ACTA PROTOZOOLOGICA

VOLUMEN IX

Fasciculi: 1-8

W A R S Z A W A 1 9 7 1

INSTYTUT BIOLOGII DOŚWIADCZALNEJ IM. M. NENCKIEGO
POLSKIEJ AKDEMII NAUK

ACTA PROTOZOOLOGICA

NOTICE TO AUTHORS

Acta Protozoologica is intended as a journal serving for the publication of original papers embodying the results of experimental or theoretical research in all fields of protozoology with the exception of purely clinical reports. The papers must be concise and will not be accepted if they have been previously published elsewhere. After acceptance by the Editors papers will be printed in the order as they have been received, in the possibly shortest time.

Papers are accepted in English, French, German and Russian. Every paper should begin with the name and postal address of the laboratory, name and the surname of the author, title in the language of the text, and translation of the title into the author's own language. The paper should be accompanied by a summary in the language of the text, not exceeding 100 words, also the translation into the author's own language. The authors speaking English, French, German, or Russian should translate the title and the summary into another one of the 4 languages accepted in the Journal. In the Russian texts also the name and the postal address of the laboratory, legends of tables, plates and text-illustrations must be translated, the translation of the summary may be somewhat more extensive, and the name of the author should be given additionally also in the Latin characters.

Manuscript should be a doublespaced typescript (30 lines on one side of a sheet) with a normal margin. No elements of the text should be fully typed in capitals nor in spaced set (only underlining with pencil is admittable). In decimal fractions points (not commas) should be used. The generally accepted abbreviations and symbols are recommended. Nomenclature must agree with the International Code of Zoological Nomenclature, London 1961. The original and one carbon copy of the whole text material should be supplied.

References must be cited in the text indicating only the author and year, thus: "Kinosita 1954 found that, etc." Only all references cited in the text should be listed. The list must be arranged as follows:

Ehret C. F. and Powers E. L. 1959: The cell surface of *Paramecium*. Int Rev. Cytol., 8, 97-133.

Gelei J. von 1939: Das äussere Stützgerüstsystem des Parameciumkörpers. Arch. Protistenk., 92, 245-272.

Titles of references are given in their original language (not translated). In papers written in English, French or German, the Cyrillic type of the Russian references is transliterated according to the international system (ISO Recommendation R 9 September 1954). This regulation is not applied to names if there exists their traditional spelling. Also author may freely choose the transliteration of his own name. In Russian papers, the Russian references are cited in Cyrillic, the others in the Latin characters, but they must be listed all together in the Latin alphabetical order.

The following material should be supplied on separate sheets: 1. the running title for the page headlines, 2. tables, 3. legends for text-figures, 4. legends for plates. Line-drawings will be published in the text, photographs and raster-figures on separate plates. No coloured photographs can be published presently. Lettering on photographs and drawings should be marked in pencil. With no regard to the language of the text, only the Latin lettering, arabic numerals or generally accepted symbols are admittable for marking on illustrations. Numbering of text-figures, plates and tables must also be marked in pencil, as well in the legends as in the text. Tables are denoted in English and in French — Table, in German — Tabelle, in Russian — Таблица. In English and French papers text-figures should be determined — Fig., in German — Abb., and in Russian — Рис. Plates are denoted in English and French — Pl., in German — Taf., in Russian — Табл.

Galley proofs are sent to the authors. Authors receive 100 reprints without covers.

Manuscripts may be submitted to each member of the Editorial Board or directly to the Office: Acta Protozoologica, Nencki Insitute of Experimental Biology. Warszawa 22, ul. Pasteura 3, Poland.

ACTA PROTOZOOLOGICA
VOL. IX WARSZAWA 1.VIII.1971 FASC. 1

Zoological Institute, University of Warszawa, Warszawa 64, Poland

Zdzisław RAABE

The morphogenetic principles of Sewertzoff, their extension and application to *Protozoa*

O zasadach morfogenetycznych Sewertzoff'a, ich rozszerzeniu i zastosowaniu do *Protozoa*

In the thirties of the current century attention and interest of morphologists were evoked by the theories of A. N. Sewertzoff concerning the different lines of changes in organs which occur in phylogenesis of animals, in the first place in vertebrates. Those lines have been determined by Sewertzoff as "principia" and so they were called after him.

In his work of 1931 Sewertzoff accepted conscientiously and broadened the following concepts: Dohrn's "principle of the change of organ function" (1875), Kleinenberg's "principle of organ substitution" (1886), Plate's "principle of the function extension" and "principle of function intensification" (1924), and finally Fedotov's "principle of physiological substitution" (1927). Accepting the above principles in his work, Sewertzoff added a number of some new own ones namely the principles of: phase fixation, extinction of mediating functions, reduction of the function number, substitution of functions, immobilization of functions, similarization of functions, and division of organs and functions. Separately are enumerated by Sewertzoff the regularities of: rudimentation and at last — of the full reduction i.e. aphanization of organs.

The principle of the primary multifunctionality of organs, as assumed by Sewertzoff should be mentioned. This means that an organ after having been already formed but not yet specialized, posesses a possibility of performing a certain number of functions. One of them becomes its main function, the others are secondary. In the subsequent development of the organ, a loss of secondary functions may occur and the organ becomes distinctly specialized. A predominance of another less important function may take place which assumes the character of the main function and even of the single one only.

It should be mentioned simultaneously that the own studies of Sewertzoff had been based on the vertebrate material or at least on *Chordata*, and when he was dealing with a foreign material, he considered the findings concerning one entirely group. Therefore in his considerations and in his formulations of new

rules or principles a postulation exists that all the organs are already existing if even in little specialized primordia. Their low specializations secures their pluripotentiality and all the processes developing in them concern the changes in their functions: transformations, extension or limitation, their mutual replacing or corroboration. It always is, in some way, a management of the existing material by evolution.

This seems to have evoked in 1950 the discussion of J. Gelei, the known Hungarian protozoologist, in his article on morphology of unicellular organisms in which he considered the morphogenetic principles of Sewertzoff. Gelei stated in the assumption of his article that "he makes no discrimination between the organelles of unicellular forms and the organs of multicellular animals". According to him "any separate part of organism having its own function is an organ and, if it is placed in the whole organism — a system of organs". Gelei tried in the first place to prove the principles of Sewertzoff on the examples of such a different and remote world as *Protozoa*. Besides this, he tried to observe and to ascertain new principles concerning the morphological evolution of protozoa which however are valid for *Metazoa* as well. As his first new principle he indicates the rule: "new organs — new functions". Subsequently follow the principles of: association of function, union of functions or organs, dissociation of functions and organs. He enclosed here "the principle of organ reduction" which was treated and discussed by Sewertzoff separately

In his supplementation of the Sewertzoff's theory and in some way in its criticism, Gelei remarked that the Russian author takes not sufficiently into consideration the qualitative changes in the organs, having in view rather the quantitative aspect. So in the process of such a close approach of single cilia, in the ciliates with an initially regular ciliature, that they begin to support mutually their action, complexes arise called by Gelei syncilia. They manifest qualitative changes. Those complexes are cirri, pseudomembranelles in the form of UM or AZM. Gelei stresses that in all those cases, an intensification of function, a qualitatively higher level of elements, is gained by their accumulation. This was overlooked by Sewertzoff.

The mutual supplementation of Sewertzoff's and Gelei's theories is especially valuable because Gelei, by taking examples mostly from the protozoan world, demonstrated that the regularities in phylogenesis of *Protozoa* and *Metazoa* often reflected in their ontogenesis being similar or the same although *Protozoa* achieve their changes by cytological differentiation of their single body cell whereas the metazoan changes are the result of differentiation of numerous cells which constitute tissues, organs and systems.

The theories of Sewertzoff and their complementation by Gelei have been slightly forgotten in the recent time. They have been neither sufficiently applied in Protozoology although their adequacy in the physiological and morphogenetic considerations is doubtless. I believe therefore useful to remind those principles i.e. the types of morphogenetic changes of organs embraced in the Sewertzoff's

concept and supplemented by Gelei as well as some further supplementations and additions which arose in my considerations and have been published previously in Polish (Raabe 1954).

In the following chapters those "principia" have been presented systematized according to sequence which seemed to me the most logical.

1. Principle of intensification of function — Plate, Sewertzoff

Here Sewertzoff has in mind the phenomena involved by the intensification of the organ function (its principal function in the first place) by the increase of the number of cells embracing this function. This rise occurs either by the increase of number of components themselves or by the progress in their construction. As example, the transformation of unicellular glands into the multicellular is cited.

Gelei was inclined to include here the cases of increase of the number and length of cilia — the locomotoric organelles of ciliates, and the extension of their contractile vacuoles system as the osmoregulatory — excretion apparatus.

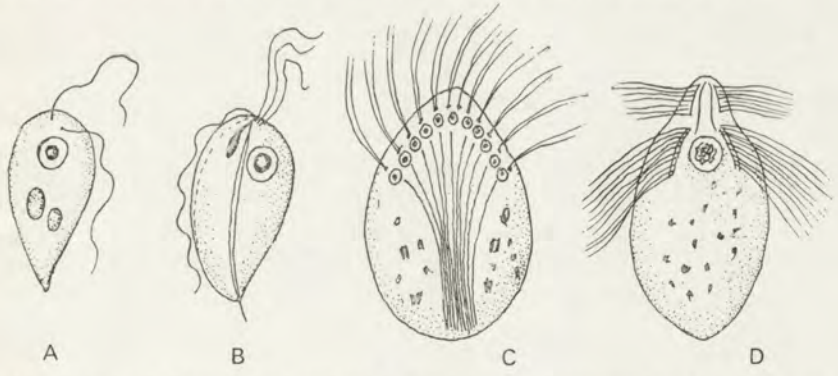


Fig. 1. Intensification of function: the flagellar apparatus in *Protomonadina* (A), *Metamonadina — Monomonadina* (B), *Metamonadina — Polymastigina* (C), *Metamonadina — Hypermastigina* (D)

I believe that if this problem would be considered more broadly, all the phenomena of organelle polymerization could be included here, e.g. multiplication of the undulipodial system in *Hypermastigina*, *Polymastigina* and *Opalinata*. Those problems have been discussed by me previously (Raabe 1964 a). I pointed out that such a polymerization by differentiation of component elements may lead to integration of such an assembly into an organ of a higher level (Fig. 1).

2. Principle of attenuation of function — Gelei

Many examples of function attenuation are discussed separately by Sewertzoff in his principal considerations in which he formulated only the principles of their progressive development. Gelei contrasted this principle to the former one. He

believes that it occurs in some cases only e.g. in weakening of the ciliary system in ciliates by reduction of cilia or cirri. The problem of reduction of the number of cirri is more complicated being in most cases accompanied by intensification of function of single cirri which results in the absence of attenuation of the function of the whole system. In my opinion, the phenomenon of transformation of dorsal cilia into stiff sensitive bristles in *Hypotricha* could find here his place.

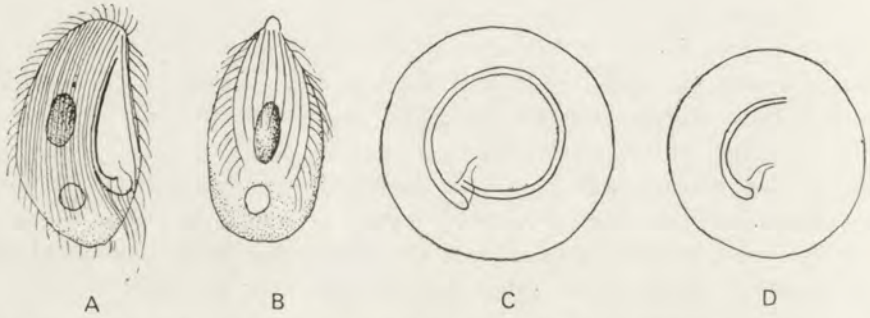


Fig. 2. Attenuation of function: reduction of the ciliature in *Thigmotricha — Hemispeiridae* (A), *Ancistrocomidae* (B), reduction of the adoral spiral in *Urceolariidae — Trichodina* (C), *Trichodinella* (D)

No doubt, all the manifestations of the reduction of locomotoric ciliature in ciliates correspond to the principle of function attenuation (e.g. in *Thigmotricha* of the range *Hemispeiridae* → *Ancistrocomidae* → *Shenophryidae* — Raabe 1967) (Fig. 2 A, B), as well as the reduction of their adoral apparatus (e.g. shortening of the adoral spiral in *Trichodinella*, *Dipartiella* etc. when compared with the full spiral of *Trichodina*, as it occurs generally in most *Peritricha* — Raabe 1963) (Fig. 2 C, D).

3. Principle of atrophy of intermediating function — Sewertzoff

Sewertzoff indicates here the atrophy of the connecting function performed by os quadratum in the mammals (*Sauropsida*) which is associated with the intensification of function of some other adjacent organs. In this example the formulation of the principle is presented not clearly. Perhaps it might be considered as a contrast to the next principle: of fixation of phases. Gelei failed to find any examples among *Protozoa* because they can scarcely be found there.

In my opinion such phenomena could be included here as the secondary atrophy of adhesive function of scopula in some non-sedentary *Peritricha* which are included however into *Mobilia* as e.g. *Opisthonecta (= Telotrochidium)* (Fig. 3).

Functioning of undulipodium of flagellates and ciliates is a suitable problem for considering the disappearance of intermediate or intermediating functions. If we assume that the helicoidal movement of undulipodium is constituted of many

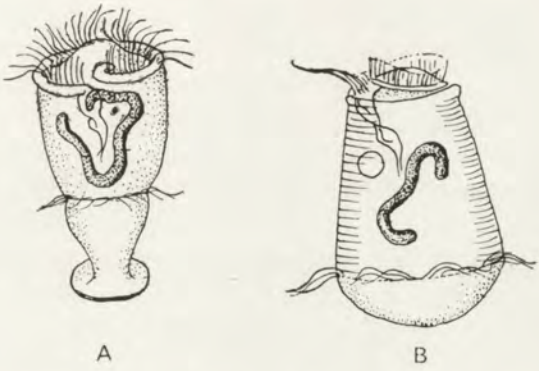


Fig. 3. Atrophy of intermediating function in *Peritricha — Sessilia* (A), *Opisthonecta* (B)

consecutive phases (Kuźnicki et al. 1970) necessary for its full locomotoric movement whereas in some *Protozoa* its movement is restricted to a uniplanar one (e.g. syncilia) — it means that it omits some of its phases.

4. Principle of fixation of phases — Sewertzoff

This extremely interesting principle concerns the fact that the function of a complex organ is constituted of many consecutive phases, some of them may become fixed as a permanent feature of the organ. So e.g. strutting of *Plantigrada* passes the phases: fool-walking, toe-walking and nail-walking phases. In *Digitigrada* only two phases, and in *Unguligrada* only one phase remained and became fixed when foot touches the ground with the nail tip. Gelei found an illustration for this principle in swimming of the ciliate *Opisthonecta (Peritricha — Sessilia)* with its aboral pole forwards so as its sedentary ancestors swam during their short free life, trying to settle with their scopula on the ground. Some more of such examples could be found in different groups of *Protozoa.*

In my opinion the principle of phase fixation may be treated more broadly, including into it the cases of fixation of the developmental phases of their ancestors

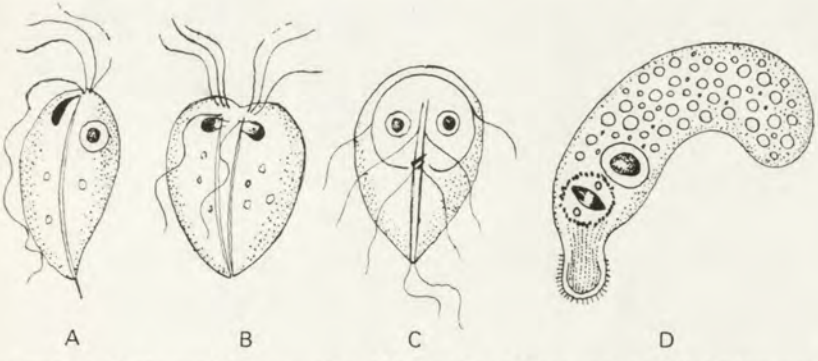


Fig. 4. Fixation of phases: *Monomonadina* (A), dividing *Monomonadina* (B), *Diplomonadina* (C), paranucleus in *Janickina* in the metaphase (D)

as a permanent character of their progeny. I mean here the symptoms of fixation of the division phase as a normal feature of derivative forms. So e.g. the flagellate of a structure type of *Monomonadina* (e.g. *Trichomonas*) has a form of a double organism during its division, with a double karyomastigont and assumes the feature of *Diplomonadina* (Fig. 4. A–C). The arrest of this phase and its fixation may account for formation in evolution such forms as *Lamblia*. A similar process was surely the base of arising of some other multiplied forms as *Polykrikos* and others.

An interesting example of phase fixation, as it seems, is provided by the so-called paranucleus in *Paramoeba* or *Janickina (Amoebozoa)* which remains permanently in anaphase in the trophic individual (Fig. 4 D).

5. Principle of substitution of organs — Kleinenberg

Sewertzoff determines this principle in this way: "an organ well developed and functioning in the ancestors of the given form, becomes degenerated in their progeny and is substituted by another organ which replaces it and assumes its functions". As an example cited is the substitution of the sinuses of the primary body cavity (*Archiannelida*) by blood vessels (*Polychaeta, Oligochaeta*) and the subsequent substitution of blood vessels by the system of lacunae (*Hirudinea*). The substitution of chorda dorsalis by vertebral column in vertebrates is a similar case.

Gelei indicates the cases of substitution of adoral kineties by plasmatic folds in some ciliates. So is in *Allosphaerium (Hypostomata)* or in *Pseudomicrothorax*.

6. Principle of physiological substitution — Fedotov

This principle concerns the cases when an organ assumes one of functions of another organ without assuming its morphological aspect, e.g. the coelom of the ophiuroid *Stegophiura* takes the function of the breeding chamber which is performed by the genital sac in the other *Ophiuroidea*.

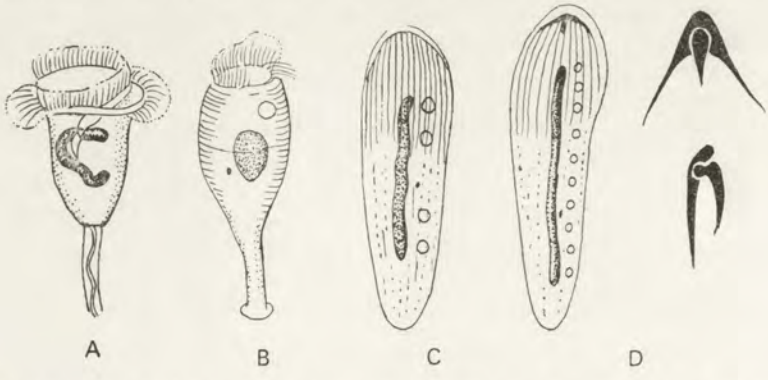


Fig. 5. Physiological substitution: stalk (A) and trunk (B) in *Peritricha — Sessilia*, thigmotactic area (C) and skeletal apparatus (D) in *Astomata*

Gelei has pointed out the substitution of the skeletal function of the supporting ectoplasmatic fibers by the thickened pellicle in *Entodiniomorpha* and the stalk of many *Peritricha* by the elongated body trunk in those which do not posses any stalk (Fig. 5 A, B). An example of physiological substitution may be as well the replacing of the thigmotactic surface area by the clinging skeletal apparatus in *Astomata* or in *Hysterocinetidae* (Fig. 5 C, D).

7. Principle of substitution of function — Sewertzoff

In the Sewertzoff's formulation this principle is as follows: "the functions of the organ are substituted by another ones which are biologically equivalent and performed by another organ situated in another region of the animal body and has

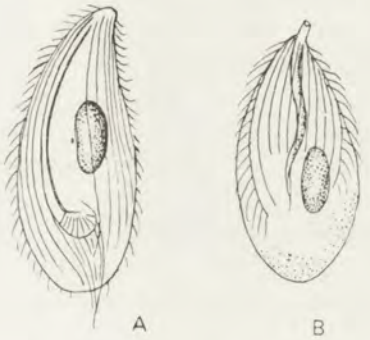


Fig. 6. Substitution of function: the primary mouth and the "bouton adhesif" in *Thigmotricha — Hemispeiridae* (A) and secondary mouth apparatus in *Thigmotricha — Ancistrocomidae* (B)

been developed of another primordium". As example Sewertzoff indicates the substitution of locomotoric function of the lizard limbs by the thoracic muscles and by the scaled covering of the ventral body side in anguis and in snakes.

Gelei indicates the substitution of trichocysts as an active defence organ in ciliates by the thick spiny pellicle of *Entodiniomorpha* as an organ of passive defence. In my opinion, an adequate example would be in this case replacing of the primary mouth in *Thigmotricha* (so called *Stomodea*) which is shifted to the posterior body end and is disappearing finally, by the new food-intake apparatus of *Thigmotricha — Rhynchodea* in the shape of a sucking snout formed by the "bouton adhesif" on the anterior body pole (Fig. 6). A similar example is the substitution of mouth by the sucking tubules in *Suctoria*.

8. Principle of reduction of the number of functions — Sewertzoff

Sewertzoff determines this principle: "simultaneously with the intensification of the main function of any organ, the general number of its functions diminishes". Examples are easy to be found. Gelei has found them among *Protozoa* as transformation of the universal cilia into the steering cilia in some undulipodia, and

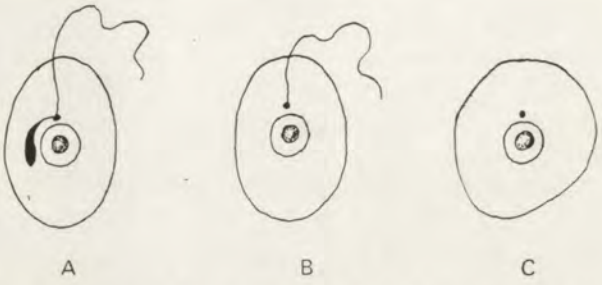


Fig. 7. Reduction of the number of functions: loss of the locomotoric function of the flagellum after the scheme of Chatton — protokinetide (A), mesokinetide (B), metakinetide (C)

atrophy of locomotoric function of cilia in the adoral system where they serve for propelling food. Another striking example following form the concept of Chatton might be cited: the basal bodies of flagella in flagellates performing simultaneously the role of centrosome keep this function as the only one in proportion of atrophy of flagellum i.e. of loss of their locomotoric role (Fig. 7).

9. Principle of extension of function — Plate

Without discussing this principle, the examples of Sewertzoff may be cited concerning the cases when the function of water and food propelling is assumed by the gills of *Lamellibranchiata* besides their respiratory function. Another case is taking the locomotoric function by antennae of *Copepoda* and *Cladocera*, or performing the function of the copulation apparatus by the ventral fin of sharks.

Gelei indicates the additional role of cilia in ciliates in propelling water and food to the mouth, and the excretion function of contractile vacuoles besides their primary osmoregulatory one. I think possible to include here the numerous cases of performing the function of food-intake, accompanied by the atrophy of mouth, by the pellicle of *Protozoa* (or cuticle of *Platyhelminthes*). This means the extension of the function of integument.

10. Principle of the change of function — Dohrn

This old principle means that: "the attenuation of the principal function of an organ and a simultaneous enhancement of the secondary one, alter the general action and shape of the organ. The secondary function becomes the principal one". Sewertzoff gave here an interesting example: the VII and IX limb of abdominal segments are transformed in insects into the ovipositor and finally into sting.

Gelei indicates the cases of loss of motoric functions by cilia and their transformation into sensory organ only, or even into a hampering or anchoring system. Michajłow speaks of adopting a clinging role by flagellum in *Euglenoidina parasitica*.

11. Principle of activation of function — Sewertzoff

This principle is represented by the cases when passive organ becomes an active one. According to Sewertzoff, this occurs when the jaw bones of reptiles especially in snakes, separate and become active.

Gelei indicates as example that the stiff stalk of some *Peritricha* may gain myonemes and become contractile. Unfortunately it is not clear which state was

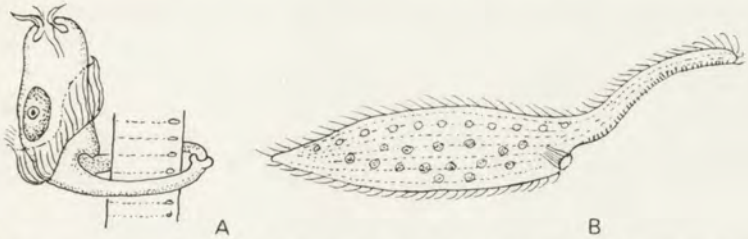


Fig. 8. Activation of function: the "staples" in Allobiophrya (A), proboscis in *Dileptus* (B)

primary and whether this examples illustrates the immobilization or activation of function. A less remote example of activation is provided by formation of "staples" on scopula of some *Peritricha* e.g. in *Ellobiophrya donacis* (Fig. 8 A) or in some *Apiosoma (= Glossatella)*. I believe that mobilization of the anterior body part as the penetrating and killing proboscis in *Dileptus* is an adequate examples (Fig. 8 B).

12. Principle of immobilization of function — Sewertzoff

Sewertzoff presents as example the coalescence of palatoquadratum with skull, and some other similar phenomena. Gelei found difficulties in finding any examples in *Protozoa*.

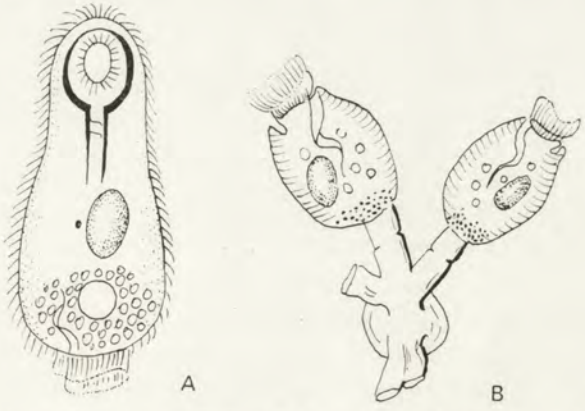


Fig. 9. Immobilization of function: stiffening of the sucker in *Cotylothigma (Thigmotricha — Hysterocinetidae)* — A, stiffening of the stalk in *Obopercularia (Peritricha — Sessilia)* — B

Raabe 1947 believes that stiffening of sucker in various ciliates by formation of skeletal structures fulfills in some way the conditions of this principle. Such a stiffening is observable e.g. in the sucker of *Cotylothigma* when compared with its exit feature in *Hysterocineta (Thigmotricha — Hysterocinetidae)* (Fig. 9).

13. Principle of similarization of function — Sewertzoff

A striking example cited by Sewertzoff is the similarization of lumbal ribs to the pectoral in lizards, accompanying the elongation of thorax and atrophy of limbs which have been the differentiating factor.

Gelei remarks that among *Protozoa* it is difficult to find cases of this principle embracing a regressive process. He indicates however the mutual similarization of cilia in *Opalina* which, in his opinion, is associated with the regression of mouth,

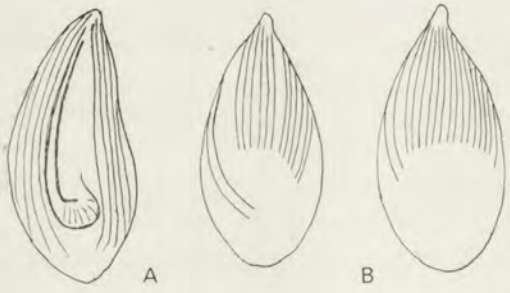


Fig. 10. Similarization of function: of the adoral kineties in *Thigmotricha — Hemispeiridae* (A) to the kineties of the thigmotactic ciliature in *Thigmotricha — Ancistrocomidae* (B)

a factor differentiating the ciliature. This Gelei's example seems not to be well chosen because there are no evidences whether the ancestors of *Opalina* posessed a distinguished adoral ciliature. This seems rather improbable. However the similarization of ciliature associated with regression of mouth occurs surely among *Astomata*. Raabe 1967 has indicated the similarization of adoral kineties, or exactly of the exadoral one, to the thigmotactic ciliature in *Thigmotricha — Ancistrocomidae* when compared with *Hemispeiridae* (Fig. 10).

14. Principle of dissimilarization or differentiation of function and organs — Raabe

This principle proposed by mi (Raabe 1954) for complementation of the principles of Sewertzoff and of Gelei, is the contrary of the principle of similarization of functions and organs. It concerns such cases when the organs constituting a given system, being similar to one another and performing similar or the same functions — differentiate, become separate and begin to perform different functions. This concerns in the first place the polymerized organs e.g. the metameric ones, as the limbs of *Arthropoda*, metameric coelomoducts of *Annelida*, metameric muscles and nerves of vertebrates.

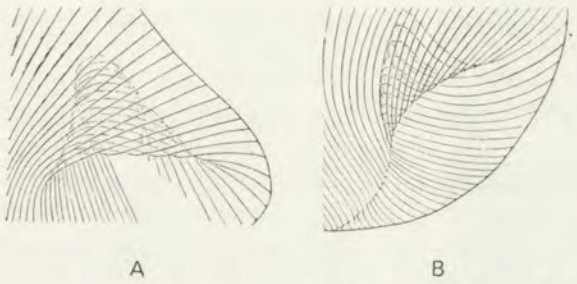


Fig. 11. Dissimilarization of function: forming of the infudibular ciliature in *Thigmotricha — Thigmophryidae — Conchophthirus* (A), *Myxophyllum* (B)

Among *Protozoa*, the most striking examples are provided by the repeating elements of the primarily uniform ciliature of ciliates — their kineties. They differentiate in different manner (Fig. 11). The description of this process would be superfluous.

15. Principle of union of functions or organs — Gelei

Gelei does not mean a complete fusion of organs into one unit but rather a topographic union of organs, mainly of their afferent or efferent ways in the form of a slight paratrematose or exactly — syntrematose. As an example from the world of *Metazoa* serves the union of elements which have arisen independently of one another as: porus excretorius and porus genitalis, or porus excretorius and mouth, and even the union of all the three apertures into the so called cloaca oralis (Mundcloake). The examples of this are provided by some *Turbellaria*. The most striking examples may be found among the sedentary organisms belonging to many different classes and even types: *Bryozoa*, *Crinoidea*, *Cirripedia*, *Ascidiae*.

Similar phenomena of formation of a common exit and inlet ducts in the form of cloaca oralis are found among *Protozoa* — in *Peritricha* (Raabe 1964 b).

16. Principle of association of activity "for the sake of harmony" — Gelei

This somewhat idealistic definition was applied by Gelei to the phenomenon when organs and functions change "entering into the unavoidable contact with other organs". As example Gelei indicates the association of ciliary apparatus with trichocysts, with the excretion pores and with the oral apparatus in ciliates. This principle has not been clearly defined and may embrace many phenomena of coordination in organisms. It may concern in fact the formation of any apparatus if we conceive this idea as an group of organs belonging to different systems which are however associated for performing one function. Such are, among others, the apparatus for food-intake in ciliates, into which are included some parts of the ciliary, myonemal, pinocytic and other systems.

17. Principle of fusion of organs into an another organ — Raabe

This principle has been proposed by me (Raabe 1954) for supplementing the former principle and for distinguishing a phenomenon which consists is the union of organs of a system into one common organ of a higher range, being compact and uniform, belonging still to the same system. Such a union is e.g. formation of the oral complex, which is uniform and undergoes subsequently evolution as a whole, by an array of limbs in *Arthropoda*. A similar example is the union of nephroducts or nephridia into the uniform kidney.

Among *Protozoa* may be indicated the union of ciliary complexes or even complexes of kineties into different types of pseudomembranelles (syncilia) like UM or AZM or cirri.

Similar examples are those which concern the union of axostyles or axodesmata in *Polymastigina (Flagellata)* into a uniform stiffening apparatus, and also the union of single resistant fibers into a compact differentiated system, like in the sucker of *Hysterocinetidae (Ciliata — Thigmotricha)* Raabe 1967.

18. Principle of division of organs and functions — Sewertzoff

The classic example of this principle, chosen by Sewertzoff, is the division of the uniform lateral fold into the paired fins in pre-vertebrates which leads to differentiation and independence of those organs. This principle concerns the essential splitting (Spaltung) of a primarily uniform organ. This is important in connection with the principle of the organs and functions dissociation.

Gelei found here a very striking and rather classic example as the division of the mononuclear system, characteristic for the majority of protozoan groups, into the heteronuclear system of ciliates. This is constituted of macro- and micronucleus, i.e. of two elements of different structure and function, however of the same origin, as well in phylogenesis as in ontogenesis. This example being so excellent, could justify by itself the formulation of organ and function division. Besides this example, *Protozoa* may supply others as well, even in the process of division of the primary uniform ciliary system into different parts performing different functions. Here however the principle of the organ and function division encroaches upon the principle of dissimilarization.

19. Principle of dissociation or separation of organs and functions — Gelei

This principle concerns not the division of single uniform organs but the separation (Trennung) of organs which have been united initially or arose in result of association and corroboration with each other. The example of Gelei is the division of cloaca into anus, sexual and urinary outlet e.g. in phylogenesis of mam-

mals. However, remarked Gelei, the isolation of cells and tissues in *Metazoa* permits the differentiation and separation of organs whereas in *Protozoa* this is impossible because of the absence of cytoplasm separation. Nevertheless, in the opinion of Gelei, the membranelles differentiating in the endoplasmic sac, contractile vacuoles etc. indicate the separation of those parts or products of cytoplasm. The recent years which had provided such a number of valuable data concerning the plasmatic structures, documented many forms of differentiation and separation of organelles and systems in the protozoan organism.

20. Principle of autonomization of functions and organs — Raabe

This principle has been introduced by me (Raabe 1954) for finding the contrary to the principle of union of functions and organs. Those cases are in question when organs comprised by a definite system, or parts of such organs, differentiate, specialize in performing a function, and acquire a morpho-physiological autonomy. Such organs are: e.g. some more independent parts of the vertebrate nervous system as: sympathetic and parasympathetic system or parts of the muscular system as the smooth muscles of the heart-muscle.

Among ciliates, a certain autonomy is manifested by the infundibular parts of ciliary system which respond e.g. in *Paramecium* to the immobilizing factors in another manner than the remaining ciliature.

21. Principle of polymerization — Dogiel, Beklemišev, Raabe

This principle has been introduced by me (Raabe 1954) into the group of principles of morphophysiological changes in organs. This principle had been presented convincingly by the theses of Dogiel and Beklemišev who tried to find one of the most essential factors of evolutionary progress in the polymerization of organs and systems. In consequence of polymerization or multiplication of the body parts or of whole organisms, metameric systems arise when similar parts appear consecutively along the main body axis. Antimeric systems arise when similar parts appear consecutively around the body axis (radial symmetry). Sometimes the polymerization, especially when it concerns some systems only, may fail to have such a regular and arranged character. Beklemišev postulates two ways of formation of metamery and antimery: arrangement of multiplied elements, and the way of a not complete, in some way supressed, division.

Besides the metamery in *Annelida*, *Arthropoda* or antimery in *Coelenterata* and *Echinodermata*, the manifestation of polymery is rather often found in *Protozoa*. It may concern single systems e.g. the osmoregulation–excretory system, the nuclear apparatus, the undulipodial system etc. It may also concern whole body parts and then it assumes the feature of a more or less distinct and arranged metamery or antimery. The evolutionary value of polymerization has been pointed out by me

in my article on the system of *Protozoa* (Raabe 1964 a). I called attention to the great role of polymerization in leading to different close and indispensable unions of multiplied elements by their differentiation and, in consequence, to the integration of polymerized system into a system of a higher range.

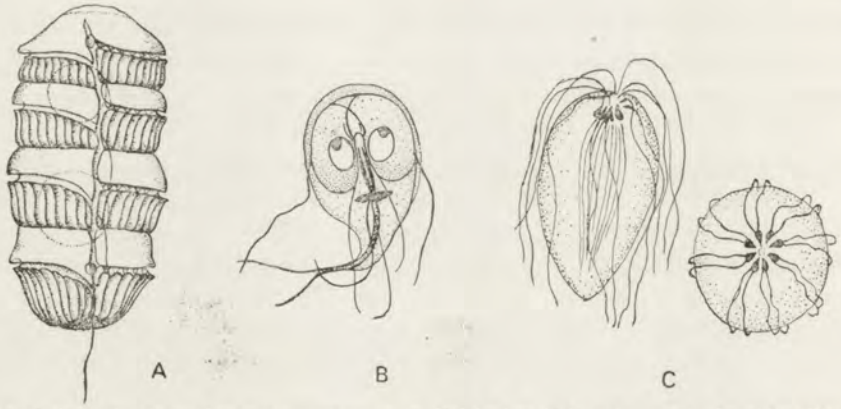


Fig. 12. Polymerization of organs: metamery in *Polykrikos* (A), antymery in *Lamblia* (B) and in *Coronympha* (C)

The manifestation of coordinated metamery among *Protozoa* is the existence of metameric flagellates as *Polykrikos* (Fig. 12 A) and the persistence of division chains in *Ceratium* for a prolonged time as well as the catenulary reproduction in many *Astomata (Ciliata)*.

Example of a more or less regular antimery is the appearing of such forms as *Diplomonadina* and *Calonymphida (Calonympha, Coronympha)* among *Flagellata — Metemonadina* (Fig. 12 B, C).

22. Principle of oligomerization of organs — Dogiel

I include as well this way of evolution, so highly stressed by Dogiel, into the principles of morpho-physiological development. Obviously, oligomerization may occur only in this case when it has been preceded by polymerization since it consists in the diminution of the number of elements occurring repeatedly many times. However this diminution may be achieved in two ways which was not clearly expressed by Dogiel.

The first way is a simple reduction leaving them in such a feature as they have occurred prior to reduction. Consequently it is an only qualitative process. This is the case of vertebra and ribs in different vertebrates, or the reduction of number of segments in many annelids or arthropods.

The second way of oligomerization is in fact leaving a reduced number of elements but not the same which were present previously, only those which arose by union

and integration of the exit elements. Consequently it is as well a qualitative as quantitative process. If e.g. metamers have joined together to form compact tagmata, the general number of repeating elements diminishes but the process of integration has occurred in every tagme and the integration of the whole organism rose to a higher level.

In *Ciliata* the first way of oligomerization of systems which have been polymerized previously manifests e.g. in the reduction of kineties number concurrently with the restriction of their motoric function and with diminution of the organism size (e.g. *Ancistrocomidae*). This is clearly seen in a number of species of the genus *Hypocomella* and presents an extreme effect in the 4 kineties in *Hypocomella quatuor* (Fig. 13).

The second way of reduction of the element number in *Protozoa* is their union and integration: polymerized undulipodia may form less numerous cirri, polymerized vacuoles — excretory systems, polymerized parabasal systems — complex but less numerous systems, polymerized food vacuoles may coalesce into endoplasmatic food sacs as in *Entodiniomorpha*.

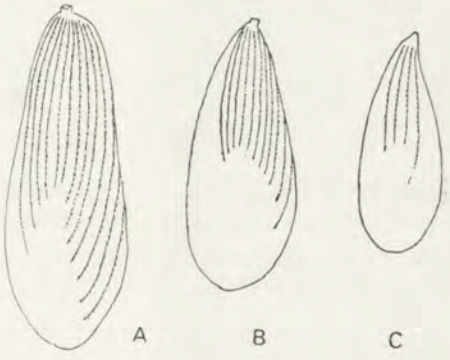


Fig. 13. Oligomerization of organs: reduction of kineties in *Ancistrocomidae (Thigmotricha)* — *Ancistrocoma* (A), *Hypocomella uniondarum* (B), *Hypocomella quatuor* (C)

I would be inclined to introduce here two contrasting morphological and functional regularities occurring in many animal groups, namely the principle of dissymmetrization and symmetrization of organs, of systems or of the whole body.

23. Principle of dissymmetrization of organs (novum)

This is an evolutionary tendency consisting in the loss of symmetric structure of the body which has been gained previously. It may be associated with differentiation mostly of specular symmetric parts, concerning their specialization, or with the adaptation of the body to the medium conditions. Dissymmetrization is expressed e.g. in differentiation of claws in many crabs (*Brachiura*), in differentiation of one arm in some *Cephalopoda* into the hectocotylized arm, displacement of internal organs (liver, heart) in vertebrates, development of one out of two paired organs

(lungs of snakes, femal reproductory organs in birds, aorta arches in mammals and birds etc.). Dissymmetrization is often associated with the spiralization of body as in *Gastropoda* or in the case of abdomen in hermit crabs (*Decapoda — Anomura*).

