitrage zur Kenntniss neiderer Thiere. Z. wiss. Zool., 1, 1–37.

S a r k a r A. and C h a k r a v a r t y M. M. 1969: Gregarines (*Protozoa : Sporozoa*) from insects. I. New cephaline gregarines of the family *Actinocephalidae.* Proc. Zool. Soc. Calcutta, 22, 17–29.

S c h n e i d e r A. 1875: Contribution a l'histoire des gregarines des invertebrates de Paris et de Roscoff. Arch. Zool. Exp. gen., 5, 493–604.

S t e i n F. 1848: Uber die Nature der Gregarinen. Arch. Anat. Physiol. 182–223.

S t e i n G. A. 1960: Gregarines of aquatic arthropods of the Lakes of Kerelia. Zool. Zh., 39, 1135–1144.

T h é o d o r i d è s J. 1955: Contribution a l'etude des parasites et phoretique de coleopteres terrestres. Vie et Milieu, suppl. 4, 302 p.

T h é o d o r i d è s J. and D e s p o r t e s I. 1966: Quelques Gregarines de Coleopteres du Laos. Protistologica, 2, 53–58.

T h é o d o r i d è s J. and Desportes I. 1967: Quatre nouvelles Gregarines parasites de Coleopteres Tenebrionides du Laos. Protistologica, 3, 147–153.

T h é o d o r i d è s J. and Jolivet P. 1959: Eugregarine parasite de Coleopteres. Explor. Prac. Nat. Albert. Bruxelles, 2, 50–59.

Triffit M. J. 1927: Some cephaline gregarines in British Myriapods. Protozoology, 3, 65–74.
Tuzet O. and Tarroux M. 1959: *Actinocephalus puisseguri* sp. n. Gregarine parasite de *Chrysocarabus panctato-auratus* Germer sous-espece *barthei*. Laponge, Vie et Milieu, 10, 408–411.
Watson M. E. 1916: Studies on Gregarines. Ill. Biol. Monogr., 2, 211–468.
Wellmer L. 1911: Sporozoen Ostprussischer Arthropoden. Sch. Physik. Oken. Gesellsch. Konigsb., 52, 103–164.

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Isospora capistrata sp. n. from a Black-headed Sibia, Leioptila capistrata Vigors

Synopsis. Isospora capistrata sp. n. (Eimeriidae) is described from the faeces of a black-headed sibia, Leioptila capistrata. Vigors collected at Darjeeling (altitude 2100 m). Trophozoites, schizonts and garmetocytes are observed in the sections of small intestine. Oocysts are 21.6 μm (mean) in diameter and sporocysts measure 14.6 μm by 9.6 μm (mean).

This is the third instalment of the series dealing with isosporan parasite from North-Eastern Himalayan bird. In course of a survey a new species of *Isospora* is found in the faeces of a black-headed sibia, *Leioptila capistrata* Vigors collected at Darjeeling (altitude 2100 m). Literature on avian *Isospora* from Himalayan region revealed that R a y et al. (1952) described for the first time five species of *Isospora* from birds obtained at Mukteswar altitude (2460 m). After a long interval, Sinha et al. (1978) recorded a species of *Isospora* from a bird caught at Darjeeling. S i n h a and S i n h a (1979) again described another species of *Isospora* from the same locality. A brief note on the occurrence of *Isospora.* in *L. capistrata* was published by Sinha et al. (1979).

Materials and Methods

Faeces of six black-headed sibia were examined and two were found to contain oocysts. Oocysts were kept in 2.5% potassium dichromate solution for sporulation at room temperature (12°C–15°C). Infected birds were killed and small pieces of intestine were fixed in Carnoy's fixative, sectioned and stained with haematoxylin-eosin as well as iron-haematoxylin. Drawings were made under camera lucida and measurements were taken.

Results

Isospora capistrata sp. n.

Type Host: *Leioptila capistrata* Vigors
Type Locality: Darjeeling (altitude 2100 m), West Bengal, India
Localization: Intestine.
Sporulation Time: 36–48 h.

