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ACTA PROTOZOOLOGICA

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The nuclear apparatus of the psammophilic ciliate *Kentrophoros fistulosum* Fauré-Fremiet: structure, divisional reorganization, and ultrastructure

Ядерный аппарат псаммофильной инфузории *Kentrophoros fistulosum* Fauré-Fremiet: строение, реорганизация при делении и ультраструктура

Introduction

Members of the genus *Kentrophoros* Sauerbrey, 1928 (syn. *Centrophorella* Kahl, 1933) are peculiar mouthless ciliates provided with only ventral ciliature and carrying obligatory ectosymbiotic sulphur bacteria on their non-ciliated dorsal side. They belong to the least studied forms among the large group of lower psammophilic ciliates (genera *Trachelocerca*, *Tracheloraphis*, *Trachelonema*, *Loxodes*, *Remanella*, *Geleia*), characterized by the so-called "primary nuclear differentiation" (Raikov 1963, 1967, 1969). These ciliates typically have small, weakly Feulgen stainable diploid macronuclei, which are unable to divide by any way; only their micronuclei divide by mitosis. A number of micronuclear division products differentiate into macronuclei some time during the cycle of each binary cell division. This restores the number of macronuclei which has decreased during the last cell division (since macronuclei are simply distributed between the daughter animals). Such a cycle of nuclear reorganization (putting aside some variations concerning the number of nuclei, the sequence and degree of synchrony of the mitoses etc.) has been discovered in all representatives of the families *Trachelocercidae*, *Loxodidae*, and *Geleiidae* where it has been looked for (for reviews see Raikov 1967, 1969).

In most species of *Kentrophoros*, the nuclear apparatus resembles that of *Remanella*, and it may be supposed that the phenomena of nuclear reorganization accompanying cell division, though not studied specially in *Kentrophoros*, must be alike in both genera. However, one species, *Kentrophoros fistulosum* Fauré-Fremiet, 1950, apparently stands apart in this genus.

In *K. fistulosum*, Fauré-Fremiet (1954) described a cycle of divisional nuclear reorganization unlike that in *Loxodes* or *Remanella* (or any other ciliate having

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diploid macronuclei). According to Fauré-Fremiet, *K. fistulosum* has a complex cycle of nuclear polyploidization alternating with nuclear fragmentation, and its nuclei are differentiated into macro- and micronuclei only at certain stages of this cycle. At first, the micronuclei undergo endomitotic polyploidization which leads to the stage of "polyenergid nuclei" (or "nuclei of type A"). This stage lacks nuclear dualism, since macronuclei previously present in the cell have already degenerated. Then, the "polyenergid nuclei" fragment into "subnuclei" which are small, dense, micronucleus-like nuclei. Fauré-Fremiet believed that some "subnuclei" differentiate into vesicular macronuclei which, however, exist no longer than one interdivision interval and rapidly degenerate; other "subnuclei" become compact micronuclei which begin a new cycle of endomitotic polyploidization. Fauré-Fremiet failed to find micronuclear mitosis in *K. fistulosum*.

Since no endomitotic polyploidization of micronuclei was ever found in any of the species of the *Trachelocercidae — Loxodidae — Geleiidae* group we studied, it seemed of great interest to re-investigate karyologically the very species of Fauré-Fremiet, *K. fistulosum*. This was done using both light and electron microscopy. An ultrastructural study of ectosymbiotic bacteria of *K. fistulosum* and of their role in nutrition of the ciliate host has been published elsewhere (Raikov 1971, 1972 a).

Until now, the ultrastructure of the nuclear apparatus has been investigated only in a few species of ciliates with diploid macronuclei, namely, in *Tracheloraphis caudatus* (Raikov et Dragesco 1969), *Trachelonema sulcata* (Kovaleva and Raikov 1970), and *Kentrophoros latum* (Raikov 1972 b). The nuclear apparatus of *Tracheloraphis caudatus* consists of many nuclear groups, four macronuclei and two micronuclei in each. The nuclear apparatus of *Trachelonema sulcata* comprises numerous free macronuclei and micronuclei. In *Kentrophoros latum*, the macronuclei and the micronuclei are assembled into several groups, each surrounded by multimembranous capsules. Published are also brief data on the fine structure of micronuclei and of free diploid macronuclei of *Loxodes* (Mashansky 1963, De Puytorac et Njiné 1970). However, no ultrastructural data are available concerning the nuclear apparatus of the type peculiar to *Kentrophoros fistulosum*, that with very compact nuclear groups where individual macro- and micronuclei are hardly seen.

Material and methods

The main material used in the present study was collected in September 1968 at the Black sea shore near Sevastopol (the Omega bay)[2]. The rest of the material was collected in November 1970 at the Roscoff Biological Station in France.

The Black sea material was fixed for electron microscopy with 2% osmium tetroxyde in a 1 : 1 mixture of 0.2 M phosphate buffer (pH 7.2) and sea water, 1–2 h on ice. Araldite sections were stained with Reynolds' lead citrate (20–30 min) and counterstained 3–5 min with a saturated solution of uranyl acetate in 90% alcohol.

[2] Collection, fixation and embedding of the Black sea material were carried out by V. G. Kovaleva, whose assistance is most gratefully acknowledged. For description of the Black sea form of *K. fistulosum* see Kovaleva 1966.

The Roscoff material was fixed on ice with 6% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 10% sucrose and 0.03% $CaCl_2$, either for 40 min or overnight. The ciliates were rinsed with cacodylate buffer containing 30% sucrose and postfixed with 2% osmium tetroxyde in the same buffer with 27% sucrose and 0.03% $CaCl_2$ (30 min on ice). Then, the ciliates were washed in four baths of cacodylate buffer with decreasing sucrose concentrations (30%, 20%, 10% and without sucrose), transferred into distilled water and pre-embedded into agar gel (to assemble the ciliates into compact groups convenient to cut). Embedding into Epon followed; the sections were stained 2–3 h with saturated aqueous uranyl acetate, then 10–15 min with Reynolds' lead citrate.

The sections were studied with JEM-5G and JEM-7 electron microscopes at the Institute of Cytology in Leningrad (Black sea material) and with Siemens Elmiskop I and IA microscopes at the Zoology Department of the University of Clermont-Ferrand (both Black sea and Roscoff material)[3].

Light microscopical research was done using both whole mounts and sections of Black sea specimens of *K. fistulosum* (collections of 1968). For whole mounts, the ciliates were fixed and attached to cover glasses with the Nisenbaum (1953) mixture; the preparations were stained with Feulgen, De Lamater's fuchsine, methyl green–pyronin or haemalum. For sections, the ciliates were fixed with sublimate — acetic acid and embedded in paraffin. The sections (5 μ) were stained with Heidenhain's iron haematoxylin, and cytochemically for DNA (Feulgen) or for RNA: with methyl green — pyronin after Brachet, with azure B after Flax and Himes (1952), or with toluidine blue after Brachet. The last three methods were accompanied by RNA digestion controls (pre-treatment of the sections with ribonuclease). For total protein, the sections were stained with mercuric bromphenol blue, and for basic proteins, with fast green at pH 8.2.

Light microscopy

Number of nuclei

Kentrophoros fistulosum is a multinucleate ciliate which has a very inconstant number of nuclei. These are assembled into numerous compact groups comprising both macronuclei and micronuclei and arranged in a series along the body (Pl. I 1–3). Both the number of groups and the number of nuclei in a group are highly variable.

The nuclear groups and their component nuclei were counted in a sample of 64 Black sea individuals of *K. fistulosum* (in whole mounts). The results are represented in Figs. 1 and 2 and in Table 1.

In this random sample, the number of nuclear groups per individual varied from 4 to 43, the distribution curve being clearly asymmetrical: with subdivision of the variation range into nine classes the second was modal, corresponding to the number of nuclear groups 6 to 10 inclusive (Fig. 1). Animals with 11–30 nuclear groups were relatively frequent, but those having more than 30 groups were rare. The number of nuclear groups is proportional to the ciliate's body size (Pl. I 1–3): small individuals always have few nuclei, and numerous nuclear groups occur only in large animals (*K. fistulosum* may attain 3 mm in length).

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[3] The author is most cordially grateful to Professor P. de Puytorac, Head of the Zoology Department of the Clermont-Ferrand University, for providing possibility to work four months in his laboratory during the winter 1970/71 and for numerous stimulating discussions. The author also acknowledges the excellent technical assistance of Mr. and Mme Guillaume, of the same laboratory.

Table 1

Composition of 899 nuclear groups in 64 Black sea individuals of *Kentrophoros fistulosum*

Mi \ Ma+MaA	1	2	3	4	5	6	7	8	9	10	11
0	1	22	9	9	1						
1	7	39	31	6	4						
2	11	38	52	488	11	75	3	22		1	
3	1	5	4	3	1	6		2	1		
4	1	7	1	11	1	5		16		2	
5											
6		1									1

Ma+MaA – the sum of adult and developing macronuclei, Mi – the number of micronuclei. Figures in the Table are frequencies of nuclear groups of the respective composition.

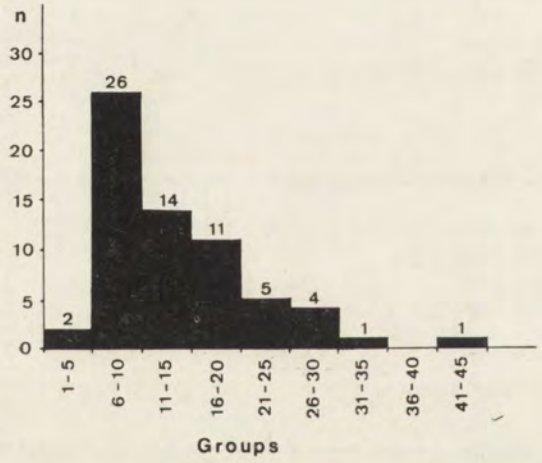

Fig. 1. Histogram of the number of nuclear groups in 64 Black sea specimens of *K. fistulosum*. Abscissae: number of nuclear groups (classes by 5 groups), ordinates: frequencies

The total number of macro- and micronuclei in each of the 64 sample individuals (with no account to how they are arranged in groups) is given in Fig. 2. This diagram shows that the total number of macronuclei per animal varied from 15 to 165 (both adult and developing macronuclei were included), and the total number of micronuclei varied from 5 to 80. A clear positive correlation exists between the number of macronuclei and that of micronuclei: the points, each corresponding to an individual, are arranged along the line representing the 2 : 1 ratio between the macronuclei and the micronuclei. No correlation coefficient was however calculated since both distributions differ from normal ones. 75% of the points are included into an elongate oval which corresponds to 25–85 macronuclei and 10–45 micronuclei (Fig. 2).

The composition of individual nuclear groups is also variable. The same 64 sample animals contained a total of 899 nuclear groups, the composition of which s shown in Table 1. It may be seen that more than a half (54%) of the nuclear

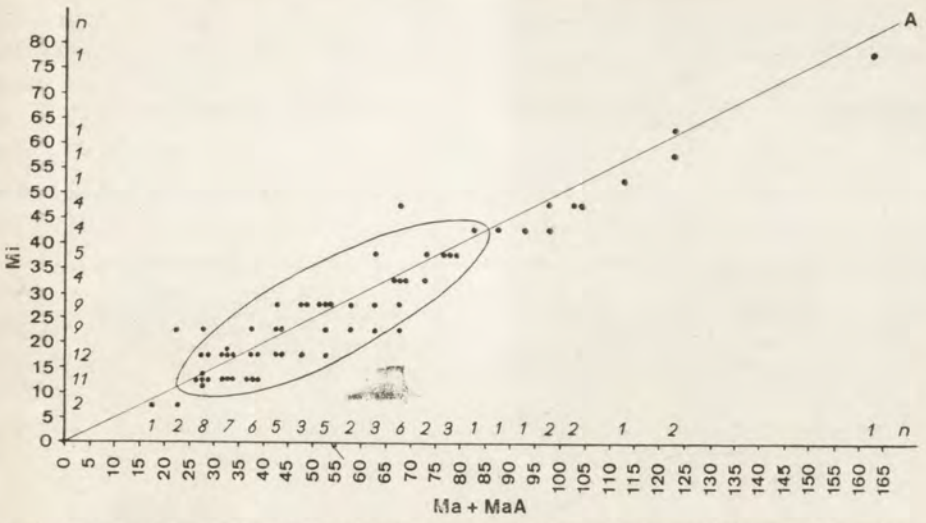

Fig. 2. Diagram showing number of adult plus developing macronuclei (abscissae), number os micronuclei (ordinates), and their correlation in 64 Black sea specimens of *K. fistulosum* (clasf intervals of 5 nuclei at both axes; n — frequencies for each class). Line 0–A corresponds to the 2 : 1 relation of macronuclei to micronuclei. Each point represents a specimen; the oval contains 75% of points

groups consisted of four macronuclei (Ma) and two micronuclei (Mi). This seems to be the "normal" nuclear group characteristic of the species (Fig. 3 d, e, g, j, k). However, groups consisting of 2 Ma and 1 Mi (Fig. 3 a), 3 Ma and 1 Mi, 2 Ma and 2 Mi, 3 Ma and 2 Mi (Fig. 3 b, l), 6 Ma and 2 Mi (Fig. 3 f, i) were also relatively frequent. About 5% of the nuclear groups consisted only of macronuclei, usually of two, but no groups including only micronuclei occurred. The maximum number of nuclei in a group which still occurred repeatedly was 8 Ma and 4 Mi, i.e., the doubled "normal" group (Fig. 3 h). Larger numbers of nuclei (up to 11 Ma and 6 Mi) were isolated and probably abnormal.

Morphology and cytochemistry of the nuclei

The micronuclei of *K. fistulosum* are oval, 2–2.5 μ in length (Fig. 3 Mi). They are sharply Feulgen-positive (Fig. 3 g–i), green after methyl green-pyronin (Fig. 3 a–f), and greenish blue after azure B (Fig. 3 j), which is characteristic of DNA. Ribonuclease does not change their stainability with basic dyes (azure B and toluidine blue); this, together with the absence of pyroninophily, indicates that the micronuclei contain no RNA. The micronuclei stain intensely for total protein (Fig. 3 l) and moderately for basic proteins (Fig. 3 k). Thus, their contents seems to be mainly deoxyribonucleoproteine (DNP).

The macronuclei of *K. fistulosum* are somewhat variable in shape (usually oval) and measure from 2.5 to 6 μ in length (Fig. 3 Ma). Spindle-shaped or sickle-shaped macronuclei sometimes occur (Fig. 3 e). A large (about 2 μ in diameter) chromatin body, which has the size and the aspect resembling those of a micronucleus, occupies the center of almost each macronucleus. Apparently this is a very large chromocenter

(Fig. 3 C). The chromocenter is Feulgen positive (Fig. 3 g–i), purely green with methyl green–pyronin (Fig. 3 a–f), greenish blue with azure B (Fig. 3 j); it contains both basic proteins (Fig. 3 k) and total protein (Fig. 3 l). Thus the chromocenter resembles a micronucleus also cytochemically.

Less frequently, macronuclei may contain two or three smaller chromocenters (Fig. 3 f); still rarer, the chromocenter splits into numerous Feulgen-positive bodies.

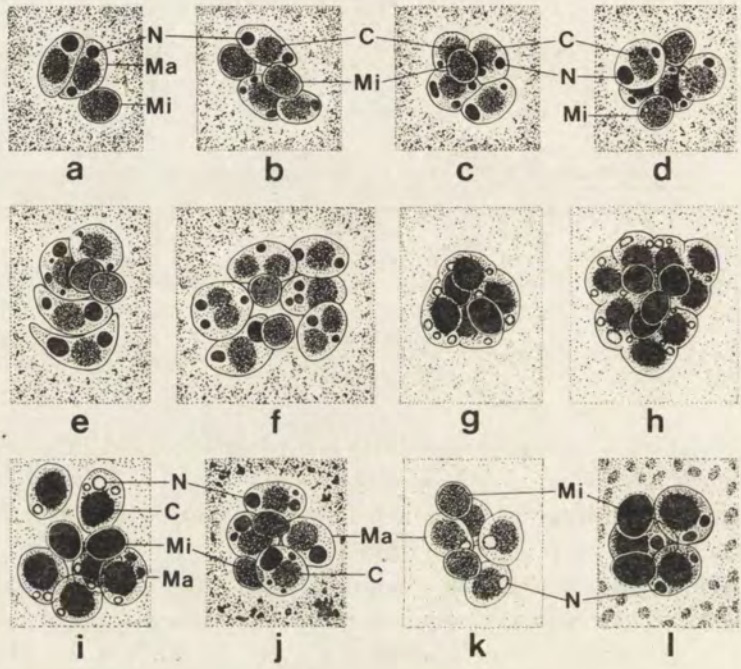

Fig. 3. Morphology and cytochemistry of the nuclear groups of *K. fistulosum*: a–f and i–l — groups with well delimited macronuclei (Ma) and micronuclei (Mi), g, h — groups with seemingly fused macronuclei ("complex nuclei"). C — chromocenters, N — macronuclear nucleoli. Staining procedures: a-f — methyl green-pyronin; g-i — Feulgen; j — azure B; k — fast green; l — mercuric bromphenol blue. Numbers of nuclei: a — 2 Ma, 1 Mi; b — 3 Ma, 2 Mi; c — 4 Ma, 1 Mi; d, e, g, j, k — 4 Ma, 2 Mi ("normal" groups); f, i — 6 Ma, 2 Mi; h — 8 Ma, 4 Mi; l — 3 Ma, 2 Mi. 3200×

The karyolymph of a macronucleus sometimes shows a faint diffuse Feulgen reaction; it stains well for total protein (Fig. 3, l). Small Feulgen-negative globules occur in the peripheral zone of a macronucleus (Fig. 3 g–i, N); these stain intensely with pyronin (Fig. 3 a–f, N) and certainly are nucleoli. Their diameter rarely exceeds 1 μ, usually it is about 0.5 μ. With azure B (Fig. 3 j) they stain violet, the colour characteristic of RNA. The basophily of the nucleoli is ribonuclease-sensitive. They contain no basic proteins (Fig. 3 k) but much total protein (Fig. 3 l).

Consequently, the macronuclei of *K. fistulosum* show an unusual relation between nucleoli and chromocenters: the former are weakly developed, the latter very conspicuous, whereas in diploid macronuclei of other members of the *Trachelocer-*

cidae — Loxodidae — Geleiidae group this relationship is exactly the opposite (see Raikov 1967, 1969).

The light microscopy is insufficient to learn how macro- and micronuclei are interconnected into groups. The groups are sometimes loose, so that the outlines of individual nuclei are distinct (Fig. 3 f, i). More frequently, however, the nuclei are more closely grouped but the boundaries between individual macronuclei are still visible (Fig. 3 a–e and j–l). Finally, in some individuals the nuclei composing each group are so compactly assembled that they appear to be fused into a common mass, and then micronuclei become difficult to distinguish from macronuclear chromocenters (Fig. 3 g, h). The micronuclei can be identified only by their sharp outlines and regular size, whereas chromocenters have more diffuse margins and variable dimensions. Probably it is this type of nuclear groups that Fauré-Fremiet (1954) held for "nuclei of type A", i.e. early stages of fragmentation of polyploid nuclei into "subnuclei" (see "Introduction"). We shall see, however, that electron microscopic data argue for another point of view.

Division and reorganization of the nuclear apparatus

Dividing individuals of *K. fistulosum* were repeatedly observed in our material (Pl. I 4). In such animals, the nuclear groups are simply partitioned between the two daughter cells and undergo no changes during this period. The nuclear reorganization leading to re-establishment of the original number of nuclear groups (which was approximately halved at cytokinesis) occurs only during the interdivision interval.

The reorganization process is asynchronous in different nuclear groups of a single animal: usually one or several but never all the groups are involved at any given moment. The main stages of reorganization of isolated nuclear groups are shown in Fig. 4. It must, however, be stressed that the proposed scheme is a somewhat arbitrary sequence of static images which thus contains an element of interpretation.

The reorganization of a normal nuclear group (consisting of 4 macronuclei and 2 micronuclei) seems to start with mitosis of its both micronuclei (Fig. 4 a). The micronuclear mitotic spindles are rhombic in shape and about 7 μ long. The metaphase chromosomes are short, elongate, and form a dense equatorial plate; in polar view (Fig. 4 a at left) their number slightly exceeds 30. The nuclear group itself usually loosens during micronuclear mitosis.

After the end of the first mitotic division of both micronuclei, the original nuclear group splits into two daughter groups, each of them usually consisting of two macronuclei and two micronuclei (Fig. 4, b). If the original number of nuclei in the reorganizing group was other than "normal", one or both daughter groups will evidently also contain more or less nuclei. Important is here the fact that we never observed division of a macronucleus.

A second (or reorganizational) division of both micronuclei of each daughter group seems to follow (Fig. 4 c); this is very characteristic of the ciliates having diploid macronuclei. This micronuclear division is also mitosis, morphologically similar to the first division mitosis. The second division obviously results in 2

macronuclei and 4 micronuclei in a daughter nuclear group; such groups actually occur in our material (Fig. 4 d).

Two of the four micronuclei of a daughter group seem to differentiate into macronuclear anlagen. The young anlagen differ from micronuclei by their larger

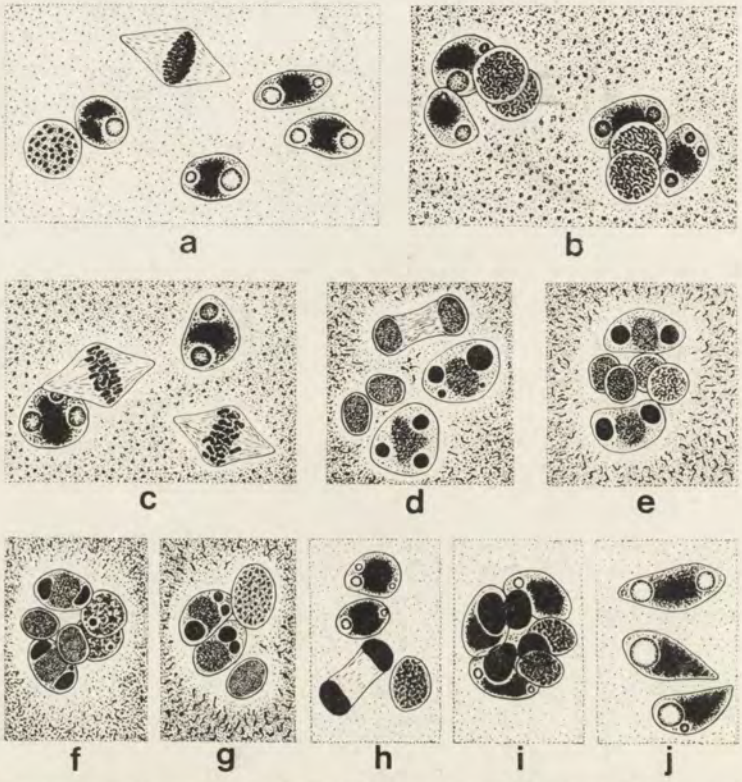

Fig. 4. Reorganization of a nuclear group in the division cycle of the ciliate: a — first mitosis of both micronuclei (Mi) showing one spindle in pole view, and four macronuclei (Ma); b — formation of two daughter nuclear groups with 2 macronuclei and 2 prophasic micronuclei in each; c — a daughter nuclear group with 2 macronuclei and metaphases of both micronuclei; d — a daughter nuclear group after the second mitosis: 2 macronuclei and 4 micronuclei, two of which are still interconnected with a telophase spindle; e — a daughter nuclear group of 2 macronuclei, 2 micronuclei, and 2 young macronuclear anlagen (at right); f — same but with more advanced anlagen showing small nucleoli (at right); g–i — abnormal reorganizing nuclear groups: g — 2 Ma, 1 Mi, and 1 macronuclear anlage (at right top), h — 2 Ma, 1 telophase of Mi, and 1 macronuclear anlage (at right bottom), i — 3 Ma, 4 Mi, and 2 macronuclear anlagen (at right); j — amicronucleate group of 3 aged macronuclei containing refringent bodies. Staining procedures: a, h, i, j — Feulgen; b, c — haemalum; d, e, f, g — methyl green–pyronin. 3200×

size and loose, spongy chromatin structure (Fig. 4 e). In older anlagen, the chromatin becomes reticular with grains of heterochromatin, and small nucleoli appear (Fig. 4 f). Such anlagen differ from adult macronuclei by the absence of the large chromocenter; this forms only much later by fusion of the heterochromatin grains into a single central body.

Nuclear groups consisting of two macronuclei, micronu twoclei, and two macronuclear anlagen which still lack large chromocenters, occurred in our material almost

as frequently as groups consisting only of adult macronuclei and micronuclei (in Fig. 2 and Table 1, the anlagen are summed up with macronuclei). This may mean that the stage of macronuclear anlagen is relatively long-lasting.

Along with stages of reorganization of "normal" nuclear groups, reorganizing groups which do not fit into the above pattern sometimes occur (Fig. 4 g–i). These may be either stages of reorganization of nuclear groups with another initial number of nuclei, or nuclear groups following an abnormal path of reorganization. Thus, Fig. 4 g shows one of the two micronuclei of a daughter nuclear group apparently developing into a macronuclear anlage still before the second mitosis. The next step of this path may be that of Fig. 4 h, where only one micronucleus undergoes the second division. This sequence of stages apparently leads to formation of a nuclear group of 3 macronuclei (Ma) and 2 micronuclei (Mi). Fig. 4 i shows a nuclear group of 3 Ma, 4 Mi and 2 macronuclear anlangen (MaA) which may have originated from a group of 6 Ma and 3 Mi [6 Ma, 3 Mi→6 Ma, 6 Mi→2 (3 Ma, 3 Mi) →2 (3 Ma, 6 Mi)→2 (3 Ma, 4 Mi, 2 MaA)].

Thus, in *K. fistulosum*, exactly as in other lower ciliates of the *Trachelocercidae—Loxodidae—Geleiidae* group which all have diploid macronuclei, the latter never divide. The restoration of the number of macronuclei occurs in our species in the same way as in these ciliates: by differentiation of some micronuclei which multiply by mitoses.

The macronuclei seem to degenerate, also asynchronously, after having passed through several cell generations. As Fauré-Fremiet (1954) also did, we observed in *K. fistulosum* a variable number of abnormal macronuclei, interpreted as "aged" ones. Such macronuclei are usually odd-shaped—flattened, disc-like, cup-shaped (Fig. 3 e at bottom), fusiform etc. Nuclear groups lacking micronuclei often consist only of aged macronuclei (Fig. 4 j). In these, the chromocenter becomes more and more diffuse and seems to fill up the entire nucleus; the karyolymph begins to stain homogeneously with Feulgen (Fig. 4 j); nucleoli usually disappear, and the degenerating macronucleus acquires the aspect of a dense, homogeneous, moderately Feulgen-positive body. Some aged macronuclei, however, contain bodies which resemble nucleoli but differ from them by greater refringence (Fig. 4 j) and absence of RNA. These bodies contain protein and may correspond to the fibrillar inclusions described below with the electron microscope.

