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Synopsis of the *Plagiotomoidea*, new superfamily (*Protozoa*)

Synopsis des *Plagiotomoidea*, une superfamille nouvelle (*Protozoa*)

Plagiotomoids, the most familiar of them being nyctotherans, are endosymbiotic spirotrichs infecting reptiles, amphibians and fishes, as well as various invertebrates, especially roaches and termites. The hallmark of the spirotrich is its adoral zone of membranelles (AZM), which runs clockwise along the left of the oral area. If asked the difference between a hymenostome and a spirotrich, you might reply, "The spirotrich has greater membranation". The *Plagiotomoidea* new superfam. consists of the *Plagiotomidae* Bütschli, 1887 and the *Clevelandellidae* Kidder, 1938, the latter having *Clevelandella* Kidder, 1938, *Paraclevelandia* Kidder, 1937 and *Metaclevelandella* Uttangi et Desai, 1963. *Plagiotomidae* contains *Plagitoma* Dujardin, 1838, *Nyctotherus* Leidy, 1849, *Nyctotheroides* (Grassé, 1928) Corliss, 1961, *Prosicuophora* de Puytorac et Oktem, 1967, *Sicuophora* de Puytorac et Grain, 1968, *Parasicuophora* Albaret, *Pseudonyctotherus* Earl, 1970, *Metanyctotherus* Albaret, 1970 *Wichtermania* n. gen. and *Kudoella* n. gen.

New information is presented here on *Wichtermania cheni* n. comb. (=*Sicuophora cheni*), similar to *W. kalii* n. comb. (=*S. kalii*), and on *Kudoella praenucleatum* n. comb. (=*Balantidium praenucleatum* Kudo et Meglitsch, 1938), along with a review of the *Plagiotomoidea*.

Plagiotomoidea belongs to the suborder *Heterotrichina* Stein, 1859 of order *Heterotrichida* Stein, 1859, subclass *Spirotrichia* Bütschii, 1889, class *Ciliatea* Perty, 1852, subphylum *Ciliophora* Doflen, 1901 of the phylum *Protozoa* Goldfuss, 1818. Honigberg et al. (1964) give these definitions: "*Spirotrichia* Bütschli, 1889. Somatic ciliature sparse in all but one order; cirri dominant feature of one order; buccal ciliature conspicuous, with adoral zone, typically composed of many membranelles, winding clockwise to cytostome; body often large". "Order 1. *Heterotrichida* Stein, 1859. Somatic ciliature, when present, usually uniform; body frequently large; some species pigmented; a few species loricate with migratory larval forms". "Suborder (1) *Heterotrichina* Stein, 1859. With characters of the order sensu stricto". While hymenostomes have the pattern 1 right undulating membrane (UM) and 3 left AZM, spirotrichs have 1–2 right UM+*n*AZM on the left.

Following Jankowski 1964, the main evolutionary direction of the relevant

heterotrichs may be *Blepharisma* (*Spirostomidae*) to *Metopus* (*Metopidae*) to *Kudoella* and other plagiotomoids with forwardly-placed buccal overtures.

At times in the past, balantidia, plagiotomids, opalinids, flagellates, amebas and other protozoa invaded higher invertebrates and vertebrates. Having adapted to the intestinal environment, only modest radiation occurred, changes being most pronounced in flagellates and ciliates invading roaches and termites. Parallel events occurred in the ciliates (thigmotrichs) of mollusks. Only opalinids, balantidia and possibly thigmotrichs are unallied to free-living species. Plagiotomoids and opalinids failed to reach the mammals, though balantidia and others did. The most successful group in numbers of individual and specific hosts, and probably in total biomass is the small, morphologically primitive flagellates. Such is the capsular sketch of the break-through to parasitism and some of its results. The general situation regarding higher categories was expressed by Simpson 1953: "The event that leads, forthwith or later, to the development of a higher category is the occupation of a new adaptive zone. As a general rule, the broader the zone the higher the category when fully developed. After initial occupation of a zone, adaptive radiation into its subzones, increased specialization by adaptation to narrower and narrower subzones and the weeding out of lineages usually follows. Occasionally it happens that a zone occupied is so narrow that significant radiation does not occur, and yet is so distinctive in adaptive type and correlated morphology of the organisms occupying it that systematists do give it higher categorial rank". This is the case with protozoan endosymbionts. Lower ranks are accorded to those organisms which have deviate — evolved — less from obvious free-living lineages, e.g., the flagellates.

The main evolutionary trend of the *Plagiotomoidea* may be: *Kudoella* and others, *Nyctotheroides*, *Nyctotherus*, *Metanyctotherus*, *Pseudonyctotherus*, *Wichtermania*, then *Prosicuophora*, *Sicuophora*, *Parasicuophora*, then *Plagiotoma*, then *Metaclevelandella* followed by *Paraclevelandia* and *Clevelandella*. Branching seems more likely than a straight line, as implied, but too much information awaits discovery. The buccal overture has shifted posteriorly and the 1–2 UM+*n*AZM has elongated to the point, perhaps, where it became inefficient, ending the *Plagiotomidae*. In *Nyctotheroides* the cytopyge commonly is to the ventral side of the posterior pole as in *Pseudonyctotherus corlissi*, though in some *Nyctotheroides* such as *N. limnocharis* it is dorsal of the pole as it is in *Metaynctotherus congoi*. In *N. curtipes* it is terminal though the canal points dorsal. Do changes in cytopyge position in any way correlate with position of the buccal overture? In *Metaclevelandella termitis* the cytopyge is close to mid dorsum and has shifted with the buccal overture. The *Clevelandellidae* begin with *M. termitis* whose kinetal suture system is the same as *Plagiotoma kempi*'s n. comb. (=*N. kempi*). The tendency of posteriorly displacing the buccal overture and cytostome continues, but with loss of the exterior AZM-peristome system so familiar to the *Plagiotomidae*. *Metaclevelandella* bridges the gap between the two families, yet in *Paraclevelandia* and *Clevelandella* the cyto-

pyge held on to its conventional position. In *Clevelandella* peristome elaboration begins anew.

Earl 1969 discussed meristic and dimensional charaters in *Nyctotheroides* and recounted plagiotomid taxonomic history in 1970. Inadvertently the works of de Puytorac et Oktem 1967, Albaret 1968 a, b and de Puytorac et Grain 1968 were omitted even though, as it happens, these workers are most responsible for raising the quality of plagiotomic descriptions to its present level or excellence. Up to 1970, all of the pertienent literature is available to the reader by using the references of this paper together with their bibliographies, including Kudo 1936 on *Nyctotherus ovalis*, Bhaskar Rao 1969 on *N. indica*, Earl and Jimenez 1969 on *N. dilleri*, Albaret 1970 a on *Metanyctotherus* and Albaret 1970 b on *Prosicuophora* and *Sicuophora*.

Evaluation of plagiotomoid characters is in a state of flux, and only recently has the suture system of the kineties taken on import, this largely due to silver impregnation techniques in the hands of the French school. Many descriptions are inadequate, especially so in comparison with the superior taxonomic results attained in the last decade — a price to be paid for progress. Progress in morphology leads to the general conclusion that the older a description, the less value it has as a point of reference. It follows then that the holotypes require redescription, particularly *Plagiotoma lumbrici* Dujardin, 1838, *Nyctotherus velox* Leidy, 1849 and *Nyctotheroides cordiformis* (Ehrenberg, 1838) Stein, 1867. As these holotypes are most probably available from their original hosts and localities, redescription can be undertaken. Still they are only nomenclatural types imbedded within the overworn typological concept. Their redescriptions will clarify neither the evolution of the plagiotomoids nor even their own genera. More importantly, a number of nyctotherans may be misclassified since most have been described with neglect of their kinetal suture systems, and additionally some undescribed species have been wrongly synonymized with *Nyctotheroides cordiformis*. For example, Higgins 1929 under the instructions of Wenrich described diverse nyctotherans, calling them variations of *N. cordiformis*. Her meticulous morphology is excellent, yet the statistics and the concepts are imperfect. It may be that *N. cordiformis* is restricted to Europe and not ubiquitous as the literature suggests.

Kidder 1937, 1938 established the *Clevelandellidae* for nine species belonging to *Clevelandella* and *Paraclevelandia*; these genera are characterized by a posterior peristome and "obviously related most closely to the family *Plagiotomidae*", according to him. The two families could be united, but since judging the gap between them is subjective they are combined as *Plagiotomoidea* n. superfam. The primary difference between them is the presence of an elongate AZM-peristome system exteriorized in *Plagiotomidae* and missing in *Clevelandellidae*. Kidder 1938 described a reorganization process in *Paraclevelandia simplex* which he considered endomixis; it seems like partially described autogamy, otherwise unreported for plagiotomoids. His Figs. 6 and 7 show spiremes — the ball-of-yarn stage — of the macronuclear

anlagen, typically homologous to that stage in *Nyctotheroides cordiformis* (see Golikova, 1965). Uttangi 1958 illustrated a stage of hemixis (or autogamy) in *N. limnocharis* (his Fig. 9), and Earl (unpublished) has noted the fertilization stage of autogamy in *N. amoroi*.

The general kinetal suture plan of the *Clevelandellidae*, according to Kidder's drawings, is radiating kineties from a ventral whorl near the contractile vacuole, accompanied by a straight dorsal suture running from the apex to short of the vertex, as in *Clevelandella hastula* (his Fig. 35) and *Paraclevelandia simplex* (Fig. 45). Dissimilar from the system in *Nyctotheroides*, it is also different from the suture pattern of *Plagiotoma* as evinced by Chakravarty's 1936, Figs. 3 and 4 of *Plagiotoma kempi* n. comb. As the suture sytem of *P. lumbrici*, the only other species, is inadequately described, Chakravarty's information on the silver-line system suffices. *P. kempi* has a whorl on the center of the left side close to the vertex from which kineties radiate, as does *Metaclevelandella termitis*. *Nyctotheroides nankingensis* Nie, 1932, and *N. curtipes* Uttangi, 1958 closely approach *Plagiotoma*, i.e,. their AZM angles are two-thirds down the cell. However they are typically nyctotheran, even to the point where *N. nankingensis* has Stein's oral bristle.

Chakravarty redescribed *Nyctotherus kempi* Ghosh, 1921 from the gastropod *Ampullaxia globosa*. It is an elongate bean-shaped spirotrich, straight along the ventrum, slightly narrower anteriorly, 150–200 × 100–110 μ, macronucleus 51 × 19 μ. The AZM extends about 72% of the length before turning sharply towards the cytostome. The AZM angle is perhaps 112°. The macronucleus is broadly elliptical, placed obliquelly (ca. 37°), "the wrong way", along the right side just anterior to the middle. The micronucleus is always located at the posterioventral tip of the larger nucleus, whose axis points to the AZM angle.

Two nyctotherans — *Nyctotherus gyoeryanus* (C et L, 1838) Stein, 1867 of larval *Hydrous piceus* (*Hydrophilidae*, *Staphylinoidea*) and *N. hylae* (Stein, 1867) Rosenberg, 1937 of *Hyla regilla* at San Francisco — because of their suture systems may not belong to *Nyctotherus*, possibly representing undescribed genera. At least they should be reworked. It is unlikely that Rosenberg found a German endosymbiont in a California frog. Rosenberg followed Ten Kate's 1927 misinterpretation of a karophore in *N. cordiformis* variety *hylae*, even though Grassé 1928 had commented that neither *N. cordiformis* nor *N. c.* var. *hylae* has a karyophore. Rosenberg's species has a karyophore, while Stein's does not. I recognize *Nyctotherus cordiformis* var. *hylae* Stein, 1867 as *Nyctotheroides hylae* (Stein, 1867) Amaro et Sena, 1967, designating Rosenberg's organism *Nyctotherus rosenbergi* n. name. It has strong, straight, left suture running central along the left face and a ventral suture from buccal overture to vertex. *Nyctotheroides hylae* (Surowiak, 1937) Amaro et Sena, 1967 of *Hyla arborea* in Poland and *Nyctotherus hylae* forma *hylae* Kifer, 1933 of the same host and location are placed in synonymy with Stein's species. *Nyctotheroides hylae pelobatidis* (Kifer, 1953) Amaro et Sena, 1967 and *N. hylae*

gigantea (Kifer, 1953) Amaro et Sena, 1967 are now elevated to *Nyctotheroides pelobatidis* n. sp. and *N. kiferi* n. sp.

Well-described, *Nyctotheroides duboisii* Künstler, 1884 from larval scarab beetles *Cetonia aurata* (=*Oryctes nasicornis*) in France belongs to an undescribed genus of the *Plagiotomidae*, because its AZM-peristome system runs dorsad from the buccal overture some 60° off the cell's main axis in a slight sigmoid curve till it ends dorsal of center on the left face, short of the apex. The buccal overture is rather forward, 20 to 25% of the cell length from the apex. Dimensions were not given. There are no karyophores on the ellipsoid macronucleus which lies central near the buccal overture at right-angles to the cell axis. *N. duboisii* has a very large dense glycogen body conforming to the apical pellicle, flat posteriorly. Künstler remarked that contrary to Stein's opinion that in all nyctotherans the contractile vacuole empties into the cytopyge, his organism had a contractile vacuole canal running posteriad from it. Higgins 1929 commented on such a canal in one of her as yet undescribed species and shows it in her Fig. 8. Grassé 1928 described and figured a nyctotheran distinct from *N. duboisii* also of *C. aurata* in France, calling it *N. duboisii*. His a dissimilar AZM-peristome system, has a karyophore and does not have a dense glycogen body. As his description and figures are excellent, it is now nominated *Nyctotherus grassei* n. sp.

Eight species are now suppressed because of inadequate descriptions: *N. haematobius* Entz, 1888 of *Apus cancriformis*, *N. parvus* Walker, 1909 of *Rana clamitans clamitans* and *R. palustris*, *N. multisporiferus* Walker, 1909 of *Cavia porcella*, *N. papillatus* Dobell, 1910 of *Bufo melanostictus* and *Rhacophorus leucomystax maculatus* and four species reported from man: *N. faba* Jacoby et Schaudinn, 1899, *N. africanus* Castellani, 1905, *N. giganteus* Krause, 1906, and *N. mazzai* Jörg, 1930.

Sicuophora cheni (Wichterman, 1934) de Puytorac et Grain, 1968 of *Rana boulengeri* (=*R. spinosa*) of Fukien, China and *S. kalii* (Uttangi, 1951) de Puytorac et Grain, 1968 of *R. curtipes*, Dharwar, India are moved to *Wichtermania cheni* n. comb. and *W. kalii* n. comb. Additionally, *Nyctotherus magnus malabarica* de Mello, 1932 is raised and moved to *Prosicuophora malabarica* n. sp.

Well-described, Gisler's 1967 *Nyctotherus* sp. of the Ivory Coast termite *Cubitermes* sp. (his Fig. 39 A) is now named *Nyctotherus gisleri* n. sp.

Four species of *Nyctotherus* are now added to those known from Arthropoda: *N. alpha* n. sp., *N. beta* n. sp., *N. gamma* n. sp. and *N. delta* n. sp. Pai and Wang 1947 originally described the first three and Semans 1939 the fourth, all as varieties or phenotypic variations of *N. ovalis* Leidy, 1850. It was weakly described, therefore Kudo's 1936 data on *N. ovalis* also from *Blatta orientalis* but from Illinois not Pennsylvania is used. Kudo found it in about 50% of hosts, usually in the anterior half of the colon. Ovoid, 90–185×62–95 μ (average: 138×79 μ versus Leidy's 136×102 μ), W/L 57%, oblong macronucleus 30–70×15–25 μ (50×20 μ), micronucleus less than 10 μ in diameter or length, *N. ovalis* has a cyst 72–106×58–80 μ (89×69 μ). Its buccal overture begins at about 40% of length. *N. velox* Leidy, 1949,

the type, of the diplopod *Nearctus americanus* (misidentified as *Julus marginatus* Say) in Pennsylvania is an elongate ovoid, 100–141×79–100 μ, having a spindle-shaped macronucleus ca. 42×19 μ with a straight peristome at near right-angles to the cell's axis. The karyophore is single, a sling hung from ventrum to dorsum. Its buccal overture begins at ca. 46% of cell length. *N. velox* does not have a glycogen storage body.

N. alpha n. sp. of the mole cricket *Gryllotalpa vulgaris* in Pehpei, China is roughly pyriform, 159±2.4×97±1.4 μ (AM±SE, N=310), W/L 61% and *r* 0.94. The apparent reason that the *r* is so high is that it is obtained from grouped data. My figures differ slightly from those of Pai and Wang 1947 as I rounded off to one micron. The ranges of *N. alpha* are 53–458×38–333 μ. It has two glycogen bodies above the macronucleus — one large oblong body, capped by a round to conical smaller body. The macronucleus is oblong to ellipsoid. The peristome slants downward and has a continuation band. Of 249 hosts, 78.2% were infected. *N. beta* n. sp. of *Periplaneta americana* at Pehpei, China, 69.7% of 89 hosts infected, is an elongate ovoid, 132±1.5×91±0.9 μ (N=337), W/L 69% and *r* 0.97. The ranges are 53–360×41–255 μ. Its two glycogen bodies are similar to those of *N. alpha*, but its macronucleus is ellipsoid to spindle-shaped and its peristome has only a slight downward slant. *N. gamma* n. sp. of *Julus* sp. at Pehpei, China is a broad ovoid, ca. 10×71 μ, its oblong macronucleus supported dorsally by two karyophores. Its anterior (of two) glycogen bodies is lens-shaped and its peristome seems shorter though similar to that of *N. beta*.

N. delta n. sp. of the hind-intestine of *Parcoblatta pennsylvanica* in Champaign County, Ohio (Seman's Fig. 44, an inadequate illustration) is ovoid, 120×91 μ, macronucleus about 45×19 μ, W/L 76%. Its oblong macronucleus is bilobate, and unlike *N. ovalis*, the peristome is slanted sharply downward.

De Mello et al. 1934 described three morphological types A, B and C, of *N. ovalis* of *Periplaneta americana* in Nova Goa, India, Chakravarty and Chaterjee 1957 described *Nyctotherus pyriformis* and commented on *N. ovalis* from *Gryllotalpa vulgaris* at Bombay, India, their plagiotomoid has a forwardly placed peristome and is now transferred to *Kuodella pyriformis* n. comb.

An alphabetical listing of the species of *Plagiotomoidea* follows as Table 1.

Table 1

Listing of *Plagiotomoidea* species (145). Numbers in parantheses indicate the number of species in the group

Clevelandella Kidder, 1938 (11)

C. augustipennis (Yamasaki, 1939) n. comb.
C. constricta Kidder, 1938
C. contorta Kidder, 1938
C. elongata Kidder, 1938
C. hastula Kidder, 1938
C. longicollis (Yamasaki, 1939) n. comb.
C. nipponensis Kidder, 1938
C. panesthiae Kidder, 1938
C. papilloris (Yamasaki, 1939) n. comb.
C. parapanesthiae Kidder, 1938
C. plantiformis (Yamasaki, 1939) n. comb.

Kudoella n. gen. (2)

K. praenucleatum (Kudo et Meglitsch, 1938) n. comb.
K. pyriformis (Chakravarty et Chatterjee, 1957) n. comb.

Metaclevelandella Uttangi et Desai, 1963 (1)

M. termitis Uttangi et Desai, 1963

Metanyctotherus Albaret, 1970 (1)

M. congoi Albaret, 1970

Nyctotheroides (Grassé, 1928) Corliss, 1961 (57)

N. amarali (Carini, 1933) A et S, 1967
N. amaroi Earl, 1970
N. amphisbaenae (Carini, 1939) A et S, 1967
N. ampullariarum (Cordero, 1928) A et S, 1967
N. bertarelli (Carini, 1939) A et S, 1967
N. breviceps (Uttangi, 1958) A et S, 1967
N. bufonis (Uttangi, 1958) A et S, 1967
N. cacopusi (Uttangi, 1961) A et S, 1967
N. ceratophis (Carini, 1940) A et S, 1967
N. cinctus (Carini, 1939) A et S, 1967
N. cochlearis (Uttangi, 1948) A et S, 1967
N. coralli (Carini, 1933) A et S, 1967
N. cordiformis (Ehrbg, 1838) Grassé, 1928
N. crossodactyli (Carini, 1945) A et S, 1967
N. cunhai (Pinto, 1926) A et S, 1967
N. curtipes (Uttangi, 1958) A et S, 1967
N. elegans (Carini, 1939) A et S, 1967
N. faberi (Carini, 1939) A et S, 1967
N. fragilis (Carini, 1939) A et S, 1967
N. fulvus (Carini, 1939) A et S, 1967
N. gamarrai (Schouten, 1937) n. comb.
N. gibber (Carini, 1939) A et S, 1967
N. gibbosus (Boisson, 1959) A et S, 1967
N. heteronucleatus (Carini, 1939) A et S, 1967
N. hylae (Stein, 1867) A et S, 1967
N. incertus (Carini, 1939) A et S, 1967
N. jaegeri (Carini, 1933) A et S, 1967
N. kiferi (Kifer, 1953) n. sp.
N. landauae (Albaret, 1968) Albaret, 1971
N. leidyi A et S, 1967
N. limnocharis (Uttangi, 1958) A et S 1967
N. loricatus (Carini, 1939) A et S, 1967
N. mogyanus (Carini, 1933) A et S, 1967
N. nankingensis (Nie, 1932) A et S, 1967
N. neivai (Otamendi, 1945) A et S, 1967
N. ochoterenai Schouten, 1937
N. ondinae (Carini, 1939 A et S, 1967)
N. ophidiae (Fantham et Porter, 1950) A et S, 1967
N. oswaldoi (Carini, 1939) A et S, 1967
N. paludicolae (Carini, 1940) A et S, 1967
N. pangasi (Tripathi, 1954, A et S, 1967
N. paulistanus (Carini, 1939) A et S, 1967
N. pelobatidis n. sp.
N. puytoraci (Albaret, 1968) Albaret, 1971
N. pyriformis (Nie, 1932) A et S, 1968
N. reniformis (Bhatia et Gulati, 1927) A et S, 1967
N. ruber (Carini, 1939) A et S, 1967
N. sandoni Earl, 1970
N. spirostomatus A et S. 1968
N. systoma (Uttangi, 1958) A et S, 1967
N. tieteanus (Carini, 1939) A et S, 1967
N. tejeraai (Pinto, 1926) A et S, 1967
N. untanha (Carini, 1940) A et S, 1967
N. vesiculatus (Boisson. 1957) A et S, 1967
N. viannai (Pinto, 1926) A et S, 1967
N. vorax (Carini, 1939) A et S, 1967
N. vulgaris (Carini, 1939) A et S, 1967

Nyctotherus Leidy, 1849 (58)

N. alpha n. sp.
N. ampullarium Cordero, 1928
N. basidentitermes Gisler, 1967
N. befaysi Tuzet et Manier, 1958
N. beltrani Hegner, 1940
N. beta n. sp.
N. boipevae Carini, 1933
N. buissoni Pinto, 1926
N. delta n. sp.
N. dilleri Earl et Jiménez, 1969
N. diplopodae Karandikar et Rodgi, 1956
N. duboisii Künstler, 1884
N. ebriensis Gistler, 1967
N. gamma n. sp.
N. gisleri n. sp.
N. gongylorrhus Karandikar et Rodgi, 1956
N. grassei n. sp.
N. gyoeranus (C et L, 1838) Stein, 1867
N. haranti Grassé, 1926
N. hormeticae Carini, 1937
N. hoyai Tuzet et Théoridés, 1956
N. indica Bhaskar Rao, 1969
N. inflatus Tuzet et Manier, 1958
N. jaboti Carini, 1938

N. kyphodes Geiman et Wichterman, 1937
N. mackinnoni Schouten, 1940
N. macrotermitis Gisler, 1967
N. madagascari Tuzet et Manier, 1954
N. mandrakae Tuzet et Manier, 1954
N. mardonii Tuzet, Manier et Vogeli-Zuber, 1952
N. mauriesi Albaret, 1970
N. mutsorae Jeekel, Tuzet, Manier et Jolivet, 1958
N. neocurtillae Carini, 1938
N. nimbani Tuzet, Manier et Vogeli-Zuber, 1952
N. obesus Tuzet et Théodoridés, 1956
N. osmodermae Zeliff, 1933
N. ovalis Leidy, 1850
N. pachybolii Tuzet, Manier et Vogeli-Zuber, 1952
N. paeninsulae Gisler, 1967
N. panesthiae Yamasaki, 1939
N. pintoi Carini, 1933
N. piscicola Daday, 1905
N. regalis Gisler, 1967
N. rhinocrici de Mello, 1953
N. rhamphidarpae Jeekel, Tuzet, Manier et Vogeli-Zuber, 1952
N. rosenbergi (Rosenberg, 1937) n. name
N. scinci de Puytorac, 1954
N. silvestrianus Kirby, 1932
N. sokoloffi Schouten, 1940
N. teleacus Geiman et Wichterman, 1937
N. termitis Dobell, 1910
N. thyropygus Karandikar et Rodgi, 1956
N. tipulae Grassé, 1928
N. trachysauri Johnson, 1932
N. travassosi da Cunha et Penido, 1927
N. uichancoi Kidder, 1937
N. velox Leidy, 1849
N. woodi Amrein, 1952

Paraclevelandia Kidder, 1937 (2)

L. brevis Kidder, 1937
P. simplex Kidder, 1937

Parasicuophora Albaret, 1968 (1)

P. mantellae Albaret, 1968

Plagiotoma Dujardin, 1838 (2)

P. lumbrici Dujardin, 1838
P. kempi n. comb.

Prosicuophora de Puytorac et Oktem, 1967 (4)

P. basoglui de Puytorac et Oktem, 1967
P. magnus (Bezzenberger, 1904) de Puytorac et Oktem, 1967
P. macropharyngeus (Bezzenberger, 1904) de Puytorac et Oktem, 1967
P. malabarica n. sp.

Pseudonyctotherus Earl 1970 (1)

P. corlissi Earl, 1970

Sicuphora de Puytorac et Grain, 1968 (3)

S. heimi Albaret, 1970
S. mabokensis Albaret, 1970
S. xenopi de Puytorac et Grain, 1968

Wichtermania n. gen. (2)

W. cheni (Wichterman, 1934) n. comb.
W. kalii (Uttangi, 1951) n. comb.

At this point allow an interjection. Why do people, myself included, make mistakes in orienting to and describing plagiotomoids on elementary matters such as left and right, dorsal and ventral? The answer is partly that the organisms are transparent. What is left is so in shallow focus, becoming tight in deep focus, somewhat analogous to a reversable coat. Photographic prints can be reversed because the negative was not printed glossy side to the light source — the matter of the negative being transparent. The figure here (Fig. 2) of *W. kalii* was originally right side and is, for convenience, now reversed. The rest of the "trouble" is that the buccal overture is seen, usually, edge on. *Tetrahymena* serves as a good model, i. e., the UM is on the right and 3 AZM on the left, but the ordinary view is left lateroventral or even ventral, whereas with plagiotomoids the illustrated view is usually left side up and

indeed in nature they seem to prefer to feed and travel on the right side. There is no factual problem here in orientation but wrong calls in the literature have confounded a plain situation. Your left is the nyctotheran left.

In Figure 1 the right side of *Nyctotheroides cordiformis*, then its left laterodorsal face, and then the right lateroventral face of *Prosicuophora basoglui* after de Puyto-

Fig. 1. The right, then left side of *Nyctotheroides cordiformis*, followed by the right lateroventral face of *Prosicuophora basoglui*, after de Puytorac et Oktem

rac et Oktem are shown. The important point is that *Nyctotheroides* have a left suture continuing over the apex while *Nyctotherus*, probably *Kuodella*, *Prosicuophora*, *Sicuophora*, *Parasicuophora* and probably *Wichtermania* have right sutures, just as *Metanyctotherus* has a major left and reduced right suture.

Diagnoses of the genera of the *Plagiotomoidea* follow

Nyctotherus (Leidy, 1849) is endosymbiotic largely in invertebrates, cell ovoid, uniformly ciliated, short anterior right suture, karyophore(s) present, compressed anteriorly, sometimes quasiglobose posteriorly, shallow ventral groove present, S-shaped AZM beginning short of the apex running into the cell at the buccal overture, continuing dorsad and inferiad into the peristome as its main component, buccal overture over one-third and less than one-half of the length down the cell, peristome contains two UM, macronucleus anterior to peristome, contractile vacuole(s) present, posterior invaginated cytopyge. Type: *N. velox* Leidy, 1849.

The other genera are now defined differentially.

Nyctotheroides (Grassé, 1928) Corliss, 1961 is like *Nyctotherus*, except that there is no karyophore or it is much reduced, the cell need not be compressed anteriorly, there is a left suture receiving slanting kineties which crosses the apex to continue on the anterior right dorsum, and it may resticted to *Anura*. Type: *N. cordiformis* (Ehrenberg, 1938) Grassé, 1928.

Pseudonyctotherus Earl, 1970 is like *Nyctotheroides*, except that it has a flattened,

funnel-shaped egestatory apparatus terminating in a grooved tubule and no contractile vacuole is present. Type: *P. corlissi* Earl, 1970.

Plagiotoma Dujardin, 1838 is like *Nyctotherus*, except that it is more elongate, the buccal overture is within the posterior third of the cell and kineties radiate from a whorl on the posterior left side. Type: *P. lumbrici* Dujardin, 1838.

Prosicuophora de Puytorac et Oktem, 1967 is like *Nyctotheroides* except that a straight suture receiving slanting kineties is on the right side interrupted by an anterior lens of kineties, its sucker. Type: *P. basoglui* de Puytorac et Okten, 1967.

Wichtermania n. gen. is like *Nyctotheroides*, except that its AZM reaches the apex and it has a flange on the right side circumscribing the cell. Type: *W. cheni* (Wichterman, 1934) n. comb.

Sicuophora de Puytorac et Grain, 1968 is like *Nyctotheroides* except that the anteriolateral concave right side is a sucker, the right *S. xenopi* de Puytorac et Grain, 1968.

Metanyctotherus Albaret, 1970 is like *Nyctotheroides*, except that it has right and left anterior sutures. Type: *M. congoi* (Tuzet, Manier et Jolivet, 1957, Albaret, 1970.

Parasicuophora Albaret, 1968 is like *Nyctotheroides*, except that there is no left suture and two right sutures are present along the buccal overture to the apex and near the vertex. Type: *P. mantellae* Albaret, 1968.

Kuodella n. gen. is like *Nyctotherus*, except that the buccal overture is near the apex and the peristome is shorter. Type: *K. praenucleatum* (Kudo et Meglitsch, 1938) n. comb.

Metaclavelandella Uttangi et Desai, 1963 is like *Plagiotoma*, except that it is cordate, the buccal overture is more posterior without the AZM running down to it and the cytopyge is shifted to the dorsum. Type: *M. termitis* Uttangi et Desai, 1963.

Clevelandella Kidder, 1938 is like *Nyctotherus*, except that there is a posterior peristome within an elongate projection, the peristome not having an external AZM running down to it; it has only a straight dorsal suture sometimes running as far posteriad as the contractile vacuole, it is pointed anteriorly and obliquely truncated posteriorly and is sometimes pyriform. Type: *C. panesthiae* Kidder, 1938.

Paraclevalandia Kidder, 1937 is like *Clevelandella*, except that there is no posterior projection. Type: *P. brevis* Kidder, 1937.

Remarks on *Kudoella* n. gen.

Balantidium as a trichostome has no membranation, thus *Kudoella praenucleatum* n. comb. is established for *B. praenucleatum* Kudo et Meglitsch, 1938. It is ovoid to pyriform with a small peristome near the apex. Its macronucleus is oblong to spindle-shaped within a karyophore. The micronucleus is ovoid. Size is ca. 66×49 μ, macronucleus ca. 30×11 μ, micronucleus 2.5–7.0 μ long, peristome length ca. 15 μ; cyst ca. 35×27 μ. Width/length is about 77%, macronuclear length/length 43% and peristome length/length 20%. Host: *Blatta orientalis* from the foundations of the old Chemistry building on the University of Illinois campus, Urbana, Ill.