T r o p h o z o i t e (Fig. 1 *1*) is ovoid measuring 5.6 μm by 2.8 μm. It has got a thin outer covering and the nucleus is subcentral. Multinucleated schizont (Fig. 1 *2*) measures 7.9 μm by 7.6 μm and contains nine nuclei.
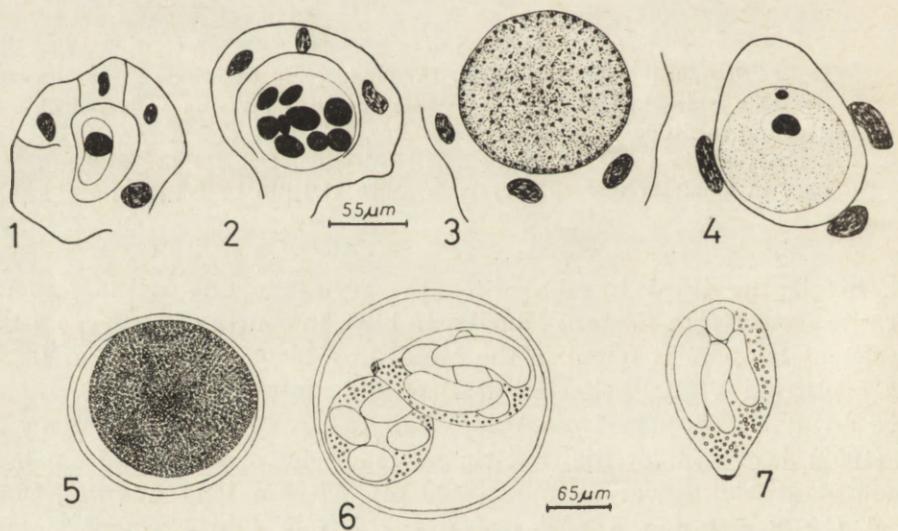
Fig. 1. *1–7.* Camera lucida drawings of *Isospora capistrata* sp. n. *1* — Trophozoite, *2* — Multinucleated schizont, *3* — Mature microgamont, *4* — Fertilized macrogamete, *5* — Unsporulated oocyst, *6* — Sporulated oocyst with two sporocysts each containing four sporozoites, *7* — A mature sporocyst. (*1–4* — in the section of small intestine, *5–7* in the faeces)

M i c r o g a m o n t (Fig. 1 *3*) is spherical measuring 13 μm in diameter and contains several small nuclei arranged at the periphery. It contains small comma shaped microgametes. A fertilized macrogamete (Fig. 1 *4*) was subspherical measuring 12 μm by 10 μm and bears two nuclei. A hallow space is present around the endosome of the fertilized macrogamete.

O o c y s t s (Fig. 1 *5–6*) are spherical, measurements twenty of them are 18.2 to 24.6 μm in diameter (mean 21.6 μm). Oocyst wall is smooth

bilayered with an uniform thickness of 0.2 μm. The distance between the two walls is 0.65 μm. Unsporulated oocyst (Fig. 1 5) is coarsely granulated. Sporulated oocyst (Fig. 1 6) develops two sporocysts each of them contains four sporozoites. An oocystic residuum a micropyle, a micropylar cap and a polar granule are absent.

A mature sporocyst (Fig. 1 7) is pyriform in shape with an anterior knob and the posterior rounded end. It is single layered, 0.13 μm in thickness and measures 11 to 16.9 μm (mean 14.6 μm) by 8.8 to 10.4 μm (mean 9.6 μm). A small stieda body and sporocystic residue, 0.3 μm in diameter, are present. Sporozoites are club shaped measuring 7.5 to 10.2 μm (mean 9 μm) by 1.8 to 2.5 μm (mean 2.2 μm).

Diagnosis of *Isopora capistrata* sp. n.