Electron microscopy

As described elsewhere (Raikov 1971, 1972 a), *Kentrophoros fistulosum* has a tubular shape: the lateral margins of its broad ribbon-like body bend towards the dorsal side until they close forming a tube. The symbiotic sulphur bacteria, attached to the dorsal non-ciliated body side, thus get inside the tube and line its internal surface (Pl. II 5). The ciliate's body properly speaking (i.e., the wall of the tube) is very thin (about 1 μ), and only along the median line the cytopasm forms a swelling which contains the nuclear groups and many food inclusions (Pl. II 5).

The latter are phagocytized symbiotic sulphur bacteria at various phases of digestion (Pl. II 5, 6; for details see Raikov 1971, 1972 a).

The nuclear groups lie immediately beneath the morphologically dorsal body surface (which is topographically internal, i.e. facing the lumen of the tube). This surface is limited with a single plasma membrane (Pl. II 6, III 7). Food inclusions are often closely apposed to the nuclei (Pl. II 6).

Unlike *Tracheloraphis caudatus* (Raikov et Dragesco 1969) and *Kentrophoros latum* (Raikov 1972 b), the nuclear groups of *Kentrophoros fistulosum* lack any common envelopes (Pl. III 7). The groups consist of closely adjacent macro- and micronuclei whose envelopes are in direct contact. But the nuclei which constitute a group always retain their individuality (Pl. III 7). No cases of true fusion of the nuclei (which would lead to formation of the so-called "complex nuclei") could be observed electron microscopically. The pictures of apparent fusion of the micronuclei, often observed with the light microscope in *K. fistulosum* (Fig. 3 g, h), as well as the observations of Fauré-Fremiet (1954) who described polyploid nuclei of "type A" (containing numerous subnuclei inside a common envelope), thus should be attributed simply to the insufficient resolving power of the light microscope.

The micronuclei

On survey micrographs, the micronuclei are distinguished by their dense, somewhat spongy contents which is doubtlessly chromatin (Pl. II 5, III 7). At higher magnifications (Pl. III 8, IV 9) this contents is seen to consist of vaguely delimited masses which seem to be chromosomes. The dense masses are separated by small lacunae filled with electron-transparent karyolymph.

The chromatin masses consist of densely packed elementary fibrils, about 100 Å thick, which seem to be deoxyribonucleoproteine (DNP) fibrils (Pl. III 8, IV 9). Under high magnfications these fibrils appear in their turn double, consisting of two parallel subfilaments about 25 Å thick (Pl. IV 9 at arrow).

Micronuclei completely lack a granular component. This is in good agreement with the absence of RNA therein (see above).

In late telophase micronuclei, the only stage of mitosis which has been observed electron microscopically, the chromatin appears as a definite skein of chromosomes or thread-like chromonemata which are approximately 0.1 μ thick (Pl. IV 10). During the passage from telophase to interphase, these threads seem to swell and cease to be clearly delimited.

The envelope of a micronucleous consists of two membranes delimiting the perinuclear space (Pl. III 7, 8, IV 9). The membranes are trilaminar but show a definite asymmetry, unlike cytoplasmic unit membranes which are symmetrical (see, e.g., the plasma membrane in Pl. IV 9). Both in the outer and in the inner micronuclear membranes, the dark layer which is turned to the perinuclear space appears thicker and denser than the other dark layer turned respectively to the cytoplasm or to the micronuclear contents (Pl. III 8, IV 9, VI 14). The perinuclear space has irregular width: it is very narrow in some places but wider in others, especially near pores. The pores in the envelope are relatively sparse, large (800–900

Å in diameter), and lack any diaphragm or central granule (Pl. III 8, IV 9, VI 14). In surface views of the envelope, no dense annuli around the pores are observed.

The nuclear side of the micronuclear envelope is lined with a layer of amorphous low contrast material pierced by some clear lacunae (Pl. III 8, IV 9, VI 14, VII 15). This layer is about 500–700 Å thick; in front of a pore it is usually continuous but sometimes thinner or pierced with a lacuna. The amorphous layer resembles the "fibrous lamina" or the "zonula nucleum limitans" occurring beneath the nuclear envelope of certain vertebrate cells (Fawcett 1966, Kalifat et al. 1967, Mazanec 1967, Patrizi and Roger 1967) and of some gregarines (Vivier 1967, Schrével 1971).

The macronuclei

Even at low magnifications, the adult macronuclei show large single chromocenters (Pl. III 7), which are absent only in developing macronuclear anlagen (Pl. IV 11, V 12).

In young macronuclear anlagen, the chromosomes are strongly despiralized and are seen in sections as vague condensations consisting of DNP fibrils. The same fibrils form a loose network in the karyolymph (Pl. IV 11). Such anlagen usually contain small electron dense nucleoli which consist mainly of fibrillar material. In older anlagen, relatively large nucleoli with fibrillar core and granular periphery are formed. Heterochromatin clumps, which are probably precursors of the future chromocenter, appear at the same time (Pl. V 12). These are skeins or reticular aggregates formed by enrolment or folding of lengths of 0.1 μ thick chromonemata. The main mass of DNP fibrils remains, however, dispersed in the karyolymph. The thickness of both the packed and the dispersed fibrils is approximately 200 Å after glutaraldehyde fixation but only some 100 Å after osmium fixation. The karyolymph contains at this stage also a considerable amount of RNP granules measuring 150–200 Å in diameter. Sometimes, macronuclear anlagen contain also small accumulations of fibrillar, material resembling those in adult macronuclei (Pl. V 12).

The nuclear envelope of the anlage is almost devoid of an amorphous layer (Pl. IV 11, V 12). This may facilitate the passage of nuclear products from the anlage into the cytoplasm through the pores.

It seems consequently that the stage of the late anlage is metabolically the most active one in the development of a macronucleous. As described below, the adult macronuclei show definite signs of lowering of their synthetic activity as compared with the late macronuclear anlage.

An adult macronucleus has an overall vesicular structure with its center occupied by a chromocenter (Pl. VI 13); it thus belongs to the so-called karyosomal type of nuclei.

The envelope of a macronucleus structurally resembles that of a micronucleus: it is two-membraned, pierced by large "open" pores, has a narrow perinuclear space and a well developed amorphous layer at its nuclear side (Pl. VI 13, 14, VIII 18, IX 20). The membranes of the macronuclear envelope show the same asymmetry as those of the micronuclear envelope (Pl. VII 15, VIII 18, IX 20).

Conspicuous structural changes of the nuclear envelopes sometimes occur in the regions of contact between the neighbour nuclei (within a nuclear group). In most cases, each of the adjacent nuclei retains its independent bimembraneous envelope, so that the septum between two nuclei consists of two cisternae (four membranes). The clearance between the envelopes of the adjacent nuclei is usually narrow (about 300–500 Å) and filled with amorphous material resembling that of the amorphous layers which line the inner sides of the nuclear envelopes. This clearance contains no additional membrane systems. Examples of this type of internuclear joints are given in Pl. II 6 (Ma to Ma joint) and Pl. VI 14 (Ma to Mi joint). Such joints usually occur also between two micronuclei (Mi to Mi).

Structural reformations of the nuclear envelopes are always limited to the surface of contact between two adjacent nuclei. A single bimembraneous cisterna of the internuclear wall may replace the two independent envelopes at a macronucleus-to-micronucleus joint (Pl. VII 15). This single cisterna thus appears to belong to both nuclei at once; it is closed along its margins, whereas free surfaces of both nuclei have envelopes of usual structure. The cisterna of the internuclear wall carries amorphous layers at its both sides, and the pores which it bears directly connect the contents of the two nuclei (in Pl. VII 15 — a macronucleus with a micronucleus). Similar structures occur also at the joint between two macronuclei, sometimes only at a portion of their contact surface (Pl. VII 17). Less frequently, a third (additional) cisterna appears in the internuclear septum (Pl. VII 16). In this case, membrane proliferation seems to take place at the margin of the contact zone. The various types of structural reformations of the nuclear envelope indicate its high lability in the contact zones between nuclei.

The center of a macronucleus is usually occupied by a large (up to 2.5 μ in diameter) electron dense body. It follows from comparison with light microscopic data (Fig. 3) that this is a giant chromocenter. The chromocenter lacks any envelope; its core is dense, and its peripheral part contains cavities and has a highly irregular outline (Pl. VI 13, VIII, 18, IX 21). Clear vacuoles sometimes occur inside the chromocenter (Pl. II 6, III, 7, VII 17).

At higher magnifications, the fine structure of the chromocenter appears similar to that of a micronucleus. The chromocenter consists of densely packed DNP fibrils which are, after osmium fixation, about 100 Å thick (Pl. VI 14, VII 17, VIII 18, IX 20). Two parallel sub-filaments are sometimes resolved in a fibril. At the periphery of the chromocenter, the same fibrils are gathered into small heterochromatin clumps which are either connected with the chromocenter (Pl. VI 13, VIII 18) or independent from it (Pl. VII 17). Finally, DNP fibrils are seen to come out of the chromocenter or of the heterochromatin clumps into the karyolymph and to form there a loose network. This seems to be the dispersed (euchromatin) part of the chromosomal set of the macronucleus. The same loose 100 Å fibrils also get in contact with nucleoli (Pl. VIII 18, IX 20).

The nucleoli of adult macronuclei of *K. fistulosum* are always peripheral (whereas in late macronuclear anlagen they are often central — see Pl. V 12). They belong to the compact type and their e'ectron density equals that of the chromocenter

(Pl. II 6, III 7, VI 13, IX 21). Nucleoli can be distinguished from the chromocenter, besides their peripheral position, by their sharp outlines and their more homogeneous structure, which depends on the smaller thickness of their component fibrils. The nucleoli usually consist entirely of densely packed filaments less than 50 Å in diameter (Pl. VIII 18, IX 20), which probably represent the fibrillar form of RNP. Only in some macronuclei sparse 130–150 Å large RNP granules occur in a single layer at the periphery of the nucleoli.

Along with nucleoli and the chromocenter, some macronuclei contain very peculiar spherical bodies consisting of spirally arranged fibers (Pl. X 22, 23). The bodies reach 1.5 μ in diameter. Their component fibers are about 80 Å thick, solid (not tubular), of medium electron density. They differ from both the DNP fibrils of the chromocenter and the RNP filaments of the nucleoli. At the periphery of the body, the fibers are looser and not so clearly spiral; their free ends protrude into the karyolymph (Pl. X 22, 23).

Only about 10% of the macronuclei seem to contain fibrillar bodies. These macronuclei are often irregularly shaped and show some signs of degeneration: their chromocenters may be fragmented, and they may lack nucleoli (Pl. X 22). However, fibrillar bodies occur also in macronuclei with apparently normal chromocenter and nucleoli, but there the bodies are smaller and less numerous. This, together with the light microscopical data presented above, allows to suppose that fibrillar bodies develop preferentially in ageing macronuclei. They may correspond to the refringent bodies observed light microscopically in aged macronuclei and lacking RNA.

The question as to the origin and mode of formation of the fibrillar bodies cannot be answered unambiguously. However, some macronuclei show structures which may be precurcors of the fibrillar bodies. These structures are intimately related to the chromocenter.

As mentioned above, small clear lacunae or vacuoles occur inside some chromocenters (Pl. VII 17). Other chromocenters contain also one or several larger vacuoles filled with reticular or vaguely fibrillar material of low electron density. Such vacuoles usually protrude the surface of the chromocenter, forming blebs which are coated externally with only a single layer of heterochromatin clumps (Pl. VIII 19).

At higher magnifications, such vacuoles are seen to contain some 80 Å thick fibers similar to those of the fibrillar bodies; they are markedly thinner than the fibrils of the chromocenter itself (Pl. IX 20). It may consequently be supposed that the fibers of the future fibrillar bodies are formed in the vacuoles of the chromocenter.

The chromatin coating of the fully grown vacuole seems to rupture, the fibrillar material getting into the karyolymph. Its structure gradually becomes more regular: at first small parallel bundles of fibers appear, which are later arranged into more or less spiral curls (Pl. IX 21). These bundles of fibers in the karyolymph are doubtless precursors of the spherical fibrillar bodies with spiral arrangement of the fibers. The bundles are often surrounded with scattered heterochromatin clumps, which may be remnants of the disrupted chromatin coating of the intrachromocentric

vacuole (Pl. IX 21). Small accumulations of fibrillar material in the karyolymph are sometimes found even at early stages of macronuclear ontogeny, e.g. in late macronuclear anlagen (Pl. V 12), where they are also associated with heterochromatin bodies. But such accumulations are much more frequent and abundant in adult and ageing macronuclei.

Discussion

The nuclear cycle

Important is the existence in *K. fistulosum* of a typical cycle of nuclear reorganization accompanying every cell division. This cycle resembles that in other members of the *Trachelocercidae — Loxodidae — Geleiidae* group; it proceeds asynchronously in different nuclear groups of a single specimen and leads to fission of each existing group into two daughter groups of approximately the same compos tion. The process of nuclear reorganization includes two successive mitotic divisions of the micronuclei and transformation of approximately a half of the products of the second micronuclear division into macronuclear anlagen.

This pattern of nuclear reorganization is most similar to that in *Tracheloraphis caudatus*, a species which also has numerous nuclear groups usually consisting each of 4 macronuclei and 2 micronuclei (Dragesco et Raikov 1966). As in *K. fistulosum*, twofold mitoses of both micronuclei followed by macronuclear anlagen formation, and fission of each original nuclear group into two daughter groups occur in *T. caudatus*. Thus, in both species, every nuclear group includes macronuclei of different age: usually two of them have been received (without any division) from the mother group, while the other two are young macronuclei formed during the last nuclear reorganization. A difference between *T. caudatus* and *K. fistulosum* also exists and is that, in the former species, all nuclear groups duplicate synchronously during cytokinesis (Dragesco et Raikov 1966), whereas in *K. fistulosum* this phenomenon is asynchronous and occurs during the interval between two cytoplasmic divisions.

We arrived thus to conclusions that differ significantly from those obtained by Fauré-Fremiet (1954) in the same species. The differences are as follows:

(a) We observed micronuclear reproduction by typical mitosis. Fauré-Fremiet failed to find mitosis in this species.

(b) We found repartition of macronuclei between daughter nuclear groups during nuclear reorganization. Fauré-Fremiet believed that macronuclei degenerated without passing through the next nuclear reorganization.

(c) We found no polyenergid nuclei of "type A", containing several identical chromatin bodies ("subnuclei") not differentiated into macro- and micronuclei, as Fauré-Fremiet did. According to our data, *K. fistulosum* has nuclear dualism at all stages of its division cycle. The "A-type nuclei" (Fig. 16–20 in the paper by Fauré-Fremiet 1954) doubtlessly correspond to very compact nuclear groups (Fig. 3 g, h in our paper), where internuclear septae are invisible with the light microscope. Probably Fauré-Fremiet failed to distinguish between micronuclei

and chromocenters of adjacent macronuclei, and believed both to be "subnuclei". It seems that he did not notice the small nucleoli adjacent to chromocenters and revealing the macronuclear nature of the latter. Electron microscopy does not confirm the existence of polyenergid nuclei of "type A", with non-differentiated subnuclei inside.

(d) We found in *K. fistulosum* nothing similar to the large "granular nuclei" which Fauré-Fremiet considered to be micronuclei undergoing endomitotic polyploidization, i.e., precursors of the nuclei of "type A" (Fig. 30–33 in Fauré-Fremiet's paper). We observed only small granular nuclei which are developing macronuclear anlagen. At no stage of the nuclear cycle, endomitotic polyploidization could be detected in our material. Also, we observed no transitional nuclear forms from "granular nuclei" to "A-type nuclei", characterized, according to Fauré-Fremiet, by gradual individualization of "subnuclei" inside them (Fig. 34–38 in Fauré-Fremiet's paper). It is difficult to understand what structure Fauré-Fremiet described under the name of "granular nuclei". Possibly they might have been accumulations of intracellular symbionts or parasites.

Thus, the cycle of nuclear reorganization in *K. fistulosum* keeps, according to all essential features, within the pattern typical of the group of lower ciliates having diploid macronuclei (reviews: Raikov 1963, 1967, 1969). Such essential features are:

(1) Inability of the macronuclei to divide, which implies their mechanical segregation at cytokinesis;

(2) Reproduction of micronuclei by repeated mitoses, and

(3) Restoration of the number of macronuclei by differentiation of some micronuclei into macronuclear anlagen.

It must be remarked that Fauré-Fremiet never considered his scheme of nuclear reorganization in *K. fistulosum* as definitely proved; on the contrary, he stressed its hypotheticity. The present reinvestigation of the same species actually showed that its nuclear reorganization phenomena are not very aberrant from those in other members of the *Trachelocercidae—Loxodidae—Geleiidae* group.

Structure of the macronuclei

The macronuclei of *Kentrophores fistulosum* belong to the same type of vesicular nuclei as those of other ciliates of the *Trachelocercidae—Loxodidae—Geleiidae* group. However, they are relatively smaller: the ratio of the macronuclear diameter to the micronuclear diameter is only 1.5–2.5 times in *Kentrophoros fistulosum*, whereas in *Loxodes* this ratio is 3–4 times, in *Remanella* 4–5 times, and in *Geleia* even 5–6 times.

The macronuclei of *K. fistulosum* contain relatively small nucleoli and a very large chromocenter. Just the reverse is the situation in all other species of ciliates with diploid macronuclei which were investigated electron microscopically (Mashansky 1963, Raikov et Dragesco 1969, Kovaleva and Raikov 1970, De Puytorac et Njiné 1970, Raikov 1972 b). In these forms (*Loxodes*, *Tracheloraphis caudatus*, *Trachelonema sulcata*, *Kentrophoros latum*), the macronuclei contain well developed

nucleoli of fibro-granular structure, and relatively small heterochromatin bodies; the main mass of their chromatin remains dispersed. Such macronuclei have a fine structural organization typical of actively functioning cell nuclei (i.e., of nuclei producing various types of RNA). According to light microscopical data, the macronuclei of the vast majority of ciliates of the genera *Trachelocerca*, *Tracheloraphis*, *Trachelonema*, *Loxodes*, *Remanella*, and *Geleia* belong to the same morphological type (Raikov 1967, 1969). Large chromocenters have been observed light microscopically only in *Trachelocerca variabilis* (Kovaleva 1966), *Tracheloraphis vermiformis*, *T. drachi*, *Trachelonema binucleata* (Raikov 1962), and *Kentrophoros flavum* (Raikov and Kovaleva 1968). Only in *Trachelocerca variabilis* the relative size of the chromocenter is comparable with that in *Kentrophoros fistulosum*; in other species mentioned above, the development of the nucleolar apparatus still exceeds that of the chromocenter.

This fact seems to mean that adult macronuclei of *K. fistulosum* are metabolically less active than macronuclei of other ciliates of the *Trachelocercidae—Loxodidae—Geleiidae* group. In *K. fistulosum*, the main mass of chromatin is condensed, i.e., must be inactive according to the current notions. Only a small portion of the chromatin fibrils are dispersed in the peripheral region of the nucleus, where they seem to contribute to the formation of the nucleoli. In adult macronuclei of *K. fistulosum*, the nucleoli are small and entirely fibrillar; such nucleoli resemble those of cells treated with actinomycin D (where DNA-primed RNA synthesis is inhibited). This also argues for low RNA synthesis rate in adult macronuclei of *K. fistulosum*.

However, there is a stage of development of a macronucleus of *K. fistulosum* which resembles, according to its ultrastructure, to the macronuclei of most other ciliates of the *Trachelocercidae—Loxodidae—Geleiidae* group. This is the late macronuclear anlage stage which is relatively long-lasting, judging by the frequency of its occurrence. The late anlage is characterized by a large central nucleolus and by strongly dispersed chromatin (Pl. V 12). The nucleolus contains both a fibrillar and a well developed granular component. It seems that this transitory phase of macronuclear development is metabolically more active than the adult macronucleus and possibly supplies the cell with the largest amount of genic products.

Thus, diploid macronuclei of various lower ciliates are not uniform as to the degree of their somatic differentiation. In other words, they may unequally deviate from the metabolically inert generative nucleus, the micronucleus. In *Kentrophoros fistulosum*, the degree of "somatization" of the macronuclei is relatively low, in most other forms it is higher. But even at low degrees of "somatization", the macronuclei completely lose their ability to divide, i.e., become irreversibly differentiated as somatic nuclei.

The fibrillar inclusions in the macronuclei

With the exception of *Loxodes*, in all species of ciliates with diploid macronuclei studied until now electron microscopically, the macronuclei contain not only chromocenters and nucleoli but also peculiar proteinaceous inclusions. In *Tracheloraphis caudatus*, these inclusions are cristalline (Raikov et Dragesco 1969), in

Trachelonema sulcata they are dense structureless spheres (Kovaleva and Raikov 1970), and in *Kentrophoros latum* they are large spheres consisting of fibrillar material (Raikov 1972 b). At least in the last two species, the material of the proteinaceous spheres has been shown to form in cavities of chromocenters. In *Trachelonema sulcata*, the spheres occur mainly in ageing macronuclei.

Macronuclear fibrillar inclusions of *K. fistulosum* belong to the same type of structures. They are certainly homologous to the macronuclear spheres in *Kentrophoros latum*: both originate in contact with the chromatin and consist of solid 80 Å thick fibers. Only the mode of fiber packing is different in the two species: in *K. latum*, the fibers are sinuous and packed without apparent order, while in *K. fistulosum*, bundles or sheets of fibers are rolled up within the fibrillar body so that the path of individual fibers is generally spiral.

The functional significance of the proteinaceous inclusions of diploid macronuclei remains obscure. We never observed passage of such bodies into the cytoplasm, neither with the light nor with the electron microscope. The spheres or the cristalloids show sometimes some basophily which is however ribonuclease-resistant (e.g. in *Trachelonema sulcata* or *Kentrophoros latum*). It may be supposed that these bodies might contain some form of RNA, firmly associated with protein and therefore RNase-resistant (Raikov 1972 b). This supposition might, for example, explain the fact of the presence of a very long living messenger RNA in *Trachelocercidae*, known from regeneration experiments (Torch 1962, 1964). If such protein-inactivated RNA is actually stored in the spheres or cristalloids of the macronuclei, it could get into the cytoplasm only several cell generations later, when the respective macronuclei become aged and are resorbed.

Other explanations of the nature of these proteinaceous inclusions are also possible, since the very presence of RNA in them remains doubtful. For instance, proteinaceous inclusions could represent an abortive mitotic apparatus, i.e., the proteins which normally (in the micronucleus) become organized into the achromatic mitotic spindle, whereas in the macronucleus, where mitosis is blocked, these proteins cannot be assembled into a spindle and/or be transformed into microtubules.

Polyploid macronuclei of higher ciliates usually contain no such inclusions. However, in some strains of *Paramecium caudatum* all the macronuclei show conspicuous achromatic zones, regularly transmitted into the daughter macronuclei during binary fission. Electron microscopy demonstrated that these zones are filled with bundles of solid proteinaceous fibers which resemble somewhat those in *K. fistulosum* but are much thicker, measuring about 250 Å in diameter (Vivier et André 1961). Their nature is not clear though they have been supposed to be intranuclear viruses (Jankowski 1961).

Fibrillar inclusions resembling those in *K. fistulosum* have been described in nuclei of reptilian primary gonocytes (Hubert 1970), amphibian oocytes (Lane 1969), and chick neurones (Masurovsky et al. 1970). In these cases the fibers are also proteinaceous, 50–70 Å thick, and contain no RNA. Their nature and functional role are unknown.

Groupwise union of nuclei and the problem of "complex nuclei"

In many ciliates possessing diploid macronuclei, the nuclei tend to unite into one or several nuclear groups. Light microscopical studies seemed to indicate that, at least in some species, the macronuclei constituting a group may even fuse into a so-called "complex nucleus", the micronuclei then getting inside the fused complex — e.g. in *Tracheloraphis phoenicopterus* or *T. striatus* (Raikov 1962), or in *Tracheloraphis prenanti* (Raikov and Kovaleva 1968). Such fusion seemed to be reversible, the "complex nucleus" breaking again into its component macronuclei during cell division and in some physiological conditions.

Electron microscope investigations of the *Trachelocercidae—Loxodidae—Geleiidae* ciliates made, however, clear that this problem is in reality much more complicated. Union of nuclei into groups apparently can be accomplished at least in two ways. First, the entire nuclear group may become surrounded with a common cytoplasmic membrane which, so to say, holds the nuclei together — e.g., in *Tracheloraphis caudatus* (Raikov et Dragesco 1969). This evolutionary path may further lead to development, around each nuclear group, of a heavy multimembranous capsule consisting of several layers of flattened cisternae (*Kentrophoros latum* — Raikov 1972 b). However, the nuclei themselves not only retain their independent envelopes of conventional structure inside the capsule, but are additionally separated by sheets of cytoplasmic vesicles.

The term "nuclear capsule", introduced by Dragesco (1960), fits well this type of nuclear complexes.

Another way of nuclear union is represented by *Kentrophoros fistulosum*. Here, the nuclear groups lack a common membrane and the more a capsule, but the nuclear envelopes themselves undergo definite changes at points of contact between adjacent nuclei.

Two most conspicuous change is the facultative disappearance of two parallel cisternae of the apposed nuclear envelopes and their replacement by a single cisterna of the internuclear wall. This cisterna belongs, so to say, to both nuclei at once, and its pores are direct communications between the karyolymphs of the two nuclei. This seems to be a definite step towards fusion of the macronuclei, i.e., towards formation of a true "complex nucleus". However, we never observed a complete disappearance of the internuclear walls in *K. fistulosum* and are unable to say whether true "nuclei complex" ever occur in this species. The light microscope allows to inspect an incomparably greater number of nuclear groups, but its resolving power is unfortunately too low to distinguish between compact nuclear groups and true "complex nuclei" which are hypothetic.

Reorganization of the nuclear envelopes of adjacent nuclei in *K. fistulosum* is certainly favored by the absence of additional separating sheets of cytoplasmic vesicles between the nuclei.

Apparently, only the second way of nuclear union into groups may ultimately lead to real nuclear fusion. Further electron microscopic studies will perhaps reveal ciliate species completely lacking septae between adjacent diploid macronuclei, i.e., species with true "complex nuclei".

Summary

The nuclear apparatus of the marine psammophilic ciliate *Kentrophoros fistulosum* Fauré-Fremiet consists of numerous (4 to 43) nuclear groups, usually comprising four diploid macronuclei and two micronuclei each. The macronuclei are characterized by single large chromocenters and by relatively small nucleoli which contain RNA. No RNA was found in the chromocenters or in the micronuclei. During cell division, mechanical segregation of the existing nuclear groups between the daughter animals occurs. The reorganization of the nuclear groups, leading to the restoration of their number, occurs during the interdivision interval. Both micronuclei of each nuclear group divide twice by typical mitosis, and the original group falls into two daughter groups (the macronuclei being segregated between them without division). A half of the micronuclei transform later into macronuclear anlagen, thus restoring the normal composition of the nuclear groups. We failed to observe in *K. fistulosum* a polyploidization — fragmentation cycle of the micronuclei supposed by Fauré-Fremiet.