Kudo and Meglitsch are now quoted in relation to the peristome and macronucleus. "The peristome is located near the anterior extremity. It is a narrow and deep cylinder... The ciliation within the cavity seem to be continuous with that of the body surface. The adoral zone of membranellae arise just inside the aperture, projecting out of the cavity in a brush form, the characteristic movement of which is in striking contrast with that of the body cilia. The membranellae lie in a longitudinal series on the dorsal left wall of the peristomal cavity, extending to the bottom of the cavity, where the cytostome is located. The membranellae are shortest at the posterior end near the cyrostome, and the longest in the middle of the peristomal cavity. These strong membranellae beat synchronously so that the entire zone functions as a single membrane". "The constancy of the position of the macronucleus in the present ciliate reminded the authors of that of *Nyctotherus ovalis*... The structure of the macronucleus of the present species is distinctly different from that of any of the known species of the genus (*Balantidium*), and resembles very closely that of *Nyctotherus ovalis* (Kudo, 1936)". Regarding type A abnormal forms, they write "The dorsal wall of the peristomal cavity carries a row of membranellae", then on type C abnormal forms "A short row of membranellae was located in the dorsal wall", meaning the right side, sufficient to indicate 1 or 2 UM.

Information on *Wichtermania cheni* n. comb. and *W. kalii* n. comb.

Information in this paragraph comes from the descriptions of *W. cheni* and *W. kalii* as given by Wichterman 1934 and Uttangi 1958. The gross morphology of these organisms is illustrated in Fig. 2. *W. cheni* measures ca. 179×121 μ having a quasitriangular macronucleus 49×26 μ, broadly rounded dorsally. The macronuclear angle to the cell's main axis is ca. 28°. It has an AZM continuation

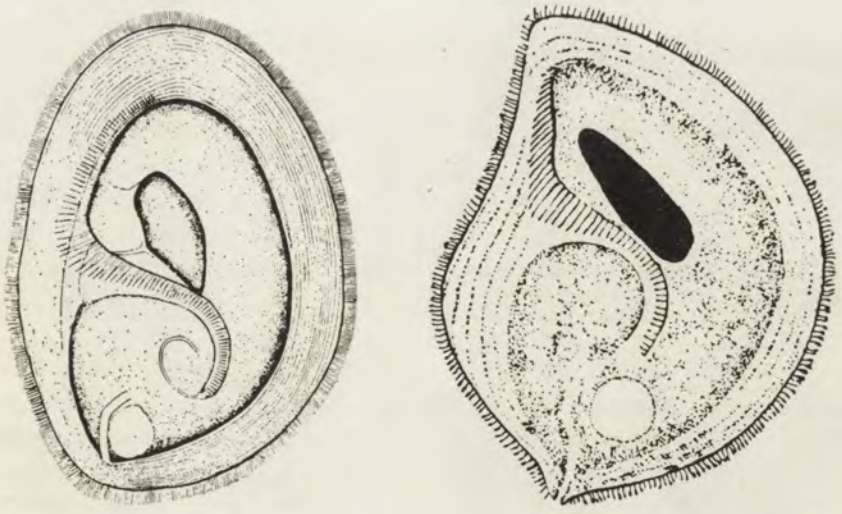

Fig. 2. The left aspects of *Wichtermania cheni* and *W. katii*, after Wichterman and then Uttangi

band extending below the cytostome. Its dorsoventrally aligned circumcellular flange is about 15 μ wide. Width/length is ca. 67%. *W. kalii* is some 280×223 μ with an avoid macronucleus 101×30 μ, which narrows ventrally. The macronuclear angle is about 41°. It has no AZM continuation band. Its flange measures 30–40 μ. Width/length is ca. 76%.

W. cheni, examined in H et E slides kindly lent by Wichterman, was accompanied by three *Balantidium* spp., a *Cepedea* and small flagellates.

From a sample of 25, dimensions (AM±SE) in microns are: length 184±7.7, width 124±3.7, macronuclear length and width 48±1.3×28±1.1, flange width 15±0.6 and AZM angle (near the anterior lip of the buccal overture) to apex 79±3.2. AZM angle to apex is 43% of the cell length, typical of nyctotherans. Width/length is ca. 67%, the mactonuclear length/length is 26% and macronuclear width/macronuclear length 59%.

W. cheni resembles a man's hat, the right flange making up the flat or slightly concave brim as the left side makes up the deep crown, some 70 μ thick. Anterior sutures could not be found, and thus I imagine the main suture as continuing dorsad from the AZM. The AZM continues beyond the apex, not true for *Nyctotherus* and *Nyctotheroides* where it stops short of the apex. The irregularly triangular macronucleus and the ovoid 5×3 μ micronucleus are encased by a strong pair of karyophores parabolically incurving to the nuclei from the anterior and posterior pellicle.

The cyst is round and crenated, measuring ca. 58 μ in diameter with an elongate ovoid macronucleus some 30×13 μ.

In cell replication, the two karyophores and the buccal overture break down completely. A constriction plane or belt forms at the buccal overture running across and up the cell at a 30° angle; concomitantly the macronucleus divides. The course of the micronucleus could not be followed with the material at hand. The peristome — cytostome to buccal overture only — degenerates in the opisthe as the AZM above the overture stays in the proter and disintegrates. Karyophores and the entire AZM-peristomal system arise de novo. This is apparently distinct from the known course of events in some species of *Nyctotheroides* and *Nyctotherus*, but similar to the progression described by Schmähl 1926 for *Bursaria truncatella*. Referring to *N. cordiformis* (?), Wichterman 1937 states that the AZM-peristomal system "is retained in the anterior daughter but undergoes a partial membranelle dedifferentiation and then redifferentiation, perhaps concomitantly". Zulueta 1916 (*Nyctotherus ovalis*) and Zeliff 1933 (*Nyctotherus osmodermae*) both mention total resorption and a de novo appearance of oral structures.

Regarding two tomonts of *W. cheni* found close to separation, identical anlagen of the AZM-peristomal system were developing parallel to the right side in both daughters. Contractile vacuoles were present in both, and the macronucleus in each was free in the cytoplasm to the right of each macronucleus. The proter was 108×76 μ and its macronucleus 40×27 μ; dimensions for its opisthe were 97×74, 37×29 μ. The constriction belt was 55 μ long.

Summary

Kuodella n. gen., other plagiotomoids, *Nyctotherus*, *Nyctotherus*, *Metanyctotherus*, *Pseudonyctotherus*, *Wichtermania* n. gen., then *Prosicuophora*, *Sicuophora*, *Parasicuophora* and *Plagiotoma* of the *Plagiotomidae* and *Metaclevelandella*, *Paraclevelandia* and *Clevelandella* make up the *Plagiotomoidea* n. superfam., an evolutionary series characterized primarily by the displacement of the buccal overture posteriorly. Taxonomic changes are effected and a list of 145 species presented, including *Nyctotheroides kiferi* n. sp., *N. pelobatidis* n. sp., *Prosicuophora malabarica* n. sp. and *Nyctotherus alpha* n. sp., *N. beta* n. sp., *N. gamma* n. sp., *N. delta* n. sp., *N. gisleri* n. sp. and *N. grassei* n. sp.

RÉSUMÉ

Kuodella n. gen., d'autres plagiotomoides *Nyctotherus*, *Nyctotherus*, *Metanyctotherus*, *Pseudonyctotherus*, *Wichtermania* n. gen., *Prosicuophora*, *Sicuophora*, *Parasicuophora* et *Plagiotoma* des *Plagiotomidae*, *Metaclevelandella*, *Paraclevelandia* et *Clevelandella* forment *Plagiotomoidea* une superfamille nouvelle, une serie evolutive characterisé essentiellement eu ce que l'ouverture bucale est déplacée posterieurement on a fait des changements taxonomiques et on a donné une liste des 145 espèces présentées sur la quelle il y a *Nyctotheroides kiferi* n. sp., *N. paleobatidis* n. sp., *Prosicuophora malabarica* n. sp. et *Nyctotherus alpha* n. sp., *N. beta* n. sp., *N. gamma* n. sp., *N. delta* n. sp., *N. gisleri* n. sp. and *N. grassei* n. sp.

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Note added in proof:

Inferostoma jankowskii Ky, 1971 of the fish *Spinibarbichthys denticulatus* of North Vietnam has been added to the *Plagiotomoidea*, neighboring *Metaclevelandella termitis*, and also added are *Nyctotherns baueri* Ky, 1971 of that fish and *N. schulmani* of *Squaliobarbus curriculus*, also of freshwater of North Vietnam. *Inferostoma* has a right sucker and a suture system different but closer to *Nyctotherus* than to *Metaclevelandella*, which it resembles in mouth position and AZM. It may partly serve to focus attention on the intestinal protozoa of bottom-feeding fishes, particularly siluroids. Fish nyctotherans are: *N. baueri*, *N. schulmani*, *I. jankowskii*, *N. piscicola*, *N. carinii*, *N. mackinnoni*, *N. dilleri* and *Nyctotheroides pangasia*.

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Ultrastructure of the cortical fibrillar systems of the marine ciliate *Helicoprorodon gigas* Kahl, 1933 (*Holotricha, Gymnostomatida*)

Ультраструктура субпелликулярных систем у морской инфузории *Helicoprorodon gigas* Kahl, 1933 (*Holotricha, Gymnostomatida*)

By now a large number has been collected of electron microscopic works dealing with the investigations into the fine constitution of the subpellicular structures of various ciliates. In those investigations a particular attention has been paid to the fibrillar components which form more or less complex systems in the space between the pellicle and endoplasm (Fauré-Frémiet et Rouiller 1955, Fauré-Frémiet et al. 1956, 1958 a, b, Fauré-Frémiet 1962, Fauré-Fremiet et Ganier 1969; Randall 1956 a, b; Randall and Jackson 1958; Noirot-Timothée 1958, 1963; Yagiu and Shigenaka 1963 a, b, 1965: de Puytorac 1958, 1959, 1961, 1963, 1964; de Puytorac and Kattar 1969; Pitelka 1961, 1963, 1965, 1968, 1970; Snigirevskaya 1964; Finley et al. 1964; Kennedy 1965; Grain 1965, 1966, 1968, 1969; Paulin 1967; Bannister and Tatchell 1968; Wessenberg and Antipa 1968; Lom and Kozloff 1969 et al.).

The confrontation of the data available enables two basic systems to be selected. First comes the ectoplasmatic fibrillar system present in all ciliates. Its particular elements represented for the most part by complexes of microtubules are connected directly or indirectly with the kinetosomes of cilia.

Apart from the ectoplasmatic system an boundary fibrillar system can be found in a number of ciliates. It constitutes a filament or fibrillar complex lying in the intermedium between the ecto- and endoplasms ("tela corticalis" according to Bretschneider, 1959).

The morphological unity between those two systems is not yet sufficiently examined and was only in few ciliates detected and already recognized.

The aim of the present work is to examine by the electron microscopy the ectoplasmatic as well as boundary fibrillar systems in the marine ciliate *Helicoprorodon gigas* Kahl, 1933 (*Holotricha, Gymnostomatida*).

We will not dwell at length on other ultrastructures of this ciliate since they were

described in detail by de Puytorac and Lattar 1969 in related to *H. gigas* species of *H. multinucleatum* having a similar constitution. A good description of *H. gigas* and basic organelles found in this protozoan by means of the light microscope is given by Raikov 1960, 1967.

Material and methods

Marine ciliates *Helicoprorodon gigas* have been collected on the littorals of the Barents Sea and the Sea of Japan[1]. For fixation the 2 per cent solution of OsO_4 buffered with veronal-acetate buffer (pH 7.3) was used. The time of fixation varied from 20 to 30 min. After fixation objects were dehydrated in solutions of ethyl alcohol in concentrations growing from 40 per cent to anhydrous one. The basic medium for capsulation of the material was araldite. Microtomic sections were examined in UEMV — 100 B electron microscope at an accelerating voltage of 75 kV. To contrast the sections use made of lead citrate (Fiske 1966) and of a saturated alcoholic solution of uranyl acetate.

Results

On the surface of the body of marine ciliate *H. gigas* longitudinal cristas can be seen and between them unpaired cilia are situated in furrow at a distance of about 7000 Å away from each other. The unpaired cilia form longitudinal ciliary rows (Pl. I 1). The structure of the undulipodia of cilia is typical. Kinetosomes of cilia are also in their structure similar to those of other ciliates already examined. Their walls are electron dense and consist of nine microtubule triplets; each of them is the continuation of a corresponding peripheral fibril of the undulipodium of a cilium. The proximal end of the kinetosome is surrounded with an electron dense material ("formation dense" according to Grain 1969) which fits tightly to its external wall. From the material a kinetodesmal filament periodically striated and two fibrils-transverse and postcilliary (radial) ones — are detached. To a certain distance the electron-dense material can accompany those structural elements (Pl. II 2–8, III 9 IV 11–12). The separation of the filament and fibrils from triplets forming the wall of kinetosome is by no means disorderly but strictly regular and in consequence of this recurs from one kinetosome to the other.

The transverse fibril originates near triplets 3–4 (using the nomenclature suggested by Grain in 1969 and also accepted on the 3rd International Congress of Protozoologists in 1969). This fibril is formed by a series of microtubules which at the proximal end of the kinetosome is connected with corresponding triplets by the electron dense material (Pl. II 2–5, 8). The fibril goes up the wall of the kinetosome and on the level of the distal end of the latter slightly deviates aside and falls to separate mictobules, 170 Å in diameter. Near the pellicle a fascicle of microtubules forming parts of the transverse fibril changes its direction abruptly and runs farther in

[1] In the Sea of Japan marine ciliates were collected, then fixed and capsulated by dr. I. B. Raikov (Institute of Cytology of the U. S. S. R. Academy of Science). Taking the opportunity the authors express their deep gratitude to dr. I. B. Raikov for the material afforded.

parallel to the surface of the ciliate body (Pl. IV 12, 13, V 14–15, VI 16), towards its fore end to the left of the ciliary row; individual microtubules may then come into contact with the membane of the pellicle. Anyway microtubules do not exceed the bounds of the kinetosomal territory and, to all appearance come to an end in the immediate proximity to the postciliary fibril (Pl. VI 16) arriving there from the left neigboring ciliary row. Apart from the set of microtubules going up as components of the transverse fibril towards the pellicle, on the microtomic longisections of kinetosomes in the zone of the same triplets (3 and 4) a filament can be seen making its way from the material downwards deep into the cytoplasm. It forms a structure resembling that of root fibril (Pl. II 2, V 14, 15), however unlike the typical root fibril characteristic of the ciliated epithelium cilia of cells of *Metazoa* (Fawcett 1961) this filament in *H. gigas* is not striated transversely. In all likelihood it is a derivative of one of the microtubules being a component of the transverse fibril (Pl. V 14, 15). The root filament recedes with its proximal end into the endoplasm. In a number of cases it can be watched to the depth of 12 000 Å.

Near triplet 7 a kinetodesmal filament (Pl. II 3–7, II 9) with the period of the transverse striation of about 200 Å originates from the dense material of the kinetosome. It goes upwards and to the right while gradually thinning and comes to an end within the limits of the kinetosome territory. In the course of advancing it is in touch with the postcilliary (radial) fibril arising from the lying ahead kinetosome of the same ciliary row (Pl. III 10).

The postciliary fibril consists of microtubules (about 100 Å in diameter) which originate in the zone of triplet 9 (Pl. II 3–8). The fibril abandons the kinetosome in the company of two electron dense processes of the coating (Pl. II 2–8) which become soon thinner and thinner thus making microtubules — its components — clearly visible (Pl. IV 11). When having been detached from the wall of the kinetosome at an angle of about 60° the postciliary fibril goes up towards the pellicle but before reaching the surface membrane it alters quietly its direction (Pl. II 2, IV 11, VI 16) and continues to run parallel to the surface of the body shaping its course backwards for the right neighboring ciliary row. This fibril of considerable length passes along several kinetosomes withdrawing to the right and, as was noticed, touching on the way the kinetodesmal filament. (Pl. III 10) of the first kinetosome lying behind.

Thus, from each somatic kinetosome in *H. gigas* a definite and constant set of ultrastructures is detached: a kinetodesmal filament, transverse and postciliary (radial) fibrils and also a root fibril running downwards into the endoplasm. Due to the well-developed postciliary fibrils the morphological unity of all those ultrastructures into one ectoplasmatic fibrillar system is accomplished. Figure 1 shows how this unity is fulfilled among particular components of the ectoplasmatic fibrillar system within the limits of one ciliary row.

Between ecto- and endoplasm in *H. gigas* the boundary fibrillar system is situated with proximal ends of the kinetosomes of cilia immersed therein. It forms something

like a sac containing the endoplasm of the protozoan. To all appearances the boundary fibrillar system is responsible for the contraction of the body of *H. gigas* so that it can be called a myoneme. The myoneme is produced by short filamentous bundle which are disposed at random throughout all its thickness. Those bundle run in all directions anastomizing with one another and forming altogether a three-dimension-

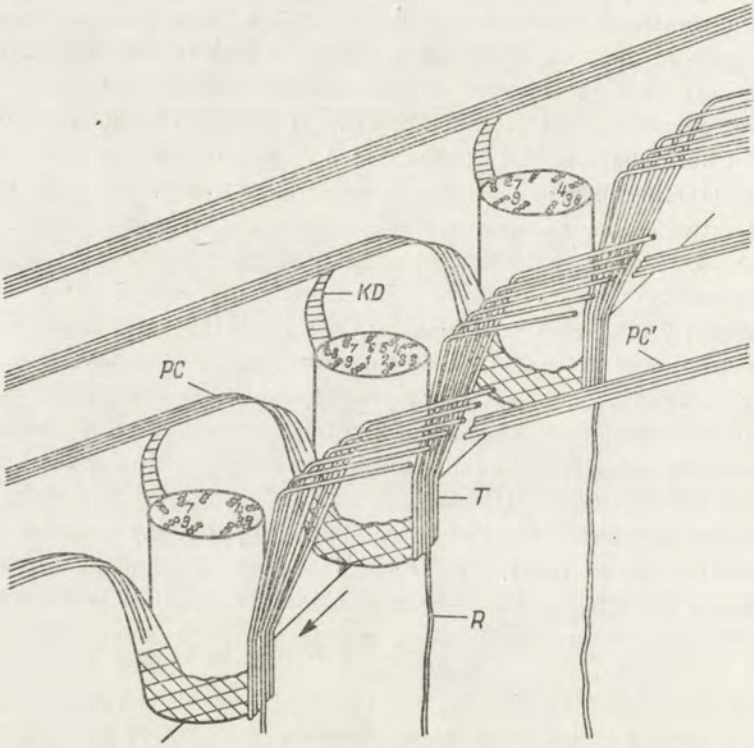

Fig 1. Diagrammatic representation of the morphological unity between kinetosomes within the range of a single row in *Helicoprorodon gigas*. KD — kinetodesmal filament; PC — postciliary (radial) fibril; T — transverse fibril; R — root fibril; PC′ — postciliary fibril coming from the neighboring ciliary row. Arrow indicates the back end of the ciliate body

al spatial network. (Pl. VII 18). No special membrane surrounding the myoneme was revealed. The thickness of the myoneme is not equal over its full length. At sections situated between the cristas of pellicle, i.e. beneath the furrow containing cilia the myoneme changes its configuration: it thickness and goes deep into the protozoan body (Pl. I 1). Thus, the kinetosomes of cilia settle down where the myoneme is the thickest (about 5000 Å) (Pl. I 1, II 2, V 15) whereas at sections between ciliary rows the myoneme is considerably thinner (about 2000 Å). At full its length the myoneme produces processes which go up towards the pellicle (sometimes reaching it) and also go down towards the endoplasm (P. I 1).

From the side of the endoplasm the myoneme is underlain by a continuous

vesicular layer (Pl. I 1). It is formed by large and small vecicles (vacuoles), the walls of which are limited by the membrane. The vesicles are divided one from another by the thin ties of the granular cytoplasm (Pl. I 1, III 9, 10, VI 16, 17). Under pellicular cristas the thickness of the vesicular layer arrives at its highest pitch (tp 20 000 Å) whereas at intervals between them it is half as thin. The vesicles are disposed in relatively good order which is particularly well visible on tangential microtomic sections (Pl. VII 19). It is not unlikely that the vesicular layer is an endoplasmatic reticulum (de Puytorac et Kattar 1969).

The processes of the myoneme go sometimes fairly deep into the vesicular layer; the ties of the endoplasm dividing the vecisles are, in their turn, in contact with the basic mass of the myoneme. Thus both the myoneme and the endoplasm of the ciliate remain in a close morphological unity with each other.

Immediately under the vesicular layer mitochondria are situated by groups (Pl. I 1). They have a constitution typical for protozoa, their cristas being presented in the form of tubules. In *H. gigas* those tubules are packed within the mitochondria into parallel rows (Pl. I 1, VI 17).

Further down the vesicular layer in *H. gigas* the strongly vacuolated endoplasm is placed with all organelles and inclusion bodies characteristic of protozoa.

Of course, between the endoplasm and ectoplasm of a unicellular organism the exchange must take place with definitely shaped inclusion bodies and intracellular structures. In all probability this exchange in *H. gigas* must be hampered to a certain extent by the presence of the continuous layer of vesicles and myoneme. Nevertheless the passage of various organized structures is successfully carried out in case of need by the break of the vesicular layer and myoneme (Pl. VI 17).

On the basis of the data obtained a scheme has been drawn (Fig. 2) which presents the location and mutual arrangement of subpellicular structures of *H. gigas* ciliate.

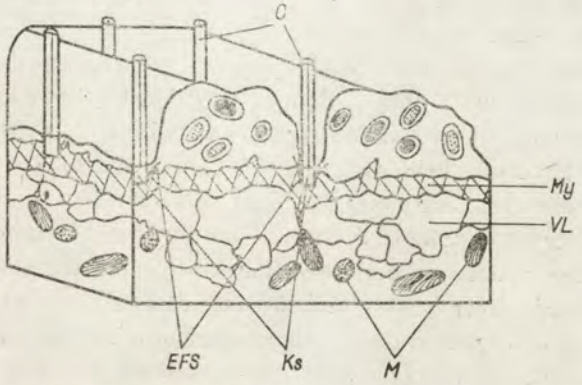

Fig. 2. Diagrammatic representation of the mutual arrangement of subpellicular structures of the *Helicoprorodon gigas* ciliate. Between pellicular cristas ciliary rows are located (C). EFS — elements of the ectoplasmic fibrillar system. Proximal ends of kinetosomes are immersed into myoneme (My) which is underlain by the vesicular layer (VL). Immediately underneath the vesicular layer separate conglomerations of mitochondria are disposed

Discussion

It is now well known from the studies (Pitelka 1968, Grain 1969) that in overwhelming majority of ciliates the same structural elements are assumed to be a basis of the organization of the ectoplasmatic fibrillar system. These are, first of all, kinetodesmal filaments (appearing in the zone of triplets 5–6–7–8), postciliary (originating at triplet 9) and transverse (beginning in the zone of triplets 2–3–4) fibrils.

There are ciliates with the kinetosomes which have not, perhaps, any sets of those structural elements (cf. Pitelka 1968) but in all such cases it is apparently necessary to reexamine anew and more thoroughly the object before definite assertion that we have to do with an extraordinary exception to the rule.

In the organization of the ectoplasmatic fibrillar system of a good number of ciliates the so-called subpellicular fibrils figure prominently. They are disposed in the epiplasm of a pellicle and run between ciliary rows along the body of the protozoan (Pitelka 1963, 1968). It appears that subpellicular fibrils have not a direct morphological contact with kinetosomes and are connected with them only by means of postciliary (radial) or transverse fibrils.

Although the sets of initial structural elements are in principle the same in the majority of cilliates, nevertheless in various species the priority in development is got by different elements in consequence of which the various types of the organization of the ectoplasmatic fibrillar system are given rise. Thus, in parasitic *Ignotocoma sabellarum* according to Lom and Kozloff 1969 kinetodesmal filaments and postciliary fibrils are rather underdeveloped, anyway they do not exceed the limits of the correspondent kinetosome territory. As to the transversal fibrils they are practically reduced. Instead, subpellicular fibrils coming into contact with postciliary ones are developed very well. This type of the organization of the ectoplasmatic fibrillar system within the group of at most developed structural elements may be termed a subpellicular type.

In *Blepharisma*, *Stentor*, *Spirostomum* and *Dileptus* (Kennedy 1965, Fauré-Frémiet et Rouiller 1955, 1958 b, Randall 1956 a, b; Randall and Jackson 1958, Dumont 1961, Yagiu and Shigenaka 1963 a, b, Finley et al. 1964, Bannister and Tatchell 1968, Grain 1968, Grain and Golińska 1969) postciliary (radial) fibrils stretching along the ciliary row for 8–10 and more neighboring kinetosomes are very well-developed. Transversal fibrils and kinetodesmal filaments do not exceed the bounds of corresponding kinetosome territory. Subpellicular fibrils are developed rather weakly and in some cases are absent at all. Such is a postciliary type of organization of the ectoplasmatic fibrillar system in ciliates.

The ectoplasmatic fibrillar system of *Helicoprorodon* may also be ranked with it. An important distinction between them is that in *Blepharisma*, *Stentor* and other *Spirotricha* the postciliary fibrils of neighboring kinetosomes go along the ciliary row in close vicinity forming a kinetodesmata-like structure called until recently a kinetodesmata (cf. Pitelka 1968, Grain 1968, 1969).

In *Helicoprorodon gigas* those fibrils go at an acute angle to the neighboring right ciliary row and never form the common structure of kinetodesmata type, in other words they are independent from one another. That is why the ectoplasmatic fibrillar system is created which differs sharply in their general scheme of constitution from that in *Blepharisma*, *Stentor* etc.

It is reasonable to suppose that the postciliary type of organization of the ectoplasmatic system occurs in various groups of protozoa in a convergent way, i.e. independently.

Once a subject of basic types of organization of the ectoplasmatic fibrillar systems in various ciliates is touched upon, mention should be made of the fact that in *Tetrahymena*, *Colpidium*, *Paramecium* and some other *Holotricha* two groups of structural elements (Pitelka 1968, Allen 1967) proved to be straight away intensely developed (and capable of exceeding the bounds of corresponding kinetosomae territory) viz., subpellicular fibrils and kinetodesmal filaments. The latter form the kinetodesmata of complex constitution. This type of organization of the ectoplasmatic system may be termed a subpellicular-kinetodesmal one.

It seems, therefore, that subsequently also other possible types of organization of the ectoplasmatic fibrillar systems will be found in ciliates, viz. kinetodesmal, transverse or complex ones in the cases when the priority in development is gained by two or even three basic structural elements of this system instead of only one. Established facts show, however, that not all imaginable combinations of those elements are possible. Thus, the formation of kinetodesmata is usually connected in ciliates with the underdevelopment of postciliary fibrils and vice versa. It is doubtful, therefore, whether a postciliary-kinetodesmal type of organization of the estoplasmatic fibrillar system exists at all.

The boundary fibrillar system like the ectoplasmatic one is constituted differently in various species of ciliates. In some of them it consists of a continuous layer, for example in *Didinium* (Yagiu and Shigenaka 1965, Wessenberg and Antipa 1968), *Dileptus* (Dumont 1961, Grain and Golińska 1969), *Metaradiophrya* (de Puytorac 1958, 1959, 1961), *Mesnilella* (Cheissin 1963, de Puytorac 1963), *Bursaria* (Snigirevskaya 1964) and also in *Helicoprorodon*. In *Stentor*, *Spirostomum* etc. on the other hand, it is composed of an aggregate of longitudinally arranged bands (Fauré-Frémiet and Rouiller 1955, 1958 a, b, Randall 1956 a, b, Randall and Jackson 1958, Yagiu and Shigenaka 1963 a, b, Finley et al. 1964, Grain 1968, Bannister and Tatchell 1968).

In some species of ciliates the boundary system is based on filaments or filiform ultrastructures such as: *Didinium* (Yagiu and Shigenaka 1965, Wessenberg and Antipa 1968), *Blepharisma* (Dembitzer and Hirshfield 1966), also in *Helicoprorodon gigas*, in such ciliates, however, as for example *Metaradiophrya* (de Puytorac 1958, 1959, 1961), *Mesnilella* (Cheissin 1963) etc. they contain microtubules.

In *Helicoprorodon gigas* both fibrillar systems remain in close morphological unity so long as kinetosomes are directly immersed into the myoneme with their proximal ends. A similar morphological picture was revealed in *Didinium* (Wessenberg and Antipa 1968) and *Dileptus* (Grain and Golińska 1969). However, also other forms of unity between those two systems are known. Thus, in *Blepharisma* (Dembitzer and Hirshfield 1966) the finger-shaped processes go upwards from the boundary fibrillar system and enter into contact with fibrils arising from kinetosomes.

Many scientists believe the system of vacuoles and tubules of the endoplasmatic reticulum which accompanies myonemes in ciliates to play an important part in the regulation of the contractile procedure (Fauré-Frémiet and Rouiller 1958 a; Yagiu and Shigenaka 1965; Dembitzer and Hirshfield 1966 et. al.). In this connection the intensive development of the vesicular layer which underlies the intermediate fibrillar system in *H. multinucleatum* (de Puytorac and Kattar 1969) and in *H. gigas* is worthy of notice. It may be supposed that the vesicular layer in those ciliates bears a relation to the accumulation and transportation of physiologically active substances necessary for accomplishing the contraction of the boundary fibrillar layer which appears to be a myoneme in those forms. The supposed function of the vesicular layer is confirmed indirectly by the continuous presence of mitochondria groups closely adjoined to the vacuoles of this layer.

Of course, better understanding of the origin and evolution of subpellicular fibrillar systems necessitates further electron microscopic investigations of them in all possible kinds of ciliates and first of all in primitive representatives of the *Holotricha* subclass.

Summary

The electron microscopic investigation into the subpellicular space in *Helicoprorodon gigas* ciliate was performed. It was proved that there are two basic fibrillar systems in this protozoan viz, ectoplasmatic and boundary (in the intermedium between ecto- and endoplasms). The greatests development in the *H. gigas* ectoplasmatic system is gained by postciliary (radial) fibrils, owing to which the morphological unity among kinetosomes of single ciliary row and among those of neighboring ciliary rows is accomplished. Transverse fibrils and kinetodesmal filaments do not exceed the bounds of corresponding kinetosome territory. The boundary fibrillar system (myoneme) is built of short anastomizing with one another filiform bundles of filaments which altogether form a three-dimensional spatial network. Underneath this system the continuous vesicular layer is situated which appears to be a derivative from the endoplasmatic reticulum. Between the ectoplasmatic and boundary systems the direct morphological unity exists so far as proximal ends of kinetosomes are immersed into the myoneme. Thus, the united subpellicular fibrillar system is organized in *H. gigas*. The paper presents various types of organization of the ectoplasmatic and boundary fibrillar systems.

РЕЗЮМЕ

Проведено электронномикроскопическое исследование субпелликулярного пространства у инфузории *Helicoprorodon gigas*. Показано, что у этого простейшего имеются две основные фибриллярные системы: эктоплазматическая и пограничная (на границе экто- и эндоплазмы). Наибольшее развитие в эктоплазматической системе *H. gigas* получают постцилиарные (радиальные) фибриллы, именно благодаря им осуществляется морфологическая связь между кинетосомами одного и соседних ресничных рядов. Поперечные фибриллы и кинетодесемальные филаменты не выходят за пределы соответствующих кинетосомальных территорий. Пограничная фибриллярная система (мионема) построена из коротких анастомозирующих друг с другом нитевидных тяжей (филаментов), которые в общей совокупности образуют трехмерную пространственную сеть. Под этой системой расположен непрерывный везикулярный слой, который, по-видимому, является дериватом эндоплазматического ретикулюма. Между эктоплазматической и пограничной системами существует прямая морфологическая связь, поскольку проксимальные концы кинетосом погружены в мионему. Таким образом, у *H. gigas* организуется единая субпелликулярная фибриллярная система. В работе обсуждаются различные типы организации эктоплазматических и пограничных фибриллярных систем у инфузорий.