In ciliates, the examples of dissymmetrization would be all the cases of differentiation of sides of the initially symmetric body e.g. in connection with the shift

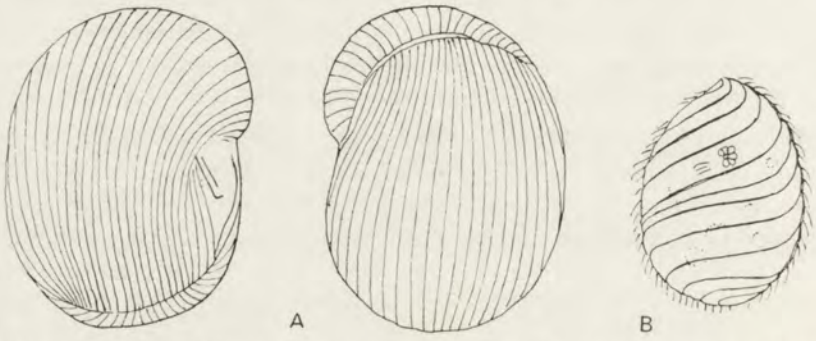


Fig. 14. Dissymetrization of organs: differentiation of the left and right body sides in *Conchophthirus* (A), spiralization in *Apostomea* (B)

of the adoral apparatus or with formation of the thigmotactic zone on one lateral side as it occurs in many *Thigmotricha — Conchophthirus* (Fig. 14 A). Examples of spiralization of the whole ciliary system are found in *Apostomea* (Chatton 1936) (Fig. 14 B) while of spiralization of the adoral apparatus — in many ciliates as well in *Holotricha* as in *Spirotricha* or *Peritricha*.

24. Principle of symmetrization of organs (novum)

This is an evolutionary tendency, not so broad as the former one, manifested in gaining symmetry by asymmetric systems e.g. by the change of the life mode or by reconstruction of the general architecture of organism. The best known examples of symmetrization are supplied by the sedentary groups which descend of bilateral or asymmetric ancestors. They develop an axial or radial symmetry. This

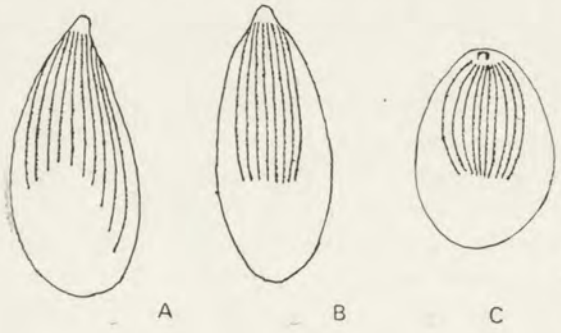


Fig. 15. Symmetrization: secondary symmetrization of the thigmotactic ciliature in *Ancistrocomidae* (*Thigmotricha*) — *Hypocomella* (A), *Enerthecoma* (B), *Hypocomina* (C)

was the case with *Echinodermata* and is expressed in a secondary way in some sedentary *Holothurioidea*.

In *Protozoa* this type of symmetrization (rather external) has been gained e.g. by *Peritricha* which descend, according to Chatton, of bilateral ancestors. Approaching to the bilateral symmetry of some systems may be perceived e.g. in the proces of formation of symmetry in the thigmotactic system of some *Ancistrocomidae (Ciliata — Thigmotricha)* (Fig. 15). This symmetry is related to the symmetry plane, perpendicular to the former one which disappeared in the earlier stages of evolutionary development (Raabe 1963).

25. Principle of reduction of organs — Gelei

Sewertzoff has discussed the rudimentation of organs and their complete atrophy or aphanization not included into the number of his principles concerning the changes in organs and in their functions. Sewertzoff found with no difficulty many examples of rudimentation and aphanization among vertebrates. The number of examples has been highly enriched by Michajłow 1960, 1968 because the material of his investigations — the parasites, supply them in a great number, in the first place, the parasites of different helminths groups. Gelei is puzzled by the negative results of his attempts to find rudimentary organs in *Protozoa*. This suggests him the conclusion that "everything which has lost its biological importance in *Protozoa* disappears soon".

In my opinion, rudimentary organs in ciliates might be found, however not functioning, or not acting in the line of their former function. As such may be considered the traces of the adoral kineties in some *Ancistrocomidae (Thigmotricha)* as e.g. in *Insignicoma* or in *Hypocomides* (Raabe 1967). The most distinct examples are here however the cases of reduction of the ciliary apparatus in *Suctoria* or in *Sphenophryidae* among *Thigmotricha*. In *Sphenophryidae*, despite the atrophy of cilia, the kineties with kinetosomes remain. In *Suctoria* — dispersed kinetosomes, not constituting a definite system are present. In tomites those systems arise a-new from the remaining primordia.

26. Principle: new activity, new functions — Gelei

This principle, so arduously postulated by Gelei, and opposed to all the others which assume that everything changes but nothing arises a-new, may be treated, equally as the other principles, which is presumably justified for the above reasons. In his discussion Gelei indicates the great role of mesoderm or mesenchyme formation and of the new organs and systems which are formed from them. Among *Protozoa* Gelei points out all those organelles which are not embraced by all the elementary and primitive organs i.e. peristome and cytopharynx (besides the pri-

mary cytostome) as well as the supplementary excretory system etc. In this way, an unusually great number of organs and organelles should be considered as new ones if the boundaries of cladus or of another broad group were trespassed.

A revue has been presented of the Sewertzoff's principles of the morpho-physiological organ changes in evolution, and of their complementation by Gelei as well as of some further additions which were brought to my mind in the course of the above considerations and were taken as well from the *Metazoa* world as that of *Protozoa*. Let us revue now their results.

The majority of those principles may be arrayed into antithetic pairs of phenomena, opposed one to the other. Those pairs indicate the possibility of two-directional course of the given evolutionary process. Indeed, a quite distinct opposition exists in the following pairs of processes:

intensification of function	— attenuation of function
fixation of phases	— atrophy of intermediating function
extension of function	— reduction of the number of functions
activization of function	— immobilization of function
similarization of function	— dissimilarization of function
association of function	— autonomization of function
separation of organs and functions	— fusion of organs and functions
division of organs and functions	— union of organs and functions
polymerization of organs	— oligomerization of organs
symmetrization of organs	— dissymmetrization of organs
reduction of organs	— new organs, new functions.

The principles: of organ substitution, physiological substitution, substitution of function and changes of function — are of their vary nature non-directional and may occur in different replaceable ways.

The number of similar pairs of phenomena might increase when considering such general phenomena as integration and disintegration, centralization and decentralization, or some special phenomena characteristic only for some animal groups. Some others contrasting evolutionary processes might also be conceived. Consequently it is the full assembly of evolutionary phenomena which are reflected in the structure and function of organisms and organs, with a sufficiently broad occurrence to be differentiated and accepted as "principles of development". This assembly is complemented by the fact that really in the majority of evolutionary processes not the only principle but several of them simultaneously are concerned. Those principles rarely or never are manifested as single ones but rather in assemblies.

If it is so and if it may be so or inversely, i.e. if the antithetic principles may always be found, if the gradually detected and formulated principles are so various

and so far from being exhausted — is their detecting, formulating and determining of any value ? Is it usefull to enlarge their number and to study in which cases the single principle and their assemblies are correct and real ?

It should be however assumed that distinguishing of the above regularities presents something more than analysis and systematization. If those principles cannot claim for a value of general and generally important evolutionary rules, nevertheless they appear in many animal groups and concern different organs consequently they represent some more general phenomena. Their extension should be proved by the examples of many other animal groups and the principles, more precisely determined. Confrontation of those principles and their groups, their comparison and contrasting permit the present more exactly the evolutionary way of the given group. It provides also a better orientation in the multidirectional character of the evolution of neighbouring groups and of the complicated branching of the evolutionary tree.

More so. The awareness of existence of those various possibilities in the individual morphogenesis and in phylogenesis of organs, enable the morphologist or morphophysiologist to pay attention to those phenomena, to analyse more effectively the conditions observed and to notice some facts which were often omitted. It contributes to an excellent amplification of the recognition apparatus, and to a comparative morphological or comparative physiological description, to enrichment of the picture of the studied phenomena. So without "erecting superfluous chapels" for the principles in discussion, we should treat them as a valuable tool of the investigation work for the morphophysiologist, as a help in orientation in many complex processes with which he has to deal. At last, they facilitate the systematization and transmission of his observations i.e. the scientifical documentation and information.

The formulation and nomenclature of those principles are of no minor, and perhaps even of major value in the systematic considerations and in phylogenetical research, not by revealing new facts and data but because, as stated above, they permit to a more exact presentation and systematization of the evolutionary trends. Especially valuable may be the characteristic of groups or illustration of their evolution by means of the notions and terms introduced in this subject.

Many times, and recently in my work on the *Protozoa* system (Raabe 1964) and in the introduction to the monograph of *Thigmotricha* (Raabe 1967), I called attention to the adequacy of dynamic and not static characterization of the taxonomic groups. Instead of speaking or writing that a group is characterized by some definite features, it is more suitable to determine that in this group definite evolutionary processes are manifested, or that definite evolutionary trends are reflected in it, occurring with different intensity and juxtapositions.

Manifestation of different evolutionary tendencies in different complexes in a given group, permits not only to characterize this group but also to reveal its internal differentiation. If in the whole group the tendencies A, B, C are present in different complexes, then the subordinate groups may be arranged according

to the manifestation of the tendencies A+B in the first, B+C in the second, C+A in the third, and A+B+C in the fourth group. In the other groups only one tendency may be present and finally in the exit group, no one of those tendencies may be, as yet, manifested.

As example of visualization of such systems is the division of *Thigmotricha* into families and the division of *Hemispeiridae — Ancistrinae* into genera, based on the specific tendencies of those groups. It should be pointed out that in the diagram of *Thigmotricha*, the intersection of 3 circles which represent 3 tendencies occurs (Fig. 16 A). Consequently they all are realized in the central area. In the diagram visualizing *Ancistrinae*, the system represents a rotatory epicycloid, and consequently

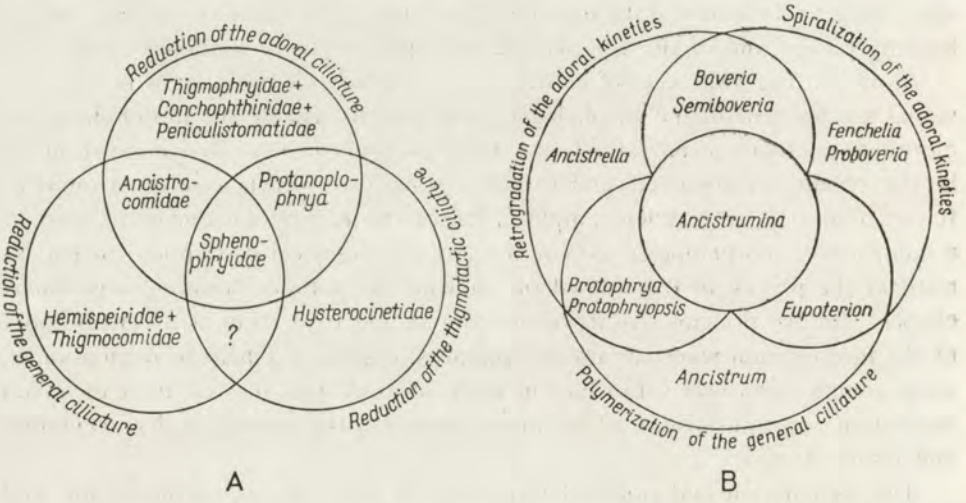


Fig. 16. Schemata of the interrelation of three evolutionary tendencies — explanation in the text

the central area represents a group which is not characterized by any of the three tendencies in question (Fig. 16 B). This method of graphic representation of interrelations, fails — unfortunately — to concern the intensity of tendencies observed in the single subordinated groups. For this purpose another diagram should have been constructed which in this case was not indispensable. My results are only one of possibilities of graphic solution, postulating some others of a better construction and clearness.

The above remarks concerning the principles of Sewertzoff and their supplementations have been presented by me to the attention and considerations of readers, seeing in them an appropriate tool in the morphophysiological, morphogenetical and evolutionary studies. The possibility of their application has been shown by the communications of Michajłow and of Raabe held on the III International

Conference in Protozoology in Leningrad, July 1969. The authors introduced in them some of the notions and formulations of regularities in evolution of *Flagellata — Euglenoidina parasitica* and *Ciliata — Thigmotricha* respectively, as they were observed by them.

Summary

Sewertzoff's morphogenetic principles and their later complements by Gelei and Raabe were discussed. They were complemented with some new principles and classified and provided with examples of protozoans world.

The author suggests quite a usefulness of those principles for phylogenetic and systematic studies, and for suitable, dynamic characterizing the higher taxons of animal world and protozoans too.

STRESZCZENIE

Poddano rozważaniu tzw. zasady morfogenetyczne Sewertzoff'a i późniejsze do nich uzupełnienia poczynione przez Geleia i Raabego. Uzupełniono je pewnymi nowymi zasadami i uporządkowano oraz zaopatrzono w przykłady ze świata pierwotniaków. Autor sugeruje znaczną przydatność przyjęcia tych zasad dla rozważań filogenetycznych i systematycznych, jak i dla dogodnego, dynamicznego charakteryzowania wyższych taksonów świata zwierzęcego, również i pierwotniaków.

REFERENCES

Beklemišev V. N. 1952: Osnovy sravnitelnoj anatomii bespozvonočnih. Moskva.
Chatton E. 1931: Essai d'un schéma de l'energide d'après une image objective et synthétique: le Dinoflagellé *Polykrikos schwartzi* Bütschli. Archo zool. ital., 16, 169–187.
Chatton E. 1936: Les migrateurs horizontalement polarisés de certaines péritriches. Mém. Mus Roy. Hist. nat. Belge, (2), 3. 910–940.
Dogiel V. A. 1929: Polymerization als ein Prinzip der progressiven Entwicklung bei Protozoen. Biol. Zbl., 49, 451–469.
Dogiel V. A. 1936: Oligomerizacija gomologičnyh organov kak odin iz processov evolucii životnyh organizmov. Archs Anat. Histol. Embryol. 15.
Gelei J. 1950: Die Morphogenese der Einzeller mit Rucksicht auf die morphogenetischen Prinzipien von Sewertzoff. Acta biol. hung., 1, 69–134.
Kuźnicki L., Jahn T. L. and Fonseca J. R. 1970: Helical nature of the ciliary beat of *Paramecium multimicronucleatum*. J. Protozool., 17, 16–24.
Michajłow W. 1960: Pasożytnictwo a ewolucja. PWN, Warszawa, 218–223.
Michajłow W. 1968: Zarys parazytologii ewolucyjnej. PWN, Warszawa 267–274.
Michajłow W. 1969: Evolution of parasitism in *Euglenoidina* parasitic in *Copepoda*. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, Nauka, Leningrad 1969, 9–10.
Raabe Z. 1947: Les voies des adaptations à la vie parasitique chez les ciliés. Annls Univ. Mariae Curie-Skłodowska, Sectio C, Lublin, 2.
Raabe Z. 1954: Morfologiczne zasady Sjewercowa w oczach protozoologa J. Geleia. Kosmos, Warszawa, 3.
Raabe Z. 1963: Systematics of the family *Urceolariidae* Dujardin, 1841. Acta Protozool., 1, 121–138

Raabe Z. 1964 a: Remarks on the principles and outline of the system of *Protozoa*. Acta Protozool., 2, 1–18.
Raabe Z. 1964 b: The taxonomic position and rank of *Peritricha*. Acta Protozool., 2, 19–32.
Raabe Z. 1967: Ordo *Thigmotricha (Ciliata, Holotricha)*. I. Acta Protozool., 5, 1–36.
Raabe Z. 1966: Les processus morphogénétiques chez les Ciliés thigmotriches. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, Nauka, Leningrad 1969, 83–85.
Sewertzoff A. N. 1931: Die morphologische Gesetzmässigkeiten der Evolution. Jena.

ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 1.VIII.1971 FASC. 2

Department of Systematic Zoology, University of Łódź, Łódź, Nowopołudniowa 14/16, Poland

Maria WOLSKA

Studies on the family *Blepharocorythidae* Hsiung. V. A review of genera and species

Badania nad rodziną *Blepharocorythidae* Hsiung. V. Przegląd rodzajów i gatunków

The aim of the present paper is to review and complete the descriptions of the genera of the family *Blepharocorythidae* Hsiung. In the case of the genus *Blepharocorys* Bundle the descriptions of particular species, considerably completed, especially with the data concerning their ciliature, are given.

A review of the genera of the family *Blepharocorythidae* Hsiung was based mainly on the own material. Only the data concerning the genera *Charonnautes* Strelkov, 1939 and *Ochoterenaia* Chavarria, 1933 were taken from the literature because their representatives were absent in the author's material.

Besides the genera known up to date the two new, monospecific genera *Raabena* Wolska, 1967 and *Pararaabena* Wolska, 1968, have been included in the family *Blepharocorythidae* Hsiung. In such way the family *Blepharocorythidae*, enriched in these two genera, presents an interesting continuous sequence of forms with clearly seen direction of development of buccal ciliature.

An analysis of the buccal and somatic ciliature may serve to make up a hypotheses on the origin of the family, its evolution and relationships, and to find out its proper position within the system of *Ciliata*.

The genus *Spirocorys* Wolska, 1969 has been tentatively included in the family *Blepharocorythidae*.

Material and methods

The material for description of the species and genus *Blepharocorys* consisted of the ciliates collected from the content of coecum and colon of a horse and from faeces of a horse and American tapir.

Nonstained protozoans, fixed in formalin, were examined with phase-contrast microscope. A part of the material, fixed in Schaudinn's solution, was stained with iron hematoxylin and Mayer's hemalaun. Most part of the ciliates was examined after impregnation with silver solution of Bielszowski and Rio Hortega (the same methods as in my previous papers).

Silver impregnation method, applied to the representatives of *Blepharocorythidae*, gave the possibility to complete and precise the generic and specific diagnoses and the characteristic of the family as well.

Results

The family *Blepharocorythidae* was created by Hsiung 1929 for the genera *Blepharocorys* Bundle, 1895 and *Charonina* Strand, 1928 (syn.: *Charon* Jameson, 1925). For the changes in the family range, before the new genera have been included by the author, see Wolska 1966 and 1967 a.

The original diagnosis of the family *Blepharocorythidae* was as follows: *Trichostomata*. Body elongated, flattened laterally; cytostome situated ventrally at the anterior body part, long oesophagus coated with cilia; the bundles of cilia confined to the anterior and posterior body ends; one contractile vacuole in the posterior body end.

Strelkov 1939 in his monography added the presence of the frontal lobe to the characteristic of the family.

The silver impregnation method allowed to precise the character of the somatic and buccal ciliature as well. It revealed also a group of kinetosomes (to which I attribute a filogenetic importance) very characteristic of *Blepharocorythidae*, situated on a protuberance of cytoplasm (over vacuole ?) and originating from the anterior group of cilia. I have called this group special kinetosomes or kineties. This group of special kinetosomes was noticed in all examined representatives of the family. The species *Ochoterenaia appendiculata* and *Charonnautes equi* were not examined but I suppose they are not the exceptions. So the presence of special kinetosomes ought to be included to the characteristic of the family. Due to silver impregnation method the character of the morphogenesis can also be estimated.

Due to silver impregnation method we can say now more about the so-called bundles of cilia. The somatic ciliature appears in a form of shorter or longer bands or zones composed of parallel kineties, oblique to the long axis of the zone and usually shorter than the length of the zone. In particular genera the ciliature of the posterior body end may be more or less abundant and is composed of two zones, the dorsal and ventral ones, or it is limited to one zone only. The buccal ciliature is also more or less abundant, not differentiated or composed of two distinct groups. Comparying the genera *Raabena*, *Pararaabena* and *Blepharocorys* we can understand the way of formation of so strange, long buccal kineties in *Blepharocorys* and to characterize the direction of development of the buccal ciliature (Wolska 1968).

In the descriptions of species and genera the denotations used in the present paper are the same as in the previous papers of the author. In *Blepharocorys* the body is laterally flattened and the edge at which the frontal lobe is situated corresponds to the dorsal side. A part of cytoplasm, surrounding the buccal overture (not cytostome) from the ventral and ventro-lateral sides is called the ventral lip. The ciliary zone situated on the ventral lip — the labial zone, *l. z.* The ciliary zone at the base of the frontal lobe — the frontal zone, *f. z.* A zone or zones of cilia on the posterior part of the body — the caudal zones, *c. z.* A protuberance of the cyto-

plasm, lying at the ventral margin of the anterior body part — the rudimental vacuole (R). The kinetosomes situated on the resting vacuole — the special kineties, *sp. k.* As in the species of the genus *Blepharocorys* the buccal ciliature is composed of two groups of cilia (Wolska 1966), situated in a deep, funnel-shaped cavity, they will be denoted — the anterior buccal zone (or a zone of short kineties), *a. b. z.* and the posterior buccal zone (or long kineties), *p. b. z.*, see scheme (Fig. 1). In the

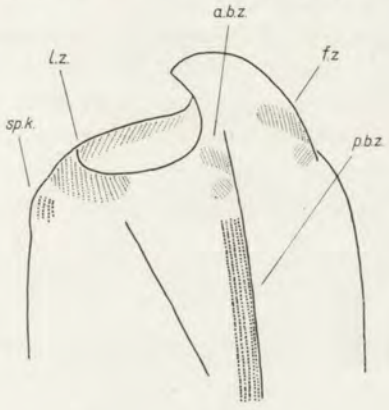


Fig. 1. Scheme of the anterior somatic and oral ciliature in the representative of the genus *Blepharocorys*

primitive species *Raabena bella*, in which the buccal ciliature is a continuation of the frontal zone, it is called the fronto-buccal zone, *f. b. z.* Nonidentificated silver impregnating element occurring in the buccal cavity of the genus *Blepharocorys*, sometimes ladder-shaped, is called an argentophilic streak.

Systematic review

Family *Blepharocorythidae* Hsiung, 1929

Body elongated, noncontractile, covered with rigid pellicle, usually laterally flattened. The buccal overture, situated in the anterior body part, leads to a vast funnel-shaped concavity. At the dorsal side the buccal overture is protected by the frontal lobe of various shape and at the ventral and lateral sides it is surrounded by the ventral lip. In the antero-ventral part of the body there is a small protuberance, the so-called rudimental vacuole. The contractile vacuole is situated in the posterior body part near the cytopyge. Strongly reduced somatic ciliature is restricted to the anterior and posterior parts of the body. On the anterior part of the body, two ciliary zones occur, one of them is situated on the frontal lobe (the frontal zone), and the other one on the ventral lip (the labial zone). On the posterior part of the body there are one or two ciliary zones (the caudal zones). The buccal ciliature is independent or connected with the frontal ciliary zone. A small group of special kinetosomes occurs on the rudimental vacuole (R).

Type of the family: *Blepharocorys* Bundle, 1895.

Genus *Blepharocorys* Bundle, 1895

The asymmetrical, noncontractile, elongated body, laterally flattened. On the dorsal side, over the buccal overture, rises the frontal lobe. The somatic ciliature is limited to the frontal, labial and one caudal zone, the last one being situated dorsally or ventrally to the cytopyge. The buccal concavity reaches, at least, to the mid-length of the body.

The buccal ciliature is composed of two groups of cilia. The nonciliated part of the buccal concavity is lined with fibers.

In the anterior part of the body, on the ventral side, there is the vacuole "R" with special kinetosomes. One contractile vacuole in the posterior part of the body, near the cytopyge.

Type of the genus: *Blepharocorys uncinata* (Fiorentini, 1890).

Blepharocorys uncinata (Fiorentini, 1890) (Fig. 2, Pl. 1–8)

Synonyms: *Diplodinium uncinatum* Fiorentini, 1890; *Blepharocorys equi* Schumacher, 1915.

Full description of the species was given by Schumacher 1915 (under the name of *B. equi*) together with the drawing of dividing protozoon. For the second time it was described by Hsiung 1930 b and synonymized with the species *B. uncinata*. Strelkov 1939, in his monography on ciliates from the intestine of *Equidae* based on vast materials, agreed with Hsiung's opinion.

Grain 1966 examined *B. uncinata* under the electron microscope; his studies concerned mainly the structure of fibers.

The protozoon is covered with rigid pellicle forming longitudinal slats. Body elongated, slightly narrowing to the posterior end. The frontal lobe surrounds the buccal overture from the dorsal side and spreads to the left side in the shape of a plate with rounded edges. On the ventral and right side the ventral lip forms a convection. The buccal concavity is in form of a funnel opening between the frontal lobe and ventral lip. It is directed backward to the dorsal side. In its final part the funnel bends towards the ventral side.

A rigid, immobile processus, in the shape of a corkscrew, protrudes from the apical part of the frontal lobe.

Heart-shaped macronucleus lies in the anterior part of the body near to the right side. Small, oval micronucleus adheres to the macronucleus, most frequently to its dorsal side. One contractile vacuole in the posterior part of the body. Cytopyge terminal at the posterior end.

Results of silver impregnation. The frontal ciliary zone lies on the dorsal side of the frontal lobe, mainly on its left side and only a small posterior portion of it passes on the right side. On the left side of the body the frontal zone reaches the base of the corkscrew-like processus. The labial zone lies on the left side of the ventral lip. Both these zones are in the shape of a stripe composed of short, parallel kineties

obliquely arranged towards the long axis of the zone (Fig. 2). The caudal ciliary zone is the same; it is situated on the left side of the protozoon, ventrally to cytopyge (Pl. I 1).

At the ventral margin, backward to the labial zone, there are some short kineties (special kineties) on a small protuberance. Their kinetosomes are big, more loosely or densely arranged here and there (Pl. I 2).

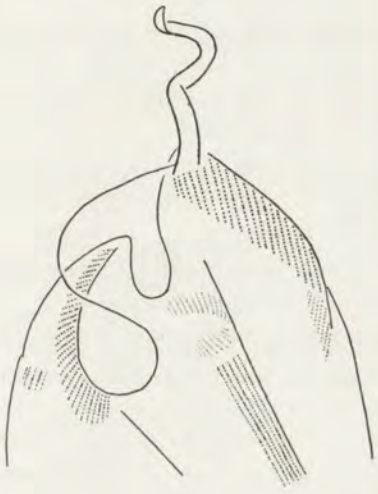


Fig. 2. *Blepharocorys uncinata* (Fiorentini, 1890); anterior part from the left side, scheme of infraciliature

Buccal ciliature is composed of two groups of cilia. In the anterior enlarged part of the buccal concavity there is a small zone of cilia, similar to the somatic zones. This ribbon-like zone runs obliquely across the left side of the concavity, passes on to the dorsal side and then to the right side (Fig. 2). This zone poorly impregnates with silver and is difficult to observe during interdivision period. It was ascertained only in a few specimens (Pl. I 3). Particular kineties are difficult to distinguish. The primordium of this zone may be seen during the division of the ciliate (Pl. I 4), then the particular kineties are less dense.

Posteriorly to this zone, on the dorsal side of funnel-like cavity, there are some long, well impregnating kineties (usually 5 were visible) with very dense kinetosomes (Pl. I 5). From these kineties run away semicircular fibers which support the non-ciliated left side of the funnel and partly pass on its right side (Pl. I 6).

Numerous fibers in the anterior part of the concavity form a very complicated pattern. Grain 1966 distinguished there a thick fiber supporting the corkscrew-like processus and a system of fibers in the frontal lobe ("lame en evantail"). He suggested that the fiber supporting the processus begins at the long oral kineties and the fibers in the anterior lobe originate from the base of the lobe.

Silver impregnated preparations indicate that the fiber of the "corkscrew" and the fibers of the frontal lobe make up one system and are not connected with kinetosomes. The fiber of the processus is formed by a thick bundle of fine fibers which

are strongly compressed inside this processus. At the base of the processus this bundle divides into three strands of more loosely arranged fibers. One of these strands runs backward along the dorsal side of the buccal concavity, approaches to the anterior buccal zone, passes on to the right wall of the concavity and surrounds the right side of the ventral lip. The second strand directs to the left part of the frontal lobe. At first it runs in a distance from the margin of the lobe, then it passes on the left margin of the ventral lip. At the top of the ventral lip both these strands meet together. The third, short strand runs to the dorsal side and ends under the kineties of the frontal lobe. These strands of fibers branch away, especially the strand entering the left part of the frontal lobe. These interlacing branches of fibers form a strongly complicated system. Independently of this system of fibers, less numerous, short fibers spread from the dorsal wall of the buccal concavity. Two of them run along the margins of the anterior oral zone. Their course, although not clear, is seen in the photograph 7 (Pl. I).

These are not all the fibers in *B. uncinata* but they will not be encountered here because the method used for the study does not allow to follow their arrangement and origin on account of their abundance and complicated network they form.

Strongly impregnating streak occurs on the left wall of the buccal funnel but not much can be said about it. This streak begins on the ventral wall in the anterior part of the funnel (Pl. I 8). The fibers are also connected with the caudal somatic zone. They go onward from the anterior margin of the zone, parallelly to the body surface.

Dimensions: body length 40–63 μ, width 18–27 μ.

Occurrence: coecum and colon of a horse (Hsiung 1930 b, Strelkov 1939). Found by the author in the coecum of a horse.

Blepharocorys jubata Bundle, 1895 (Fig. 3, Pl. II 9–12)

Body egg-shaped in outline, the posterior end more tapered than the anterior one. Rigid pellicle with longitudinal slats. The frontal lobe bends on the ventral side and forms a convex roof over the buccal overture. The right margin of the lobe is smooth, the left one has two denticles (Fig. 3). The ventral lip forms a convexity on the right body side. Backward to it there is a protuberance of the vacuole "R". Cytopyge at the posterior end, somewhat shifted dorsally. Near to it lies the contractile vacuole. Oval macronucleus is situated in the anterior part of the body to the right from the buccal concavity and more closely to the dorsal side. Small, rounded micronucleus lies in a depression in the macronucleus (mostly on the ventral side of Ma). The buccal concavity in form of a funnel slightly narrowing and directed dorsally.

Results of silver impregnation. The frontal ciliary zone is rather large and situated mainly on the left side of the frontal lobe. Only a portion of it passes over the dorsal edge of the frontal lobe to the right side and ends in a small depression.

The labial zone surrounds the margin of the buccal overture ventrally and partly its left and right sides. The posterior margin of the zone lies at the same level on the right, ventral and left sides while the anterior margin ascends on the ventral side and descends on the left and right sides according to the shape of the buccal overture. The frontal and labial zones are composed of parallel kineties, oblique to the long axis of the zone. On the surface of the "R" vacuole there are some (5–6) short kineties with big kinetosomes — the special kineties (Fig. 3). The caudal zone is small and situated ventrally to the cytopyge within a small depression on the left side of the body. Sometimes a part of cilia of this zone, somewhat longer and situated more dorsally, may cling together to form a bundle — syncilium (Pl. II 10).

Small anterior oral zone, composed of short kineties (Pl. II 11, 12), is situated dorsally in the anterior part of the buccal concavity. This zone is inconspicuous but well visible in dividing specimens. Five long oral kineties go backward from the anterior zone, along the dorsal and right side of the buccal concavity. These kineties strongly impregnate with silver. Four of them make up a compact group, they run parallelly to the end of the buccal concavity. One kinety, somewhat shorter, bends to the ventral side (Fig. 3). Four long kineties in their final segments approach each other and turn out to the ventral side. Left, ventral and partly right wall of the

Fig. 3. *Blepharocorys jubata* Bundle, 1895; anterior part from the left side, scheme of infraciliature

infundibulum is lined with semicircular fibers beginning at one of the long kineties. On the dorsal wall of the anterior part of the buccal concavity begin other fibers which run to the frontal lobe. The left denticulated margin of the frontal lobe is supported by the group of strongly impregnating fibers. A narrow argentophilic streak lies on the ventral wall of the buccal concavity.

Dimensions: body length 39–61 μ, width 19–25 μ.

Occurrence: coecum and colon of a horse (Hsiung 1930 b, Strelkov 1939), colon of a zebra (Strelkov 1931). Found by the author in the coecum of a horse.

Blepharocorys valvata (Fiorentini, 1890) (Fig. 4, Pl. II 13–16)

Synonyms: *Entodinium valvatum* Fiorentini, 1890; *Blepharocorys microcorys* Gassovsky, 1919.

Body elongated with somewhat narrowed posterior end and concave dorsal side. The anterior part of the body ventrally truncated and provided with the triangular frontal lobe at the dorsal side. Cytopyge at the posterior end, somewhat shifted to the dorsal side; it is situated posteriorly to the caudal ciliary zone lying in a small depression.

Oval macronucleus lies in the anterior body part to the right of the buccal concavity. Rounded micronucleus is pressed into a depression in the macronucleus. Contractile vacuole in the posterior body part near to the cytopyge. Buccal concavity slightly bent in form of S. The protuberance of "R" vacuole slightly marked.

Results of silver impregnation. The frontal ciliary zone, in form of a ribbon of oblique kineties, runs nearly transversally over the base of the frontal lobe in a shallow depression. Its larger segment is situated on the left side and the shorter one on the right side (Fig. 4). The labial zone reaches the right margin of the buccal overture up to the base of the frontal lobe.

Fig. 4. *Blepharocorys valvata* (Fiorentini, 1890); — anterior part from the left side, scheme of infraciliature

Special kinetosomes are grouped in few thick and short kineties (Fig. 4).

The caudal zone, similarly as the other somatic zones, is composed of short kineties; it is small and situated in a depression (Pl. II 14).

The anterior oral zone well developed and elongated in comparison with the species described above. It is usually well seen after impregnation. The long axis of the zone is nearly parallel to the body axis (Pl. II 14, 15). The long oral kineties differ somewhat from those described in *B. jubata*. They begin near the anterior zone on the dorsal side and pass directly to the ventral side of the buccal funnel. One of these kineties is very short and somewhat distant from others. Similarly as in other species the nonciliated wall of oral funnel is supported by semicircular fibers (Pl. II 16).

Dimensions: body length 41–67 μ, width 22–36 μ.

Occurrence: coecum and colon of a horse (Hsiung 1930 b, Strelkov 1939). Found by the author in the colon of a horse.

Blepharocorys angusta Gassovsky, 1918 (Fig. 5, Pl. V 17)

Ciliate with very slender body, the ventral and dorsal margins almost without convections. The body widest in the anterior part gradually tapering backward. The frontal lobe strongly bent ventrally. Oval macronucleus is situated in the anterior part of the body near to the ventral margin, micronucleus pressed into a depression in the anterior part of the macronucleus. The shape of the buccal concavity similar to that in *B. valvata*. Cytopyge at the posterior body pole, somewhat shifted to the dorsal side. Contractile vacuole in the posterior body part.

Results of silver impregnation. The character and arrangement of the anterior somatic and buccal zones as well as of the fibers connected with them bear strong resemblance to those in *B. valvata*. Special kinetosomes are grouped in few very short, strongly impregnating kineties (the same in *B. valvata*). The anterior buccal zone well developed and situated in similar way as in *B. valvata*. Long oral kineties begin on the dorsal wall of buccal funnel and run to the ventral wall (Fig. 5).

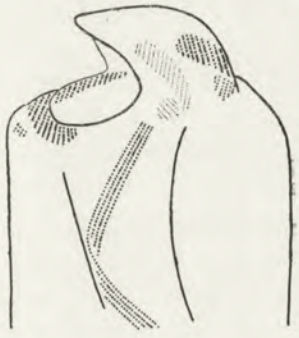


Fig. 5. *Blepharocorys angusta* Gassovsky, 1918; anterior part from the left side, scheme of infraciliature

Small caudal ciliary zone situated terminally on the left side of the body.

Dimensions: body length 45–65 μ, width 14–31 μ.

Occurrence: colon of a horse (Hsiung 1930 b), colon of a horse, donkey, and zebra (Strelkov 1939). Found by the author in the colon and faeces of a horse.

Blepharocorys curvigula Gassovsky, 1918 (Fig. 6, Pl. III 20–29)

The largest species within the genus *Blepharocorys*. Body oval in outline (Pl. III 20). The frontal lobe strongly bent to the ventral side. Oval macronucleus in the anterior body part, closely to the right and dorsal sides. Micronucleus pressed into a depression in the ventral side of the macronucleus. Contractile vacuole at the

posterior pole. Cytopyge situated dorsally in relation to the caudal ciliary zone. Buccal concavity strongly bent ventrally in its final part and overpassing the mid-length of the body. Its structure is complicated. Two parts may be distinguished. The calyx-shaped anterior part is situated directly behind the buccal overture near to the right side of the body. This part is connected with another one which is tubular in shape. The tubular part runs backward to the dorsal side (Fig. 6). In its distal segment the tube turns back to the ventral side.

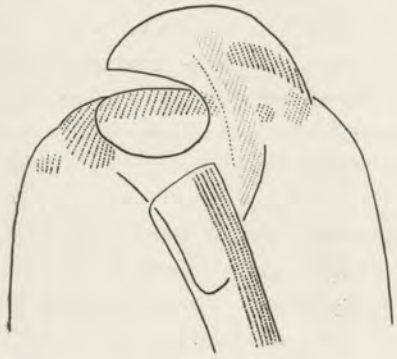


Fig. 6. *Blepharocorys curvigula* Gassovsky, 1918; anterior part from the left side, scheme of infraciliature

Results of silver impregnation. The frontal ciliary zone lies at the base of the frontal lobe, mostly on the left side. The labial zone is ribbon-like elongated and situated mostly on the right margin of the buccal overture. Only a part of it overpasses to the left side. Special kinetosomes constitute a group of 5–6 kineties. They are conspicuous and well impregnating with silver. The caudal zone, in form of a long ribbon, lies on the left side of the ciliate body, in a depression.

On the dorsal wall of the anterior, calyx-shaped part of the buccal concavity very long anterior oral zone (a zone of short kineties) occurs. It runs in arc along the dorsal wall (Fig. 6). Usually it is well impregnating with silver (Pl. III 21). The internal edge of the arc is accompanied by single kinety shown in the scheme Fig. 6. So clearly as in the drawing I saw it only once (Pl. III 22). The primordium of this kinety is well visible in dividing specimens (Pl. III 23). Probably this kinety separates from the group of long oral kineties. Such separation of one kinety from the group of long oral kineties was seen also in *Blepharocorys jubata* and *B. valvata*. But here the separated and shortened kinety has been shifted to the group of short anterior oral kineties. In the fully developed ciliates it adheres tightly to the zone of short kineties and is difficult to be distinguished from others. The ciliate in the Pl. III 22 is a postdividing specimen and the kinety, surrounding the anterior zone, did not yet reach its proper position.

Long oral kineties begin in the tubular part of the buccal concavity. At first they run on its dorsal wall (Fig. 6), then in the distal part they run in a loose spiral along the walls.

The nonciliated wall of the tubular part is supported by semicircular fibers. In the most part of the wall they are dense and parallel to each other, while in the distal part their arrangement becomes more complicated. In the calyx-like part of the concavity the fibers also form a complicated net. A band of fibers supports the frontal lobe. Another fiber runs near the right margin of the buccal overture.

At the ventral and left margin of the buccal overture there is a formation of parallel rods, intensively impregnating with silver and getting down to the buccal concavity (Pl. III 25). Possibly they correspond to the "ladder" (Wolska 1966). This rod formation becomes gradually narrower and disappears in deeper part of the calyx-shaped concavity. In the tubular part the argentophilic streak is visible (Pl. III 24) but I could not ascertain whether it is an elongation of the rod formation.

Frequently in the dorsal part of the caudal zone the cilia cling together into a bundle called cirrus by Strelkov 1939. On account of the presence of such bundle Strelkov distinguished *B. curvigula* f. *curvigula* and *B. curvigula* f. *cirrata*.

But this bundle can not be regarded as true cirrus because there are no group of kinetosomes corresponding to cirrus in the caudal zone. The spaces between the kineties are the same in the whole caudal zone. This bundle of cilia in *B. curvigula* is an unstable formation bearing the character of syncilium. It may be more or less compact. It is evident that the compactness of the bundle is variable (Pl. VI 26–29).

Dimensions: body length 49–76 μ, width 23–36 μ.

Occurrence: colon of a horse (Hsiung 1930 b); colon of a horse, donkey, and zebra (Stelkov 1939). Found by the author in the colon and faeces of a horse.