The micronuclei contain densely packed DNP fibrils which are about 100 Å thick and gathered into several bundles (chromosomes). The macronuclear chromocenters, which may be very large, have a similar fine structure but without apparent subdivision into bundles. The macronuclear nucleoli are almost completely formed by fibrillar RNP, except the young macronuclei whose nucleoli are larger and contain also the granular component, and whose chromatin is more dispersed. In older macronuclei, proteinaceous inclusions of unknown functional significance are formed. These consist of spirally packed curved fibrils, about 80 Å thick, which seem to be synthesized inside the vacuoles of the chromocenters.

Both the macronucleus and the micronucleus are surrounded by a flattened cisterna of the nuclear envelope, perforated with large pores and lined at the nuclear side by an amorphous layer. However, at the boundary of two adjacent nuclei the two parallel cisternae are sometimes replaced by a single cisterna of the internuclear wall which belongs to both nuclei at once. This may represent an initial stage of fusion of the components of the nuclear group into a "complex nucleus".

РЕЗЮМЕ

Ядерный аппарат морской псаммофильной инфузории *Kentrophoros fistulosum* Fauré-Fremiet состоит из многочисленных ядерных групп (от 4 до 43), обычно включающих каждая 4 макронуклеуса диплоидного типа и 2 микронуклеуса. Макронуклеусы характеризуются одиночным крупным хромоцентром и сравнительно мелкими ядрышками, содержащими

РНК. В хромоцентрах, а также в микронуклеусах РНК не обнаруживается. При делении инфузории происходит механическое распределение имеющихся в наличии ядерных групп между дочерними особями. Реорганизация ядерных групп, ведущая к увеличению их числа, происходит в промежутке между двумя делениями особи. Оба микронуклеуса каждой ядерной группы делятся дважды путем типичного митоза, и вся группа подразделяется на 2 дочерние группы (макронуклеусы при этом распределяются без деления). Половина микронуклеусов превращается затем в зачатки макронуклеусов, восстанавливая тем самым нормальный состав ядерных групп. Мы не наблюдали у *K. fistulosum* цикла полиплоидизации-фрагментации микронуклеусов, существование которого предполагал Форе-Фремье.

Микронуклеусы содержат плотно упакованные фибриллы ДНП толщиной около 100 Å-иногда собранные в толстые тяжи (хромосомы). Аналогичную ультраструктуру (но без обособленных тяжей) имеет и хромоцентр макронуклеуса, достигающий очень крупных размеров. Ядрышки макронуклеусов мелкие, почти целиком состоят из фибриллярной формы РНП; однако, в молодых макронуклеусах ядрышки крупнее и включают также и гранулярный компонент, а хроматин более диспергирован. В стареющих макронуклеусах обнаруживаются белковые включения неясного функционального значения, состоящие из завитков спирально уложенных фибрилл толщиной около 80 Å. Предшественники этих фибрилл, по-видимому, синтезируются в полостях хромоцентра, а затем выходят в кариолимфу макронуклеуса.

Как макронуклеусы, так и микронуклеусы окружены цистерной ядерной оболочки, которая пронизана крупными порами и подостлана со стороны кариолимфы аморфным слоем. На границе двух контактирующих ядер (в пределах одной ядерной группы) их ядерные оболочки тесно сближены и разделены лишь тонким слоем аморфного материала, но при этом обычно сохраняют типичное строение. Однако, иногда две параллельные цистерны независимых ядерных оболочек заменяются здесь единственной цистерной межъядерной перегородки, принадлежащей сразу обоим ядрам. Это может быть начальной стадией слияния компонентов ядерной группы в "сложное ядро".

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EXPLANATION OF PLATES I–X

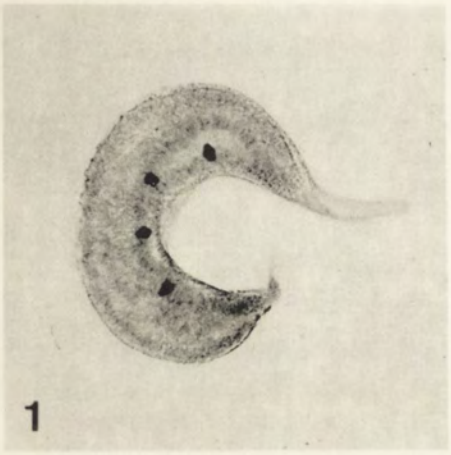
Light micrographs of *Kentrophoros fistulosum* (Pl. I)

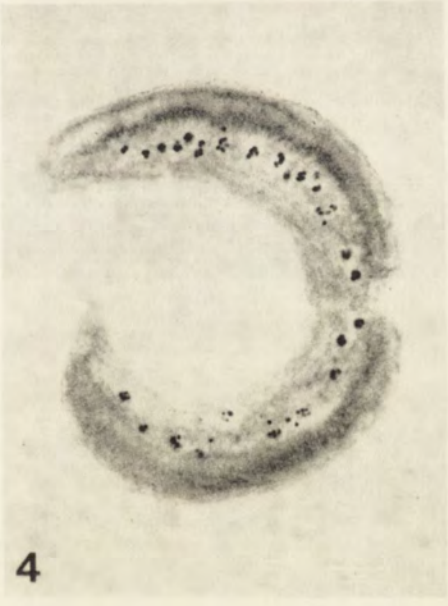
1–3: Specimens of different size and number of nuclear groups, Feulgen — light green (1, 2 — 400×, 3 — 280×)
4: Dividing animal, hemalum, 280×

Electron micrographs of sections of *K. fistulosum* (Pl. II–X)

Abbreviations used: AL — amorphus layer beneath the nuclear envelope, B — bacteria, C — chromocenter, Cs — chromosomes, Ema — macronuclear envelope, Emi — micronuclear envelope, ER — endoplasmic reticulum, F — food inclusions, FV — vacuoles with fibrillar contents in the chromocenter, FB — fibrillar bodies in macronuclei, FM — fibrillar material in the macronuclear karyolymph, G — Golgi apparatus, H — heterochromatin clumps, IW — internuclear wall, Ma — macronucleus, Mi — micronucleus, N — nucleolus, P — pore of the nuclear envelope, PM — plasma membrane, V — clear vacuole in the chromocenter

5: Part of a transverse section of *K. fistulosum* showing the ciliate's tubular body with symbiotic bacteria inside the tube and nuclei in the median cytoplasmic swelling. 4500×
6: Dorsal body surface in the region of the median swelling showing a macronucleus with adjacent food inclusions, the plasma membrane, and symbiotic sulphur bacteria. 27 000×
7: Nuclear group showing 3 macronuclei and 2 micronuclei, localized just beneath the plasma membrane of the dorsal body side. 16 500×
8: Micronucleus and adjacent parts of a macronucleus and of a second micronucleus. 54 000×
9: Part of a micronucleus showing a pore in its envelope and a trilaminar structure of the plasma membrane. Paired chromatin subfilaments at arrow. 120 000×
10: Late telophase of a micronuclear mitosis showing re-formation of the amorphous layer under the envelopes of the daughter nuclei; a portion of a macronucleus at bottom. 30 000×
11: Young macronuclear anlage showing sectioned chromosomes and a small nucleolus. 43 000×
12: Later stage of development of a macronuclear anlage showing a large nucleolus, a heterochromatin body, and a fibrillar inclusion. 45 000×
13: Adult macronucleus with a chromocenter in central position and a peripheral nucleolus. 31 000×
14: Joint between a macronucleus and a micronucleus showing intact nuclear envelopes of both nuclei. 64 000×
15: Joint between a macronucleus and a micronucleus showing the single cisterna of the internuclear wall. 105 000×
16: Appearance of an additional third cisterna between the nuclear envelopes in a joint between two macronuclei. 92 000×
17: Reorganization of the nuclear envelopes in a joint between two macronuclei: fusion of independent envelope cisternae into the single cisterna of the internuclear wall. The macronucleus shows the chromocenter with a vacuole, heterochromatin clumps, and a nucleolus. 62 000×
18: Part of a macronucleus showing the chromocenter, a finely fibrillar nucleolus, and the nuclear envelope with "open" pores. 77 000×
19: Part of a macronucleus with a fibrillar vacuole inside the chromocenter. 46 000×
20: Fibrillar vacuole in the macronuclear chromocenter, a nucleolus, and the macronuclear envelope with a pore. 72 000×
21: Macronucleus showing accumulations of fibrillar material in the karyolymph. 28 000×
22: Macronucleus showing fragmented chromocenter and four fibrillar bodies. 30 000×
23: Fibrillar body and part of the chromocenter. 66 000×





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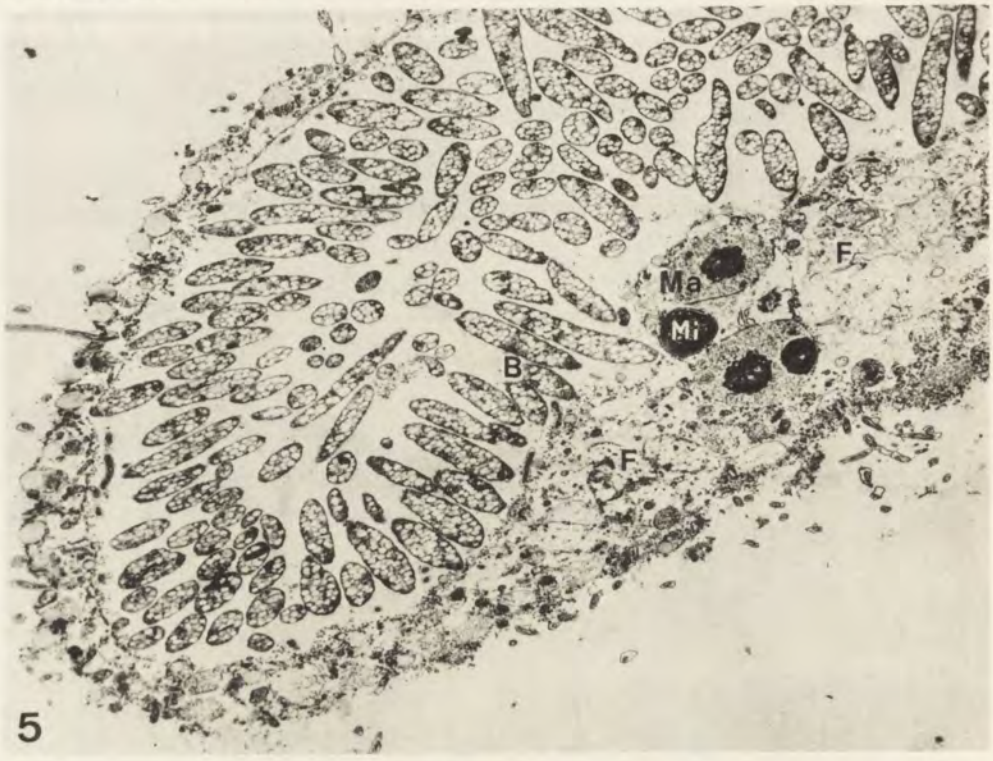

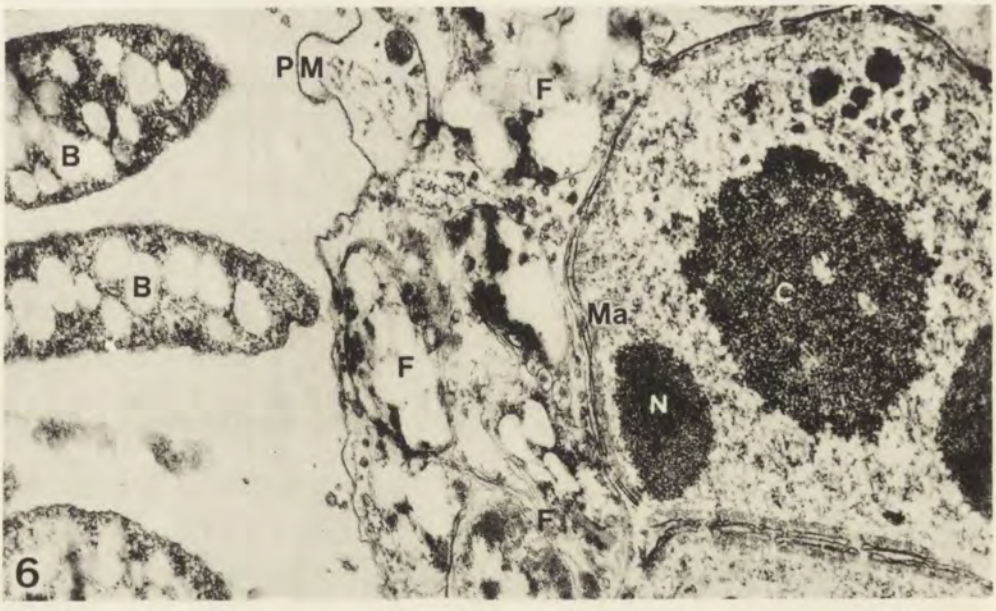

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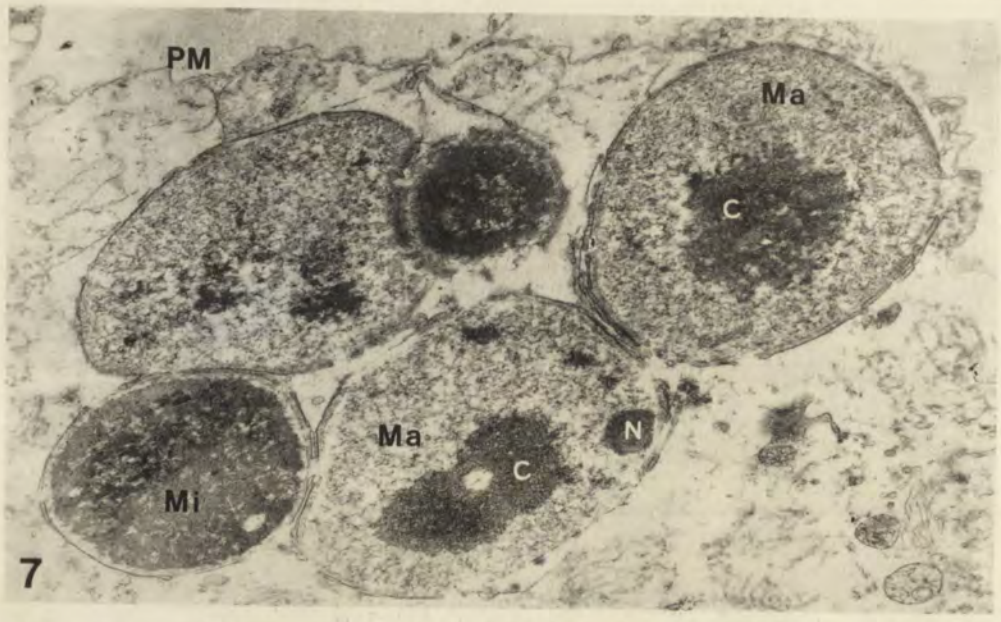

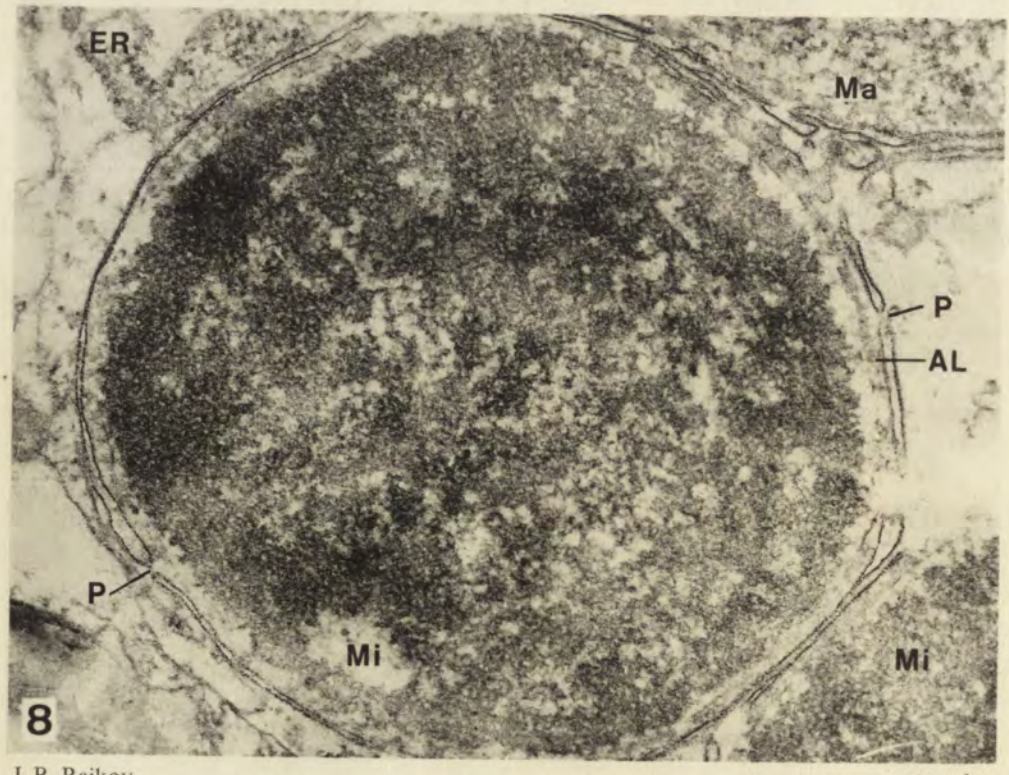

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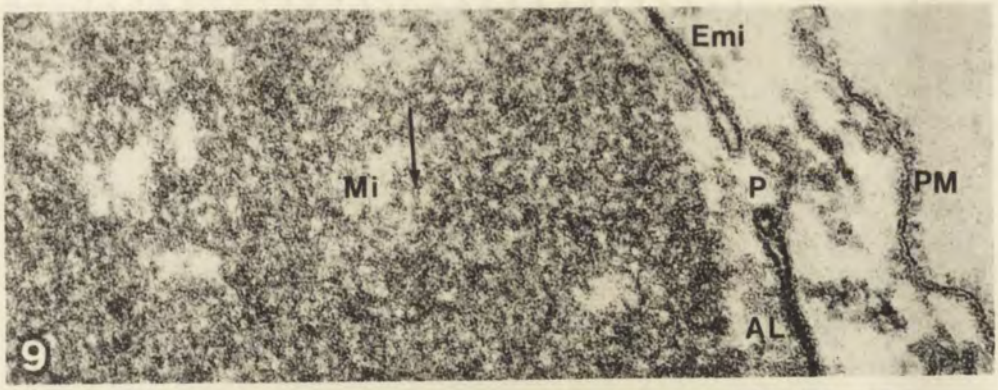

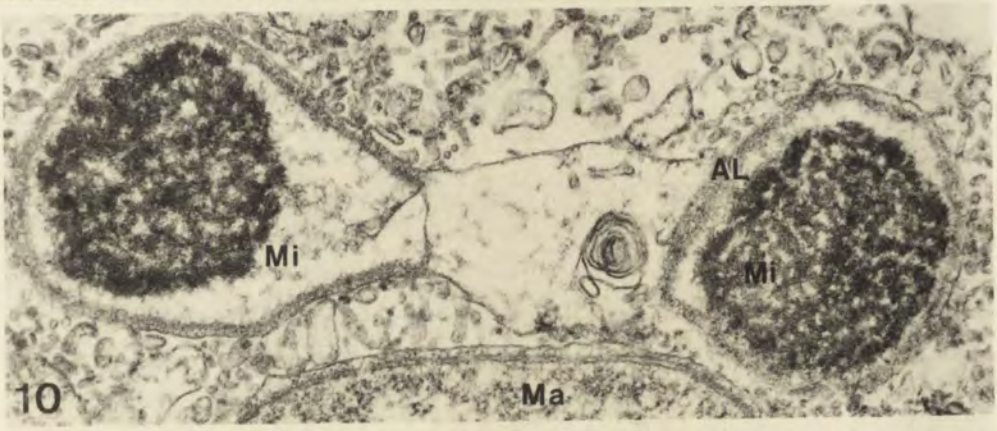

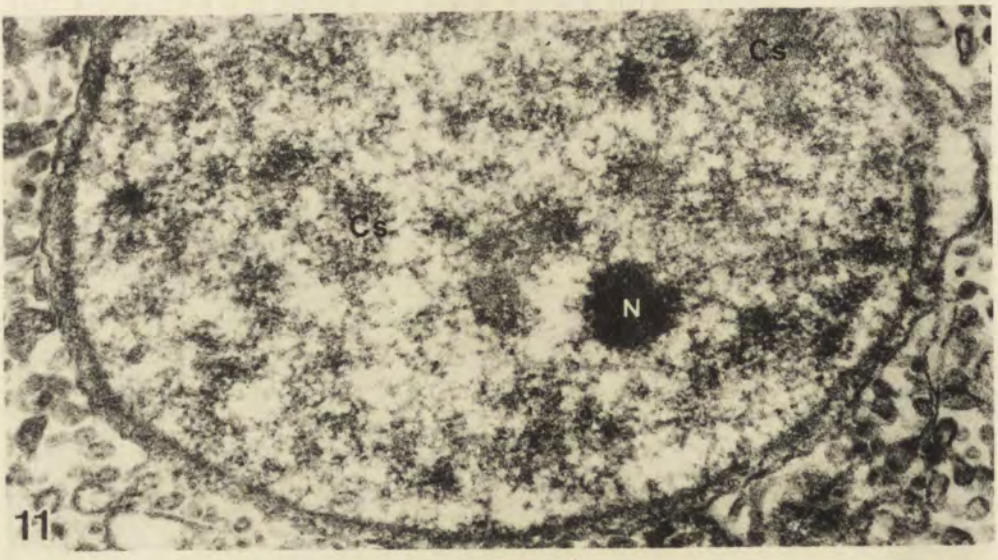

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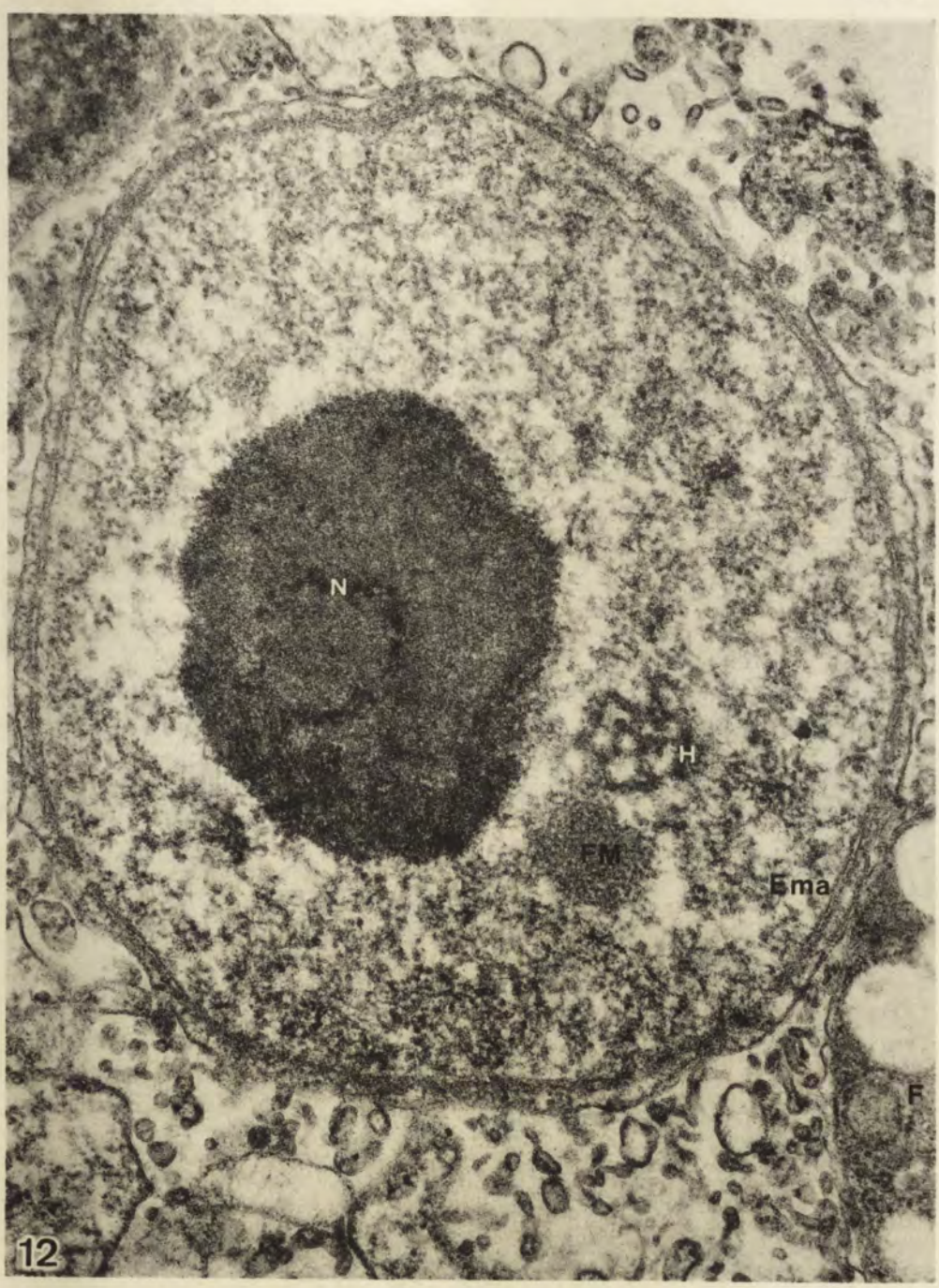