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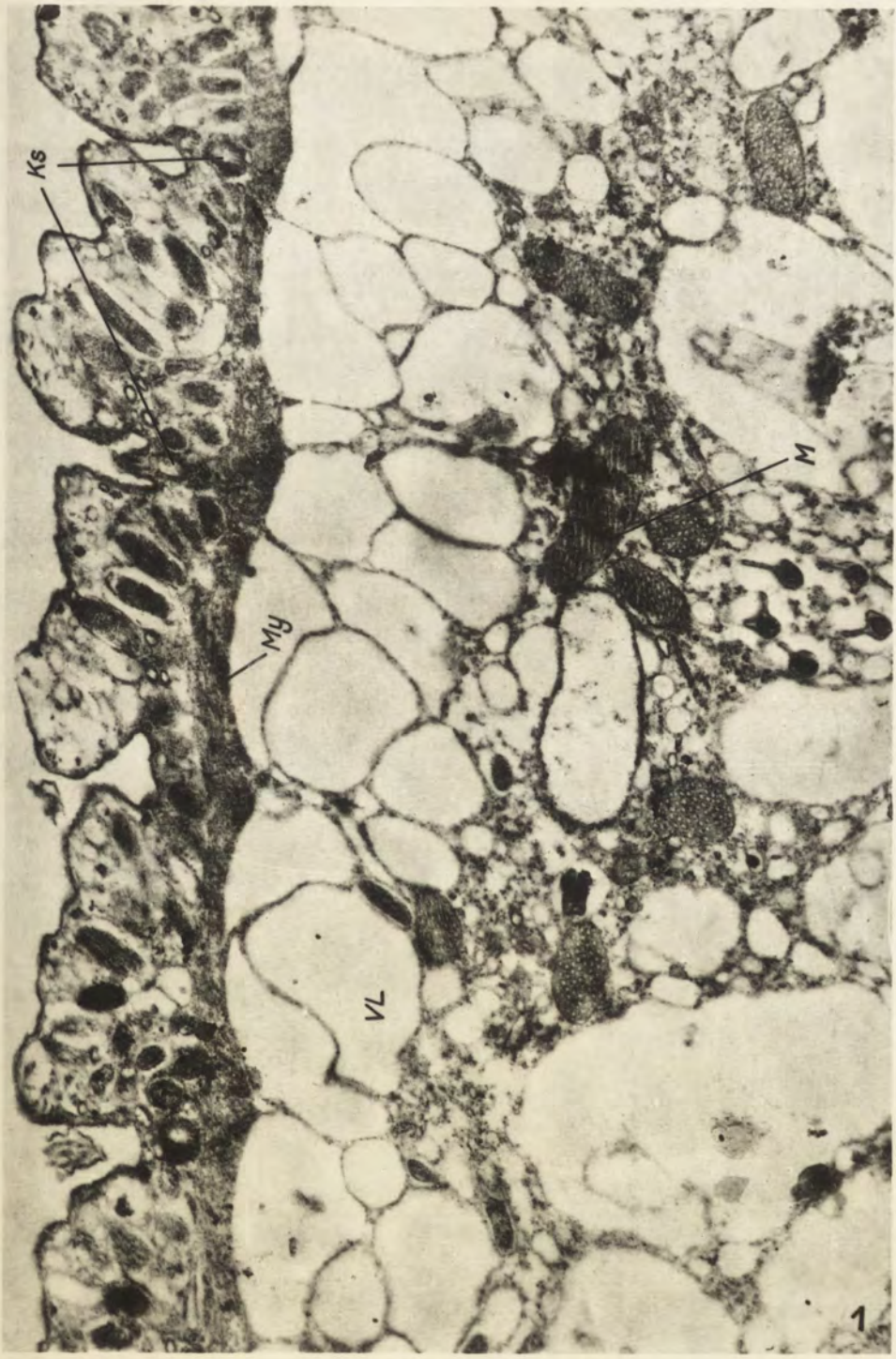
EXPLANATION OF PLATES I–VII

Helicoprorodon gigas Kahl, 1933

1: Cross section through the surface of the body of the *Helicoprorodon gigas* ciliate. Under ectoplasmatic cristas in the intermedium between ecto- and endoplasms the myoneme (My) is located which is underlain by the vesicular layer (VL). Immediately beneath this layer separate conglomerations of mitochondria (M) can be seen. Proximal ends of kinetosomes (Ks) are immersed into myneme. ×19 000.
2: Longisection through the cilium. Proximal end of kinetosome is surrounded by electron dense material which fits tightly to its external wall. ×60 000
3–7: Cross sections through kinetosome made on various levels of its longitudinal axis (from proximal to distal end). KD — kinetodesmal filament; PC — postciliary fibril; T — transverse fibril. ×60 000
8: Tangential section through the surface of the body of the ciliate. Two rows of kinetosomes with sets of fibrils can be seen. At the right — fore end of the body, at the left — back end of the body of ciliate. ×40 000
9: Oblique longisection through the surface of the body of the ciliate. Arrow indicates the dense material of the kinetosome from which postciliary fibril (PC) and periodically striated kinetodesmal filament (KD) are detached. ×37 000. At the left below — the dense material of the kinetosome; due to great magnification the periodic striation of kinetodesmal filament can be seen. ×70 000
10: Oblique longisection through the surface of the body of the ciliate. Arrows indicate the contact points of post-ciliary fibrils (PC) with kinetodesmal filaments (KD) of the same ciliary row. ×45 000
11–13: Longisections through the kinetosomes of cilia. The withdrawal of fibrils from the dense material can be seen. Postciliary fibril (PC) consisting of four microtubules withdraws to the left. Transverse fibril (T) falling to separate microtubules leaves to the right. ×80 00
14–15: Longisections through the kinetosomes of cilia. Root fibrils (R) moving down into the endoplasm of the protozoan can be seen. They appear to be derivatives from microtubules being parts of transverse fibrils (T). PC — postciliary fibril. ×60 000
16: Oblique longisection through the surface of the body of the ciliate. To the left of the kinetosome postciliary fibril (PC) goes away, to the right — transverse fibril (T) which comes to an end within the limits of the kinetosomatic territory in the immediate proximity to the postciliary fibril (PC′) arriving from one of the kinetosomes of a neighboring ciliary row. ×43 000
17: Longisection through the surface of the body of the ciliate. It is shown how the structures morphologically finished (trichocysts — Tr) pass through the vesicular layer (VL) and myoneme (My) of the protozoan. ×2100.
18: Oblique cross section through the myoneme (My) of the ciliate. Short, disorderly disposed filamentous boundles of which it is composed can be seen. ×70 000
19: Tangential section through the vesicular layer of the ciliate. Well-ordered arrangement of vesicles is shown. Arrows point out intervals between cristas over which pellicle fissures lie. ×30 000.



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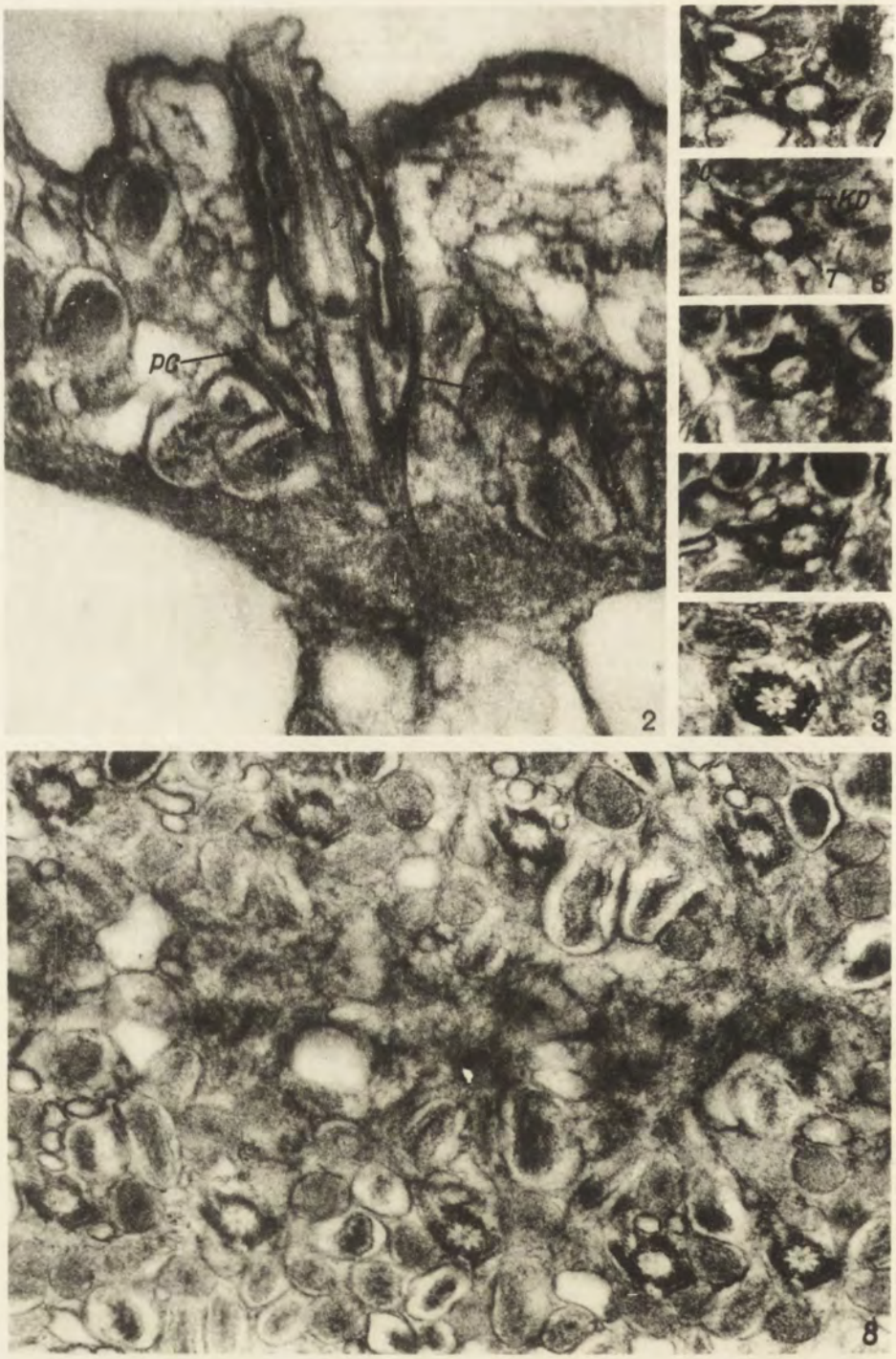

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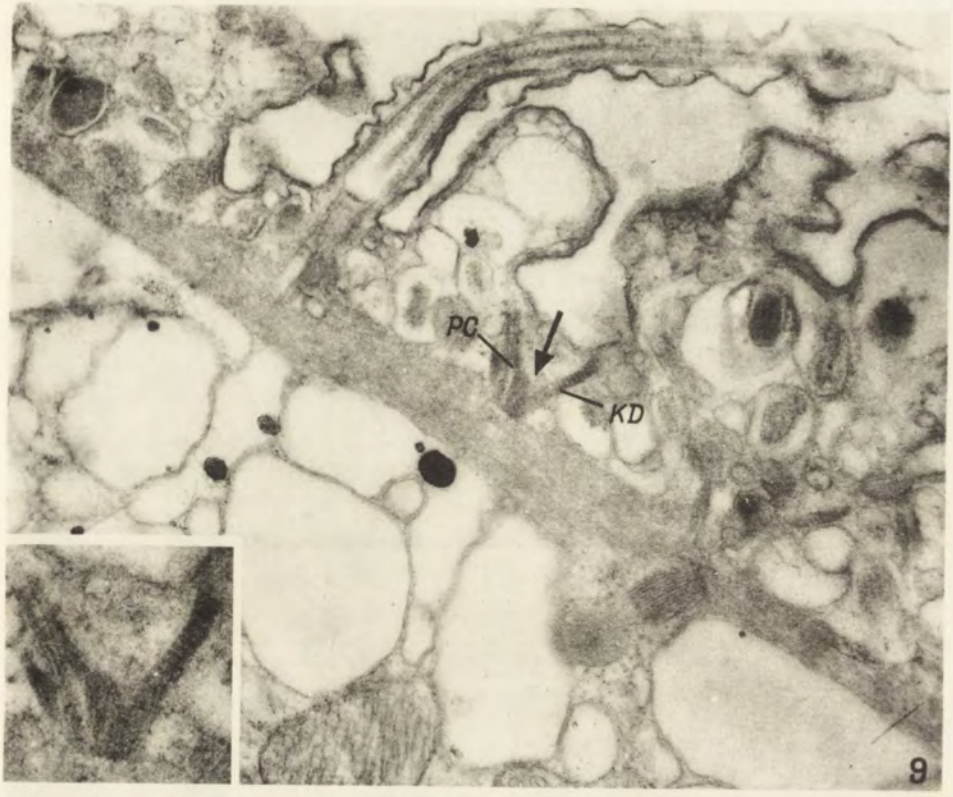

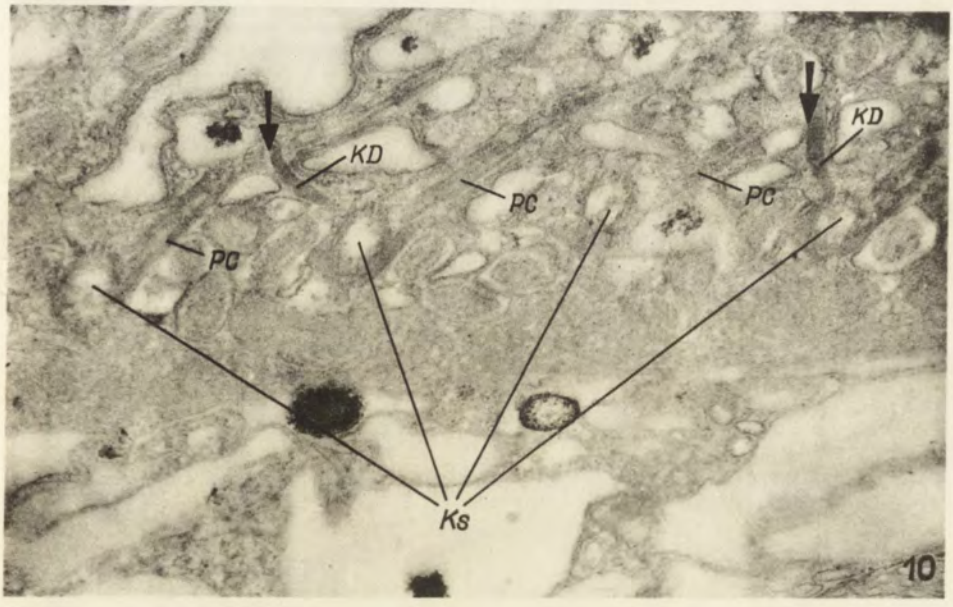

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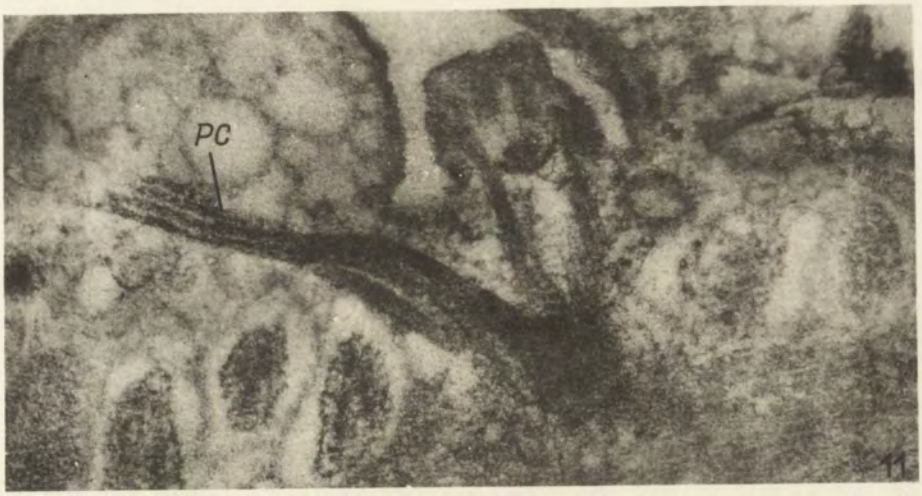

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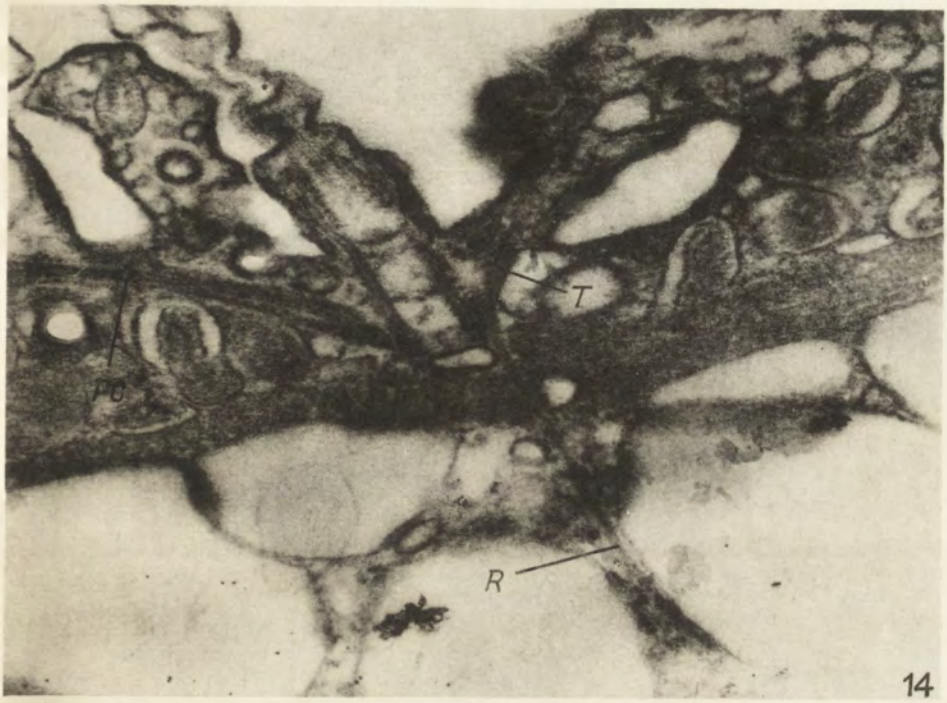

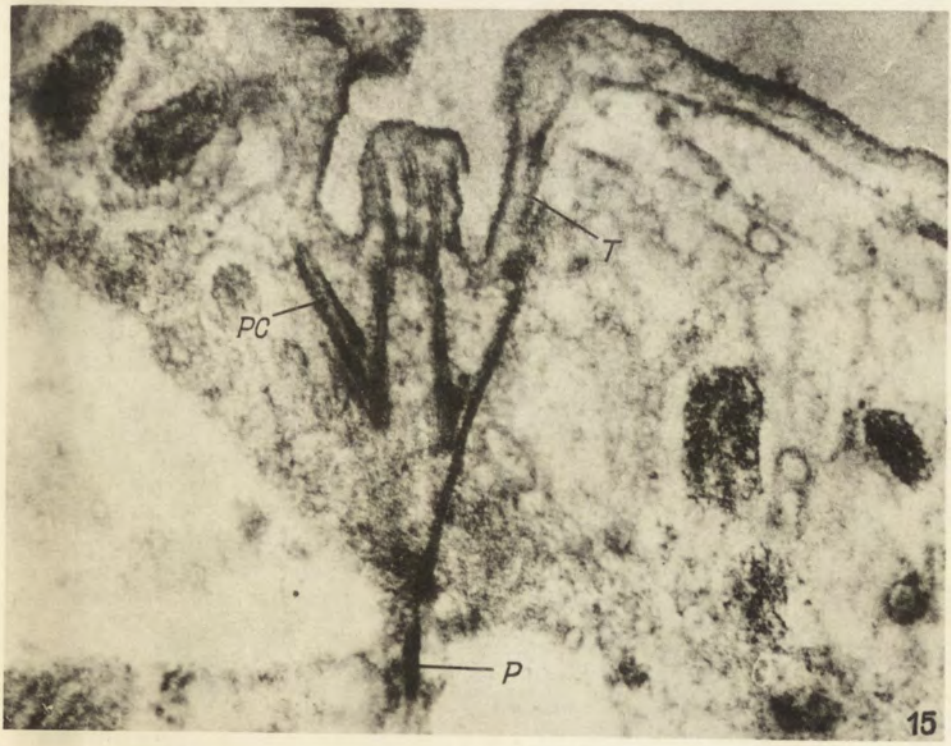

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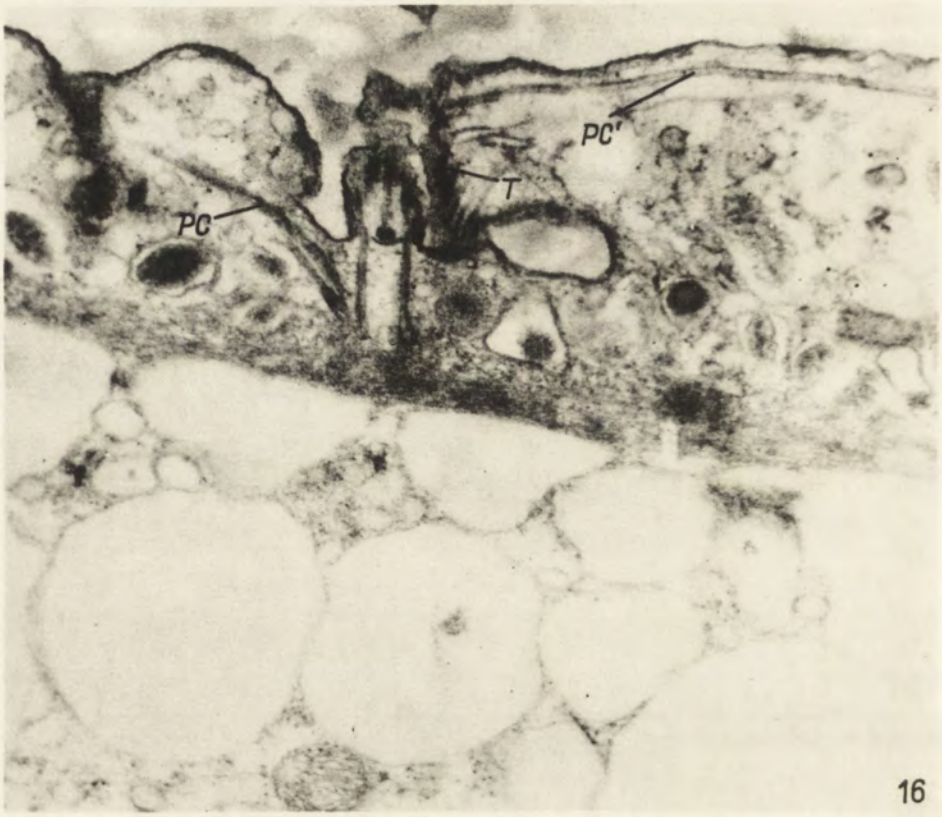

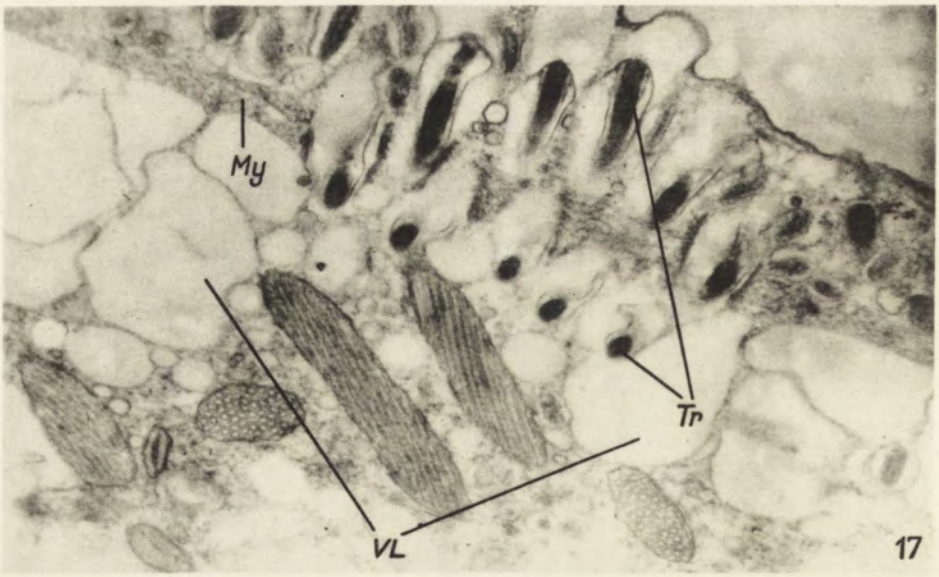

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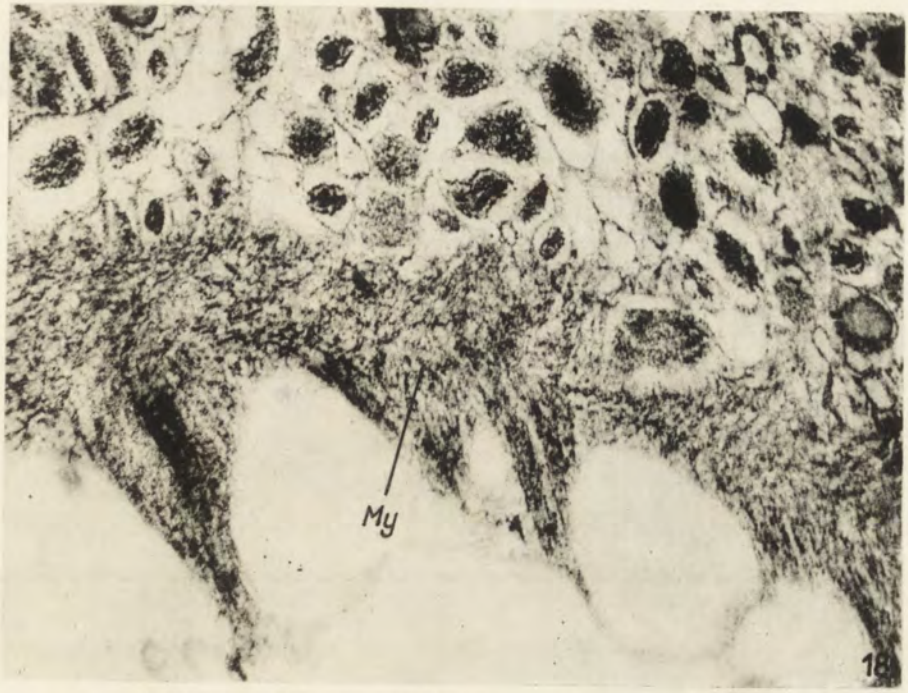

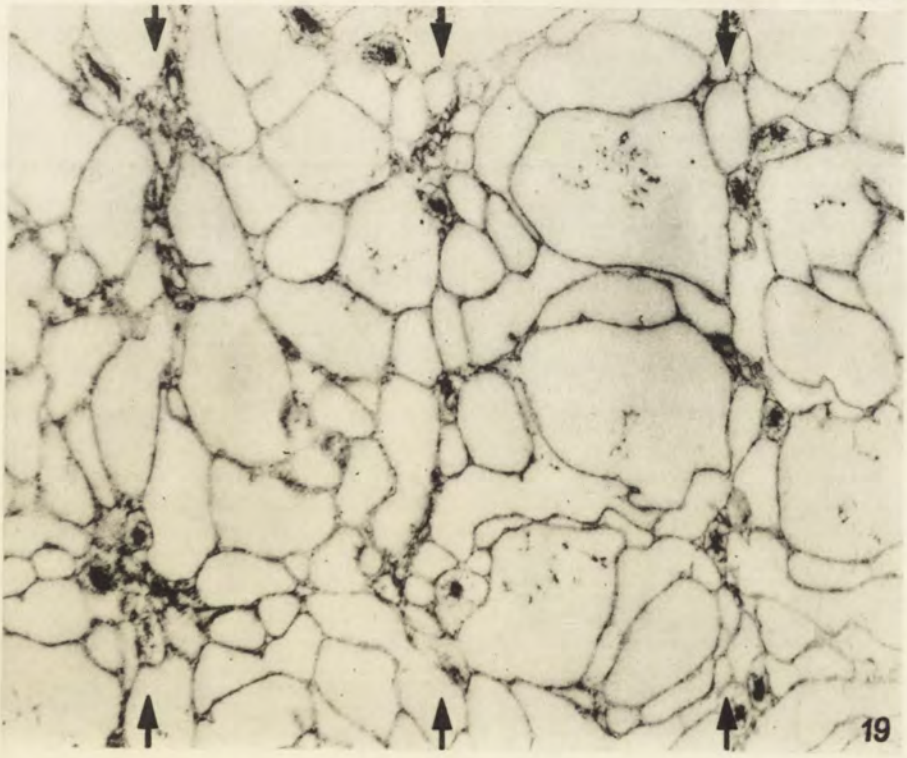

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The cytological sequence of events in the binary fission of *Didinium nasutum* (O. F. M.)[4]

La consécution cytologique des événements dans la fission binaire de *Didinium nasutum* (O. F. M.)

Recently renewed interest into the ultrastructural cytology and feeding of *Didinium nasutum* has resulted in a better appreciation of ultrastructure and function of protozoan prey ingestatory processes (Wessenberg and Antipa 1968, 1970). The visualization of the ingestatory process as studied with scanning electron microscopy was initially observed in our laboratory (Small and Marszałek 1969). A related analysis is presented here concerning the sequence of events observed via scanning electron microscopy of the normal binary fission of *Didinium nasutum*. Ciliogenesis follows a normal sequence whose totality may be expressed as a pattern of division. The pattern illustrates well the concept of morphogenetic gradients. Further, the retardation of ciliary outgrowth can be observed in specified areas. Polarity as well as a somewhat specialized assymetrical radial symmetry are obvious. This study further illustrates the utility of scanning electron microscopy for studying dynamic life processes like cell division.

Materials and methods

Didinium nasutum (O. F. Müller) was originally obtained from a commercial supplier (Carolina Biological Supply Company). They were maintained by feeding on *Paramecium multimicronucleatum* obtained from the same supplier. Following a period of 24–48 h after feeding, observation through a binocular dissecting microscope (×50) revealed cells with additional bands of ciliation which was presumptive that binary fission was taking place. All of the cells, whether in obvious division states or not, were quickly washed in millipore filtered culture fluid and subsequently fixed in Parducz' fixative for the initial steps of the Small–Marszalek fixation technique. The remainder of the steps of preparation have been described previously (Small and Marszalek 1969).

Freeze dried, coated specimens were observed in the Stereoscan Mark II a scanning electron

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microscope (Cambridge Instrument Company)[5]. The specimens were examined with a 20 KV accelerating voltage and a 100 μ final aperture. Working distances between 6–2 mm were employed for all magnifications recorded. The condensor lens was overpeaked by several major steps in order to obtain the smallest cross-sectional diameter spot size. This factor is of important in obtaining the necessary resolution for observing cytological structures and thus permitting the interpretation of biological events from static micrographs.

From a series of cells examined with the SEM a sequence of stages was readily established. An additional advantage of using SEM is that for the first time cell surfaces, other than those first examined from a single specimen, could also be observed by simply rotating the same specimen with a different tilt angle to the whole preparation. In this fashion cells could be observed in several different positions (i.e., anterior and posterior) to determine the extent, if any, that the division process had on the external organization of organelles visible with the SEM.

Results

The results of this study are summarized in Plates I–V and they will be discussed sequentially.

Plate I. Trophic cell morphology. Presented here are a series of figures to illustrate normal morphology of the cell not undergoing division for the sake of later comparisons that will be make. Pl. I 1 illustrates a whole cell with the prominent girdles of cilia as well as kinetally oriented but non-locomotory clavate cilia visible between the two girdles. The posterior girdle exhibits a metachronous arrangement of its cilia as previously demonstrated by Parducz. Of interest is the fact that the two girdles are not in a synchronous metachronous arrangement. The 300 fold increased depth of field at this magnification compared with similar magnification using photon optics systems is clearly demonstrable here. Pl. I 2 at a magnification of ×11 500 illustrates clearly that the girdles of cilia are single files, the posterior margins of which contain, even in the trophic cell, cilia not fully differentiated as locomotory organelles. On one side of the rotund cell two special compact fields of clavate cilia may be found (Pl. I 3). These fields are generally 5 kineties in width, the shortest row always to the viewer's left; the longest row always the fourth to the right. The length of the rows is not absolutely constant but the proportionate length of the rows appears to be relatively constant from one cell to another. If one tips posteriorly such a cell a few degrees, the proboscis region becomes clearly evident (Pl. I 4). The proboscis has no presumptive cytostomal depression, cleft, or ridge. The structural nature of the cortex underlying this anterior region has been worked out in detail earlier by Wessenberg and Antipa 1968, and the scanning micrograph supports the view that ribbons of microtubules course anteriorwards from each ciliated kinetal segment of the ciliated girdle. Also evident is the biased, angular

[5] The Cambridge Instrument was purchased with funds from the University of Illinois Research Board, and grants USPH FR 07030 and NSF GA 1239 (W. W. Hay, principal investigator). We thank Dr. B. V. Hall, Director, University of Illinois Center for Electron Microscopy, for use of the instrument housed in the Central Facility, and Mr. Leroy Dreyer for maintainance of the instrument. Mr. James Koepisch is also acknowledged for his help with preparation of photographic plates.

position of the anterior regions of each kinety that comprises a girdle. The same or similar cell when tipped posteriorly reveals the number and disposition of the expulsion vesicle pores (Pl. I 4). Apparent membranes occlude the oriface of some whereas others appear to be open, so that a double circular membrane is present surrounding the margins of these pores whose bases thus appear black. These pores are numerous, 14 in this single micrograph, and in other specimens have at times been found to occur as two sets (not illustrated). In either case they occupy a posterior polar position as evidenced by the linear ridges or kinetal arrangements leading to them. They are abutted by a large field of presumed clavate cilia which on close inspection are interposed between ridges. The external ridges are presumed to overly the underlying rows of microtubular ribbons. There is no evidence of a cytologically distinctive cytoproct in *Didinium*.