Blepharocorys cardionucleata Hsiung, 1930 (Fig. 7, Pl. II 18–19)

The ciliate is similar to the proceeding species on account of the shape of the ventral lobe and buccal concavity strongly bent ventrally. Macronucleus cordate in outline. Micronucleus pressed in a depression in the macronucleus, sometimes

Fig. 7. *Blepharocorys cardionucleata* Hsiung, 1930; anterior part from the left side, scheme of infraciliature

in its medial part, sometimes on the dorsal side. Cytopyge at the posterior body pole, shifted to the dorsal side. Contractile vacuole in the posterior body part, near to the cytopyge.

Results of silver impregnation. The anterior somatic zones are arranged similarly as in *B. curvigula*. Special kinetosomes, strongly impregnating, occur on a small protuberance of cytoplasm (Fig. 7, Pl. II 19). The caudal zone occupies mainly the left body side, partly overpassing to the right side.

The anterior buccal zone well developed, broad. The position of the long axis of this zone is different in comparison with that in *B. curvigula*. Usually in the medial part it is nearly perpendicular to the long body axis of the ciliate. Single kinety surrounds the margin of this zone (Fig. 7) similarly as in *B. curvigula*. This kinety, difficult to see in the interdivision period, is clearly seen during the division of the ciliate. The long oral kineties begin on the dorsal wall of the funnel and run around it in a loose spiral. Numerous semicircular fibers are regularly aranged. In the anterior part of the buccal concavity the net of fibers is complicated similarly as in other species.

Dimensions: body length 40–54 μ, width 22–31 μ.

Occurrence: colon of a horse (Hsiung 1930 b, Strelkov 1939). Found by the author in the faeces of American tapir from the Zoological Garden in Łódź.

Genus *Ochoterenaia* Chavarria, 1933

The genus closely related with *Blepharocorys* Bundle. Body elongated, asymmetrical, laterally flattened. The frontal lobe occurs at the anterior part of the body and the finger-like processus at the posterior pole. The buccal concavity is directed to the dorsal side. Four groups of somatic cilia occur: two in the anterior and two in the posterior part of the body. Macronucleus in the anterior body part at the

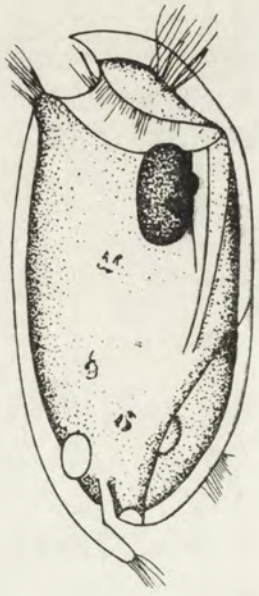


Fig. 8. *Ochoterenaia appendiculata* Chavarria, 1933, after Chavarria 1933

dorsal side. Micronucleus in a depression of the macronucleus. Cytopyge at the posterior body pole. One contractile vacuole in the posterior part of the body.

Strelkov 1939 regarded this genus to be identical with *Blepharocorys* Bundle. In my opinion it is not justified. The characteristic formation of the posterior body end (finger-like processus) and 2 groups of cilia on the posterior part of the body differentiate this genus from *Blepharocorys*.

Type of the genus and single species: *Ochoterenaia appendiculata* Chavarria, 1933 — a parasite of the intestine of a horse (Fig. 8).

References: Chavarria 1933.

Genus *Charonnautes* Strelkov, 1939

Body elongated, asymmetrical, round in cross section. The cilia are arranged in two lateral zones around the buccal overture shifted to the ventral side. There is also the parietal zone of cilia. Two bundles of cilia are situated laterally on the posterior body part. The position of the macronucleus in cytoplasm is unstable,

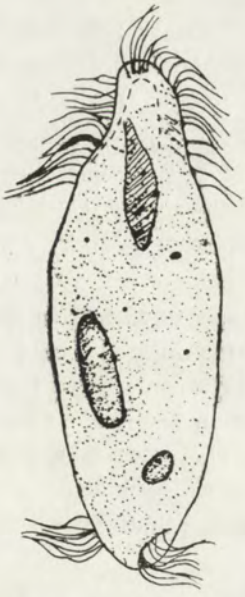


Fig. 9. *Charonnautes equi* Hsiung, 1930, after Hsiung 1930 b

the micronucleus lies at the side of the macronucleus. One contractile vacuole in the posterior body part. The frontal lobe feebly developed.

Type of the genus and single species: *Charonnautes equi* (Hsiung, 1930) — a parasite of the intestine of a horse (Fig. 9).

Synonyms: *Charon equi* Hsiung, 1930; *Charonina equi* (Hsiung, 1930) Hsiung, 1931.

References: Hsiung 1930 a, Strelkov 1939.

Genus *Charonina* Strand, 1928

Synonym: *Charon* Jameson, 1925

Body asymmetrical, elongated, laterally flattened. Small frontal lobe in form of a protuberance at the antero-dorsal part of the body. The somatic ciliature reduced to two anterior groups of cilia (on the frontal lobe and around the buccal overture) and two caudal groups on the posterior end of the body. Oral ciliature formed by one ciliary zone. Nonciliated wall of the buccal concavity supported by

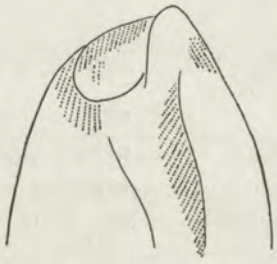


Fig. 10. *Charonina ventriculi* (Jameson, 1925); anterior part from the left side, scheme of infraciliature

the fibers. Oval macronucleus situated usually in the anterior part of the body, near to the dorsal side. Small, rounded micronucleus pressed into the macronucleus. One contractile vacuole in the posterior part of the body. Cytopyge terminal.

Type of the genus: *Charonina ventriculi* (Jameson, 1925) — a parasite of the rumen of cattle (Fig. 10).

Synonyms: *Blepharocorys bovis* Dogiel, 1926; *Blepharocorys ventriculi* (Jameson, 1925).

References: Wolska 1967 b.

The species *Charonina nuda* described by Hsiung 1932 from the rumen of Chinese cattle was removed from the family *Blepharocorythidae* by Strelkov 1939. The author agrees with Strelkov's opinion in the sense that there is too scarce information about this species to place it in the proper position in the system of *Ciliata*. The description given by Hsiung is very insufficient and the characters distinguished by him do not allow to classify the species in the family *Blepharocorythidae*, although such possibility can not be excluded.

Genus *Raabena* Wolska, 1967

Body asymmetrical, laterally flattended. Vast buccal overture in the anterior part of the body, somewhat shifted to the left side. Frontal lobe feebly developed, situated at the dorsal side of the body. Four somatic ciliary zones — two in the anterior and two in the posterior body part. Buccal ciliature connected with the somatic one. Contractile vauole (?) on the ventral side in mid-length of the body. Elongated macronucleus situated in mid-length of the body, nearly to the dorsal

Fig. 11. *Raabena bella* Wolska, 1967; anterior part from the left side, scheme of infraciliature

side. Micronucleus in a depression of the macronucleus. Rudimental vacuole with the group of special kineties in the antero-ventral part of the body. Cytopyge at the posterior pole.

Type of the genus and single species. *Raabena bella* Wolska, 1967 — a parasite of the intestine of Indian and African elephants (Fig. 11).

References: Wolska 1967 b.

Genus *Pararaabena* Wolska, 1968

Body laterally flattened, elipsoid in outline. Buccal overture on the left side of the anterior body part. Leaf-like processes at the posterior end of the body. Frontal lobe feebly developed. Small rudimental vacuole situated on the ventral side of the anterior part of the body. Four somatic ciliary zones — two in the anterior

Fig. 12. *Pararaabena dentata* Wolska, 1968; anterior part from the left side, scheme of infraciliature

and two in the posterior part of the body. Few short special kineties on the "R" vacuole. Buccal concavity with independent ciliature represented by one ciliary zone. Elongated macronucleus situated in the mid-length of the body, near the dorsal side. Two contractile vacuoles (?) at the ventral margin of the body.

Type of the genus and single species: *Pararaabena dentata* Wolska, 1968 — a parasite of the intestine of Indian elephant (Fig. 12).

References: Wolska 1968.

Genus *Spirocorys* Wolska, 1969

The genus tentatively included to the family *Blepharocorythidae*.

Pear-shaped body with narrowed and twisted anterior end. Nonciliated spiral groove proceedes the buccal concavity which is in the shape of bent funnel opening at the left side of the body, in one third of the body length.

Somatic ciliature limited to four groups of cilia. Preoral group is situated at the anterior edge of the spiral groove, while the postoral group — at the posterior edge of the groove. The other two groups occur in the posterior part of the body.

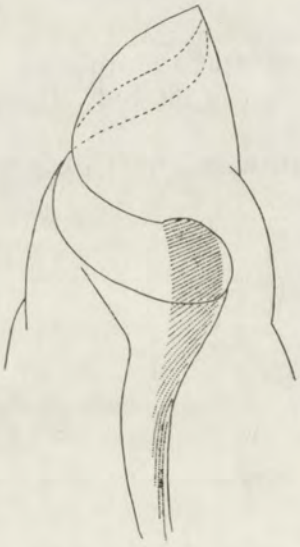


Fig. 13. *Spirocorys indicus* Wolska, 1969; scheme of the oral infraciliature from the left side

On the right body side, backward to the postoral zone, some special kineties occur. Buccal concavity with the ciliature on the right wall and semicircular fibers on the nonciliated wall. Oval macronucleus situated in the enlarged part of the body. Small, round micronucleus lies near the macronucleus.

Type of the genus and single species: *Spirocorys indicus* Wolska, 1969 — a parasite from the intestine of Indian elephant. (Fig. 13).

References: Wolska 1969, 1970.

Some general conclusions on the family *Blepharocorythidae* Hsiung will be presented in the next part of the paper.

Summary

In the present paper the review of the genera: *Blepharocorys* Bundle, *Ochoterenaia* Chavarria, *Charonnautes* Strelkov, *Charonina* Strand, *Raabena* Wolska, *Pararaabena* Wolska, and *Spirocorys* Wolska, belonging to the family *Blepharo-*

corythidae Hsiung, are given. The descriptions of species of the genus *Blepharocorys* Bundle (*B. uncinata*, *B. jubata*, *B. valvata*, *B. angusta*, *B. curvigula*, and *B. cardionucleata*) are also included. Special attention is paid to the infraciliature of the mentioned species.

STRESZCZENIE

Praca zawiera przegląd rodzajów: *Blepharocorys* Bundle, *Ochoterenaia* Chavarria, *Charonnautes* Strelkov, *Charonina* Strand, *Raabena* Wolska, *Pararaabena* Wolska i *Spirocorys* Wolska należących do rodziny *Blepharocorythidae* Hsiung. Praca zawiera także opisy gatunków z rodzaju *Blepharocorys* Bundle (*B. uncinata*, *B. jubata*, *B. valvata*, *B. angusta*, *B. ucrvigula* i *B. cardionucleata*) ze szczególnym uwzględnieniem infraciliatury.

REFERENCES

Bundle A. 1895: Ciliate Infusorien im Coecum des Pferdes. Z. wiss. Zool., 60, 284–350.
Chavarria M. Ch. 1933: Estudios protistologicos. II. *Ochoterenaia appendiculata* gen. n., sp. n. nuevo infusorio del intestino del caballo (*Equus caballus* Linn.) de Mexico. Ann. Inst. Biol. Univ. Méx., 4, 191–196.
Dogiel V. 1926: Une nouvelle espèce du genre *Blepharocorys*, *B. bovis* n. sp. habitant l'estomac du boeuf. Annls Parasit. hum. comp., 4, 61–64.
Fiorentini A. 1890: Intorno ai protisti dell'intestino degli equini. Boll. sci., Pavia, 12, 7–17.
Gassovsky G. 1918: K mikrofaunie kišečnika lošadi. Trud. petrogr. obsc. estest., 49, 30–36.
Grain J. 1966: Etude cytologique de quelques Ciliés Holotriches endocommensaux des Ruminants et des Equidés. Protistologica, 2 (1), 59–141, 2 (2), 5–51.
Hsiung T. S. 1929: A survey of the protozoan fauna of the large intestine of the horse. J. Protozool., 16, 99.
Hsiung T. S. 1930 a: Some new ciliates from the large intestine of the horse. Trans. Am. microsc. Soc., 49, 34–41.
Hsiung T. S. 1930 b: A monograph on protozoa of the large intestine of the horse. Iowa St. Coll. J. Sci., 4, 4–259.
Hsiung T. S. 1931: The protozoan fauna of the rumen of the Chinese sheep. Bull. Fan. meml. Inst. Biol., 2, 29–43.
Hsiung T. S. 1932: A general survey of the protozoa fauna of the rumen of the Chinese cattle. Fan. meml. Inst. Biol., 3, 87–107.
Jameson A. P. 1925: A new ciliate *Charon ventriculi* n. g., n. sp. from the stomach of ruminants Parasitology, 17, 403–405.
Schumacher I. C. 1915: On *Blepharocorys equi* sp. nov., a new ciliate from the caecum of the horse. Univ. Calif. Publ. Zool., 16, 95–106.
Strand E. 1928: Miscelanea nomenclatorica zoologica et paleontologica I and II. Arch. Naturgesch. 92, 30–69.
Strelkov A. 1931: Über die Fauna des Colon beim Zebra. Zool. Anz., 94, 37–54.
Strelkov A. 1939: Prakticeskie infusorii iz kišečnika neparnokopytnych sem *Equidae*. Uchen. Zap. leningrad. pedagog. Inst. Gercena, 17, 1–262.
Wolska M. 1966: Study on the family *Blepharocorythidae* Hsiung. I. Preliminary remarks. Acta Protozool., 4, 97–103.
Wolska M. 1967 a: Study on the family *Blepharocorythidae* Hsiung. II. *Charonina ventriculi* (Jameson). Acta Protozool., 4, 279–283.
Wolska M. 1967 b: Study on the family *Blepharocorythidae* Hsiung. III. *Raabena bella* gen. n., sp. n. from the intestine of the Indian elephant. Acta Protozool., 4, 285–290.
Wolska M. 1968: Study on the family *Blepharocorythidae* Hsiung. IV. *Pararabena dentata* gen. n., sp. n. from the intestine of Indian elephant. Acta Protozool., 5, 219–224.
Wolska M. 1969: *Spirocorys indicus* gen. n. sp. n. cilié holotriche de l'intestin de l'élephant des Indes. Progress in Protozoology. Abstr. Third int. Congr. Protozool., Nauka, Leningrad 1969, 285.
Wolska M. 1970: *Spirocorys indicus* Wolska, 1969 ciliate holotriche from the intestine of Indian elephant, its systematic position. Acta Protozool., 8, 143–148.

EXPLANATION OF PLATES I–III

Blepharocorys uncinata (Fiorentini, 1890)

1: General view from the left side
2: Anterior part of the body from the right side. Special kinetosomes are visible
3: Optical section. The anterior buccal zone is visible
4: Dividing specimen. The primordium of the anterior buccal zone is visible in the opisthe
5: Anterior part of the body, optical section. Long oral kineties are visible
6: Anterior part of the body, optical section. Semicircular fibers are visible
7: Optical section. Branching of the fibers of the corkscrew-like processus
8: Optical section of the buccal concavity, the argentophilic streak is visible

Blepharocorys jubata Bundle, 1895

9: Left side view
10: Long syncilium in the caudal ciliary zone
11: Anterior part of the body from the left side, optical section. The anterior oral zone is visible
12: The same from the right side

Blepharocorys valvata (Fiorentini, 1890)

13: General view from the left side
14: Optical section. Two buccal zones are visible
15: Anterior part of the body from the right side, optical section. Two buccal ciliary zones are visible
16: Anterior part of the body, optical section. Semicircular fibers are visible

Blepharocorys angusta Gassovsky, 1918

17: General view from the left side

Blepharocorys cardionucleata Hsiung, 1930

18: Heart-shaped macronucleus is visible
19: Anterior part of the body. Special kinetosomes are visible posterior to the labial zone

Blepharocorys curvigula Gassovsky, 1918

20: General view from the left side
21: Right side view. The somatic zones and the anterior buccal zone are visible
22: Anterior part of the body from the left side, optical section. Single kinety adjoining to the anterior buccal zone is visible
23: A part of dividing specimen seen from the right side. Semicircular fibers and the primordium of single kinety are visible in the opisthe
24: Isolated buccal apparatus. The argentophilic streak is visible
25: A part of the buccal apparatus, optical section. The anterior buccal zone and ladder-like structure are visible
26: The caudal zone with syncilium
27: Posterior part of the body, not very compact syncilium is visible
28: Dispersed syncilium
29: Specimen in the early stage of division. Compact syncilium is visible in the caudal ciliary zone

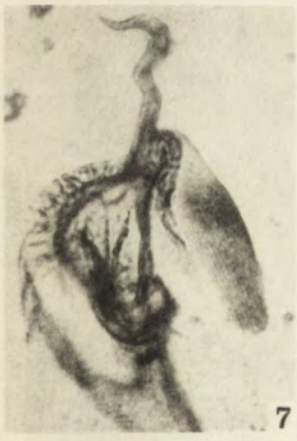
1, 4, 9, 10, 13, 14, 17, 18, 20, 21, 24, 26–29 — 800× 2, 3, 5–8, 11, 12, 15, 16, 19, 22, 23, 25–1600×


1

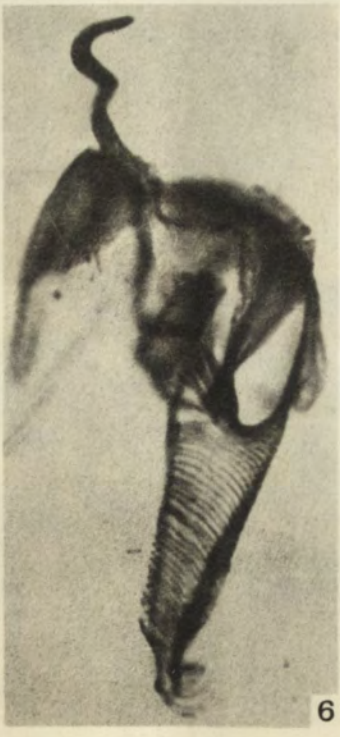

2


3


4


7


5


6


8

M. Wolska auctor phot.


9


10


11


12


13


14


15


17

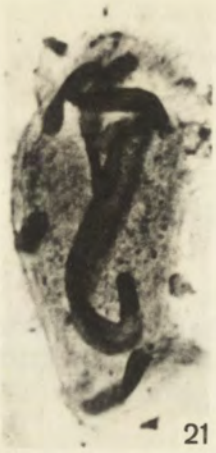

18


19


16

M. Wolska auctor phot.


20


21


24


25

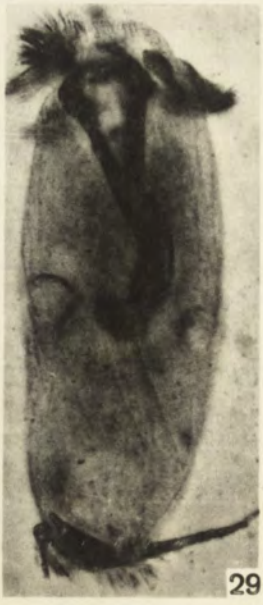

22


23


26


27


28


29

M. Wolska auctor phot.

ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 1.VIII.1971 FASC. 3

Facultad de Ciencias Biologicas Universidad de Nuevo Leon, Monterrey, N. L., México

Paul R. EARL

Hegneriella dobelli gen. n., sp. n., (*Opalinidae*) from *Bufo valliceps* and some remarks on the systematic position of the *Opalinidae*

Hegneriella dobelli gen. n., sp. n., (*Opalinidae*) du *Bufo valliceps* et quelques remarques sur la position sistèmatique des *Opalinidae*

Hegneriella dobelli gen. n., sp. n., an opalinid protozoan having only one nucleus, is here inscribed to Robert W. Hegner and Clifford C. Dobell, major contributors to the study of enterozoa. Its host is *Bufo valliceps* of Terrebonne Parish, Louisiana.

Enterozoic protozoa reported from *Bufo valliceps* are: *Balantidium* sp., *Nyctotheroides cordiformis*, *Leptotheca ohlmacheri*, *Trichomitus augusta*, *Retortamonas dobelli*, *Opalina obtrigonoidea*, *Zelleriella intermedia cuneata*, *Z. intermedia*, *Z. elliptica*, *Z. louisianensis*, *Z. pfitneri* and *Z. valliceps* (see Metcalf 1923, Walton 1946 and Chen 1948). In this study 3/40 hosts (acquired in October, 1968, protozoa being killed and fixed in October and November) had no protozoa, 9 flagellates only, 17 *Nyctotheroides cordiformis* (?), 24 *Zelleriella* spp., 8 *Opalina* sp. A, 6 *Opalina* sp. B, 11 *Opalina* sp. C and 4 *Hegneriella dobelli* gen. n., sp. n. All harbored nematodes. Except for 3/40, all hosts harbored flagellates. A number of similar *Zelleriella* spp., possibly 4, were encountered (see Pl. I 6) which could not be discriminated, i.e., matched to recorded descriptions. *Opalina* sp. A, ovoid, measures 78×53 µ; *Opalina* sp. B measured 125×70 µ, and *Opalina* sp. C 181×60 µ. The latter two species belong to the obtrigona complex.

In order to add *Hegneriella dobelli* gen. n., sp. n., to the *Opalinidae*, the superclass definition is emended to:

Cilia in oblique rows over the entire body surface; cytostome absent; one, two or many isomorphic nuclei; inter- or perikinetal binary fission; some life cycles may involve syngamy with anisogamous gametes; all endosymbiotic.

Honigberg et al. 1964 had defined the group as:

Superclass II. *Opalinata* Corliss et Balamuth, 1963

Cilia in oblique rows over entire body surface; cytostome absent; two to many

nuclei of one type; nuclear division acentric; binary fission generally interkinetal, thus usually symmetrogenic; known life cycles involve syngamy with anisogamous flagellated gametes; all parasitic.

Material and methods

A one-step stain-fixative was used routinely to produce temporary mounts: dilute azure B in glutaraldehyde buffered with phosphates to pH 4. At low pH in dilute concentration (below 0.1%), azure B differentiates nucleic acids metachromatically, DNA staining green and RNA blue-purple. Azure B-glutaraldehyde is made up double strength so that when added to an equal volume of 0.6% saline containing live organisms, the final concentrations are: azure B 0.025% and glutaraldehyde 6% at pH 3.8 to 4.2. Glutaraldehyde, available as a 50% solution by volume, is placed in a vessel to make up a 12% solution and distilled water added to about 70% of final volume. Then, with constant stirring and checking with pH paper and a pH meter, saturated dibasic and/or monobasic phosphate (K or Na) is slowly added until pH 4 is attained. Now add 10% of final volume as a 10×solution of azure B in distilled water (0.5 g/100 ml). Next water is added to final volume. Phosphate-buffered glutaraldehyde solutions at various hydrogen ion concentrations are attained by trial-and-error additions of phosphate since different lots give varying results. Also some solutions may develop a slight precipitate on standing, but this has never interfered with fixation or staining.

Rectal contents is diluted with 0.6% saline and examined directly and/or after adding an equal amount of azure B-glutaraldehyde fix-stain. A drop is placed on a slide and a coverslip added. All operations, including measuring and photographing, can be carried out with such temporary mounts. Loss of organisms is avoided, and time and labor are saved, however organisms are not cleared and heavy cytoplasmic purple staining may interfere with vision. A permanent record can be maintained photographically. For comparative purposes a few slides were made: fixation with 6% glutaraldehyde at pH 7.5, ethanol dehydration to 85%, drop on albuminized coverslips, then dip in 0.5% celloidin in 95% ethanol with a trace of ether in Columbia jars, hydrate and stain with chromosome red (Gurr 1965), dehydrate to 95% ethanol and mount in Diaphane (Wills Corp., Rochester, N.Y.). Kodak High Contrast and Agfa IFF 35 mm films were used and developed in Kodak D-19.

Results

Hegneriella is like *Zelleriella*, exept that it has one very large nucleus which is a prolate spheroid.

Typical trophozoites of *Hegneriella dobelli* gen. n., sp. n. (Pl. I 1) measure 98× ×61 μ and the nucleus 34×16 μ. An interphase nucleus is shown in Plate I 2. Like all other opalinids, *H. dobelli* is asymmetric and parabolic anteriorly. The longitudinal axis of the adult runs through the cell at an angle of ca. 26° to the direction of the kineties. The larger area on one side of this axis has a ratio of ca. 1.2 to the smaller. The angle of the nuclear long axis to the cell's long axis is ca. 34° and to the kinetal direction ca. 52°. In the mitotic cycle the nucleus, a prolate spheroid moves to 115° from the direction of the kineties (see Pl. I 3).

The length: breadth ratio is 1.6, and nuclear length: breadth is 2.1. The ratio of nuclear length to cell length is 2.9.

The number and spacing of kineties varies as the organism grows, true also for opalinids in general. The endospherules are conspicuous, round to irregular.

Plate I 3 shows a specimen in telophase. Cytokinesis is interkinetal, as evinced by the anterior notch (viewer's right), Cytokineses is interkinetal in *Opalina* generally and always so in *Zelleriella*. *Protoopalina* divides perikinetally and I suspect *Cepedea* does also. The cell shown in Pl. I 3 has a nucleus 89×26 μ. It measures 113 μ along the arm of an angle 70° to the kinetal direction (normal length) and 149 μ perpendicularly (width for the trophozoite). Another dividing cell measured the same way (81×132 μ) had 2 nuclei 26×14 and 23×17 μ, and its apical notch had scarcely progressed beyond that of the cell depicted.

Table 1

Dimensions of *Hegneriella dobelli* gen. n., sp. n. in microns (N = 100)

	Range	Arithmetic mean	Standard deviation	Standard error	Coefficient of variation in %
Cell length	62–133	98.2	23.1	2.3	24
Cell width	50– 75	61.0	14.0	1.4	23
Nuclear length	30– 42	34.1	5.7	0.6	6
Nuclear width	13– 21	16.2	4.2	0.4	4

A cyst and a juvenile are shown in Plate I 4 and 5. The juvenile is 65.5×30.0 μ and has a 13.5×14.3 μ nucleus. I have never seen a "juvenile" of *Zelleriella* and think of such forms as typical of *Opalina*, though in that case multinucleated. A *Zelleriella* sp. of *Bufo valliceps* is illustrated in Pl. I 6 for comparison.

The dimensions of adult *H. dobelli* are shown in Table 1.

Discussion

Pinto 1926 described a uninucleate *Opalina* which Amaro 1968 assigned to *Zelleriella*, believing Pinto's specimens abberants. Indeed, abberant *Zelleriella* specimens are taxonomically prejudicial to the validation of a uninucleate genus. When a uninucleate *Zelleriella* is sighted, it seems safe to assume that the nucleus is very close to or in mitosis. *Hegneriella*'s nucleus replicates just before cytokinesis as does the classic generalized cell. *Zelleriella*'s two nuclei are distributed one to each daughter cell whereupon mitosis takes place to regain the normal binucleate condition. Chen 1948 wrote: "After each division, each daughter contains one metaphase nucleus. Finally, the nucleus completes its division, giving rise to two daughter nuclei — the normal binucleate condition thus restored in each daughter

animal". Incidentally, this is also true for the nuclear cycle of *Protoopalina*, though that genus divides perikinetally.

Further, it is well known that *Zelleriella* and *Protoopalina*, the two binucleate genera, may have asynchronous mitosis and cytokinesis occasionally so that daughter cells eventuate with the wrong number of nuclei, commonly four or eight. However, anomalous conditions in *Zelleriella* seem irrelevant to the genus *Hegneriella*.

* * *

The balance of this discussion concerns cytological data vis à vis the systematic position of the *Opalinidae*. Protozoan opalinids are astomatous, bilaterally, asymmetric, diploid endosymbionts of ectothermic hosts, having one kind of nucleus and bearing cilia.

Opalina is the only genus reported for gametes and cysts, though *Cepedea* (Lwoff and Valentini 1948) produces large 75 μ cysts in culture and *Hegneriella* has cysts (this paper). Also I have noted 15×14–15 μ uni- and binucleate cysts in *Cepedea* spp.

Opalina and *Cepedea* seem to produce some cysts continually, yet *Hegneriella*, *Zelleriella* and *Protoopalina* may not. Sexuality is only recognized in *Opalina* and evidently circumstances that may induce it have not yet been encountered for the other genera. Do they pass through the stages of synkaryon formation ?

Brumpt 1915 accurately worked out the life cycle of *Opalina*, including reinfection cysts. Wessenberg 1961 confirmed this cycle inclusive of micro- and macrogemetes and syngamy, but the karyology of synkaryon formation and the events leading to and from in are not actually established. Events surrounding meiosis, all of the *Opalinidae* assumed diploid, take place in the first two weeks of infection following the ingestion of cysts by tadpoles. Uni- and binucleate gamonts have spindle-shaped nuclei considerably larger than those of trophozoites. There may be two meiotic divisions as micro- and macrogametes have a single nucleus smaller than that of any other stage. Sukhanova and Nilova 1965 reported that in tadpoles some binucleate opalinids replicate rapidly to produce multinucleate cells, trophozoites, synthesizing DNA at a high rate, 11.5% of nuclei synthesizing DNA and 6% RNA compared with trophozoites from frogs synthesizing DNA at 3.5% and RNA at 42%. This seems to be interesting information on the protrophozoite (protrophont in Wessenberg's terms, called young trophonts by Sukhanova and Nilova) versus trophozoite, rapid versus slow expansion of nucleoplasm. A cell differentiated to a gamont cannot likely change its career and become a trophozoite.

The zygote resulting from syngamy in the tadpole encysts and when voided is ready to repeat: gamont, gametes, zygote, zygocyst in a new host, or the zygote can develop into a trophozoite. Sukhanova 1961 reported that nuclei in gametes strain less intensely for DNA than those of trophozoites. This is most likely due to a reduction of ploidy and gametes are almost certainly haploid. However a reduction in DNA and/or nuclear size does not positively indicate reduction in ploidy

though this is implied. Chromosomes have only been counted in the genus *Zelleriella* (see Chen 1948, and Ivanić 1933) thought that *Cepedea dimidiata* has six or eight beaded chromosomes with one, two or even three mucleoli. I have estimated six in a *Cepedea* sp. with two or three mucleoli (unpublished). In the latter case, rather uncertain, at least there is a low number of 1–3 μ chromosomes.

Chen 1948 found 12 pairs, 24 chromosomes in the five *Zelleriella* spp. he studied intensively, yet this number is not universal for the genus as he reported one with 38 chromosomes. The two chromatids are mirror images, and there are four or six nucleoli constant per species. A haploid race he studied had three nucleoli. All chromosomes have contromeres, J-shaped when off center and V-shaped when central.

Chromosomes in *Opalina* are small, difficult and have never been counted. This difficulty might be reasonably circumvented by counting nucleoli, usually four or fewer in the trophozoite; in some species I have seen only one. How many nucleoli-generating chromosomes are there, and is each a member of a homologous pair ? Nucleoli persist throughout mitosis making chromosome counts difficult unless a strain like Feulgen's is used. Further nucleoli can fuse and there may be strands between them. The chromosomal events are at the root of the meiosis problem in the *Opalinidae*, redundant definitionally, but still the heart of the matter. How many reduction divisions occur and where ? Does the binucleate gamont, or indeed the uninucleate gamont, have tetrads formed by synapsis of homologous chromosomes. Why should there be two nuclei in the gamont anyway ? Are the products of the binucleate gamont all of one kind or half micro- and half macrogametes ? Isolations in slide cultures should provide answers.

Aside from initial infection events in tadpoles, two other stages in the hosts' life cycle are especially interesting: metamorphosis of tadpoles and the breeding adult. Wichterman 1937 found conjugating nyctotherans only in metamorphosing tadpoles and termed this stage a period of crisis for endosymbionts, especially so since such hosts do not feed during the transition from herbivore to carnivore. In frogs, El Mofty and Smyth 1969 found that the percentages of *Opalina* cysts, small forms and trophozoites vary markedly throughout the breeding season. What happens to opalinids in these host stages ?

The five genera of the *Opalinidae* are: *Hegneriella*, *Zelleriella*, *Protoopalina*, *Cepedea* and *Opalina*. All nuclei are isomorphic, as stated. These genera are defined later in this discussion.

The first three genera commonly have nuclei over 15 μ long which are prolate spheroids, whereas the latter two have small nuclei less than 15 μ which are spheres, disks or ovoids.

The cylindrical shapes of *Protoopalina* and *Cepedea* are highly similar as are the flat outlines of *Hegneriella* and *Zelleriella*. *Opalina*, though flat, presents a very wide variety of outlines. Cytokinesis is transverse in the cylindrical and longitudinal

in the flat genera; *Opalina also* exhibits transverse division. In that genus, apparently an asynchronous series of mitoses leads to cytokinesis; this does not seem true for the other multinucleate genus, *Cepedea*. It shows a peak of mitotic activity at cell division. The single nucleus of *Hegneriella* reaches anaphase before cytokinesis begins. The two nuclei of *Zelleriella* and *Protoopalina* each replicate in a daughter cell, i.e., mitosis is underway in the mother cell so that after cytokinesis each daughter nucleus is near metaphase.

None of these facts really helps in establishing evolutionary relationships among opalinids or for them among protozoa. Little in opalinid characteristics recommends them to the flagellates as they are not flagellated in the accepted sense. It may be better to state that small forms are ciliated other than go to the trouble of calling them multiflagellated. This issue is now old hat. They are not ciliates as the hallmark of the ciliate is its indispensable macronucleus. Evolutionary positioning still maintains its subjective component.

Multinucleation, taken as an advanced character, may have arisen through suppression of cytokinesis by a microbe-produced toxin.

The opalinid evolutionary order might be: *Hegneriella*, *Zelleriella*, *Protoopalina*, *Cepedea*, *Opalina* or *Hegneriella*, *Zelleriella*, *Protoopalina*, *Cepedea* and *Hegneriella*, *Zelleriella*, *Opalina*.

Opalina Corliss et Balamuth is now removed from the *Sarcomastigophora* and the opalinids raised to *Opalinida* n. subphylum, a move implying they are neither flagellates nor ciliates.

The hierarchy then is:

Subphylum Opalinida subphylum novum, with the present emended definition of the superclass.
Superclass *Opalinata* Corliss et Balamuth, 1963.
Order *Opalinida* Poche, 1913, with the same characters.
Family *Opalinidae* Claus, 1874, with the same characters.
Subfamily *Hegneriellinae* subfamilia nova, flat with one or two nuclei.
Hegneriella gen. n., flat with one nucleus.
Zelleriella Metcalf, 1920, flat with two nuclei.
Subfamily *Protoopalininae* Metcalf, 1920, spindle-shaped with two or more nuclei.
Protoopalina Metcalf, 1918, spindle-shaped with two nuclei.
Cepedea Metcalf, 1920, spindle-shaped with many nuclei.
Subfamily *Opalininae* Metcalf, 1920, flat with many nuclei.
Opalina Purkinje et Valentin, 1835, flat with many nuclei.

Subfamilies previous to the addition of *Hegneriella* were: *Protoopalinae (Protoopalina, Zelleriella)* and *Opalininae (Opalina, Cepedea)*. *Zelleriella* may have arisen from *Hegneriella* and *Opalina* from either, as *Cepedea* probably arose from *Protoopalina*.

Summary

Hegneriella dobelli gen. n., sp. n. is described and assigned to *Hegneriellinae* n. subfamily which includes *Zelleriella*. It is like *Zelleriella* except that it has one nucleus. Opalinids are distinct from both flagellates and ciliates and here assigned to *Opalinida* n. subphylum containing the single family *Opalinidae*, with three subfamilies: *Hegneriellinae*, *Protoopalinae* with *Protoopalina* and *Cepedea*, and *Opalininae* with *Opalina*. Multinucleation may have repeatedly arisen via biotoxins, and changes in basic cell shape are less easily explained.

RÉSUMÉ

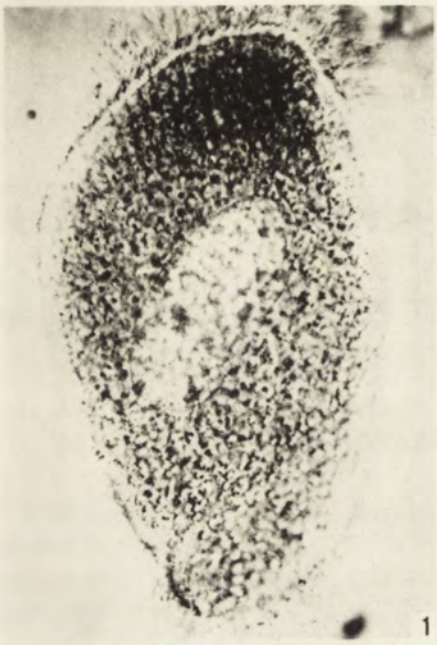
On a décrit *Hegneriella dobelli* gen. n., sp. n. et on l'a classifié comme appartenant à la subfamille de *Hegneriellinae* qui comprend aussi le *Zelleriella*. L'espéce ressemble à *Zelleriella* avec cette exception qu'il possède un nucléus seulement. Les Opalinides diffèrent des Flagellés et Cilliates et on les a classifiés ici comme appartenant aux *Opalinida* n. subphylum qui contient aussi l'unique famille *Opalinidae* avec trois subfamilles: *Hegneriellinae*, *Protoopalinae* avec *Protoopalina* et *Cepedea* et *Opalininae* avec *Opalina*. La présence de plusieurs nucléus peut etre expliqué par l'effet des biotoxines mais le changement de la forme essentielle des cellules peut être moins facilement expliqué.

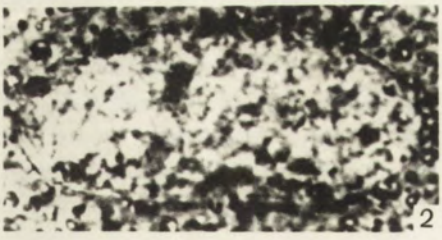
REFERENCES

Amaro A. 1968: Personal communication.
Brumpt E. 1915: Cycle évolutiv des opalines. Bull. Soc. Path. exot., 6, 397–404.
Chen T. T. 1948: Chromosomes in *Opalinidae (Protozoa, Ciliata)* with special reference to their behaviour, morphology, individuality, diploidy, haploidy, and association with nucleoli. J. Morphol., 83, 281–357.
El Mofty M. M. and Smyth J. D. 1969: Endocrine control of encystation in *Opalina ranarum*. Exp. Parasitol., 25, 185–199.
Gurr E. 1965: Rational use of dyes in biology and general staining methods. Williams and Wilkins, Baltimore, 422.
Honigberg B. M. 1964: A revised classification of the phylum *Protozoa*. J. Protozool., 11, 7–20.
Ivanić M. 1933: Zur Auflärung der Kernverhältnisse und der Kernteilung bei der im Enddarme der gemeien Erdköte (*Bufo vulgaris* Laur.) lebenden Opaline, *Cepedea dimidiata* Stain. Arch. Protistenk., 80, 1–35.
Lwoff A. et Valentini S. 1948: Culture du flagellé opalinide *Cepedea dimidiata*. Annls Inst. Pasteur, Paris, 75, 1–7.
Metcalf M. M. 1923: The opalinid ciliate infusorians. Bull. U. S. natn. Mus., 87, 465–634.
Pinto C. 1926: Estudos sobre ciliados. Bol. Inst. Brasil. Ci. 2, 219–224.
Sukhanova X. M. 1961: Cytochemical investigations of life cycles in some species of *Opalinidae*. Progress in Protozoology, Proc. First int. Congr. Protozool., Prague 1961, Publ. House Czechosl. Acad. Sci., Prague 1963, 296.
Sukhanova X. M. and Nilova V. K. 1965: An autoradiographic study of nucleic acid synthesis in the life cycle of *Opalina ranarum* Ehrbg. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. int. Congr. ser. No. 91, 202.
Walton A. C. 1946: Protozoan parasites of the *Bufonidae (Amphibia)*. Trans. Ill. St. Acad. Sci., 39, 143–147.
Wessenberg H. 1961: Studies on the life cycle and morphogenesis of *Opalina*. Univ. Calif. Publ. Zool., 61, 315–370.
Wichterman R. 1937: Division and conjugation in *Nyctotherus cordiformis* (Ehr.) Stein (*Protozoa, Ciliata*) with special reference to the nuclear phenomena. J. Morphol., 60, 563–611.