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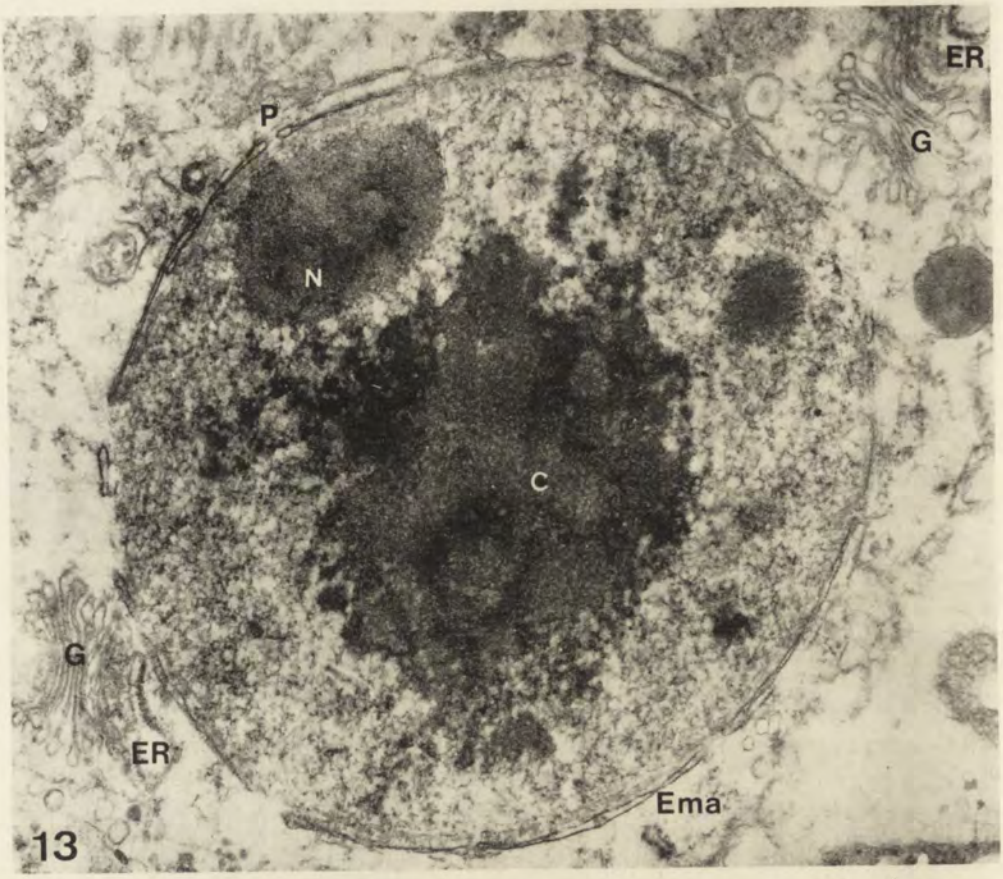

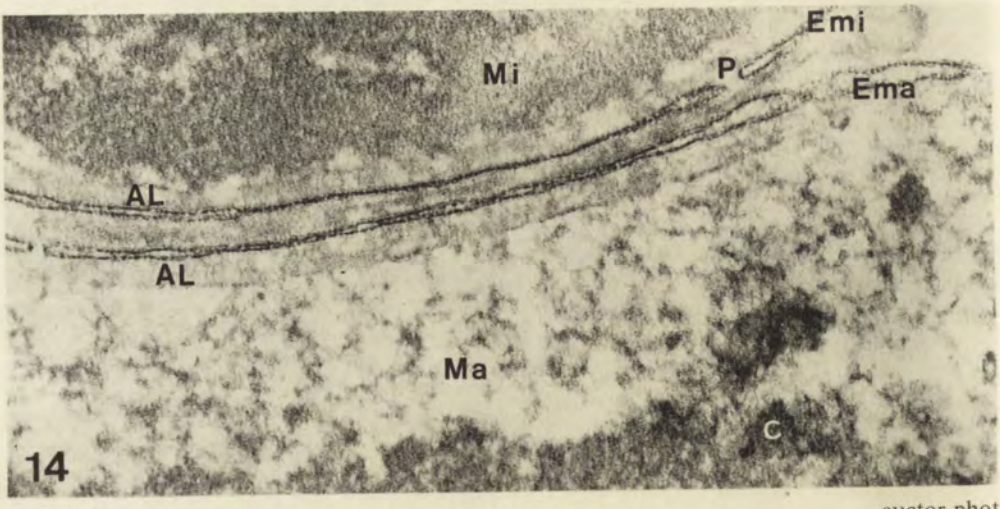

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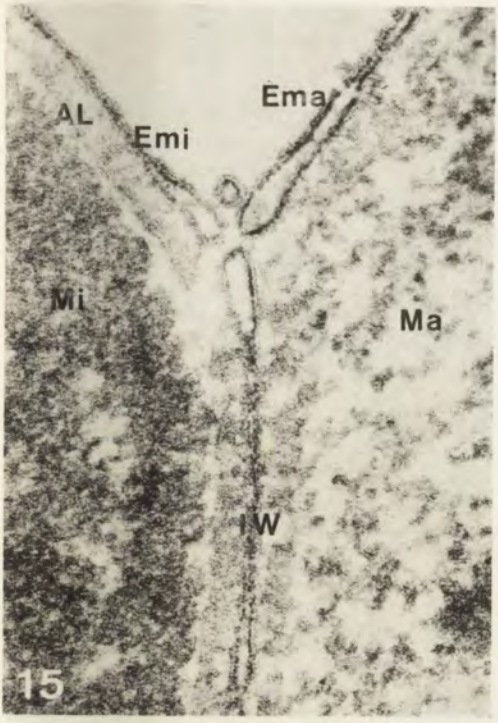

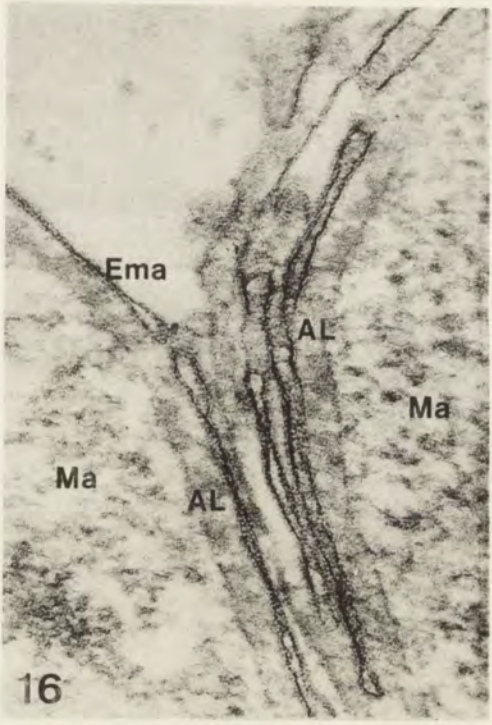

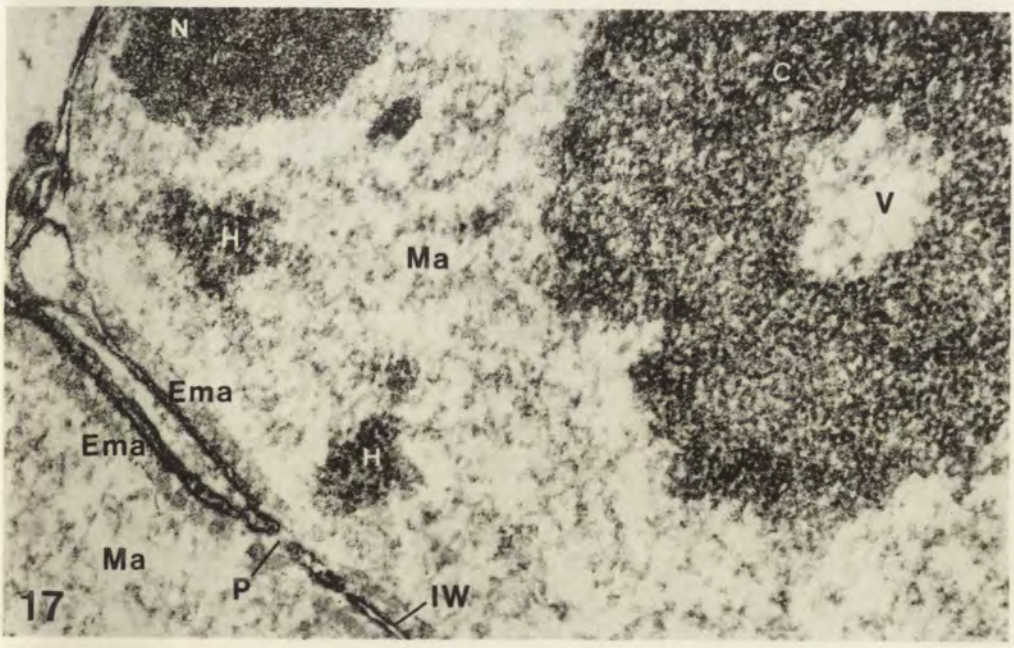

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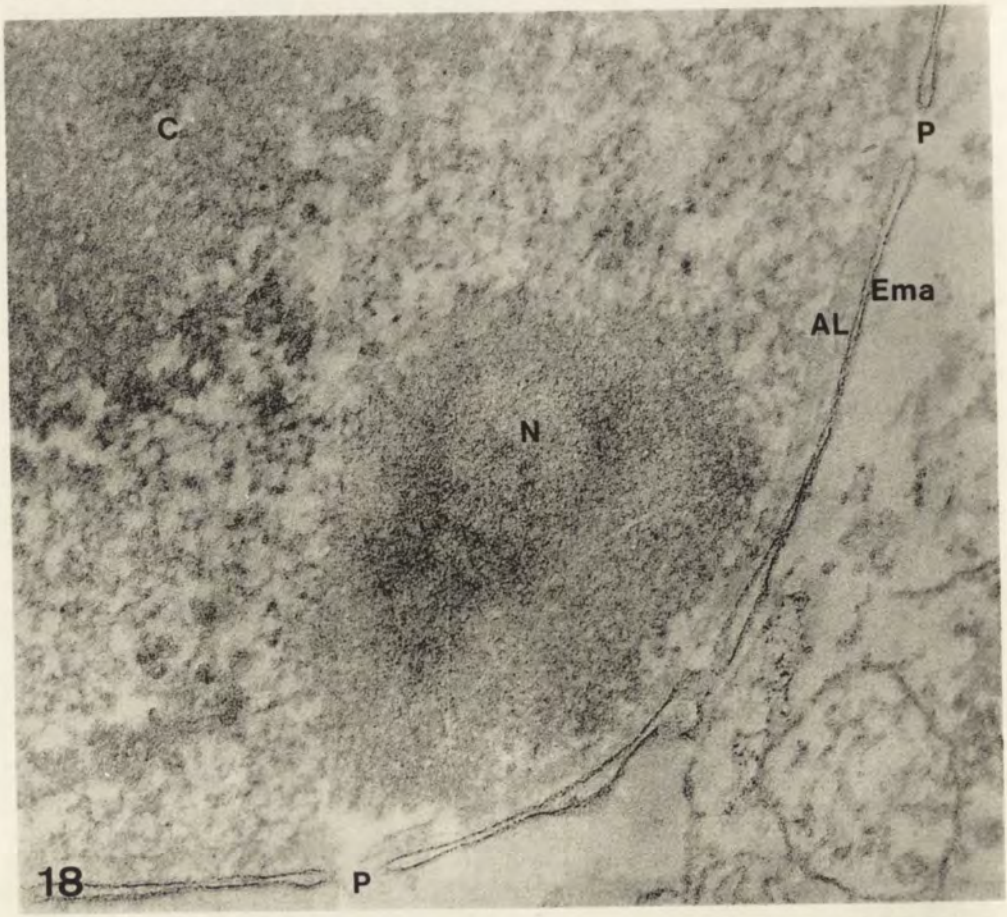

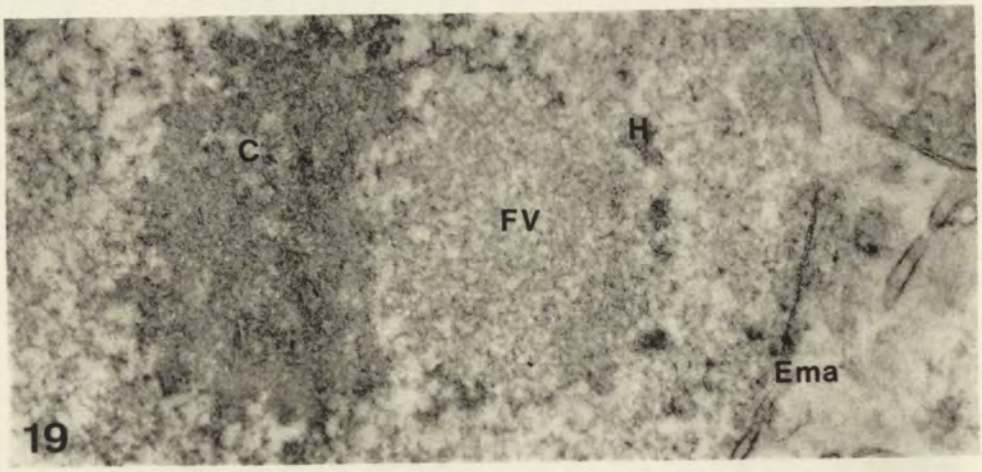

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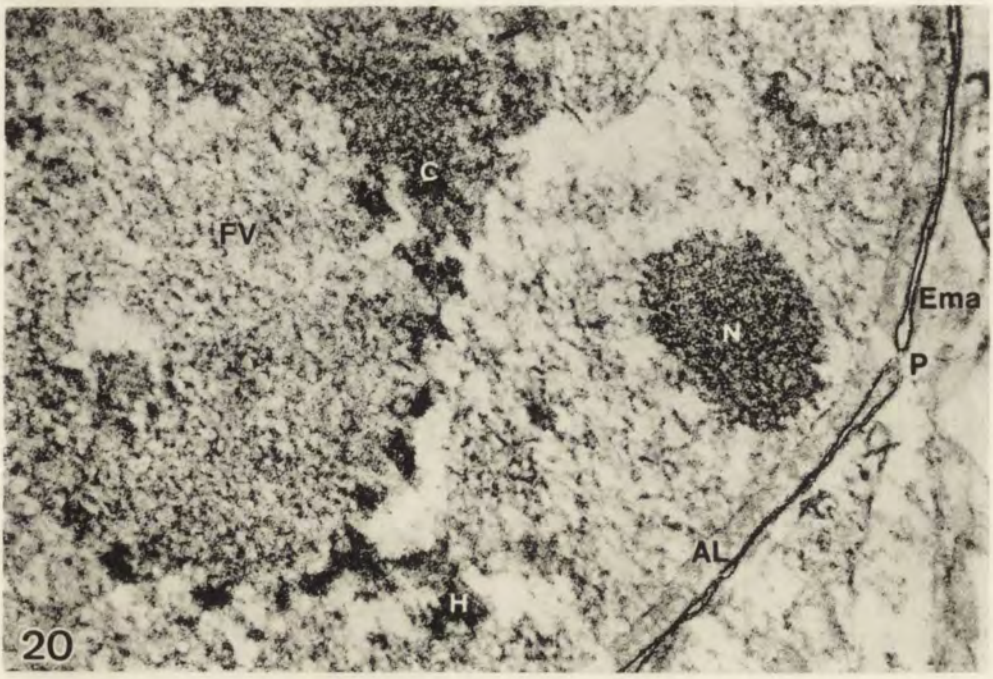

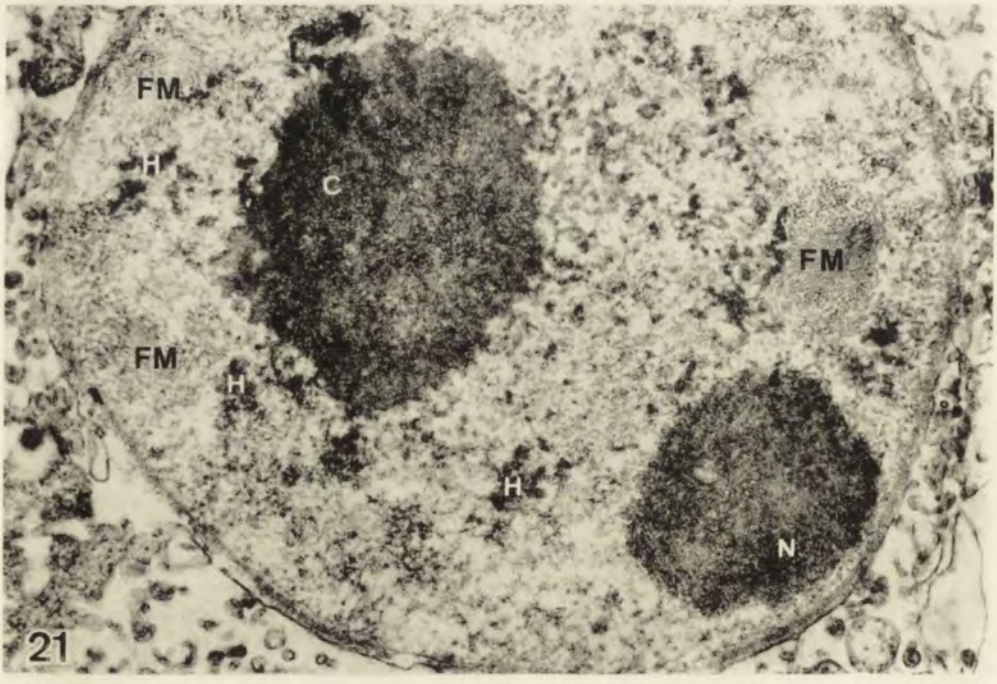

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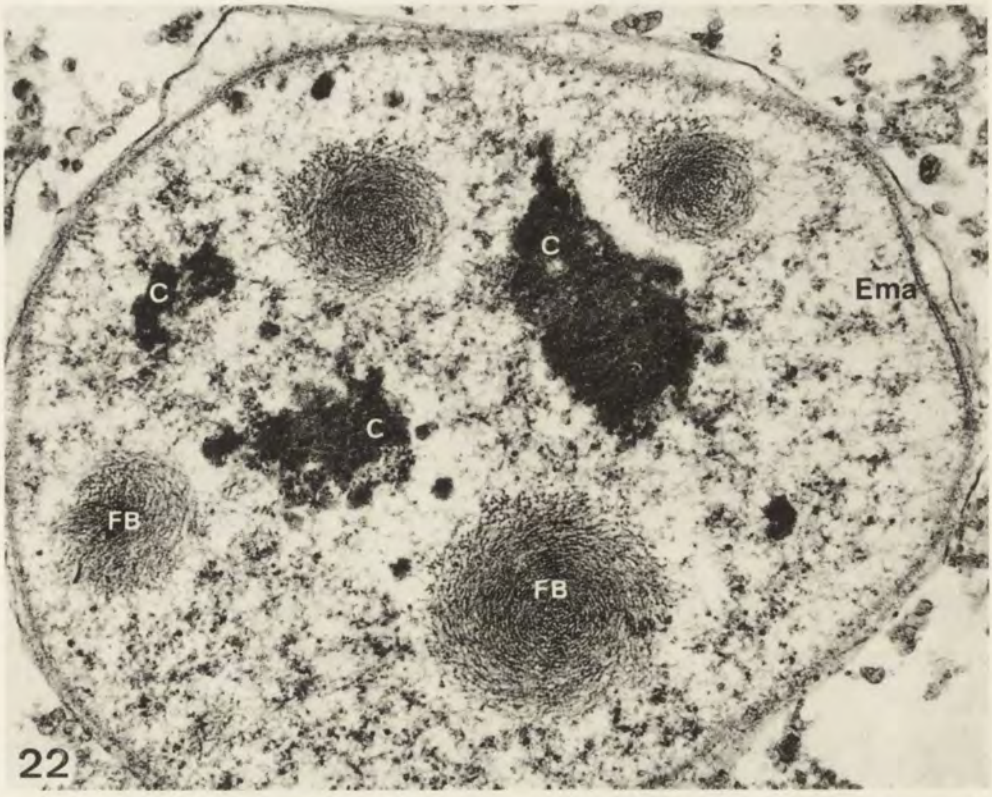

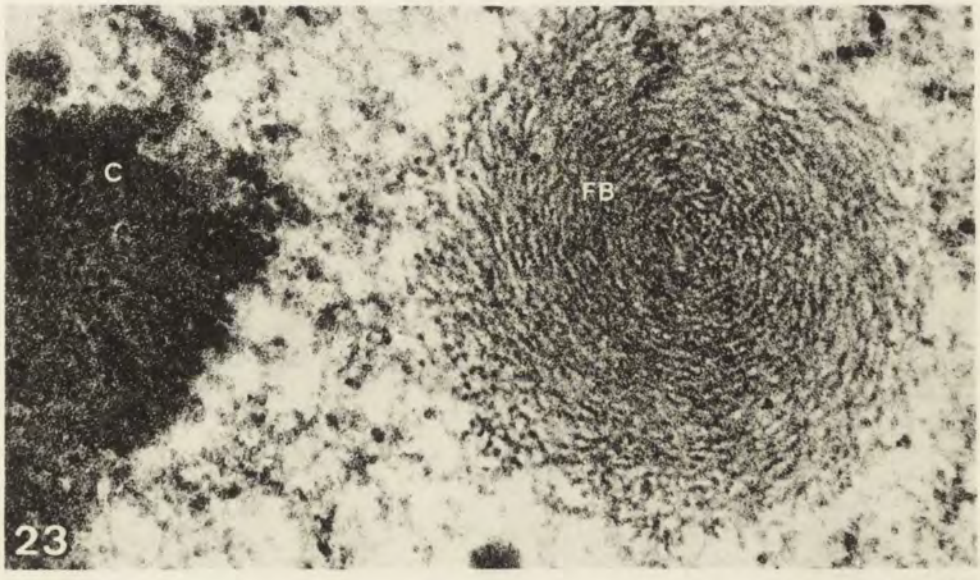

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Localization of primordia during cortical development in *Keronopsis rubra* (Ehrbg., 1838) (*Hypotrichida*)

Lokalizacja zawiązków powierzchniowych w czasie rozwoju *Keronopsis rubra* (Ehrbg., 1838) (*Hypotrichida*)

The problem of participation and role of the preexisting structures in the cortical development of ciliates is a central question in the study of morphogenesis. The view is still accepted that kinetosomes and other fibrillar structures play an essential role in the process of morphogenesis (to quote only Lwoff 1950, Sonneborn 1963, 1970, Wise 1965, Tuffrau 1969, Frankel 1972).

Experimental study of the morphogenesis of the hypotrich ciliates supplied evidence, that the preexisting structures may take part in morphogenesis, but their role is limited in such processess as the determination of the site where primordia of the new ciliature are to be formed.

Jerka-Dziadosz and Frankel 1969 showed in *Urostyla weissei* that the preexisting cirri located in the regions morphogenetically competent are involved in the formation of the primordia of new cirri. The kinetosomes from their basal plates disaggregate and become incorporated into the primordium. A similar phenomenon was described by Grimes 1972 in *Oxytricha fallax*.

It has been demonstrated as a result of the experimental analysis that the presence of preexisting cirri in the loci of primordia formation is not necessary for the primordia to appear (Jerka-Dziadosz 1972 a), and that microsurgical removal of a part of the cell causes a change in the localization of primordia in relation to the old ciliature (this shifting may cause that other or none of the old cirri take part in development (Jerka-Dziadosz and Frankel 1969)).

In most ciliates the oral primordia originate closely to and with connection to a particular kinety which is related to the old oral apparatus (Tartar 1967). The connection between the oral primordium and one (or several) kinety would not only be topographical, but also structural — in the meaning of the continuity of kinetosomes (Lwoff 1950). It has been also well documented in many holotrichs and heterotrichs that the oral primordium may shift along the stomatogenic kinety (Suzuki 1957, Suhama 1961, Frankel 1960, 1969, Hashimoto 1964).

In hypotrichs, where the character closest to a kinety exhibits only the dorsal ciliary rows, it is impossible to speak about the stomatogenic kinety. It has been

demonstrated that in *U. grandis* and *U. cristata* during division the oral primordia of the proter and opisthe are formed along the meridian which passess through the central region of the ventral surface (Jerka-Dziadosz 1972).

In this paper the author presents a study on a possibility of meridional movements of the formation sites of the oral and cirral primordia. The formation of primordia during division, reorganization and regeneration after transection were studied. It has been observed, that during these processes the oral primordium may originate at different levels of the central meridian called stomatogenic meridian. The participation and role of preexisting structures located along the stomatogenic meridian are discussed. The pigmented hypotriche *Keronopsis rubra* was chosen for this study, since its morphology and morphogenesis appear to be very similar to that in *U. cristata*, however, the very simple somatic ciliature does not complicate the pictures of the primordial structures.

Materials and methods

The study was carried out on two strains of *Keronopsis rubra* which differ slightly in the number of the frontal and marginal cirral rows. The first strain was collected from the pond of Sadyba in Warsaw in Summer 1970, while the second was collected in Spring 1971 from the Jeziorka river near Warsaw, Poland. The ciliates were maintained in Pringsheim solution medium and fed with *Tetrahymena pyriformis*, according to the method described previously for *U. weissei* (Jerka-Dziadosz and Frankel 1969). It is very difficult to maintain good prospering culture, since the organisms are very sensitive to changes in the culture conditions and susceptible to infection by the parasitic suctorians *Sphaerophrya* sp.

The studies were based on preparations stained with protargol according to the method used previously (Jerka-Dziadosz and Frankel 1969). Cells were cut by hand using a sharp microknife. Fragments were fixed at different intervals between 2–4 h after operation.

Determination of the species was difficult, since none of the descriptions in Kahl's book (Kahl 1934) does fit exactly to our specimens. The ciliate seems to be most similar to *Keronopsis rubra* (Ehrbg., 1838). Wallengren 1902 described briefly the morphogenesis during division in related species *Holosticha rubra* (Ehrbg., 1838). Rühmekorf 1935 worked with other species *K. flava* and *K. flavicans*.

Results

Morphology of morphostatic individuals

Figure 1 shows the schematic drawing of the ventral surface of *K. rubra*. The length of the animal is about 130–170 μm. The ciliates show a dark pink to purple colouring. The intensity of colour depends on the culture conditions.

The ciliature of the ventral surface consists of the oral ciliature with the adoral zone of membranelles — AZM and two undulating membranes — UM, and the frontal, ventral transverse and marginal cirri.

The AZM rounds up the anterior portion of the cell, and passes on the left side to the ventral surface, surrounding the opening to the peristome. The number of the membranelles in AZM varies. Most frequently there are 35 membranelles.

Under the pellicle the peristome extends as a funnel directed toward the posterior end (Pl. I 2). On the right side of the buccal cavity there are two undulating membranes (Pl. I 2). The outer one is longer and is situated at the edge of the peristomal lip. The inner UM lies on the inner surface of the lip.