Plate II is a single figure of the earliest evidence we could find of impending division. Just anterior to the posterior ciliary girdle is a slight indentation that encircles the cell at this plane. We presume this to be the presumptive fission furrow since it is recurrent in all well fixed cells to the end of cytokinesis. In the anterior hemisphere, equidistant from the anterior parental girdle and the presumptive fission furrow are a row of regularly arranged, linear bumps whose position in accompanying later stages must represent the sites of ensuing ciliogenesis for the proter's posterior ciliary girdle. No such development can be seen in the posterior parental hemisphere.

The next stage is seen in Pl. III by a combination of a low magnification survey micrograph and the accompanying montage at four times the magnification (Pl. III 7, 8). By this stage ciliogenesis has begun in the anterior parental hemisphere (proter's) new girdle whereas in the posterior parental hemisphere (opisthe's region) only the regularly arranged presumptive sites of ciliogenesis are visible. Within the proter's developing girdle, differentiating cilia are longest in the anterior part of the girdle, and progressively decrease in length posteriad. To be also noted is the proximal position of the expulsion vesicle pores on this side which lacks the field of clavate cilia.

In Pl. IV two cells at low magnification are compared at approximately similar times in the sequence (Pl. IV 9, 12) with higher magnification views of the regions of active ciliogenesis. Note especially that they are of different sides of the cell surface since in only one the compact clavate field (and its development) are seen. As a continuation from the former stage in Pl. III ciliogenesis continues in the proter's posterior hemisphere so that in Pl. IV 10 four long cilia are now present within a single row. However, additional cilia, not fully developed, are present in the posterior portion of these rows. From left to right and vice versa, it is not possible to determine special length of any row. Indeed the whole process appears symmetrical. In a similar fashion the outgrowth of cilia in the opisthe's anterior girdle appears to follow a similar course, except that anterior to the longest differentiating cilium per row, a single shorter cilium is visible (Pl. IV 11).

For the developing clavate field in the presumptive posterior hemisphere of the developing opisthe, the field can be seen to be perfectly aligned with the parental opisthe field. To be especially noted, however, is that the outgrowth of cilia within the emerging presumptive opisthe's posterior girdle is inhibited or lagging when compared with the ciliary outgrowth on either side of the clavate field region.

As illustrated in Pl. V 14, 15 the outgrowth of cilia continues so that by the time some rows within the proter's posterior girdle possess ten cilia per row, a metachronous locomotory pattern is evident. Further evident in Pl. V 15 are intrakinetally placed pores, presumed to be sites of mucocysts. Here, as well as in the Pl. V 16 cell, the presumptive fission furrow is still evident although not much altered from the earlier stage in Pl. II.

By the time the new ciliary girdles of both proter and opisthe are complete, cytokinesis then ensues with some torsion (Pl. V 17). The clavate fields are no longer in direct anterior posterior line with one another. The depression to the viewer's left in the posterior hemisphere as well as in the anterior hemisphere are the sites in which the expulsion vesicle pore cluster may be found. Based on observations of protargol stained cells from the same culture (not included here), by this time micronuclear division is complete and macronuclear division is near completion. The basic cytoplasmic and karyoplasmic architecture (except for the opisthe proboscis) is nearly completed. The opisthe proboscis is the last structure to differentiate with the ultimate separation of the two filial products.

Discussion

Presented here in as few micrographs as possible is an interesting series of cells, frozen in the very act of dividing. The photographs (with all but Pl. I 1 and Pl. II excepted) are all contact prints so that the magnification, resolution and depth of field is that of the microscope and not of a photographic enlarger. The views allow one for the first time to successfully follow a sequence of ciliogenesis heretofore impossible without elaborate three dimensional reconstructions from serial sectioned material, the likes of which for *Didinium* studies have been nonexistent. Earlier studies of *Didinium* cytoarchitectural changes with division (Fauré-Frémiet 1945, Dragesco 1966) were unable to follow the course of ciliogenesis.

Ciliogenesis follows a very simple pattern or gradient of development: anterior ciliary differentiation precedes posterior both within a girdle and between the two developing girdles. No right to left or left to right gradient is apparent so, the gradient appears to symmetrically pass posteriorly except for its retardation in the regions of the compact clavate fields. If considered biochemically from the standpoint of lack of substrate availability, this observed retardation should be expected.

The external evidence of change is minimal to the pre-existent parental architecture as it passes through the period of transition to ultimately become redistributed to the two filial fission products. Our published observations confirm the fact that *Didinium nasutum* does feed during most stages of binary fission, so parental-proter cytostomal architecture persists in a manner similar to various suctoria, chaeniid and colepidid rhabdophorine gymnostomes but dissimilar to the loss of cytostome-cytopharynx noted in cytophorine gymnostomes, hymenostomes, scuticociliates, and perhaps many other related groups.

The alteration via selective regression of the parental ciliature of the two parental ciliary girdles appears to remain unaltered as they become the two separate anterior girdles for both proter and opisthe. For those micrographs of dividing cells which we have thus far examined, no visible changes are detectable.

The early appearance of a presumptive fission furrow that persists without external evidence of a change perhaps signifies a multitude of internal re-organizational changes taking place. Certainly the complete development of a proboscis is not a simple task when one considers the morphological details of its internal organization. The whole development including both sites of origin and growth of the proboscis microtubule systems is unknown. To be sure, the proboscis itself is the last major organellar complex to differentiate following the completion of cytokinesis and separation of the two filial products. The torsion through which these cells pass as they are separating appears to be manifest in the spiral ridged anterior end of the cell and in the proboscis itself (see P. I 4).

The pores of the expulsion vesicle for this strain of *Didinium nasutum* do not appear directly posterior to the compact clavate field in either the trophic cells nor in the fissionts. In this respect our organisms of North American origin differ from those illustrated by Dragesco (1966, figs. 14–15).

The position of the pores to one side of the posterior apex of the cells would lead one to suspect perhaps, that a cytoproct could be found in the remaining space. Such is not the case, since the other posterior areas appear to possess the ridge pattern with unevenly distributed clavate cilia comparable to the remainder of the somatic cell surface anterior to the posterior ciliary girdle. Furthermore, the expulsion vesicle itself occupies the whole internal posterior region of the cell. The peculiar, specific *Didinium* diet is similar in many respects to the specificity of the diet of ciliate ingesting suctoria which also lack a cytoproct. In our estimation, based on the evidence at hand as well as twenty other posterior views of *Didinium*, no cytoproct exists.

The pores with respect to the two compact clavate fields do appear to give the cell an assymmetry, yet this appears to be superficial to the symmetrical anterior-posterior developmental gradient.

In conclusion, the course of externally visible cytological events may be summarized as follows:

Major events in sequence	Normal trophant morphology
1	Presumptive cytokinetic fission furrow In the anterior presumptive ciliary girdle of the proter
2a	Presumed kinetosome proliferation in anterior posterior linear sequence within all kineties
2b	anterior to posterior ciliary outgrowth
2c	metachrony in new ciliary girdle In the posterior presumptive ciliary girdle of the opisthe
2b	Presumed kinetosome proliferation in anterior posterior linear sequence within all kineties
2c	anterior to posterior ciliary outgrowth
2d	metachrony in new ciliary girdle
3	Cytokinesis
4	New cytostome-proboscis differentiation for the opisthe

The letter subscripts a, b, c, and d designate the total developmental sequence simultaneity, whereas the developmental sequence within an anlagen is set off by indentation of the series of events.

Summary

Didinium nasutum (O. F. M.) has been observed via scanning electron microscopy during binary fission. Organellogenesis proceeds in an orderly manner within a ciliary organelle. The observed sequence of events includes alignment of kinetosomes followed by the sprouting of cilia. The anteriormost cilia appear first. Their outgrowth is gradual. The opisthe anterior ciliary girdle forms slightly after that of the proter anterior girdle has been initiated. Visible evidence of a presumptive cytokinetic fission furrow appears very early in the sequence, although cytokinesis itself does not appear until after both new ciliary girdles have completely formed.

RÉSUMÉ

On a observé la division de *Didinium nasutum* (O. F. M.) en utilisant le «scanning» microscope électronique. La genèse des organelles ciliare s'accomplit d'une façon ordonnée. L'alignment des kinetosomes est suivi par la pousse des cils. Les cils les plus anterieures apparaissent les premier. Ils apparaissent d'une façon graduelle. La bande ciliaire antérieure de l'opisthe se forme un peu après que la bande anterieure de proter a éte initiée. La trace visible d'un sillon de division enformation apparait tres tôt bien que la cytokinese comme telle n'apparait pas avant que les deux nouvelles bandes ciliares ont été complètement formées.

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EXPLANATION OF PLATES I–V

Didinium nasutum (O. F. Müller)

Plate I. Trophic cell morphology

1: The whole cell viewed laterally. Note the metachronous pattern of the cilia in the posterior girdle. ×800. Original magnification ×500
2: The base of the anterior girdle in higher magnification. ×11 500
3: The compact clavate ciliary field, anterior hemisphere of cell. ×2590
4: The proboscis and a portion of the cell membrane system anterior to the anterior ciliary girdle. × 235
5: The posterior end of a cell. Note both open (black centres) and membrane bound closed expulsion vesicle pores. ×2170

Plate II. 6: Early division stage. ×600. Original magnification on the plate, ×500

Plate III. Slightly later early division stage

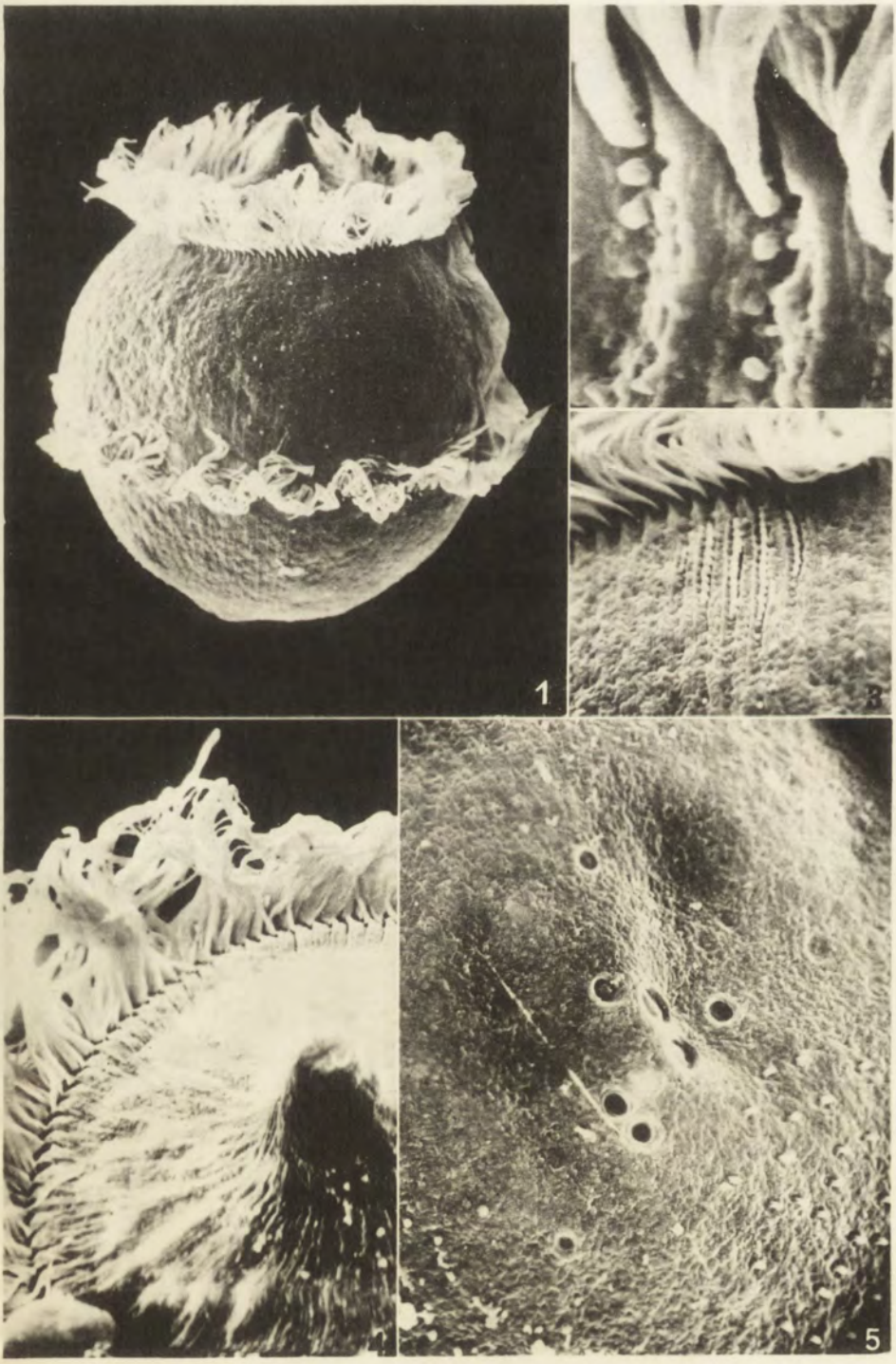
7: Whole cell, low magnification. ×510
8: Higher magnification, ×2040, to illustrate in detail the initiation of ciliogenesis in the anterior (proter's) new girdle and the alignment of presumed kinetosomes in the site at which the posterior (opisthe's) new girdle will appear.

Plate IV. Two cells in different view at slightly later time in the sequence

9: Whole cell, low magnification. ×578
10: Higher magnification of cell in IV 9. New ciliary girdle for the presumptive proter. ×5610
11: Higher magnification of cell in IV 9. New ciliary girdle for the presumptive opisthe. ×5610
12: Another whole cell at same stage but with the compact field of clavate cilia present. ×578
13: Enlargement of posterior region of the same cell as IV 12 to illustrate the development of the opisthe posterior compact field of clavate cilia. ×2210

Plate V.

14: Whole cell at later stage with metachronous waves now demonstrable in the new proter's posterior girdle. ×510
15: Higher magnification of same cell illustrating the exact number and single file organization of the cilia within the new girdle. ×2040
16: Whole cell at slightly later stage illustrating metachronous waves in both newly differentiated girdles and presence of the presumptive cytokinetic fission furrow. ×720
17: Later stage of whole cell in which cytokinesis is evident. ×425



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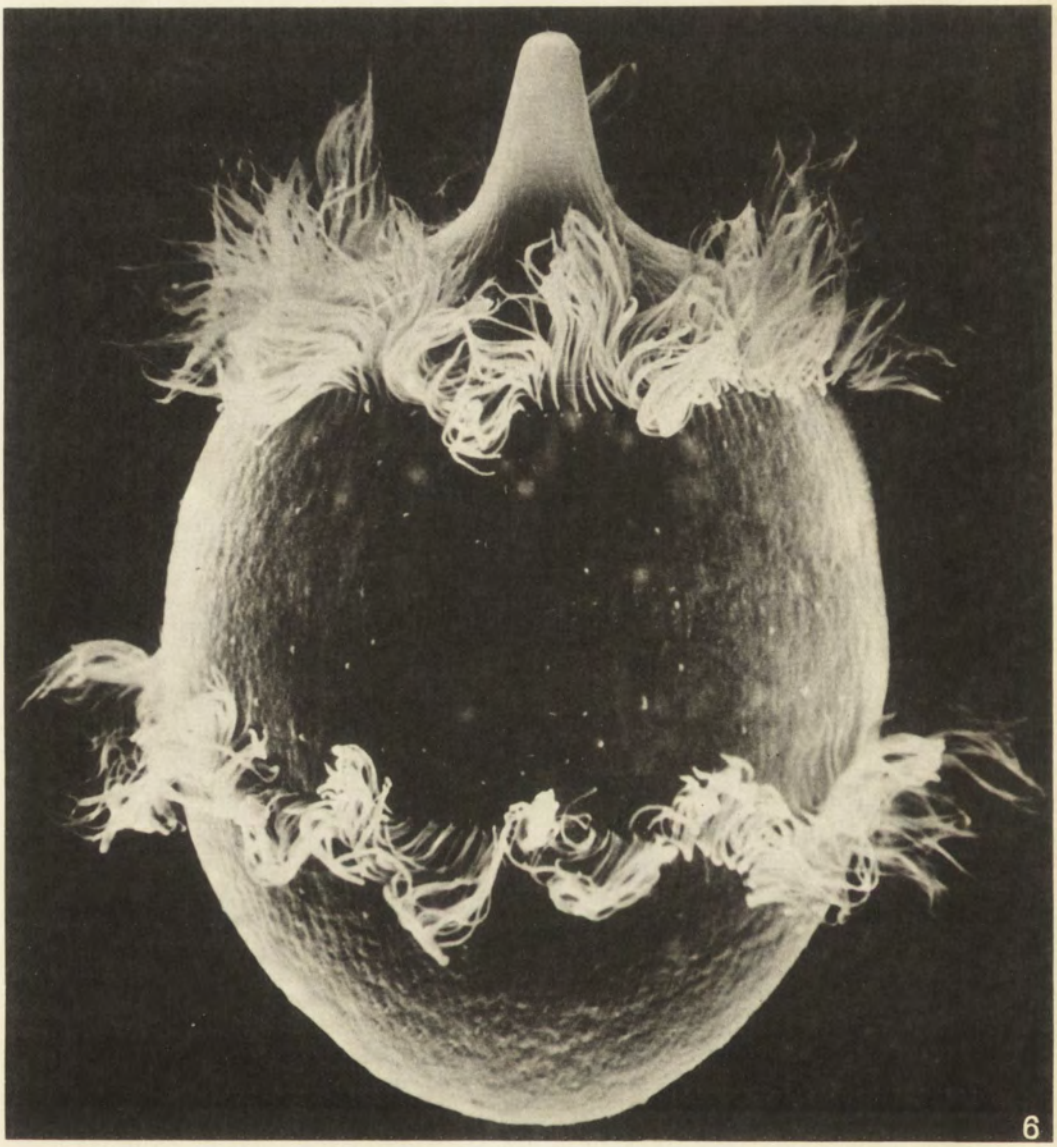

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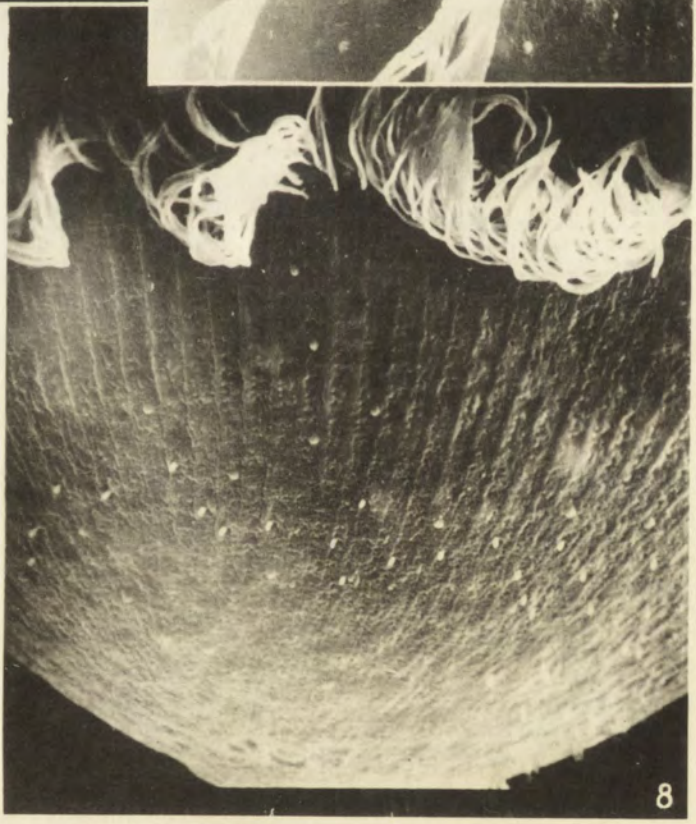

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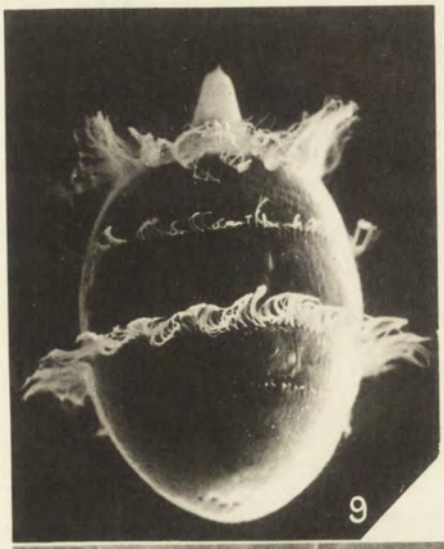

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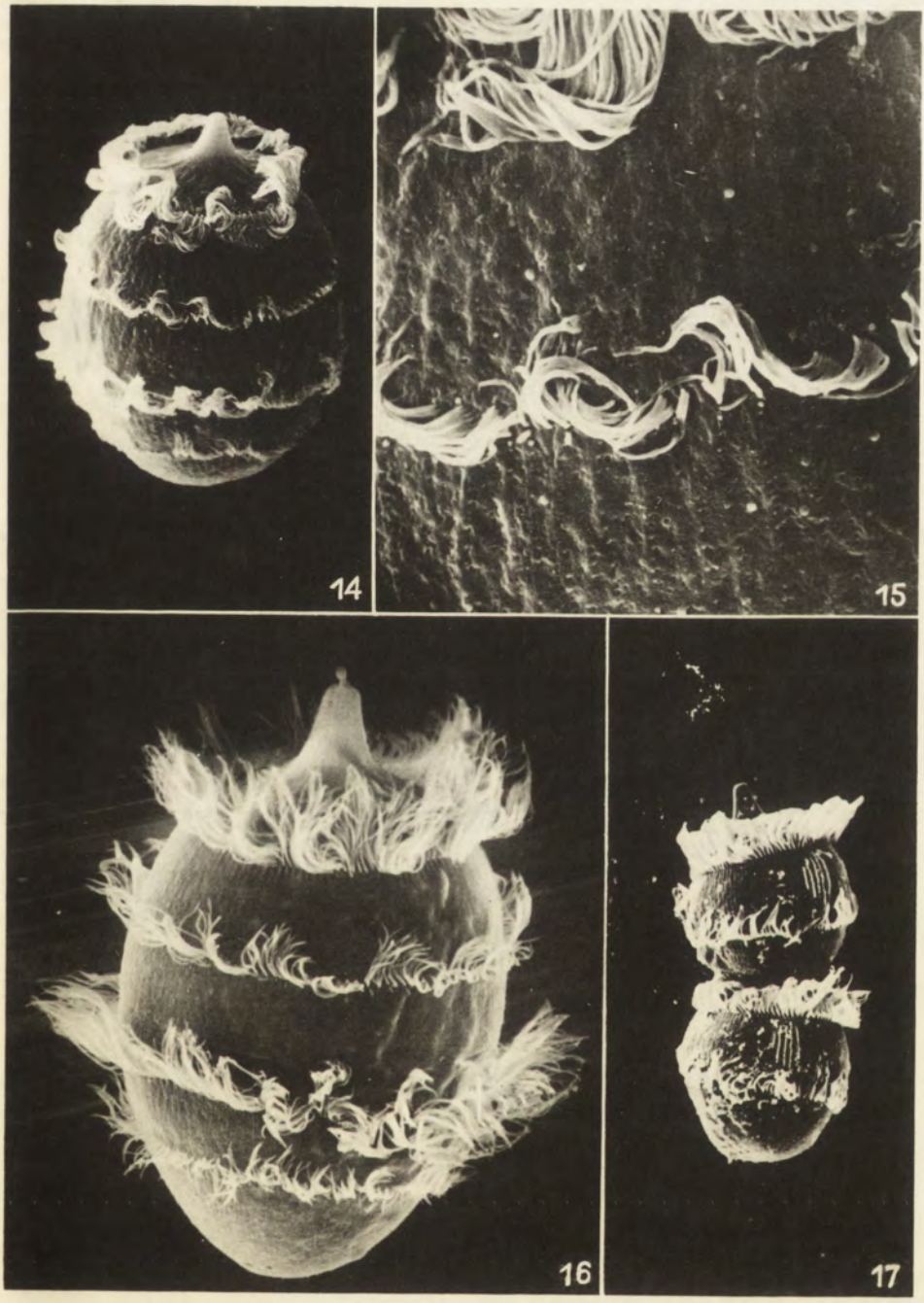

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Studies on stomatogenesis in *Dileptus* (*Ciliata*, *Holotricha*) in the course of division processes

Badania nad stomatogenezą *Dileptus* (*Ciliata*, *Holotricha*) w trakcie procesów podziałowych

The studies carried out previously on the morphology of *Dileptus* (Grain et Golińska 1969, Golińska 1970) have provided reasons to postulate that the ventral band of proboscis is a differentiated paracytostomal lip, a sort of protrusion of this lip, elongated and reaching the tip of proboscis. In favour of this speaks the fact that as well the ventral band as the lip around the cytostome are rimmed by non-ciliated kinetosomes, each of them giving a transverse fiber directed towards the cytostome.

The studies on stomatogenesis in the course of regeneration in *Dileptus cygnus* (Golińska et Grain 1969) permitted to establish the main stages of this process. As the first stage of the regeneration stomatogenesis, the resorption of the old ciliature should be considered. Later on the proliferation of kinetosomes follows and their immediate arrangement, at first in the region of the new proboscis, and then in the region of the paracytostomal lip.

The obtained results indicated that the ciliature around the cystostomal lip and the ventral band of proboscis are formed — at least in part — in the course of stomatogenesis. Consequently we have here to do most probably with a specialized oral ciliature. However, it was difficult to state what parts of the ciliature are produced from the new-arising kinetosomes. It was suggested that this concerns in the first place the non-ciliated kinetosomes. The aim of the present study is the precise determination of the ciliature cathegories which arise in the course of division of *Dileptus*.

Another problem in question concerns the apical position of the mouth in *Dileptus*. If the ventral band was differentiated peristomal lip, then the mouth of *Dileptus* would reach as far as the tip of proboscis. It might be compared in the best way to the mouth of *Spathidium* with its oblique course and a simultaneous apical position (Wenzel 1955). The fact that the process of division is initiated by formation of the primordium of the future proboscis in the dorsal side (Golińska

and Doroszewski 1964), speaks also in favour of the view that the mouth of *Dileptus* has an apical position. This becomes explicable when we assume that the new mouth of *Dileptus* begins to differentiate from its dorsal part i.e. from the tip of the new proboscis. The ciliates with the anterior localization of the mouth — as the majority of *Gymnostomata-Rhabdophorina* and some forms of *Trichostomata* — produce in division a paracytostomal ciliature on the periphery of the body directly below the division furrow (Fauré-Frémiet 1955, Grain 1964, 1966). This has been stated at least in the forms known from the scarce studies. If the paracytostomal ciliature of *Dileptus* arose in a similar way, it would provide evidence speaking in favour of the apical position of its mouth.

The last problem considered in the present study is the structure of the division furrow and structures or ultrastructures accompanying it.

Material and methods

For the present investigations, 3 species of *Dileptus* were used: *D. cygnus* (Clap. et Lachm.), *D. anatinus* Golińska and *D. visscheri* Dragesco. The methods of culture were described in the previous publications. For observations in the light microscope *D. cygnus* and *D. anatinus* were used. The dividing individuals of *D. cygnus* were stained by osmofication (Grain et Golińska 1969). The individuals of *D. anatinus* were stained with iron haematoxylin according to the Parducz's method (1952).

For the electron microscopic study all three species were used. Material was fixed with a solution of OsO_4 in phosphate buffer of pH 7.2. Material was embedded in Epon, section were contrasted with uranyl acetate followed by lead citrate according to the Reynolds (1963) procedure. The japanese JEM-7a microscope was used.

Results

The course of division process is essentialy the same in the three *Dileptus* species studied. The rate of division and the sequence of appearing structures are the same, differences were found only in the amount of the appearing elements.

The paracytostomal ciliature in *Dileptus* is very specialized and differentiated in different sectors. It may be divided into the following groups: the ciliature of the right side of the ventral band of proboscis, the ciliature of the left side of the ventral proboscis band, and the ciliature around the cytostome lip (Fig. 1). It has been stated that all the groups of the paracytostomal ciliature arise in division, in a close connection with the division furrow, on the periphery of the *Dileptus* cell.

A scheme of construction of the oral parts is represented in Fig. 1. The dotted area marks the places of the previous connection with proter. This area is surrounded on all sides by the paracytostomal ciliature, consequently in division all the parts of the oral apparatus are formed directly near the division furrow.

In the adult individual, near the right side of the ventral band of its proboscis, two rows of the so-called feeding cilia are present i.e. the right paracytostomal kinety, and a second row of cilia, parallel to it and with the same densification

of kinetosomes. A pair of kinetosomes of the paracytostomal kinety have a common transverse fiber on the inner non-ciliated kinetosome as well as another common postciliary fiber on the outer kinetosome with cilium. To the right, towards the dorsal side, rows of somatic cilia enter the proboscis, their kinetosomes being distributed much more rarely than in the rows of feeding cilia. The inner row of

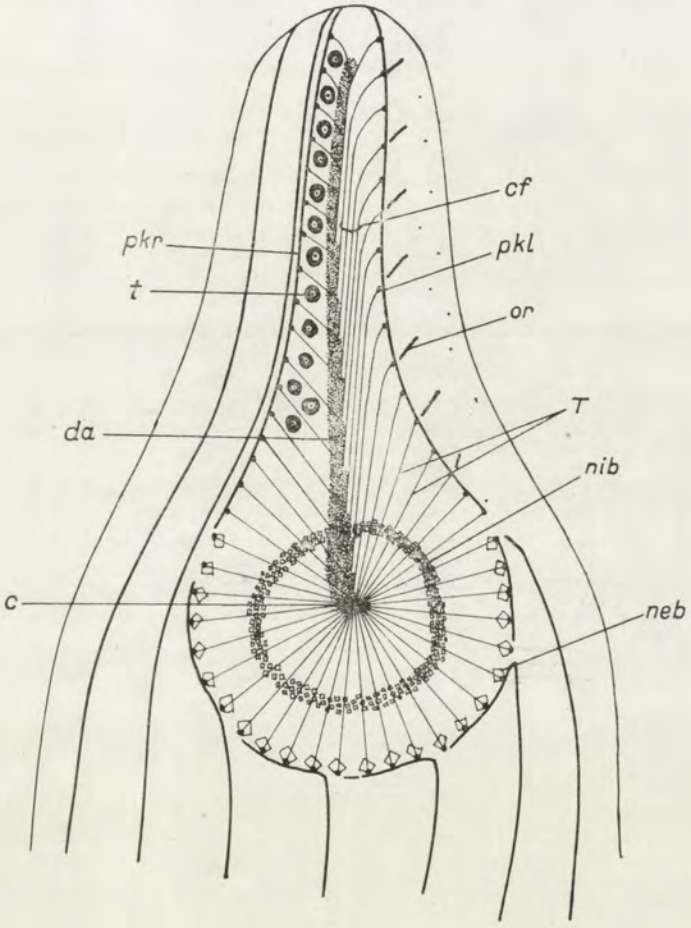

Fig. 1. Scheme of the buccal apparatus of *Dileptus*. Place of junction with proter in the former division is marked. da — dotted area, place of junction with proter in the proceeding division, c — cytostome, cf — central fiber, or — oblique row, t — toxicysts, T — transverse fiber, pkr — right paracytostomal kinety, pkl — left paracytostomal kinety, neb — nemadesma of external basket nib — nemadesma of internal basket

feeding cilia, which we call here the right paracytostomal kinety, is invisible in the light microscope. It is composed of a dense range of kinetosome pairs, its inner kinetosome being deprived of cilia and connected witht the transverse fiber.

The left side of the ventral band is provided with feeding cilia composed of the

left paracytostomal kinety as well as of short oblique ciliary rows. The left paracytostomal kinety is constructed like the mirror reflection of the right one, the transverse fibers running from the non-ciliated kinetosomes are directed toward the middle of the ventral band, they diverge and run toward the cytostome producing the central fiber of proboscis.