Oocysts spherical, 18.2 to 24.6 μm (mean 21.6 μm), oocyst wall smooth, bilayered, 0.2 μm in thickness, oocystic residuum, micropyle, micropylar cap, polar body absent, sporocysts pyriform, single layered, 0.13 μm in thickness, 11 to 16.9 μm (mean 14.6 μm) by 8.8 to 10.2 μm (mean 9.6 μm) stieda body, sporocystic residue present, sporozoites club shaped 7.5 to 10.2 μm (mean 9 μm) by 1.8 to 2.5 μm (mean 2.2 μm).

Discussion

I. capistrata sp. n. resembles superficially *I. corvae* and *I. garrulae* Ray et al. (1952) because of bilayered oocyst and without a micropyle but again differs sufficiently on account of oocystic residuum which is absent in the present parasite. *I. bengalensis* M a n d a l and C h a k-r a v a r t y (1964) also draws some affinity but again can be differentiated due to the presence of a micropyle. Moreover, the parasite under report does not fit any other known species of the genus and is considered new. The species name is given after the specific name of the host bird. The holotype and paratypes will be deposited to the National Zoological Collection of Zoological Survey of India, Calcutta.

REFERENCES

M a n d a l A. K. and C h a k r a v a r t y M. M. 1964: Studies on some aspects of avian coccidia (*Protozoa : Sporozoa*) 2. Some new species of the genus *Isospora* Schneider, 1881. Proc. Zool. Soc., (Calcutta) 17, 35–45.

Ray D. K., Shivnani G. A., Oommen M. and Bhaskaran R. 1952: A study on the coccidia of some Himalayan birds. Proc. Zool. Soc. Bengal. 5, 141–147.
Sinha C. K., Sinha S., Chattoraj N., Bandyopadhyay S. and Ghosh K. 1978: *Isospora ceylonensis* sp. n. from a grey-headed fly catcher, *Culicicapa ceylonensis calochrysea* Oberholser. Acta Protozool. 17, 503–507.
Sinha C. K., Sinha S., Chattoraj N., Ghosh K. and Bandyopadhyay S. 1979: Record of an *Isospora* Schneider, 1881, from a black-headed sibia. *Leioptila capistrata* Vigors. Proc. 66th Ind. Sc. Cong. p. 113.
Sinha C. K. and Sinha S. 1979: *Isospora concinnus* sp. n. from a Sikkim red-headed tit, *Aegithalos consinnus rubricapillus* (Ticehurst). Acta Protozool. 18, 455–460.

ANNOUNCEMENT

Third International Congress of Ecology will take place in Warsaw, Poland, 5–11 September 1982, under the auspices of: International Association for Ecology (INTECOL), Ecology Section, International Union for Biological Sciences (IUBS) and Polish Academy of Sciences.