The frontal cirri are located on the frontal area, anteriorly and to the right from the UMs. There are three large cirri behind the anterior portion of the AZM, and

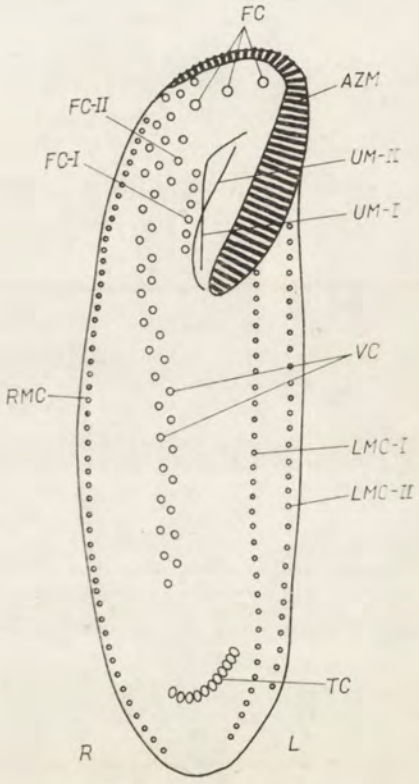

Fig. 1. Cortical anatomy of the ventral surface of *Keronopsis rubra*
Abbreviations: AZM — adoral zone of membranelles, FC — frontal cirri behind the AZM, FC-I and FC-II — two longitudinal rows of frontal cirri, LMC-I and LMC-II — two left marginal row of cirri, RMC — right marginal cirri, TVC — transverse cirri, UM-I — inner undulating membrane, UM-II — outer undulating membrane, R — right side of the animal, L — left side of the animal

two short longitudinal rows running parallel to the UMs. Close to the outer undulating membrane is located the first row of frontal cirri It consists of about 5 cirri. The second row of the frontal cirri is shifted slightly to t he right and anteriorly. It contains about 3–4 cirri. In strain I some of the cells failed to have the second row of frontal cirri. The ciliates from strain II in the first row possessed 7–8 cirri, in the second row 4–5 cirri. About 34 ventral cirri are arranged in two parallel rows running from the anterior portion of the cell to the transverse cirri, which are disposed in a short transverse row in the posterior region of the cell. The transverse row contains about 10 cirri. Those three groups of cirri: frontal, ventral and transverse will be subsequently determined as the FVT complex.

The groups of rows of marginal cirri cover the spaces on the left and right side from the FVT complex. There is one row on the right side with about 40–55 cirri in it, and two parallel rows on the left side. The first one, which is situated closer

to the FVT complex contains about 35 cirri, the second, disposed on the margin of the cell possess about 30 cirri. In some cells from the strain II there are 3 marginal rows on the left side.

The dorsal surface is covered by three longitudinal rows of single cilia. These rows extend continuously from the anterior to the posterior end of the animal.

Development of primordia during division

Proter

Stomatogenesis of the proter begins with disaggregation of the inner undulating membrane. On the internal margin of the lip an extensive proliferation of new kinetosomes takes place. As a result, the longitudinal field of closely packed kinetosomes is formed (Pl. I 3, upper arrow). This field makes the primordium of the anterior AZM. The primordium is separated from the old AZM by a space of about several micrometers. Next to this primordium and parallel to it, another parallel field of kinetosomes is formed. This field incorporates the outer undulating membrane and becomes the primordium of the undulating membranes for the proter (Pl. I 4, left arrow). The UM primordium joins the AZM primordium in their posterior part.

The AZM primordium grows anteriorly and to the right assuming a slightly diagonal, central position in the anterior part of the dividing cell (Pl. I 6). In the anterior part of the primordium new membranelles start to differentiate. Simultaneously with differentiation of new membranelles from the primordium, in the old AZM the resorption of old membranelles occurs. The old AZM is resorbed successively beginning from its most posterior part. After the ventral part of the old AZM is resorbed, the new AZM moves to the left and takes its final position. This process is finished at the fifth stage of division when the coalescent Ma starts to divide (Pl. II 12).

Within the UM field two undulating membranes and one frontal cirrus are differentiated. This process starts from the anterior part of the field (Pl. II 9). The completion of the new anterior mouth is finished after the two daughter cells became separated. The process of differentiation of the new AZM, UMs and frontal cirrus No. 1 is very similar to the same processes described in *U. grandis*, and *U. cristata*.

Development of the primordia of the FVT complex begins with the disaggregation of the frontal cirri from the row adjacent to the outer UM. These cirri are incorporated into the field in which the kinetosomes very soon become arranged in a short diagonal streaks (Pl. II 7, 8). The detailed manner of the FVT primordia formation was not followed. Within the FVT field about 24 streaks are formed. They are arranged in a ladder-like pattern (Pl II 8, 9) quite similar to that described in *U. cristata* and *U. grandis*. Differentiation of new cirri within the FVT primordium initiates at the anterior part. The first two streaks form the frontal cirri, the middle part of the ladder differentiate into the ventral cirri. In the posterior portion of the primordium the transverse cirri differentiate. The old ventral cirri are pushed to the right, and are resorbed after the new cirri have differentiated.

The primordia of the marginal cirri on the right side of *K. rubra* originate within old marginal row. Several old cirri disaggregate at the beginning of this process, and produce short files of cilia. This way of formation of marginal primordium is very similar to that described in *U. weissei* by Jerka-Dziadosz and Frankel 1969. The primordium grows anteriorly and posteriorly overlapping the old marginal cirri.

The primordium of the left marginal cirri originates in a quite different way. Both left marginal rows of cirri are formed within the old inner marginal row. The pattern of formation of the left marginal primordium is similar to that described in *U. cristata* (Jerka-Dziadosz 1972). The primordium of the left marginal cirri originates as a longitudinal field of kinetosomes. This process starts with a disaggregation of several old cirri from the left inner marginal row (Pl. II 7, V 25). The field then differentiates into two longitudinal marginal rows (Pl. II 10, 12, V 27). The most left row of old marginal cirri is not involved in morphogenesis and becomes resorbed after division.

The mode of formation of dorsal primordia was not followed.

Opisthe

The formation of the oral primordia of the opisthe in *K. rubra* proceeds in a very similar way to that noticed in the other lower hypotrichs described by Tuffrau 1969 and Jerka-Dziadosz 1972 b.

The development of the oral primordium of the opisthe initiates a little earlier than in the proter. The first sign of stomatogenesis is the appearance of small groups of kinetosomes around and to the left of the ventral cirri from the left ventral row (Pl. I 3). There is an extensive proliferation of new kinetosomes in this region and a longitudinal field is formed as a result. This is the AZM primordium of the opisthe. In the first stage of division the AZM primordium of the opisthe is situated at the same meridian as the AZM primordium of the proter (Pl. I 3). This meridian is situated to the right from the old, parental AZM.

After having been formed the AZM primordium of the opisthe moves to the left from the ventral cirri, which remain unaffected. During the movement of the AZM primordium the process of differentiation of new membranelles within the primordium occurs. This process starts from the anterior part of the primordium (Pl. I 5, 6). In close vicinity of the same ventral cirri which formed the AZM primordium, proliferation of new kinetosomes occurs, and gradually the UM primordium appears. The oral complex of the opisthe moves then to the left and situates posteriorly to the old parental AZM (Pl. I 4, 6). At this stage the oral primordia of the proter and the opisthe are located at two different meridians (Fig. 2).

The differentiation of AZM and UMs proceeds in the same fashion as in the proter. Within the UMs field two undulating membranes and one frontal cirrus are formed (Pl. II 9).

The primordium of the FVT complex of the opisthe is formed between the UM primordium and the left ventral cirral row. At first it appears as a kinetosomal field, then differentiates into streaks in a ladder-like form (Pl. I 5, II 9). From this primordium the frontal, ventral and transverse cirri differentiate.

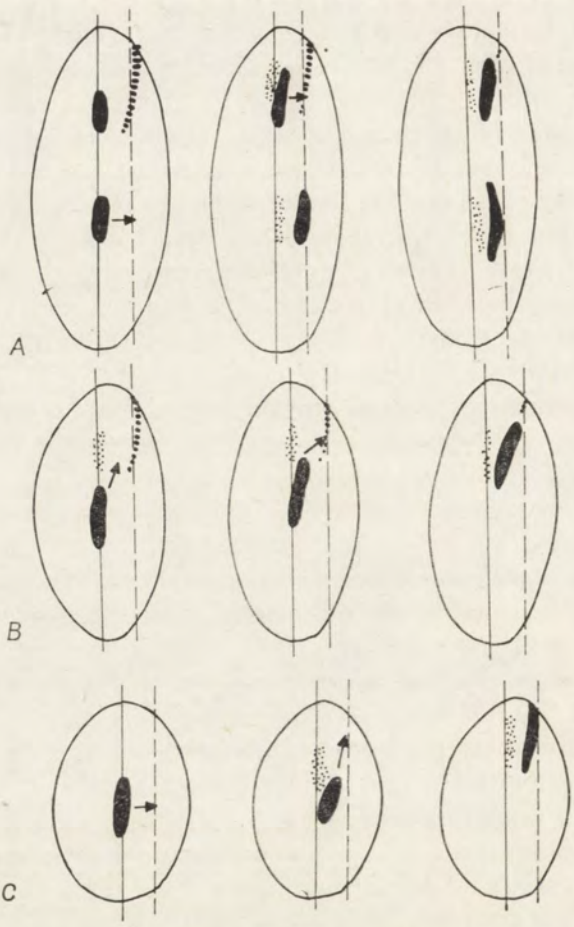

Fig. 2. Schematic representation of the localization and morphogenetic movements of oral primordia during division, reorganization and regeneration in *Keronopsis rubra*. The solid longitudinal line represents the stomatogenic meridian, the dotted longitudinal line represents the adult AZM meridian. The solid black field represents the AZM primordium, the dotted field represents the UM primordium. The arrows indicate the directions of morphogenetic movements, A — division, B — physiological reorganization and promers, C — opimers

The marginal primordia are formed at the same time and in similar way as in proter (Pl. II 7, 10).

The changes in the nuclear apparatus during division will be briefly described. During the first stage of morphogenesis in every fragment of macronucleus the replication band can be observed. That means that the morphogenesis starts at the late S phase of the macronuclear DNA synthesis (Jerka-Dziadosz and Frankel 1970). When the replication band passess through macronuclei, each one rounds up (Pl. II 7) and then the macronuclei start to coalescence into one unit. The division of MA and mitosis of Mi occur at the fifth stage of division (Pl. II 11, 12), when the new cirri are well developed.

Development of primordia during reorganization

Reorganization, known also as a physiological regeneration has been described in many ciliates by various authors. It is a process of replacement of the oral apparatus as in *Stentor* and *Tetrahymena* (Tartar 1961, Frankel 1960, 1969) or the replacement of oral and somatic ciliature as in *Stylonychia*, *Oxytricha* and *Urostyla* (Dembowska 1938, Hashimoto 1963, Jerka-Dziadosz 1963, 1965, Jerka-Dziadosz and Frankel 1969). Cortical reorganization in *Keronopsis rubra* involves a replacement of all the oral and somatic ciliature.

Morphogenesis begins with the proliferation of kinetosomes at two regions of the ventral surface. As a result two kinetosomal fields are formed (Pl. III 13, 15). The anterior field is formed within the inner undulating membrane. This is the primordium of the new UMs. On the same meridian, more posteriorly, close to several ventral cirri from the left row, the second kinetosomal field is formed (Pl. III 13). This is the primordium of the new AZM. The AZM primordium grows and moves gradually toward the anterior end and takes its place between the old AZM and the field of the UMs primordium (Pl. III 15, 16). During the movement of the AZM primordium, the old AZM becomes broken and resorbed. This process is visible on Plate III 16.

Differentiation of new membranelles within the AZM primordium occurs during the morphogenetic movement. At the same time the new UMs differentiate within the UM field. When the UM and AZM gain the parallel arrangement the whole oral complex shifts leftward and take its final position.

The formation of the FVT primordium initiates with the disaggregation of the old frontal cirri from the row nearest the UM. The kinetosomes from these cirri proliferate and join the kinetosomes formed simultaneously close to the anterior part of the AZM primordium. This field then organizes itself into kinetosomal streaks which are situated parallel to the primordium of the UM placed anteriorly and primordium of the AZM placed posteriorly (Pl. III 16). This primordium differentiates into the frontal, ventral and transverse cirri in the same way as in the proter and opisthe (Pl. III 17).

Formation of marginal cirri occurs in the same fashion as in the proter or opisthe.

The macronucleus remains dispersed during reorganization of cortical organelles in *K. rubra*. In some specimens during the first stage of reorganization the replication band in the macronuclei can be seen (Pl. III 14).

In other specimens no replication bands were observed. That means that both kinds of cells: G_1 and S may enter the process of reorganization. During the later stages of reorganization each fragment of Ma rounds up, but no coalescence of the macronuclear material occurs.

Development of oral primordia during regeneration

Promer

After transection of morphostatic individual in the middle of the cell (at the plane where normally the division furrow is formed), two fragments are obtained.

Each fragment is covered by the same ciliature as the future proter and opisthe at the beginning of divisional morphogenesis. It was expected therefore, that stomatogenesis of proter and promer (anterior fragment) should proceed according to the same pattern. The study of the formation of oral primordia in promers has revealed that the pattern of the formation of the oral primordia differs significantly from that in the anterior product of division, and is very similar to that in reorganization.

The first sign of cortical regeneration is the formation of small groups of kinetosomes close to the cirri from the left ventral cirral row (Pl. IV 18). The proliferation of new kinetosomes in this region continues and spreads anteriorly (Pl. IV 19, 20, 21). The anterior portion of the AZM primordium moves and locates itself between the old AZM and the UMs being transformed into the new UM primordium. The UM primordium is formed within the inner undulating membrane (Pl. V 27). The fate of the outer undulating membrane is unclear. It is probably resorbed, but it is also possible that it becomes incorporated into the UM field. When the AZM primordium gains the position parallel to the UM primordium, the whole oral complex moves to the left and takes the place of the resorbed old AZM (Pl. V 26, 27). Differentiation of the AZM and UMs from primordia proceed in the same manner as in the proter during division.

The formation of the FVT primordia initiates with the disaggregation of the old frontal cirri from the frontal row situated closely to the old UM. A longitudinal field of kinetosomes is formed at the frontal area. This field joins the kinetosomes formed to the left of the anterior part of the AZM primordium at the early stage of development (Pl. IV 20, 21, V 24). This longitudinal field is then organized into the kinetosomal streaks and later differentiates into the frontal, ventral and transverse cirri (Pl. V 25, 26).

The second type of the anterior fragments studied were small promers, which contained only the frontal area (the line of operation ran anteriorly to the cytostome, near the middle region of the old AZM). In such small fragments (Pl. IV 22, 23) both UM and AZM primordia originate within the inner undulating membrane. At the first stage a longitudinal field of kinetosomes is formed. Within this field the primordium of AZM occupies the posterior part, and the UM primordium the anterior part. Parallel to this primordium of the oral complex, the primordium of FVT orginates around the frontal cirri from the first row (Pl. IV 23). During development the AZM primordium grows and moves to the left on the spot of the resorbed old AZM.

The spatial organization of the primordia of the oral complex in small anterior fragments is very similar to the spatial organization of primordia in small posterior fragments (cf. Pl. IV 22, 23, V 29, 30).

Opimer

Cortical development in the posterior fragments of *K. rubra* resembles of this process in the posterior products of division, i.e., in opisthes.

At the beginning of morphogenesis, the proliferation of new kinetosomes sets in on the middle of the ventral surface of the fragment, close to the ventral cirri of

the left ventral cirral row (Pl. VI 28). The first formed AZM field appeared as a longitudinal field of kinetosomes. Very soon at the anterior portion of this field differentiation of new membranelles occurs.

Anteriorly to the AZM primordium, also close to the ventral cirri the primordium of the UM is formed. This kinetosomal field grows anteriorly. Both primordia the AZM and UM primordium move apart from the ventral cirral row. In their former place another longitudinal field of kinetosomes is formed. This is the primordium of the FVT complex (Pl. VI 30, 31, 32). During the next stages of development the AZM primordium moves forwards and to the left, until it rounds up the anterior border of the fragment. The UM primordia differentiate into two undulating membranes, the FVT field forms kinetosomal streaks and then differentiate into cirri (Pl. VI 32, 33). The marginal primordia are formed as in reorganization.

It is worth pointing out here that in contrast to the situation which exists in the promer, where in the process of formation of the FVT primordia the old frontal cirri are involved, in opimers and opisthes none of the old cirri disaggregate into primordia. Only at the late stages of development, when new cirri differentiate within the old ventral row 2–3 old cirri are absent at the point where the FVT streaks move posteriorly and to the right (Pl. VI 25 arrow).

The formation of primordia in the large opimers, in which part of the mouth has been left, proceeds in a way more resembling reorganization. The primordium of the AZM is formed as in opimers, but the UM primordium develops with the participation of the remnants of the old inner UM. Both primordia are located at the same meridian.

In the morphogenetic processes described above in *K. rubra* the following regularities could be observed:

(1) During cortical development the oral primordia originate always at the same central meridian of the cell or its transverse fragment.

(2) There are morphogenetic movements of the newly formed primordia from the place where they originate to their final position in the adult cell. The extent of these movements is different in different types of morphogenesis (binary fission, reorganization, regeneration).

(3) In all types of morphogenesis the activation of several old cirri from the left postoral ventral cirral row take place. In their vicinity the whole oral apparatus (opisthe, opimer) or only one part of the oral apparatus (promer, reorganizer) may be formed.

(4) When the reorganizing cell possess the intact oral apparatus within its inner undulating membrane the oral primordium (or part of it) is formed. The old AZM is always resorbed.

(5) The primordia of the somatic cirri as well as the oral primordia are formed as kinetosomal fields, and are subsequently arranged into appropriate structures. In this respect *K. rubra* is very similar to *U. cristata.*

(6) Some parts of the old oral and somatic ciliature are involved in the process of formation of primordia. The participation of old ciliature in morphogenesis is not the same in different development situations.

The following summary shows what old structures are used in the formation of primordia: (a) AZM — in all morphogenetic processess remains inactive and is resorbed in the late stages of development. (b) The inner UM is always incorporated into the kinetosomal field formed close to it. This field is a part of the oral primordium. (c) The outer UM is incorporated into the UM field in the proter. (d) The frontal cirri from the first row, as in *U. cristata*, are incorporated into the FVT primordium in proters, promers and reorganizers. (e) From the ventral cirri — only those situated on the middle of the central meridian of the whole cell or fragment are activated and used as generative centers for kinetosomal proliferation. Some of the ventral cirri are incorporated into the FVT streaks in their posterior regions. (f) The most frontal cirri, the second row of frontal cirri, the transverse cirri and the most left row of marginal cirri do not take part in the morphogenesis.

Discussion

The study on localization of the oral primordia in different developmental situations supplies interesting material for discussion of the problem of the determination of the sites of formation of the oral primordia in hypotrich ciliates.

The localization and development of the oral and the somatic primordia in *Keronopsis rubra* is very similar to that described by one of the authors (Jerka-Dziadosz 1972) in *U. grandis* and *U. cristata*. In all the three species the existence of a stomatogenic meridian has been observed. On this meridian the initiation of the kinetosome proliferation for both the AZM and UM primordia take place. In all three species the old AZM is not involved in development and is resorbed at a late stage of division. In the species in question a morphogenetic movements have been noted. These movements are most distinct in *K. rubra* and will be discussed here (Fig. 2).

During division the kinetosomal fields which give origin to the AZM and UM primordia are formed along one and the same meridian and later differentiate and move to their final position. The movements of all primordia do not occur simultaneously, but successively, in proportion to the appearance of the primordia in question. First, the AZM primordium for the opisthe moves to the left and is situated on the same meridian as the maternal AZM. At this time the oral primordia of both offspring are located on two different meridians. Later, after the old AZM has been resorbed, the new oral ciliature for the proter also moves to the left and takes a position on the same meridian as the AZM of the opisthe. The proliferation of new kinetosomes occurs continuously during the morphogenetic movements of the primordia, and new kinetosomes are added even when new membranelles are formed (Grimes 1972), in each part of the dividing cell, the AZM and UM primordia are situated parallel to one another.

In contrast to this, during reorganization the two parts of the newly formed oral ciliature are situated along one line of the central meridian and at some distance from each other (Fig. 2, second row of drawings). The same situation occurs in

regenerating fragments. The UM primordium is always situated anteriorly to the AZM primordium. The distance between these two structures is proportional to the size of the fragment.

The heterogeneity of the two parts of oral structures formed during reorganization is a phenomenon of general character. It occurs as well in those hypotrich ciliates in which the anterior AZM persists unchanged during division, as in *U. weissei* and *Stylonychia mytilus*, and also in those in which the original AZM is replaced during division as in *U. cristata* and *K. rubra* (Dembowska 1938, Jerka-Dziadosz 1964, Jerka-Dziadosz and Frankel 1969).

It is interesting that in such anterior fragments which are identical to proters with respect of the amount of ciliature, the oral primordium develops according to the reorganization pattern rather than divisional.

This phenomenon occurs also in both types of hypotrichs. Promers of *U. weissei* (similarly and promers of *K. rubra*) form the oral primordium according to the reorganization pattern. Morphogenesis in the posterior fragments occurs according to the same pattern as in opisthes. As it follows from the summary presented in Fig. 2, in all three types of morphogenesis the site of primordia formation appears to be at the central meridian on the ventral surface. This is called the "stomatogenic meridian". In the whole normal cell of *U. grandis*, *U. cristata* and *K. rubra* this meridian covers the inner undulating membrane and part of the ventral row of cirri. The proliferation of new kinetosomes usually starts in close vicinity to these structures and this region of the cell gains morphogenetic competency first.

The activation of other region of the surface takes place in relation to the oral primordia formed at first. As a result the primordia are located proportionally to the whole cell and to the oral primordium. As was mentioned in the introduction, removal of preexisting cirri from places already morphogenetically competent does not affect the formation of the cirral primordia in *U. weissei* (Jerka-Dziadosz 1972 a). The study of Hashimoto 1964 on induced excystment in *O. fallax* has shown that the site of formation of the oral primordia during excystment covers the size of the formation of the oral primordia during reorganization. On the other side Grimes in ultrastuctural studies on morphology of cysts of *O. fallax* failed demonstrate any kinetosomes (Grimes unpubl.). That means, that neither kinetosomes nor other elements of preexisting structures (cirri, UMs) are responsible for the determination of the site and formation of the primordia.

The studies now in progress, on the regeneration of lateral fragments and regeneration of fragments with stomatogenic meridian damaged by laser irradiation will possibly provide new data to the problems of cortical determination and differentiation in ciliates.

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Summary

Formation of oral and cirral primordia during binnary fission, physiological reorganization and regeneration after transverse sections were investigated in *Keronopsis rubra*.

The oral primordia appear in the central meridian, no matter what preexisting ciliary structures (UM, ventral cirri) are present on this meridian.

The topographical relationships between different elements of primordial structures, and their morphogenetic movements were studied. The directions and magnitude of the morphogenetic movements depends on the developmental situation and on the size of fragment studied.

STRESZCZENIE

Zbadano tworzenie zawiązków oralnych i zawiązków cirri somatycznych w procesach podziału, reorganizacji fizjologicznej i we fragmentach powstałych po przecięciach poprzecznych u *Keronopsis rubra*.

Stwierdzono, że zawiązki oralne powstają wzdłuż środkowego południka ciała, niezależnie od tego, jakie preegzystujące struktury rzęskowe (UM, cirri wentralne) znajdują się na tym południku.

Badano zależności topograficzne różnych elementów zawiązków oraz ich ruchy morfogenetyczne. Kierunek i wielkość ruchów morfogenetycznych zależy od sytuacji rozwojowej i wielkości fragmentu.

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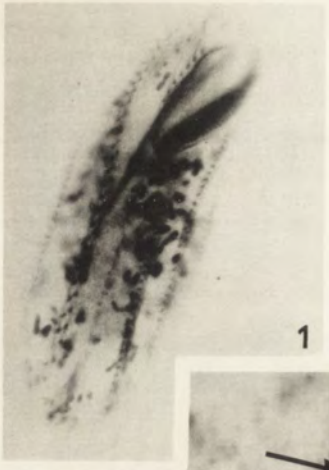
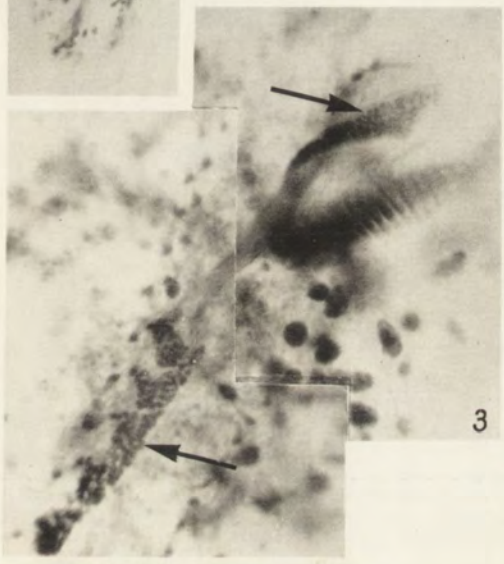
EXPLANATION OF PLATES I–VI

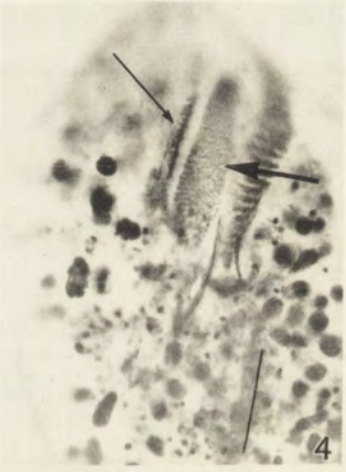
Photographs of protargol impregnated specimens of *Keronopsis rubra* engaged in cortical morphogenesis associated with cell division (3–12) reorganization (13–17) and regeneration (18–33). All photographs are printed at the same magnification and so that the anterior end is up and animals left correspond to the viewers right. The magnification (in parentheses) indicates that of the microscopic objective

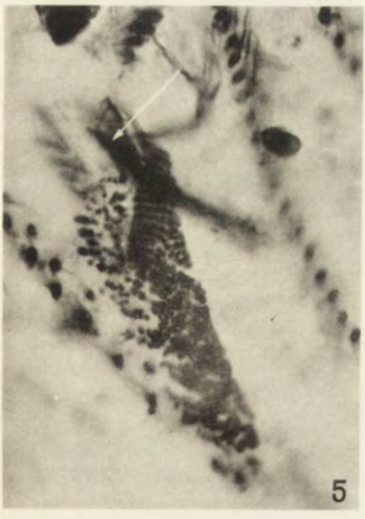
1: The general view of the ventral surface of morphostatic cell (40×)
2: The anterior portion of the morphostatic cell. Note the position of the frontal cirri close to the UM. The arrow indicates the region where the first kinetosomes for the primordium of AZM would appear (100×)
3: The 1st stage of division. The upper arrow indicates the kinetosomal field formed within the inner undulating membrane. The lower arrow points to the AZM primordium for the opisthe (100×)
4: The cell at 2nd stage of division. The AZM primordium of the proter is well organized (right arrow). The UM primordium is developing (left upper arrow). The line in the posterior region marks the position of the posterior AZM (100×)