Around the cytostome lip runs a very dense row of non-ciliated kinetosomes connected with nemadesmae of the external basket and with the transversal fibers directed toward the cytostome. Those kinetosomes are bound into pairs with the neighbouring kinetosomes of the curved ventral rows, however, this connection seems to be less close than that in the pairs of paracytostomal kineties. The non-ciliated row around the lip presents possibly the continuation and junction of the left and right paracytostomal kineties. The inner basket of nemadesmae has no connections with kinetosomes in the adult individual.

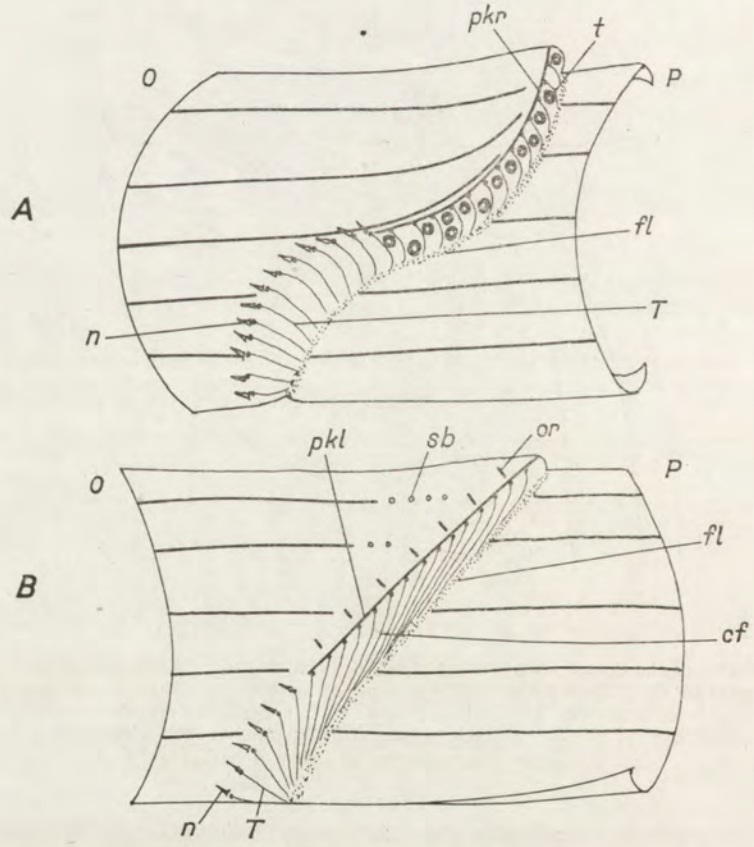

Fig. 2. Scheme of the course of division furrow in *Dileptus*. A — plain of the right side, B — plain of the left side, pkl — left paracytostomal kinety, pkr — right paracytostomal kinety, t — toxicysts, fl — fission line, sb — sensory bodies or — oblique row cf — central fiber n — nemadesma T — transverse fiber, P — proter, O — opisthe

The course of the furrow and the segments where every ciliature group arise are represented in Fig. 2. Remarkable is the oblique course of the furrow which was discussed in the previous paper (Golińska and Doroszewski 1964) and shifting of the primordium of the cytopharyngeal complex to the right side. This primordium mentioned previously as a small convexity on the ventral side of the animal, proved to be a convex band which passes on the edge directly into the primordia of the right and left sides of proboscis respectively.

The differentiation of primordia in division, similarly as in the process of regeneration (Golińska et Grain 1969) proceeds irregularly on the periphery of the cell. The process of differentiation proceeds from the dorsal to the ventral side on both lateral aspects of the animal. Consequently the successive development stages of pimordia may appear simultaneously — the younger one nearer the ventral and the older nearer the dorsal side.

Division furrow on the right side, primordium of the right side of the ventral band

Observation in the light microscope permit to distinguish the first stage of division as the so-called dorsal primordium (Golińska and Doroszewski 1964). It is a small convexity on the dorsal side, into which toxic trichocysts from endoplasm are included (Doroszewski and Golińska 1967). This primordium extends gradually towards the ventral side, the neighbouring kineties become initially curved (Pl. I 1) and then interrupted (Pl. I 2). In this area deprived of cilia, the paracytostomal kinety appears at once as a very dense row (Pl. I 2, 3). Evidently all its components are especially formed in the course of division. The next, equally dense, row of feeding cilia is constructed in division from the consecutive curved segments of kineties of the right side. The images obtained indicate that at the earlier stages, those curved kinety segments are formed from the rarely dispersed kinetosomes (Pl. I 3). At later stages they become as dense as in the paracytostomal kinety (Pl. I 4). It may be postulated that the equalization of kinetosome density in the row occurs by proliferation and inclusion of new kinetosomes between those existing previously. Between the paracytostomal kinety and the posterior segments of the proter kineties there is a stripe of the future ventral band of proboscis (Pl. I 5). Toxicysts are included into it since the very early stages of development.

The pictures gained in the electron microscope indicate that at the early development stages, the primordium is exclusively an ectoplasmic differentiation: the microfibrillar layer being a boundary between ecto- and endoplasm, fails even to bend below the distinct convexity which is produced by the primordium (Pl. II 6). This layer presents evidently not an obstacle for the toxic trichocysts passing into the primordium. In the very early primordia — at the stage of loosening and interruption of kineties observed in the light microscope — the convex primordium contains the structures which indicate the occurrence as well of resorption of ciliature as of proliferation of kinetosomes at this period. The pictures are identical with

those gained in the studies on regeneration (Golińska et Grain 1969). The process of resorption involves presumably pushing apart of kineties, the proliferation of kinetosomes concerns only the paracytostomal kinety.

When observing the arranging of two rows of feeding cilia, the electron microscopic images indicate that each of them is placed in its groove. The internal row i.e. the paracytostomal kinety shows at once its characteristic structure: the internal kinetosome is non-ciliated, transmitting its transverse fiber to the area of the ventral band between the trichocysts, and the external, neighbouring kinetosome with a cilium connected with the former. Growth and differentiation of the new-formed ciliature occur very quickly, the transitory stages have not been observed, neither has a sort of the anarchy field characteristic for the other ciliates. The process of differentiation follows immediately the process of proliferation although the neighbouring parts of primordium lying nearer the ventral side are less advanced, not ready. Initially the newly formed kinetosomes are not yet fixed with their bases in the microfibrillar layer (Pl. II 6). In the external row of feeding cilia, cases of proliferation may often be found, leading possibly to densification of this row. The orientation of kinetosomes in both rows of feeding cilia is the same. the transverse fibers leave it toward the same side (Pl. II 7).

At the early stages, the boundary between proter and opisthe is not marked by the presence of any structure. The kinetosomes of the posterior parts of proter reach as far as the margin of the ventral band. They stick however with their proximal ends in the microfibrillar layer, what indicates that they do not undergo proliferation. In the later primordia, the microfibrillar layer becomes interrupted under the arising band. Beneath the primordium, under the territory of opisthe a densification of microfibrills is formed, surrounded by single-walled vesicles. Similar structures exist under the advanced primordia of the left side of the proboscis band as well as under the primordium of the cytopharyngeal complex (Pl. VI 21, 22). On the territory of proter, no swellings of the microfibrillar layer are observed in the place of its rupture. Such condensation of microfibrils, connected also with vesicles, was found in the regenerating fragments however at much earlier stages of stomotogenesis, namely during the rupture of the microfibrillar layer, prior to proliferation (Golińska et Grain 1969).

Furrow on the left side, primordium of the left side of the ventral band of proboscis

The pictures gained in light microscope, of formation of feeding cilia on the left side of proboscis are in their initial phases identical with those gained for the right side. Rupture and spreading of ciliary rows (Po. III 8) are here also observed, as well as formation of swelling which constitutes the primordium of the left side the ventral band and appearing of the paracytostomal kinety in the form of a very dense row of cilia (Pl. III 9). The difference consists in the lack of inclusion of toxic

trichocysts into the primordium, and a different manner of formation and arrangement the remaining feeding cilia.

Around the paracytostomal kinety, in the course of division, numerous cilia appear, dispersed at random (Pl. III 9) which may be formed anew or originate from the ends of somatic kineties. At the later stages of development, those kinetosomes become arranged into short rows perpendicular to the paracytostomal kinety (Pl. III 10). In the course of the final formation of proboscis they assume an oblique position. In such a row, the number of kinetosomes is mostly in harmony with the number characteristic for the give species or sometimes exceeds it slightly. Sometimes between proter and opisthe a thin undulating line (Pl. III 9) is visible which represents most probably the primordium of the central fiber of proboscis i.e. the boundary of the left side of ventral band.

The pictures gained in the electron microscope indicate that in this case, the early primordium occupies only the territory of ectoplasm (Pl. IV 14), the microfibrillar layer limiting the endoplasm becomes interrupted in the later stages of primordium differentiation (Pl. VI 22). Appearing of the paracytostomal kinety is — of course — preceded by resorption and proliferation of ciliature. All the kinetosomes of this kinety seem to be formed anew in the course of stomatogenesis. The transversal fibers leaving this kinety bend near the anterior edge of opisthe in central fiber of the future proboscis. Between the bundles of transverse fibers no toxic trichocysts occur, only the mucine trichocysts are numerous.

Possibly the kinetosomes of the future oblique rows — at least a part of them — are formed in the division process. It is interesting that the initial orientation of kinetosomes of the future oblique rows differs from that of the kinetosomal pairs of paracytostomal kinety: the transversal fibers leave them in opposite directions (Pl. IV 12). Since in the mature individuals the kinetosomes of all this part of feeding cilia are directed in the same manner (Pl. IV 11), the change of orientation of kinetosomes of the oblique rows should occur in stomatogenesis. It has not been stated however whether this change takes place in the course of arrangement of kinetosomes into rows or during the change of orientation of those rows from the perpendicular to the oblique ones.

Right and ventral part of the furrow — structure of primordia of the cytopharyngeal complex

In the light microscope, very little information may be gained concerning the formation of primordia of paracytostomal structures. Staining with haematoxylin reveals a clear stripe with no cilia on the ventral side and on a part of the right side (Pl. V 18) which joins into a full circle both primordia of the ventral band. This stripe forms an insignificant convexity and seems not to be limited by any ciliary system. At this stage, bending of the ventral rows does not appear, they simply interrupt on the boundary of the cilialess field. The changes of shape of the primordium

from the cilialess stripe into the circular paracytostomal field proceeds parallel with the constriction of the division furrow and seems to be in a close connection with this process. Nemadesmae of the double basket may be observed only in very late primordia when it has become a final double basket (Pl. V 17).

In electron microscope, consecutive images of formation of the paracytostomal structures may be observed. In the region of cilialess stripe, the first stages of the primordia formation look identically as it has been previously described for primordia: resorption of ciliature, then formation of new kinetosomes, which are cilialess and transmitting a long transverse fiber in the direction of proter. Similarly as in the adult individual (Grain et Golińska 1969) the paracytostomal kinety is so distinct around the cytostome as it is around the ventral band. Cilialess kinetosomes of this part of the mouth are formed in the course of division but have no distinct union into pair with the neighbouring ciliated kinetosomes. Those ciliated kinetosomes, if they have proliferated in the course of stomatogenesis, this should occur very late or bending of the ventral rows occurs at those later stage.

The cilialess kinetosomes present a row — invisible in the light microscope — being the posterior margin of the cilialess stripe. Presumably they are a continuation and junction between the right and left paracytostomal kineties. Besides the transverse fibers, the non-ciliated kinetosomes produce also the nemadesmae of pharyngeal baskets, similarly as in the course of regeneration (Golińska et Grain 1969).

In the early stages, the non-ciliated kinetosomes are connected with rather thin nemadesmae (Pl. V 15) which are separated from kinetosomes and shifted forwards, initiating the formation of the internal basket. At the next stage, possibly near the same kinetosomes arise thick nemadesmae of the external basket. They do not separate from kinetosomes (Pl. V 16). Both processes take place prior to the constriction of the division furrow. The final effect of this stage is the formation of a double palisade of nemadesmae: one of its arrays at the base of the non-ciliated band, the other approximately at its middle. At the boundary with the proter, the ends of the transversal fibers are present, they meet concentrically at the subsequent stages and enter the future cytostome. Those later stages, associated with the constriction of furrow, are simultaneously connected with the closure of both palisades producing the internal and external basket of the cytopharyngeal complex.

Subsequent differentiation of mouth, development of the division furrow

Formation of the division furrow sets on at the moment when virtually all the parts of the paracytostomal ciliature have been differentiated. At this moment, *Dileptus* is the widest in its equatorial part, and — in *Dileptus cygnus* — begins the condensation of macronucleus (Golińska 1965). In the course of cytokinesis a small groove which separates the cytostomal primordia from the proter territory becomes deeper. Any special differentiations which might involve the constriction of the furrow have not been stated. The development of furrow, similarly as the

development of primordia proceeds very irregularly at the periphery of the *Dileptus* cell, being always most advanced on the dorsal side. The constriction of the furrow on the dorsal side is accompanied by coalescence of both primordia of the ventral band (Pl. VI 20). An allometric growth of the separated part of the new proboscis should be simultaneously expected, since it is always longer that it would result after a simple cutting off by the furrow. The boundary between the right and left side of the ventral band of the new proboscis runs initially in the groove. A trace of such groove may be found sometimes in the interphaseal individuals (Pl. IV 13).

In the course of separation of the new proboscis by the furrow, a field of sensory bodies arises leftwards of its tip (Pl. VI 19). Presumably they are formed by transformation of normal cilia, although the possibility of their formation in the course of division cannot be excluded. In electron microscope, the sensory bodies of opisthe are identical with those of proter: microtubules of cilia are always dispersed at random (Grain et Golińska 1969, Golińska et Grain 1969). In proportion as the proboscis develops, all its sensory bodies appear on its surface.

Since the division furrow is most advanced in its development on the dorsal side of the animal body, the place of separation of proter from opisthe occurs nearer the ventral side, mostly on the territory of the paracytostomal lip, sometimes at the base of the ventral band of proboscis.

Proliferation of kinetosomes

Proliferation of kinetosomes occurs most possibly in the course of the whole life cycle of *Dileptus*. As mentioned above, an intense proliferation on the territory of the primordia of the paracytostomal structures may be observed in the course of division. On the remaining body regions of the dividing *Dileptus* — except the mouth of proter — proliferation of kinetosomes may also be observed. This process seems to take place at quite different places of the cell and is not very intense. The duplication of the somatic kinety number in *Dileptus* takes place most possibly prior to appearing of cytostomal primordia, because this is always accompanied by a very dense disposition of somatic kinetosomes.

In the interphaseal individuals of *Dileptus*, pictures of proliferation near the external nemadesma basket were found (Pl. VII 24) as well as among the somatic ciliature (Pl. VII 23).

The pictures of kinetosomal proliferation in electron microscope indicate that this process takes always place in ectoplasm — the old kinetosomes being raised considerably over the microfibrillar layer (Pl. VII 23, 26). At the base of old kinetosomes, some new ones are formed, they shift then towards the surface, grow and organize fibers. It has not been stated whether there exist cellular regions in which the proliferation of kinetosomes would occur more frequently then in the others. Possibly the new kinetosomes are incorporated between the old ones more or less regularly on the whole body surface.

Consequently all the cathegories of ciliature are formed in division, however this may occur in interphase, as well presumably at a lower degree. A high capability of *Dileptus* for changes of length and thickness of proboscis in interphase should be stressed as well as the possibility of necessary changes in the number of oral kinetosomes of all the cathegories. This follows from the capability mentioned above.

Discussion

The aim of the studies on stomatogenesis in *Dileptus* has concerned three problems as mentioned in the introduction: 1. should the ciliature around the mouth of *Dileptus* be considered as the oral ciliature; 2. is the position of mouth of *Dileptus* really apical ? and 3. what is the structure of the region of the division furrow?

It is generally accepted that the feature of the oral ciliature — besides the complexity of its structure — is its formation connected with the non-ciliated structures of the mouth. In favour of the existence in *Dileptus* of a specialized oral ciliature speaks the fact that it is formed in the course of division in connection with the nemadesma baskets, and that its formation is indispensable for arising of the pharyngeal structures. As it has been described previously, the formation of this ciliature especially in the course of regeneration (Golińska et Grain 1969) and the high degree of complexity of structure of this ciliature (Grain et Golińska 1969) strongly support the postulation put forward. On the other hand, significant differences may be found between the character of the oral ciliature of other ciliates and the parapharyngeal ciliature of *Dileptus*. E.g. the oral primordia of *Stentor* are sufficient for the formation of a limited number of membranells. This number fails to increase in interphase (de Terra 1966). More so, the increase of the oral apparatus is preceded by reorganization (Schwartz 1935) which consists in formation of new primordia. *Dileptus* forms its oral ciliature gradually, the repeated elements of the paracytostomal ciliature arise in the course of elongation of the new proboscis, keeping the same densification of the kinetosomes of the paracytostomal kinety. Since in the moment of fission of the progeny, the proboscis of opisthe is much shorter than that of proter, consequently we should expect the possibility of shift of proliferation of the paracytostomal ciliature far to the period of interphase. Unfortunately, it was not possible to ascertain whether in the time of elongation of proboscis new structural elements i.e. pairs of kinetosomes with transverse fibers are formed at different places along the proboscis or only on both sides of the base of the ventral band. The ciliature around the cytostomal lip may also undergo proliferation, as it has been stated in the course of the present study. Consequently it may be presumed that even the number of nemadesmae of the external basket may increase. It is doubtfull that the number of nemadesmae of the internal basket might undergo changes in the course of interphase.

The possibility of proliferation of kinetosomes during interphase has been stated for the somatic ciliature of ciliates of different groups (Evans and Corliss 1964,

Fauré-Frémiet 1948) although sometimes it is doubled in the course of division (Porter 1960, Williams and Scherbaum 1959). Proliferation of the somatic ciliature may also occur only in the region of the furrow, in the course of division (Roque 1957). As it has been stated, the somatic ciliature may augment by new kinetosomes as well at interphase as at division. This process is characterized by a low intensity, and possibly may occur at different places of the cell. Unfortunately it is not known whether there exists a phase in the life cycle when the proliferation of oral as well as somatic ciliature would be impossible. Now it should be stated that the difference between the oral and somatic ciliature in *Dileptus* consists mostly in the fact that the oral ciliature undergoes a highly intensified proliferation in the time of stomatogenesis.

As to the problem of the apical position of mouth in *Dileptus*, the kineties called here paracytostomal should be discussed first. Their name has been introduced for stressing their resemblance to the paraoral kinety of other ciliates, without suggesting the identicity of those two structures. The pictures gained in the study of division permit to imagine the structure of the region surrounding the ventral proboscis band as follows: the paracytostomal kinety is only single, being bend on the tip of proboscis in such manner that the transverse fibers are always directed toward the cytostome. In the course of stomatogenesis, the whole kinety is oriented according to the system of the right side whereas on the left side it penetrates from the dorsal side between proter and opisthe, preserving its system of kinetosomal pairs and their fibers. The other parts of feeding cilia are formed presumably in a close connection with the somatic ciliature as well on the right as on the left side. The fact, that a change takes place in the orientation of the kinetostomes of the oblique rows from the "somatic" into the "oral" kinetosomes is highly interesting and unusual in the morphogenetic processes.

It was often stressed in the description of stomatogenesis of *Dileptus* that all the mouth parts arise closely beneath the division furrow and their development and differentiation seem to be connected with the development of the furrow. The formation of the new ciliature under the furrow and on the periphery of the whole body was also stated in some *Gymnostomata* (Grain 1966) and *Trichostomata* (de Puytorac et Grain 1965, Fauré-Frémiet 1955, Wolska 1965). In all those ciliates the mouth is situated apically. In favour of the apical position of mouth in *Dileptus* speaks the fact that in division all the elements of the paracytostomal ciliature form a full circle under the division furrow. It seems possible to assume the ventral band as a differentiated part of the paracytostomal lip and the *Dileptus* mouth itself — as situated apically.

Some features of stomatogenesis — similar as in *Dileptus* — may be found in *Nassula*. So the basket nemadesmae arise in *Nassula* (Tucker 1970) in connection with the kinetosomes of the paraoral kinety, initially arranged into one row, they shift to produce a basket at later stages. In *Dileptus*, nemadesmae are formed in connection with the kinetosomes being continuation of the paracytostomal kinety,

they are initially arrayed to form a fence, and detach themselves from kinetosomes to form the internal pharyngeal basket. Besides, the formation of baskets themselves occurs in *Dileptus* on the ventral side as well, although on a very long band.

The formation of division furrow in *Dileptus* seems to be tightly connected with the development of the oral primordia. There are however no special structures which might be responsible for cytokinesis. The numerous data on the ultrastructure of the division furrow concern also *Metazoa*. Presently the concept of "contractile ring" put forward by Marsland and Landau 1954 seems to be the most adequate. This hypothesis is supported by detection of microfibrillar rings under the furrow of segmenting eggs. The function of constricting the furrow was ascribed to those structures (Szollosi 1968, Tilney and Marsland 1969). Mercer and Wolpert 1958 found below the furrow a swelling of the layer composed of fine granular material and of membranous tubules, they ascribed however to this structure no function in constricting the furrow. If really this role may be performed by the microfibrillar material, so in *Dileptus* the constriction of the furrow would be the function of the ecto- and endoplasmic layer. However special swellings of this layer directly under the furrow were not found in the course of division. Such a swelling is formed beneath the primordium. Perhaps in connection with the stiff transverse fibers coming here from the furrow, it might perform a role in the cytokinesis of *Dileptus*. This postulation may be verified by an experimental analysis of division process. For this kind of study *Dileptus* presents a very convenient material.

Summary

The studies of stomatogenesis in division of *Dileptus cygnus* (Clap. et Lachm.), *D. anatinus* Golińska and D. *visscheri* Dragesco proved that all the cathegories of ciliature around the cytostomal lip and the ventral band of proboscis are formed in the course of division and arise directly beneath the division furrow on the periphery of the ciliate body. No special differentiations were revealed to which the role in constricting the furrow might be ascribed. The problem of existence of the oral ciliature and the type of structure of *Dileptus* are discussed.

STRESZCZENIE

Badania nad stomatogenezą podziałową gatunków *Dileptus Cygnus* (Clap. et Lachm.). *D. anatinus* Golińska i *D. visscheri* wykazały, że wszystkie kategorie orzęsienia wokół wargi cytostomu i bandy wentralnej proboscis są wytwarzane w trakcie podziału i powstają bezpośrednio poniżej bruzdy podziałowej na obwodzie ciała orzęska. Nie wykryto specjalnych zróżnicowań, którym możnaby przypisać zaciskanie bruzdy. Autorka dyskutuje zagadnienie istnienia orzęsienia oralnego i typu budowy *Dileptus*.

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EXPLANATION OF PLATES I–VII

Ciliature round the division furrow on the right side of *Dileptus cygnus*, seen in light microscope on osmified preparations

1: Dorsal primordium (a), neighbouring cilia rows become bent
2: Kineties are interrupted, the right paracytostomal kinety is marked between them (pkr)
3: Right paracytostomal kinety (pkr) is distinctly seen, onset of formation of the second row of feeding cilia from the bent segments of lateral rows (fc)
4: Density of array of kinetosomes is the same in the right paracytostomal kinety (pkr) as in the second row of feeding cilia (fc)
5: Same stage as in 4, the intensely stained toxic trichocysts (t) occupy the space between the internal row of feeding cilia (pkr) and the posterior part of proter (P)

Division primordia on the right side of *Dileptus cygnus*. Electron microscope photogram

6: Early primordium, a pair of kinetosomes are seen: a ciliated and non-ciliated of the right paracytostomal kinety (pkr) and a part of kinetosome of the second row of feeding cilia (fc). Microfibrillar endo-ectoplasmic layer (mf) is at a distance from the bases of kinetosomes. Toxic trichocysts stained dark (t). ×11 600
7: Slightly later stage oblique section. Transverse fibers (T) leave the non-ciliated kinetosome of paracytostomal kinety in the same direction as the kinety of the neighbouring row of feeding cilia. ×18 000

Ciliature around the division furrow of the left side of *Dileptus cygnus* seen in light microscope on osmified preparations

8: Rupture of kineties and formation of non-ciliated band.
9: Disorderly arrangment of kinetosomes near the left paracytostomal kinety (pkl), a trace of the central fiber of future proboscis (cf)
10: Disposition of kinetosomes into short "membranelles" (or) near the left paracytostomal kinety (pkl)

Electron microscopic images of the division primordia on the left side of *Dileptus*

11: Array of kinetosomes on the left proboscis side of an adult individual of *D. visscheri*, transverse fiber (T) is directed to the same side near the pair of kinetosomes of the paracytostomal kinety (pkl) as near one of kinetosomes of the oblique row. Central fiber (cf) and toxic trichocysts (t) are visible. ×20100
12: Array of kinetosomes in primordium of the left proboscis side of *D. visscheri*. Transverse fiber (T) of the pair of kinetosomes of paracytostomal kinety is directed to the opposite side to that of neighbouring kinetosomes. ×17 000
13: High magnification of the central fiber of *D.* cygnus. In the pellicle, over the fiber a groove is seen, presumably a trace of union of both parts of the ventral band of proboscis. ×50 000
14: Central fiber (cf) in the proboscis primordium of *D. visscheri*. Microfibrillar layer (mf) is between the primordium and endoplasm. ×24 000

Division primordium of cytopharyngeal complex

15: Early mouth primordium of *D. anatinus*, comprising only one row of nemadesmae (n) presumably designated for the internal basket. Electron microscope. ×26 000
16: Late mouth primordium of *D. visscheri*, the non-patent cytostome forms a slight convexity (c), both rows of nemadesmae are seen: this of the external basket (en) and of the internal one (in). Electron microscope ×20 000
17: Light microscope photograms representing the mouth primordium of *D. anatinus*. Upper arrow indicates the nemadesmae of internal basket, the lower — of the external
18: Bright horizontal band is seen, formed by primordium of the non-ciliated cytopharyngeal complex on the ventral side of *D. anatinus*

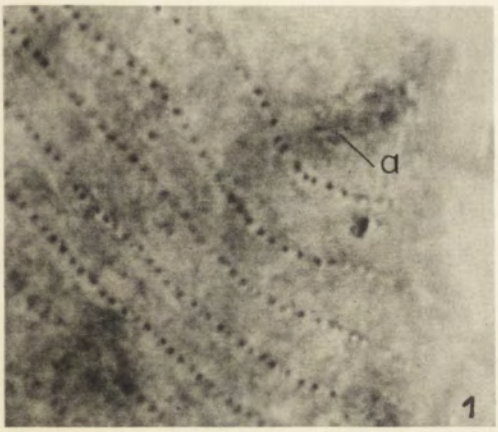
Differentiation of primordia, coalescence of parts of proboscis and change in microfibrillar layer

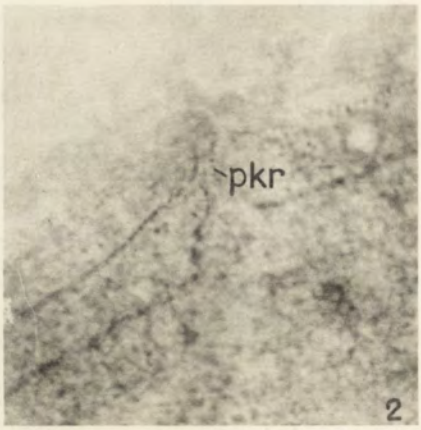
19: Dorsal side of *D. anatinus*. The formed part of the ventral band of proboscis is straightened (vb), on its left side sensory bodies (sb) are arrayed on the prolongation of the normal cilia rows
20: The straighthened part of the newly formed ventral band of proboscis in *D. anatinus* is seen
21: Rupture of the microfibrillar layer beneath the mouth of *D. anatinus*. (n) — nemadesme of the external basket of mouth (mf) — forms a big ball in the region of opisthe beneath the place of rupture. The ball is accompanied by numerous smooth vesicles. Electron microscope ×12 000

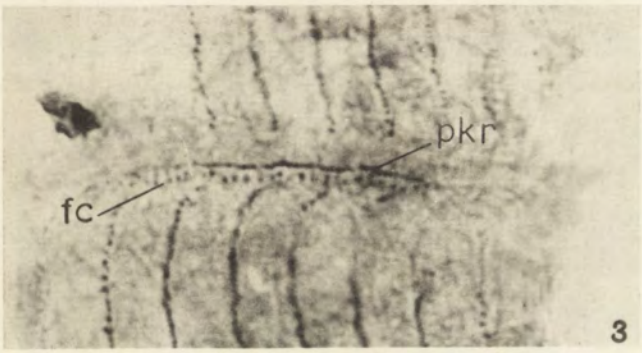
22: Analogical ball of microfibrils beneath the primordium of left side of proboscis of *D. anatinus*. Smooth vesicles are also seen. Electron microscope ×22 000

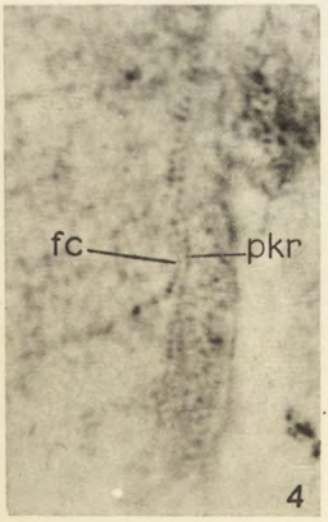
Pictures of kinetosomes proliferation in the cell of *D. anatinus*

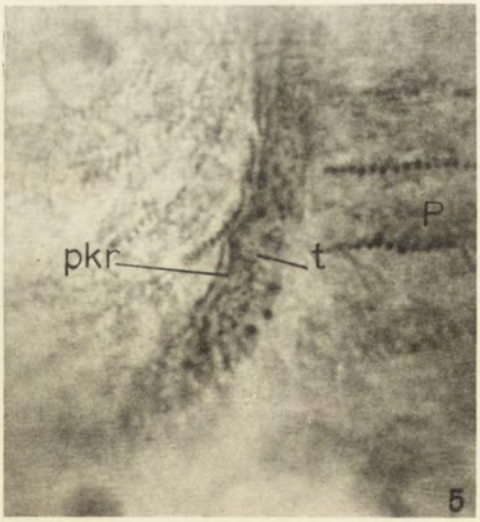
23: Proliferation of somatic ciliature in the course of interphase. New kinetosome (k) placed in relation to the old one in position of centriole ×24 700
24: Proliferation at the external basket in the course of interphase. New kinetosome is shorter than the old one and placed at an angle (n) nemadesmae ×40 900
25: Proliferation of somatic ciliature in the course of division. New kinetosome near the kinetodesma of the old kinetosome, no accompanying fibers ×22 000
26: Same as in 25. New kinetosome near the microfibrillar layer (mf), the old one is above ×24 600











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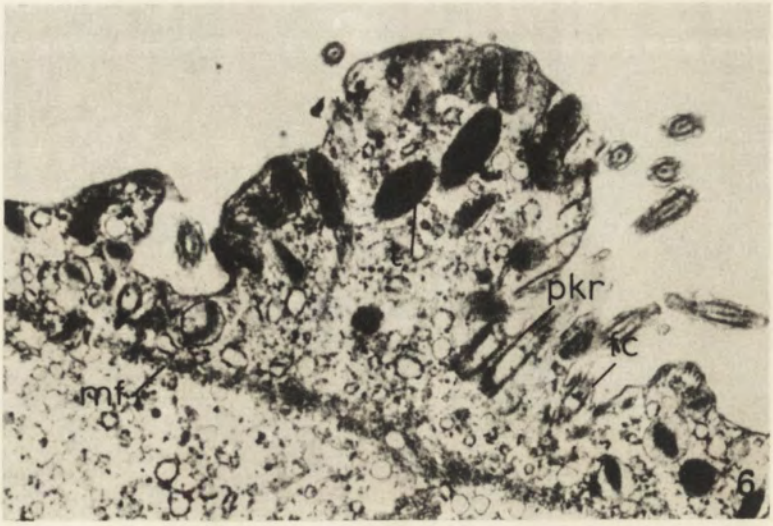