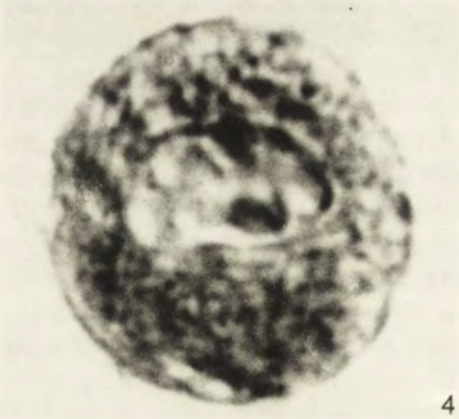
EXPLANATION OF PLATE I

1: Trophozoite of *Hegneriella dobelli* gen. n., sp. n. showing shape kineties, the massive nucleus, some of its nucleoli, and cytoplasmic endospherules. Azure B. ×700
2: Interphase nucleus of *H. dobelli* showing some nucleoli and many RNA-rich endospherules in the cytoplasm. Azure B. ×1770
3: Telophase specimen of *H. dobelli* showing notch at apex on viewer's right. Left side of nucleus seems larger as right portion is falling out of focus. Azure B. ×465
4: Cyst of *H. dobelli* showing nucleus of typical adult shape with a concentration of nucleoleic material above lower nuclear membrane. Azure B. ×930
5: Juvenile of *H. dobelli* showing ring of RNA around its nucleus, and the falx as a clear anterior line. A bleb is evident on the right posterior pellicle which is a fixation artifact. Azure B. ×790
6: *Zelleriella* sp., typical of *Zelleriella* spp. of *Bufo valliceps*. Burr's chromosome red. ×700


1


2


3


4


5


6

ACTA PROTOZOOLOGICA
VOL. IX WARSZAWA, 1.VIII.1971 FASC. 4

Zoological Institute, University of Warszawa, Warszawa 64, Poland

Maria S. SOŁTYŃSKA

Morphology and fine structure of *Chilodonella cucullulus* (O. F. M.). Cortex and cytopharyngeal apparatus

Morfologia i ultrastruktura *Chilodonella cucullulus* (O. F. M.). Korteks i aparat cytofaryngealny

Chilodonella cucullulus is a dorsoventrally flattened ciliate, with the cytostome situated on the ventral side of the body. According to Corliss 1961 these are traits characteristic of the *Cyrtophorina*. The morphology and morphogenesis of this protozoan have been studied by Fauré-Fremiet 1950, Dobrzańska-Kaczanowska 1965, Radzikowski 1966, Kaczanowska and Kowalska 1969, Kowalska and Kaczanowska 1970. These studies have revealed that both the dorsal and the ventral sides of the cell are provided with an argentophilic net work and intravital observations indicate the presence of surface alveoli with a liquid contents. The ventral side is ciliated, on the dorsal one there is a single kinety. The ventral ciliature is differentiated into somatic and oral (circumoral and preoral kineties). The somatic kineties are rigid skeletal structures as each of them separates as a whole, when digitonin treated. The pattern of somatic kineties is asymmetric, the right side kineties bending left in the front part of the cell. There the left side and the right side kineties are separated by the obliquely running preoral kinety. Oral kineties originate during the postdivisional and postconjugational morphogenesis from fragments of the somatic kineties. The ciliary beat of these kineties is different from that of the somatic kineties, it resembles rather that of membranelles.

The cytostome is situated on a naked circumoral field. The basket surrounding the cytostome slit consists of 10–14 trichites (nemadesmata). The thickened top end of each nemadesma is furnished with a stalked pair of triangular bodies, pointing inwards — the dentes (= small nemadesmes of Kaczanowska and Kowalska 1969). Inside the basket runs the cytopharyngeal tube. During conjugation the oral field is the place of attachment of conjugants.

On the ventral side the silver impregnating contractile vacuole pores are visible. These are permanent structures of a strictly determined and stable position in relation to the kineties.

In specimens impregnated with silver after Chatton–Lwoff granular structures become visible on the dorsal side.

Out of the present knowledge of the structure of *Chilodonella cucullulus* arise the following problems:

1. Are there any structures responsible for the flattened shape of the cell.

2. Are differences in movement of oral kineties (arising from fragments of somatic kineties) based on differences in their structure.

3. Are the physiological peculiarities of the oral field (it being the site of phagocytosis as well as of conjugational contact) due to its morphological particularities.

The answer to these questions is to be looked for in the fine structure of the superficial part of the cell, of the cortex. Preliminary results have pointed to the existence of some structures, determining the shape of the ventral side (Sołtyńska 1969).

Cortex of a ciliate is the superficial part of the cell and at the same time the body wall. In *Paramecium* Sedar and Porter 1955 refer as cortex to the peripheral layer of cytoplasm, known also as ectoplasm or ectosark, containing the fibrillar systems, cilia and their kinetosomes, trichocysts and mitochondria. On the outer side the cortex is bordered by the covering layer, the pellicle, on the inner side the boundary with the endoplasm is less visible. Ehret and Powers 1959, Pitelka 1968 use the term cortex to cover the pellicle and the superficial layer of cytoplasm. In that later sense it will be used here. The ciliate cortex consists of surface units called "ciliary corpuscles" (Ehret and Powers 1959), "cortical units" which are the base of cortex reproduction (Dippell 1965) or "kinetosomal territories" the morphogenetic centre of which are the kinetosomes (Pitelka 1968, 1969).

The terms proximal and distal are used here according to Grain 1969. The proximal end of a kinetosome, elements of the cytopharyngeal apparatus etc. is the end imbedded in the cytoplasm, the distal end — the one near to the cell surface.

Numeration of triplets in a kinetosome is in accord with the standard established by the III International Congress on Protozoology in Leningrad, 1969. The triplet situated in the posterior part of kinetosome, at the front-rear axis of the kinety, bears number 1, the one at which arise the postciliary fibrils bears number 9.

Material and methods

Stocks of *Chilodonella cucullulus* marked B and K have been isolated in March 1967 from a mass culture coming from a sewage plant in Józefów near Warsaw. They were maintained in tap water at room temperature and fed every other day yeast suspension. In the cultures were also some flagellates, serving as additional food for *Chilodonella*. The ciliates were inoculated at 4 days intervals. Individuals directly from the cultures as well as starved ones have been studied.

Live organisms were examined by means of phase contrast microscope.

For light microscopy the animals were silver impregnated after Chatton–Lwoff (modification after Corliss 1953) and protargol silvered after Tuffrau 1967, but instead of in gelatin they were embedded in a glycerin–albumin mixture.

For electron microscopy ciliates from two-days old cultures were taken and treated the following ways:

a. Material was centrifuged, fixed for 30 min at 4°C in 5% glutaraldehyde, adjusted to pH 7.2 with phosphate buffer, then in 1% osmium tetroxide in the same buffer for 1 hr at 0°C.

b. Organisms were fixed in 2% glutaraldehyde, adjusted to pH 7.3 with cacodylate buffer for 45 min at room temperature, and next in 1% osmium tetroxide adjusted to pH 7.3 with Michaelis buffer with saccharose for 30–45 min at 0°C. After fixation they were embedded in 1.2% agar.

c. Ciliates were fixed in 1% osmium tetroxide in Michaelis buffer with saccharose for 30–40 min at 0°C.

Fixed material has been dehydrated the standard way and embedded in Epon 812. Thin sections were made with a LKB-880 microtome, stained with uranyl acetate and lead citrate, examined and photographed in a JEM 6A electron microscope.

Besides, a series of sections 1 μ thick has been made from the Epon-embedded material. These sections were stained with 1% toluidin blue for light microscopy purposes.

Results

Observations by light microscopy

General morphology

An interdivisional individual of *Chilodonella* is 80–113 μ long, 27–63 μ wide and 17–30 μ thick (measurements of 30 specimens from stock B).

The dorsoventrally flattened *Chilodonella cucullulus* exhibits a marked difference in shape and in stability of shape of both sides, the dorsal and the ventral one. The dorsal side is less or more convex depending on whether the individual in question is starved or fed full. The ventral side is always entirely flat, but changes in width. Starved animals are narrow, well fed are wider (Pl. I 1, 2). It is the interkinetal distances that change, and they do not change equally throughout the whole organism. In narrow specimens the left-side kineties lie close to each other, and are a little bent to the inside. Thus a narrow specimen is no longer elliptic, but kidney shaped. The right side kineties are both in narrow and in wide individuals spaced similarly.

The 1 μ sections reveal a folding of the ventral side. The kineties lie in furrows, lined by the cytoplasmatic folds (Pl. II 2, 3). The height of the interkinetal folds remains stable along the cell axis.

Movement

Chilodonella cucullulus moves along a highly peculiar path, consisting of a series of successive semicircular sections. This is probably due to the right side kineties turning left in the front of body (Fig. 1 A). In ciliates moving around in dense food suspension or passing grains of sand, flexions of the ventral side can be observed. All these flexions occur along the lateral or along the front margin of the body, i.e. they are always interkinetal, never perkinetal.

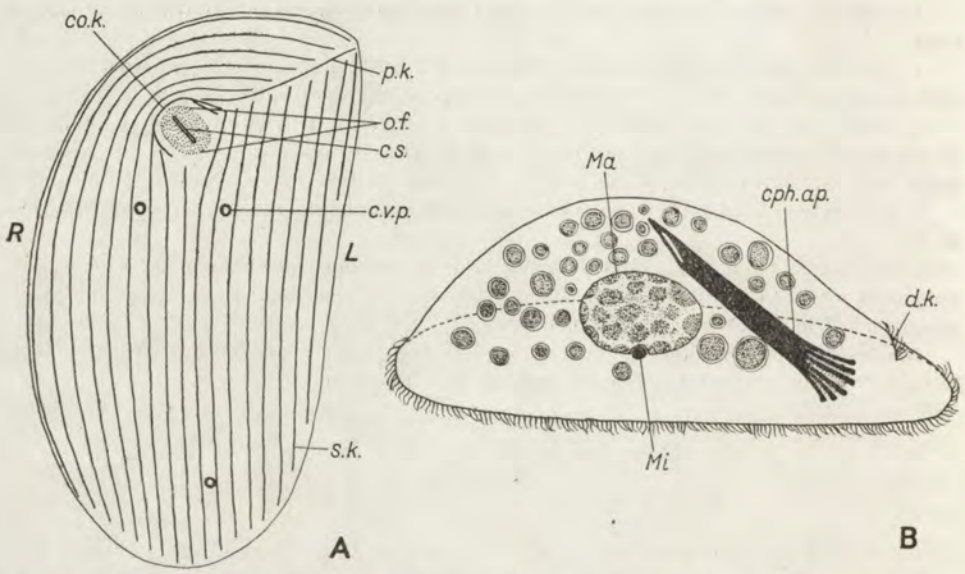


Fig. 1. *Chilodonella cucullulus*. A — Diagrammatic ventral view. B — Diagrammatic lateral view. Abbreviations used: *a.* — arms, A, B, C, — microtubular fibrils of triplet, *ax.* — axosome, *b.* — bacterium, *c.f.* — central fibrils, *cil.* — cilium, *co. k.* — circumoral kinety, *c. m.* — cell membrane, *cph. ap.* — cytopharyngeal apparatus, *cph. t.* — cytopharyngeal tube, *cs.* — cytostome, *c. w.* — cartwheel structure, *c. v.* — contractile vacuole, *c. v. p.* — contractile vacuole pore, *d.* — dentes, *d. g.* — dense granule, *d. k.* — dorsal kinety, *d. s.* — dorsal side, *ep.* — epiplasme, *ex. m.* — extracellular material, F — front, *i. a. m.* — inner alveolar membrane, *kd.* — kinetodesmal fibril, *ks.* — kinetosome, L — left side, *lam.* — lamella, *l. m.* — lateral margin, *m.* — mitochondria, *Ma* — macronucleus, mf. a. — microfibrillar annulus, *mtb. b.* — microtubular band, *Mi* — micronucleus, *nd.* — nemadesma, *n. ks.* — noncilliated kinetosome, *o. a. m.* — outer alveolar membrane, *o. f.* — oral field, *p. a.* — pellicular alveole, *pc.* — postcilliary fibrils, *p. f.* — periferal fibrils, *ph.* — phagoplasme, *p. k.* — preoral kinety, *p. s.* — parasomal sac, R — right side, Re — rear, *s. k.* — somatic kinety, *sk. sh.* — subkinetal sheet, *t.* — transverse fibrils, *t. d.* — transverse dense fibrils, *t. mtb.* — transverse microtubular fibrils, *t. p.* — terminal plate, *v. s.* — ventral side

The ciliary beat in somatic kineties display the usual metachronic waves, the movement of cilia of oral kineties is membranella-like (in accord with Radzikowski 1966).

Cell surface

In ciliates silvered after Chatton–Lwoff, and kept for several days in da Fano solution, the argentophilic network becomes visible (Pl. I 3–5, 8, 11). Its meshes on both the dorsal and the ventral side are polygonal. In starved individuals, when the interkinetal distances diminish, the polygons change shape, they become elongated (Pl. I 5).

In protargol silvered preparations a network is sometimes visible, that looks like a negative of the network from Chatton–Lwoff preparations. The meshes are dark, the rims light (Pl. I 3). The oral field is devoid of the argentophilic network. If silver impregnated after Chatton–Lwoff, all the oral field is dark (Pl. I 6, 10).

The network appears beyond the limits of the field, marked off by the insertions of the cytopharyngeal apparatus (Pl. I 11).

Somatic ciliature

The number of somatic kineties of the ventral side varies in stock B from 18 to 21, and in stock K from 16 to 19. When silver impregnation after Chatton–Lwoff is applied, dark, rounded bodies, of a diameter smaller than that of a kinetosome, appear near to almost every kinetosome, at its right side (Pl. I 6, 10). They are found also in the vicinity of kinetosomes of the dorsal kinety (Pl. I 7). In protargol preparations these structures are not visible. Along the ventral kineties there are instead dark bands, lying in closest proximity of the kinetosomes (Pl. I 9).

Oral ciliature

The oral field is in front and on the right side encircled by 2 or 3 circumoral kineties, out of which the inner ones are shorter. The preoral kinety runs from the oral field obliquely forwards (Fig. 1 A). In material, silvered the Chatton–Lwoff technique, each of these kineties appears as consisting of several rows of dark bodies, lying closely to each other (Pl. I 6). No dark, protargol staining bands have been detected in the proximity of these kineties (Pl. I 9). Cilia of the circumoral kineties are longer than those of the somatic kineties (Pl. II 22).

Cytopharyngeal apparatus

On the oral field the cytostome is visible as an obliquely running slit (Pl I 6, 9–11). In whole mounts the length of the slit is 5.8–6.5 μ. Around the cytostome appear the distal ends of nemadesmata, belonging to the cytopharyngeal apparatus. This apparatus runs into the cytoplasm, directed rearwards and upwards, to the dorsal side, and opens above the macronucleus (Fig. 1 B, Pl II 12–16), less frequently under it. During food intake it can be observed, that the oral field bulges out and then sinks again in relation to the plain of the ventral side. This is also visible in cross sections and in longitudinal sections (Pl. II 12, 16), The cytopharyngeal apparatus consists of the innermost cytopharyngeal tube and of the basket of nemadesmata, surrounding it. In the upper third these parts lie at a distance, and the palisade of nemadesmata is calyciform. Below they come closer together, and between them appears some material, poorly staining with toluidin blue (Fig. 2, Pl. II 12, 19). In the lower portion, till the proximal end of the cytopharyngeal apparatus, the tube and the basket are in close contact. Total length of the cytopharyngeal apparatus is 24–33 μ. The number of nemadesmata is variable, in our stock K it amounts to 14–15, in stock B it is 10–12. To the distal end of each nemadesma there is attached a pair of small, triangular bodies, the dentes, pointing inwards, to the cytostome (Fig. 2 a; Pl. II 16, 17). These structures lie at the level of the distal end of cytopharyngeal tube, surrounding the cytostome, or a little lower (Pl. II 12, 16), which indicates, that they may change their relative position during

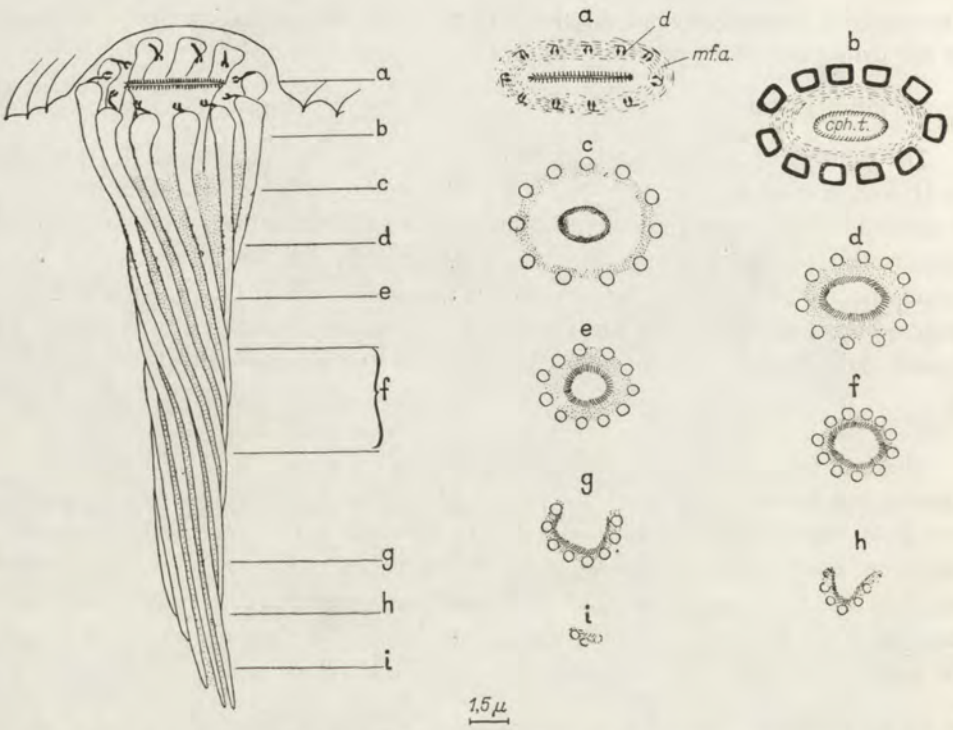


Fig. 2. *Chilodonella cucullulus*. Diagrammatic reconstruction of cytopharyngeal apparatus. Left — three-dimensional reconstruction from a series of 1 μ cross-sections. Right (a–i) — cross-sections at successive levels, based on observations made by light and electron microscopy. Explanations as in Fig. 1

food intake. Between the insertion of cytopharyngeal tube in the cell surface and the attachment place of the dentes there is a prominent cytoplasmatic fold — the lip (Pl. II 12–16). The nemadesmata are club-shaped, much elongated. Their distal ends are swollen, trapezoid in cross section, the longer base of the trapezium being 1.3–1.4 μ long (Fig. 2 b, Pl. II 12, 16, 17). Proximally they taper to a circular cross section, their diameter dropping to 0.6 μ (Fig. 2 c–h, Pl. II 18–21). In the upper part of the basket the nemadesmata are not interconnected, they form an elliptic ring, its longer axis being 8–10 μ (Fig. 2 b). Proximally the nemadesmata come close together, the basket becomes circular in cross section, and between the nemadesmata appears some material, poorly staining with toluidin blue (Fig. 2 c, Pl. II 15). In the region, where nemadesmata lie close to the cytopharyngeal tube their course, oblique to the basket axis, is discernible in longitudinal sections (Pl. II 14). The same is sometimes visible in protargol stained preparations. The oblique arrangement of nemadesmata was revealed also by a spatial reconstruction of cytopharyngeal apparatus from a series of 1 μ sections (Fig. 2). In living ciliates and in whole mounts, baskets without this oblique arrangement of nemadesmata, have

also been detected (Pl. II 24). Nemadesmata, forming the palisade, are not equal in length. About 24 μ from the distal end of basket some of them terminate, and from this point on the termination of successive nemadesmata is noted. Thus the ring of nemadesmata gets more and more incomplete (Fig. 2 g–i, Pl. II 20, 21). At the same level the wall of the cytopharyngeal tube also partially disappears. This way arises an elliptic opening, situated at an acute angle to the basket axis, of the length of 4–6 μ, rarely more (Pl. II 12, 15).

The lumen of cytopharyngeal tube changes along its axis. At the cytostome it is elliptic in cross section (Fig. 2 b). Farther inwards it grows more and more circular (Fig. 2 c–f), at 4.5–6 μ from the distal end the diameter is about 2.5 μ. At the region, where nemadesmata draw close to the tube, the lumen of tube widens (Fig. 2 d, Pl. II 13). Still further, as the diameter of basket decreases, the diameter of tube decreases also, to widen again at the proximal end, just above the opening of the cytopharyngeal apparatus.

Contractile vacuole

In preparations silvered after Chatton–Lwoff the contractile vacuole pores appear as rings (Fig. 1 A, Pl. I 10), as described by Kaczanowska and Kowalska 1969. In cross sections, stained with toluidine blue, around the contractile vacuole there is a zone of light cytoplasm (Pl. II 23).

Observations by electron microscopy

In the surface layer of *Chilodonella cucullulus* cell two morphologically differentiated regions are discernible, viz. the somatic and the oral one (Sołtyńska 1969).

Somatic cortex

Pellicle

The pellicle of *Chilodonella cucullulus* of both the ventral and the dorsal sides consists of three membranes of the unit membrane type, similarly as in many other ciliates, e.g. *Paramecium* (Pitelka 1965), *Tetrahymena* (Pitelka 1961), *Euplotes* (Gliddon 1966), thus corresponding with the pellicle of the cortex type C, according to the classification by Fauré-Fremiet and André 1968 b. The outer pellicular membrane — the cell membrane — covers the entire cell, with cilia and all the depressions of cell surface, parasomal sacs, contractile vacuole pores etc. included. Directly under the cell membrane lies the membrane, constituting the outer wall of pellicular alveoles. The third membrane lines the inner side of alveolar lumen. The shape of alveoles depends on the used fixative. In material fixed with glutaraldehyde the alveoles are more flat, then in that fixed with osmium, in accord with Pitelka 1965.

Inside the alveoles there are electron dense granules, part of which forms a layer, adjacent to the inner alveolar membrane. The rest of them is scattered in the lumen of alveole, and interconnected to form a meshwork pattern. A similar structure, referred to as "spongy" has been described in *Nassula elegans*, and cytochemically acid polysaccharides have been detected there (André and Fauré-Fremiet 1967). Such structure has been found also in *Ignotocoma sabellarum* (Lom and Kozloff 1969). Under the outer alveolar membrane of *Chilodonella cucullulus* there are aggregations of electron-dense material, probably fibrous in structure (Pl. III 25, 26, 28).

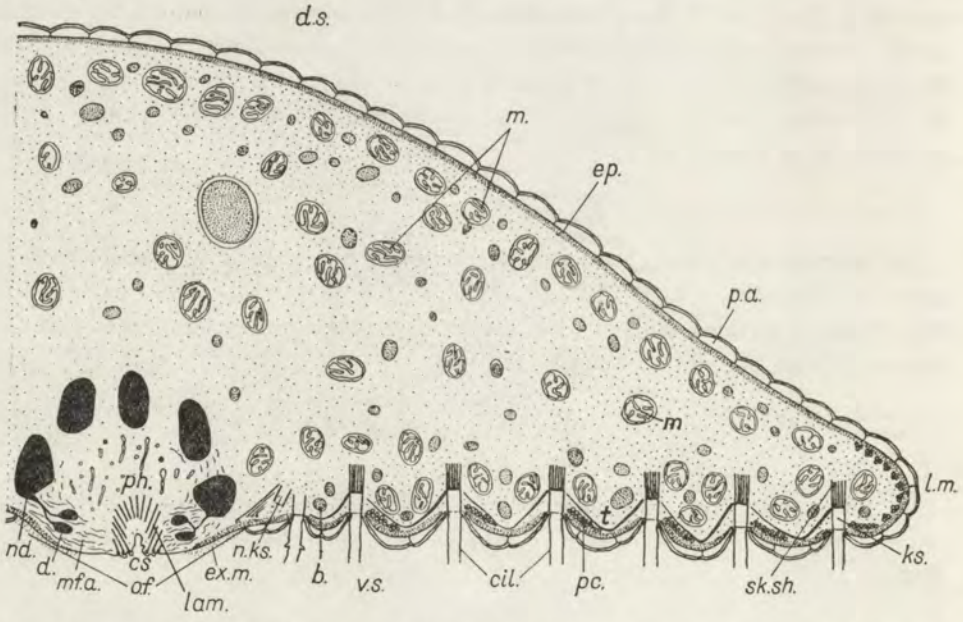


Fig. 3. Schematic diagram of cortex organization in different regions of the cell of *Chilodonella cucullulus*, transverse section. Explanations as in Fig. 1

Alveoles of two neighbouring interkinetal spaces may touch in the axis of the kinety, between the kinetosomes, or to one or the other side of the axis. The arrangement of alveoles in relation to the kinetosomes is also not at all regular. A single alveole may touch either two or three kinetosomes (Pl. IV 33). Close to the kinetosome the alveoles reach up to the rim of the depression in cell surface, surrounding the kinetosome, referred to by Pitelka 1961 as the circumciliary depression.

Epiplasm

Directly under the inner alveolar membrane is a layer of cytoplasm of medium electron density, named epiplasm by Fauré-Fremiet 1962. The epiplasm is here about 50 mμ thick, and in glutaraldehyde fixed material it looks sometimes as if it had a laminar texture. These layers are of different electron density (Pl. V 36,

VI 39), similarly as in *Nassula elegans* (André and Fauré-Fremiet 1967). The epiplasm is discontinuous in the area of parasomal sacs, contractile vacuole pores, cytostome and probably also of cytopyge. Partial discontinuities are noted at the cilium kinetosome border (see below).

Below the epiplasm the ribosomes are observed, arranged parallel to it, often in the form of polysomes, as in *Tetrahymena* (Allen 1967).

Subpellicular fibrils

On the dorsal side of the animal no fibrillar structures have been found. On the ventral side there are only the systems of microtubular and periodic fibrils, which start at the proximal ends of kinetosomes (see below). Along the lateral margin of the cell runs a band of microtubular subpellicular fibrils, that have no connection with any kinetosomes. These fibrils are arranged in small complexes, presumably triads (Fig. 3, Pl. III 26, 27).

Cilium

Cilia of the somatic kineties are of the standard type. Their walls are made up of the cell membrane, covering the whole body of the ciliate. The fibrils running along the cilium are microtubules, and are arranged according to the typical pattern, i.e. two axial fibrils and 9 pairs of peripheral fibrils (Fig. 4).

The proximal ends of axial fibrils stick in the axosome (Pl. IV 30), the distal ends reach farther towards the end of cilium, than do whole pairs of peripheral fibrils (Pl. IV 32). In material fixed with glutaraldehyde connections of the axial fibrils are visible (Pl. V 37), the "central sheath" of Gibbons and Grimstone 1960. From the axial fibrils to the peripheral ones run radii ("spokes" of Gibbons and Grimstone 1960), consisting of finely granular material, with peculiar swellings (Pl. IV 31).

The peripheral fibrils run along the cilium, pass through the terminal plate, which marks the boundary between the cilium and the kinetosome, and reach down to the proximal end of kinetosome. Between the outer walls of fibrils and the cell membrane there is a finely granular material (Pl. IV 31). In the distal part of the cilium the peripheral fibrils end at varying levels. Most often one fibril of the pair ends sooner than the other (Pl. IV 32). On fibril A of each peripheral pair there are the arms, described by Gibbons and Grimstone 1960. The arms are unequal, the outer is crooked, the inner one straight, pointing obliquely inwards (Pl. IV 31). This phenomenon has been described in *Tetrahymena*, and probably has some physiological importance (Allen 1968). The filaments, connecting the inner arm of fibril A with the fibril B of the adjacent pair (described in *Tetrahymena* by the same author), are also visible here (Pl. IV 31). Similar connections are noted as well between the outer arm of fibril A and the fibril B (artifacts ?).

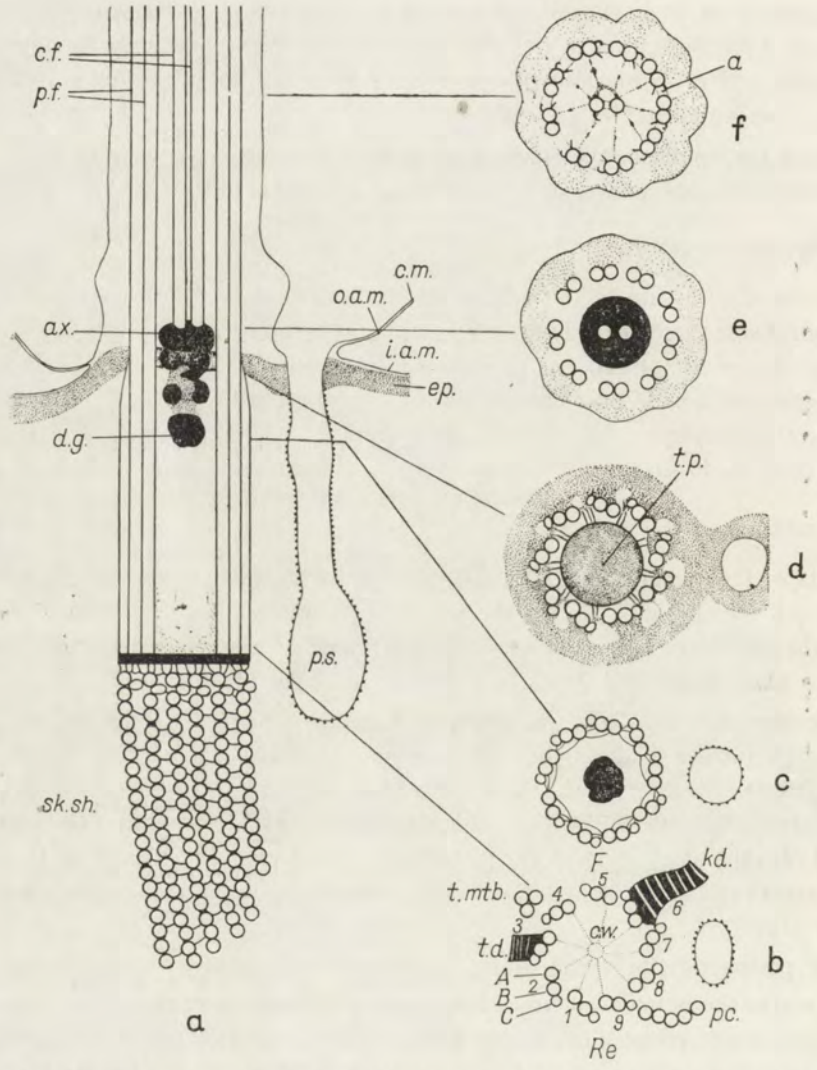


Fig. 4. *Chilodonella cucullulus*. Schematic representation of a kinetosome, cilium and complex of kinetosome-based fibrils, a — longitudinal section of kinetosome and cillium, b, c, d, — cross-sections of kinetosome, as viewed from inside the cell, e, f — cross-sections of cillium. Explanations as in Fig. 1

Kinetosome

The length of kinetosomes, taken from the terminal plate to the bottom, made of electron-dense material and closing the proximal end, is 420–520 μ, the diameter is from 200 μ to 270 μ.

The fine structure of kinetosomes in *Chilodonella cucullulus* does not substantially differ from that of other ciliates, as reported by Grain 1966. The kinetosome

wall consists of 9 triplets of microtubules. These triplets skew inwards clockwise, when viewed from inside the cell (Fig. 7, Pl. IV 35). Out of the fibrils A, B, and C, composing each triplet, the outermost and thinnest fibril C ends at the level of terminal plate, at the distal end of the kinetosome.

In longitudinal sections of the kinetosomes the limits of the terminal plate are discerned with difficulty. The only visible structure, cutting transversely through the whole lumen of the kinetosome, is the plate, immediately underlying the axosome (Fig. 4 d, Pl. V 36, 37). In cross sections of this region a plate of material of uneven electron density is noted, occupying a central position. This plate communicates through fine radii with the medium electron-dense material, lying outside the triplets. This material in turn communicates with the epiplasm, which at this level reaches the kinetosome (Pl. IV 29, 33). In *Paramecium aurelia* (Hufnagel 1966, 1969) in isolated fragments of pellicle the terminal plate was found to be a continuation of epiplasm. There it consists of a central part, of the radii, passing between the triplets, and of the outer annulus. In other words it is a plate, provided with perforations, through which the peripheral fibrils pass from the kinetosome into the cilium.

Below the terminal plate there are dense granules, 1–5 in number, connected to each other and to the terminal plate (Fig. 4, Pl. V 36, 37), not exceeding half the length of the kinetosome. Granules of this type are found in many ciliates, in varying numbers and positions (Grain 1969).

Near to the proximal end of the kinetosome lies the axial structure ("cartwheel structure" of Gibbons and Grimstone 1969), with its radii of finely granulated material, running from it to the triplets (Fig. 4 b, Pl VI 40). At the proximal end of the kinetosome is a layer of electron-dense material, which forms its bottom (Fig. 4, Pl. V 37, VI 40).

Kinetosome-based fibrils of the somatic kinety

The somatic kinety is a single row of irregularly spaced kinetosomes, linked together by a band of microtubules the subkinetal sheet. From the proximal end of each kinetosome run a number of fibrils: the kinetodesmal fibrils, the postciliary fibrils and the complex of transverse fibrils.

1. The kinetodesmal fibril in *Chilodonella cucullulus* is striated, with alternating narrow and wide striae (Pl. V 36). It is attached to triplet 6, the attachment being direct, as is typical of this fibril (Pl IV 35). The zone of attachment covers about 1/5 of the kinetosome length. From there the kinetodesmal fibril proceeds usually to the right of the kinety axis, anteriad and towards the cell surface as in other ciliates. The angle between the kinety axis and the kinetodesmal fibril changes along the run of the latter. Just at the kinetosome it is about 70–80°, then the kinetodesmal fibril turns more towards the course of the kinety axis, and the angle falls to 45–25°. In this animal the kinetodesmal fibril is short, reaching but the level of the kinetosome

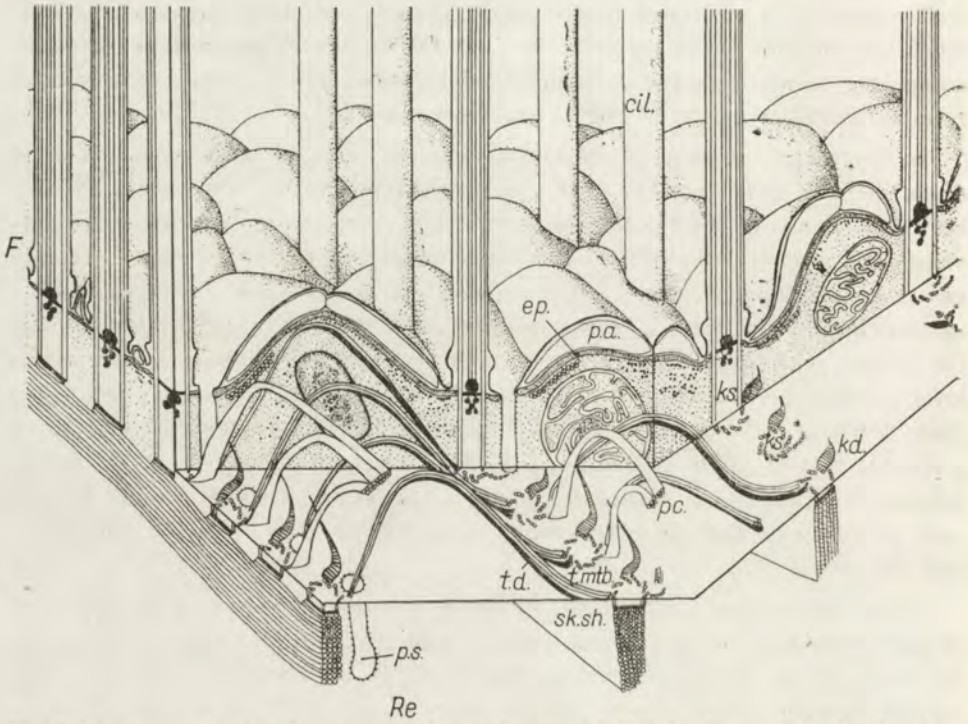


Fig. 5. Stereogram showing the structure of ventral cortex of *Chilodonella cucullulus*. Explanations as in Fig. 1

just in front of it, always at a distance from its kinetodesmal fibril (Fig. 4 b, 5, 6, Pl. III 25).

2. The postciliary fibrils have been described in *Tetrahymena* by Pitelka 1961. These are microtubules, arranged so as to form a ribbon, originating close to triplet 9, and attached to it by electron-dense material (Grain 1969). In *Chilodonella cucullulus* the postciliary fibrils form at their base a row of 5–6 fibrils (Fig. 4 b, Pl. IV 35), proceeding, as in other ciliates, rightwise, upwards and rearwards. The single row changes meanwhile into a double-row cluster, to join other such clusters, running from next kinetosomes, and to form together a double-row ribbon, parallelling the kinety (Pl. V 37, VI 38). This ribbon includes 20–30 microtubules. Thus each particular cluster of 5–6 fibrils has to pass 4–6 kinetosomes. As the interkinetosomal distances vary from 0.4 μ to 0.75 μ, the length of microtubules is probably not less then 1.6 μ and not more than 4.5 μ. At their mother kinetosomes the postciliary fibrils rise upwards, so as to reach the level of epiplasm just behind the parasomal sac. They join the left, kinety-facing side of the ribbon, and in tangential sections two or three of them are visible under the cell surface (Fig. 5, Pl. IV 29, VI 38). Hence the conclusion, that the double-row arrangement of fibrils, composing the ribbon, where fibrils of both rows form a succession of triads, is a result

of prior regroupment of fibrils on their way from the kinetosome to the ribbon.

In the oral region the postciliary fibrils, similarly as the kinetodesmal ones, do not cross the zone of microfibrils, associated with the buccal apparatus (Fig. 6, Pl. III 25).

3. Transverse microtubular fibrils run to the left, at right angles to the kinety axis, directed towards the cell surface, and towards the adjacent kinety. Such fibrils,

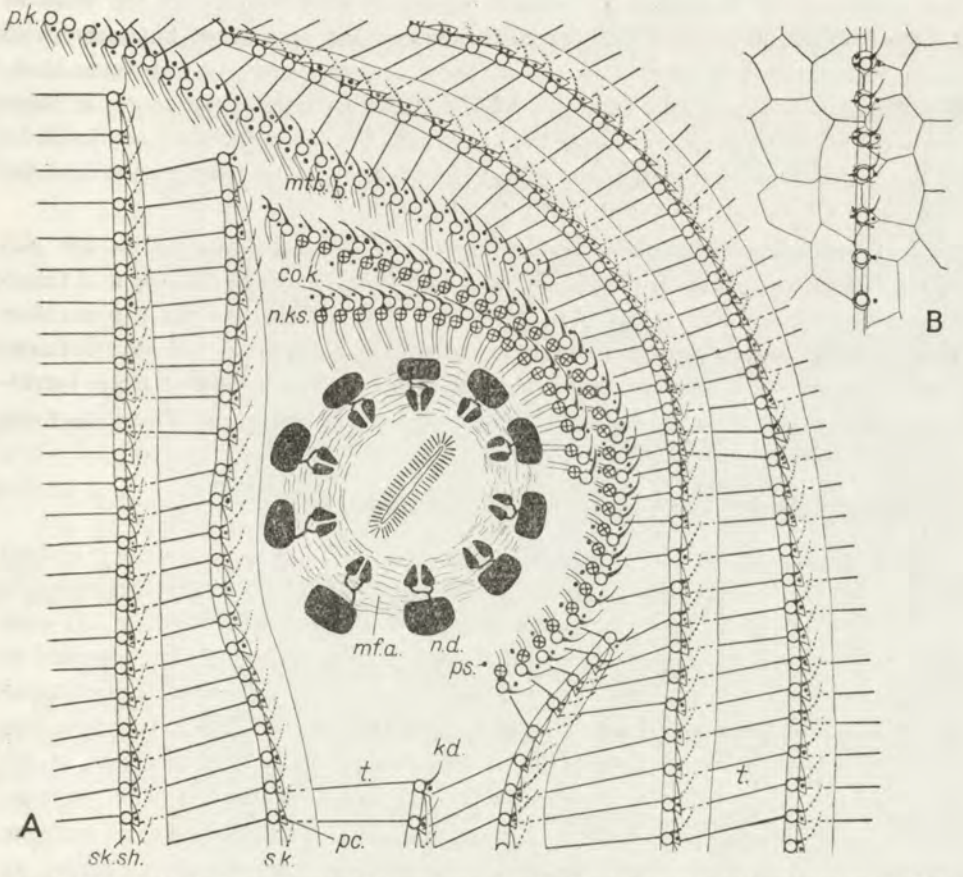


Fig. 6. Schematic diagram showing the structure of subpellicular layer in the oral region of *Chilodonella cucullulus*. A — oral region, disposition and structure of somatic, preoral and circumoral kineties. B — kinetosomal territories of a somatic kinety with pellicular alveoles superimposed. Explanations as in Fig. 1

described in *Tetrahymenida* by Pitelka 1961, occur also in many other ciliates (Grain 1969).