5: The dividing cell at 2nd stage of division. Note the beginning of differentiation of new membranelle within the AZM field of opisthe. The FVT primordium is being formed. The white arrow points an artefact (100×)
6: The cell at late stage 3rd of division. Note the position of both new AZMs and the old AZM (arrows) (100×)
7: The cell at late 3rd stage of division. The posterior oral and FVT primordia are well developed. The arrow indicates the marginal cirri forming a field (40×)
8: High magnification of the anterior part of the dividing cell. The arrow indicates the FVT field formed around the old frontal cirri (100×)
9: High magnification of the posterior part of the dividing cell. The kinetosomal streaks of the FVT can be clearly seen. The right arrow indicates the frontal cirrus formed within the UM field. The left arrow indicate kinetosomes of the anterior FVT streaks froming ventral cirri (100×)
10: The cell at 4th stage of division. The arrows indicate the two rows of left marginal cirral rows (100×)
11: Nuclear apparatus of cell at 5th stage of division (40×)
12: The ventral surface of a cell at 5th stage of division. The new ciliature is fully formed (40×)
13: The reorganizing cell at 1st stage of development. The arrow points to the AZM primordium (100×)
14: Nuclear apparatus of the reorganizing cell. Note the replication bands (100×)
15: The same object as on phot 14. The arrows indicate the anterior (upper arrow) and posterior (lower arrow) parts of the oral primordium. Note also the beginning of dispersion of marginal cirri at the level of the lower arrow (100×)
16: Reorganizing cell at 3rd stage. Note the position of the FVT complex. The old AZM is in the process of resorption (100×)
17: Reorganizing cell at 4th stage. The FVT, UM, AZM and marginal primordia are well developed (100×)
18: Promer at the 1st stage of regeneration. The arrow indicates the new kinetosomes close to the cirri from the left ventral row (100×)
19: Regenerating promer. The primordium of AZM is well developed and moves anteriorly (100×)
20: Regenerating promer. Note the kinetosomal field extending anteriorly and rightwards from the primordium of the AZM (100×)
21: Regenerating promer. The AZM primordium is shifting anteriorly (100×)
22: Small regenerating promer. Note the position of the new oral complex. The old UMs are incorporated to the primordium (100×)
23: The same fragment as on the phot. 22 focused at the surface. The right upper arrow indicates the UM field. The right lower arrow indicates the AZM primordium. The left arrow indicates the FVT field. Note the second intact row of frontal cirri
24: The regenerating promer at the same stage of development as that on the phot. 21. Note the formation of kinetosomal field to the left on the inner undulating membrane and disaggregating frontal cirri next to the outer UM. The membranelles of the old AZM are at the beginning of resorption (100×)
25: The regenerating promer at the 4th stage of regeneration. The old AZM is partly resorbed. The right arrow indicates the disaggregated old marginal cirri from which the kinetosomal streak is being formed. The left arrow indicates the region of the ventral cirral row where the FVT primordium growing posteriorly has incorporated 4 old ventral cirri (Compare with the phot. 9, left arrow) (100×)
26: Regenerating promer at late 4th stage of development. The new transverse cirri can be seen in the posterior part of the FVT primordium (100×)
27: Regenerating promer at 4th stage of development. The upper arrow indicate the remnants of the original AZM. The lower arrow indicate the forming two left marginal row of cirri (100×)
28: Regenerating opisthe at first stage of development. The arrows indicates the primordium of AZM
29: The regenerating opimer. Longitudinal field of kinetosomes is seen in the middle of the fragment. The upper arrow indicates the UM primordium. The lower arrow points to the AZM primordium (100×)
30: Regenerating opimer at 2nd stage of development. The upper right arrow indicates the UM primordium, and the left arrow marks the position of the primordium of FVT complex (100×)
31: The regenerating opimer at 3rd stage of development role the incorporation of old ventral cirri adjacent to the posterior of primordia
32: The regenerating opimer at 3rd stage of development. In the right anterior part of the AZM primordium differentiating membranelles of AZM are seen
33: The regenerating opimer at 4th stage of development. The AZM has moved anteriorly. The arrow marks the interruption in the rows of ventral cirri, where the posterior FVT streaks have developed (100×)









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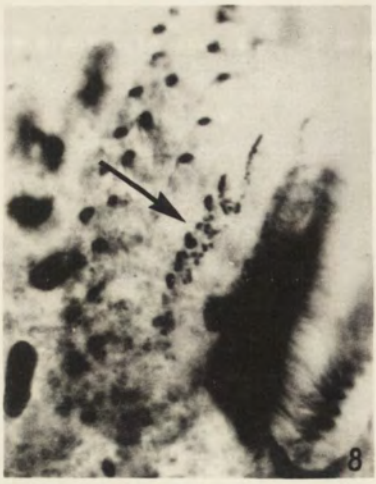

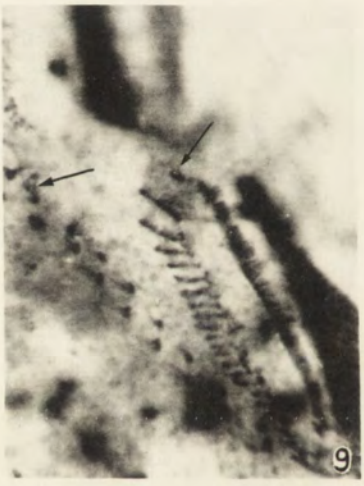

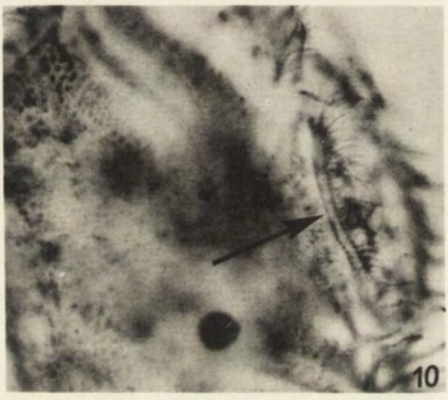

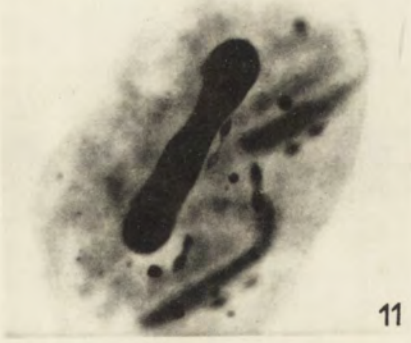

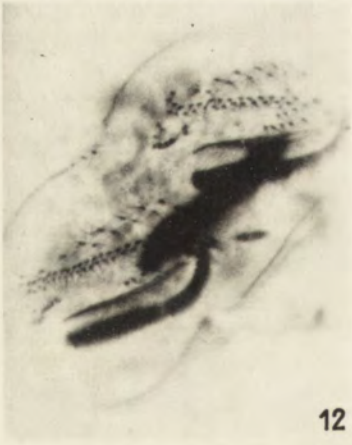

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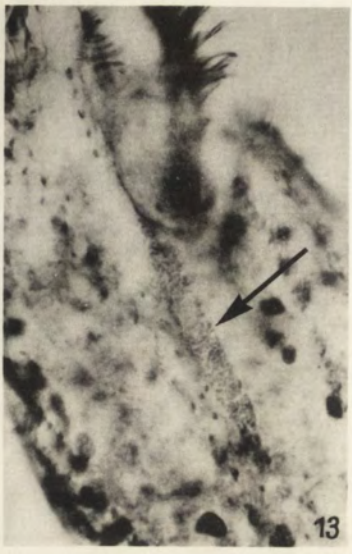

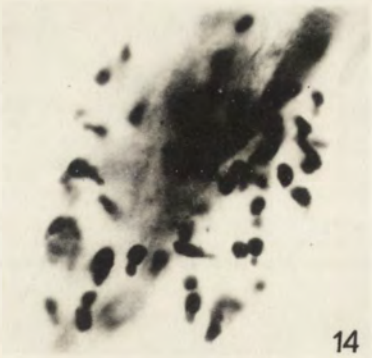

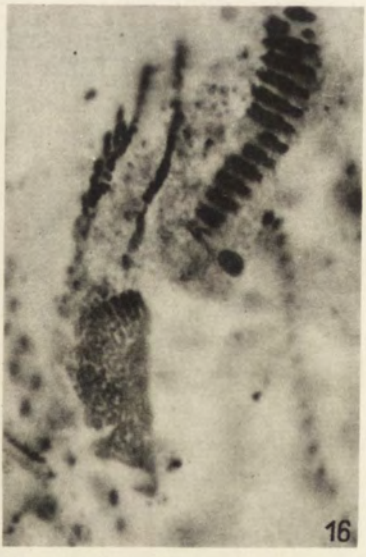

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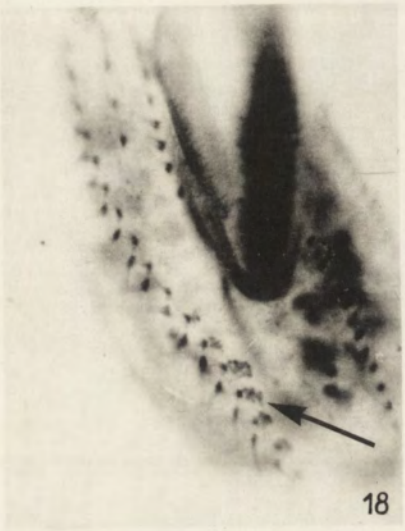

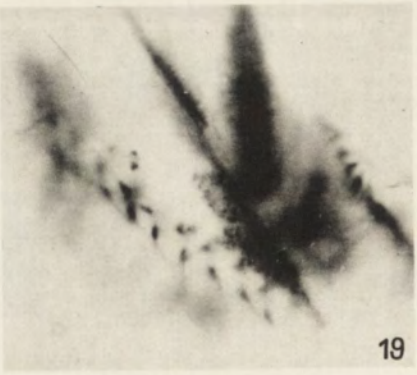

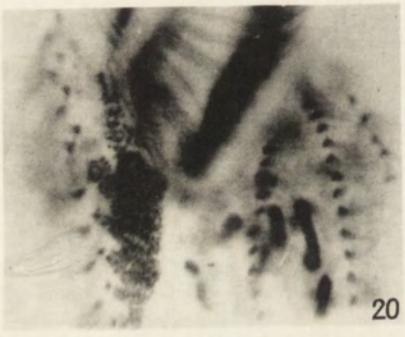

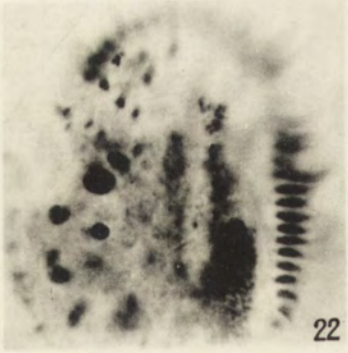

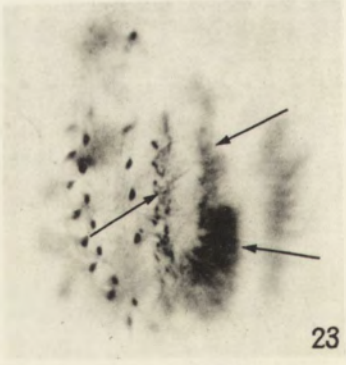

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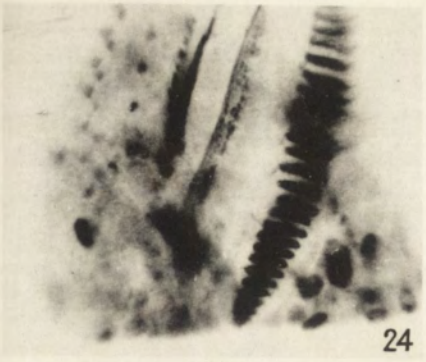

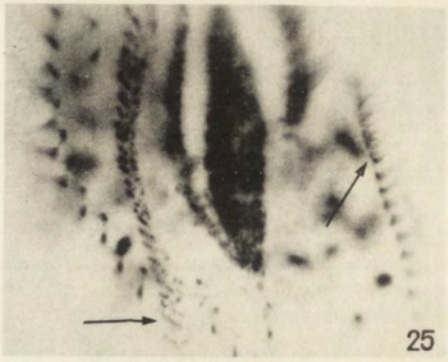

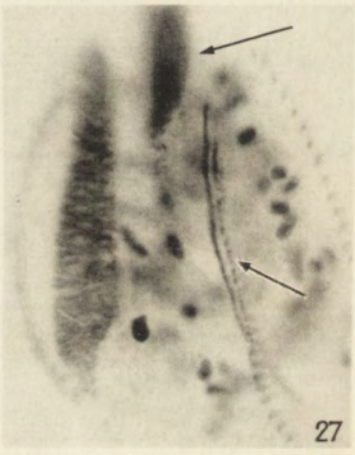

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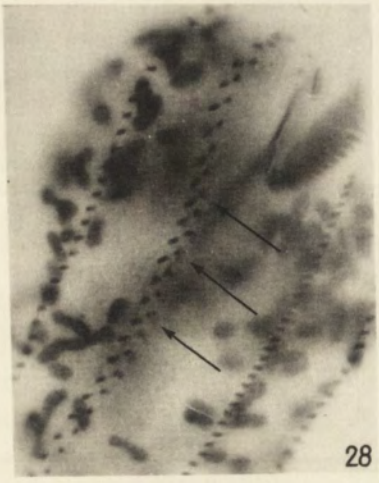

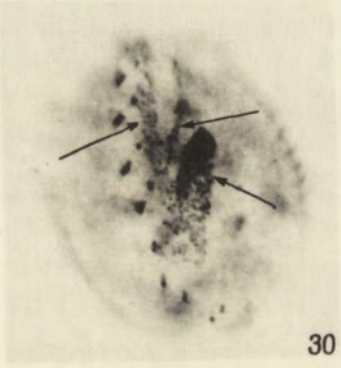

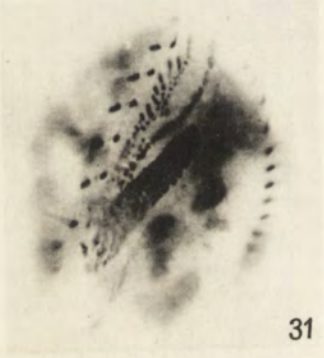

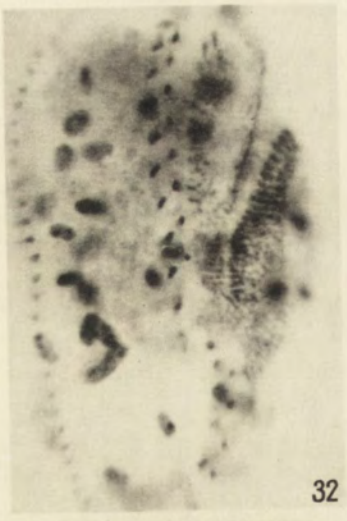

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ACTA PROTOZOOLOGICA

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M. ANWAR

Isospora erithaci sp. n. (*Protozoa, Eimeriidae*) from the redbreast (*Erithacus rubecula*)

Isospora erithaci sp. n. (*Protozoa, Eimeriidae*) из малиновки (*Erithacus rubecula*)

Since the first description of *Isospora* from passerine birds by Labbé 1893, a number of investigators (Yakimoff and Gousseff 1936, 1938, Yakimoff and Matschoulsky 1936, 1938, Boughton 1937, Carpano 1937, Chakravarty and Kar 1944, 1947, Ray et al. 1952, Schwalbach 1959, and Anwar 1966) have described several species from this large class of birds. With some exceptions all descriptions are based on oocyst morphology. The present investigation is a description of a new species from the redbreast.

Materials and methods

All the birds used for this study were trapped at the Imperial College Field Station, Ascot, Berkshire.

The trapped birds were transferred to a single canary cage and the droppings were examined under the microscope. Coccidial oocysts were recovered from all but one. For further study, the faecal sample of each bird was placed in 2.5% potassium dichromate in a petri dish and was oxygenated by stirring vigorously twice a day. The sporulated oocysts were identified as *Isospora*. The measurements were made to the nearest fifth of a division of a micrometer eye piece. This was felt to be the limit of discrimination.

In order to study the endogenous stages two birds were killed, and the endogenous phases were located in the region of the duodenum behind the gizzard. Infected regions were fixed in formol-sublimate or Carnoy's fluid, sectioned and stained with Heidenhain's iron haematoxylin, or with Himes and Moriber trichrome stain.

Results

In fourteen infected birds coccidial oocysts of the genus *Isospora* were recovered. A distinct type of oocyst was observed and complete measurements were made of at least 200 oocysts.

Isospora erithaci sp. n.

The oocysts were spherical (Pl. I 5 and Fig. 1 F) and measured 12.5–26.2 μ with a mean of 16.6 μ. The oocyst wall was a single layer, colourless and smooth and was ~0.9 μ thick. Micropyle and oocystic residuum were absent, but there were

one or two splinter-like polar granules. The sporocysts were pyriform 12.5–15.2 μ× ×7.6–10.6 μ with a mean of 14.0 μ×9.0 μ. Stieda body was thick and formed a lid-like cap (Pl. I 6). The sporocystic residuum was present as a compact mass. The sporozoites had a random arrangement within the sporocyst; they were elongated, pointed at the anterior and rounded at the posterior ends. Refractile globules were present at both ends. The sporulation time was 48 h at 25°C.

The endogenous stages took place above the host cell nuclei, between the nuclei and the brush border of the epithelial cells (Pl. I 1–4).

The smallest endogenous stage was a uninucleate round body measuring 3.0 μ×3.0 μ (Fig. 1 A). It was impossible to correlate this body with asexual or sexual stages as natural infections were examined.

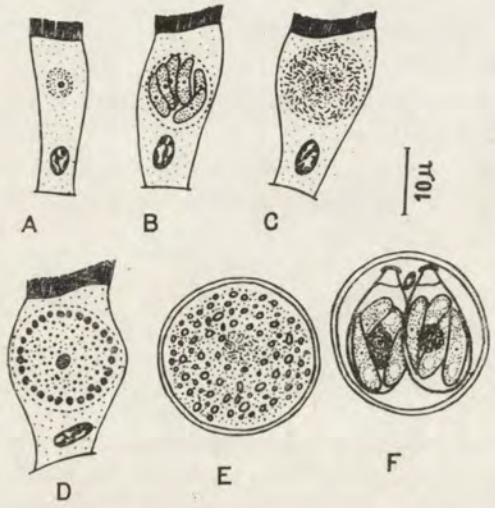

Fig. 1. Line drawings of stages of *Isospora erithaci* sp. n. A — uninucleate body, B — schizont, C — microgametocyte, D — macrogamete with "plastic granules", E — unsporulated oocyst, F — sporulated oocyst

The early schizonts were rounded and contained eight nuclei before the separation into merozoites. The mature schizonts measured 9.7 μ×8.0 μ and produced 6–8 crescent-shaped merozoites measuring 6.0 μ×2.5 μ (Pl. I 1 and Fig. 1 B). This was the only kind of schizont observed. Since the naturally infected birds were examined the number of asexual generations was not ascertained.

Development of the sexual stages also took place above the host cell nuclei. After the merozoites penetrated the host cell, they rounded off. The developing gametocytes were similar to young schizonts. They developed further into microgametocytes and macrogametes. The microgametocytes underwent repeated nuclear division without cytoplasmic separation. The nuclei of the mature microgametocytes were considerably smaller than those of schizonts. They finally separated at the surface into spindle-shaped microgametes, leaving a scattered residual cytoplasmic body (Pl. I 2 and Fig. 1 D). The mature microgametocytes measured 12.5 μ×11.6 μ. The macrogametes remained uninucleate during growth. The "plastic granules" were laid down at a very early stage appearing peripherally in the cytoplasm (Pl. I 3 and Fig. 1 E). The mature macrogametes were 13.5 μ×13.5 μ.

Discussion

To date there is no report of *Isospora* from the redbreast, though a number of *Isospora* have been recorded from different species of passerines. With a few exceptions, all these descriptions are based on the morphology of the oocysts. In some reports the discovery of oocysts in a new host has resulted in the description of a new species. Levine and Mohan 1960, however, suggested that as many supposed species were incompletely described, it was better to retain only the name *I. lacazei* for all species infecting passeriformes. Subsequent work (Anwar 1966), showed that, as Levine and Mohan had predicted, some parasites were clearly different from *I. lacazei*. There is now no doubt that *I. lacazei* is not the only species of *Isospora* in passerine birds.

Among the recent investigators, Schwalbach 1959 studied the morphology of oocysts from different passeriformes. He described new species solely on the basis of oocyst characteristics. The size of the oocysts of *I. erithaci* sp. n. is similar to those of *I. ficedulae* Schwalbach, 1959, a parasite of *Ficedula hypoleuca*. The new species differs, however, in the smaller size of sporocyst which in Schwalbach's parasite was 17.0 μ × 11.0 μ. Another difference is in the arrangement of the sporozoites which Schwalbach described as close together, near the residual body. Furthermore, I have not observed the square-shaped sporoblast of *I. ficedulae*. *I. erithaci* also differs from the parasites described by Claassen 1923 who gave the old name *I. lacazei* to an *Isospora* of *Carduelis spinus* although the size of oocyst differed from *I. lacazei* (Labbé, 1893). In the endogenous phases Claassen found more than one type of schizont which Anwar 1966 suggested was evidence that more than one species was present.

Thus *I. erithaci* and *I. ficedulae* have oocysts of similar dimensions. There are also superficial resemblances between *I. erithaci* and the parasite reported by Claassen. However, morphology of the oocysts of *I. erithaci* provides clear characters differentiating it from these other species.

Diagnosis of *I. erithaci* sp. n.

Oocyst spherical, 12.5–26.2 μ. Oocyst wall a single layer, smooth, colourless and ~0.9 μ thick. Sporocyst pyriform 12.5–15.2 μ × 7.6–10.6 μ with a compact residuum. Stieda body thick and cap-like. One or two polar granules present in sporulated oocyst. The only schizont observed contains 6–8 crescent-shaped merozoites. Microgametes spindle-shaped and macrogametes with "plastic granules".

Habitat: Epithelial cells of the duodenum.

Host: *Erithacus rubecula* (*Aves*, *Passeriformes*).

Locality: Silwood Park, Sunninghill, Berkshire, England.

Summary

Isospora erithaci sp. n. is described from the redbreast *Erithacus rubecula*. Oocysts are spherical, measuring 12.5–26.2 μ. The oocyst wall is colourless, smooth and consists of one layer. The micropyle and the oocystic residuum are absent,

but there are one or two splinter-like polar granules. The sporocysts are pyriform, with a thick Stieda body forming a lid-like cap. Internal stages are found in the epithelial cells of the duodenum. The only type of schizont seen contains 6–8 crescent-shaped merozoites. The microgametes are spindle-shaped and the macrogametes have obvious "plastic granules".

РЕЗЮМЕ

Описан новый вид *Isospora* — *I. erithaci* — из малиновки *Erithacus rubecula*. Ооцисты сферические, размером 12.5–26.2. Стенка ооцисты бесцветная, гладкая, однослойная. Micropile и остаточное тело ооцисты отсутствуют, однако имеются одна или две палочковидные полярные гранулы. Спороцисты грушевидной формы, толстое штидевское тельце образует крышку в виде колпачка. Стадии эндогенного цикла обнаружены в клетках эпителия двенадцатиперстной кишки. Щизонты, которых удалось наблюдать автору при спонтанном заражении малиновок, содержали 6–8 полулунных мерозоитов. Микрогаметы имеют форму веретена, макрогаметы содержат "пластиноидные гранулы".

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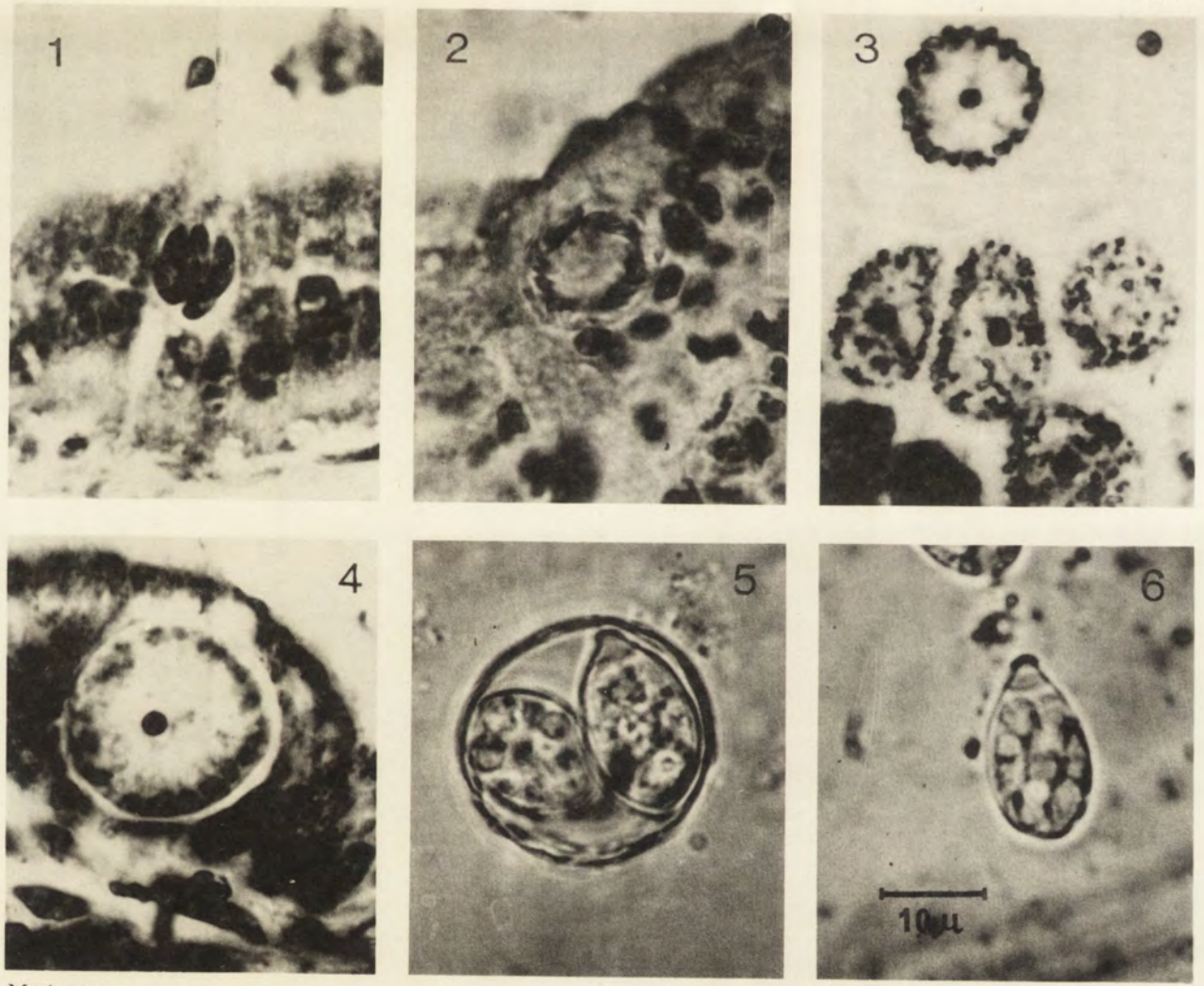
EXPLANATION OF PLATE I

1–6: Photomicrographs of stages of *Isospora erithaci* sp. n.,
1: Fully developed schizont
2: Microgametocyte
3: Macrogametes (mature and immature)
4: Zygote in host cell
5: Sporulated oocyst
6: Sporocyst



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Paul R. EARL

Hegneriella cheni sp. n. and *Opalina wessenbergi* sp. n. (*Protozoa*)

Hegneriella cheni sp. n. et *Opalina wessenbergi* sp. n. *(Protozoa)*

Hegneriella cheni sp. n.