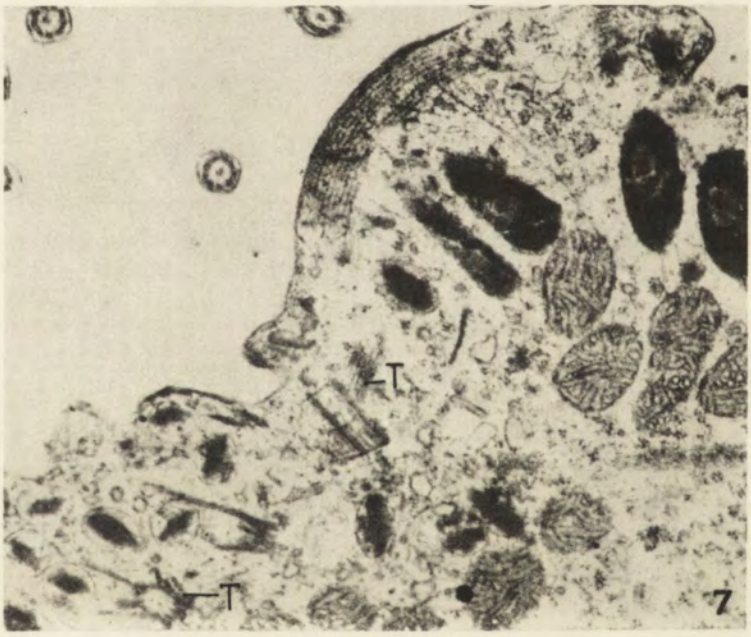

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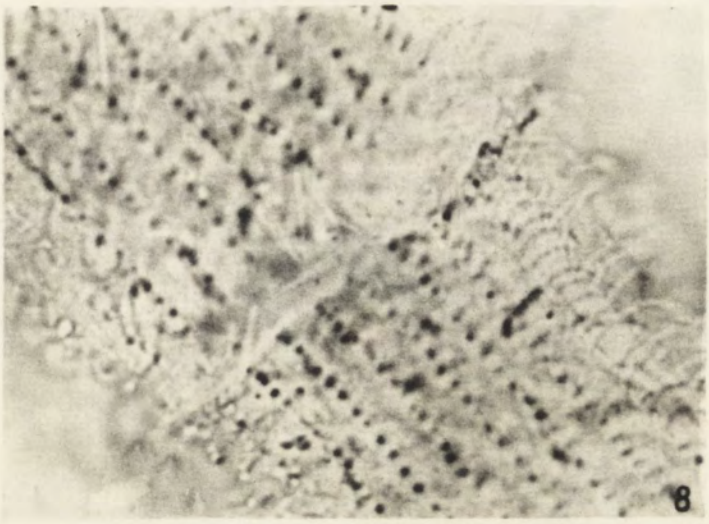

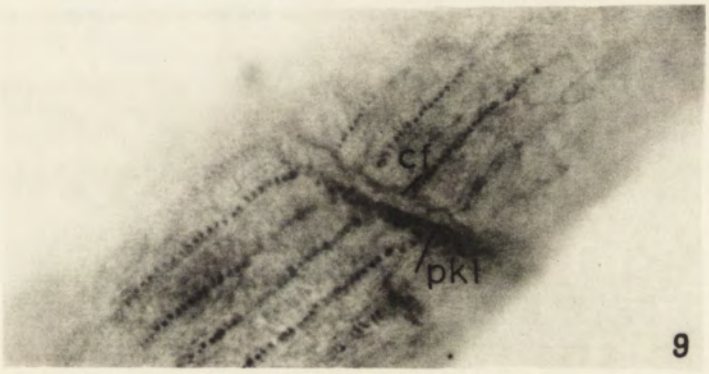

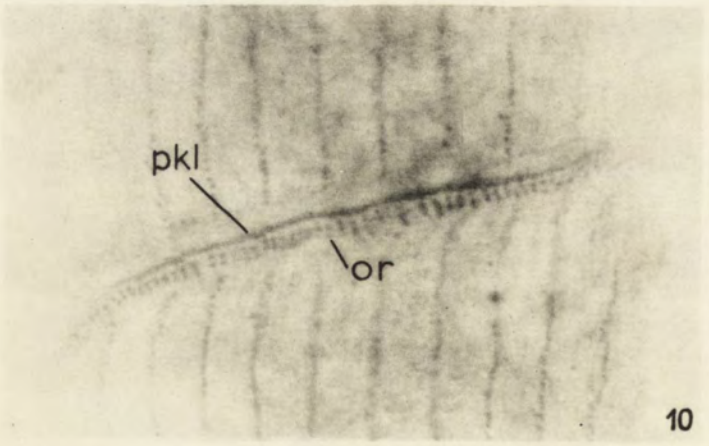

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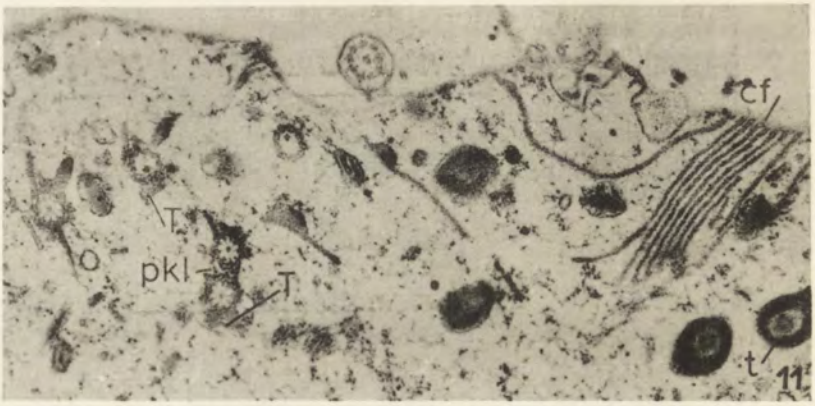

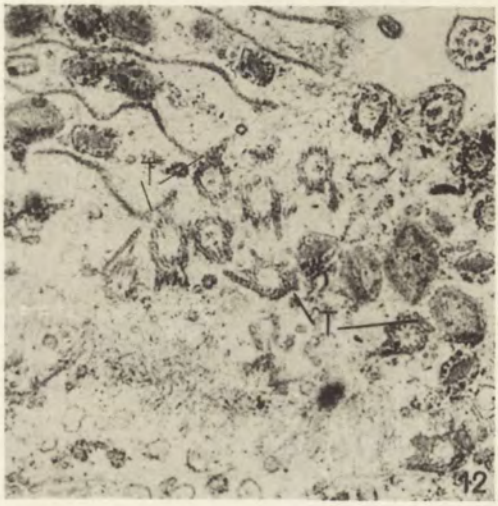

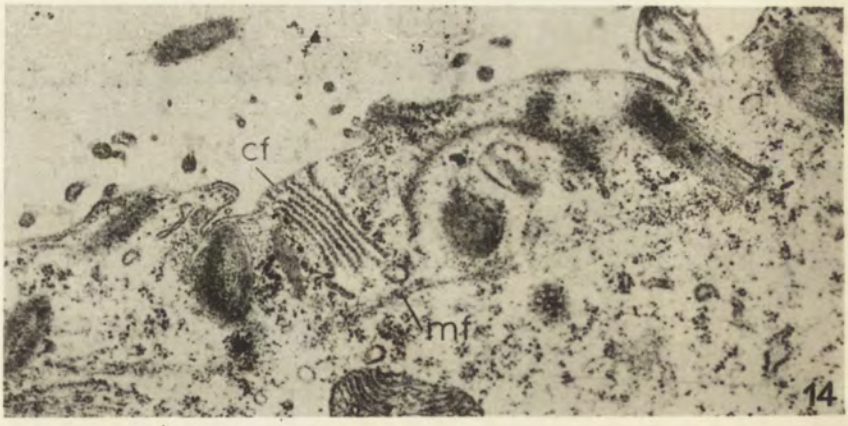

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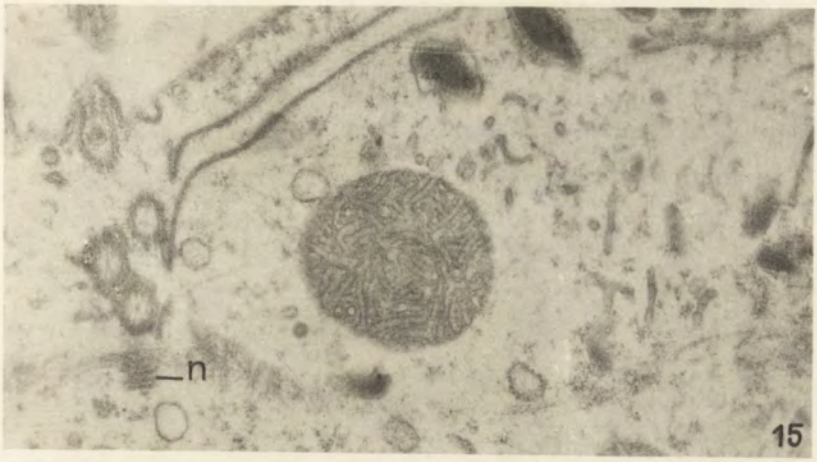

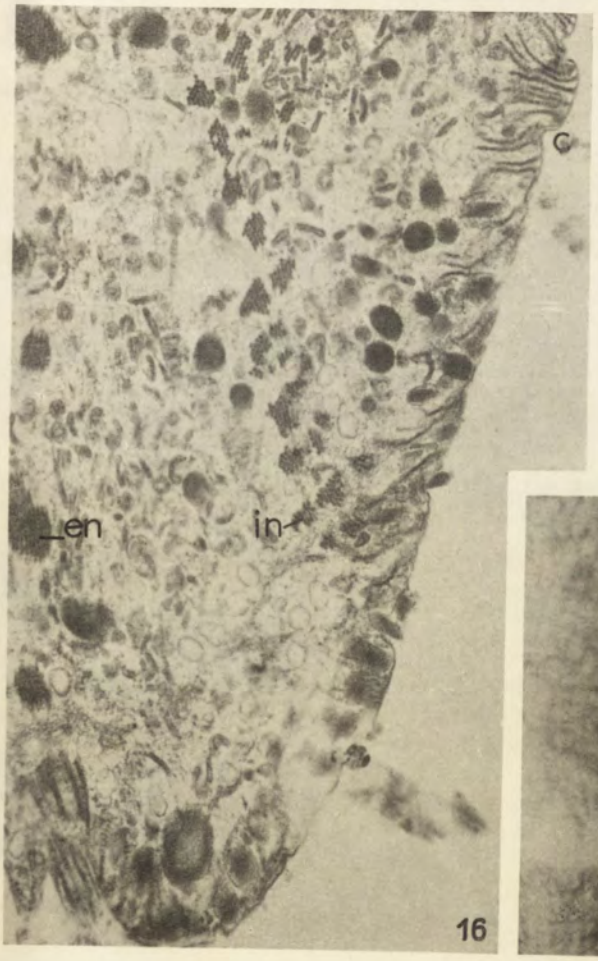

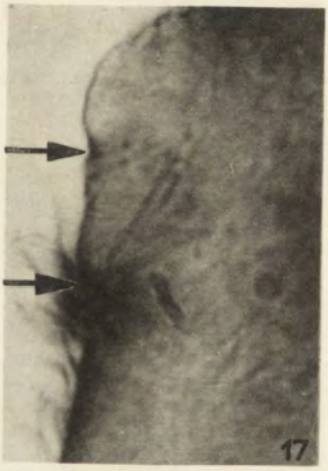

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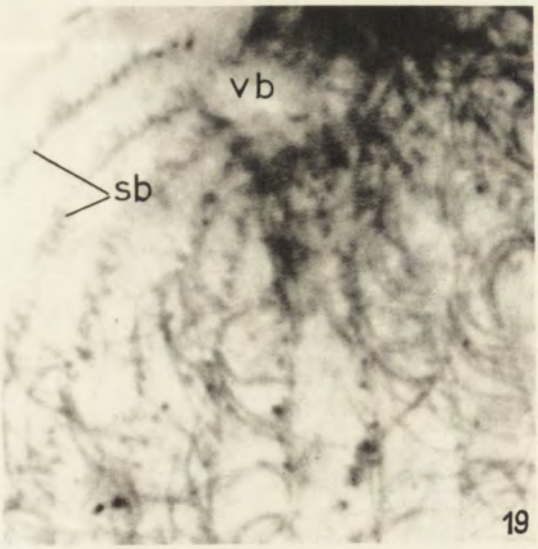

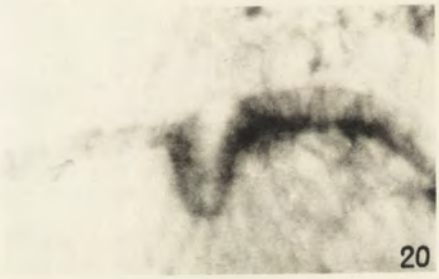

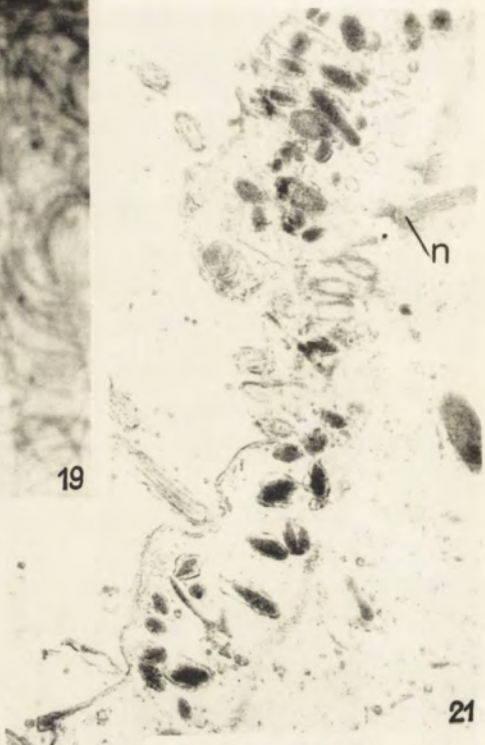

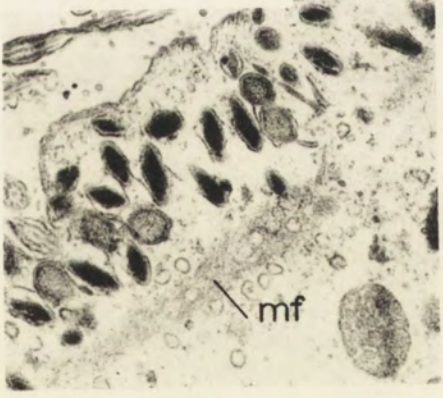

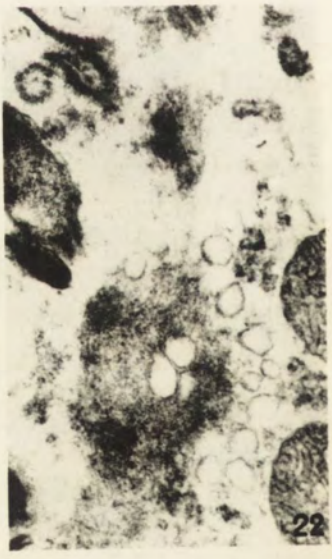

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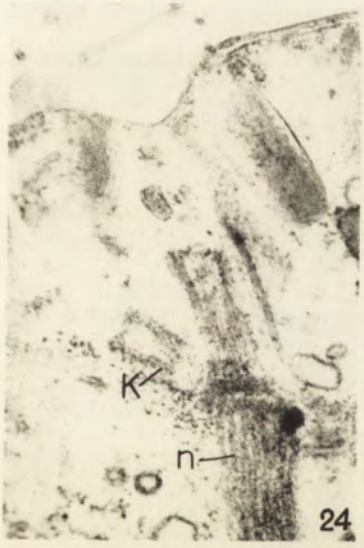

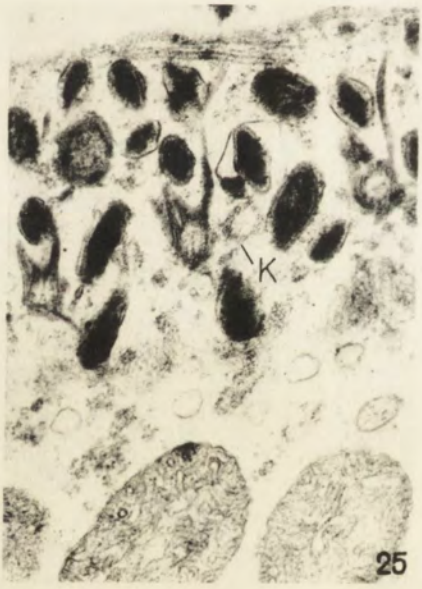

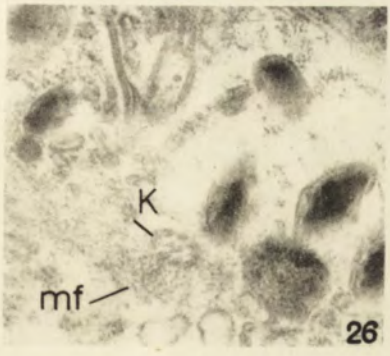

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Cultivation of chicken coccidia in chick embryos. II. Experiments with *Eimeria brunetti*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. acervulina* and *E. maxima*

Культивирование кокцидий кур на куриных эмбрионах. II. Опыты с *E. brunetti*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. acervulina* и *E. maxima*

In our previous papers (Shibalova 1968, Shibalova et al. 1969, Shibalova and Korolev 1970) it was reported that *Eimeria tenella* completed its endogenous development, with production of oocysts, in the chorioallantoic membrane (C. A. M.) of chick embryos following the inoculation of the allantoic cavity with sporozoites obtained in vitro. No development was obtained following oocyst or spore inoculation of chick embryo.

Long 1965, 1966 successfully cultivated on chick embryos four of six chicken coccidia examined (*E. tenella*, *E. brunetti*, *E. mivati* and *E. necatrix*). The other two species — *E. acervulina* and *E. maxima* — failed to develop under similar conditions. Of the four species whose development was followed in chick embryo, only the former three completed both asexual and sexual phases of the life cycle, whereas the growth of *E. necatrix* was confined to asexual development only.

The present paper gives the results of attempt to infect embryos with *Eimeria brunetti*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. acervulina* and *E. maxima*.

Material and methods

Local strains of eimerian oocysts used in the experiments are maintained at the Laboratory of Protozoology of the Institute of Poultry Diseases in Leningrad by Dr. A. A. Taldrik. These were obtained by inoculation of coccidia-free chickens with single oocysts of either species — *E. brunetti*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. acervulina* and *E. maxima*, resp. Methods of excystation of sporulated oocysts, preparation of the inoculum and embryo inoculation have been described in detail in our previous paper (Shibalova and Korolev 1970).

8–9 day old chick embryos of Russkaya Belaya Cross-288 strains were used in the experiments. Two kinds of inoculum were prepared: the one consisting of sporulated oocysts or spores, in doses between 10 000 and 60 000, and the other containing sporozoites, in doses between 100 000 and 800 000. The total volume of the fluid injected never exceeded 0.2–0.25 ml, each inoculum being accompanied with 2000 i.u. of penicillin and streptomicin injection.

The injected embryos were examined every 24 h throughout the experiment. The C. A. M., allantoic fluid and various organs and tissues of embryos were examined microscopically with a subsequent fixation of the material in the Stiva fluid and 10% formol; paraffin sections were stained with haematoxylin-eosin stain.

Results

Sufficient numbers of viable sporozoites of *E. brunetti*, *E. necatrix* and *E. praecox* were obtained within the first hour, rarely 1.5 h following excystation procedure. Sporozoites of *E. mitis*, *E. acervulina* and *E. maxima* were most numerous a little later, however, never exceeding 3 h after the excystation started.

Only the in vitro-hatched sporozoites appeared to be suitable inoculum. Attempts to induce the development of the parasite by the introduction of sporulated oocysts or spores of either species into the allantoic cavity of embryos were unsuccessful.

Experiments with *Eimeria brunetti*

The whole life-cycle of *E. brunetti* was completed in 8 day old embryos. Doses of inoculated sporozoites varied from 100 000 to 250 000. The most severe infection of embryos occurred when 200 000–250 000 sporozoites were given. Three of twenty embryos inoculated with 250 000 sporozoites died within 4–5 days after inoculation. Parasites found in the rest 17 embryos developed normally and corresponded morphologically to those in vitro growing stages of *E. brunetti*.

Trophozoites were traced at 36–48 h. First- and second-generation schizonts were seen within 68–80 and 92–144 h, resp., macrogametes and microgametocytes first appeared at 120 h. Oocysts could be found 144, 168 and 192 h following inoculation, with the peak being reached on 7 and 8 day. All the stages of endogenous development of *E. brunetti* are shown on Pl. I 1–5. The oocysts produced in the C. A. M. and chorioallantoic fluid sporulated normally and being fed to sensitive host induced brunetti-coccidiosis.

Experiments with *Eimeria necatrix*

E. necatrix sporozoites completed the life-cycle in the C. A. M. of 8–9 day old embryos. Doses between 50 000 and 100 000 sporozoites gave poor infections, better results were obtained with inoculations of 200 000–250 000 sporozoites. Higher doses, between 500 000 and 800 000, resulted in deaths of embryos.

In the sporozoite-injected embryos one-nuclear schizonts were first seen in the C. A. M. at 48 h; multinuclear and segmented first-generation schizonts were seen at 72–96 h, second-generation schizonts at 120 h. Sexual stages and oocysts were seen on day 6, 7 and 8. Oocysts appeared in increasing numbers on day 7, 8 and even 9 (Pl. II 6–10). Oocysts recovered from the allantoic cavity sporulated normally and produced characteristic infection when inoculated into 2 week old chickens.

Experiments with *Eimeria praecox*

The whole endogenous development of the parasite was traced in 8–9 day old embryos inoculated with 150 000–250 000 sporozoites of *E. praecox*. The intensity of infection varied with the inoculum size.

Non-mature schizonts-trophozoites appeared as early as at 24 h (Pl. III 11). Simultaneously, non-developed sporozoites were also seen. Schizonts, morphologically similar to first-generation schizonts of *E. praecox*, were found at 48 h (Pl. III 12), and those similar to second-generation schizonts at 80–120 h (Pl. III 13). At this time, numerous free merozoites released from the latter could be seen in allantoic fluid.

Sexual generations of *E. praecox* were seen 120 h following inoculation (Pl. III 14,15). The first oocysts appeared on the 6th day, their number increasing by the 7–8 day after inoculation, persisting up to the 9th day. The oocysts sporulated normally and produced coccidian lesions when fed to coccidia-free chickens.

Experiments with *Eimeria mitis*

Sporozoites of *E. mitis* in doses between 150 000 and 250 000 were introduced into the allantoic cavity of embryos aged 8 days. The starting development of the sporozoites in the C. A. M. was observed 36–48 h after inoculation. One- and multinuclear schizonts, morphologically similar to corresponding stages, in vivo, were seen within 60–120 h. Sexual stages appeared at 120–140 h. Single oocysts were first seen at 114 h and then increased in number on the next 2–3 days. Photomicrographs of shizogonous and sexual stages developed in the C. A. M. are shown in Pl. IV 16–20.

Oocysts recovered from the C. A. M. underwent normal development and produced characteristic mitis-infection when inoculated into susceptible host — 2 week old chickens.

Experiments with *Eimeria acervulina* and *E. maxima*

Sporozoites of either species (doses between 100 000 and 800 000) were inoculated into embryos aged 8–9 days. A total of 80 embryos used in the experiments were examined during 12 days (up to the time of hatching), after inoculation. No infection was recorded. No parasites were seen either in the C. A. M., or in amniotic membranes and other sites of the embryo.

The infectivity of the material for inoculation (oocysts, spores and sporozoites) was examined by infecting 2-week-old chickens with positive results. The latter displayed characteristic acervulina- and maxima-coccidioses, responsibly.

Discussion

In previous communications (Long 1965, 1966, Shibalova 1968, Shibalova et al. 1969, Shibalova and Korolev 1970) it was shown that sporozoites but not spores (sporocysts) or oocysts of *Eimeria* of the fowl appeared to be the only suitable material for infecting chick embryos. Long 1966 reported that sporocysts could also infect the embryo, although the infection was detected with difficulty.

At present the total of eight species of *Eimeria* of chicken have been studied for their ability to develop in the chorioallantois of the chick embryo: *E. tenella*, *E. brunetti*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. mivati*, *E. acervulina* and *E. maxima*. Of these only the two last mentioned species failed to develop outside the host body, i.e. in chick embryo. Long 1966 suggested that the two species (*E. acervulina* and *E. maxima*) which are unable to develop in the caeca are also unable to grow in the chorioallantois, and vice versa. This was a curious suggestion that seemed likely to be true, since the other four species examined by that author (*E. tenella*, *E. brunetti*, *E. mivati* and *E. necatrix*) have been shown experimentally to complete their endogenous cycle in the caeca of chicken (Horton-Smith and Long 1965, 1966). The fact that *E. necatrix* in Long's experiments failed to develop beyond late schizogony did not make his idea less interesting. The ability of this species to complete its sexual development in the C. A. M. of chick embryo has been demonstrated in our experiments.

It in of interest to touch upon these experiments, since the results obtained somewhat differ from those reported earlier by Long 1966. Our local strain of *E. necatrix* completed its whole life-cycle in the C. A. M. of chick embryo. The oocysts recovered from embryos sporulated normally and produced specific infections when fed to susceptible host. We are inclined to think that the failure Long experienced in attempts to induce the whole life-cycle of this parasite probably was due to some events involving strain peculiarities. In this respect, it would be of much interest to exchange strains of *Eimeria* between our two laboratories.

Working with *E. tenella*, *E. brunetti* and *E. mivati* Long 1966 found sometimes remarkable delay in the duration of developmental stages of the life-cycle. Unlike in our experiments, only slight delay was followed in the life-cycles of *Eimeria* examined. This difference can also be put down to strain divergence of the species examined.

The unability of *E. acervulina* and *E. maxima* to develop in chick embryo remains still an open question. Our experiments with *E. mitis* and *E. praecox* — two other species earlier not investigated whose natural endogenous development in the chicken does not involve caecal localization (Joyner 1958, Tyzzer et al. 1932) — demonstrated their fair growth in the C. A. M. of chick embryo with production of oocysts. Thus, the suggested relation between caecal localization (even partial) of the parasite in the host and its embryo development may coincide not always. Nevertheless it

is true that the in vivo adaptation of the parasite may involve these peculiar physiological, biochemical, nutritional and other requirements that could or could not be satisfied during embryo development.

Summary

Attempts were made to infect chicken embryos with six local strains of *Eimeria* — *E. brunetti*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. maxima* and *E. acervulina*. Sporulated oocysts or spores of the above *Eimeria* inoculated into the chorioallantois of embryos failed to initiate coccidian development.

Sporozoites of four species (*E. praecox*, *E. brunetti*, *E. necatrix* and *E. mitis*) were able to complete their whole endogeneous cycle in the chorioallantoic membranes. Oocysts that developed in the infected embryos sporulated normally and induced infections in 15 day old chickens.

Under similar conditions, sporozoites of *E. maxima* and *E. acervulina* underwent no further development in the inoculated embryos.

РЕЗЮМЕ

Изучено 6 местных штаммов куриных кокцидий (*Eimeria brunetti*, *E. necatrix*, *E. praecox*, *E. mitis*, *E. maxima*, *E. acervulina*) на куриных эмбрионах.

Введенные в хориоаллантоисную полость куриного эмбриона спорулированные ооцисты или споры, указанных видов кокцидий не развивались и не вызывали заражения эмбрионов.

Спорозоиты *E. praecox*, *E. brunetti*, *E. necatrix* и *E. mitis* продолжали свое развитие в хориоаллантоисных оболочках и заканчивали там эндогенный цикл. Все фазы развития соответствовали таковым при развитии в цыпленке.

Ооциты, полученные из зараженных эмбрионов каждого из описанных видов в отдельности, нормально спорулировали и при введении 15 суточным цыплятам вызывали их заражение.

Спорозоиты *E. maxima* и *E. acervulina*, введенные в эмбрион, не давали дальнейшего развития и не заражали эмбрионы.

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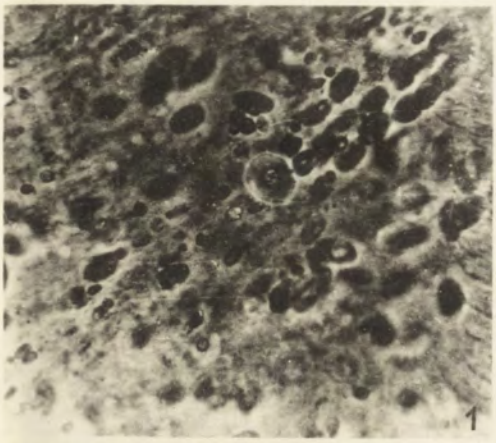
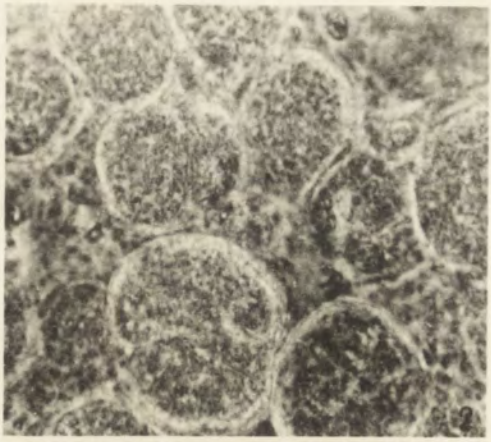
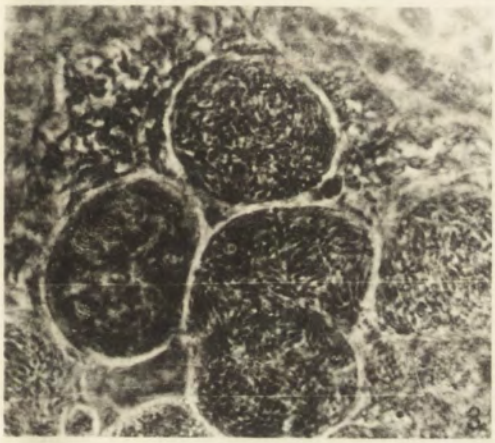
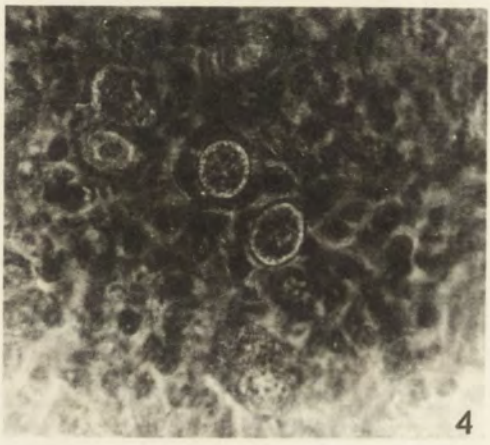
EXPLANATION OF PLATES I–IV

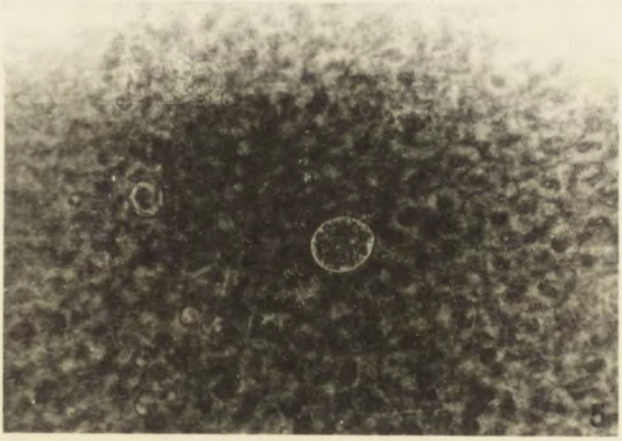
1: Trophozoites of *Eimeria brunetti* in the C. A. M. 36 h after inoculation. ×1510
2: First generation schizonts of *E. brunetti* in the C. A. M. 68 h after inoculation. ×1510
3: Second generation schizonts of *E. brunetti* in the C. A. M. 120 h after inoculation. ×1510
4: Sexual stages of *E. brunetti* in the C. A. M. 144 h after inoculation. ×1510
5: Oocysts of *E. brunetti* in the C. A. M. 168 h after inoculation. ×1510
6: Trophozoites of *Eimeria necatrix* in the C. A. M. 48 h after inoculation. ×1500
7: First generation schizonts of *E. necatrix* in the C. A. M. 72 h after inoculation. ×1500
8: Second generation schizonts of *E. necatrix* in the C. A. M. 120 h after inoculation. ×1500
9–10: Sexual stages and oocysts of *E. necatrix* 144–168–192 h after inoculation. ×1500
11: Trophozoites of *Eimeria praecox* in the chorioallantoic membrane C. A. M. 24 h after inoculation. ×1500
12: First generation schizonts of *E. praecox* in the C. A. M. 48 h after inoculation. ×1500
13: Second generation schizonts of *E. praecox* in the C. A. M. 96 h after inoculation. ×1500
14–15: Sexual stages and oocysts of *E. praecox* in the C. A. M. 144–168 h after inoculation. ×1500
16: Trophozoites of *Eimeria mitis* in the C. A. M. 36 h after inoculation. ×1500
17: First generation schizonts of *E. mitis* in the C. A. M. 60 h after inoculation. ×1500
18: Second generation schizonts of *E. mitis* in the C. A. M. 120 h after inoculation. ×1500
19–20: Sexual stages and oocysts of *E. mitis* in the C. A. M. 120–144 h after inoculation ×1500.