In *Chilodonella cucullulus* these fibrils arise at the left side of the kinetosome, at triplet 4, separated from it by electron-dense material. At the kinetosome base they are arranged in a compact bundle (not row, as in other ciliates) of three or four microtubules (Fig. 4 b, Pl. IV 35, VI 38). From the kinetosome base they proceed to the

left and upwards, usually at right angles to the kinety axis, towards the adjacent kinety. Rising towards the epiplasm they run at first parallel to the neighbouring dense fibril, discussed below (Pl. IV 35). Reaching the epiplasm, they contact this dense fibril (Pl. VI 43), proceed further towards the adjacent kinety as a complex of both types of fibrils, and terminate near to the beginning of postciliary fibrils (Pl. VI 42) or, more rarely, at the beginning of kinetodesmal fibrils. All their way the transverse fibrils remain a compact bundle of microtubules, in the form of triad or tetrad. Because of differences in number and spacing of kinetosomes in adjacent kineties it happens, that not all the kinetosomes receive transverse fibrils from the kinety next to the right (Pl. VI 42). Hence sometimes the course of these fibrils is not exactly perpendicular to the kinety axis, but somewhat skew rearwards — in the middle and hind part of the cell, or forwards — in the front part, where the right side kineties curve left (Fig. 6, Pl. VIII 48).

4. Transverse electron-dense fibrils, with visible striation, arises at the left side of the kinetosome, at the triplet 3, i.e. the next one rearwards to the origin of transverse microtubular fibrils (Fig. 4 b, Pl. IV 35). It is attached to the triplet either directly or through electron-dense material. Identification of the actual way of attachment is prevented by the obscuring effect of striation. The transverse dense (periodic ?) fibril runs parallel to the microtubular transverse fibrils, and then joins them (Pl. VI 41, 43).

Subkinetal sheet

In somatic kineties, underlying and contacting the kinetosomes, there is a sheet of microtubules, connecting all the kinetosomes within the kinety. This sheet consists of six rows of fibrils, neatly arranged and interconnected with desmata. At each kinetosome arises the upper layer of that sheet, i.e. six microtubules, attached to the proximal end of kinetosome. The attachment occurs through the plate of electron-dense material, forming the kinetosome bottom (Fig. 4 a, Pl. V 37). It seems, that the bottom plate does not contact the microtubules directly, but by means of numerous desmata. Tubules of the upper layer are also interconnected with desmata.

The layer proceeds rearwards, and the microtubules slide under or between the fibrils, arising under the next kinetosome (Pl. VI 39, 40). Somewhat farther to the rear the lower fibrils lie exactly under the upper ones, and in direct contact with them, so as to form distinct, compact rows. Thus the number of fibrils is increasing backwards, but the increase is not very regular. In particular rows of the sheet the number of tubules may be different, e.g. from left to right: 10, 10, 12, 9, 8, 7, or 21, 21, 20, 19, 18, 15. Maximum number of fibrils per row noted was 21. Hence it may be concluded, that either the length of fibrils is variable, or that not always six tubules arise under one kinetosome. The latter situation, though, was never observed. Maximum length of a single fibril has accordingly to be equal to 21 interkinetosomal distances. As the kinetosomes are spaced at 0.65 μ on the aver-

age, maximum length of a single subkinetal microtubule can be estimated at around 14 μ.

The subkinetal sheets correspond to the protargol-staining bands, running along the somatic kineties.

Parasomal sac

Close to the kinetosomes of somatic and oral kineties, as well as of the dorsal kinety, are found invaginations of the cell membrane, penetrating deep into the cytoplasm — the parasomal sacs. In the somatic kineties there is always one parasomal sac to each kinetosome, at a fixed position to the right of the kinetosome, behind the kinetodesmal fibril, before the postciliary fibrils (Fig. 5, 6, Pl. IV 29). Their position in the preoral and the oral kineties will be discussed later.

The parasomal sacs are variably deep. Maximum noted depth was 600 mμ. They may sink deeper than the proximal end of kinetosome (Pl. V 37). The diameter of sacs is similarly variable. Even along a single kinety in an interdivisional specimen there are big differences, without any recognizable regularity (Pl. IV 34). The diameter of each sac is different at different levels (Fig. 4).

The sac wall consists of the cell membrane, lined on the cytoplasmatic side with electron-dense granules, arranged in one layer (Pl. IV 34). Around the distal part of the sac the epiplasm exhibits annular swellings (Pl. III 25), similar to those described in *Paramecium* (Metz et al. 1953, Pitelka 1965, Hufnagel 1966, 1969).

The bodies, silver impregnating after Chatton et Lwoff, and situated to the right of the kinetosomes, correspond to the parasomal sacs, as far as their position is concerned.

Mitochondria

In *Chilodonella cucullulus* the mitochondria are scattered throughout the cytoplasm. In the superficial cytoplasm they lie near to the kinetosomes in the interkinetal spaces. Their sections are circular or elliptic in shape, with numerous twisted tubular cristae, such as are typical of free-living, active ciliates (Vivier 1966).

In glutaraldehyde-fixed specimens mitochondria are in general spherical, and in the middle of matrix a conglomeration of electron-dense material is visible (Pl. III 25, Pl. XI 55, 57). When osmium tetroxide fixation is applied, mitochondria are usually elliptic, and no such conglomeration can be detected (Pl. XII 61, 62).

Bacteria

Bacteria are found both in the superficial cytoplasm and in the middle of the cell. Around the bacterial cells no cytoplasmatic membrane is noted, separating them from the ciliates cytoplasm, irrespective of the used fixative (Pl. III 25, Pl. XI 57). Presumably the absence of vacuolar membranes can not be ascribed to faulty fixation, as such membranes are found intact around ingested yeast cells. Hence

the conclusion, that these must be symbiotic bacteria. Symbiotic bacteria have already been described, as living in the cytoplasm of many ciliates (de Puytorac 1967).

Cortex of the oral field

Over the oral field the pellicle consists of one membrane only, the cell membrane. The outer surface of this membrane is covered with material of medium electron density (Pl. IX 50–52). Under the membrane lies the epiplasm. Neither the extracellular material, nor the epiplasm reach the rim of cytostome. Both are absent at the insertion of cytopharyngeal tube into the cell membrane (Pl. IX 50, 52). Out of the fibrous structures there are in the cortex of the oral field the microfibrils, spreading in the superficial layer of cytoplasm around the cytostome (Pl. IX 50, 51).

Oral ciliature

Cilium

Cilia of the preoral and the circum oral kineties have their central and peripheral fibrils arranged according to the standard pattern. Cross sections of distal ends of cilia reveal no central fibrils, and deficiencies in the ring of peripheral fibrils. Cell membrane, covering these cilia, forms digitiform or, less frequently, lobate protrusions (Pl. VII 46). In some sections these protrusions seem to form bridges, connecting the cilia.

Kinetosome

In the circum oral kineties, beside cilia-bearing kinetosome, there are nude kinetosome, lacking cilia. The ciliated kinetosomes are 360–380 mμ long, the non-ciliated ones 260–320 mμ, both as measured in glutaraldehyde material.

In the ciliated kinetosomes the terminal plate is of identical build as in somatic kineties. The electron-dense granules, lying under the terminal plate, may be more numerous, say, 7 in number, filling up to 2/3 of the length of kinetosome (Pl. VII 44). No plate of electron-dense material has been found at the proximal end.

The non-ciliated kinetosomes, shorter than the ciliated ones, do not reach the level of epiplasm, and their proximal ends are sunken deeper in the cytoplasm, than those of ciliated kinetosomes (Pl. VII 44, 47).

Organization of circum-oral kineties

Ciliated and nude kinetosomes alternate along the kinety, and are somewhat shifted to the sides, so as to form two rows in a zig-zag pattern. The outer in relation to the cytostome, i.e. the right one consists of ciliated kinetosomes, the left one of nude ones (Pl. VII 44, 46, 47). Kinetosomes of both rows are connected, forming pairs. In each pair the nude kinetosome is in front, its triplets 9 and 8 being in con-

tact, by means of electron-dense material, with triplets, 5, 4 and 3 of the right, cilated kinetosome, situated to the rear (Pl. VII 45).

At the kinetosomes arise microtubular and periodic fibrils.

At the proximal end of a nude kinetosome, on the left, close to triplet 4, arise 6 microtubules. These proceed as a one-layer band to the left and upwards, up to the cell surface (Pl. VII 47).

In one single case a periodic fibril has been detected, at triplet 6, on the right side of a nude kinetosome, but its course could not be ascertained (Pl. VII 45). Judging from the structure and the typical place of attachment, it must be a kinetodesmal fibril.

From the proximal end of a ciliated kinetosome (the right, hind one in a pair) starts but a single fibril, as it seems. Having arisen at triplet 6, on the right side of the kinetostome it runs forwards and upwards (Pl. VII 45). Being conspicuously periodic it may be classified as kinetodesmal fibril.

At the distal ends of kinetosomes no interkinetosomal connecting structures have been observed.

Parasomal sacs are noted on both sides of each circum oral kinety, to the right of a ciliated kinetosome, and to the left of a nude kinetosome (Fig. 6, Pl. VIII 46). During the division, in the newly formed circumoral kineties of the future opisthe, the same disposition of parasomal sacs on both sides of a double-row kinety is observed (Pl. XII 59).

Organization of the preoral kinety

The preoral kinety is a single row of kinetosomes, each bearing the same set of kintosome-based fibrils. To the right, at triplet 6, is noted the beginning of kinetodesmal fibril, which follows next the conventional course forwards, to the right and towards the cell surface (Pl. VIII 48). Left of the kinetosome arise microtubules, making a six-tubule band, which does not join other such bands (Pl. VIII 48, 49). The exact position of their origin in relation to kinetosome has not been identified. There are two parasomal sacs to each kinetosome, one at each side of the kinety.

The pattern of pellicular alveoles around the preoral and the circum oral kineties is the same as at the somatic kineties.

Arrangement of all the ventral kineties of *Chilodonella cucullulus*, together with the associated fibrillar complexes and parasomal sacs is diagrammatically pictured in Fig. 6.

Buccal apparatus

Here belong the oral kineties, discussed above, the cytostome, cytopharyngeal tube, nemadesmata and a peculiar cytoplasmatic region, the phagoplasm.

Cytostome

The cytostome is a slot-like depression of the cell membrane (Pl. IX 53). On the outer surface of this membrane there is no material of medium electron density, such as is coating the rest of the oral field. There is no epiplasm here, the cell membrane contacting directly the cytoplasm. The cytostome rim is visibly folded (P. IX 51–53).

Cytopharyngeal tube

Cytopharyngeal tube is the inner duct of the cytopharyngeal apparatus (Pl. IX 51). It is composed of about 50 lamellae, any one of which consists of about 20 microtubules, fitted tightly to each other in a plane (Pl. X 54). The number of microtubules per lamella is not stable, some lamellae are narrower than other ones. The distal end of each lamella is overlaid with electron-dense material, anchored in the layer of microfibrills, which underlies the cell membrane around the cytostome. This material reaches the inner surface of cell membrane, which in this region is folded (Pl. IX 50, 52).

Below the distal end of cytopharyngeal tube there are structures, connecting the outer (in relation to tube axis) margins of lamellae. These are electron-dense granules, three to five in number, tied to the lamellae with desmata (Pl. IX 52, Pl. X 54).

The inward edges of lamellae lie free in the cytoplasm. Lamellae are not exactly radial in position, but skew inwards counterclockwise, when viewed from outside the cell, and are somewhat bent, some of them more, some less (Pl. X 54). In the middle part of basket, where a nemadesmata approach the cytopharyngeal tube, the connections between lamellae disappear (Fig. 2 d Pl. XI 55).

The lumen of cytopharyngeal tube changes from elliptic at the distal end of basket to circular in the middle of its length. Close to the proximal end of tube ring of lamellae is deficient, as some of the lamellae fall out (Fig. 2 g–i, Pl. XI 57).

Nemadesmata

The cytopharyngeal tube is surrounded by a palisade of nemadesmata. Each nemadesma is a bunch of microtubules, parallelling each other, interconnected and arranged in a fixed, hexagonal pattern (Pl. X 54, 54 A). In the electron-dense walls of microtubules, subfibrils are discernible. The desmata are also not homogeneous in their electron density (Pl. X 54 A). Distal ends of nemadesmata are covered with caps of electron-dense, not homogeneous material (Pl. IX 50, 51). From the distal end of nemadesma towards the cell surface runs an electron-dense string, carrying the pair of dentes. The fine structure of the dentes and their stalk is obscure. All the dentes, stalks and nemadesmata tops stick in the layer of microfibrils, situated just below the epiplasm. (Pl. IX 51). Besides the distal end caps, nemadesmata have no coating, consist solely of microtubules. At their outer surfaces, facing outside the basket, there is material of medium electron density, observed among

the marginal microtubules (Pl. X 54). As towards the proximal end the nemadesmata draw closer to each other, the hexagonal pattern turns loose on their inner and lateral surfaces. Nemadesmata decrease in diameter, and freed microtubules fill the spaces between nemadesmata and between them and the cytopharyngeal tube (Fig. 2 d–f, Pl. XI 55). There still are desmata between the microtubules but these connections lack regularity. The layer of free tubules unites both elements of cytopharyngeal apparatus in a compact whole, which does not get loose even when the cytoplasm is swollen, due to faulty fixation (Pl. XI 56).

Nearing the proximal end of basket the ring of nemadesmata opens, and the number of microtubules per nemadesma drops visibly (Fig. 2 g–i, Pl. XI 57).

The cytoplasm, filling the distal half of cytopharyngeal apparatus corresponds structurally to the phagoplasm, as described by Fauré-Fremiet 1961 b. It contains numerous, membrane-bound vesicles (Pl. III 25, Pl. IX 50, 51). In the tube lumen and in the space between tube and palisade, and even outside the nemadesmata, but close to them, there are tubules of a rather complex structure. They show an outer triple membrane and a narrow tubule inside in cross-sections, and striation in longitudinal sections (Pl. X 54). These tubules are identical with those, found in *Ch. uncinata* by Pyne 1970. In the proximal half the cytoplasm does not differ in structure from that, filling the rest of the cell, it even contains mitochondria (Pl. XI 55).

Contractile vacuole pore

The excretory organellum of *Chilodonella cucullulus* consist of elements typical of any ciliate: the excretory pore on the cell surface, the canal, connecting the pore with the vacuole, the vacuole proper and the nephridial tubules, discharging into it.

The pore is surrounded by pellicular alveoles (Pl. XII 60). They do not reach the rim of pore and the free space around it is covered only by the cell membrane, which dips into the cytoplasm to make the wall of the canal. The epiplasm does not reach the pore rim either. The wall of discharge canal is lined with fibrillar structures (Pl. XII 61).

The pore is constantly gaping, presumably due to the surrounding epiplasm, and to the almost regularly radial arrangement of walls of neighbouring pellicular alveoles. Changes in intravacuolar pressure result in but minor changes in pore diameter.

Discussion

When comparing data, obtained by silver impregnating techniques with the results of electron-microscopical observations, the following may be concluded.

The argentophilic meshwork corresponds to the contiguous rims of pellicular alveoli, in accord with de Puytorac 1959, Ehret and Powers 1959, Pitelka

1961, Dippell 1962. Dark polygons, appearing in some protargol silvered preparations, correspond to the contents of pellicular alveoles, presumably fluid, as suggested by intravital observations of Kaczanowska and Kowalska 1969.

As the interkinetal distances diminish in starved specimens, the meshes of silverline system become elongated and narrow. Hence the stratum, underlying the alveoles, i.e. the epiplasm, can be presumed to exhibit marked elasticity.

All the structures, which darken, when silvered after Chatton et Lwoff, viz. kinetosomes, cytostome border, contractile vacuole pores and parasomal sacs, share one common trait, i.e. the absence of pellicular alveoles. Hence the conclusion that it is the alveoles, which form a barrier, hindering the penetration of silver ions into the cell. Silver is reduced here either in the epiplasm (annulus around the contractile vacuole pore), or in the terminal plate of kinetosome, which is a prolongation of epiplasm, or in the peripheral cytoplasm (parasomal sacs, cytostome border). It may be presumed therefore, that dark granules of unknown ultrastructure, observed on the dorsal side of *Chilodonella cucullulus* (Kaczanowska and Kowalska 1969), are discontinuities of alveolar cover, and perhaps of epiplasm, too (cuticular pores ? mucocysts ?) (Pl. I 7).

Cortex

The ciliate cortex serves both to protect the cytoplasm from harmful environmental agents and to preserve the proper shape of cell (Pitelka 1965).

When treating *Chilodonella cucullulus* with a toxic agent (formalin) it was found (Kink 1970), that conjugating pairs are more resistant than non-conjugants. In conjugants it is the oral field, that is the contact zone. It is also the only major area, devoid of alveoles, which otherwise cover the entire body except for pores and parasomal sacs. Blocking of this unprotected surface is presumably the cause of higher resistance of conjugants. It is safe to assume, that pellicular alveoles form a barrier against penetration of toxic substances into the cell.

The role of cortical elements in keeping the body form is plainly indicated by experiments with *Blepharisma* and *Paramecium* (Asterita and Marsland 1961). *Blepharisma*, its cortex consisting of one membrane, the cell membrane only (Kennedy 1965), is much easier disfigured with proteolitic enzymes, polysaccharidases, high pressure etc. than is *Paramecium*, furnished with pellicular alveoles (Pitelka 1965).

Lom and Kozloff 1969 have also stressed the role of alveoles in preservation of body form. Besides the pellicle, of importance are here also the epiplasm and subpellicular fibrils (Pitelka 1965, Lom and Kozloff 1969).

Four different structural types of cortex can be discerned in *Chilodonella cucullulus*, determining differences in stability of form: cortex of the oral field, dorsal cortex, cortex of the lateral body margin and the ventral cortex.

1. The cortex of the oral field has no pellicular alveoles and almost half of its area is free of epiplasm. Hence the plasticity of the oral field, indispensable in phagocytosis of bigger food particles. Microfibrils of the oral field link the elements of cytopharyngeal apparatus, thus coordinating their movement at phagocytosis. If they were contractile, they could even effect these movements. Both these functions are possible for microfibrils.

Material of medium electron density, covering the cell membrane, serves probably for protection, and possibly also for adhesion of food particles. It may effect the adhesion of partners during the preliminary phase of conjugation, too.

2. The dorsal cortex is built of the pellicular alveoles and of epiplasm. As these are the only shape-supporting structures of the dorsal side, the cortex is markedly elastic here. The body form is influenced by macronucleus and basket, which bulge the cell surface out (Fig. 1 B). Convexity of the dorsal side depends upon the number of food vacuoles.

3. Cortex of the lateral body margin besides pellicular alveoles and epiplasm contains also strands, located in the peripheral cytoplasm and built, as it seems, of microtubular triads.

Microtubules are accredited with various roles, that of support, of secretion, passage of impulses, movement coordination, transport of cell fluids, in morphogenesis, in cell contractility (opinions on this subject are reviewed by Grimstone 1966, Grim 1967, Pitelka 1968, Grain 1969). Moreover, there is no evidence to the effect, that all microtubules should perform the same function. Contrariwise, in different microtubulues, when treated with various chemical agents, different reactions were noted by Behnke and Forer 1967. These authors managed even to base upon such data a classification of microtubules into four classes.

Anyway the function of mechanical support is the one most frequently ascribed to microtubules (Grim 1967, Pitelka 1968, Grain 1969). Allen 1967 makes the cortical microtubules of *Tetrahymena* (basal microtubules excepted) responsible for keeping the body in shape, because of their comparatively high rigidity. The same role has been experimentally proven for the axopodial microtubules in *Actinosphaerium* (Tilney et al. 1966).

The shape-stabilizing effect increases, when microtubules are grouped in strands, ribbons etc. A microtubular triad seems to be the simplest organization of a supporting structure. Triads have been found in the layer of subpellicular microtubules in dorsal cortex and in the body margin of *Euplotes* (Gliddon 1966, Grim 1967, Tuffrau et al. 1968, Fauré-Fremiet and André 1968 a).

Disposition of microtubules along the cell margin results in dorso-ventral flattening of cell. Microtubular strands running along the cell circumference were found also in blood platelets of man and rat (Behnke and Zelander 1966).

4. The ventral cortex is provided, out of the supporting structures with pellicular alveoles, epiplasm and kinetosome-based fibrils. The ventral cortex is not

homogenous, because kinetosomal territories of somatic, circumoral and preoral kineties are different.

Somatic kineties

Kinetosomal territory is a small area around a kinetosome or a pair of kinetosomes, which is (or are) its organization centre. It is characterized by a complex of kinetosome-based fibrils (Pitelka 1969). The ciliary corpuscle, as defined by Ehret and Powers 1959 should besides include the parasomal sac, which is regarded as a constant and important element (Dippell 1965, Hufnagel 1969).

The kinetosomal territory of a somatic kinety in *Chilodonella cucullulus* contains the typical set of fibrils: the kinetodesmal fibril, postciliary fibrils and transverse fibrils.

The kinetodesmal fibril is short and leaves the kinetosome almost at right angles to the kinety axis, similarly as in *Ignotocoma* (Lom and Kozloff 1969).

Postciliary fibrils are arranged so as to form triads.

Transverse fibrils form a complex of two types of fibrils: a triad or tetrad of microtubules and a dense (periodic ?) fibril. A similar fibrillar complex, oriented at right angles to the kinety, was described in *Conchophthirus* (Antipa and Small 1968), and a dense fibril, being the sole transverse fibril, was found in *Ignotocoma* (Lom and Kozloff 1969).

In *Chilodonella cucullulus* the outer limits of a kinetosomal territory are not marked on the cell surface by any distinct alveolar pattern, as is the case in *Paramecium*, *Hyalophysa*, *Didinium* and *Condylostoma* (Pitelka 1969). Most often a single alveole contacts two or three territories. The parasomal sac has a fixed position to the right of the kinetosome.

Kinetosomal territories of a somatic kinety are in *Chilodonella cucullulus* connected through the subkinetal sheet, linked to each kinetosome. The subkinetal sheet in *Ignotosoma sabellarum*, as described by Lom and Kozloff 1969, is composed of two rows of fibrils, which do not contact the kinetosomes. A similar sheet underlies the kineties in *Ancistrocoma pelseneeri*, and seems to be connected with the kinetosomes through desmata (Khan 1969 Pl. III 8).

Kinetosomal territories of adjacent kineties are loosely linked (not all) through the transverse fibrils of the right territory, terminating close to the origin of postciliary fibrils of the left territory.

Configuration of fibrils of a territory is asymmetric already at the spot of contact (direct or indirect) with the kinetosome, as is typical of all ciliates, and determined by the inner polarity and asymmetry of kinetosome (Pitelka 1969). In a number of *Holotricha* Grain 1966 described the kinetodesmal fibril as being a prolongation of an imaginary spiral radius, running through the triplet, at which this fibril starts. In *Chilodonella cucullulus* there is no pronounced relation between the orientation of fibrils, passing from the kinetosome to the right and left, and that of spiral radii

(Fig. 7). The only geometrically regular course is that of subkinetal fibrils, which start and proceed always at right angles to the kinetosome axis. The number of these fibrils — 6 per layer — is the lowest number, at which every triplet is contacted by one at least fibril. Similarly in *Ignotocoma sabellarum* (Lom and Kozloff 1969) fibrils of the subkinetal sheet are organized in layers of 6 microtubules each.

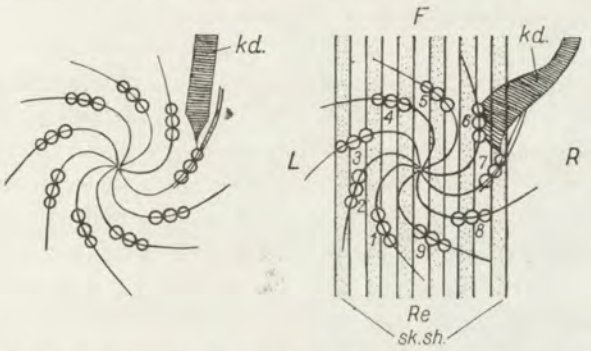


Fig. 7. Schematic drawing to show the orientation of kinetodesmal fibril, as seen from inside the cell. A — in Gymnostomes, after Grain 1966. B — in *Chilodonella cucullulus*, with outlines of subkinetal microtubules added. Explanations as in Fig. 1

On the assumption, that all kinetosome-based fibrils are (possibly besides other functions) supporting the cortex and keeping kinetosomes and cilia steady, preventing any shifting or rotating movement of the kinetosome (Allen 1967), this complex of fibrils should be regarded as a single functional entity of complementary elements.

In *Chilodonella cucullulus* and *Ignotocoma sabellarum* the shortness of kinetodesmal fibril is probably compensated for by the presence of an accessory supporting structure, the subkinetal sheet. Similar short kinetodesmal fibrils were found in *Colpidium* (Cheissin and Mosevitch 1962), *Tetrahymena* (Metz and Westfall 1954), *Glaucoma* (Pitelka 1961) and *Ophryoglena* (Roque et al. 1965), always accompanied by not kinetosome-based subpellicular microtubules.

Kinetosomes of the somatic kineties in *Chilodonella cucullulus* are kept steady at their distal ends by the terminal plate, contacting the epiplasm, and at the proximal ends by fibrils, running right and left, and by the subkinetal sheet, so that in relation to other kinetosomes no movement of the kinetosome is possible. This immovability does not prevent the ciliary beat to be of the metachronal-wave type, though Grim 1968 thought it important in coordination of ciliary beat into the membranella-like type.

Ultrastructure of the subkinetal sheet indicates its considerable rigidity, resulting from the orderly packing of interconnected microtubules. That much can be deduced also from the fact, that in moving animals any flexure of the ventral side is possible only in the interkinetal spaces. Every somatic kinety is an integrated whole,

integrated by a straight and rigid structure (the subkinetal sheet), and the morphological effect of this is the flat shape of the ventral side of *Chilodonella cucullulus*.

Preoral kinety

The outer limits of the territory, similarly as in somatic kineties, are not marked off by the arrangement of pellicular alveoles.

The fibrillar complex is different from that of a somatic territory. It is only the kinetodesmal fibril, which is recognizable at its usual position, to the right, at triplet 6. The strand of 6 microtubules at left does not correspond either in position or in course to any part of the fibrillar complex of a somatic kinetosomal territory.

In the preoral kinety there are two parasomal sacs to each territory. This double number does not indicate here any process of territory multiplication, being under way, as is the case in *Paramecium* (Dippell 1965). Two sacs per territory is standard number in interdivisional specimens, and the territories themselves are even smaller than the somatic ones.

As to the morphogenesis of preoral and circumoral kineties divergent descriptions have been published. According to Fauré-Fremiet 1950 and Radzikowski 1966 these kineties are detached anterior fragments of somatic, postoral kineties, which subsequently migrate around the newly forming buccal apparatus up to their normal positions. According to Kaczanowska and Kowalska 1969 morphogenesis of these kineties consists of resorption of somatic ciliature in the region to be occupied by oral ciliature of the future opisthe. Then ciliated kinetosomes are rapidly formed de novo, and organized into membranella-like arrays. Rotation of these membranellae follows, accompanied by allometric growth of somatic kineties of the right side.

Noirot-Timotheé and Lom 1968 demonstrated, that parasomal sacs are the site of pinocytosis. Bradbury 1965 suggested, that in *Opisthonecta* oxygen diffusion through cuticular pores occurs at a much higher rate, than through the pellicular membranes. These pores are similar in structure to the parasomal sacs.

The oral kineties arise at the time of intense morphogenesis of new kinetosomal territories, of intense cortical growth. Doubling of the number of sacs may be of importance in covering the higher energetic demands (gas exchange, pinocytosis) of this active period, the more so as the cytostome is not working yet.

Circumoral kineties

The structure of circumoral kineties being poorly known it is difficult to discuss their kinetosomal territories in detail. The double-row arrangement of kinetosomes, with the outer row of ciliated, and inner row of non-ciliated ones, resembles the pattern, found in UM of *Tetrahymena* (Nilsson and Wiliams 1966), and in the haplokinety of *Opisthonecta henneguyi* (Bradbury 1965).

Besides the different composition of fibrillar complexes in kinetosomal territories of the somatic, preoral and circumoral kineties, these kineties differ in inter-

kinetosomal distances and hence in length of kinetosomal territories, in size of kinetosomes, length of cilia and in structure of the distal ends of cilia.

In somatic cilia of *Chilodonella cucullulus* the peripheral fibrils terminate earlier, than do the central ones, and not all at the same level at that, similarly as in other ciliates: *Ophryoscolex*, *Diplodinium* (Roth and Shigenaka 1964), *Euplotes* (Roth 1956), *Isotrichia* (Roth 1964). In circumoral cilia, which are longer, than the somatic ones, it is the central fibrils, that terminate earlier, below the peripheral ones, similarly, as is the case with *Flagellata* (Gibbons and Grimstone 1960) and with some *Metazoa* (Elliptio, Anodonta, Satir 1965). Hence the assumption, that the structure of the distal end of cilium is not a stable character of this undulipodium but depends upon its length, and this in turn would suggest, that the length of central fibrils is to some extent limited and stable.

The size of kinetosome is probably related to the size of cilium. In *Chilodonella cucullulus* kinetosomes of the circumoral cilia are shorter, than those of somatic ones. Similarly in *Diplodinium* (Roth and Shigenaka 1964) the cilia, which are bigger in diameter, have shorter kinetosomes. In *Tetrahymena* kinetosomes of somatic kineties and those of membranelles do not differ visibly (Satir and Rosenbaum 1965).

Differences in structure between the oral and somatic kineties and between their respective kinetosomal territories confirm the results of Kaczanowska and Kowalska 1969 as regards the morphogenesis of oral kineties. These authoresses have stated that new kinetosomes "... are quickly equipped with cilia and form membranellar structures", even before their migration and rotation. Now, the kinetodesmal fibril is here orientated the same way as in somatic kineties, i.e. to the right and forwards. Hence the question, whether the kinetosome-based fibrils of circumoral kineties arise before the morphogenetic rotation of these kineties. It may be so, because the migrating kinetosomes are already ciliated. Studies on the formation of new kinetosomes in somatic kineties of *Tetrahymena* (Allen 1969), have revealed, that the complex of fibrils, associated with the proximal end of kinetosome, arises in close contact with it, before the kinetosome has attained its final position in the kinety. Consequently another question arises, whether the kinetodesmal fibril can originate on the kinetosome's left in order to take the usual position after rotation. If that were the case, the kinetosomes, according to its actual position in this or that cortical region, would not only display different organizing potentialities, but would even change its polarity (on the assumption, that "... position of fibrillar appendages at their origin around the kinetosome are specified by the kinetosome's inherent polarity and asymmetry" — Pitelka 1969).

Studies on morphogenesis of oral kineties and on growth and regeneration of somatic kineties can, because of the presence of subkinetal sheet in the latter ones, yield valuable information on the formation and lysis of fibrils in kinetosomal territories.

The fine structure of cortex of different regions of cell surface in *Chilodonella cucullulus* indicates the function of mechanical support of pellicular alveoles, epiplasm and subpellicular fibrils.

The most elastic cortex is found in the oral field, covering the cytostome and its close vicinity. It resembles in structure the cortices of top elasticity, the contractile cortices of *Spirostomum* (Finley et al. 1964, Daniel and Mattern 1965, Grain 1968), of *Stentor* (Randall and Jackson 1958, Grain 1968) and of proboscis in *Dileptus cygnus* (Grain and Golińska 1969). In all these ciliates, similarly as around the cytostome of *Chilodonella cucullulus*, these are no pellicular alveoles and no epiplasm. The cortex exhibits contractility only when furnished with a microfibrillar mat, accompanied by peculiar vesicles (Grain and Golińska 1969). Whether such vesicles are associated with cortical microfibrils about the cytostome of *Chilodonella cucullulus* is not known.

A cortex devoid of alveoles is not necessarily elastic. The lack of alveoles can be mechanically compensated for either by the presence of various membrane-bound vesicles in the peripheral cytoplasm, as were noted in *Pseudoprorodon lieberkuhni* and *P. niveus* (Fauré-Fremiet and André 1968 b), or by a high development of subpellicular cytoplasm, the "lamina corticalis" in *Pseudomicrothorax dubius*, *P. agilis* (Fauré-Fremiet and André 1967) and *Drepanomonas dentata* (Prelle 1968).

Thus in non-contractile ciliates diverse cortical structures may substitute or assist each other in supporting the cell shape.

A comparison of structure of the nude dorsal side in *Chilodonella cucullulus* and in *Ignotocoma sabellarum* (Lom and Kozloff 1969) makes apparent, as was suggested among others by these writers, the supporting role of subpellicular microtubules. The dorsal side of *Chilodonella cucullulus*, devoid of such structures, is variable in shape, while that of *Ignotocoma*, provided with 5 microtubular layers, is quite stable.

Microtubules, packed in orderly arrays, like the subkinetal sheets of *Chilodonella* or the cirrus-based nemadesmata of *Euplotes*, (Tuffrau et al. 1968), seem to be an important factor, determining the flat shape of the ventral side of these ciliates, due to their course, parallelling the cell surface.

Cytopharyngeal apparatus

The pharyngeal basket is characteristic of all gymnostomes (Fauré-Fremiet 1961 a). Grain 1969 made the distinction between two principal types — the basket proper ("la nasse") and the pseudobasket ("la pseudonasse"), according to, respectively, the presence or absence of an microfibrillar annulus, connecting the distal ends of nemadesmata. According to this classification the basket of *Chilodonella cucullulus* is a basket proper. It resembles very much that of *Chlamydodon pedarius* (Kaneda 1962). In both species this apparatus consists of a single, outer

palisade of nemadesmata and of a cytopharyngeal tube, built of lamellae. Distal ends of nemadesmata are covered with caps of dense material, and bear additional structures. In *Chlamydodon* each nemadesma bears one "triangular trichite" with walls of electron-dense material and centre similar in structure to the surrounding cytoplasm. In *Chilodonella* each nemadesma bears two stalked dentes, dense throughout. In both species at the level, where cytopharyngeal tube approaches the palisade, there appears between them a connecting material in the form of microtubules as in *Chilodonella*, or in the form of a "reticulate structure of many filaments", as in *Chlamydodon*. It may be assumed, that these were also microtubules, but difficult to recognize as such, equally in nemadesmata (trichites), lamellae and in the spaces between them, because Kaneda fixed his material in 1% osmium tetroxide.

Lamellae of the cytopharyngeal tube in *Chilodonella* are comparable in structure to the rod lamellae of *Nassula* (Tucker 1968), or to lamellae of the suctorial tube in *Ancistrocomidae* (Lom and Kozloff 1968).

In *Chilodonella* no connections between nemadesmata and the proximal ends of kinetosomes were detected, such as were described in several ciliates, e.g. *Prorodon viridis* (de Puytorac 1964), *P. palustris* (de Puytorac and Savoie 1968). Similarly as in *Chilodonella*, no connections of this kind were noted in *Didinium* (Yagiu and Shigenaka 1965), where some of the nemadesmata are situated in a non-ciliated region. In *Ancistrocomidae* (Lom and Kozloff 1968) there are no nemadesmata and the suctorial tube, which has no connection with kinetosomes, consists of lamellae, each being a single layer of microtubules, and of an outer wall of several layers of microtubules.

This outer wall has been regarded as homologous with the gymnostome nemadesmata. Thus it seems probable, that nemadesmata and other complex microtubular structures may arise without any contact with kinetosomes. In *Chilodonella cucullulus* the palisade of nemadesmata is encircled in half by kinetosomes of the circumoral kineties, in the other half by kinetosomes of several somatic kineties. The latter ones, as was discussed above, contact the subkinetal sheet with the entire surface of their proximal ends. This contact excludes any possibility of accessory connections with nemadesmata of the basket.

Function of cytopharyngeal apparatus

Data on morphology and fine structure of cytopharyngeal apparatus, presented above, are static ones, still they bear implications as to the possible ways of functioning of this apparatus. As the lamellae of cytopharyngeal tube are interconnected in the distal part, and nemadesmata are interconnected somewhat below, the only possible route to be followed by the food vacuole leads through the phagoplasm, along the lumen of cytopharyngeal tube.

Series of 1 μ sections give some hints as to how the cytopharyngeal apparatus works. In some the cytostome bulges out, and nemadesmata parallel the axis, or skew

just a little (Pl. II 16), in others nemadesmata skew markedly and cytostome lies at a level with their distal ends (Pl. II 14). It may be presumed, therefore, that in *Chilodonella cucullulus*, similarly as in *Nassula* (Tucker 1968), during food intake nemadesmata change their inclination towards the basket axis.

The only observed structure, which could likely be contractile, is the microfibrillar annulus around the cytostome. Its action would displace the distal ends of nemadesmata, thus effecting the changes of inclination, mentioned above.

The diameter of lumen of cytopharyngeal tube as compared with the diameter of food particles (yeast cell about 3 μ), indicates that this lumen has to widen during phagocytosis. Basing upon the morphology it may be presumed, that the cytostome slot opens wide, when taking in food, and the folds of cytostome rim straighten. Lamellae of the cytopharyngeal tube, being bound to the cytostome rim by dense material, probably follow that movement. They possibly rotate around their outer edges, so that the inner edges move outwards, thus enlarging the tube lumen, similarly as works an iris diaphragm.

Acknowledgements

I wish to express my profound gratitude to Professor Dr Zdzisław Raabe for his advice and encouragement.

The most helpful and valuable criticism on the part of Docent Dr Władysław Bartoszewicz is hereby gratefully acknowledged.

Many thanks are due to Dr Jan Kozubowski, who did all the technical work on electrophotograms.

Summary

In the cortex of *Chilodonella cucullulus* (O. F. M.) differences were found between the oral field and the rest of cell surface, which are of functional importance. The oral field is devoid of pellicular alveoles and underlain by epiplasm on the periphery only. The cortex is thus highly elastic, which is indispensable in phagocytosis. The cell membrane is covered with material of medium density.

In the somatic body surface, the structure of the subpellicular layer is diversified, determining the cell shape. Three structural types of cortex are discerned:

The dorsal cortex, elastic, without any subpellicular fibrils.

Cortex of the lateral cell margin, containing a strand of microtubules.

The ventral cortex (oral field excepted), supported by the fibrillar complexes of kinetosomal territories.

In somatic kinetosomal territories kinetodesmal and postciliary fibrils of typical build are found, besides a peculiar complex of transverse fibrils and the subkinetal sheet. The latter is integrating the kinety, connecting all the kinetosomes.

In the preoral kinety there are two parasomal sacs to every kinetosomal territory. In circumoral kineties ciliated and non-ciliated kinetosomes were found, each with a parasomal sac. Kinetosome-based fibrils of the oral kineties differ from those of the somatic ones.

STRESZCZENIE

W korteksie *Chilodonella cucullulus* (O. F. M.) stwierdzono różnice w budowie pola okołogębowego i somatycznej części ciała orzęska, związane z funkcją tych okolic. Na polu okołogębowym brak alweoli pellikularnych, a epiplazma występuje tylko na jego peryferiach, co powoduje elastyczność korteksu, niezbędną przy procesie fagocytozy. Błonę komórkową okrywa materiał średniej gęstości elektronowej.

W somatycznej części ciała *Ch. cucullulus* występują różnice w budowie warstwy subpellikularnej, warunkujące kształt komórki. Wyróżniono 3 typy organizacji korteksu:

Korteks strony grzbietowej, elastyczny, bez włókien subpellikularnych.

Korteks bocznej krawędzi komórki, zawierający pasmo włókien mikrotubularnych.