Host: *Bufo valliceps* collected by T. T. Chen in Texas (locality unknown) in July, 1935; H and E slides, kindly lent by R. Wichterman in July, 1970.

Accompanied by a *Zelleriella* sp. (Pl. I 3), *H. cheni*, ca. 142×71 μ with a nucleus 37×17 μ, is easily separated from *Hegneriella dobelli* Earl, 1971 by its dimensions and nuclear differences. A sample of 50 of the *Z.* sp. averaged 190×89 μ, nuclei 22×15 μ, internuclear distance from their centers 46 μ and anterior nuclear center to apex 44 μ. *H. dobelli*, ca. 98×61 μ, nucleus 34×16 μ, is also from *B. valliceps*, but from Terrebonne Parish in southern Lousiana.

H. cheni has evidently been bypassed and was possibly considered an abberant *Z.* sp. Depicted in Pl. I 1, 2, 3, its dimensions are given in Table 1.

Table 1

Hegneriella cheni sp. n., dimensions in microns (N=50)

	Range	Arithmetic mean	Standard error	Percent of length
Length	86.0–168.0	142.0	2.2	100
Width	44.0–89.0	70.7	0.7	50
Nuclear length	27.0–50.0	36.6	0.7	26
Nuclear width	12.3–24.0	16.9	0.3	12
Nucleus to apex	42.0–100.0	68.6	1.8	41

Data for *H. dobelli* is placed in parentheses after that of *H. cheni*. The latter's nuclear axis is ca. 16° (34°) to the cell axis. Width is 50% (62%) of length, and nuclear width 46% (48%) of nuclear length, just as nuclear length is 26% (35%) of cell length. The cyst of *H. cheni* is ca. 30×25 μ and its nucleus about 14×9 μ. The cyst nucleus contains a diffuse nucleolar mass surrounded by 2–3 μ of clear space, possibly in part an artifact.

Two *H. cheni* were found in metaphase: 168×87 (42×15) and 134×61 μ (34×17 μ). Along with spindle fibers, a loose highly-structured strand of nucleolar material was evident in each case, though chromosomes could not be seen. Another metaphase had 12 irregular to round nucleoli and a strand enclosing two more bits of nucleolar material off to one side. Nucleoli are active throughout the cycle with fragmentation and fusion seemingly continuous so that counts are not positive. *H. cheni* probably has 3 oblong nucleoli, whereas *H. dobelli* has about 10. Two anaphases measured 149×70 (43×13) and 100×67 μ (40×19 μ). These nuclei had rotated some 20° more away from the cell's axis, whereas in *H. dobelli* the replicating nucleus rotates a full 90°.

Opalina wessenbergi sp. n.

Host: *Bufo speciosus* collected at Waco, Texas by O. Sanders, April 15, 1971. Azure B slides.

Having a width: length ratio of ca. 0.40, averaging 424.4±17.7×157.1±27.1 μ, this typical, large, *obtrigona*-type opalinid has 4–5 spherical to conoid nuclei. A mature trophozoite in depicted in Pl. I 4. Length and width ranges are: 87.5–600.0× ×67.5–215.0 μ. For more detail on *O. wessenbergi*, see Earl (1971).

It was compared with *O. gigantea* Metcalf, 1923 of *B. speciosus* (=*B. compactilis speciosus* of Texas; U. S. Nat. Mus. slide 16605). Only 19 specimens were available on this badly faded slide. The nuclei of this *ranarum*-like opalinid are over 6 μ, and it averages 443.3±10.8×296.9±6.3 μ with ranges of 263.0–517.0×130.1–353.5 μ. *O. woodhousei* Metcalf, 1923 of *B. woodhousei* cannot be compared because the description is poor.

Summary

Hegneriella cheni sp. n. and *Opalina wessenbergi* sp. n. are described from Texas toads, *Bufo valliceps* and *B. speciosus* respectively.

RÉSUMÉ

On a décrit deux nouvelles espèces de porasites de crapauds: *Hegneriella cheni* sp. n. de *Bufo valliceps* et *Opalina wessenbergi* sp. n. de *Bufo speciosus*.

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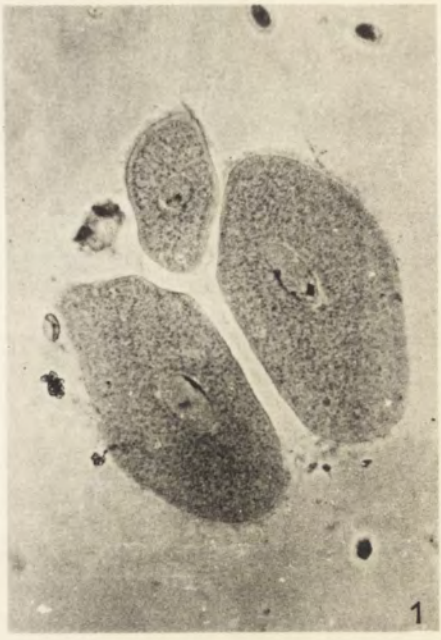
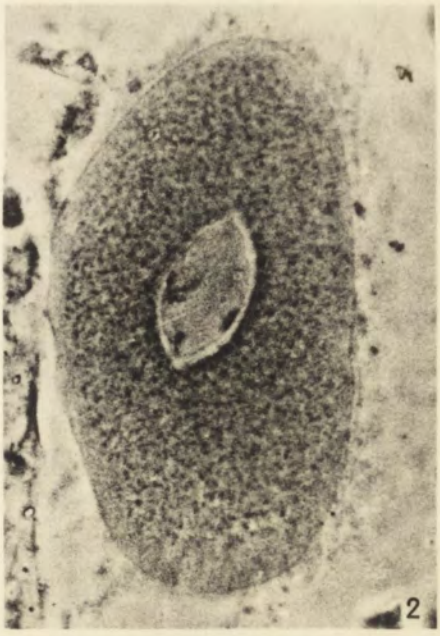
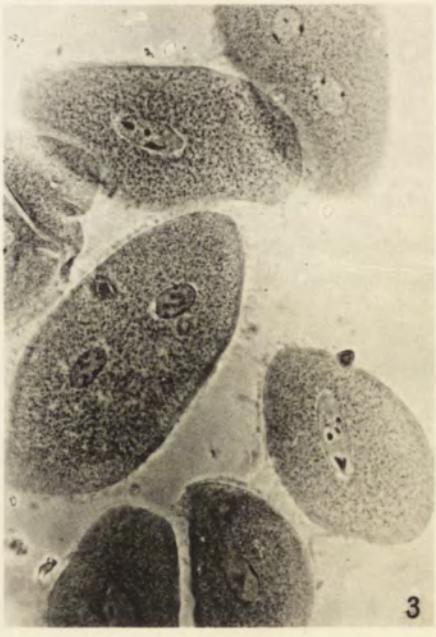
EXPLANATION OF PLATE I

1: Two typical trophozoites and a juvenile of *Hegneriella cheni* sp. n. H and E; 500×
2: Early prophase in *H. cheni*. Strong cytoplasmic (RNA?) synthesis is evinced by the staining reaction around the nucleus. H and E; 900×
3: *H. cheni* specimens and the accompanying *Zelleriella* sp. (binucleate). H and E; 600×
4: Mature trophozoite of *Opalina wessenbergi* sp. n. Azure B; 300×







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On the occurrence of *Trichodina domerguei* subsp. *megamicronucleata* Haider (*Ciliata, Peritricha*) on *Eudiaptomus zachariasi* Poppe (*Calanoida*)

Występowanie *Trichodina domerguei* subsp. *megamicronucleata* Haider (*Ciliata, Peritricha*) na *Eudiaptomus zachariasi* Poppe (*Calanoida*)

Only scarce information is available as concerns the occurrence of *Trichodina* on various representatives of *Calanoida*. For the first time these ciliates were found, by Dogiel 1940 on *Diaptomus* sp. from the environs of Leningrad. Occurrence of *Trichodina* on *Diaptomus vulgaris* was noted by Šramek-Hušek 1953 in some ponds from Czecho-Moravian highland and from southern Czechoslovakia. Lom 1960 observed *Trichodina* on *Diaptomus castor*, *Eudiaptomus gracilis* and *Diaptomus vulgaris* from Blatna near Prague (Czechoslovakia). Haider 1964 noted the occurrence of these protozoans on *Diaptomus* sp. and *Eudiaptomus* sp. from the environs of Dechsendorf (Mfr., FRG).

According to the opinion of some authors, Dogiel 1940 and Lom 1960 among others, on *Calanoida* occur the same species of *Trichodina* as on fish. On the other hand Haider 1964 supposes that these ciliates belong to different species. It is worthy to note that many complications in taxonomy of *Urceolariidae* occurring on *Calanoida* are a result of incomplete descriptions of species and different methods used in study on these ciliates (e.g., Dogiel 1940 and Šramek-Hušek 1953 used hematoxyline stained preparations while Lom 1960 used silver impregnation method). The present paper deals with the data on the occurrence of *Trichodina* on *Eudiaptomus zachariasi* Poppe. The specific identity of these ciliates is discussed.

Material and methods

While observing living plankton from fish ponds, situated in the territory of the Department of Fish Culture, Institute of Inland Fishery, in Żabieniec, numerous *Trichodina* were found on some specimens of *Eudiaptomus zachariasi* Poppe. This phenomenon was observed twice, in July 1968 and in July 1969, in fry ponds. *E. zachariasi* was one of the main components of zooplankton in these ponds. In other seasons of the year the ciliates were not observed.

In order to compare the morphology of these ciliates with fish parasites trichodinids occurring on *Gasterosteus aculeatus* L. were collected in the same ponds and season. Silver impregnation method after Klein was applied for detailed qualitative and quantitative studies on collected ciliates.

Results and discussion

The intensity of infection of calanoids by Trichodina, counted on 20 infected specimens of *E. zachariasi*, amounted to ciliates per one host specimen on average. The specimens of calanoids nearly covered with ciliates were also observed. In such cases the number of ciliates many times overstepped the average.

Close examination of these ciliates has shown that they fit well the description of *Trichodina domerguei*, having a characteristic white spot in the centre of adhesive disc. The adhesive disc is not very large, it is surrounded by a border membrane about 4 μ wide. Denticles, in shape of a very wide sickle, are ended with rather blunt tip. Rays are pointed, their length is almost the same or slightly shorter than length of blade. They are directed toward the geometrical centre of the disc or slightly deviated to the right (Pl. II 5). There are usually 8 radial pins for one denticle. The centre of adhesive disc does not impregnate with silver when Klein's method is applied, but remains in form of a white spot (Pl. II 5, 6, 7). The body dimensions are as follows: body diameter 40–65 μ (mean 52.8 μ), diameter of adhesive disc 28–37 μ (mean 31.9 μ), diameter of denticulate ring 15–21 μ (mean 17.5 μ), number of denticles 15–23 (mean 18.6). Adoral zone of cilia forms a spiral about 380°, its shape is similar to cordate (Pl. I 1).

As it was mentioned *Trichodina* occurring on *Calanoida* had been described by some authors but only Lom 1960 gave the photographs of silver impregnated specimens. The ciliates collected by the present authors on *E. zachariasi* fit well the photographs measurements of Lom's specimens. The only difference concerns the shape of adoral spiral, being cordate in our specimens and rounded in those drawn by Lom. It may be a result of inaccuracy in drawing.

Lom 1960 included the ciliates, found by on calanoids, to the species *Trichodina domerguei* f. *latispina* Dogiel, 1940, this relating them with fish parasites. Actually this form is regarded as distinct nominal subspecies *T. domerguei* subsp. *domerguei* Wallengren, 1897. The present authors, comparing the trichodinids from the skin of *Gasterosteus aculeatus*, collected during the same season and in the same ponds in Żabieniec, came to the conclusion that they represent two distinct forms. The differences between them are demonstrated on photographs (Pl. I and II). These differences concern the shape of denticles, structure of adhesive disc centre, shape of adoral spiral and dimensions of particular parts of the body (Table 1, Fig. 1). One could suppose that these differences are due to various host species on which these ciliates occur, but the ciliates collected on *Gasterosteus aculeatus* in Żabieniec do not differ from the representatives of *T. domerguei domerguei* from various hosts and environments, from tadpoles of *Bufo bufo* and *Rana temporaria* as well as from fishes *Nerophis ophidion*, *Gobius minutus* and *Pungitius pungitius* (Raabe 1959 a, b, Lom and Stein 1966). Morphological differences as well differences in measurements between *Trichodina* from *E. zachariasi* and *T. domerguei domerguei* do not permit to accept Lom's opinion about the identity of the species of *Trichodina* occurring on calanoids and fish. In contrast, these data suggest that both compared forms of *Trichodina* ought to be regarded as distinct species.

Table 1

Comparison of morphometric data of *Trichodina domerguei* subsp. *megamicronucleata* from *Eudiaptomus zachariasi* Poppe and *Trichodina domerguei domerguei* from *Gasterosteus aculeatus* L. in Żabieniec

Parasite	Host	Location	No. of specimens measured	Body diameter µ	Diameter of adhesive disc with border membrane µ	Adhesive disc diameter µ	Denticulate ring diameter µ	Length of denticle µ	Number of denticles	Adoral spiral
T. domerguei subsp. *megamicronucleata*	*Eudiaptomus zachariasi* Poppe	body surface	20	52.8* / 40–65**	39.9 / 36.0–44.0	31.9 / 28.0–37.0	17.5 / 15.0–21.0	9.8 / 9.0–11.5	18.6 / 15–23	about 380°
T. domerguei subsp. *domerguei*	*Gasterosteus aculeatus*	skin	20	68.9 / 60.0–90.0	51.1 / 41.0–65.0	43.3 / 35.0–58.0	27.6 / 21.0–36.0	12.8 / 11.0–15.0	26 / 22–30	about 400°

* mean values of measurements.

** ranges of measurements.

All measurements are made on adult specimens of *Trichodina*.

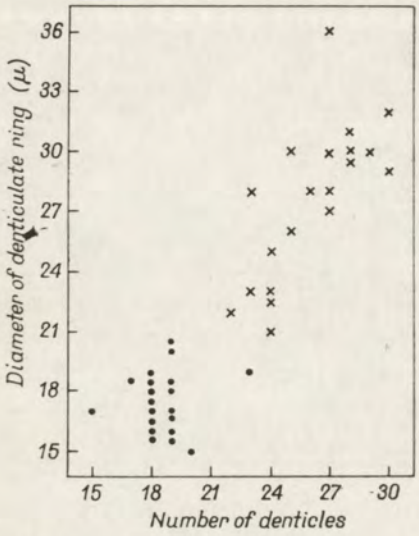

Fig. 1. Comparison of denticulate ring and number of denticles between *Trichodina domerguei megamicronucleate* Dogiel from *Eudiaptomus zachariasi* Poppe (●) and *T. domerguei domerguei* Wallengren from *Gasterosteus aculeatus* L. (×)

The morphometric data concerning the *Trichodina* collected by us were compared also with the measurements of the ciliates from *Calanoida* given by other authors (Table 2). Our specimens correspond with these described by Haider 1964 being

Table 2

Some morphometric data on *Trichodina* from *Calanoida* according to various authors

Parasite (original name)	Body diameter μ	Adhesive disc diameter μ	Denticulate ring diameter μ	Number of denticles	Author
T. domerguei f. *megamicronucleata* on *Diaptomus*	43* / 38–48**	—	less than 37	22 / 19–25	Dogiel 1940
T. domerguei f. *diaptomi* on *Diaptomus vulgaris*	40–70	30–40	20–28	16–27	Šramek-Hušek 1953
T. domerguei f. *latispina* on *Diaptomus vulgaris* and *Eudiaptomus gracilis*	48 / 37–55	31 / 27–38	17 / 13–21	19–20 / 16–22	Lom 1960
T. domerguei subsp. *megamicronucleata* on *Diaptomus* and *Eudiaptomus*	50.4–73.4	33.6–53.5	19.8–30.6	19–22	Haider 1964

* mean values of measurements.
** ranges of measurements.

at lower range of dimensions given by this author. This fact may be explained by another dates of collection of the material. Our *Trichodina* were collected in summer while those of Haider in late autumn. Dates of collection of the material are important for any comparison of measurements on account of seasonal variability observed in these ciliates (Kazubski and Migała 1968).

According to Haider's opinion the *Trichodina* occurring on fish and those on *Calanoida* are different. He separates the *Trichodina* from calanoids into a distinct subspecies *T. domerguei* subsp. *megamicronucleata* including *T.* (*Cyclochaeta*) *domerguei* f. *diaptomi* described by Šramek-Hušek 1953 into its synonyms. Trichodinids from calanoids, described by Lom 1960 under the name *T. domerguei* f. *latispina*, ought to be referred also to this subspecies.

The present authors share this opinion and classify the *Trichodina* collected by them into the species *T. domerguei* subsp. *megamicronucleata* Haider 1964 including *Eudiaptomus zachariasi* Poppe to the hosts of this species. It may be expected that the list of hosts of this species will increase, as there not many papers concerning these ciliates and host species not in all cases are determined.

Taking into account some differences, among others the differences in dimensions of the *Trichodina* described by Lom 1960 and by us in the present paper in comparison with the *Trichodina* described by other authors (Šramek-Hušek 1953) as well as differences in biology of particular species of *Calanoida*, a supposition is justified that more than one species of *Trichodina* occur on calanoids. However, further study on this problem is necessary.

Summary

Numerous *Trichodina* were observed on *Eudiaptomus zachariasi* Poppe (*Calanoida*) in fish ponds in Żabieniec near Warszawa.

It was ascertained, after detailed morphological and biometric examination, that these ciliates belong to the species *Trichodina domerguei* subsp. *megamicronucleata* Haider, 1964.

STRESZCZENIE

Stwierdzono występowanie licznych *Trichodina* na *Eudiaptomus zachariasi* Poppe (*Calanoida*), w stawach karpiowych w Żabieńcu koło Warszawy.

Na podstawie szczegółowych badań morfologicznych i biometrycznych oraz na podstawie danych literatury zaproponowano określenie badanych orzęsków jako *Trichodina domerguei* subsp. *megamicronucleata* Haider, 1964.

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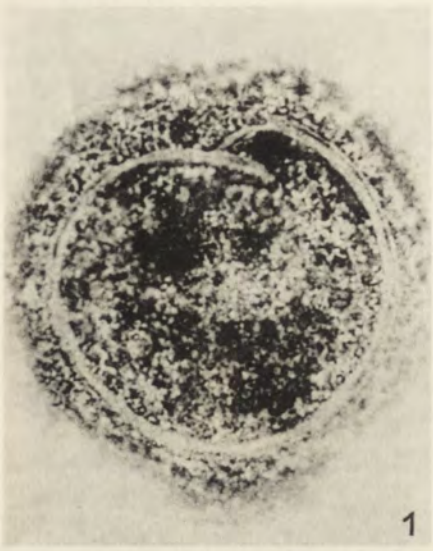
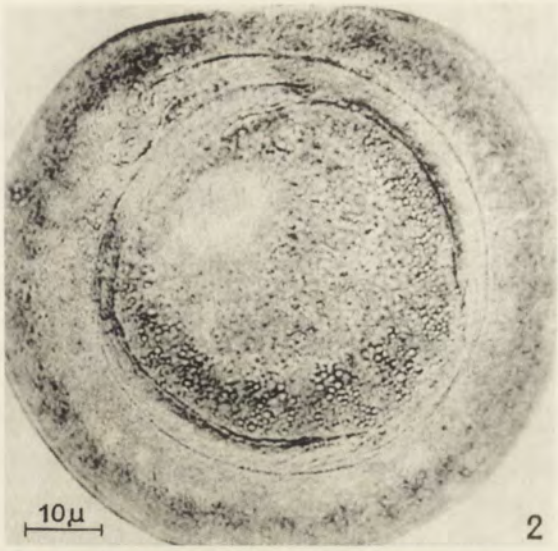
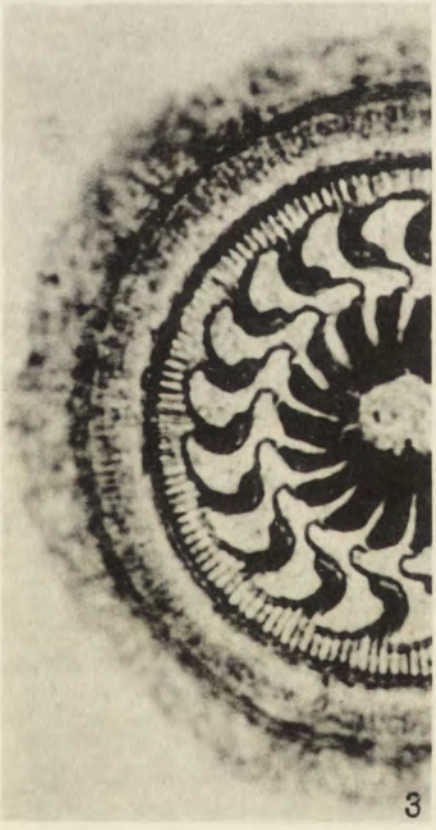
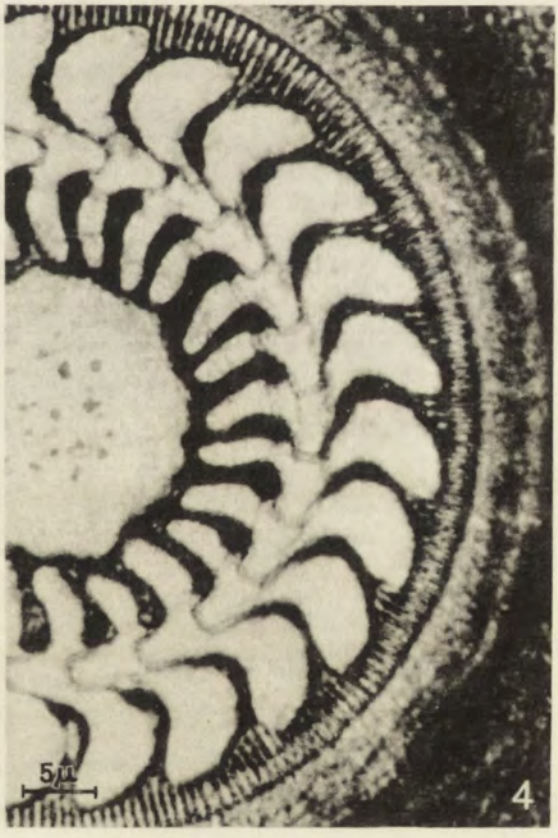
EXPLANATION OF PLATES I–II

1: Adoral spiral in *Trichodina domerguei* subsp. *megamicronucleata* Haider, 1964 from *Eudiaptomus zachariasi* Poppe
2: Adoral spiral in *Trichodina domerguei* subsp. *domerguei* (Wallengren, 1897) Haider, 1964 (syn. *T. domerguei* f. *latispina* Dogiel, 1940) from *Gasterosteus aculeatus* L.
3: Part of the adhesive disc of *T. domerguei* subsp. *megamicronucleata* from *E. zachariasi* in greater magnification
4: Part of the adhesive disc of *T. domerguei* subsp. *domerguei* from *G. aculeatus* in greater magnification
5, 6, 7: Specimens of *T. domerguei* subsp. *megamicronucleata* collected on *E. zachariasi*
8, 9, 10: Specimens of *T. domerguei* subsp. *domerguei* collected on *G. aculeatus*
Microphotographs 3 and 4 in magnification 2000×, other microphotographs in magnification 1000×









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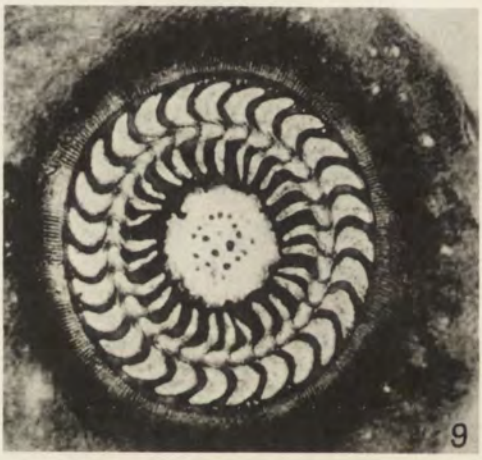

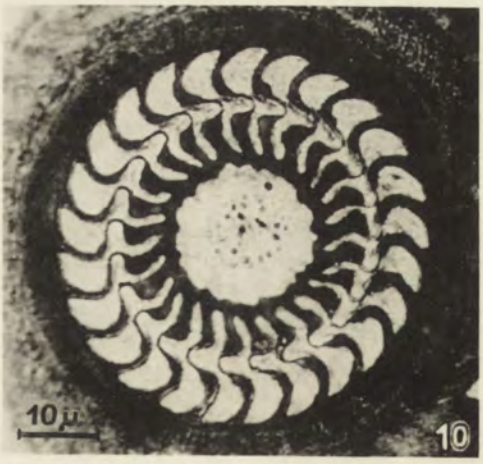

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The in vitro experiments on the fatal infection of *Amoebida* (Kent, 1880) by pathogenic bacterium

Infekcja *Amoebida* (Kent, 1880) wywołana przez patogenną bakterię

Small free-living, cysts forming, amoebae are widely distributed in soil, fresh or salt water and in sewage effluent. The organisms occur in their natural habitat in large number in the encysted state (Cutler et al. 1922, Singh 1946, 1949, Brzezińska-Dudziak 1954).

The cystic condition enable the amoeba to survive periods of dessication or lack of food. Under favorable conditions excystation occurs and the amoeba emerges from the cyst (Crump 1950, Drożański 1961, 1970, Dudziak 1955, Kunicki-Goldfinger et al. 1957, Singh et al. 1956, Singh et al. 1958, Wagtendonk 1955). The active life is confined to the ameboid form. However, predominantly ameboid organisms of some of the *Schizopyrenidae* are able to synthesise a kinetic apparatus at same stage of their life cycles and transform to the flagellates. Therefore two or three forms complete life cycle of *Amoebida*: cyst, amoeba and in some genera flagellate (Chang 1958, Schuster 1963, Outka 1965, Singh and Das 1970).

Although some strains of amoebae may be cultivated in laboratory condition in axenic or synthetic media (Adam 1959, Balamuth and Outka 1962, Band 1959, 1962, Cailleau 1933 a and b, Drożański 1963 c, Reich 1935) in natural habitat amoebae are bacteria feeders. Few years ago, there was found in a raw culture of amoebae freshly isolated from soil a new bacterial parasite which caused a fatal infection of the host (Drożański 1956).

The aim of the present work was to check whether fatal infection by the parasite is restricted to amoebae from the familie of *Hartmannellidae* or whether the spectrum covers also amoebo-flagellates and *Endamebidae*.