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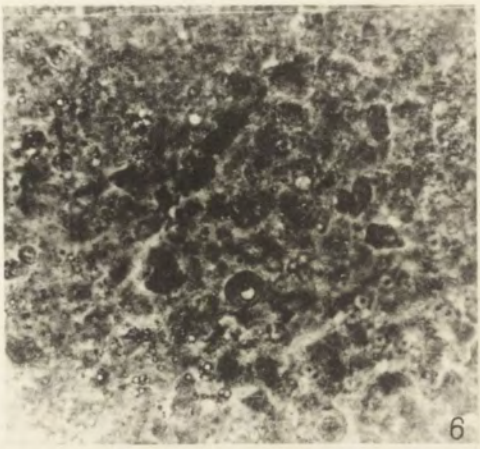

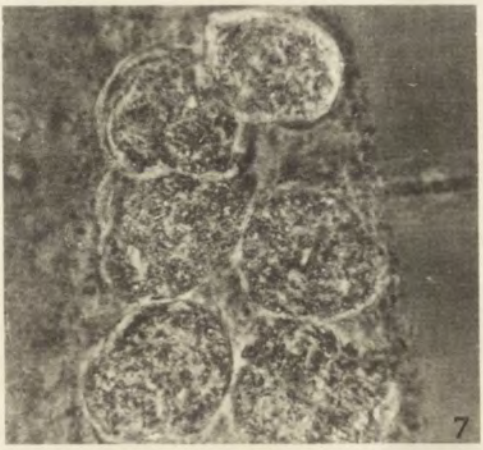

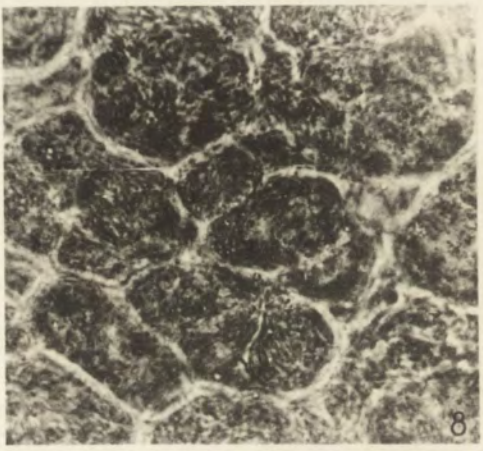

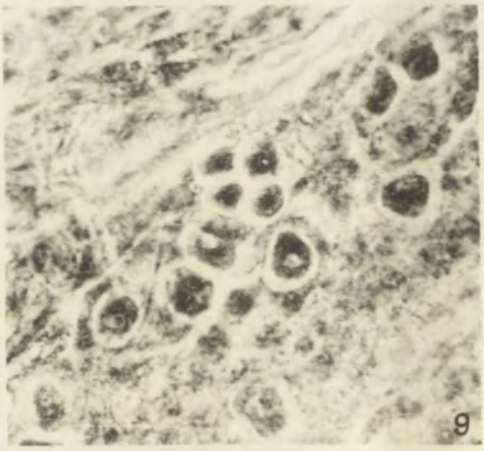

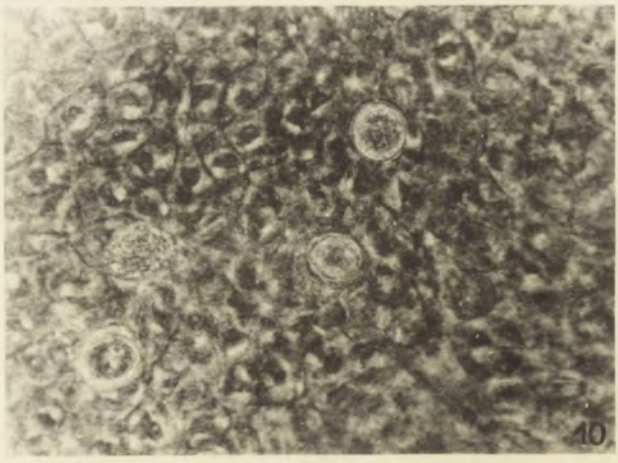

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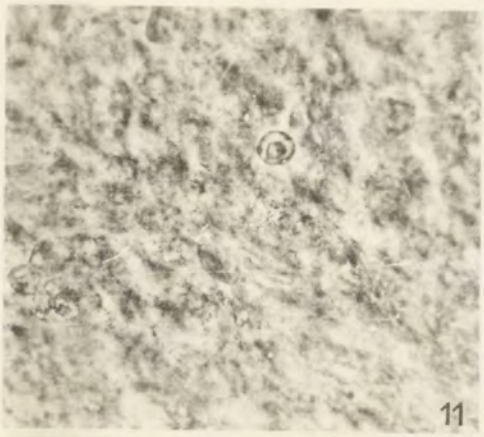

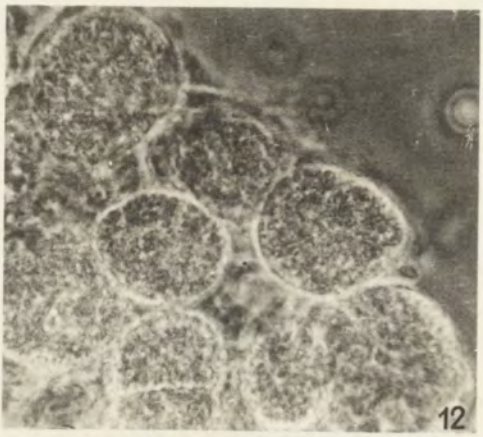

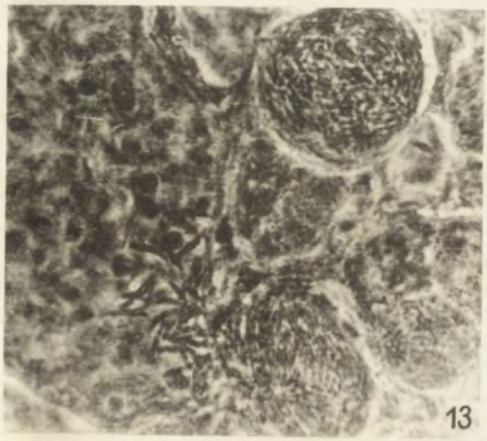

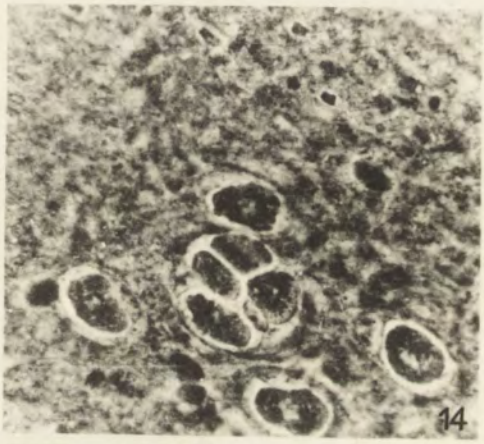

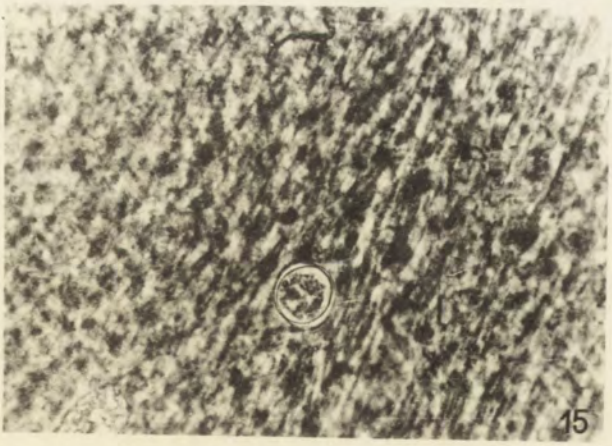

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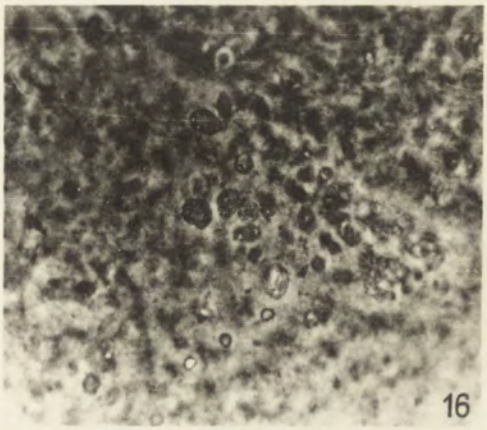

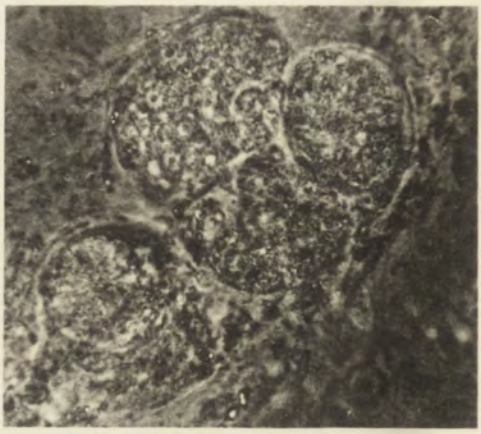

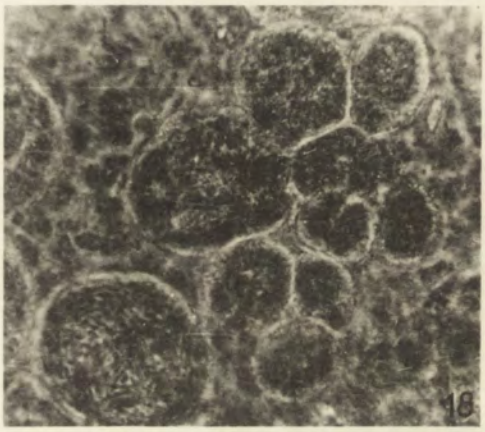

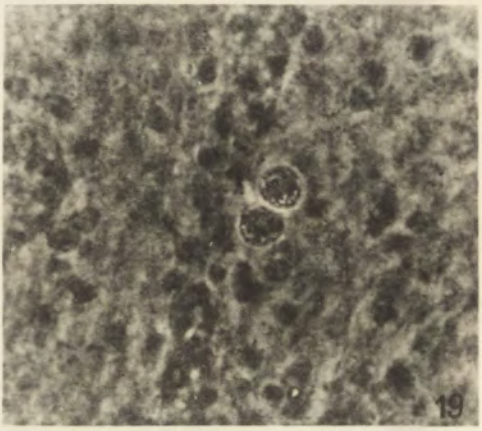

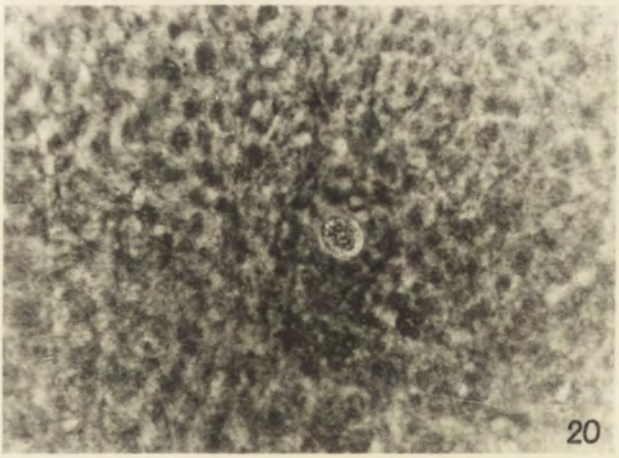

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The influence of definite ionic medium on the negative chemotaxis in *Stentor coeruleus*

Wpływ określonego środowiska jonowego na zjawisko chemotaksji ujemnej w *Stentor coeruleus*

In the former researches on chemotaxis, the "range of chemotactic sensitivity" to some chemical compounds had been stated in ciliates (Dryl 1952, 1959 a, b, 1961, 1963, Pietrowicz-Kosmynka 1971). The term "chemotactic sensitivity" applied in those studies has corresponded to the different degree of intensity of the chemotactic reaction. In *Stentor coeruleus* in which the chemotactic sensitivity to the ions of biological significance was mostly studied, a negative chemotactic reaction was detected ranging from a strong down to a weak one, or to the so called neutral chemotaxis.

The notion "chemical sensitivity" seems however to require a further analysis. It should be ascertained whether there exist some other measurable parameters signalizing such changes in the sensitivity of the cell besides the various degree of intensity of chemotactic reaction. If they exists really, then whether their variability is correlated with the course of chemotactic reaction and in what manner it occurs. Those problems are the subject of the present study.

For solving them the following experiments and observations were carried out:

1. The study of chemotactic sensitivity to quinine hydrochloride an organic compound with strong negative chemotactic properties even in sublethal concentrations.

2. The study of the character of chemotaxis evoked by quinine hydrochloride in the media of different concentrations of ions K^+, Na^+, Ca^{++}, Mg^{++}; since quinine in devoid of ionic properties — it proved to be very useful for this series of experiments.

3. Observation of *S. coeruleus* behaviour in the media of different concentrations of the above ions (see above).

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* Submitted in partial fulfillment of the requirements for the degree of Doctor of Biological Science in Nencki Institute of Experimental Biology under the guidance of Prof. dr. Stanisław Dryl.

Material and methods

As material for experiments *Stentor coeruleus* was used. A dense culture of this ciliate was kept in the Pringsheim's medium supplied every day with the ciliate *Tetrahymena pyriformis*. For experiments and observations the culture material was densified on special nylon sieves.

The results presented in the first and second part of this study have been mostly based on the method of macrophotographic quantitative analysis of chemotaxis (Dryl 1958, 1959) with a modification of the author (Pietrowicz-Kosmynka 1971 a). The assumptions serving as base for evaluation of quantitative results have been described in the same publication.

The observations of behaviour of *Stentor*, measurements of its length and rate of its movement were carried out every time under a binocular and light microscope as well as on the photograms of no less than 100 individuals in each test. The length of living animal was defined on photograms exposure time 1/100 sec). The photographic registration of paths which were the base of defining the character and rate of the ciliate movement, was performed during 3 and 5 second exposure.

Experiments

Chemotactic sensitivity of *St. coeruleus* to quinine

The chemotactic sensitivity to quinine hydrochloride was studied at different concentration of this compound. The concentration increased progressively so that every next was twice higher than the preceeding one. The quantitative results demonstrating the course of reaction are presented in Table 1.

At the concentration 0.0002%, the statistic differences between the number of ciliates in the control C and in the test T are not essential, consequently the chemotaxis is neutral. The threshold of sensitivity at which the negative chemotaxis appears first, is at conc. 0.0004%. The number of individuals responding to quinine hydrochloride increases with the gradual rise of concentration of this compound.

Table 1

Chemotactic sensitivity of *Stentor coeruleus* to quinine

concentr. in %	$m^C \delta$	$m^T \delta$	%nR	significance of difference between T and C
0.0002	59.0±2.0	58.5±2.3	99.3	sing.
0.0004	67.5±4.7	47.3±4.2	70.1	sign.
0.0009	58.8±3.0	37.0±0.3	62.0	sign.
0.001	52.5±0.7	24.4±1.3	46.4	sign.
0.003	58.1±2.9	13.3±2.3	22.8	sign.
0.005	68.7±3.8	8.8±0.4	12.8	sign.
0.007	52.1±5.9	4.3±0.1	8.1	sign.
0.009	60.7±4.3	2.0±0.6	3.3	sign.

C – control field, T – test field, m – arithmetic means from 10 experiments, δ – standard deviations, %nR – percentage of non-responding individuals to chemotactic stimulus

At conc. 0.009% the number of individuals not responding to this stimulus by escape, amounts 3.3%. The gradual intensification of negative chemotaxis is seen in the diagram (Fig. 1).

The results of experiments on chemotactic sensitivity of *Stentor* to quinine proved that this compound may be applied as a chemical stimulus evoking a typical negative chemotactic reaction.

In all the following experiments, a 0.007% solution of quinine hydrochloride was applied for evoking this phenomenon.

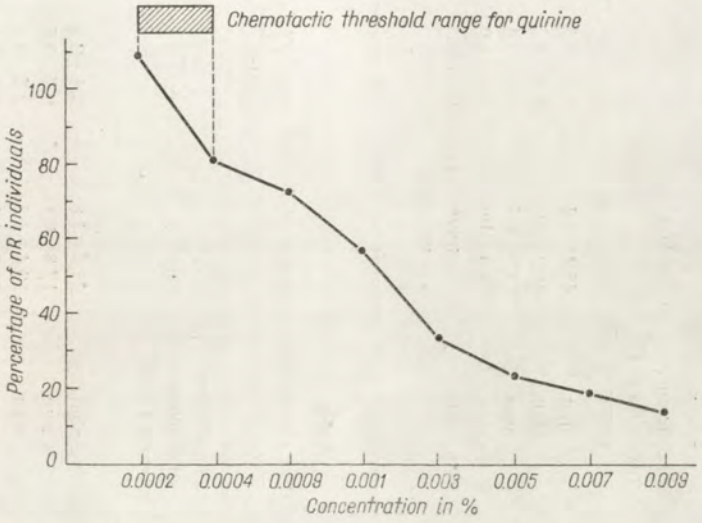

Fig 1. Chemotactic sensitivity of *Stentor coeruleus* to quinine

Character of chemotaxis reaction in the media of different concentrations of ions K^+, Na^+, Ca^{++}, Mg^{++}

Ciliates were subjected to the test of chemotactic sensitivity to 0.007% quinine after having been adapted previously to the media of definite concentrations of chlorides of potassium, sodium, calcium and magnesium. Each cycle of experiments was performed twice at 5 min interval and after a 45 min long adaptation of ciliates to the given ion. As solvent for the 0.007% solution of quinine chlorhydrochloride served always the solution of the ion studied at a definite concentration prepared with 1mM/l Tris/HCl+1mM/l $CaCl_2$. As a control for evaluation of the chemotactic sensitivity to quinine after adaptation to the ion studied, was in every case the solution 0.007% of quinine prepared with 1mM/l Tris/HCl+1mM/l $CaCl_2$. This solution served also as solvent for every concentration of the ions studied.

It became evident that in the case of adaptation of ciliates to the media of different concentration of K^+ (Table 2) — the chemotactic sensitivity to quinine

Table 2

Influence of K^+ ions on the negative chemotactic response of *Stentor coeruleus*

concentr. in mM/l KCL	after 5' of adaptation				after 45' of adaptation			
	m C δ	m T δ	%nR	significance of difference between T and C	m C δ	m T δ	%nR	significance of difference between T and C
0	24.0±3.8	1.9±0.3	8.0	sign.	45.4±4.4	3.6±0.2	8.1	sign.
1	36.0±3.4	1.8±0.2	5.0	sign.	48.5±3.2	16.9±3.2	35.0	sign.
2	15.4±0.3	13.5±0.9	87.6	sign.	17.7±0.3	18.4±0.4	104.0	no sign.
4	51.4±2 5	52.7±1.7	102.7	no sign.	28.9±1.9	31.2±1.0	108.0	no sign.
8	42.5±1.8	46.7±2.0	110.0	no sign.	experiments unfeasible			
16	experiments unfeasible				experiments unfeasible			
32	experiments unfeasible							

Explanations — see Table 1.

Table 3

Influence of Na^+ ions on the negative chemotactic response of *Stentor coeruleus*

concentr. in mM/l NaCl	after 5' of adaptation				after 45' of adaptation			
	m C δ	m T δ	%nR	significance of difference between T and C	m C δ	m T δ	%nR	significance of difference between T and C
0	54.9±2.9	4.7±0.1	8.6	sign.	23.7±1.8	2.1±0.3	9.1	sign.
2	46.6±1.8	0 ⁸±0.0	1.7	sign.	17.8±0.1	0.7±0.0	3.9	sign.
4	56.4±2.7	1.9±0.1	3.4	sign.	50.4±2.3	3.5±0.2	6.9	sign.
8	46.6±1.1	2.0±0.1	4.3	sign.	33.0±1.1	2.5±0.1	7.6	sign.
16	77.4±3.5	11.2±0.3	14.4	sign.	18.9±0.6	2.6±0.4	13.7	sign.
32	45.4±2.0	44.5±1.3	98.1	no sign.	17.3±0.6	17.0±0.1	102.0	no sign.

Explanations — see Table 1.

Table 4

Influence of Ca^{++} ions on the negative chemotactic response of *Stentor coeruleus*

concentr. in mM/l $CaCl_2$	after 5' of adaptation				after 45' of adaptation			
	m C δ	m T δ	%nR	significance of difference between T and C	m C δ	m T δ	%nR	significance of difference between T and C
0	81.0±2.1	7.2±0.2	9.0	sign.	54.0±0.8	4.6±0.1	8.5	sign.
2	82.1±4.1	3.4±0.4	4.1	sign.	85.5±4.4	3.4±0.2	4.0	sign.
4	83.6±5.0	1.0±0.1	1.2	sign.	80.6±6.8	6.1±0.3	7.0	sign.
8	121 9±7 7	3.3±0.2	2.7	sign.	144.2±8.9	10.0±0.3	7.0	sign.
16	68.1±1.6	4.4±0.2	6.5	sign.	41.3±2.8	6.9±0.4	17.7	sign.
32	71.5±3.4	19.3±0.6	26.9	sign.	38.2±1.1	16.2±0.4	48.8	sign.

Explanations – see Table 1.

Table 5

Influence of Mg^{++} ions on the negative chemotactic response of *Stentor coeruleus*

concentr. in mM/l $MgCl_2$	after 5' of adaptation				after 45' of adaptation			
	m C δ	m T δ	%nR	significance of difference between T and C	m C δ	m T δ	%nR	significance of difference between T and C
0	23.3±0.9	1.8±0.1	7.9	sign.	66.5±1.6	5.5±0.4	8.4	sign.
2	53.9±2.4	0.6±0.0	1.3	sign.	65.5±1.1	2.3±0.7	3.5	sign.
4	29.4±1.9	0.8±0.0	2.7	sign.	33.8±1.3	0.8±0.0	2.4	sign.
8	42.3±2.2	9.2±0.2	21.7	sign.	26.4±1.1	4.5±0.5	17.0	sign.
16	16.9±0.5	5.6±0.1	33.1	sign.	24.8±1.4	19.0±0.4	76.6	sign.
32	29.6±1.1	30.9±1.7	104.4	no sign.	experiment unfeasible			

Explanations – see Table 1.

falls inversely to K^+ concentration in solution. The reaction of negative chemotaxis to quinine extincts completely in the presence of 3 mM/l KCl in medium (after a 5 min long lasting adaptation).

Quantitative study of chemotactic reaction at high concentrations of KCl proved impossible became of slackening of movement after 5 min of adaptation at concentrations over 16 mM/l as well as after 45 min of adaptation in the concentrations over 8 mM/l in the medium.

Consequently in the case of adaptation to KCl, essential changes appear in the negative chemotaxis reaction to quinine in the ciliates studies. They are manifested in the gradual extinction of sensitivity to this stimulus.

The Na^+ ions of medium influence the character of chemotaxis reaction beginning with the NaCl concentration of 32 mM/l (Table 3). In these conditions *S. coeruleus* shows complete absence of sensitivity (neutral chemotaxis) to quinine. Lower concentrations of NaCl in medium fail to change the negative character of chemotactic response. The duration of adaptation to the medium containing sodium ions fails to evoke any changes either.

Adaptation of *S. coeruleus* to $ClCl_2$ (Table 4) in medium independently of its duration, does not change the specificity of the stimulus action. Only the high concentrations of $CaCl_2$ 32 mM/l after 5 min long adaptation as well as the concentration over 16 mM/l after 45 min of adaptation, evoke a slight fall of chemotactic sensitivity which is manifested by a higher number of individuals non-reacting to the stimulus.

Mg^{++} ions (Table 5) in medium involve the changes of chemotactic reaction character only at high concentrations. The inclination to diminish the sensitivity to the quinine stimulus rises proportionally to the value of $MgCl_2$ concentration in medium. The result indicate a full neutralization of *S. coeruleus* to quinine at the concentration 32 mM/l. 45 min long-lasting adaptation for the consecutive concentrations of $MgCl_2$ in medium involves a significant slowing of movement at 32 mM/l and the experiment becomes unfeasible.

The presence of ions of biological significance in medium — especially at high concentrations — acts upon the changes in the reception of the strong chemotactic stimulus i.e. quinine. The studies carried out indicate also the different action of the ions studied (Fig. 2).

The most essential changes are evoked by K^+ ions beginnig already with the concentration 1mM/l. In the presence of potassium, ciliates cease to respond to strong quinine stimulus by the negative chemotactic reaction. The influence of Na^+ and Mg^{++} ions changes the character of the negative chemotactic reaction just at 32 mM/l. Then the chemotactic sensitivity disappears. The presence of Ca^{++} in medium fails to involve essential changes in the negative chemotactic reaction.

The duration of adaptation to the definite ionic medium does not influence the change in the reaction character. Only a certain tendency to the rise of its manifestation is observed in the ciliates adapted previously for 5 min.

In the course of experiments, distinct changes in the behaviour of ciliates were observed. They were especially striking in the medium containing K^+ ions. Those changes concerned: the character of movement, rate and degree of contraction of ciliates. This suggested a certain dependence of chemotactic sensitivity to the quinine stimulus and the changes of ciliate behaviour on a definite ionic medium.

For this reason, the next series of experiments has been devoted to more precise observation of *S. coeruleus* behaviour in the ionic composition discussed above.

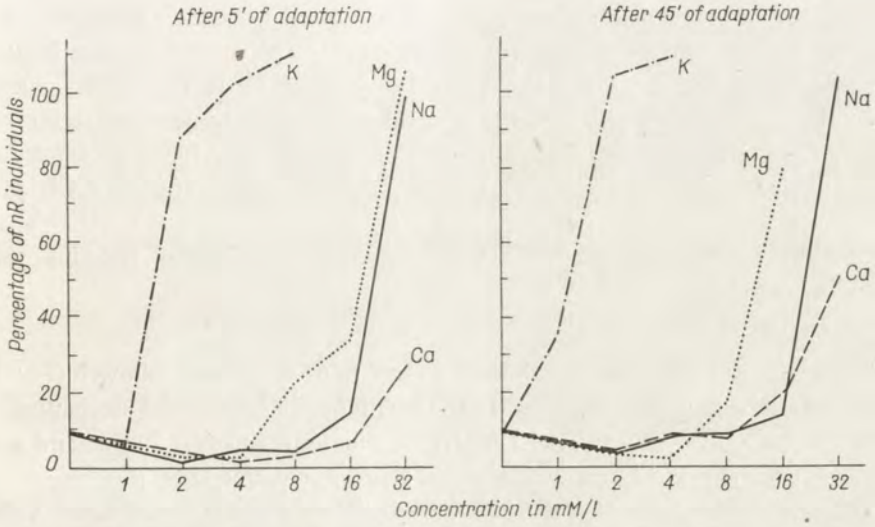

Fig. 2. Influence of ion medium on the chemotactic reaction of *Stentor coeruleus*

Behaviour of *Stentor coeruleus* in the media of different concentration of ions K^+, Na^+, Ca^{++}, Mg^{++}

The aim of these observations was to catch all the changes in the character of movement, evoked by the chemical composition of medium, as related to the normal movement. The manner of movement in the medium containing 1 mM/l Tris HCl+ 1 mM/l $CaCl_2$ at ph 7.2 was assumed as a normal behaviour. (See Fig. 4 and the detailed description at the end of the present chapter). The ciliate length amounts in this solution 250–300 μ and the rate of movement fluctuates within the limits 1800–2000 μ/sec.

The changes of behaviour of *Stentor* in single media are represented in schemes and diagrams, related to the corresponding measurements in the control medium at pH 7.2. Measurements were executed within the first 5 min after placing the ciliates into the solution studied. The character of the paths is illustrated in schemaitc drawing and in photograms.

K^+ ions

The behaviour of *S. coeruleus* was studied at different concentrations from 0.25 mM/l to 64 mM/l of KCl (Fig. 3 a).

Since the very beginning the ciliates move much slower than in the control solution. They show a tendency to elongate and slowing their movement in proportion as the KCl concentration rises in the medium. Beginning with the 8 mM/l KCl solution, ciliates shrink immediately after introduction of this substance and relax — gradually (after 5 min) to their maximal length.

The distinct slackening of movement is associated with the appearance of the so-called periodic ciliary reversal (PCR), which takes place after 30 min long stay in 1 mM/l KCl (see Fig. 6 and the detailed description at the end of this chapter).

In the 2 mM/l KCl solution, the reversal assumes continuous character — the so-called continuous ciliary reversal (CCR) of the type A. After a prolonged stay in this concentration which evokes another form of several appears CCR of the type B (see Fig. 5, and the description at the end of the chapter). At this state, ciliate manifest a distinct fall of sensitivity to the mechanical stimuli (shaking, touching with microneedle etc.).

Na^+ ions

The behaviour of ciliates in solutions from 1 to 64 mM/l was followed (Fig. 3 b).

At concentrations 1 mM–16 mM/l the length of ciliates remains unchanged and amounts 300 μ on average. At 32 mM/l the ciliate shrinks to 200 μ, and at the concentration 64 mM/l assumes a spherical shape of a diameter 80 μ.

The rate of the ciliate movement in NaCl distinctly differs from the norm. Ciliates move much slower. At concentration 32 mM/l the rate falls abruptly.

The character of movement shows also essential changes at this concentration. The ciliary reversal of a continuous character appears. The movement is gradually slower till ciliates are immobilized and desintegrate after several hours. This is preceded by inversion of pharynx, lateral flattening of the body, casting away of cilia of AZM and emission of pigment.

At concentration 64 mM/l ciliates show some spontaneous contractions shrink immediately and desintegrate. All the above aspects of desintegration occur at this concentration at an accelerated rate.

Ca^{++} ions

The behaviour of ciliates at different concentrations of $CaCl_2$ was followed from 0.25 to 64 mM/l (Fig. 3 c). Calcium acts distinctly stimulating on the rate of movement, especially at concentrations 1 mM–8 mM/l. At higher concentrations the movement rate reaches its norm again and falls distinctly at 32 mM/l. At 64 mM/l ciliates become immobilized.

The character of ciliate movement at high concentrations of $CaCl_2$ resembles to the so-called partial reversal (PaCR). This type of change of the movement character is in this case considered as premortal disturbances.