Korteks strony brzusznej (z wyjątkiem pola okołogębowego), wzmocniony systemem włókien terytoriów kinetosomalnych.

W somatycznych terytoriach kinetosomalnych stwierdzono kinetodesmę i włókna postciliarne o budowie typowej dla orzęsków oraz nietypowy kompleks włókien transwersalnych i pasmo podkinetosomalnych włókien mikrotubularnych. To ostatnie łączy wszystkie kinetosomy, integrując kinetę.

W kinecie przedgębowej są po 2 woreczki parasomalne na terytorium kinetosomalne. W kinetach okołogębowych występują kinetosomy urzęsione i bezrzęse, a przy każdym z nich woreczek parasomalny. Struktury włókniste w kinetach gębowych są odmienne niż w somatycznych.

REFERENCES

Allen R. D. 1967: Fine structure, reconstruction and possible functions of components of the cortex of *Tetrahymena pyriformis*. J. Protozool., 14, 553–565.

Allen R. D. 1968: A reinvestigation of cross-sections of cilia. J. Cell Biol., 37, 825–831.

Allen R. D. 1969: The morphogenesis of basal bodies and accessory structures of the cortex of the ciliated protozoan *Tetrahymena pyriformis*. J. Cell Biol., 40, 716–733.

André J. et Fauré-Fremiet E. 1967: Lésions cytoplasmiques provoquées chez un cilié par un tentaculifére parasite. Protistologica, 3, 121–126.

Antipa G. A. and Small E. B. 1968: The somatic cortex of *Conchophthirus*. J. Protozool., 15, suppl., 8

Asterita H. and Marsland D. 1961: The pellicle as a factor in the stabilization of cellular form and integrity: effects of externally applied enzymes on the resistance of *Blepharisma* and *Paramecium* to pressure-induces cytolysis. J. cell comp. Physiol., 58, 49–55.

Behnke O. and Forer A. 1967: Evidence for four classes of microtubules in individual cells. J. Cell Sci., 2, 169–192.

Behnke O. and Zelander T. 1966: Substructure in negatively stained microtubules of mammalian blood platelets. Expl Cell Res., 43, 236–239.

Bradbury Ph. C. 1965: The infraciliature and argyrome of *Opishonecta henneguyi* Fauré-Fremiet. J. Protozool., 12, 345–363.

Chatton E. et Lwoff A. 1935: La constitution primitive de la strie ciliaire des infusoires. La desmodéxie. C. r. Séanc. Soc. Biol., 118, 1068–1072.

Cheissin E. M. and Mosevich T. N. 1962: An electron microscope study of *Colpidium colpoda* (*Ciliata, Holotricha*). Arch. Protistenk., 106, 181–200.

Corliss J. O. 1953: Silver impregnation of ciliated protozoa by the Chatton–Lwoff technic. Stain tech., 28, 97–100.

Corliss J. O. 1961: The Ciliated Protozoa: characterization, classification, and guide to the literature. Pergamon Press, Oxford.
Daniel W. A. and Mattern C. F. T. 1965: Some observations on the structure of the peristomial membranelle of *Spirostomum ambiguum*. J. Protozool., 12, 14–27.
Dippell R. V. 1962: The site of silver impregnation in *Paramecium aurelia*. J. Protozool., 9, suppl. 24.
Dippell R. V. 1965: Reproduction of surface structure in *Paramecium*. In: Progress in Protozoology, Abstr. Second int. Conf. London 1965, Excerpta med. int. Congr. ser. No. 91, p. 65.
Dobrzańska-Kaczanowska J. 1965: Studies on morphology of *Chilodonella cucullulus* O. F. M. 1786. In: Progress in Protozoology, Abstr. Second int. Conf. London 1965, Excepta med. int. Congr. ser. No. 91, p. 211.
Ehret C. F. and Powers E. L. 1959: The cell surface of *Paramecium*. Int. Rev. Cytol., 8, 97–133.
Fauré-Fremiet E. 1950: Mécanismes de la morphogénèse chez quelques Ciliés Gymnostomes Hypostomiens. Archs Anat. microsc. Morph. exp., 39, 1–14.
Fauré-Fremiet E. 1961 a: Les Ciliés *Cyrtophorina* et leur diversification morphologique. C. r. Acad. Sci., 252, 3912–3916.
Fauré-Fremiet E. 1961 b: Le cytoplasme stomopharyngien des Ciliés Cyrtophores. C. r. Acad. Sci., 253, 357–362.
Fauré-Fremiet E. 1962: Le genre *Paranassula* Kahl (*Ciliata, Cyrtophorina*). Cah. Biol. mar., 3, 61–77.
Fauré-Fremiet E. et André J. 1967: Etude au microscope electronique du cilié *Pseudomicrothorax dubius* Maupas. J. Protozool., 14, 464–473.
Fauré-Fremiet E. et André J. 1968 a: Structure fine de l' *Euplotes eurystomus* (Wrz.). Archs Anat. microsc. Morph. exp. 1, 53–78.
Fauré-Fremiet E. et André J. 1968 b: L'organisation corticale des *Ciliata*. C. r. Acad. Sci., 266, 487–490.
Finley H. E., Brown Ch. A. and Daniel W. A. 1964: Electron microscopy of the ectoplasm and infraciliature of *Spirostomum ambiguum*. J. Protozool., 11, 264–280.
Gibbons J. R. and Grimstone A. V. 1960: On flagellar structure in certain flagellates. J. biophys. biochem. Cytol., 7, 697–716.
Gliddon R. 1966: Ciliary organelles and associated fibre systems in *Euplotes eurystomus (Ciliata, Hypotricha)*. I. Fine structure, J. Cell Sci., 1, 439–448.
Grain J. 1966: Etude cytologique de quelques Ciliés Holotriches endocommensaux des Ruminants et des Equides. Protistologica, 2, 59–141.
Grain J. 1968: Les systèmes fibrillaires chez *Stentor igneus* Ehrenberg et *Spirostomum ambiguum* Ehrenberg. Protistologica 4, 27–35.
Grain J. 1969: Le cinétosome et ses dérivés chez les Ciliés. Anneé Biol., 7, 53–97.
Grain J. et Golińska K. 1969: Structure et ultrastructure de *Dileptus cygnus* Claparède et Lachman 1859, Cilié Holotriche Gymnostome. Protistologica, 5, 269–291.
Grim J. N. 1967: Ultrastructure of pellicular and ciliary structures of *Euplotes eurystomus*. J. Protozool., 14, 625–634.
Grim J. N. 1968: Functional analysis of the infraciliature in complex ciliary structures of ciliate *Protozoa*. Expl. Cell Res., 53, 459–470.
Grimstone A. V. 1966: Structure and function in *Protozoa*. A. Rev. Microbiol., 20, 131–150.
Hufnagel L. A. 1966: Fine structure and DNA of pellicles isolated from *Paramecium aurelia*. In: Electron Microsc., Abstr. VIth int. Congr. Electron Microsc. Kyoto 1966, R. Uyeda, (ed.) Maruzen Co. Ltd., Tokyo, 2, 239.
Hufnagel L. A. 1969: Cortical ultrastructure of *Paramecium aurelia*. Studies on isolated pellicles. J. Cell Biol., 40, 779–801.
Kaczanowska J. and Kowalska D. 1969: Studies on topography of the cortical organelles of *Chilodonella cucullulus* (O. F. M.) I. The cortical organelles and intraclonal dimorphism. Acta Protozool., 7, 1–15.
Kaneda M. 1962: Fine structure of the oral apparatus of the Gymnostome ciliate *Chlamydodon pedarius*. J. Protozool., 9, 188–195.
Kennedy J. R. Jr. 1965: The morphology of *Blepharisma undulans* Stein. J. Protozool., 12, 542–561.
Khan M. A. 1969: Fine structure of *Ancistrodoma pelseneeri* (Chatton et Lwoff) a rhynchodine thigmotrichid ciliate. Acta Protozool., 7, 29–47.
Kink J. 1970: The influence of formalin on morphogenesis of *Chilodonella cucullulus* (O. F. M.). Acta Protozool., 8, 203–209.
Kowalska D. and Kaczanowska J. 1970: Studies on cortical organelles position in *Chilodonella cuculus* (O. F. M.). II. Topographical relations of the total number of kineties to the disposition of CVPs. Acta Protozool., 7, 181–192.

Lom J. and Kozloff E. N. 1968: Observations on the ultrastructure of the suctorial tube of Ancistrocomid ciliates. Folia parasitol., 15, 291–308.
Lom J. and Kozloff E. N. 1969: Ultrastructure of the cortical regions of Ancistrocomid ciliates. Protistologica, 5, 173–192.
Metz C. B., Pitelka D. R. and Westfall J. A. 1953: The fibrillar systems of ciliates as revealed by the electron microscope. I. *Paramecium*. Biol. Bull., 104, 408–425.
Metz C. B. and Westfall J. A. 1954: The fibrillar systems of ciliates as revealed by the electron microscope. II. *Tetrahymena*. Biol. Bull., 107, 106–122.
Nilsson J. R. and Williams N. E. 1966: An electron microscope study of the oral apparatus of *Tetrahymena pyriformis*. C. r. Trav. Lab. Carlsberg, 35, 119–141.
Noirot-Timothée C. et Lom J. 1968: Organisation du cortex chez *Trichodinopsis paradoxa* (*Peritricha Mobilina*) et valeur founctionelle des sacs parasomaux comme sites de pinocytose. J. Protozool., 15, suppl. 41.
Pitelka D. R. 1961: Fine structure of the silverline and fibrillar systems of three Tetrahymenid ciliates. J. Protozool., 8, 75–89.
Pitelka D. R. 1965: New observations on cortical ultrastructure in *Paramecium*. J. Microsc., 4, 373–394.
Pitelka D. R. 1968: Fibrillar systems in *Protozoa*. In: Research in Protozoology. T. T. Chen, (ed.), Pergamon Press, Oxford–New York, 3, 280–388.
Pitelka D. R. 1969: Fibrillar structure of the ciliates cortex: the organization of kinetosomal territories. In: Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, Nauka, Leningrad 1969, 44–46.
Prelle A. 1968: Ultrastructures corticales du Cilié Holotriche *Drepanomonas dentata* Fresenius, 1858. J. Protozool., 15, 517–520.
Puytorac P. de 1959: Nouvelles observations sur l'argyrome des ciliés Astomes, par l'emploi du microscope électronique. C. r. Ass. Anat., 46, 675–679.
Puytorac P. de 1964: Quelques aspects de l'ultrastructure du Cilié *Prorodon viridis* Ehrb. Kahl. Acta Protozool., 2, 147–151.
Puytorac P. de 1967: Bactéries et Ciliés. Botaniste, sér. L. 351–358.
Puytorac P. de et Savoie A. 1968: Observations cytologiques et biologiques sur *Prorodon palustris* nov. sp. Protistologica, 4, 53–59.
Pyne Ch. K. 1970: High resolution electron microscopic studies on the complex tubules of the Gymnostome ciliate *Chilodonella uncinata*. In: Microscopie Électronique, Abstr. VIIth Congr. int. Microsc. Électron., Grenoble 1970, 3, 401.
Radzikowski S. 1966: Study on morphology, division and postconjugation morphogenesis in *Chilodonella cucullulus* (O. F. Müller). Acta Protozool., 4, 89–95.
Randall M. J. T. and Jackson S. F. 1958: Fine structure and function in *Stentor polymorphus*. J. biophys. biochem. Cytol., 4, 807–830.
Roque M., Puytorac P. de et Savoie R. 1965: *Ophryoglena bacterocaryon* sp. n., Cilié Holotriche Péniculien (cytologie, ultrastructure, cycle). Archs Zool. exp. gén., 105, 309–344.
Roth L. E. 1956: Aspects of ciliary fine structure in *Euplotes patella*. J. biophys. biochem. Cytol., 2, 235–240.
Roth L. E. 1964: Motile systems with continuous filaments. In: Primitive Motile Systems in Cell Biology. R. D. Allen and N. Kamiya (eds.), New York, Academic Press, Inc., 527–548.
Roth L. E. and Shigenaka Y. 1964: The structure and formation of cilia and filament in rumen *Protozoa*. J. Cell Biol., 20, 249–270.
Satir P. 1965: Studies on cilia. II. Examination of the distal region of the ciliary shaft and the role of the filaments in motility. J. Cell Biol., 26, 805–826.
Satir P. and Rosenbaum J. L. 1965: The isolation and identification of kinetosome-rich-fractions from *Tetrahymena pyriformis*. J. Protozool., 12, 397–405.
Sedar A. W. and Porter K. R. 1955: The fine structure of cortical components of *Paramecium micronucleatum*. J. biophys. biochem. Cytol., 1, 593–604.
Sołtyńska M. 1969: The cortex of *Chilodonella cucullulus*. In: Progress in Protozoology, Abstr. Third int. Congr. Protozool. Leningrad 1969, Nauka, Leningrad 1969, 74–75.
Tilney L. G., Hiramoto Y. and Marsland D. 1966: Studies on microtubules in *Heliozoa*. III. A pressure analysis of the role of these structures in formation and maintenance of the axopodia of *Actinosphaerium nucleophilum* (Barret). J. Cell Biol., 29, 77–95.
Tucker J. B. 1968: Fine structure and function of the cytopharyngeal basket in the ciliate *Nassula*. J. Cell Sci., 3, 493–514.
Tuffrau M. 1967: Perfectionnement et practique de la technique d'imprégnation au Protargol des Infusoires Ciliés. Protistologica, 3, 91–98.
Tuffrau M., Pyne Ch. K. et Haller G. de 1968: Organisation de l'infraciliature chez quelques ciliés Hypotriches. Protistologica, 4, 289–302.

Vivier E. 1966: Variations ultrastructurales du chondriome en relation avec le mode de vie chez des Protozoaires. In: Electron Microsc., Abstr. VIth int. Congr. Electron Microsc. Kyoto 1966, R. Uyeda, (ed.), Maruzen Co. Ltd. Tokyo, 2, 247.
Yagiu R. and Shigenaka Y. 1965: Electron microscopy of the ectoplasm and proboscis in *Didinium*. J. Protozool., 12, 363–381.

EXPLANATION OF PLATES I–XII

Chilodonella cucullulus (O. F. M.). Structure and ultrastructure

1: Starved individual. Visible inward curving of left somatic kineties. Silvered after Chatton–Lwoff. ×650
2: Individual fed full. Silvered after Chatton–Lwoff. ×800
3: Fragment of ventral body surface. Interkinetal spaces show the network with meshes dark inside. Protargol silvered. ×2400
4: Fragment of ventral body surface of a fed individual, with the argentophilic network showing. Silvered after Chatton–Lwoff, kept long in da Fano fluid. ×2400
5: Fragment of ventral body surface of a starved specimen, same region as in phot. 4. Meshes of the argentophilic network are somewhat elongated. Silvered after Chatton–Lwoff, kept long in da Fano fluid. ×3000
6: Fragment of ventral body surface from the front part of cell. In somatic kineties the parasomal sacs are visible close to the kinetosomes (short arrow). In the preoral and circumoral kineties several rows of silver-impregnated bodies are visible. The cytostome slot in the middle of oral field (long arrow). Silvered after Chatton–Lwoff. ×1800
7: Fragment of dorsal body surface from the front part of cell, with the dorsal kinety showing. Silver-impregnated surface structures are visible (arrow). Silvered after Chatton–Lwoff. ×1800
8: Argentophilic network of the dorsal side. Silvered after Chatton–Lwoff, kept long in da Fano fluid. ×2400
9: Fragment of ventral side in the oral region. Visible distal ends of nemadesmata and of cytopharyngeal tube. Along the somatic kineties a dark band (arrow). Protargol silvered. ×1800
10: Oral region. Note the darkening of oral field around the cytostome slot. Arrow points the contractile vacuole pore. Silvered after Chatton–Lwoff. ×2000
11: Oral region. Note the argentophilic network, surrounding the oral field. Silvered after Chatton–Lwoff, kept long in da Fano fluid. ×2000
12–16: Longitudinal sections of cytopharyngeal apparatus. Epon embedded, stained with toluidine blue
12: The oblique inner opening of apparatus (arrow). ×2000
13: Dilatation of cytopharyngeal tube lumen (arrow). ×2000
14: Note the position of nemadesmata, skew in relation to the basket axis. ×2400
15: Between the nemadesmata, below their distal ends note the material, poorly staining with toluidine blue (arrow). ×2000
16: Between cytopharyngeal tube and nemadesmata note the material poorly staining with toluidine blue (arrow). Cytostome bulges out above the distal ends of nemadesmata. ×2000
17–21: Cross-sections of cytopharyngeal apparatus. Arrows point to the inner opening. Epon and toluidine blue. ×2000
22: Tangential section of oral field. Cilia of circumoral kineties are longer (arrow), than those of somatic ones. Epon and toluidine blue. ×2000
23: Cross-section of cell. Note grooves of cell surface, with kinetosomes in them. *c.v.* — contractile vacuole, surrounded by a zone of cytoplasm, poorly staining with toluidine blue. Arrow points the contractile vacuole pore. Epon and toluidine blue. ×1000
24: Palisade of nemadesmata. Silvered after Chatton–Lwoff, kept long in Champy fluid. ×2000
25: Cross-section of the animal, about the oral field. Above ventral side, lower left — dorsal side, right — fragment of cytopharyngeal apparatus. Note the parasomal sac to the right of kinetosome. In the matrix of mitochondrium a conglomeration of electron-dense material (arrow). 5% glutaraldehyde. ×15 800
26: Oblique section through the lateral cell margin. Note the subpellicular microtubules (arrow) 2% glutaraldehyde. ×50 000
27: Tangential section of the lateral cell margin. Note the subpellicular microtubules (arrow) 5% glutaraldehyde. ×55 000
28: Cross-section through the pellicle. Note the conglomeration of finely granular, electron-dense material inside the pellicular alveole (arrow). 2% glutaraldehyde. ×50 000

29: Cross-section of somatic kinetosomes at the level of terminal plate. Note the electron-dense material between the triplets and epiplasm. Arrow points to the parasomal sac. 5% glutaraldehyde. ×47 000

30: Cross-section of a cilium at the level of axosome. 5% glutaraldehyde. ×47 000

31: Cross-section of cilia. Arrow points to the crooked outer arm at fibril A. Note connections between both arms (inner and outer) of fibril A and the fibril B. 5% glutaraldehyde. ×47 000

32: Cross-section of somatic cilium, near its distal end. Note the pair of central fibrils and single peripheral fibrils. 5% glutaraldehyde. ×47 000

33: Section of a somatic kinety at the level of cilium (kinetosome boundary). Between the kinetosomes note limits of contiguous pellicular alveoles. In the lower part of picture at each kinetosome is a complex of transverse fibrils. 1% osmium tetroxide. ×45 000

34: Tangential section of a somatic kinety at half length of kinetosomes. Arrow points to the dense granule in the middle of kinetosome. Note differences in size of parasomal sacs. 5% glutaraldehyde. ×27 000

35: Tangential section of a somatic kinety at the level of proximal ends of kinetosomes. Note the complex of kinetosome-associated fibrils (*pc.*, *kd.*, *t. d.*, *t. mtb.*). 5% glutaraldehyde. ×55 000

36: Longitudinal section through somatic kinetosomes and cilia. In the kinetosome at right note the connection of terminal plate and epiplasm. Epiplasm with marked laminar structure. Kinetodesmal fibril (*kd.*) shows periodicity. Arrow points to the polysomes. 5% glutaraldehyde. ×47 000

37: Longitudinal section of a somatic kinetosome and cilium, cutting across the subkinetal sheet. 5% glutaraldehyde. ×73 000

38: Oblique section through a somatic kinety. Note the postciliary fibrils, running from each kinetosome, to join the ribbon of them, parallelling the kinety. 1% osmium tetroxide. ×47 000

39: Longitudinal section of a somatic kinety. Note the rows of subkinetosomal microtubules joining the subkinetal sheet. 5% glutaraldehyde. ×31 000

40: Tangential section of a somatic kinety, cutting through the proximal ends of kinetosomes and deeper, through the subkinetal sheet. Arrow points to the electron-dense material of kinetosome bottom. 1% osmium tetroxide. ×30 000

41: Oblique section of a somatic kinety, cutting longitudinally the transverse fibrils 5% glutaraldehyde. ×47 000

42: Tangential section of a somatic kinety. Note the course of transverse fibrils. 1% osmium tetroxide. ×10 000

43: Section of cortex along the interkinetal space. Note complexes of transverse fibrils (arrow). The upper part from microtubules, overlying the dense fibril. 5% glutaraldehyde. ×36 000

44: Longitudinal section of kinetosomes of a circumoral kinety. Arrow points to a non-ciliated kinetosome. Note the subkinetal sheet of a neighbouring somatic kinety. 1% osmium tetroxide. ×27 000

45: Tangential section of circumoral kineties at the level of proximal ends of kinetosomes. In the upper part of picture note transverse fibrils, running from kinetosomes of the neighbouring somatic kinety. Arrow points to the periodic fibril (kinetodesmal ?) of a nude kinetosome. 5% glutaraldehyde. ×70 000

46: Section of cilia of circumoral kineties. Note the digitiform protrusions of cell membrane, covering the cilium. At the bottom of picture note the kinetosome-based fibrils. 5% glutaraldehyde. ×25 000

47: Longitudinal section of the circumoral kinety. Non-ciliated kinetosomes (*n. ks.*) sit deeper in the cytoplasm. Microtubules (*mtb. b.*) running from proximal ends of non-ciliated kinetosomes proceed steeply upwards. 5% glutaraldehyde. ×70 000

s8: Tangential section of the preoral kinety. On either side of each kinetosome the parasomal 4acs are showing. At lower left a fragment of circumoral kinety (*co. k.*) and kinetodesmal fibrils (*kd.*), running from its kinetosomes, at lower right the subkinetal sheet of a somatic kinety (*sk. sh.*). In the upper part of picture note the transverse fibrils (*t.*) of a somatic kinety, proceeding anteriad towards the preoral kinety. 1% osmium tetroxide. ×47 000

49: Section of ventral cortex, just in front of the oral field, showing fragments of preoral (*p. k.*), somatic (*s. k.*) and circumoral kineties. Under the epiplasm note the strands of microtubules (*mtb. b.*), running from kinetosomes of preoral kinety. 5% glutaraldehyde. ×47 000

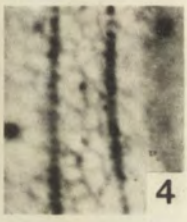
50: Longitudinal section of oral field, parallel to cytostome slot and close to it. Note the electron-dense material, covering distal ends of lamellae of cytopharyngeal tube (arrow). 1% osmium tetroxide. ×20 000

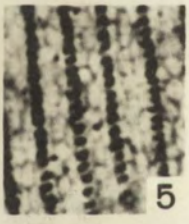
51: Cross-section of cytopharyngeal apparatus near its distal end. Note the microfibrillar annulus (*mf. a.*). Outside the oral field pellicular alveoles are visible. 1% osmium tetroxide. ×12 000

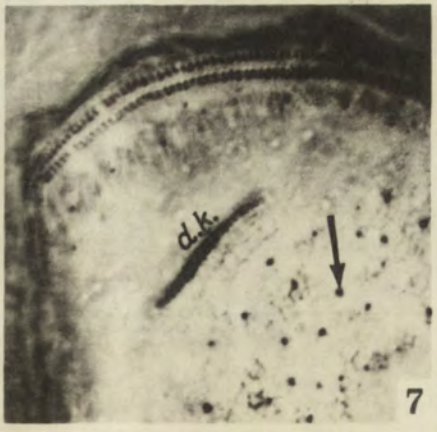
52: Section through the vicinity of cytostome. Outside the zone of lamellae insertions note the extracellular material (*ex. m.*) and the epiplasm (*ep.*). 5% glutaraldehyde. ×25 000

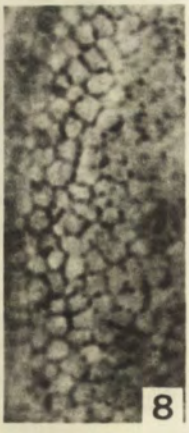
53: Section through the cytostome slot. 5% glutaraldehyde. ×38 000
54: Fragment of a cross-section of cytopharyngeal apparatus. Note the connecting granules between lamellae of cytopharyngeal tube (thin arrow). Thick arrows point to the complex tubules. 5% glutaraldehyde. ×73 000
54 A: Fragment of a cross-section of nemadesma. Note the subfibrils composing walls of microtubules. 5% glutaraldehyde. ×200 000
55: Fragment of a cross-section of cytopharyngeal apparatus about half of its length. In the lumen of cytopharyngeal tube note the mitochondrium with a conglomeration of electron-dense material 5% glutaraldehyde. ×45 000
56: Section of cytopharyngeal apparatus after faulty fixation. Note the intact consistence of the whole complex. 1% osmium tetroxide. ×12 000
57: Section through the proximal end of cytopharyngeal apparatus. In the cytoplasm note mitochondria and symbiotic bacteria. 5% glutaraldehyde. ×26 000
58: Section of circumoral kinety of the opisthe of a dividing specimen. Kinetosomes of the upper row, more distant from nemadesma, are ciliated. 1% osmium tetroxide. ×19 000
59: Section of circumoral kineties of the same individual, as in phot. 58, but more superficial, above the distal ends of non-ciliated kinetosomes. Parasomal sacs showing on either side of kineties. 1% osmium tetroxide. ×19 000
60: Section through contractile vacuole pore. 1% osmium tetroxide ×21 000
61: Section through the excretory canal. Under the cell membrane note the fibrillar structures (arrow). 1% osmium tetroxide. ×21 000
62: Mitochondria. No conglomerations of electron-dense material. 1% osmium tetroxide. ×30 000

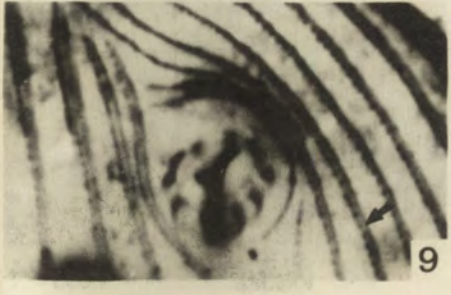
Abbreviation used: *a.* — arms, A, B, C — microtubular fibrils of triplet, *ax.* — axosome, *b.* — bacterium, *c. f.* — central fibrils, *cil* — cilium, *co. k.* — circumoral kinety, *c. m.* — cell membrane *cph. ap.* — cytopharyngeal apparatus, *cph. t.* — cytopharyngeal tube, *cs.* — cytostome, *c. w.* — cartwheel structure, *c. v.* — contractile vacuole, *c. v. p.* — contractile vacuole pore, *d.* — dentes, *d. g.* — dense granule, *d. k.* — dorsal kinety, *d. s.* — dorsal side, *ep.* — epiplasme, *ex. m.* — extracellular material, F — front, *i. a. m.* — inner alveolar membrane, *kd.* — kinetodesmal fibril, *ks.* — kinetosome, L — left side, *lam.* — lamella, *l. m.* — lateral margin, *m.* — mitochondria, *Ma* — macronucleus, *mf. a.* — microfibrillar annulus, *mtb. b.* — microtubular band, *Mi* — micronucleus, *nd.* — nemadesmos, *n. ks.* — nonciliated kinetosome, *o. a. m.* — outer alveolar membrane, *o. f.* — oral field, *p. a.* — pellicular alveole, *pc* — postciliary fibrils, *p. f.* — periferal fibrils, ph. — phagoplasme, *p. k.* — preoral kinety, *p. s.* — parasomal sac, R — right side, *Re* — rear, *s. k.* — somatic kinety, *sk. sh.* — subkinetal sheet, *t.* — transverse fibrils, *t. d.* — transverse dense fibrils, *t. mtb.* — transverse microtubular fibrils, *t. p.* — terminal plate, *v. s.* — ventral side



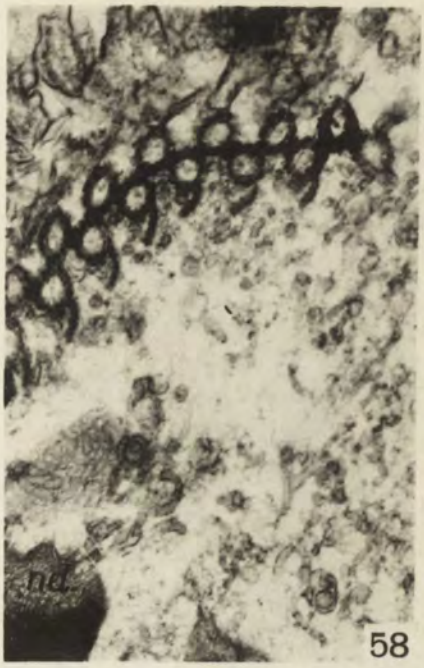


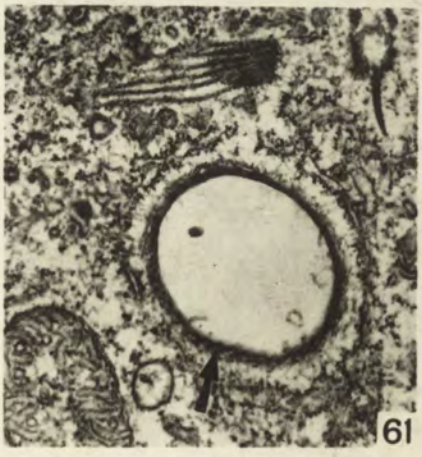


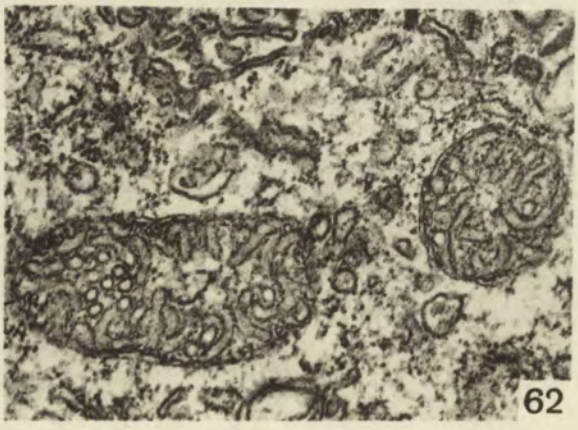




M. S. Sołtyńska auctor phot.







M. S. Sołtyńska auctor phot.

ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 1.VIII.1971 FASC. 5

Zoological Institute, University of Warszawa, Warszawa 64, Poland

Janina KACZANOWSKA

Studies on topography of the cortical organelles of *Chilodonella cucullulus* (O. F. M.). III. Morphogenetic movements, regional multiplication of kinetosomes and cytokinesis in normal dividers, and after phenethyl alcohol treatment

Studia nad topografią organelli kortykalnych *Chilodonella cucullulus* (O. F. M.). III. Ruchy morfogenetyczne, lokalne namnażanie się kinetosomów i cytokineza u podziałowców normalnych i po działaniu alkoholu fenetylowego

Sites determination, morphogenetic movements and cytokinesis are major problem of divisional morphogenesis of ciliates (Hanson 1967, Tartar 1968). These events are clearly involved in divisional morphogenesis of the ciliate protozoan *Chilodonella cucullulus* (O. F. M.).

Sites determination and model of induction was presented previously (Kaczanowska 1970). In the course of this work the amazing fact was stated that the new posterior contractile vacuole pore CVP-3'' of future opisthe is differentiated in the vast majority of cases, anteriorly to the position of old one i.e. CVP-3 of parental individual (Fig. 3). This fact seems paradoxal. It would mean that the posterior part of cell cortex (posterior to old CVP-3) decreases during cell generation, if the new posterior CVP-3'' of opisthe is differentiated more anteriorly. There is no trace of resorption or contraction in this region and the existence of morphogenetic movements leading to changes in configuration of organelles on ventral surface of opisthe is expected.

Morphogenetic movement and the sequent migration of the ciliated segments of kineties in the organizing opisthe were previously described (Fauré-Fremiet 1950, Radzikowski 1966, Kaczanowska and Kowalska 1969). The same events were also analyzed in related species e.g. Deroux et Dragesco 1968. The mechanism of these movements are totally unknown. All these description were restricted to oral part of the forming opisthe.

In this paper the morphogenetic movements and associated changes in topography of cortical organelles during division are re-examined. The received result suggest that morphogenetic movement of cortical structures is not limited to the

oral area of incipient opisthe but concerns also somatic ciliature and lasts until cell separation (i.e. it accompanies cytokinesis itself).

The changes in topography of cortical organelles can be attained by an allometric growth of some part of the cell cortex, which rearranges the cortical structures, or by an autonomic movement of some structures which actively change their positions. Therefore the calculation should be made for elimination of the allometric growth as the reason for changes in topography. This estimations are presented in this paper.

Chilodonella is particularly favorable object for this kind of study, because all events described here take place on the flat ventral surface. In this respect all measurements can be made on one scale photomicrographs or camera lucida drawings.

An application of the drug PEA (phenethyl alcohol) allowed to disrupt the normal sequence of morphogenesis impairing some morphogenetic movements without impairing differentiation and induction. These experiments reinforced the hypothesis of active movements during cytokinesis.

Regional miltiplication in one somatic kinety after its morphogenetic movement can be interpreted as the new activation of synthesis after relocation. The same events were described in the case of traumatic disturbances in somatic ciliature of *Chilodonella* (Dobrzańska-Kaczanowska 1965).

Material and methods

The stocks B 1 and S of *Chilodonella cucullulus* were used. Stock S was used only in one experiment on total ingrowth of cell surface. The method of cultivation and details of morphology are described previously (Kaczanowska and Kowalska 1969). All samples were tested during the vegetative phase of the stocks.

Following methods employed in this paper are enumerated according to the sequence of chapters:

1. Description of divisional morphogenesis is based on some observations made in phase contrast microscope. It was completed by silvered specimens after Chatton and Lwoff method (Corliss 1953). On silvered specimens the counting of number of oral segments, and counting of kinetosomes in kinety A_4 were made. On this basis the sequence of events during cell division was deduced.

2. Comparison of the sizes of the ventral surface of future proter and opisthe cells were performed on camera lucida drawings of silvered dividers. For such kind of measurements only specimens with pronounced fission line (i.e. with disruption of all somatic kineties) were selected.

3. Estimation of ratio of the growth of the ventral surfaces during one ontogenesis (i.e. from one to subsequent division) were calculated from the mean value of cell surface of 7–13 cells fixed at the same time after division. Details of experiments were described previously in experiments on the ciliates of the same ages (Kaczanowska and Kowalska 1969).

4. Estimation of the minimal ingrowth of the cell surface during cytokinesis which would be sufficient high for bringing out the proper ratio of surfaces between arising proter and opisthe were based on calculation made on drawings of the silvered specimens. As concerns the surface of whole specimen and of organizing proter and opisthe, and geometrically needed part of surface

of opisthe, all these measurements were made by cutting the images of area out of the drawings and weighing them following de Terra 1969.

5. The growth of the ventral surface was measured during cytokinesis of single dividers. The specimens were selected from samples in dissecting microscope. The selected specimens were in the stage of morphogenetic movements but before cytokinesis. The cell was immediately place in 0.01% methylcellulose and covered by a cover-slip. The excess of fluid was removed by a filter blotter up to the moment when the cell can freely though slowly swim but it cannot turn over. Such preparation was emarginated by paraffin oil for avoiding further evaporation. This procedure was shortened to about 3 min. The cell were observed and periodically photographed in phase contrast microscope (Leitz synchronized photographic camera, film Ilford FP4/22 DIN). The series of photomicrographs in the same magnification (Pl. I 1–6) were subsequently used for measurements of total size and ingrowth of ventral surface from the first sign of furrowing up to final cell separation. Twelve cells photographed from the beginning up to the end of cytokinesis were employed in this study.

Two additional remarks should be added here. First, that the timing of cytokinesis of photographed cells was greatly delayed in comparison to the control. The recorded time for 18 controls specimens observed in dissecting microscope was limited to 8–12 min. The same period in photographed cells lasted up to 12–23 min. This delay is obvious in respect to data on influence of pressure on time of cytokinesis e.g. Macdonald 1967. Some cases the more delayed cytokinesis, or total inhibition of the cytokinesis were observed and eliminated. In all specimens (even delayed) if cytokinesis was accomplished, the morphology of the offsprings were not disturbed. It was presumed then that from the morphological point of view the condition of experiments do not influence on shaping during cytokinesis.

The second remarks concerns the observed flattening of the photographed ciliates, particularly during the end of cytokinesis. It seems possible that prolonged period of cytokinesis and flattening could cause an error in the estimation of the ingrowth during cytokinesis. However, both cautions concern rather the possibility of an over-estimation of the ingrowth rather than under-estimation. It can be admitted that observed cytokinesis was normal in the sense that it gives morphologically normal descendants. In these limits the received results are valid.

It should be add that the ingrowth of the cell cortex is only understood as the increase of surfaces.

6. Phenethyl alcohol stock solution (0.2% v/v) was prepared freshly each time in sterilized culture medium. In these experiments the double strength solution was slowly mixed in the equal ratio to the medium of mass culture, or isolated cell. Final concentration varied between 0.07–0.1%. After desired time the samples were fixed and silvered. Calculations were made on drawings. The single isolated cells and recovery were observed after gradual washing in fresh drug-free medium and observed in vivo.

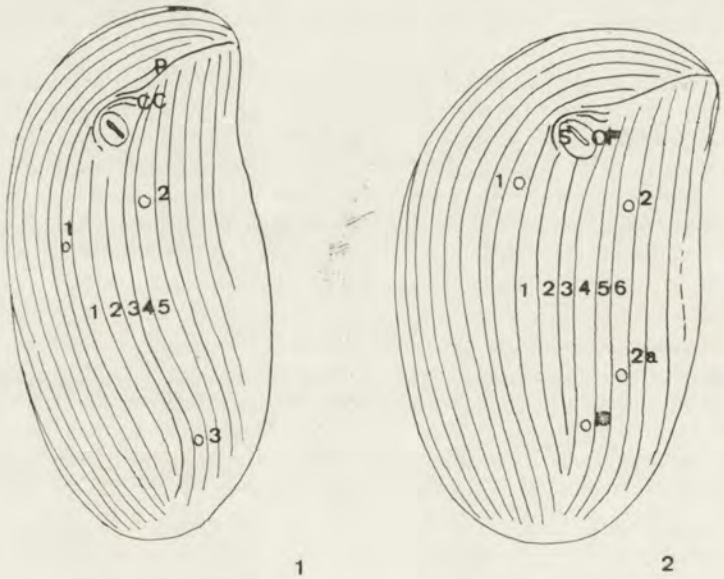
Results

Sequence of cortical events during bipartition of *Chilodonella cucullulus* (stocks B_1 and S)

Divisional morphogenesis of *Ch. cucullulus* was described previously in many respects (e.g. more recently Fauré-Fremiet 1950, Radzikowski 1965, 1966, Kaczanowska and Kowalska 1969, Kaczanowska 1970). Only the major outlines important for the further calculations will be recalled here. All used abbre-

viations are followed after Radzikowski 1966 and Kaczanowska and Kowalska 1969.

A map of kineties and cortical structures on the flat ventral surface of nondividers is illustrated in Fig. 1 and 2. The number of kineties may vary, but the pictures illustrate the most typical topography (Kowalska and Kaczanowska 1970). The disposition of contractile vacuole pores (CVPs) is different in the cell which was the proter during last division in comparison with the cell, which was the opisthe. So, the terms proter and opisthe will be applied to the cells which are postdividers with typical and undoubtful pattern (Kaczanowska and Kowalska 1969).