Material and methods

Organisms

(a) Amoebae. The studies reported in this paper were conducted on 11 strains of following free-living amoebae: *Hartmannella rhysodes* Singh, 1952 and *Hartmannella* (*Acanthamoeba*) *castellanii* — Duglas, 1930 (Volkonsky, 1931) (obtained from dr W. Balamuth, Department of Zoology, University of California). *Hartmannella astronyxis* Ray and Hayes, 1954 (received from dr O. W. Heal, the Nature Conservancy Merlewood Research Station, Grange over Sands). *Hartmannella*

(*Mayorella*) *palestinensis* Reich, 1933 and *Tetramitus rostratus* Perty (sent from the Culture Collection of *Algae* and *Protozoa* at the Botany School, Cambridge University, England). *Schizopyrenus russelli* Singh, 1952 and *Didasculus thorntoni* Singh, 1952 (originated by dr B. W. Singh, Central Drug Research Institute, Lucknow, India). *Naegleria gruberi*, strain 1518 and *Naegleria gruberi* "Singh" (obtained from dr F. Schuster, Argone National Laboratory, Biology Division, Argonne, Ilinois). *Entamoeba moshkowskii* (obtained from dr H. Hirszlerowa, Institute of Marine Medicine, Gdańsk, Poland). One not classified amoeba strain G 10, isolated from soil, was also used in this paper.

The organisms were maintained in monoxenic culture with *Aerobacter aerogenes* as the food source. Strains were grown also in axenic cultures. Amoebae were subcultured monthly and stored at room temperature.

(b) Bacterial parasite of amoebae. Bacterial parasite infecting amoebae was found accidentally in a culture of amoebae isolated from soil (Drożański 1956). The organism was purified from other bacteria present in raw culture by several transfers of lysate infected amoebae on new monoxenic amoeba cultures. The purification of pathogenic bacteria was succesful owing to the ability of infecting amoebae to migrate not only along the streak of *Aerobacter aerogenes* but also over the sterile agar surfaces (Drożański 1963 b). The pure culture of pathogenic bacteria was maintained on axenic culture of *H. castellanii* grown in peptone-yeast-glucose liquid medium, or on the suspension of amoebae in physiological saline. The axenic cultures or amoeba suspensions in saline soon after infection with parasites underwent lysis but the parasite was able to survive for a long period of time (Drożański 1963 a). The parasite was subcultured monthly on axenic culture of *H. castellanii*.

Media and conditions for the cultivation and infection of amoebae

(a) Monoxenic culture. Experiments on the infectivity of the bacterial parasite were carried out with monoxenic cultures of all amoebae studied. The method of growing small free-living amoebae on the surface of non nutrient agar supplemented with edible living bacteria developed by Singh (1941, 1950) was utilized. Non nutrient agar poured on the Petri plates after solidification was cut into small blocks (⌀ 1.5 cm). One drop of the suspension of 18 h old culture of *A. aerogenes* or *Staphylococcus aureus* (about 2×10^9 cells/ml) and 10 to 50 amoeba cells were layered on the surface of the agar blocks. At least six blocks inoculated with one strain of amoeba were transferred on two slides and incubated in a humid chambers at 28°. The number of amoebae on each block of agar was counted immediately on the block under a low power of microscopy. Enumeration of amoebae was repeated every 3 h during the first 12 h, then at 8 h intervals up to the stage of encystment. After various periods of time, usually after 8 to 12 h from the inoculation, cultures on three blocks were infected with the known concentration of the parasite and the process of infection was observed under the microscope.

Entamoeba moshkowskii failed to grow on the surface of the agar block and this organisms was cultured in a liquid medium with mixed bacterial flora plus rice starch and horse serum.

(b) Axenic culture. Axenic cultures of *T. rostratus* and *N. gruberi* were obtained in a peptone-yeast-liver extract (PYL) medium to which heat killed *Escherichia coli* (1 min in 100°) was added. This medium was prepared according to Brent (1954) and Schuster (1961) for *T. rostratus* and *N. gruberi* respectively. *H. rhysodes*, *H. castellanii*, *H. palestinensis* and strain G 10 were also cultivated axenically in peptone-yeast-glucose medium. The composition of this medium was described

elsewhere (Drożański 1969). Number of the amoebae cells per milimeter of axenic cultures was counted in Büchner hemacytometer. Titre of the parasite was calculated by the dilution technique. To estimate the unit of infectivity, the culture of the parasite after complete amoebae lysis was diluted in logarithmic progress with physiological saline. From the least dilutions 6 samples of 1 ml each, were added to the amoeba suspension and after 3 days of incubation the dilution end point was determined by scoring each incubation as positive or negative. Enumerations of intracellular bacteria during the first hours of infection were carried out as described by Chang et al. (1967). The pathological changes of infected amoebae were observed under the contrast phase microscope. Fixed preparations stained with the Macchiavello method were also applied (Conn et al. 1951)

Results

Infection of *Hartmannella castellanii* suspended in physiological saline

Three days old cultures of *H. castellanii* grown axenically were harvested by centrifugation at 120× g. Pellets of amoeba were gently resuspended in sterile amoeba saline prepared after Band (1959). 20 ml of amoebae suspension in 100 ml Erlenmayer flasks containing about 7.0×10^6 cells per mililiter, were infected with 2×10^7 of infective units and incubated on shaker at 28°. At various intervals samples were removed from the cultures and the number of amoebae and degree of their infection were checked. Results of these experiments showed (Fig. 1) that during first hours after infection parasites disappeared from the culture fluid and accumulated inside amoebial food vacuoles. Two hours after infection, as it was revealed in preparations stained by Macchiavello method, about 85 per cent of amoeba cells were infected with parasite. Food vacuoles soon after food intake shrank and the parasites were tightly surrounded by vacuole membranes. During this stage of infection, as it was judged under the contrast phase microscope, the infected amoebae behaved as healthy one. Between second and fourth hour from the infection the number of the parasites per one food vacuole remained constant but the volume of the food vacuoles increased. After the lag phase which lasted about 6 h, the increase of parasites number per food vacuole was noted. 7 hours following the infection of amoebae, the rapid multiplication of the parasites in food vacuoles was observed. During this explosion of parasite multiplication food vacuoles filled up with the parasites frequently fused together (Pl. I 1, 2).

The increase of the number of the parasites inside food vacuoles caused morbid changes in infected cells. 16–18 hours upon the infection the overcrowded food vacuoles underwent disruption, the differentiation between ecto- and endoplasm disappeared and the amoeba cells took a ball shaped form. Soon after these changes amoeba were lysed (Pl. I 3, 4) and parasites were liberated. The parasites from 36 h old lysate were used in further experiments in which the sensitivity of other amoebae to the infection were tested.

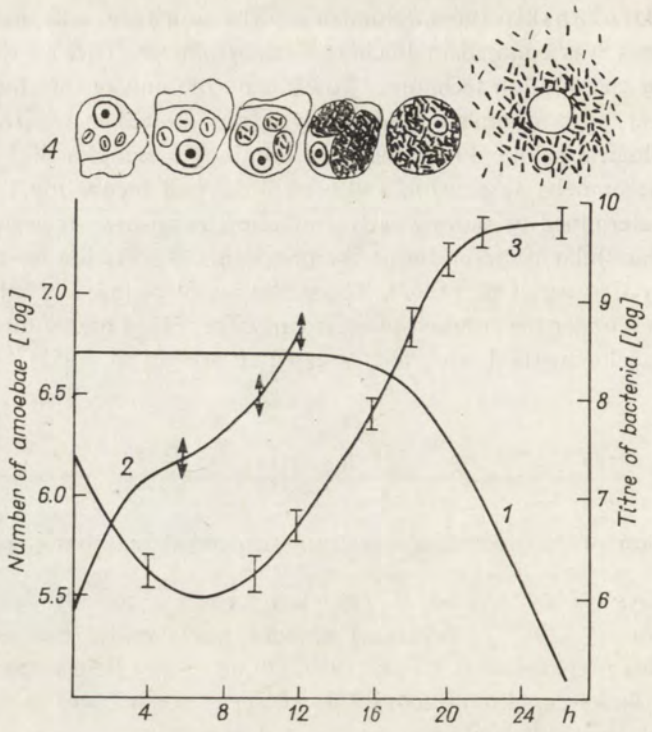


Fig. 1. Infection of *Hartmanella castellanii* suspended in physiological saline by pathogenic bacterium. 1 — number of amoeba cells, 2 — number of bacterial parasites inside food vacuoles, 3 — number of bacterial parasites outside amoeba cells, 4 — morphological changes of amoeba cell during infection

The infection of amoebae grown in monoxenic culture

T. rostratus, *N. gruberi* and *D. thorntoni* grow with the distinct lag phase (Fig. 2) due to the transformation to the flagellate stage. Amoeba to flagellate transformation was induced in a fraction of the cells by addition of a drop of bacterial suspension in water. The logarithmic phase of growth lasted about 4, 6, 7 and 9 h

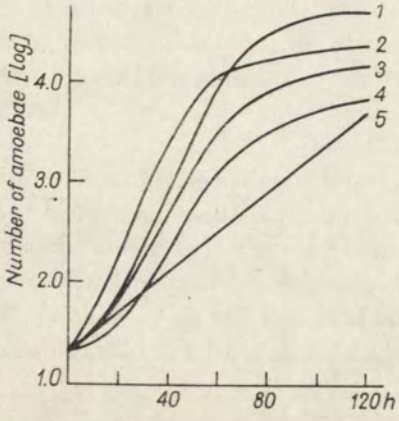


Fig. 2. Growth of amoebae on solid medium in monoxenic cultures with *Aerobacter aerogenes* as food source. 1 — *Tetramitus rostratus*, 2 — *Naegleria gruberi*, 3 — *Didasculus thorntoni*, 4 — *Schizopyrenus russeli*, 5 — Hartmanellid amoebae

for *T. rostratus*, *D. thorntoni*, *S. russeli* and *Hartmannellidae* amoebae, respectively. After a shorter period of slower growth followed the logarithmic growth phase, the increasing number of the amoebae was interrupted by mass encystment. Amoeboflagellates *T. rostratus*, *N. gruberi* and *D. thorntoni* encysted readily between second and third day, *S. russeli* on fourth and *Hartmannellidae* after six days of incubation. The duration of the logarithmic growth phase and the time after which encystment occurred were significantly influenced by the amount of bacterial food supplied and the humidity of the agar surface. However, the genetic properties of the species played the most important role in the formation of a pattern of growth.

Amoebae from the family of *Hartmannellidae* as well as that from *Schizopyrenidae* grown in monoxenic cultures were sensitive to the infection with the parasitic bacteria and under suitable conditions were lysed on second or third day after infection. However, cultures infected with the parasites, behaved differently depending on: the species, the time of infection, the number of the parasite cells added and the humidity of the culture.

Differences between species on infection

Results showed that the parasite was not closely linked to the *Hartmannellidae* amoebae but also infected these other organisms, although the infection of some amoeboflagellates and especially *N. gruberi* was less efficient. Cultures of *Hartmannellidae* and *S. russelli* infected at the start with 1 or 2 parasites cells per agar block or during the whole logarithmic phase and even one day before mass encystment with 5 to 10 parasites per amoeba cell underwent complete lysis. To destroy 50–80% of *D. thorntoni* or *T. rostratus* in their logarithmic phase of growth about 10^3 parasites were needed per agar block. *N. gruberi* required the addition of approximately 10^6 parasites per agar block to lyse the young culture.

The differences between species in their response to the bacterial infection may by due not only to the duration of the logarithmic phase or to the ability of some amoebae for flagellate transformation but also to the way on which the bacteria are taken in and digested. *Hartmannellidae* in contrast to *Schizopyrenidae*, were able to agglutinate of flagellated bacteria (Pl. I 5, 6) and to form a "tail" on the functionally posterior "trophic" end (Ray 1951, Drożański 1956). The dense clumps of agglutinated bacteria were ingested with the help of special type of "cup like" pseudopodia (Ray 1951, Drożański 1961, 1963 c).

Such pseudopodia were formed by *Hartmannellidae* amoebae even in liquid axenic culture deprived of bacteria (Pl. II 7, 8) but never could be observed in *Schizopyrenidae*. The bacteria were ingested by *Schizopyrenidae* by cicrcumfluens as shown on Pl. II 9, 10.

Infection of amoebae grown in axenic culture

Axenic cultures of *H. rhysodes*, *H. castellanii* and *H. palestinensis* grown on rotary shaker at 28° in a silicone coated Erlenmayer flasks with the mean generation time of approximately 20 h.

On the third day after inoculation when the yield of amoebae reached about 2×10^6 cells per ml, cultures were infected with 5×10^6 to 1×10^7 of infecting units of the parasite. As results of infection cultures of amoeba underwent complete lysis within 24 to 36 h (Fig. 3). Total destruction of amoebae was also observed with

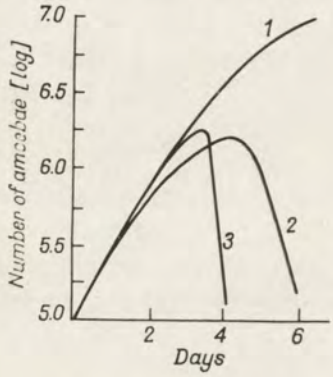
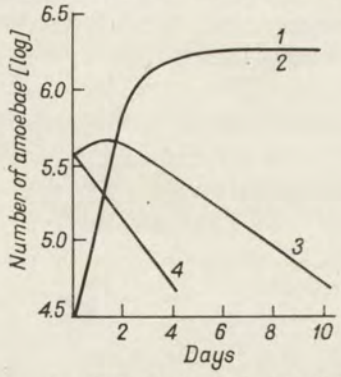


Fig. 3. Infection of *Hartmanella castelanii* in axenic culture. 1 — control, 2 — culture of amoebae infected with one infective units/ml, 3 — culture of amoebae infected with 5×10^6 of infective units/ml

one infective unit per ml but the time period from infection to lysis lasted 3 to 4 times longer.

The degree of oxygenation of amoeba cultures had a great influence on the rate of amoeba multiplication as well as on the rate of their lysis after infection. In the insufficiently aerated, static deep, cultures infected with parasites, many thread-like bacterial forms instead of rods were observed. This may indicate that at a low concentration of oxygen the division of bacterial parasite was inhibited.

Axenic cultures of amoeboflagellates *N. gruberi* and *T. rostratus* infected with living parasites underwent also lysis (Fig. 4 and 5). Cultures were selected for studies on the sensitivity to infection while the organisms were still in the logarithmic growth phase. Results of these experiments showed that *N. gruberi* grew in axenic cultures, similarly as in monoxenic conditions, was also more resistant to infection



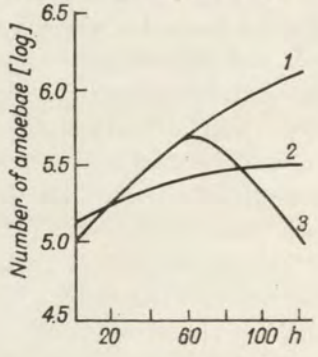


Fig. 4. Growth of *Naegleria gruberi* in Schuster's medium supplemented with: 1 — heat-killed *Escherichia coli* (1 mg per ml), 2 — heat-killed parasite cells (1 mg per ml), 3 — heat-killed *Escherichia coli* $+1\times10^6$ of infective units/ml, 4 — live cells of the parasites (0.5 mg per ml)

Fig. 5. Growth of *Tetramitus rostratus* in Brand's medium supplemented with: 1 — heat-killed *Escherichia coli* (1 mg per ml), 2 — heat-killed parasite cells (1 mg per ml), 3 — heat-killed *Escherichia coli* (1 mg per ml) $+1\times10^3$ of infective units per ml

from other amoebae studied. Axenic cultures of *T. rostratus* infected with parasites during logarithmic phase of growth in contrast to *N. gruberi* were completely lysed as were amoebae from the family of *Hartmannellidae*. *E. moshkowskii* grew well with even thick suspension of the parasite. The insensitivity of this organism to infection was probably due to the anaerobic condition of growth.

The second object was to explain why the parasites, engulfed by amoebae as normal prey, are not then digested by the host's enzymes and used as were many other bacteria. It was found that parasites killed by heating at 100° for 2 min were engulfed by *H. castellanii* as normal but supported very poor growth compared with a control using killed *E. coli* as food source (Fig. 6). *T. rostratus* grew only on

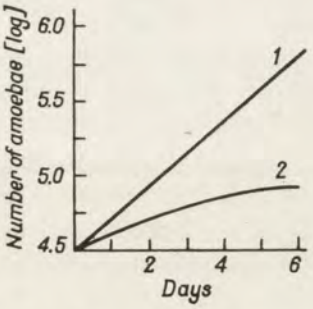


Fig. 6. Growth of *Hartmanella castellanii* in physiological saline supplemented with: 1 — heat-killed *Escherichia coli* (1 mg per ml), 2 — heat-killed parasite cells (1 mg per ml)

organic medium supplemented with suspension of heat killed *E. coli* or *S. aureus* cells. Dead parasites added to the Brant's medium instead of edible bacteria also failed to support growth of this amoeboflagellate (Fig. 5). Microscopic observations showed that the inability of the heat-killed parasites to support good growth of *H. castellanii* was due to incomplete digestion; ball-shaped remains of the parasites accumulated not only in the food vacuoles but also in the medium. This shows that at least some part of the parasite, perhaps its cell wall, is not broken down by the amoebae. *N. gruberi* was grown axenically in organic medium supplemented with heat-killed *E. coli* (1 mg of dry weight per ml). In some experiments dead *E. coli* cells were replaced in Schuster's peptone-yeast-liver extract medium by equal amount of heat-killed parasite cells. Results of these experiments (Fig. 4) showed that cells of heat-killed parasites were completely digested by *N. gruberi* and supported growth to the same extent as when suspension of heat-killed *E. coli* cells was added. Therefore *N. gruberi* in contrast with *H. castellanii* and *T. rostratus* was being able to digest the dead parasite cells completely and this would account for the lowered efficiency of infection in *Naegleria*. The higher resistancy of *N. gruberi* to infection with bacterial parasite might be caused by the different composition of digestive enzymes.

Discussion

Amoebae play an important role in the limitation of the number of bacteria in their natural environment. The predator-prey relationship has an influence on the soil fertility and on natural purification of polluted water from *Salmonella*, *Shigella*

and other pathogenic bacteria (Singh 1949, Chang 1958). However, some strains of aerobic free-living amoebae classified by Singh (1952) to the genus *Hartmannella* or *Naegleria* have been shown to be facultatively pathogenic for man and other animals and even fatal brain infection were reported (Culbertson et al. 1959, 1968, Carter 1968, Cerva et al. 1969, Dwivedi and Singh 1965). The potentially pathogenic free-living amoebae producing a highly fatal human or animal diseases were isolated from soil, water of lakes and swimming pools.

Small free-living amoebae are now intensively studied in several laboratories but little is known about their biology and their taxonomy is still debated (Adam 1964, 1969, Page 1967 a and b, Singh and Das 1970). In this laboratory studies on the relationship between amoebae and a specific bacterial parasite have been carried out for many years. In previous papers infection process in *Acanthamoeba* (*Hartmannella*) *castellanii* and *Hartmannella rhysodes* together with 41 other unclassified amoebae from soil and fresh water reservoirs were described by Drożański (1963 b). The parasite causing fatal infection were found not to grow on any of the bacteriological media examined which were devoid of living amoebae. This was so even with a suspension of disrupted amoebae. The parasite might be propagated only in the presence of live amoebae. The parasites were not infective for chick embryos, frogs, mice, guinea pigs and rabbits (Drożański 1963 a). Although the morphology of the parasite as well as the mureine (mucopeptide) present in their cell walls (Drożański 1972) suggest it is a bacterium, it was not possible to classify it for the lack of nutritional tests — it did not correspond with any species of bacterium described in Bergey's Manual of Determinative Bacteriology (Breed et al. 1958).

Results of the experiments presented in this paper on the sensitivity of *H. castellanii*, *H. rhysodes*, *H. astronyxis*, *H. palestinensis*, *N. gruberi*, *T. rostratus*, *D. thorntoni*, *S. russeli* and *E. moshkowskii* to the infection were carried out only in the laboratory condition. The bahaviour of the infected amoebae in monoxenic and axenic cultures showed that besides of *E. moshkowskii* which live anaerobicaly, all other amoebae could be infected and underwent lysis. However, some of them (for instance *N. gruberi*) were less sensitive to the infection. This may by due to the shortlasting logarithmic phase of growth or to the differences between species of amoebae in the composition of digestive enzymes. The way on which prey is ingested by amoebae may also have some influence.

Summary

Studies on the sensitivity of some amoebae from the families of *Schizopyrenidae*, *Hartmannellidae* and *Endamoebidae* to the infection caused by the pathogenic bacterium were carried out. It was shown that the bacterial parasite found by Drożański (1956) in a raw culture of amoebae was a pathogen not only for small free-living amoebae from the family of *Hartmannellidae* but also was able to infect amoeboflagellates from the family of *Schizopyrenidae*, although the infection was less efficient. *Entamoeba moshkowskii*, a naked, anaerobic amoeba, was insensitive to infection.

STRESZCZENIE

Przeprowadzono badania nad wrażliwością ameb z rodziny *Schizopyrenidae*, *Hartmannellidae* i *Endamoebidae* na infekcję powodowaną bakteryjnym pasożytem. Wykazano, że bakteria ta wyosobniona przez Drożańskiego (1956) ze świeżo założonej hodowli ameb glebowych jest potencjalnym patogenem nie tylko dla ameb z rodziny *Hartmannellidae*, ale również dla ameb z rodziny *Schizopyrenidae*. Wrażliwość na infekcję była jednak różna u poszczególnych gatunków ameb.

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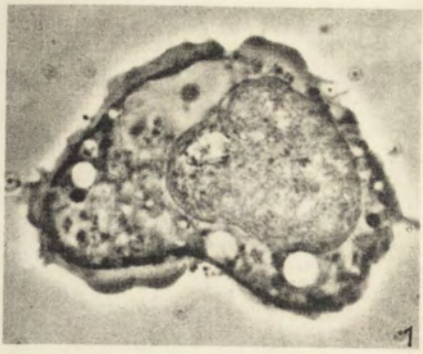
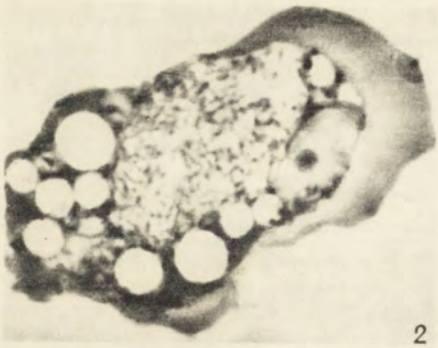
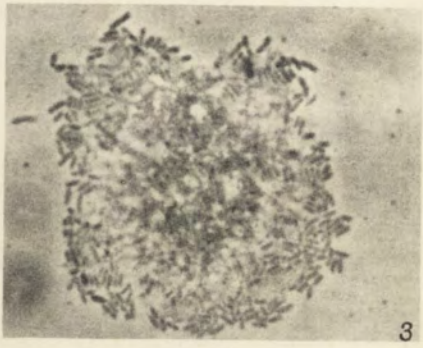
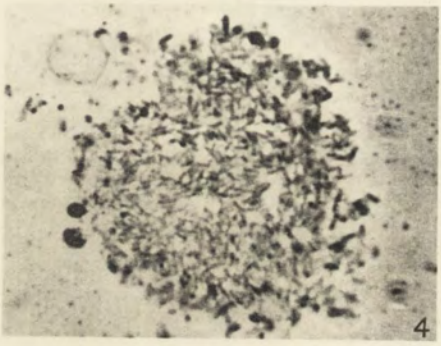
EXPLANATION OF PLATES I–II

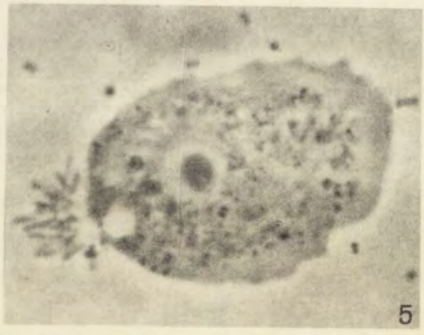
1–2: Trophic form of *Hartmanella castellanii* each with one large food vacuole filled up with the parasite cells
3–4: Final effect of the infection. Amoebae underwent disruption and pathogenic bacteria were liberated
5–6: Trophic forms of amoebae with dense clump of agglutinated bacterial parasites and with large number of food vacuoles filled up with cells of pathogenic bacterium
7–8: Trophic forms of strain G 10 of soil amoeba, successive stages of formation of "cup like" pseudopodia for food intake
9–10: Successive stages of *Escherichia coli* cells intake by trophic forms of *Schizopyreneus rısseli*

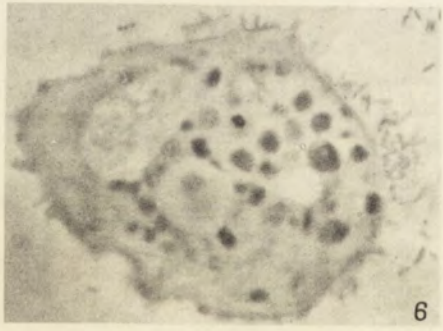












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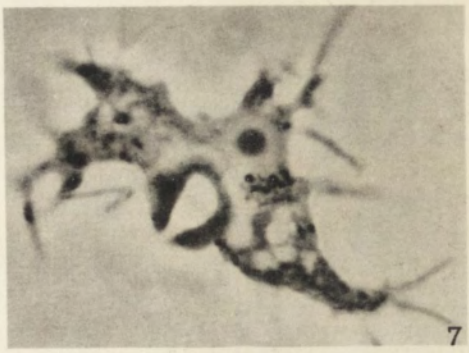

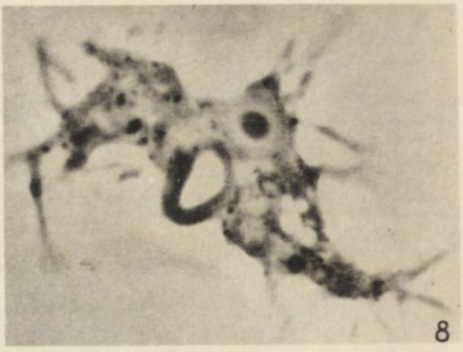

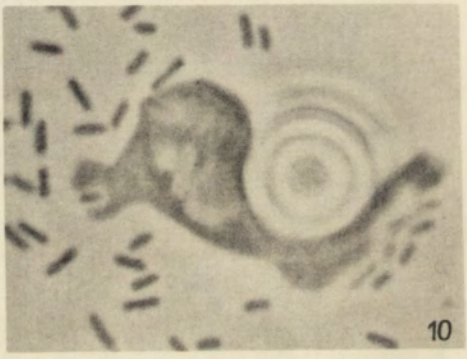

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