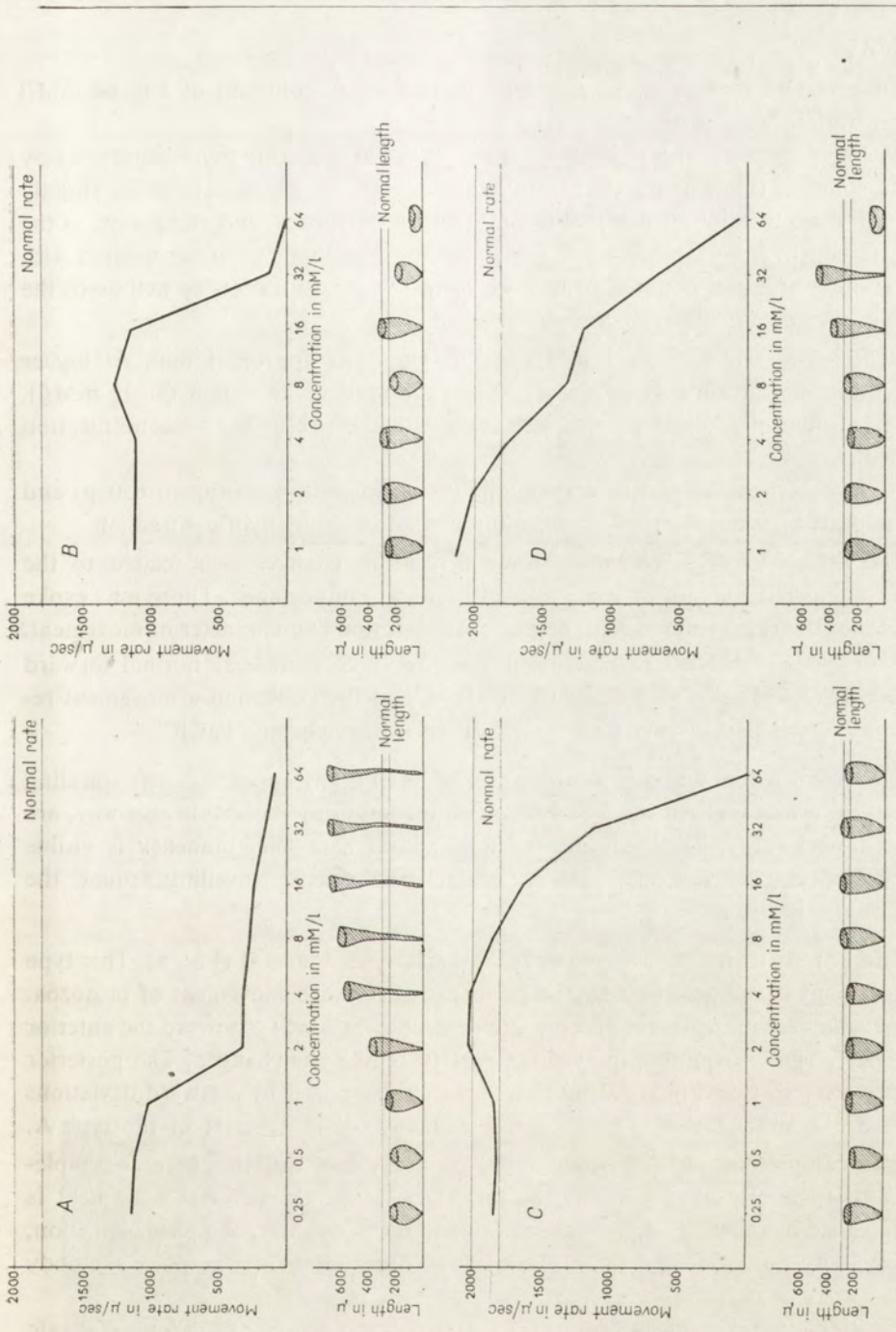

Fig. 3. Changes in the rate of movement and length of body of *Stentor coeruleus* in different ion media A — K^+ ions, B — Na^+ ions, C — Ca^{++} ions, D — Mg^{++}

Mg^{++} ions

Observations and measurements were carried out in solutions of 1 to 64 mM/l concentrations (Fig. 3 d).

The medium with $MgCl_2$ involves appearing of PCR during first seconds of stay in the solution (see Fig. 6), slackening of the movement which is however slightly faster in concentrations 1 mM/l and 2 mM/l than in control, and elongation of the body in concn. from 8 mM/l to 32 mM/l. All those phenomena are correlated with one another and proportional to the rise of Mg^{++} concentration as well as to the time of the ciliate exposure in this medium.

PCR appears already at 1 mM/l $MgCl_2$ and lasts approx. 1 min. At higher concentrations the time of its durations is prolonged up to 5 min (at 32 mM/l). The frequency of reversal periods rises also with the rise of Mg^{++} concentration in medium.

At 32 mM/l occurs a considerable elongation of protozoon (up to 650 μ) and the holdfast becomes bent. After 10 min the ciliate dies in slight contraction.

The behaviour of *S. coeruleus* shows perceptible changes — as related to the norm — under the action of some ions of biological importance. Those ions evoke changes of the rate of movement, degree of contraction and character of movement.

Four types of movement have been observed in *S. coeruleus*: normal forward movement (NCM) two forms of CCR (type A and B), PCR, and a movement resembling to the partial ciliary reversal marked in the scheme "PaCR".

The normal forward movement — NCM (Fig. 4) appears as left spiraling pattern of swimming. All the cilia on the protozoon body moving in this way, are directed backwards. The beat direction of adoral zone membranelles is visible under binocular microscope, showing metachronal waves travelling around the edge of the peristome.

The continuous ciliary reversal — CCR type A and B (Fig. 5). This type of movement is characterized by the permanent backward movement of protozoa. All the cilia beat in the direction opposite to the normal one i.e. toward the anterior body part. The left-spiraling movement pattern remains unchanged. The posterior end performs so-called penetrating movement characterized by sideward deviations which evoke spiralization. This is the continuous ciliary reversal of the type A.

After certain time, of CCR ciliates move gradually slower till they become completely motionless and anchor to the bottom. Slowing of the reversal movement is accompanied by the gradually weaker rotation of the body till its complete extinction, as well as by the gradually diminishing sidewerd deviations of the posterior body end.

The individuals kept for a long time in high concentrations of K^+ ions, elongate considerably, their holdfast becomes flattened and twisted. Contortion and bending of the holdfast together with the extinction of rotation movement and of sideward

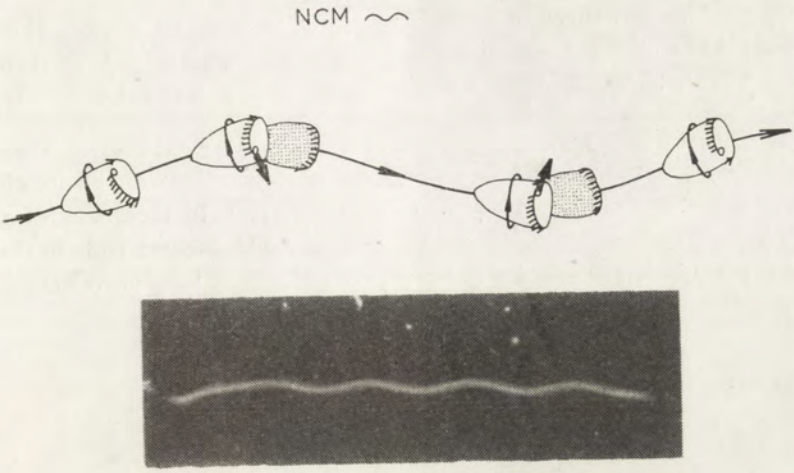

Fig. 4. Normal forward movement (NCM) of *Stentor coeruleus*

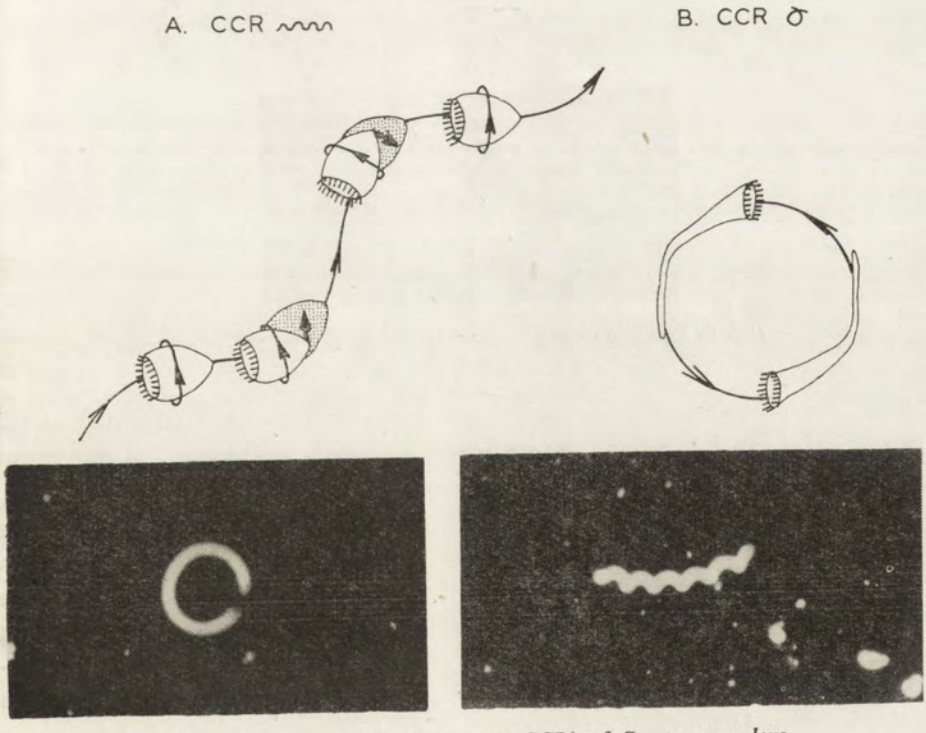

Fig. 5. Continuous ciliary reversal (CCR) of *Stentor coeruleus*

deviations, produce the effect of movement along an arch or circles. The wavy movement of AZM cilia becomes gradually weaker and long periods of stifffening are observed. This corresponds to the continuous ciliary reversal of the type B.

The periodic ciliary reversal — PCR (Fig. 6). This type of movement is characterized by alternating periods of forward movement — with all its element described above — and short periods of backward retreat. In these conditions the rotation movement is not observed and the cilia of AZM become stiff. In the case of repeating periods of backward movement, the effect of zig-zag movement is produced.

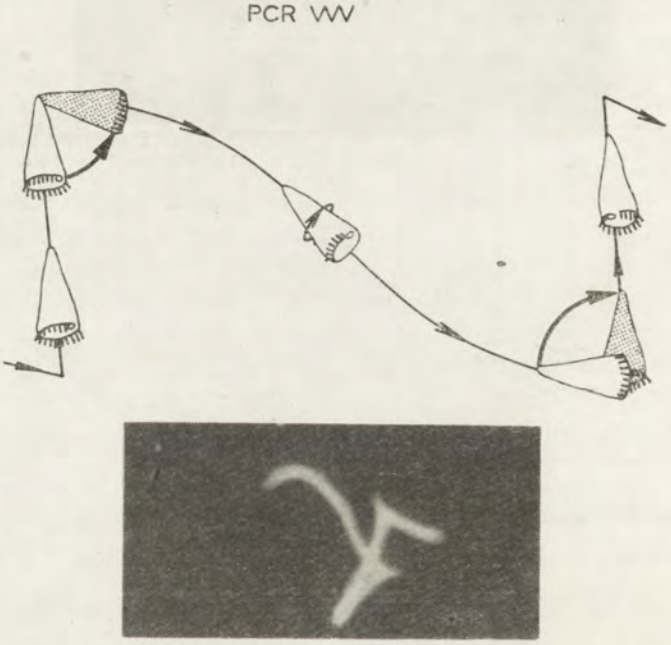

Fig. 6. Periodic ciliary reversal (PCR) of *Stentor coeruleus*

Since the movement type marked by the symbol PaCR is not an actual partial ciliary reversal but just resembling to it, the discussion of this type of movement is to be omitted in this study.

The changes of movement character are associated with the changes of the movement rate. E. g. an abrupt slowing was observed after the action of Na^+ ions during transition from NCM to CCR, or of K^+ ions with simultaneous extinction of rotation movement in reversal and in transition from the form A into B. With this regard, the action of K^+ ions (slowing of movement) and Ca^{++} ions (acceleration of movement), are distinctly contrasting. The changes of protozoan length are also associated with the rise of concentration of ions in the medium. As a rule —

except the action of Na^+ ions — parallel to the rise of concentration, occurs the elongation of protozoa which attains its maximum in the case of K^+.

On the general diagram (Fig. 7), the effects of different ions are presented, using the symbols introduced by Dryl (1961 b) and by Grębecki (1965). It might be presumed that the sequence of appearing types of movement reactions cited above, depends on specificity of various ions.

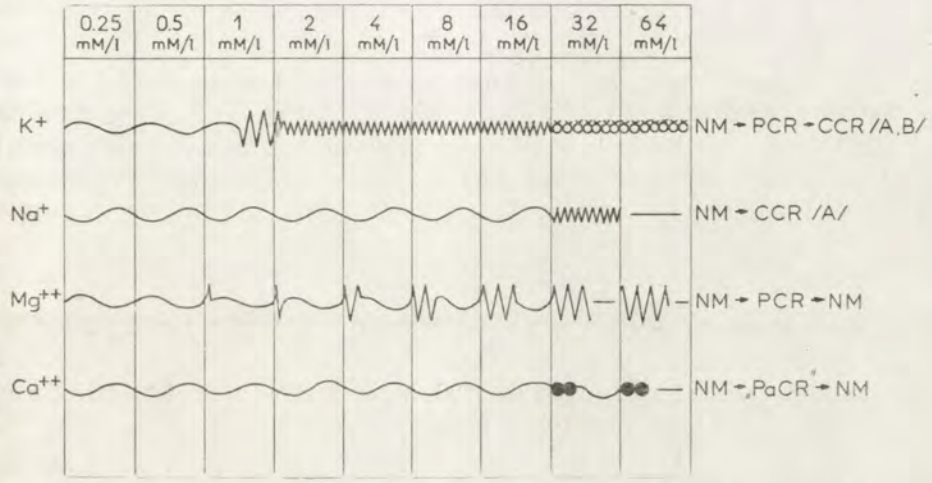

Fig. 7. Influence of ions on the change of movement character of *Stentor coeruleus*

The comparison of results of experiments on the influence of ion medium upon the chemotactic reaction, and of results of observation of *S. coeruleus* behaviour in various ion media — permitted to draw the following conclusions.

The presence of 1 mM/l KCl in medium evokes PCR after 30 min. In connection with this phenomenon, after a 45 min long adaptation to this medium, the sensitivity to quinine disappears in a great number of individuals. Together with the appearance of continuous reversal at concentration 2 mM/l KCl — the negative chemotaxis to quinine appears in a gradually lower number of individuals, till it disappears completely after a 45 min long adaptation. This is associated with transition from CCR type A (after a 5 min long adaptation) to the type B (after a 45 min long adaptation). In the concentration 4 mM/l of KCl in medium, when the continuous reversal of both types may be observed, no chemotactic phenomena take place. No statically essential differences exist between the number of individuals in T and that in C (Table 2). A considerable slowing of movement in the concentrations from 8 to 32 mM/l KCl is the reason why experiments on chemotaxis in this medium are not possible, because protozoa being in the state of continuous reversal of type B move at one place.

The results of the study on chemotaxis to quinine in ciliates subjected to the action of other ions, should be interpreted in the same way.

The absence of chemotactic sensitivity in the presence of Na^+ ions occurs only in concentration 32 mM/l. This is connected again with appearing of continuous reversal in all the ciliates in the samples studied.

The quantitative data concerning the extinction of sensitivity in a gradually higher number of individuals beginning with the concentration of 8 mM/l $MgCl_2$, is also associated with the phenomenon of periodic reversal since the time of its duration increases proportionally to the rise of concentration of Mg^{++} ions in medium.

The extinction of chemotactic sensitivity to quinine in a certain number of individuals in the medium with Ca^{++} ions at high concentrations is connected with the onset of premortal disturbances of movement in ciliates, since it is not a typical partial ciliary reversal. This problem will be presented more extensively in discussion.

Discussion

The early observations on *Paramecium* indicated already that the characteristic changes in the behaviour of this ciliate, as reversal of ciliary movement, are involved by K^+ and Na^+ ions (Jennings 1897, 1899, Mast and Nadler 1926, Oliphant 1938, Kamada and Kinosita 1940). Besides, reversal may last from several to over ten minutes. It is known as well that reversal is the reflection of the change of excitation state. Since the moment when it was ascertained that appearing of ciliary reversal is accompanied by depolarization of the cell membrane, the time of duration of ciliary reversal became even the measure of the state of excitability of *Paramecium* (Jahn 1962).

The responses of *S. coeruleus* to K^+ and Na^+ ions are somewhat different. Appearing of CCR in the presence of K^+ in medium occurs already at 2 mM/l KCl (in presence of $CaCl_2$). The disturbance of movement does not disappear after a certain time (as it occurs in *Paramecium*) but lasts till the protozoan death, changing only its form (from type A to type B CCR). CCR appears under the action of Na^+ ions at 32 mM/l and has an equally continuous character as under the action of K^+. It should be mentioned that the persisting character of the continuous reversal in *Stentor* has been observed by Merton (1923) and by Dierks (1926). However a gradual extinction of reversal is not observed in *S. coeruleus*, being so typical for *Paramecium* (Dryl 1952, 1959, Jahn 1961, 1967). Consequently the evaluation of the excitability state of *Stentor* by duration of CCR is not possible because of permanent character of CCR in this ciliate.

Perhaps some other measurable parameters could serve for this aim, namely: the character of movement, rate of movement or the length of protozoan body.

Besides CCR which is characteristic in the presence of univalent ions in medium, PCR was observed in the presence of Mg^{++} ions and after a more or less prolonged stay in lower concentrations of KCl. This manner of movement was characterized

by short alternate periods of reversal and of normal movement. During short reversal period the rotation movement never appeared as a result of stiffening of AZM. It should be noticed that PCR observed in *Paramecium* (Dryl 1961, 1964) after the action of Ba^{++} ions in presence of Ca^{++} ions, was characterized by much shorter periods of alternation of normal and reversal movement.

In the ciliate studied at high concentrations of Ca^{++}, some changes in the character of movement were observed which suggested appearing of motile behaviour similar to partial reversal (PaCR) observed by Grębecki (1965), and Kuźnicki (1966) in *Paramecium*. However a more exact analysis of this phenomenon fails to support this suggestion, because it was ascertained that higher concentrations of Ca^{++} (32, 64 mM/l $CaCl_2$) act toxically on *S. coeruleus* by osmotic way, similarly as it occurs in the case of *Paramecium* (Dryl 1961). According to Tartar 1961 the powerfully beating cilia of AZM in *Stentor*, may easily be destroyed. A partial impairment of this very important motor apparatus — which performs the role of steering organelle — may evoke some disturbance of motion similar to PaCR. The ciliates moving in a definite manner, swam with the rate corresponding to the character of this movement. The most significant slackening of movement was observed in presence of K^+ in medium (this was associated with occurrence of CCR type B). The animals moved most quickly in concentrations 4 mM/l and 8 mM/l of $CaCl_2$.

The change of contracture degree under the action of different concentrations of alkaline metal ions indicates also a strong influence of K^+ ions on the contractility of myonemes proportionally to the concentration of these ions in the medium, *Stentor* becomes more relaxed than normally and — in consequence — its body length increases.

A question arises, how should be explained the extinction of chemotactic sensitivity in a definite ion medium, in the light of results of the above experiments.

A complete extinction of the negative chemotactic response to quinine is observed in the presence of K^+ ions already at concentration of 2 mM/l. Ciliates in state of ciliary reversal induced by potassium are not able to respond to the next chemical stimulus like quinine as it was noticed even at low concentration (1 mM/l KCl) after a 45 min adaptation to KCl. The correlation between the extinction of sensitivity and appearing of reversal (which is the sign of changed excitability state) is in this case most striking.

The other three ions Ca^{++}, Mg^{++}, Na^+ exert influence on the change of sensitivity only in high concentrations. In the case of Na^+, the absence of sensitivity coincides also with the moment of manifestation of CCR. On the other hand, the action of Mg^{++} is connected with the occurrence of PCR which becomes gradually prolonged in proportion as the concentration rises. This becomes clear after the comparison of results of the chemotactic reaction preceded by a 5 min long adaptation in $MgCl_2$ and a 45 min adaptation to this compound. Ca^{++} ions fail to influence essentially the chemotactic sensitivity. The extinction of sensitivity in a certain

number of individuals at concentration 32 mM/l $MgCl_2$ should be considered as a sign of a premortal state of ciliates.

A more prolonged time of adaptation to the conditions of medium evoking any form of reversal or acting toxically, cause always the rise of number of individuals in which extinction of sensitivity to chemical stimuli is manifested. It is presumed that the individual properties of ciliates i.e. their physiological condition, phase of their life cycle, are decisive factors of participation in the process of adaptation to the changed medium.

The above experiments carried out on *Stentor coeruleus* proved that the presence of certain ions in medium changes distinctly the character of response to chemical stimuli. However in every case there occurs a coincidence of extinction of chemotactic sensitivity and the reversed character of movement. On the other hand, it is known that ciliary reversal is a universal equivalent of changes in the state of excitability of the cell in response to external stimuli (Yamaguchi 1960, Kinosita, Dryl and Naitoh 1964, Kinosita, Murakami and Yasuda 1965, Naitoh 1966). It should be concluded that the change of sensitivity to a definite chemical substance depends on the state of excitability of the cell, associated with the influence of ionic medium which presumably involves changes in the ionic equilibrium in the cell membrane of protozoan.

Summary

The aim of experiments was the study of the effects of some ions of biological significance on the course of negative chemotactic reaction towards 0.007% solution of quinine hydrochloricum.

Experiments carried out on *S. coeruleus* proved that the presence of some ions in medium changes distinctly the character of reaction to chemotactic stimuli. Concentrations of KCl 2 mM/l or higher cause complete extinction of negative chemotaxis reaction to quinine. The other ions (Ca^{++}, Mg^{++}, Na^{+}) involve a complete extinction of sensitivity only at high concentrations.

Changes in the behaviour of protozoa were observed under the action of various ions in external medium. Three forms of movement in *S. coeruleus* could be distinguished: normal movement, continuous ciliary reversal of type A and B and periodic ciliary reversal. It was ascertained that the extinction of chemotactic sensitivity is always correlated with the reversed beat of body cilia.

STRESZCZENIE

Praca ta jest kontynuacją badań nad zjawiskiem chemotaksji pierwotniaków. Eksperymenty miały na celu przebadanie wpływu niektórych jonów o znaczeniu biologicznym na przebieg reakcji chemotaksji ujemnej.

Do wywołania tejże użyto (w oparciu o ocenę wrażliwości *S. coeruleus* na ten związek) 0,007% roztwór chlorowodorku chininy.

Eksperymenty przeprowadzone na *S. coeruleus* dowiodły, że obecność pewnych jonów w środowisku wyraźnie zmienia sposób reakcji pierwotniaków na bodziec chemiczny jakim jest chinina. Najsilniej działają jony K^+. Począwszy już od stężenia 2 mM powodują zupełny zanik reakcji chemotaksji ujemnej na związek chininowy. Świadczy to o zaniku wrażliwości chemotaktycznej pierwotniaków. Pozostałe 3 jony (Ca^{++}, Mg^{++}, Na^+) powodują zanik wrażliwości tylko w wysokich stężeniach. Zaobserwowano ponadto zmiany w sposobie zachowania się pierwotniaków pod wpływem różnego środowiska jonowego. Opisano trzy sposoby poruszania się *S. coeruleus*: ruch normalny NCM, rewersję rzęskową ciągłą CCR typu A i B oraz rewersję rzęskową okresową PCR.

Następnie porównano wyniki obserwacji nad zachowaniem się badanego orzęska z danymi o wpływie różnego środowiska jonowego na zjawisko chemotaksji ujemnej. Okazało się, że zanik wrażliwości chemotaktycznej jest zawsze skorelowany z rewersyjnym charakterem ruchu.

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Size and shape of cold- and heat-treated specimens of *Tetrahymena pyriformis* (GL)

Hideg- és meleg-kezelt *Tetrahymena pyriformis* GL egyedeinek méretei

The synchronizing of the cell division in the case of *Tetrahymena* was first worked out by *Scherbaum* et al. 1958, but it was soon found that the temperature treatment used during the synchronization procedure changed the life cycle of the cells and produced an effect similar to effects of some specific chemical compounds. Some investigations have shown that changes take place both on the effects of heat and cold (Scherbaum 1956, Scherbaum et al. 1958 and Scherbaum 1960).

Authors who had dealt with the synchronous division of *Tetrahymena* usually mention in their reviews that the cell-sizes increase during the repeatedly applied shocks whereas during the synchronous division, when the animals divide simultaneously, the cell-sizes become normal. Authors also pointed out that heat-treatment for synchronization (either cold- or heat-shock effects) have the same effects like some chemical substances with specific character. It is known that the animals have normal size and shape 5 h after the last heat-treatment. However, these data became known mainly on these basis of the total volume of cells as well as the number of cells in the culture-medium. So far the quantitative data concerning the volume of one animal, are not available. Considering the changed metabolism during the treatment, it is highly probable that besides the DPNH oxidase and lactic acid-oxidase enzymes, other enzyme-systems could also be damaged or inactivated for a certain period.

On the basis of all above mentioned facts the author found necessary to examine on the shape and size the changes of *Tetrahymena* caused by heat or cold effects.

Methods

For cultivation of *Tetrahymena* generally accepted methods were used. The animals were maintained in liver- and brain culture-medium (Furgason 1940) in which they survived for a long time without any transfer to fresh medium.

Single cell cultures were subjected to temperature treatment. Prior to experiments the animals were — at three days intervals — five times inoculated, using 0.85 per cent aqueous peptone culture medium (Borbély 1961) After the fifth inoculation the culture was started anew from one animal. Then the animals in 1 ml culture medium were inoculated into test-tubes containing 10 ml peptone

culture medium each. The culture medium had been previously sterilized and the cold and heat-treatment of the animals was applied on the fourth day after inoculation. All the cultures, including the controls, were incubated in darkness at temperature 25°C (±0.3). On the fourth day after inoculation 15 of the test-tubes containing the culture medium and cell population were placed in boiling water for 4 sec. The temperature of culture medium raised to 39°C in 4 sec. Most of the animals subjected to the heat-shock died during the treatment; only about 10–15 per cent of them survived. The heat-treated animals were then replaced into a thermostat with 25°C and after 3 h the survivors were transferred into fresh peptone solution. The other 15 test-tubes with animals in culture medium were placed into a deep-freezer at 25°C for 30 min. The frozen cultures were first placed in melting ice and 3 h after melting of the ice they were replaced into fresh peptone solution. Parallel to the treated cultures untreated controls were incubated at 25°C.

24 h after the treatment the animals were transferred into plates and their motion was paralyzed by some drops of 1% $NiSO_4 \cdot (NH_4)_2So_4 \cdot 6H_2O$ solution, then they were covered. The width and length of 300 heat- and cold-treated specimen as well as of the controls *Tetrahymena* were measured. The measurements were performed by means of ocular micrometer with 0.01% exactness at 600-fold magnification. The measurements were done on eight subsequent days after the treatment comparing the changing size of the treated animals with that of the controls. The sizes measured on each day were also controlled.

Results

Considering the results of our experiments we compared the length and width of the controls as well as that of the heat- and cold-treated animals. Details of results may be seen in the Table 1. The length and width differences between the mean values of each day were found in most cases significant.

Discussion

The experimental results have shown that the size of cells, the length- and width-values of the treated animals considerably differed from those of the non-treated ones.

Cold and heat- treatments cause not only changes in the size of the animals, but their multiplication intensity was also changed as well as the activity of some enzymes (Németh 1964). It is well known from the literature that temperature effects of shock treatment applied for synchronous division cause also significant biochemical changes in the animals. After heat-treatment the animals contain glycogen in great quantity (Sonneborn 1947), but the amount of high energy phosphates is reduced. During temperature treatment the volume of nucleus is also growing.

Some authors have found that the production of structural proteins was interrupted during the treatment. When the animals were replaced to optimum temperature the synthesis started again (Christensson 1949). During temperature-treatment the soluble proteins in the cells — being most sensitive upon temperature effects turned into insoluble proteins, thus manifesting the protective ability of the animals. This has been proved by experiment with carbon and sulphur radio isotopes (Scherbaum 1960). The investigations on metabolism proved that a heat-sensitive system in animals is damaged by temperature treatment. This heat-sensitive system was

Table 1

Length and width of control, cold-treated and heat-treated *Tetrahymena* with the mean, the standard error, the variation coefficient (V%) and degree of freedom

Numbers of days		Control		Cold-treated		Heat-treated		Cold-treated		Heat-treated	
		Tetrahymena pyriformis						Significance of the length and width of *Tetrahymena* (t test)			
		mean and standard error	V%	mean and standard error	V%	mean and standard error	V%	t	p	t	p
1	length	39.99±0.01	6.63	23.66±0.02	8.75	26.96±0.02	6.62	48.72	0.01	41.46	0.01
	width	16.95±0.01	2.71	11.98±0.01	3.70	13.27±0.01	3.67	22.92	0.01	17.09	0.01
2	length	39.38±0.01	6.02	25.43±0.02	6.79	31.36±0.02	11.99	46.18	0.01	21.90	0.01
	width	16.97±0.01	1.98	10.51±0.01	3.78	13.40±0.91	2.37	31.49	0.01	20.06	0.01
3	length	40.03±0.01	5.83	34.49±0.02	6.40	33.38±0.10	10.64	28.35	0.01	18.92	0.01
	width	17.32±0.01	2.13	14.47±0.01	2.48	16.12±0.02	6.33	14.10	0.01	4.84	0.01
4	length	38.44±0.01	5.42	34.14±0.02	6.11	35.92±0.01	10.06	18.35	0.01	10.46	0.01
	width	17.30±0.01	3.41	15.15±0.01	1.90	16.28±0.21	4.62	10.89	0.01	4.18	0.01
5	length	39.92±0.01	6.98	34.75±0.02	6.11	37.76±0.10	10.60	16.71	0.01	5.99	0.01
	width	17.26±0.01	2.98	14.20±0.01	2.59	15.06±0.01	3.28	15.41	0.01	1.05	0.05
6	length	39.83±0.01	7.83	38.27±0.02	7.03	38.09±0.11	10.99	4.75	0.01	4.69	0.01
	width	17.32±0.01	2.63	15.15±0.01	2.61	14.29±0.01	2.63	11.10	0.01	1.52	0.05
7	length	38.95±0.01	4.90	38.49±0.02	5.61	38.91±0.10	10.34	1.67	0.05	2.56	0.01
	width	17.16±0.01	1.89	15.19±0.01	1.87	14.50±0.01	2.00	38.51	0.01	49.35	0.01
8	length	38.94±0.01	5.60	38.72±0.02	5.92	38.96±0.10	10.12	0.65	0.05	0.06	0.05
	width	17.30±0.01	2.15	17.20±0.01	2.14	15.75±0.01	3.05	1.63	0.05	8.21	0.01

found in *Tetrahymena* to be identical with DPNH oxidase and lactic acid oxidase (Scherbaum 1957). Several other enzyme systems were also damaged, like succinic acid, glutamic acid and lactic acid dehydrogenase (Németh 1964).

After high temperature treatment the protein synthesis in the animals is starting again, but at lower rate at normal cultivation conditions. The sensitive soluble protein content during the treatment turns into insoluble proteins causing a single division in animals after the temperature shock, but due to decreased protein synthesis the cells remain smaller and grow slower than the controls. The reduced rate of division is progressively coming back to the normal level by the seventh day (Németh 1964) and parallel with it the protein synthesis as well as the structural protein synthesis caused by temperature treatment also become normal, producing the specific cell structures which determine both the shape and size of the cells. This characteristic shape of the cell attains a value similar to that of the control on the seventh or eigth day.

Summary

Cultures of *Tetrahymena pyriformis* have been treated by cold and heat shock. A part of the animals has been frozen quickly together with the culture media, while the other part has been treated by heat. The temperature applied in the former case was —25°C, and that in the latter +39°C. The frozen cultures have been stored in the deep freezer for 30 min; the heat treatment lasted 4 sec. The size of the surviving animals has been measured in both cases and compared to the controls that have been kept at 25°C. The length and width of animals have been measured through 8 subsequent days after the treatment. The changes have been found most marked on the first day following the treatment. The length of the animals both in the cold and heat-treated group decreased to nearly the half. The width of the animals has been found to decrease also significantly. The two values reached the size of the controls, after a gradual increase, by the 8th day.

The results are given in the mean-values of the sizes of 300 *Tetrahymena* cells in each groups (controls, heat and cold treated animals).

ÖSSEFOGLALÁS

Vizsgálataink során *Tetrahymena pyriformis* tenyészeteket kezeltünk hideg és meleg- shock hatással. Az állatok egy részét a tenyészfolyadékkal együtt hirtelen megfagyasztottuk, a másik részét meleggel kezeltük. Az elözö esetben az alkalmazott hömérséklet —25°C volt, az utóbbi esetben +39°C. A fagyasztott kulturák 30 percig álltak a mélyhütöben, a melegkezelés 4 másodpercig tartott. Mindkét esetben a tulélö állatok nagyságát mértük meg a 25°C hömérsékleton tartott kontrol állatokkal együtt. A kezeléstkövetöen nyolc napon át megmértük az állatok hosszuságát és szélességét. A változás legszembeötlöbb a kezelés utáni elsö napon. Mind a hideg-mind a melegkezelt állatok hoszusága csaknem a felére csökkent. A szélesség értéke hasonló módon jelentösen kisebb. A két méret fokozatosan növekedve a nyolcadik napon eléri a kontrol állatok méreteit. Az eredményeket 300 *Tetrahymena* mérési eredményeinek a középértékeiböl adtuk meg, mind a kontrol, mind a hömérsékelettel kezelt állatok esetében.

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