A major theme of the Congress will be: "Ecology as a basis for environmental management and human welfare". The Congress will consist of six main plenary sessions with related concurrent sessions and also of the special symposia organized by INTECOL Working Groups: A. Processes integrating ecosystems and landscape. A.1. Energy flow as the factor integrating environmental units. A.2. Mechanisms of transport, exchange and cumulation of matter in environmental units. A.3. Biocenotic and population codependences between ecosystems. A.4. Ecology of contact zones, ecotones and barrier zones between ecosystems. A.5. Problems linked with study of multiecosystemal units. A.6. Techniques of studying large scale ecological systems (including modelling). B. Advancements in the understanding of organismal interactions in ecological systems. B.1. The role of parasites in ecological system. B.2. Ecology and ethology — a synthetic approach. B.3. Demography of plants. B.4. Predator-prey relationships. B.5. Life history strategies and tactics. B.6. Evolutionary ecology and genetics. C. Restoration and stabilization of environmental quality. C.1. Theory and practice of rehabilitation methods applied to aquatic system. C.2. Ecological problems of the landscape reconstruction. C.3. Ecological monitoring as a programme of control and supervision over the qualitative state of environment and its components. C.4. Protected environments and biosphere conservancies. C.5. Ecological consequences of extinction. C.6. Strategies to achieve stability in ecological system. D. Ecological basis of intensifying biological production. D.1. The impact of altering the environment and technology on productivity and stability of agricultural and forest systems. D.2. The consequences of control of pest species on production and environmental quality. D.3. The environmental consequences of crop-rotation, selection and landscape planning; ecological guidelines for agriculture and forest landscape planning. D.4. The ecological significance of traditional and alternative agriculture. D.5. The impact of agricultural activities on patterns of energy and matter cycling in temperate and tropical systems. D.6. The consequences of aquaculture on ecology of aquatic systems. E. Technological impact of man on environment. E.1. Transport, cycling and accumulation of toxicants and pollutants in environment. E.2. Impact of pollutants on various ecosystems; structure and function. E.3. Bioindication of pollutants on organismic and ecosystem levels. E.4. Salinisation of soil and waters. E.5. Radioactive contamination of environment. E.6. Patterns, processes and strategies of ecosystem recovery from stress. F. Utilisation of ecological expertise in resource management and regional planning. Sessions for following INTECOL Working Groups are planned: G.1. Statistical ecology. G.2. Physiological ecology (on data transfer from laboratory to field study and vice versa). G.3. Urban ecology. G.4. Environmental education. G.5. Aquatic ecology (on wetlands). G.6. Granivorous birds. G.7. Special symposium on "Ecology of polar regions".

Each session will be organized by Conveners appointed by the Steering Committee. Oral contributions (15 min.) after selection made by Conveners, and posters' presentations as well as scientific films will form the main possibilities

for active participation. The Official Congress language is English. Registration fees: participants — 100 US $, accompanying persons — 60 US $, students — 60 US $. First Announcement containing all needed details together with Preliminary Application Form will be distributed in the beginning of 1981. All correspondence and request for scientific information should be sent (by airmail) to: Congress Secretariat of III INTCOL Congress, Institute of Ecology, Dziekanów Leśny, 05-150 Łomianki, Poland, or by telex 817378 IE PAN PL.

In preparation:

J. Kaczanowska and T. Garlińska: Endocytosis and Exocytosis of Doublets of *Paramecium tetraurelia* — J. C. Gutierrez, A. Torres and J. Perez-Silva: Excystment Cortical Morphogenesis and Nuclear Processes during Encystment and Excystment in *Laurentiella acuminata* (*Hypotrichida, Oxytrichidae*) — N. Ricci and R. Banchetti: Nuclear Phenomena of Vegetative and Sexual Reproduction in *Oxytricha bifari* Stokes (*Ciliata, Hypotrichida*) — E. Wyroba, G. Bottiroli and P. Giordano: Autofluorescence of Axenically Cultivated *Paramecium aurelia* — S. Fabczak: Electrical Properties of Cell Membrane in *Stentor coeruleus*. III. Influence of changes in Temperature on the Membrane Permeability to Ions — H. Rebandel, A. Gierczak and A. Karpińska: Toxic Action of Colistin and Penicyllins V and G on *Tetrahymena*. I. Lethal Effect and Influence on Multiplication — N. K. Sarkar and D. P. Haldar: Cephaline Gregarine (*Protozoa : Sporozoa*) *Ramicephalus olivacus* sp. n. Parasite of an Odonate *Ceriagrion olivacum* Laidlow from India — N. K. Sarkar and D. P. Haldar: Cephaline Gregarine *Tetractinospora victoris* gen. n. sp. n., Parasite of an Odonate *Ceriagrion coromandelianum* (Fabricius) from India — J. Weiser, C. Kalavati and B. V. Sandeep: *Glugea nemipteri* sp. n. and *Nosema bengalis* sp. n., Two New Microsporidia of *Nemipterus japonicus* in India — J. J. Lipa and J. Bartkowski: Light and Electron Microscope Study of *Amblyospora* (*Thelohania*) *californica* (Kellen et Lipa) Microsporidia in Larvae of *Culex tarsalis* Coq. (*Culicidae*)

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