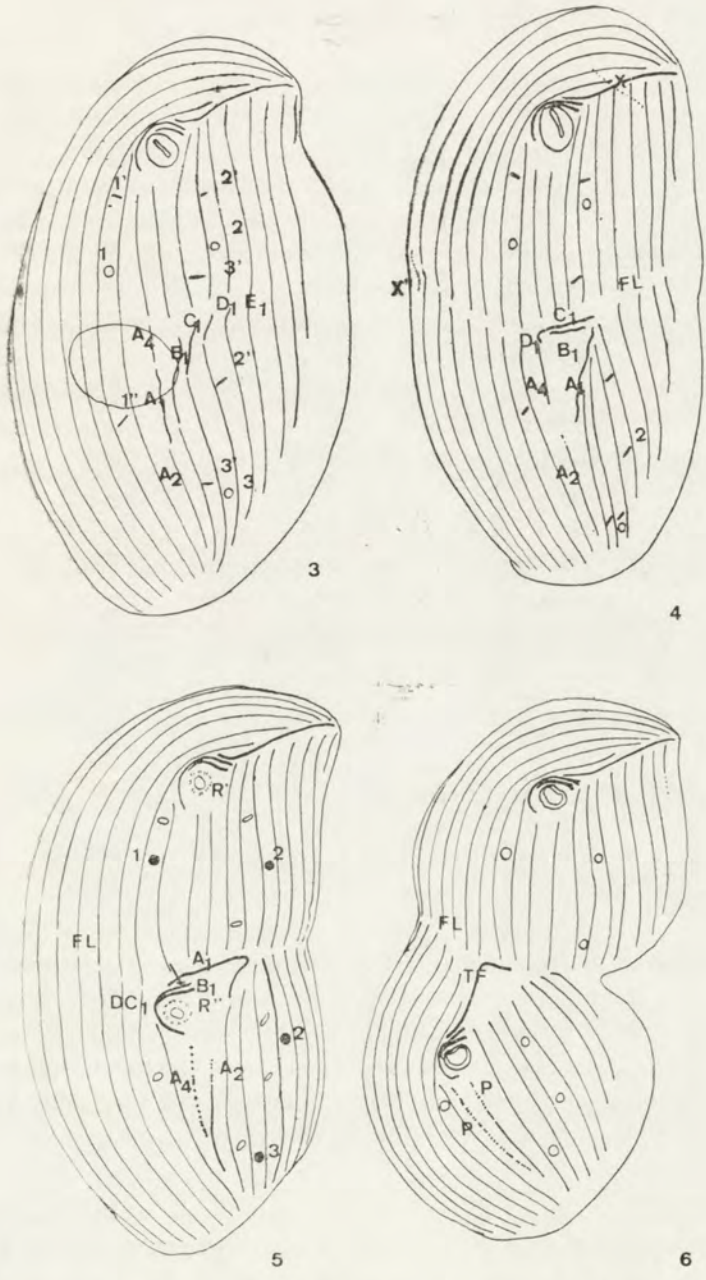


1 2

Figs. 1–2. *Chilodonella cucullulus* (O. F. M.), patterns of the cortical organelles on the ventral surface. 1 — typical proter cell, 2 — typical opisthe cell. Abbreviations: CC — circumoral kineties, OF — oral field, P — preoral kinety, S — oral slit, 1–5 — kineties of the sector between anterior contractile vacuoles in proter, and 1–6 in opisthe, 1, 2, 2a, 3 — denominations of contractile vacuole pores after Kaczanowska and Kowalska 1969

The early stage of division is represented in Fig. 3. The oral segments for the tuture opisthe are differentiated in this stage. These segments are named according fo Radzikowski 1966 as A_1, A_3, B_1 and D_1, or even E_1. The phase contrast microscope shows the first sign of the formation of anlagen of the oral basket for the opisthes in vicinity of segment B_1. Nevertheless there are no traces of oral anlagen in the silvered preparation during this stage.

The next stage (Fig. 4) involves the translocation of the oral anlagen and migration of all oral and one somatic (namely A_4) in the oral region of the incipient opisthe. The rotation of segments begins from the small segments D_1, or is eventually preceded by E_1. Both segments turn to the right, and next C_1 and B_1 follow this



Figs. 3–6. *Chilodonella cucullulus* (O. F. M.), morphogenetic movements. 3 — early divider (proter), 4 — morphogenetic movement of the oral kineties of incipient opisthe cell. 5 — migration of kinety A_4. Early furrowing. 6 — advanced cytokinesis. Abbreviations: A_1, A_2, A_4, B_1, C_1, D_1, E_1, DC_1 — kineties involved in first phase of morphogenetic movement, denominations after Radzikowski 1966. FL — fission line, P — sites of intensive kinetosome multiplication, R′, R″ — oral baskets (rosettes) of future proter and opisthe respectively, TF — triangular field, X — inherited dorsal kinety of proter, X″ — dorsal kinety of future opisthe, 2, 3 — denominations of contractile vacuole pores of parental individual, 1′, 2′, 3′ — of incipient proter, and 1″, 2″, 2 a″, 3″ — of incipient opisthe after Kaczanowska and Kowalska 1969

direction. These fragments and the oral anlagen migrate to the right and after some time a little posteriorly. At the same time, the somatic segment A_4 migrates only posteriorly between the pre-existing somatic ciliature of the future opisthe. After this, kinety A_1 is involved in this whirl. A_1 migrates forward and a little to the right. So this kinety encircles anteriorly the kineties B_1 and C_1. The same event was also observed in *Ch. uncinata* contrary to the former description Dobrzańska-Kaczanowska 1963. The translocation of kineties is accompanied in many cases by the junction of the gliding segments E_1, D_1, C_1. This statement is based upon the counting of the number of oral kineties in this and the later stage of bipartition. The resorption of D_1 and E_1 and the possibility of an additional lengthening of segment C_1 are not clearly excluded but seems highly unlike (Table 1). In some cases the segment A_3 was also observed following description of Radzikowski 1966.

Table 1

Number of oral segments of *Chilodonella cucullulus* during divisional morphogenesis

Number of segments	Induction	Morphogenetic movement	Cytokinesis
3	3	34	23
4	28	17	2
5	27	2	—
6	2	—	—
Total	60	53	25

The somatic segment A_4 in the first stage of migration consists of 14–18 kinetosomes crowded one after another (Pl. II 7). The segment is rather short migrating posteriorly it increases the number of kineties between the anterior vacuoles of the future opisthe. On the other hand, this ingrowth of one additional kinety in the somatic set complements the total number of kineties of the future opisthe. Former lack of one kinety is caused by the fact that one extreme left very short kinety falls entirely to the proter and it is not represented in part of the surface organizing into the future opisthe.

Cytokinesis (Fig. 5 and 6) begins with the first sign of furrowing appearing on the right margin of the ventral surface (Pl. I 1). An indentation observed on the right margin of the cell is always positioned anteriorly to the furrowing on the left margin. Furrowing on the left margin appears a little later in comparison to the right one (Pl. I 2). The cytokinesis is very irregular. At first the constriction between two descendants has the form of a broad V letter (Fig. 4). During next stages of morphogenetic movements segment B_1 and CD_1 encircle the arising oral basket of the opisthe cell. The segment A_3 eventually is pushed posteriorly to the oral apparatus.

During cytokinesis a rarefaction of kinetosomes of kinety A_4 is observed. The total number of kinetosomes arises very little. Each of kinetosomes is now separated from its neighbours by a distinct space. Therefore kinety A_4 becames longer. The same rarefaction of kinetosomes comprises the second postoral kinety A_2 (Pl. II 8). in anterior its portion. In the later stages of cytokinesis or even after cell separation the multiplication of kinetosomes can be observed in both segments A_4 and A_2. Some of the very well silvered kinetosomes are clearly doubled (Pl. II 9). In some cases as many as three kinetosomes form one group. There is an impression that smaller (probably new) kinetosome is formed anteriorly to old one. Gradually in postdividers the number of kinetosomes arises and about two hours attains normal density of kinetosomes in these kineties (Table 2). The same process was also observe in postconjugational morphogenesis too.

Table 2

Number of kinetosomes in segment A_4 of *Chilodonella cucullulus* during divisional morphogenesis

Stage	Number of kinetosomes in A_4	Remarks
Morphogenetic movement	14–20	quiescent
Cytokinesis	17–45	rarefaction and multiplication
Postdividers (1 hr old)	39–50	normal growth

The fate of oral segment A_1 was followed during cytokinesis. The anterior tip of A_1 encircles anteriorly the circumoral segments CD_1 and B_1 and it halts. However, the posterior portion of A_1 moves forward. In result, it gives the arched shape of this kinety. At the upper angle of this arch, the naked triangular field is shown (TF in Fig. 6 and Pl. II 8). The further progress of furrowing is intimately bound with the pronounced incurvation of the right somatic kineties in the supraequatorial region of the parental cell. The curvature of the right kineties of the organizing opisthe is much stronger than slight bowing of the posterior ends of right kineties of the organizing proter. Then the assymetry of the events on both sides of the division furrow is sharply accentuated.

The final shaping of the anterior preoral part of the opisthe cell is based upon the migration of the left somatic kineties forward into the triangular field TF. A_1 is straightened now, and it takes place just in between the right and the left somatic kineties. The existence of autonomic movements of the left somatic kineties is still unproven. Following experiments indirectly corroborate this interpretation.

Ratio of the areas of the ventral surfaces of the incipient proter and of incipient opisthe during the various stages of division of parental cell

The results presented here (Table 3) show a great ununiformity of proportions between areas in the stage of morphogenetic movements. This disproportion and ununiformity of ratio decreases in the specimens more advanced in bipartition. In the moment of cell separation in majority of cases the area of proter and opisthe is the same. They have the same shape too.

Table 3

Proportion of areas of future proter and opisthe of *Chilodonella cucullulus* as measured on the cell surface of dividers drawn in the same scale

Stage	Proter			Opisthe			
	mean	max.	min.	mean	max.	min.	ratio
Induction	23.09 (16)	42.0	15.0	26.36 (16)	38.6	20	0.80
Morphogenetic movement	24.70 (15)	35.8	17.2	25.93 (15)	38.0	21.2	0.94
Early furrowing	27.47 (22)	38.5	15.6	27.24 (22)	38.5	15.6	1.01
Advanced furrowing	29.3 (41)	38.5	24.2	28.80 (41)	38.8	23.8	1.04

Note. In parentheses number specimens examined.

Theoretically it can be supposed that the ingrowth of cell cortex during cytokineses leads to the uniformity of the sizes and shapes of the offsprings. To prove or disprove this assumption two experiments and one estimation were undertaken.

Growth of the ventral surface during ontogenesis

The results of three experiments presented here (Fig. 7) give a general picture of the changes of the mean sizes of ventral surface during ontogenesis. The first and last period of ontogenesis are characterized by the increased level of ingrowth. An innacuracy of the cell synchrony excludes however the possibility of evaluating these recordings in the last period of cytokinesis. It is not clear whether this ingrowth is sufficiently high during cytokinesis to carry out demanded equality of areas of the daughter cells (Fig. 7. Table 4).

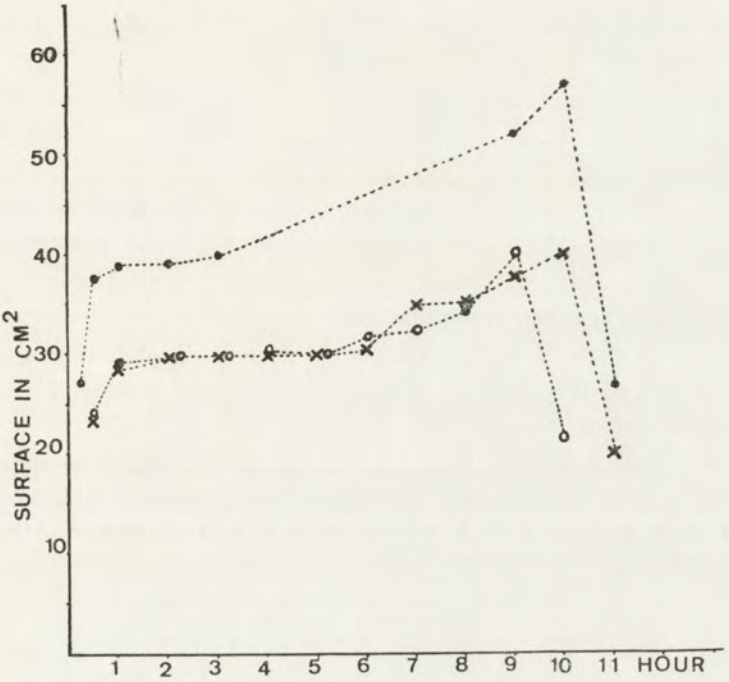


Fig. 7. The course of cell surface growth in B_1 (lower curves) and S stocks of *Chilodonella cucullulus* as observed in three experiments performed from one to the subsequent cell division. Each point represents one sample examined

Table 4

Growth of the cell surface of *Chilodonella cucullulus* in postdividers. Strain B_1 (mean generation time 11 hr) as measured on specimens drawn in the same scale

Time after cell separation	Surface			Ingrowth during experiment
	mean	max.	min.	
15 min	27.63 (40)	40.0	25.5	
60 min	38.60 (30)	53.5	26.3	28.5%

Note. In parentheses number specimens examined.

Estimation of the minimal ratio of ingrowth of the ventral surface area during cytokinesis which is sufficient to equalize the areas of the incipient proter and opisthe

The assumption was made, that the equal sizes of two descendants is carried out exclusively by the ingrowth of the ventral area during cytokinesis. In other words assumption was made that the autonomic rearrangement of the structures does not exist besides of the oral segments.

The way of the speculation leading to estimation of the minimal demanded ingrowth of the ventral surface is as follows:

a. During the period before furrowing, but after the first phase of morphogenetic movements of the oral segments made, the areas of incipient proter and opisthe are unequal.

b. The fission line at this stage does not confirm the shape of the anterior part of opisthe. The area of the incipient opisthe is devoid of its anterior part.

c. The ingrowth of this area during following stages of cytokinesis should form the anterior part of incipient opisthe.

d. The ratio of this ingrowth should shape the anterior, preoral part of opisthe. This shape should be the same in the case of proter and opisthe. This condition conforms morphological observations.

e. The total area of the ventral surface of the future opisthe composed of the really existing surface of incipient opisthe and the supposed, additional preoral part should be equal to the area of the ventral surface of the proter at the moment of the cell separation.

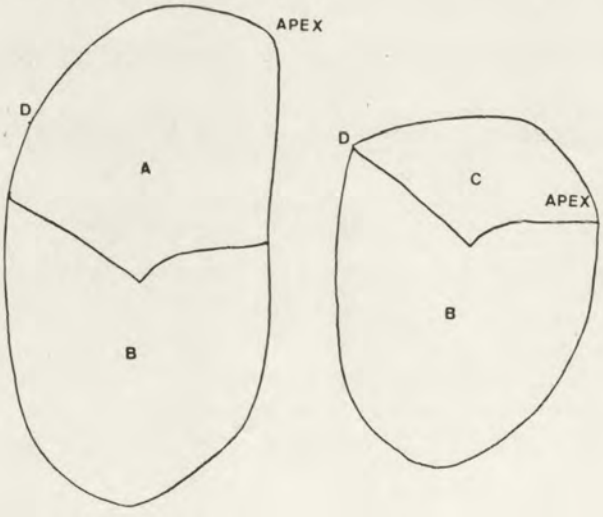


Fig. 8. Model of estimation of minimal ingrowth demanded during cytokinesis of *Chilodonella cucullulus*. A — surface of future proter, B — surface of future opisthe, C — minimal surface of the preoral part for opisthe, D — right margin of fission line

The Figure 8 diagrammatically illustrate the way of estimating the ingrowth. The equation of the total ingrowth can be expressed in the following form:

$$\text{Total ingrowth} = 2(B+C) - (A+B)$$

Table 5 gives the rate of the ingrowth calculated.

The conclusion can be drawn that minimal ingrowth of the ventral surface drops

from the very high value at the beginning of cytokinesis (mean value 39.7%) gradually to 0% at the moment of cell separation. In other words during very short period a great ingrowth about 40% is demanded. This estimation was compared with the ingrowth observed in single cell during cytokinesis.

Table 5

Ratio of ingrowth demanded for shaping of equal sizes of future proter and opisthe of *Chilodonella cucullulus* as estimated in % of ingrowth from the stages examined. (Explanation in text)

Stage	Ingrowth		
	mean	max.	min.
Morphogenetic movement before furrowing	139.7 (13)	162.9	127.6
Various stages of furrowing	—* (41)	125.0	101.7

Note. In parentheses the number specimens examined.
* Calculation of mean value without any sense.

Ingrowth of the cell surface during the period of cytokinesis measured on single cells

The calculation made on 12 cells which successively passed the cytokinesis shows that the total ingrowth does not exceed 7% of total ventral surface. The mean value for all experiments is limited to 4.5%. This value corresponds time from the start of observation up to cell division, then there is about 3 min after the beginning of furrowing. This 3 min can corresponds up to 30% of time of cytokinesis. If 4.5% of the ingrowth of the ventral surface corresponds to 70% of time, the whole ingrowth can be calculated as 6.4% of the ventral surface. This result is only a little lower in comparison to results of Table 3 between the earlier and latter stages of cytokinesis which are calculated about 7–8%. In any case discrepancy between the value estimated (about 40% of the ingrowth of the ventral surface) and existing one, about 7–8% is very striking.

Indirectly, it leads to the supposition that existing autonomic movements and not only the ingrowth shape descendants during cytokinesis.

Pattern of morphogenesis after PEA treatment

Wille 1966 reported alterations in the course of bipartition of *Paramecium* after PEA (phenethyl alcohol) treatment. He established that some kinds of the malformations are related strictly to the period of morphogenesis when the shock of PEA was applied. In earlier phases, he observed, the cases of resorption of oral

apparatus and inhibition of normal events in Ma, in the further — abnormal topography and eventually doublet condition. Wille supposed that the later phenomena are the effects of the suppression of the ingrowth of cortical units and/or suppression of synthesis of the new kinetosomes.

The case of *Chilodonella* is particularly advantageous for testifying whether the primary action of PEA suppresses synthesis of kinetosomes or ingrowth of the cell cortex. The special regional multiplication of kinetosomes in A_4 gives such opportunity. By the comparison of the ranges of areas of normal and treated dividers in the same stage of morphogenesis conclusion can be drawn about the influence of PEA on the ingrowth of cell surface. At last, the types of anomalies can be compared in the cases of *Paramecium* and *Chilodonella*.

The effects of PEA on mass culture

Table 6 presents the results. In the case of affected cells, the drawings were made. In the case of dividers, doublets and altered cells the number of kinetosomes in A_4 was recorded. Among 451 tested cells the great majority — 405 does not exhibit clear malformations. In the cells affected two types are distinguished:

Doublets: Fig. 9, Pl. II 10. The doublets in many respects are similar to dividers during cytokinesis. However doublet state of protozoan can be easily recognized by the abnormal shape of cell body, abnormal topography of opisthe's

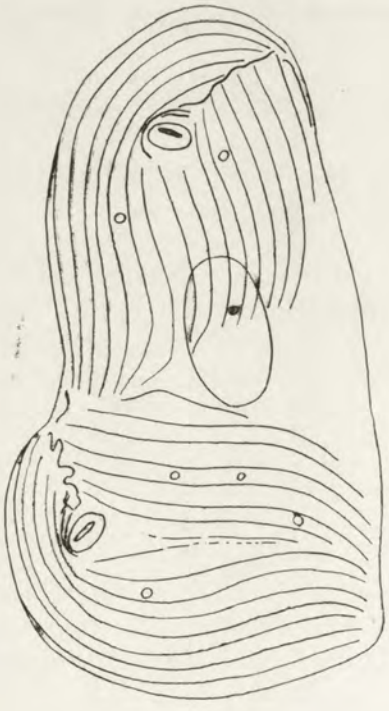


Fig. 9. *Chilodonella cucullulus* (O. F. M.). Pattern of doublet after PEA treatment as observed after 18 hr

preoral kinety A_1, the absence of TF field and fully completed organellogenesis (well developped CVPs and membranous system encircling the oral slits of oral baskets).

The step of alterations in morphology are very different. All of them accomplished resorption of old, induction of new oral baskets and CVPs and morphogenetic movement of two circumoral kineties and A_4. In two heavier affected specimens, the rarefaction of kinetosomes of A_4 and proliferation is inhibited. In all specimens the second phase of morphogenetic movements were disturbed. Preoral kinety o, the opisthe's member of doublet A_1 is always snaky and long, often fragmentatedf TF field is not formed and left kineties are often in abnormal dispositions.

On contrary the degree of inhibition of advancement in cytokinesis, division of Ma and separation of daughter Ma may vary from normal to very altered situation. Similar variations is observed in the topography of CVPs. In some cases as in Pl. II 10 abnormal topography is observed in competent sectors. There is an impression that at least in some cases, new oral baskets do not function and formation of nutritive vacuoles is stopped. The most of doublets exhibit the normal sizes in comparison to dividers. In five of them, however, very small surface was observed. It is not clear, whether there is the cases of halt of the ingrowth or the effect of the starvation.

Abnormal singlets: The second group of anomalies concerns cells in the great majority classified as opisthes. These cells have an abnormal preoral part of the ventral surface and the snaky character of the preoral kinety. In some cases deviations in the state of macronucleus are detected. In general these opisthes have

Table 6

Analysis of cortex pattern of *Chilodonella cucullulus* after PEA treatment and controls examined in silvered preparations

Experiment exposure time, concentration of PEA	Number of cells							
	normal		abnormal singlets				doublets	total
	proter	opisthe	proter		opisthe			
			s.a.	h.a.	s.a.	h.a.		
24 hr, 0.1%	28	33	6	—	5	9	10	91
24 hr, 0.08%	95	59	2	1	22	10	2	191
4–5 hr, 0.08	85	62	1	1	7	10	3	169
Total	208	154	9	2	34	29	15	451
Control	52	47	1	—	4	—	—	104

very similar appearance to the posterior member of the doublet cell. It is necessary however to remark that in control group of cells untreated by PEA a few cases with a little, snaky character of the preoral kinety were also recorded. So this anomaly was recorded in Table 6 as "heavy alteration" in the cases of cells affected in a way never observed in the control cells, and "slightly affected" when the alteration was less pronounced.

The effect of PEA on isolated cells

This study was performed in the aim to test, whether the action of PEA influences directly the process of migration of the structures and process of cytokinesis or indirectly the metabolism, or the property of the ciliate cytoplasm and cortex. In the first case, an immediate and strong effect should be exerted if shock is applied during cytokinesis itself. The cells entering into period of cytokinesis can be tested in such way, because it is known (Silver and Wendt 1967, Burns 1968) that PEA very well and quickly penetrated through the membrane system, particularly in higher concentrations. In the second case the pulse shock applied in preparatory phase of cell, before cytokinesis unables to perform properly the processes characteristic for the stage of cytokinesis.

The results can be summerized as follows:

1. Nondividers (without predividers) after PEA treatment are stopped in division by at least 10–12 hr. They do not exhibit any malformations. This delay in the start of subsequent divisional morphogenesis was observed in the cells kept continuously in 0.1% PEA, as well as after a short 10 min shock of about 0.12% PEA followed washing in fresh medium without drug.

2. Thirty cells isolated in the course of cytokinesis and placed in 0.1 and 0.15–0.2% PEA all of them completed cell division and in stronger solution died after about 15–20 min.

3. Isolated 30 cells recognized under dissecting microscope as predividers or early dividers were treated 10 min by about 0.15% PEA and next replaced in fresh medium. Out of them 14 successfully completed the bipartition during up to 3 hr. The other cells were greatly delayed, and one of them after 18 hr were found in the doublet chains. This case proves that the 10 min treatment of 0.15% is sufficient in bringing out the morphological effects of injury.

4. The observations made on isolated cells after 5 hr of 0.1% PEA treatment and replaced in the fresh medium without the drug indicate, that doublets are unviable, while the other isolated cells are able to give the normal clone.

The rare cases of the heavy altered proters are not clearly understood. May be, there are the products of the division of altered parental opisthe.

Discussion

The received results concern three problems: the ingrowth of cell cortex and proliferation of kinetosomes, the morphogenetic movements and furrowing. All these phenomena influence the shaping and are involved in divisional morphogenesis.

The ingrowth of cell cortex

Intensive ingrowth of the number of kinetosomes and of the cell surface presumably in equatorial region of parental cell was observed in early predividers of

Chilodonella (Fig. 7 and Kaczanowska 1970). This ingrowth is continued in further stages of cell division and in the postdividers (Table 4). These data confirm the various evidences on other ciliates (Cameron and Prescott 1961, Frankel 1960, Ehret and de Haller 1963, Sonneborn 1963, Gillies and Hanson 1968). However, during rather short period of cytokinesis of *Chilodonella* this ingrowth is unsufficient to bring out proper shaping of the preoral part of opisthe. The existence of autonomic morphogenetic movements is proposed as an additional mechanism for form regulation. The ingrowth of cell surface in any case regulates the equal sizes of proter and opisthe which are in various ratio in the early induction stage.

It is supposed that the ingrowth of cell surface accompanying furrowing region as one can observed dislocation of ventral kinety X'' displaced on the dorsal surface of opisthe during cytokinesis. It is unknown, however whether whole ring of furrowing is formed of the new surfaces, as observed in various dividing cells (Rappaport and Ratner 1967, Buck and Krishan 1965, Selman and Perry 1970). In any case, this ingrowth was not indispensable factor for cell division in experiments on re-furrowing of *Stentor* (Tartar 1968).

Proliferation of kinetosomes

The fate of kinety A_4 could be specified as a case of regional kinetosome proliferation which accompanies the course of morphogenesis in specific time and loci. The course of events during proliferation in A_4 is characteristic for the later stages of morphogenesis. This fact reminds the second local wave of kinetosomes synthesis observed in postdividers in *Tokophrya* (Millecchia and Rudzińska 1969). Somatic segment A_4 migrates as one unit. In the initial phase the existence of complex of fibrillar interkinetosomal structures in this kinety can be suspected after Sołtyńska 1969 description. The question however arises about the mechanism and about the ultrastructure of kinety A_4 during migration, rarefaction and proliferation of kinetosomes. Received silvered pictures are consistent with the description of cortical units in early division in *Paramecium* (Dippell 1965, Gillies and Hanson 1968, Selman and Jurand 1968). There is an impression that before proliferation each kinetosome is quite isolated to the others and that all connections among them disappeared. This point should be verified in electron microscope. Particularly intrigueing in this respect is the fate of transverse fibrils and subkinetosomal fibrils (Sołtyńska 1971).

Morphogenetic movements

Morphogenetic movements begins from the rotation of the oral segments. After it, this movement is still continued and oral basket and two circumoral kineties of forming opisthe are shifted backwards while the posterior end of preoral kinety A_1 and left somatic kineties are shifted forwards into the region of furrowing. This

process is visualized in Fig. 10. This drawing is the result of superposition of four outlines of four specimens of the same sizes of ventral surfaces drawn in the same scale which were in different stages of cytokinesis. The displacement of oral basket, oral kineties and CVPs is clearly shown. The more obscure shift in CVP-3 posterior represents more posterior displacement of this pore in more advanced stages. The second phase of morphogenetic movements causes the changes of the configuration of kinetome and CVPs. Kineties are pulled anteriorly while CVPs are less shifted

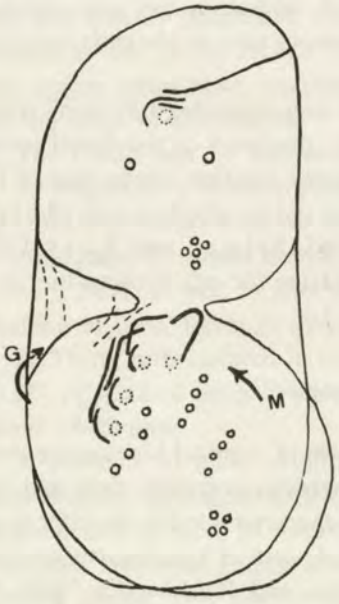


Fig. 10. Outlines of four specimens of *Chilodonella cucullulus* drawn in the same scale in different stages of furrowing. Abbreviations: G — supposed growth of cell surface displacing dorsally kinety X'', M — morphogenetic movement of left somatic kineties

forwards or even rest in the same place (so they are shifted posteriorly in relation to the kinetome). This changes in topography are regulated during early stage of subsequent cell division. In such way the existing paradox that CVP-3'' of opisthe is differentiated anteriorly to the position of the old one can be understood.

The changes in configuration of kinetome and CVPs are known. In *Paramecium* King 1954 described active morphogenetic movement of CVPs. On contrary Porter 1962 reported shift of the oral apparatus and vestibular kinetome during cytokinesis of *Paramecium*. Similar events are reported in other ciliates too, but there is no data on ultrastructure of them during their migration. For example Sołtyńska 1971 described in *Chilodonella cucullulus* subpellicular microtubules on the lateral margin of the ventral surface. It would be interesting to learn about their fate and role during moulding of ventral surface of opisthe.

The migration of kinetal segments has the character of a whirl. It can be supposed that there is some kind of center around which the organelles turn to the right. It is worth mentioning that at least two other morphogenetic events are radially oriented in *Chilodonella* namely formation of radial oral baskets and radial induction of

CVPs (Kaczanowska 1970). It is not clear if one point which shifts or three separate geometric points are geometric centers of these radial induction and arrangement of the structures. The question then arises if these geometric centers are only geometric points, or they play the role of the organizing centers. The role of this center could be compare by analogy to organizing role played by blastoporus in polarized amphibian embryo.

Developmental character of migration of oral kineties in *Chilodonella* and related species were pointed out by Deroux et Dragesco 1968. In this respect very important is discovery of Sołtyńska 1971 that preoral kinety (A_1) and circumoral kineties are polarized in nondividers according to polarity of the rest of ciliature. She found that kinetodesmas and parasomal sacs are oriented in the same way in somatic and oral ciliature (Sołtyńska 1971 Pl. VII 47). The urgent question then arises if these kineties are synthesized in reverse polarity in early stages of division and their rotation leads to normal polarization according to polarity of the rest of cortex. The same questions concern the radial symmetry of oral baskets. Tucker 1970 described the rolling up of cytopharyngeal units preceding formation of radial oral anlagen in *Nassula*. In *Chilodonella* of special interest is the formation of the oral basket of proter in respect to the absence of any changes in the state of oral ciliature.

Mechanism of morphogenetic movement is totally unknown. Even more it is very hard to imagine what kind of changes of ultrastructure can be associated with it. The moving segments are ciliated and seem to form separate units with their interkinetosomal connections. Where is the place of boundaries of pulled or pushed elements ? What are the forces pulling or pushing these structures and what determines the ends of movement ? These are the most principal questions without any, even hypothetic, explanations. Porter 1962 thinks that morphogenetic movements of oral apparatus of *Paramecium* is bound with the changes in rigidity in suture region. It was observed that during morphogenetic movements of oral kineties, there is regional more viscous state of cortex, and the cortex seems more plastic. So the biophysic state of cortex also can be involved in this process.

Cytokinesis and effect of PEA on morphogenesis

During furrowing the second phase of morphogenetic movements are associated with furrowing. Preoral part of cortex of the opisthe is shaped during this stage. Shaping of the preoral part of opisthe consists of: the backward displacement of the oral basket and circumoral kineties (1), the shift forward the left somatic ciliature (2), the ingrowth of the cell cortex, dorsally displacing kinety X'' (3), the forward migration to apex posterior end of kinety A_1 (4), and constriction of the fission line (5).

In the case of morphogenesis without the shaping anew of the preoral part of cortex i.e. in exconjugants, all these events are absent except the apical migration

of the kinety A_1 along the disappearing old one. This kinety in this case has the snaky character and triangular field is not formed (Radzikowski 1966). Therefore this migration of the preoral kinety A_1 is an independent process to cytokinesis.

The experiments with the use of PEA are quite consistent to results received on *Paramecium* (Wille 1966). The resorption of oral apparatus was not observed in *Chilodonella* in contrast to *Paramecium* but this kind of process can be easily omitted in our experiments not focussed on this problem. PEA brings out various effects on cell metabolism (Berrah and Konetzka 1962, Rosenkranz et al. 1964, Silver and Wendt 1967, Plagemann 1968, Burns 1968, Higgins et al. 1969, Bostock 1970). It is not clear what kind of processes are most sensitive in *Chilodonella*. In general it can be admitted that initial phases of divisional morphogenesis gives all or none inhibition and morphological anomalies are revealed only in further stages of division. The deviation of the course of morphogenesis of the cell treated by PEA is produced even by pulse shock in the early stage of cell division but the morphological effects are revealed during later stages. It seems rather sure that the proliferation of kinetosomes and the ingrowth of the cortex are not the most disturbed process. The immediate effect of PEA on the cytokinesis seems excluded if even in the presence of high dose of PEA the cytokinesis is completed. Produced anomalies concerns the processes in cytoplasm and cortex if the construction and separation of Ma and furrowing and morphogenetic movements are the most disturbed processes.

The various degrees of anomaly (doublets or abnormal opisthes) seems to indicate that the effect of PEA can be expressed partially. In many cases an abnormal cytokinesis can be completed with a great delay. In such a way the abnormal opisthes can arise. It can be concluded that rather the synthesis or proper aggregation of prerequisite substances for furrowing, constriction or displacement of the structures is more affected that the execution of the work performed during cytokinesis.

From the morphological point of view, it is interesting to observe that different course of morphogenesis in proter and opisthe during cytokinesis is visualized by alteration of morphology after PEA which is more easy distinguishable in the class opisthes or in opisthe member of doublet, than in the class of proters, though the differences in the sensivity of metabolism in both classes are not expected.

Acknowledgements

I wish to thank Professor Doctor Gerard de Haller for helpful suggestions during the work, reading manuscript and generous hospitality during my work in Department of Protozoology and Parasitology of University of Geneve.

I am much indebted to Professor Doctor Zdzisław Raabe Director of Institute of Zoology of Warsaw University for reading of manuscript and valuable criticisms and discussions.

I am also grateful to Doctor Maria Sołtyńska, who gave me manuscript of her work prior to publication and to Doctor Perret from Department of Comparative Physiology of University of Geneve, who helped me to photograph isolated cells during furrowing.

Summary

During divisional morphogenesis active migration of some cortical structures, local multiplication of kinetosomes in migrating kinety and course of cytokinesis was described in *Chilodonella cucullulus* (O. F. M.).

Morphogenetic movements were observed in incipient opisthe of dividing cell and have the character of a whirl in clockwise direction. There is at least three important morphogenetic processes observed during division of *Chilodonella* which are radially oriented namely: CVPs induction, oral baskets formation and morphogenetic movements in organizing opisthe. Question arises if geometric centers of radially oriented processes are really existing organizing centers.

After phenethyl alcohol treatment only further steps of divisional morphogenesis are disturbed. In effect, the abnormal opisthes and doublets arise after exposure to PEA during preparative phase of divisional morphogenesis.

STRESZCZENIE

W trakcie morfogenezy podziałowej *Chilodonella cucullulus* (O. F. M.) istnieje aktywne przemieszczanie się struktur kortykalnych, lokalne namnażanie się kinetosomów oraz szereg zmian w topografii w trakcie cytokinezy.

Aktywne przemieszczanie się struktur występowało jedynie w organizującej się tylnej komórce potomnej (opistorze). Ruchy te mają charakter wiru.

W trakcie morfogenezy podziałowej *Chilodonella* co najmniej trzy ważne procesy, a mianowicie: indukcja otworków wodniczek tętniących, tworzenie się koszyczków gębowych i wirowy ruch struktur w opistorach są zorientowane promieniście. Powstaje pytanie, czy geometryczne punkty środkowe promieniście zorientowanych procesów nie są rzeczywiście istniejącymi centrami organizacyjnymi.

Pod wpływem działania alkoholu fenetylowego zakłócone są późniejsze stadia morfogenezy podziałowej. Jeżeli zadziałamy PEA w trakcie przygotowań komórki do podziału, w efekcie otrzymać możemy dublety i anormalne opistory.

REFERENCES

Berrah G. and Konetzka W. A. 1962: Selective and reversible inhibition of DNA synthesis in *E. coli* H. J. Bact., 83, 738

Bostock C. J. 1970: The effect of 2-Phenyl ethanol PE on the DNA synthesis of *Schisosaccharomyces pombe*. J. Cell Sci., 7, 523–530.

Buck R. C. and Krishan A. 1965: Site of membrane growth during cleavage of amphibian epithelial cells. Expl Cell Res., 38, 426–429.

Burns V. W. 1968: Effect of phenethyl alcohol on yeasts cells. J. cell. Physiol., 72, 97–109.

Cameron I. L. and Prescott D. M. 1961: Relations between cell growth and cell division. V. Cell and macronuclear volumes of *Tetrahymena pyriformis* HSM during the cell life cycle. Expl Cell Res., 23, 354–361.

Corliss J. O. 1953: Silver impregnation of ciliated protozoa by Chatton–Lwoff technic. Stain tech., 28, 97–100.

Deroux G. et Dragesco J. 1968: Nouvelles données sur quelques ciliés holotriches cyrtophores a ciliature ventrale. Protistologica, 4, 365–403.

Dippell R. V. 1965: Reproduction of surface structure in *Paramecium*. In: Progress in Proto-

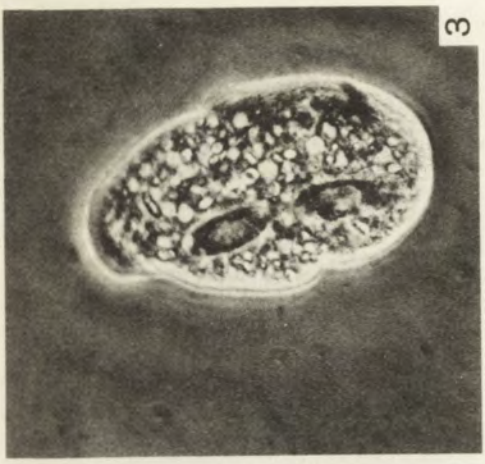
zoology. Abstr. Second int. Conf. Protozool., London 1965, Excerpta med. int. Congr. ser. No. 91.
Dobrzańska-Kaczanowska J. 1963: Comparaison de la morphogenèse des Ciliès: *Chilodonella uncinata* (Ehrbg.), *Allosphaerium paraconvexa* sp. n. et *Heliochona scheuteni* (Stein). Acta Protozool., 1, 354–392.
Dobrzańska-Kaczanowska J. 1965: Studies on morphology of *Chilodonella cucullulus* O. F. M., 1786. In Progress in Protozoology. Abstr. Second int. Conf. Protozool., London 1965, Excerpta med.. int. Congr. ser. No. 91, 211.
Ehret C. F. and de Haller G. 1963: Origin, development and maturation of organelles and organelle systems of the cell surface in *Paramecium*. J. Ultrastruct. Res. Suppl., 6, 1–42.
Fauré-Fremiet E. 1950: Mécanismes de la morphogenèse chez quelques Ciliés *Gymnostomes hypostomiens*. Archs Anat. microsc. Morph. exp., 39, 1–14.
Frankel J. 1960: Morphogenesis in *Glaucoma chattoni*. J. Protozool., 7, 363–376.
Gillies C. G. and Hanson E. D. 1968: Morphogenesis of *Paramecium trichium*. Acta Protozool., 6, 13–31.
Hanson E. D. 1967: Protozoan development. In: M. Florkin and T. Scheer (eds.), Chemical Zoology, Vol. I Protozoa G. W. Kidder (ed.), 397–539. Academic Press.
Higgins M. L., Shaw T. J., Tillman M. C. and Leach F. R. 1969: Effect of phenethyl alcohol on cell culture growth. II. Isolated cell components and lysosomal enzymes. Expl Cell Res., 56, 24–29.
Kaczanowska J. 1970: Topography of cortical organelles in early dividers of *Chilodonella cucullulus* (O. F. M.) Acta Protozool., 8, 231–250.
Kaczanowska J. and Kowalska D. 1969: Studies on topography of the cortical organelles of *Chilodonella cucullulus* (O. F. M.) I. The cortical organelles and intraclonal dimorphism. Acta Protozool., 7, 1–15.
King R. L. 1954: Origin and morphogenetic movements of the pores of the contractile vacuoles in *Paramecium aurelia*. J. Protozool., 1, 121–131.
Kowalska D. and Kaczanowska J. 1970: Studies on topography of the cortical organelles of *Chilodonella cucullulus* (O. F. M.) II. Topographical relations of the total number of kineties to the disposition of CVPs. Acta Protozool., 7, 181–192.
Macdonald A. G. 1967: Delay in the cleavage of *Tetrahymena pyriformis* exposed to high hydrostatic pressure. J. cell. Physiol., 70, 127–131.
Millecchia L. L. and Rudzińska M. A. 1969: Replication of basal bodies in suctorian. *Tokophrya infusorium*. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, Nauka, Leningrad 1969, 100–101.
Plagemann P. 1968: Phenethyl alcohol: Reversible inhibition of synthesis of macromolecules and disaggregation of polysomes in rat hepatoma cells. Biochim. biophys. Acta. 155, 202–218.
Porter E. D. 1962: A theory of morphogenetic migration in *Paramecium aurelia*. J. Protozool., 9, suppl. 96, 26.
Radzikowski S. 1965: Changes in the heteromeric macronucleus in division of *Chilodonella cucullulus* (Müller). Acta Protozool., 3, 233–238.
Radzikowski S. 1966: Study on morphology, division and postconjugation morphogenesis in *Chilodonella cucullulus* (O. F. Müller). Acta Protozool., 4, 89–95.
Rappaport R. and Ratner J. H. 1967: Cleavage of sand dollar eggs with altered patterns of new surface formation. J. exp. Zool., 89–100.
Rosenkranz H. S., Carr H. S. and Rose H. M. 1964: Phenethyl alcohol and messenger RNA. Biochem biophys. Res. Commun., 17, 199–201.
Selman G. G. and Jurand A. 1968: The formation of cell organelles during the fission cycle of *Paramecium aurelia*. J. Protozool., 15 suppl., 37.
Selman G. G. and Perry M. M. 1970: Ultrastructural changes in the surface layers of the newt's egg in relation to the mechanism of its cleavage. J. cell Sci., 6, 207–229.
Silver S. L. and Wendt L. 1967: Mechanism of action of phenethyl alcohol: breakdown of the cellular permeability barrier. J. Bact. 93, 560–566.
Sołtyńska M. 1969: The cortex of *Chilodonella cucullulus*. In: Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, Nauka, Leningrad 1969, 74.
Sołtyńska M. 1971: Morphology and fine structure of *Chilodonella cucullulus* (O. F. M.). Cortex and cytopharyngeal apparatus. Acta Protozool., 9, 49–82.
Sonneborn T. M. 1963: Does preformed cell structure play an essential role in cell heredity. In: Nature of Biological Diversity. Allen (ed.), 165–223.
Tartar V. 1968: Micrurgical experiments on cytokinesis in *Stentor coeruleus*. J. exp. Zool., 167, 21–36.

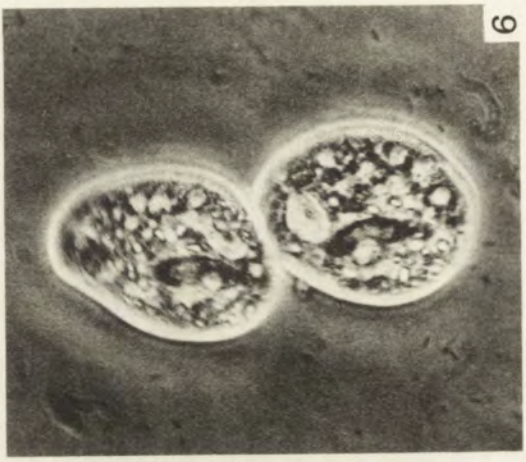
Terra de N. 1969: Differential growth in the cortical fibrillar system as the trigger for oral differentiation and cell division in *Stentor*. Expl Cell Res. 56, 142–154.
Tucker J. B. 1970: Morphogenesis of a large microtubular organelle and its association with basal bodies in the ciliate *Nassula*. J. cell Sci., 6, 385–429.
Wille J. J. 1966: Induction of altered patterns of cortical morphogenesis and inheritance in *Paramecium aurelia*. J. exp. Zool., 191–214.

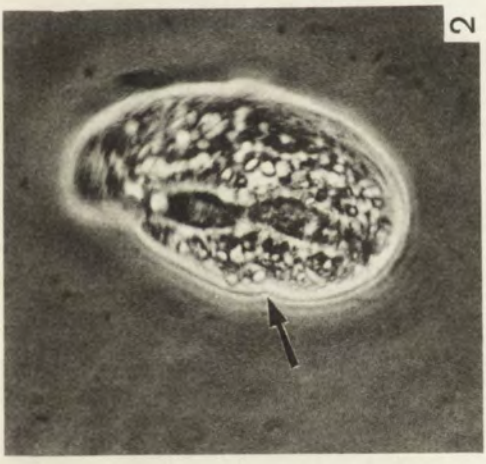
EXPLANATIONS OF PLATES I–II

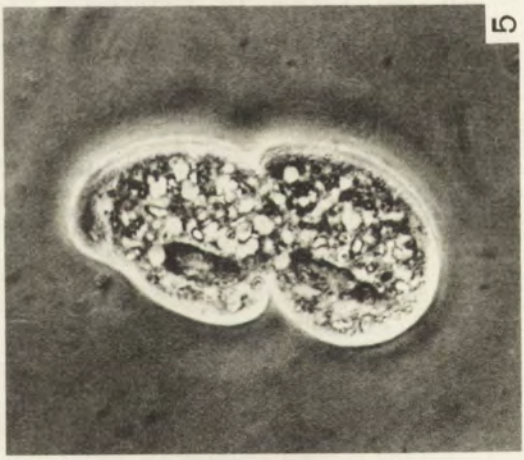
Chilodonella cucullulus (O. F. M.)

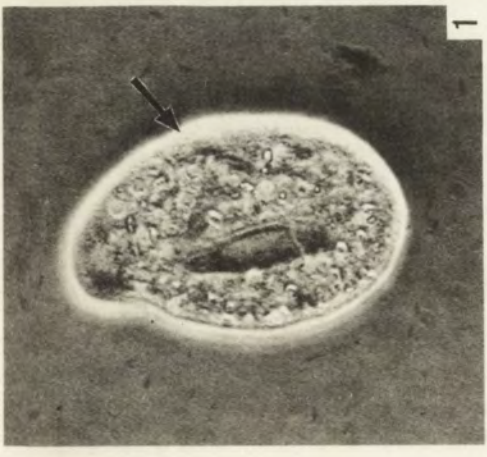
1–6: Sequential phases of furrowing of single cells of *Ch. cucullulus*. Phase contrast photomicrographs
7: Migration of kinety A_4
8: Cleavage of *Chilodonella cucullulus*. Proliferation of kinetosomes in kineties A_4 and A_2 (early stages)
9: Opisthe postdividers. Advanced stages of proliferation of kinetosomes in kineties A_4 and A_2
10: Doublet of *Ch. cucullulus* after PEA treatment. Specimen fixed after 5 hours. Abnormal CVPs disposition in competent sectors. Well developed CVPs and lips of oral baskets
7–10: Specimens silvered after Chatton et Lwoff method

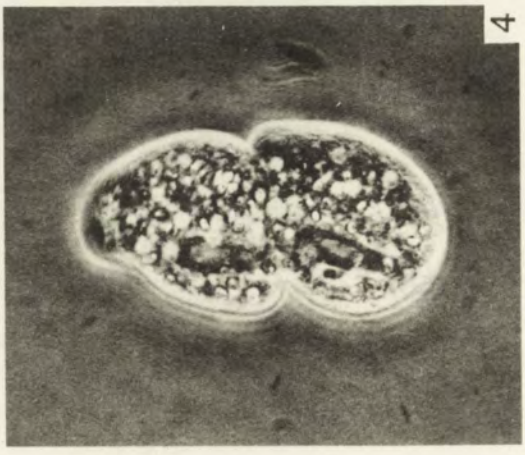






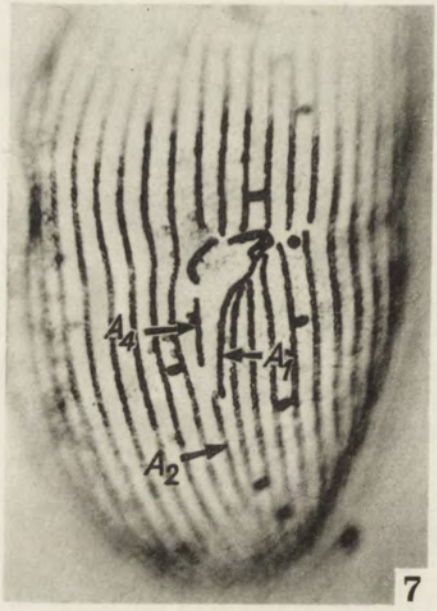






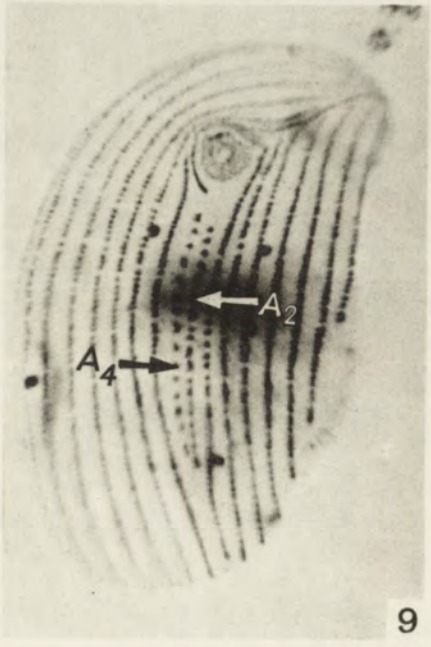
J. Kaczanowska

auctor phot.



7

8



9

10

J. Kaczanowska auctor phot.

ACTA PROTOZOOLOGICA
VOL. IX WARSZAWA, 1.VIII.1971 FASC. 6

Zoological Institute, University of Warszawa, Warszawa 64, Poland

Andrzej KACZANOWSKI

Opalina ranarum Purkinje et Valentin: meiosis and dimorphism of nuclear behaviour during meiosis

Opalina ranarum Purkinje et Valentin: meioza i dymorfizm zachowania się jąder w czasie meiozy

Copulation of gametes of an anisogamous type occurs during the life cycle of *Opalina ranarum* Purkinje et Valentin. This was first reported by Neresheimer 1907 and was confirmed by Wessenberg 1961, who made the most detailed investigation of this problem. Infective cysts are produced by palintomy in the hindgut of the adult frog and following infection these develop into gamonts followed by macro- and microgametes. Microgametes are very narrow cells, which swim in an easily distinguishable manner.

Chen 1936 a, b, 1948 established that nuclei of *Zelleriella*, another genus of Opalinids, consist of a diploid set of chromosomes. This would lead one to expect pregamic meiosis in this group of protozoa.

Material and methods

To examine this question, 2 to 3 week old tadpoles were exposed to cysts of *Opalina ranarum*. These tadpoles were kept sterile until the moments of infection. Formation of microgametes and copulation of these with microgametes was observed 2 to 5 days after the time of infection. Zygocysts of the first generation were initially observed at 3 days after infection.

During this period smears containing gamonts and gametes were prepared and fixed using formalin alcohol (1 : 9). The further procedure consisted of drying and staining by the Feulgen method followed by counterstaining with toluidin blue after Kaczanowski 1968.

Results

Bivalents became visible in some nuclei 2 or 3 days after infection thus far they have not been observed at later time. Pl. I 1 and 2 depict bivalents in late prophase of the first meiotic division. Clear chiasmata are visible (arrows). The number of bivalents is 8 or 9, further studies would be necessary to make a detailed estimation.

In many cases meiosis occurs in specimens containing two or more nuclei. Invariable, however, only one nucleus was observed in meiosis while the other(s)

appeared inactive. For example, one nucleus (a) in Pl. I 2 is in meiosis while another nucleus (b) remains in interphase without any sign of division.

It seems probable that the observed dimorphism of nuclear behaviour during meiosis reflects some important regularity but more observation is needed to establish the phenomenon firmly. Moreover it is not clear only one nucleus per cell is capable of entering meiosis, or whether there is asynchrony such that another nucleus (or nuclei) will enter meiosis with some delay.

With respect to the number of chromosomes, the present data are in agreement with those of previous study by Kaczanowski 1968, who estimated the diploid set in trophonts at about 18 chromosomes. This is consistent with the statement that meiosis in *Opalina ranarum* is pregamic. Chromosomes of *O. ranarum* are rather small and separate chromatids were not distinguished. So can be a little doubtness if tetrads are present and chiasmata are followed by crossing over.

Summary

Pregamic meiosis occurs in *O. ranarum*. In many cases meiosis occurs in specimens containing two or more nuclei. Invariable however, only one nucleus was observed in meiosis, while the other(s) appeared inactive.

STRESZCZENIE

Wykazano, że u *O. ranarum* występuje pregamiczna meioza. W wielu przypadkach obserwowano ten proces u gamontów zawierających 2 lub więcej jąder. Wówczas tylko jedno jądro podlegało meiozie, podczas gdy pozostałe było(y) nieaktywne.

REFERENCES

Chen T. T. 1936 a: Observations on mitosis in Opalinids (*Protozoa, Ciliata*). I. The behaviour and individuality of chromosomes and their significance. Proc. natn. Acad Sci., 22, 594–601.

Chen T. T. 1936 b: Observations on mitosis in Opalinids (*Protozoa, Ciliata*). II. The associations of chromosomes and nucleoli. Proc. natn. Acad. Sci., 22, 602–605.

Chen T. T. 1948: Chromosomes in *Opalinidae (Protozoa, Ciliata)* with special reference to their behaviour, morphology, individuality diploidy, haploidy and association with nucleoli J. Morph., 83, 281–385.

Kaczanowski A. 1968: Mitosis and polyploidy in nuclei of *Opalina ranarum*. Experientia, 24, 846–847.

Neresheimer E. 1907: Die Fortpflanzung der Opalinen. Arch. Protistenk., suppl. I. 1–42.

Wessenberg H. 1961: Studies on the life cycle and morphogenesis of *Opalina*. Univ. Calif. Publ. Zool., 61, 315–370.

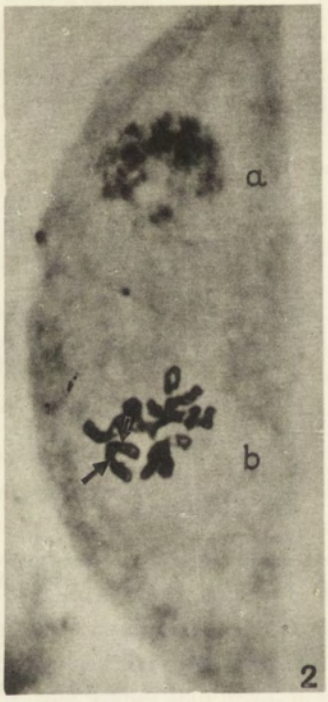
EXPLANATION OF PLATE I

Opalina ranarum Purkinje et Valentin

1: Late prophase of the first meiotic division in one gamont containing only one nucleus. Arrows indicate chiasmata. ×2000

2: Meiosis in gamont containing two nuclei. Meiosis occur only in nucleus (a), while nucleus (b) has interphasal character. Arrows indicate chiasmata. ×2000

1



2

A. Kaczanowski auctor phot.

ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 1.VIII.1971 FASC. 7

Department of Biology, New York University, Washington Square College, New York, N. Y. and Department of Zoology and Physiology, University of Nebraska, Lincoln, Nebraska, U. S. A.

J. D. LUTTON and B. W. McCASHLAND

The effect of isoriboflavin and cyanide on growth and respiration in *Tetrahymena pyriformis*

L'effect de l'isoriboflavine et de la cyanide sur l'accroissement et la respiration dans *Tetrahymena pyriformis*

Adaptation of growth and respiration of *Tetrahymena pyriformis* against cyanide inhibition by exposure to increasing concentrations of cyanide has been accomplished (McCashland 1955 a, b, 1956). Furthermore, it has been proposed that cyanide adaptation in *Tetrahymena* is a physiological adjustment rather than the result of mutation or selection. Alterations in glucose, acetate, and amino acid metabolism have been demonstrated in cyanide adapted cells (McCashland and Steinacher 1962). In addition, the amount of intracellular riboflavin was reported to be almost twice as great in cyanide adapted cells as in normal cells. McCashland considered that a possible explanation for the mechanism of cyanide resistance may involve increased flavoprotein activation with molecular oxygen.

In view of the possible action of isoriboflavin on flavoproteins (Emerson and Tishler 1944), and the role of flavoproteins in respiration and growth, experiments were undertaken to determine the response of normal and adapted *Tetrahymena* to isoriboflavin and cyanide.

Materials and methods

Tetrahymena was established as a clone and cultured axenically in synthetic medium (Kidder et al. 1954) and in darkness at 25°C. Parent cultures were maintained in 5 ml of medium, contained in silicone stoppered 15 × 125 mm pyrex test tubes, by 0.1 ml transfers to fresh medium every 5 days. Details of the adaptation procedure, in which cyanide concentration is gradually increased to a maintenance level of 10^{-4} M KCN, have been described (McCashland 1955 b, 1956). These cells were designated as ACN, differing from normal (N) cells not so exposed. In a similar manner, cultures were adapted to isoriboflavin ($IsoB_2$) (Calbiochem) at 40 times the riboflavin level of the medium (1.08×10^{-5} M $IsoB_2$). These cells were designated as AB4.

Cultures were counted by use of a Klett-Summerson colorimeter with a blue filter (No. 42) according to the methods described previously (Elliott 1949). Readings were taken on 72 hr cultures, and a reading of 60 was equivalent to a culture population of 100,000 cells/ml, which is the equivalent of 0.48 mg dry wt/ml.

In order to obtain large uniform numbers of cell samples for growth tests, a Fisher Volustat and a 12-flask replicator were employed (McCashland 1963). All growth experiments were conducted with 72 hr cultures growing at 25°C.

Respiration was measured manometrically at 25°C by the direct method of Warburg. Cultures were grown in 50 ml Erlenmeyer flasks containing 20 ml of medium, and were re-suspended in conditioned medium to give the desired cell concentration for experiments. Warburg flasks of approximately 5 ml were used, and the main compartments contained 1.7 ml of cell suspension, the side arms 0.05 ml of 8 N H_2SO_4, and the center wells 0.05 ml of KOH or KOH–KCN solutions. Results are presented as the $Q0_2$, calculated from the dry weight of known numbers of washed cells.

Results

Growth

It was found that $IsoB_2$ tends to inhibit growth of normal *Tetrahymena*. $IsoB_2$ was added to give concentrations of 1.08, 1.62, 2.16, and 3.24×10^{-5} M respectively (40, 60, 80, and 120 times the riboflavin concentration in the culture medium). Preliminary experiments revealed that 10–25% inhibition could be obtained with $IsoB_2$ concentrations ranging between 1.08 and 2.16×10^{-5} M. Similar growth tests were conducted employing riboflavin rather than $IsoB_2$, and it was found that there was little effect upon growth with increasing concentrations (Table 1).

Table 1

The effect of $IsoB_2$ and riboflavin on growth of normal (N) *Tetrahymena*. Colorimeter values are the average of 20 cultures for each condition ( ) = % of control

Isoriboflavin concentration $\times 10^{-5}$ M				
Control	1.08	1.62	2.16	3.24
52.5 (100.0)	47.0 (89.6)	41.5 (79.1)	40.0 (76.5)	32.0 (61.3)
Riboflavin concentration $\times 10^{-5}$ M				
40.0 (100.0)	40.0 (100.0)	40.0 (100.0)	40.0 (100.0)	40.0 (100.0)

Table 2

The effect of 1.08×10^{-5} M $IsoB_2$ and 10^{-4} M KCN on growth of normal (N), $IsoB_2$-adapted (AB4), and cyanide-adapted (ACN) *Tetrahymena*. Colorimeter values are the average of 24 cultures in KCN and 48 cultures at control conditions and in KCN and $IsoB_2$ combined ( ) = % of control

Cell	Control	KCN	$IsoB_2$	KCN+$IsoB_2$
N	64.7 (100.0)	39.2 (60.7)	58.4 (90.3)	35.9 (55.4)
AB4	65.8 (100.0)	37.9 (57.5)	60.2 (91.5)	36.8 (55.8)
ACN	59.6 (100.0)	40.3 (67.7)	49.4 (82.7)	37.8 (63.5)

Results in Table 2 indicate the effect on growth by 10^{-4} M KCN, 1.08×10^{-5} M $IsoB_2$, and by combination of cyanide and $IsoB_2$ on N, AB4, and ACN cells. Growth inhibition by 10^{-4} M KCN is 39.3, 32.3, and 42.5% in N, ACN, and AB4 cells

respectively. Cumulative growth inhibition is effected by combination of cyanide and $IsoB_2$ in N and AB4 cultures, but to a lesser degree in ACN cultures.

Respiration

Addition of 1.08 and 1.62×10^{-5} M $IsoB_2$ initially to normal cells stimulates respiration 14 and 25% respectively. Further addition of 2.16 and 3.24×10^{-5} M gave 5 and 35% inhibition respectively (Table 3).

Table 3

The effect of $IsoB_2$ on respiration of normal *Tetrahymena* as determined by the direct method of Warburg. $Q0_2$ values are the average of 8 determinations. ( ) = % of control

Isoriboflavin concentration $\times 10^{-5}$ M				
Control	1.08	1.62	2.16	3.24
26.8 (100.0)	30.5 (114.0)	33.5 (125.0)	25.8 (96.4)	17.5 (65.4)

Table 4

The effect of 10^{-4} M KCN and 1.08×10^{-5} M $IsoB_2$ on respiration of normal (N), $IsoB_2$-adapted (AB4), and cyanide-adapted (ACN) *Tetrahymena* as determined by the direct method of Warburg. $Q0_2$ values are the average of 12 determinations. ( ) = % of control

Cell	Control	KCN	$IsoB_2$	KCN+$IsoB_2$
N	27.8 (100.0)	21.7 (78.2)	31.1 (112.5)	25.7 (92.7)
AB4	15.8 (100.0)	12.9 (81.6)	18.5 (117.0)	17.8 (112.7)
ACN	20.6 (100.0)	18.5 (90.0)	22.4 (109.0)	18.9 (91.8)

Experiments indicate that $IsoB_2$-adapted cells, normal cells, and cyanide-adapted cells are stimulated upon addition of 1.08×10^{-5} M $IsoB_2$ (Table 4). Furthermore, addition of 10^{-4} M KCN inhibits respiration in each of the cultures. Respiration by $IsoB_2$-adapted cells is stimulated by the presence of both cyanide and $IsoB_2$, and is inhibited slightly in normal cells but to a greater degree in cyanide-adapted cells.

Discussion

All concentrations of $IsoB_2$ tested produce an inhibitory effect on growth of normal *Tetrahymena*. In addition, growth inhibition occurs with AB4 and ACN cells exposed to 1.08×10^{-5} M $IsoB_2$. This is not surprising in view of the action if $IsoB_2$ (Emerson and Tishler 1944). It is of interest that ACN cells are inhibited to a greater degree than N or AB4 cells (18 vs 10%). This may be explained if one considers that the ACN cell has adapted a respiratory mechanism that is more dependent upon a $IsoB_2$ sensitive system. In this respect, when ACN cells are compared with N or AB4 cells, they are less sensitive to KCN (32 vs 40–45%), but more sensitive to $IsoB_2$ added separately or in combination. Furthermore, AB4

cells, grown for 72 hrs, demonstrate no increased resistance to KCN or $IsoB_2$ added separately or in combination (similar to N cells).

Stimulation of respiration by 1.08 and 1.62×10^{-5} M $IsoB_2$, is not unusual in view of the many inhibitors that demonstrate a similar effect in low concentrations (Borst and Slater 1961, Hemker 1964, and Lardy et al. 1958). It is of interest that ACN cells are the least sensitive to cyanide inhibition (10%), followed by AB4 (18%) and N cells (22%). This suggests that physiological adaptation may have occurred, and that adapted cells may involve different respiratory mechanisms, or various degrees of dependence on different respiratory mechanisms. In one case, the ACN cell may depend more predominately on a mechanism by-passing the cyanide sensitive system. On the other hand, the AB4 cell may have a tendency not to by-pass the cyanide sensitive system as extensively as the ACN cell. Since cumulative addition of $IsoB_2$ and KCN overcome respiratory stimulation in both N and ACN cells (8–9% inhibition), but not in AB4 cells (13% stimulation), suggests the possibility that several adaptive mechanisms may be involved. As in growth experiments, respiration of the ACN cell is more sensitive to $IsoB_2$ added individually or cumulative with cyanide, suggesting that the ACN cell has adapted a respiratory mechanism which is more sensitive to $IsoB_2$.

Apparently the AB4 cell has a respiratory mechanism that is slightly less sensitive to KCN, as compared with N cells, yet is less capable of overcoming respiratory stimulation by $IsoB_2$ when added cumulative with cyanide. In view of the cumulative effect of KCN and $IsoB_2$, it may be that adaptive respiratory mechanisms in ACN and AB4 cells involve different pathways, or more specifically a $IsoB_2$ sensitive system in ACN cells, and a $IsoB_2$ resistant system in AB4 cells. Indeed, if the AB4 cells are less sensitive to $IsoB_2$, then one might expect less respiratory inhibition upon cumulative addition with KCN, as is the case.

In conclusion, one may infer that various degrees and types of physiological adaptation may be involved with AB4 and ACN cells.

Summary

Isoriboflavin ($IsoB_2$) at 10^{-5} M inhibits growth of normal (N), cyanide adapted (ACN), and to a lesser degree isoriboflavin adapted (AB4) *Tetrahymena pyriformis*. KCN inhibits growth of N, AB4, and to a lesser degree ACN cells. Cumulative addition of KCN and $IsoB_2$ results in greater growth inhibition in all cell types as compared with inhibition by either inhibitor added separately. KCN at 10^{-4} M inhibits respiration of N, AB4, and ACN cells by 28, 18, and 10% respectively. Initial addition of $IsoB_2$ stimulates respiration in all cell types, and is overcome by cumulative addition with cyanide in N and ACN cells but not in AB4 cells. It is inferred that various degrees and types of physiological adaptation may be involved with AB4 and ACN cells.

RESUME

L'isoriboflavine ($IsoB_2$), à 10^{-5} M, prohibe l'accroissement de (N) normale, la cyanide adaptée (ACN) et, à un moindre degré, l'isoriboflavine adaptée (AB4) *Tetrahymena pyriformis*. Ls KCN prohibe l'accroissement de N, AB4 et, à un moindre degré, les cellules ACN. L'addition cumulative de KCN et d'$IsoB_2$ a pour resultat un arrêt plus radical de l'accroissement dans tous les types de cellules si on le compare à l'arrêt provoqué par chaque inhibiteur ajouté séparement. La KCN à 10^{-4} M prohibe la respiration de N, AB4 et ACN cellules par 28, 18 et 10 pour cent respectivement. L'addition initiale d'$IsoB_2$ stimule la respiration dans tous les types de cellules; elle est surmontée par l'addition cumulative avec la cyanide dans les cellules N et ACN, mais pas dans les cellules AB4. On peut en conclure que divers degrés et divers types de l'adaptation physiologique peuvent s'appliquer aux cellules AB4 et ACN.

REFERENCES

Borst P. and Slater E. C. 1961: The site of action of 2,4-dinitrophenol on oxidative phosphorylation. Biochim. biophys. Acta, 48, 362–379.

Elliott A. M. 1949: A photoelectric colorimeter for estimating protozoan population densities Trans. Am. microsc. Soc., 68, 228–233.

Emerson G. A. and Tishler M. 1944: The antiriboflavin effect of isoriboflavin. Proc. Soc. exp. Biol. Med., 55, 184–185.

Hemker H. C. 1964: Inhibition of adenosine triphosphatase and respiration of rat liver mitochondria by dinitrophenols. Biochim. biophys. Acta, 81, 1–8.

Kidder G. W., Dewey V. C. and Heinrich M. R. 1954: The effect of non-ionic detergents on the growth of *Tetrahymena*. Expl. Cell Res., 7, 256–264.

Krebs H. A. 1935: Metabolism of amino acids. III. Deamination of amino acids. Biochem. J., 29, 1620–1644.

Lardy H. A., Johnson D. and McMurry W. C. 1958: Antibiotics as tools for metabolic studies. I. A survey of toxic antibiotics in respiratory phosphorylative and glycolytic systems. Archs Biochem. Biophys., 78, 587–597.

McCashland B. W. 1955 a: A study of the mechanism for cyanide in *Tetrahymena*. Ph. D. Thesis, University of Nebraska.

McCashland B. W. 1955 b: Adaptation by *Tetrahymena pyriformis* to potassium cyanide. I. Adaptive reversal of cyanide inhibition of growth. J. Protozool., 2, 97–100.

McCashland B. W. 1956: Adaptation by *Tetrahymena pyriformis* to potassium cyanide. II. Adaptation against respiratory inhibition. J. Protozool., 3, 131–135.

McCashland B. W. 1963: The nature of cyanide adaptation in *Tetrahymena pyriformis* W. Growth, 47–56.

McCashland B. W. and Steinacher R. H. 1962: Metabolism changes in *Tetrahymena pyriformis* W adapted to potassium cyanide (27924). Proc. Soc. exp. Biol. Med., 11, 789–793.

ACTA PROTOZOOLOGICA
VOL. IX WARSZAWA, 1.V.III.1971 FASC. 8

Laboratory of General Parasitology, Department of Parasitology, Polish Academy of Sciences, Warszawa 22, Pasteura 3

Stanisław L. KAZUBSKI

Some aspects of applying the photo method for investigating the morphology of ciliates

Niektóre aspekty zastosowania metody fotograficznej do badań nad morfologią orzęsków

In the course of intense research on the morphology of ciliates I came across upon numerous problems which I should like to discuss and also to present some suggestions of my own. Even a superficial review of the papers on morphology of ciliates shows that their photo material mainly illustrates the author's thesis or presents some specimens selected from his examined material.

Broad application of photograms as a direct research method in protozoology is rather seldom encountered, although this method is frequently used in other disciplines. An exception is the research on ultrastructures by means of electron microscope where the photograms are almost the only convenient materials. But it seems that the photograms may be widely used precisely in protozoology and particularly in research on the morphology of ciliates.

Morphological research faced the necessity of comparing in an objective way two or more specimens investigated. Of course, the best results may be obtained by means of simultaneous observation of all investigated specimens. This is impossible to be done when the object extends over the entire field of vision. Then more than one microscope should be used. I apply with success the photo method in my research on morphology of ciliates. It consists in elaborating the large series of photographs of specimens taken in the identical scale. This method permits a simultaneous comparison of some scores or even several hundreds of protozoans. Also it makes possible a detailed examination of the patterns of investigated organisms and all its variability.

A simultaneous application of a definite magnification scale (a photo picture should be in definite scale to the photographed specimens) simplifies matters.

In my work I only use magnifications 1000×, 2000× or 5000×. It has two good points. On the one hand it enables the measurement of random element of protozoans on the photos by means of an ordinary milimeter ruler without the use of any additional calculations. I only need to remember that 1 mm corresponds to 1 μ at the magnification 1000× and 2 mm and 5 mm corresponds to the same

argeness in the other magnifications respectively. On the other hand, the introduction of standard magnifications permits easily a maximal objective comparison of different species investigated at different periods of time.

The photos graduated in this manner may be obtained in a very simple way. At the beginning of a film one needs to take a picture of a micrometric measure and then in the course of copying magnify it to the required size. In addition, a permanent graduation of the film in metric (absolute) units is obtained. In my practice I use a Zeiss micrometric measure ("object micrometer") which contains a 1 mm section divided into 100 parts, 10 μ each. Then you have to match the small line obtained through the magnification with those drawn on the milimeter paper (which are in a distance of 10, 20 or 50 mm one from another) in order to obtain the photograph magnified by 1000×, 2000× or 5000×.

The photos may also serve a perfect basis for making drawing through their copying. The drawings through this technique are very precise and render faithfully all details of the original.

The methods mentioned in the present paper are particularly convenient for the investigations on ciliates impregnated with silver (especially impregnated with the Klein's method) when rather complicated pattern of cortical structures is revealed. This methods were mainly used by me in this investigations (Kazubski 1965, 1969, 1970, Kazubski and Migała 1968).

Summary

The photo method used by the author in his investigations on morphology of ciliates has been presented. It is based upon the comparative researches of photographs taken in the same definied scale. These photos are taken from the films firmly graduated by the photographing of micrometric measure.

STRESZCZENIE

Przedstawiono metodę fotograficzną stosowaną przez autora w jego badaniach nad morfologią orzęsków. Polega ona na porównawczych badaniach fotografii wykonanych w tej samej, określonej skali. Fotografie te są wykonywane z filmów trwale wyskalowanych przez fotografowanie linijki mikrometrycznej.

REFERENCES

Kazubski S. L. 1965: The development of skeletal elements in *Trichodina*. Progress in Protozoology, Abstr. Second int. Conf. Protozool., London 1965, Excerpta med, int. Congr. ser. 91, 1965, 221–222.

Kazubski S. L. 1969: Seasonal variability in ciliates and its consequences. Progress in Protozoology, Abstr. Third int. Congr. Protozool., Leningrad 1969, Nauka, Leningrad 1969, 369–370.

Kazubski S. L. 1970: Morphological variability of *Semitrichodina sphaeronuclea* (Lom, 1956). Acta Protozool., 8, 251–260.

Kazubski S. L. and Migała K. 1968: *Urceolariidae* from breeding carp — *Cyprinus carpio* L. in Żabieniec and remarks on the seasonal variability of trichodinids. Acta Protozool., 6, 137–160.

THE JOURNAL OF PROTOZOOLOGY

Volume 17

Number 1

Number 2

DEDICATED TO DR. RICHARD P. HALL, 1900–1969

Other Papers

PROTISTOLOGICA

Tome VI

Fascicule 1

Fascicule 2

ARCHIV FÜR PROTISTENKUDE

Band 112

Heft 4

BOOKS RECEIVED

Mario F. CANELLA. **Sur les organelles ciliaires de l'appareil buccal de hyménostomes et autres ciliés. Révision critique de questions controversées de morpho-physiologie, stomatogenèse, phylogenèse et taxonomie.** (Avec 24 figures dans le texte).

Annali dell'Università di Ferrara (Nuova Serie). Sezione III — Biologia Animale. Supplemento al volume III. 1970, pp. 235.

Avant propos. Iconographie de l'appareil oral des Ophryoglènes (*Hymenostomatida, Ophryoglenina*). Qu'est-ce qu'un peniculus selon Gelei? Le péniculus et le « quadrulus aberrant » d'un Cilié pan-tropical: *Neobursaridium gigas*. Un cas troublant: le gros et unique «péniculus» de *Frontoniella marina* Roque. Subtilités byzantines sur les soi-disant « péniculi » de trois espèces de *Lembadion*. — L'imaginaire « retour à la stomatogenèse somatique » de *Turaniella* et le prétendu pouvoir morphogène des cinéties. Les structures buccales et les soidisant « péniculi » de *Frontonia, Disematostoma* et *Stokesia*. Ce qu'on a imaginé sur l'origine des *Peniculina* (avec ou sans péniculi) et sur le rôle joué par *Clathrostoma*. Une innovation sub iudice: les *Scuticociliatida* et leurs « membranoïdes 2. Des « semi-péniculi » aux organelles adoraux à une seule rangée de cils. Les structures subsidiaires de l'apareil buccal des *Loxocephalidae* et les notions de « polymérisation » et de « auxomorphisme ». Les organelles buccaux des Thigmotriches *Arhynchodina*: multiplicité de patterns, connaissances insuffisantes, généralisations arbitraires. Aussi les *Astomatida* sont-ils des Thigmotriches ? Thèses diamétralement opposées sur les relations phylétiques et la position taxonomique des *Rhynchodina*. Comment doit-on envisager les organelles buccaux des Péritriches. Panorama des spéculations phylogénétiques sur les Péritriches. Ce que nous apprennent les micrographes électroniques. Appendice. À propos de *Turaniella vitrea*. Des éclaircissement à propos de *Peniculistoma (Conchophthirus, Morgania, Kidderia) mytili*. Les *Entodiniomorphida* sont-ils des Holotriches ? Surprenantes observations sur la morphologie et la morphogenèse des structures buccales de *Loxodes*. On n'a pas encore démontré que *Faurella thermalis* appartient aux *Parameciidae*. Le « pentalogue » des Ciliatologistes formulé par Corliss. Quelques extraits des Principles of Protistology de Dobell. Bibliographie citée.

Karl G. GRELL., **Protozoologie.** Zweite Auflage. Springer-Verlag, Berlin-Heidelberg-New York. Mit 422 Abbildungen. VIII, 511 Seiten Gr. — 8°. 1968 Gebunden DM 98,—, US 27.00. Titel-Nr.: 0330.

Einleitung. Abgrenzung und Begriff. Morphologie: Das Cytoplasma (Das Grundplasma: Die Strukturen). Die Zellhülle. Der Zellkern (Ruhekern und Chromosomen, Kernteilung, Kerndualismus und Polygenomie). Fortpflanzung: Zweiteilung. Vielteilung. Knospung. Befruchtung und Sexalität: Gametogamie. Autogamie (Gamontogamie mit Gametenbildung ohne Gametenbildung, Conjugation). Rückblick. Generationswechsel. Vererbung: Mutabilität. Kreuzungsversuche (Haplonten, Diplonten). Modifikabilität und Zellvererbung. Bewegung: Ortsveränderung (Pseudopodien Geisseln une Wimpern, Fehlen von Bewegungsorganellen). Gestaltveränderung. Verhalten. Ernährung (Permeation, Pinocytose, Phagocytose). Parasitismus und Symbiose: Protozoen als Parasiten und Symbionten von Protozoen. Formenübersicht: Klasse: *Flagellata* (Ordnung: *Chrysomonadina Cryptomonadina, Phytomonadina, Euglenoidina, Dinoflagellata, Protomonadina, Diplomonadina, Polymastigina, Opalinina*). Klasse: *Rhizopoda* (Ordnung: *Amoebina, Testacea, Foraminifera, Heliozoa, Radiolaria*). Klasse: *Sporozoa* (Ordnung: *Gregarinida, Coccidia*). Klasse: *Ciliata* (Ordnung: *Holotricha, Peritricha, Spirotricha, Chonotricha, Suctoria*). Anhang: *Cnidosporidia*. Veröffentlichungen: Zusammenfassende Darstellungen. Einzelarbeiten und Werke aus Nachbargebieten Filme. Sachverzeichnis. Gattungen und Arten.

DREWS G. **Mikrobiologisches Praktikum fur Naturwissenschaftler.** Springer-Verlag, Berlin–Heidelberg–New York. Mit 51 Abbildungen. VIII, 214 Seiten Gr.-8°. 1968. Geheftet DM 14.80, US 4.10. Titel-Nr.: 1535.

Heidelberger Taschenbucher, Band 84. REHM H.-J. **Einfuhrung in die industrielle Mikrobiologie.** Mit 96 Abb. XII, 241 Seiten. 1971. Berlin–Heidelberg–New York: Springer-Verlag ISBN: 3-540-05157-0

Fasciculi praeparati:

Z. Raabe: Ordo *Thigmotricha (Ciliata — Holotricha)*. IV. Familia *Thigmotrichidae* — M. Wolska: Studies on the family *Blepharocorythidae* Hsiung. VI. Phylogenesis of the family and the description of the new genus *Circodinium* gen. n. with the species *C. minimum* (Gassovsky, 1918) [Badania nad rodziną *Blepharocorythidae* Hsiung. VI. Filogeneza rodziny i utworzenie nowego rodzaju *Circodinium* gen. n. z gatunkiem *C. minimum* (Gassovsky, 1918)] — M. A. Khan: Ultrastructure of *Ancistrumina nucellae* Khan, an arhynchodine thigmotrichid ciliate [Ultrastructure de *Ancistrumina nucellae* Khan, un Ciliate arhynchodine thigmotrichide] — H. Tamar: *Mesodinium fimbriatum* Stokes, 1887, a ciliate with bifurcated and barbed cirri [*Mesodinium fimbriatum* Stokes, 1887 ein Ciliat mit gezweigten Borsten] — M. A. Musajev and S. G. Ismailov: Endogenous stages of the life cycle of *Eimeria schamchorica* Musajev et Alijeva, 1961 (*Sporozoa, Coccidia*) the parasite of *Meriones erythrourus* Gray [[Эндогенные стадии жизненного цикла *Eimeria schamchorica* Musajev et Alijeva 1961 (*Sporozoa, Coccidia*). Парасита краснохвостовой песчаники] — D. Pietrowicz-Kosmynka: Chemotactic effects of cations and of pH on *Stentor coeruleus* [Wrażliwość chemotaktyczna orzęska *Stentor coeruleus*]

SUBSCRIPTION

price is $ 12 for one volume consisting of four parts appearing quarterly
Place your order with your bookseller or directly with:

Export and Import Enterprise „RUCH"
Warszawa, Wronia 23, Poland

Cable: Exprimruch, Warszawa
Bank Account: Bank Handlowy S.A. Warszawa

In the East-European countries the subscription orders are to be placed with the local agencies for press distribution.

Państwowe Wydawnictwo Naukowe
(PWN — Polish Scientific Publishers)
Warszawa

Warszawska Drukarnia Naukowa, Warszawa, ul. Śniadeckich 8. Zam. 686/70
Wklejki światłodrukowe wykonały Zakłady Graficzne „Ruch", Warszawa, ul. Ludna 4

Cena zł 35,—

Fasciculi: