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Remarks on the principles and outline of the system of *Protozoa*

Uwagi o podstawach i zarysie systemu *Protozoa*

Recent years brought again many general discussions on the system of *Protozoa* — this distinct subregnum of the animal kingdom, mostly considered also as one of its types. No doubt, those generalizations were stimulated by the progress of protozoology in those years as well as by publication of the magnificent volume of Grassé's work treating of *Protozoa* yet with a non-rigorous approach to the system. The tentative classification worked out mostly by the American authors and presented on the International Conference of Protozoologists in Praha 1961, the discussion of Corliss 1961 and at last the recent article of Cheissin and Poljansky 1963 — tend towards the construction of a system possibly approaching the natural.

I take opportunity to share in this discussion, having in view not so much the revision of the system or alteration of its details, as the reconsideration of the general problems concerning evolution, in conclusion the *Protozoa* classification, and finally — the rank of this subregnum and of its subordinate groups (types, classes) as related to other superior taxonomic unities of the animal world.

The rank of the *Protozoa* taxon and of subordinate groups

The division of the animal kingdom into two subregna: *Protozoa* and *Metazoa* evokes in general no doubts and objections. It is generally assumed that those groups are distinctly separated from each other and that their evolution has been effected within them in a similar manner but along different paths. The evolution of *Metazoa* is characterized by the histological differentiation i.e. differentiation of many cells into ensembles: tissues, organs, systems, performing different functions and assuming various features. The evolution of *Protozoa* is characterized by the cytological differentiation i.e. differentiation of a really one cell of their body, by forming its parts and organelles performing different functions and having a different features. Those both forms of evolutionary differentiation run in some way parallelly to each other; organelles are analogues (although not homologues) of the organs. The living matter follows similar paths of differentiation and integration undepending on its division into cells or on lack of it (Raabe 1954).

However, in spite of their sometimes high organization, *Protozoa* being full independant organisms, do not cease being cells, the more so when we consider the different forms of *Metazoa* cells. *Protozoa* are cells since their structure — notwithstanding their secondary differentiations — corresponds to the cell structure. Simultaneously *Protozoa* are indepedent, full-potential organisms, with their own development cycles and own history of evolution. This position of remaining only a cell and becoming an organism is determining the evolution of *Protozoa*: formation by them specialized parts of the cell — organelles — differentiating gradually more and more, multiplying and simultaneously gradually integrating the organism.

This variety (and in some way the parallelism) of *Protozoa* and *Metazoa* development indicate their equal position in the system of the animal kingdom; equal as well in the taxonomic meaning as in the phylogenetic, since we cannot assume that *Protozoa* are organisms remaining „at foot of the animal genealogical tree" as Haeckel wished. In their evolution from the with *Metazoa* common ancestors, *Protozoa* reached not less far although along different paths — more so — not along one path but along numerous diverging tracks.

Recognizing the taxons of *Protozoa* and *Metazoa* for equivalent, the great majority of zoologists and even protozoologists assume the uniformity of *Protozoa* on the rank of type (phylum, cladus), consequently distinguishing in the subregnum *Protozoa* only one phylum of the same name, whereas *Metazoa* are usually divided into numerous phyla. No doubt, the perception of essential differences between organisms and qualifying them to separate systematic groups of a high rank, initiated in the history of human knowledge from organisms bigger and closer related by their structure to the animals well known to man and also to himself. After all, in the last century more distant organisms which at first were placed in a „common bag" were individualized. Distinct phyla arose, comprising few forms and occupying positions between the phyla known before; the former „collective" phyla were desintegrated into separate unities, indicating even their distant mutual position (e.g. the former type „*Vermes*" or even the more narrowed type „*Scolecida*"). In fact — the phyla formed in this way are not equivalent as to their content, i.e. as to the number of forms included into them, and to the number or gradation of their subordinate groups. Yet they are — at least this is the zoologist's tendency — equivalent as to the validity of their distinctive characters; e.g. the phylum *Chaetognata* although not numerous is equivalent to *Echinodermata* or *Chordata*.

In contrast to this, *Protozoa* still remain a single phylum in the concepts of taxonomists as if their subordinate groups were not distinguished by sufficiently deep and valid differences!

The practical taxonomic consequence of accepting the uniformity (or rather not accepting or not paying attention to the differences among *Protozoa*), is the difficulty of forming their system and ascertaining the adequate gradation of their superior and subordinate taxons. Whether the author proceeds from below upwards — i.e. after having stated genera, families and orders tries to embrace them into classes and superior unities — or if he proceeds downwards — i.e. after having divided *Protozoa* into subphyla and classes tries to include into them orders and families — in both cases he finds that he is „lacking" ranks of taxons. This is the reason of introducing all super- or sub-

taxons, categories uniting some of them and differentiating the others. Such a procedure introduces an unusual confusion!

It should be agreed that *Protozoa* are a highly differentiated group, differentiated in extension as well as in depth, i.e. comprising organisms which evolution proceeded in various directions and in each direction reached different distances; it underwent different and variably numerous aromorphoses. It should be expected that the eventual phyla among *Protozoa* would embrace not the organisms being on a same degree of development or proceeding in their evolution in a same direction — but rather „tufts" or „bundles" of evolutionary paths, possibly nearing one another, possibly of a common source, but somewhat diverging and not equally advanced.

Besides, it should be postulated that the morpho-physiological differences between the organisms united into those distinct types would be at least of the same rank as the differences separating the types of *Metazoa*. Of course, this would be possible only approximately because — no doubt — even among *Metazoa* the boundaries and differences e.g. between *Annelida* and *Arthropoda* are much less significant than e.g. between *Echinodermata* and *Chordata*, considering just those related phyla.

Tendency to distinguish several types (phyla, cladus) in *Protozoa* is not new. Lameere 1932 separated 8 groups of a cladus rank: *Flagellata, Infusoria, Mycetozoa, Sporozoa, Amoebina, Foraminifera, Radiolaria* and *Axopoda*. Raabe 1948 suggested recognition of 3 types: *Mastigiae, Sarcodina* and *Ciliata*. Grassé and co-workers distinguished 5 subphyla: *Rhizoflagellata, Actinopoda, Sporozoa, Cnidosporidia* and *Ciliata* (p. 39, 129), including numerous classes. Finally, Wurmbach 1962 distinguished as many as 6 „Kreise" of an over-class rank, and namely: *Flagellata, Sarcodina, Telosporidia, Cnidosporidia, Sarcosporidia* and *Ciliata*.

In all the above classifications *Ciliata*, respectively *Ciliophora*, occupy a separate position, which is expressed in the division of *Protozoa* into *Cytomorpha* and *Cytoidea* (Hatschek 1911), into *Plasmodroma* and *Ciliophora* (Doflein 1902), or into *Homocaryota* (Biocca 1956) and *Heterocaryota* (Piekarski 1954). In all cases, *Cytoidea, Ciliophora* or *Heterocaryota* coincide exactly with the term *Ciliata* (*Opalinata* excluded and *Suctoria* included), if we contrast with them all other *Protozoa*. Besides, any clear connections between *Ciliata* and other *Protozoa* fail to exist, especially since *Opalinata* has been finally excluded of them. So the distinctness of *Ciliata* and possibility recognizing them as a separate type would not be objected even by the most arduous adherent of the types theory of the passed century. For the above reasons I support my former view of considering *Ciliophora* (= *Ciliata*) as a separate phylum of the animal world.

There remains still open for discussion the problem of another group of *Protozoa*: organisms defined by the names *Plasmodroma, Cytomorpha* or *Homocaryota*, and in this way opposed to *Ciliata*. Many authors (recently Cheissin and Poljansky 1963) treated all that group as a single subphylum. Grassé 1952 divided them into 4 separate subphyla: *Rhizoflagellata, Actinopoda, Sporozoa* and *Cnidosporidia* ascribing in this way a rank over subphylum to the whole group.

I fully agree with the authors who believe that *Rhizopoda* and *Flagellata* are bound by so many morphological, cytological and developmental elements that it is not possible to find a strict limit between them. Nevertheless, in my

opinion this may rather concern more primitive forms with flagella, yet keeping (or gaining?) the ability of producing pseudopodia. As to the less primitive forms, it may be easily decided whether they represent rather the *Rhizopoda* type or that of *Flagellata*. In my opinion, producing such flagelloidal forms as gametes and especially microgametes — cannot prove a direct connection with *Flagellata* since this type of microgametes occurs also in very remote groups of the plant and animal world. In some *Rhizopoda* flagelloidal trophic forms appear as well as — in some *Flagellata* — the amoeboidal stages. Only those forms may evoke doubts.

Generally, among *Plasmodroma* two distinct groups are evident, their evolution tending in different directions although no doubt being initiated from common ancestors.

One group, or rather a bundle of groups, are organisms of a plastic body able to produce pseudopodia of different kind, often producing skeletal elements (exterior or interior), organic or mineral. Those groups are: *Amoebozoa, Foraminifera, Radiolaria, Heliozoa,* evidently modified *Cnidosporidia* and some smaller groups. For this branched group I suggest keeping the name *Sarcodina* and treating it as a phylum of the animal world belonging to the subregnum *Protozoa.*

The second group, or rather several groups again — are organisms of a body well defined, able to produce and keep undulipodia, i.e. one pair or more of flagella (or cilia). Those groups are the eutrophic *Phytomastigina* and the heterotrophic *Zoomastigina* (together with *Opalinata*) of a very various type of flagellar apparatus and the parasitic *Sporozoa* (= *Telosporidia*). For this group, more homogenous, I suggest the new name *Mastigota*, n. nov.[1] treating it also as a phylum of the subregnum *Protozoa.*

In this way I postulate to recognize *Protozoa* as a subregnum of the animal world, highly differentiated and embracing 3 groups of rank of types (phylum, cladus), namely: *Mastigota, Sarcodina,* and *Ciliata.* It seems desirable to discuss those essential and deep evolutionary trends and their resultants which separated those three phyla and gave them this high rank.

Morphophysiological polymerization in *Protozoa*

Without regard which group, or rather which primitive forms of such a group, we recognize as really primitive forms of the divergent development of *Protozoa,* and independently from the number of phyla distinguished in *Protozoa,* different degree of morphological organization and of differentiation of the cell-body will be found in many *Protozoa* groups. Nearly in any of the distinguished groups, more primitive forms may be indicated, usually of a simple structure (although the simple structure may also be result of a secondary regression) and some forms more advanced in their evolutionary development. In every group this primitivity or advancement may be proved by different criteria. The most distinct transformations seem to be those which may be defined by the term of morphophysiological polymerization, leading often to a seemingly unexpected result — to a stronger integration of the organism. This evolutionary path — through polymerization and inte-

[1] I want to change the name „*Mastigiae* Chatton", used by me previously (Raabe 1948), as in my significance and extent it does not correspond to those given by Chatton 1925.

gration — seems to be a „beaten" and very essential track as well in *Protozoa* as in *Metazoa* (D o g i e l 1928).

Among *Protozoa* the most distinct evolutionary paths in this respect are those which concern the complication of the nuclear and undulipodial apparatus.

As to the problem of differentiation of the nuclear apparatus I wish to refer to the former theory of H a r t m a n n 1911 who, following S a c h s, introduced a very fortunate idea of energide for determination of nucleus (haplo- or polyploidal) and of the subordinate to it (or at any rate connected with it) territory of cytoplasm. Like the majority of cells of the *Metazoa* body, the majority of *Protozoa* are monoenergides in which the whole territory of cytoplasm is bound to one nucleus — in trophozoits haploidal or diploidal, in the zygote always diploidal, in gametes always haploidal. The form of monoenergide is dominating in the majority of *Mastigiae* and *Rhizopoda* and is accompanied by a comparatively insignificant complication of the organism.

Yet very numerous *Protozoa* remain single cells coated by cytolemma or pellicle, however, they contain not one but several or even a higher number of equal (or sometimes unequal) nuclei, of a haplo- or diploidal character. Such organisms are among *Flagellata: Diplomonadina* (*Lamblia* etc.), *Polymastigina, Opalinata,* numerous *Amoebozoa, Foraminifera,* some *Heliozoa,* plasmodia of *Myxosporidia.* They are organisms corresponding in the structure of their nuclear apparatus to the scheme of the polyenergide.

Those two schemes — monoenergide and polyenergide — are not sufficient to embrace all the forms of complication of the nuclear apparatus in the cell body of the protozoon. The type represented by *Ciliata* with their differentiated and highly complex nuclear apparatus, escapes from this pattern. Ciliates are characterized not only by the nuclear dualism but also by the fact that macronucleus is really a polyploidal structure and in many cases even highly polyploidal. Nevertheless single genoms in Ma are not lying side by side but form a compact combination, in which mitotic processes are masked and manifest sometimes as endomitosis. In some forms, more primitive in regard to their structure and evolutionary advancement of their nuclear apparatus (*Loxodes, Trachelocerca* — R a i k o v 1957) the macronuclei have no ability of division but arise de novo derivating of the micronuclei in any division of the individual; in more advanced forms, they are able to division. During the conjugation and after its completion, the nuclear apparatus undergoes far reaching transformations, reproducing itself really from the synkaryon, with subsequent differentiation and complication.

The nuclear apparatus of *Ciliata* presents in this way not the sum of single diploidal nuclei but their complex and differentiated system. I suggest the term h y p e r e n e r g i d e for determination of such a system of the nuclear apparatus in the cell-organism.

In consequence, the evolutionary complications among *Protozoa* concerning the system of nuclear — or rather plasmo-nuclear apparatus would consist in the transformation of the, no doubt, primitive form of monoenergide either towards the polyenergide by polymerization (in *Plasmodroma*), or towards hyperenergide by polymerization and integration (in *Ciliata*).

As to the problem of complication of the motor system, it seems right to take advantage of the highly fortunate and correct approach of C h a t t o n 1931 in his theory of kinetide and, before all, to refer to his theories of the

development of the kinetide in *Flagellata*. From the primitive form of kinetide (monokinetide with a single flagellar apparatus consisting of one or several flagella with basal corpuscles and one blepharoplast) Chatton traces the origin of organisms with two or many kinetides of this type (consequently polykinetides), or also of organisms with a compact complex of kinetides (the hyperkinetides).

To the monokinetides belong the lower *Flagellata*: besides *Phytomastigina*, also *Protomonadina*, *Choanoflagellata* and the more primitive *Polymastigina* (e.g. *Trichomonadina*), as well as the flagellate stages of *Rhizomastigina* and the flagellate gametes of other *Plasmodroma*. They correspond either to the form of protokinetide or mesokinetide or metakinetide (with the lost flagellum as e.g. the parasitic form of *Leishmania*) — in the Chatton's scheme.

The form of polykinetide is represented classically by the higher *Flagellata* as *Diplomonadina* (diplokinetides) and other *Polymastigina*; temporarily this form is represented by the division stages of more primitive *Flagellata* so that it may be assumed that occurrence of e.g. *Diplomonadina* is a fixation of a division phase of some *Trichomonadina* (fixation of phases — Sewertzoff 1931).

The form of hyperkinetide is represented by several groups: by *Hypermastigina* with their integrated undulipodia, complex and highly developed common parabasal apparatus (sensu Janicki 1915), and also by *Opalinata* and, at last, by *Ciliata* with an advancing, although expressed in different ways, integration of the ciliary apparatus.

In the *Metazoa* tissues, spermatozoa and cells of the flagellate epithelia are monokinetides, cells of ciliary epithelia — hyperkinetides. According to the concept of Chatton, the majority of *Metazoa* cells followed the line of reduction of the ciliary apparatus in their development, i. e. following the line: mesokinetide, metakinetide, and in the plant cells rather akinetide. Leaving aside the correctness of Chatton's generalizations it should be stressed that this line of development is characteristic for organisms included here to *Sarcodina*.

Consequently the evolutionary complications among *Protozoa* concerning the undulipodial motor system, would consist in the transformation of the primitive monokinetide form either towards the polykinetide by means of polymerization, or towards the hyperkinetide by means of polymerization and integration, or at last by the reduction of the kinetide.

An interesting image presents the comparison of those two groups of evolutionary tendencies concerning the nuclear and motor apparatus. *Protomonadina* are monoenergides-monokinetides, *Polymastigina* are polyenergides-polykinetides, besides, the coincident multiplication (antimerization) of the whole karyomastigont (Janicki 1915) is here occurring. *Hypermastigina* are monoenergides-hyperkinetides, *Opalinata* are polyenergides-hyperkinetides, and at last, *Ciliata* should be looked upon as hyperenergides-hyperkinetides. *Protozoa* without undulipodia (metakinetides) are monoenergides (the majority of *Amoebozoa*) or polyenergides (*Foraminifera*, *Pelomyxa*, plasmodia of *Myxosporidia* etc.).

In both evolutionary processes discussed here, occurs a plain coincidence of paths: polymerization of organelles and later on (or simultaneously with it) their integration accompanied by differentiation of components. This process

of integration cannot be thoroughly identified with oligomerization of the organs so extensively discussed by Dogiel 1947, being their reduction or diminution of their number. A multiplied and integrated organ presents a qualitatively different structure than a single or only multiplied one.

Besides those two tracks of the *Protozoa* evolution, some others paths of polymerization and integration, may be pointed out concerning other organelles and occurring not so commonly. They are manifesting in different manner in various groups and concern different systems. The following examples may be given:

Polymerization of osmoregulative-excretory organs, i. e. contractile vacuoles (e. g. in *Ciliata: Astomata, Entodiniomorpha*), and their integration into contractile canals.

Polymerization of skeletal structures: shells of *Foraminifera,* skeletal elements of *Radiolaria* and *Acantharia,* skeletal lamellae of *Ophryoscolecidae* (Dogiel 1929, 1951).

Polymerization of digestion organs — vacuoles in many groups and their integration into digestion zones in *Peritricha* or endoplasmatic sacks in *Entodiniomorpha,* etc.

Polymerization of the elements of the ambihymenial system (Raabe 1963), and — before all — of the AZM in *Ciliata* (especially in *Spirotricha*) as well as the polymerization of entire zones of membranellae in *Entodiniomorpha* (as to the state in *Elephantophilus* Kofoid 1935).

In all those respects, the course of development may be not uniform and not parallel, similarly as it was demonstrated in the case of multiform mutual relations of the nuclear and undulipodial systems. For instance the groups of a more primitive form of the food ingesting system (the plesiomorphic feature) may show a high development of the skeletal system (the apomorphic feature). The more so the comparison of those different parameters contributes to a high differentiation of groups, and to characterization of the evolutive tendencies within them, and also to a possibly adequate construction of their system, approaching the natural one and illustrating the evolution of *Protozoa.*

Concept of „somatization" in *Protozoa*

It was ascertained above that the evolutionary development of *Protozoa* followed the direction of cytological differentiation of single cell, representing their body, and consisted essentially in differentiation of this cell-plasm into elements specializing in performing definite functions and assuming definite features. This way of differentiation and integration of different parts resulted in changes in various organelles and systems. Those changes were interlinking and combining in different ways and in different degree.

Nevertheless, in the evolution of *Protozoa* tendencies are observable reaching beyond the limits of this cytological differentiation and causing objection in recognizing the protozoon as a single cell, even of a polyenergide or hyperenergide character. Hence the morphology of *Protozoa* (before all *Ciliata*) is treated sometimes as histology and not cytology. Often the attention is called to the fact that some development stages of some *Protozoa* should be looked upon as multicellular organisms, at least as some *„Mesozoa"* (*Myxosporidia, Actinomyxidia*).

In the fundamental evolutionary processes, which led to development of forms not typically unicellular, lies, in my opinion, the tendency of differentiation of the generative elements from the somatic, similar to that which — among others — influenced the evolution of *Metazoa*.

This tendency, this evolutionary phenomenon, I suggest to call by the term somatization, i. e. by one term, although I realize that its essence and effects use to be different and seamingly have little of common.

In what form can I imagine this somatization?

I assume (and possibly I am not isolated in this view) that the primitive protozoan organisms of the flagellate rank of structure, reproducted by division without sexual processes. The division was probably mitotic ensuring the correct transmission of the genom. The sexual process was initiated by union of such two organisms into a zygote being the only diploidal stage of their development which after a shorter or longer time produced next organisms by means of division accompanied by meiosis (postgamic meiosis). In this step of development the trophic individuals equaled in their chromosomal structure the gametes and were haplonts. Some *Flagellata* (e. g. *Chlamydomonas* and many others), remain on this step of development till now. A similar phenomenon occurs also in *Sporozoa*. It might be presumed further that the zygote period became prolonged for some reasons; the presumably more vital diploidal phase gradually protracted. Organisms in this phase had a definite mode of life, nourished and became the vegetative stages of development. The transitory diploidal phase of the zygote was stabilizing and prolonging — according to the law of the fixation of phases (Sewertzoff 1931). Finally such a diploidal trophic organism gained the ability of division by mitosis, consequently the ability of asexual reproduction, depriving the haploidal (sexual) organisms of this capacity. A segregation took place: the trophic individual became diplont, and yet its division connected with meiosis results in haploid gametes (the progamic meiosis) which after copulation form the zygote dividing or developing into diploidal trophic individuals.

Presumably — at first commonly, and now in a great number of cases — the trophic organism transformed into a gamont divided entirely producing gametes; its whole living matter was exhausted for their production. The same occurred in the vegetative division; there were no remaining organized elements, aging or necrotic.

And namely here, on the top of the evolutionary branches of different groups of *Protozoa* apper the trophic forms or gamonts not entirely, and not without remnant, used for production of the subsequent forms or the next generation. Here, besides the transmittable generative substance, some structures appear coating only that what is hereditary and transmittable. They fail to continue from generation to generation, or they propagate themselves apart from the hereditary elements.

Such a differentiation of the substance into the vegetative and generative is mostly accompanied by the differentiation of nuclei into vegetative and generative ones and finally by the differentiation of energides or cells. A certain differentiation of nuclei may be found in some *Foraminifera* (*Rotaliella* — Grell 1957); it occurs and manifests in the processes of agametes formation in which always the generative nuclei take part. This phenomenon is manifested much more plainly in *Myxosporidia* where it occurs in two stages: in the differentiation of nuclei (energides) into the somatic and generative (giving

sporoblasts) and in the differentiation of cells in sporogenesis into the vegetative cells of the envelope and of the polar capsules, and into the generative energides of the planont (sporoplasm). In those both phases of development the mortal soma appears besides the germinative element continuing the life of the species. This is the reason of the frequent stressing the similitude of *Myxosporidia* or *Actinomyxidia* to *Metazoa* (G r e l l 1956, G r a s s é 1960). The similitude is involved namely by somatization conforming the protozoan organism and its life cycles to those of *Metazoa*.

Besides, it seems possible that the acceptation, developing and extending of the somatization concept in *Protozoa* would also help to elucidate the derivation and the phylogenetic interrelations of *Orthonectidae* and *Dicyemidae* which cannot find their right place in the system.

Similar signs of somatization can — as it seems — be detected in *Gregarinida*, manifested as well in the development of their trophozoits as in the processes occurring among syzygites and — before all — in the process of gametes and spores formation in which not the entire gamont body is used. A certain „reliquat somatique" (C h a t t o n 1929, G r a s s é 1962) remains with hypertrophic nuclei and no chromocentres.

It is not clear to me whether somatization may be suspected to occur in the development of the body of the higher multinuclear *Opalinata* as compared to their less complex binucleate forms; here also the trophic individual is especially well developed and the processes of the palintomic division, as yet not sufficiently investigated, show many specifities not consistent with the general schemes.

In all those cases, somatization is — as it seems — a character of groups which are assumed as very highly specialized, as the last links of developmental ranges, as summits of the phylogenetic branches...

Quite different form of somatization occurs in *Ciliata*. Here besides the generative nucleus — micronucleus, the vegetative polyploidal macronucleus arises. In some processes, even in division, the generative nucleus may not participate (amicronucleate races) but its presence and activity is conditionning the sexual processes. In the vegetative processes the role of macronucleus seems to be undeniable but some genetic properties of it cannot be overlooked. Numerous vital processes — before all the very complex morphogenetic processes in the cortical system — prove to be high autonomic as well in the division periods as in regeneration. The cortical system is differentiating in a high degree and simultaneously is integrating, especially in the more specialized groups (D o b r z a ń s k a - K a c z a n o w s k a 1963). That is why *Ciliata* fail to conform to the general scheme of the cell and the methods of investigating them are looked upon as rather histological than cytological.

The somatization of *Ciliata* is manifesting most strikingly in the sexual processes — in conjugation. The conjugant is not a gamete, it is a hermaphroditic gamont, producing one gamete of each sex: the masculin migrating pronucleus (sometimes it is evident that it represents a gamete and not a naked nucleus — D o g i e l 1924) and the feminine stationary pronucleus. In the soma of each conjugant there remains (except pronuclei) merely the dispersed chromatin substance of Ma and of the three quarters of the divided Mi. The exchange of pronuclei is a process which might be confronted with the insemination in *Metazoa;* copulation, even typical, is the fusion of both gametes — pronuclei — into the synkaryon or rather the zygote. This zygote containing

the new set 2n of chromosomes (half derivating of each partner) is falling after the copulation in the soma of the post-conjugant deprived of an organized chromosomal apparatus, and is subordinating it to itself in the process of reorganization in one or in several divisions. This subordination of the soma by the new genom is proved namely by those reorganization processes as e. g. formation of a new crown of hooks in the post-conjugant of *Trichodina* sp. sp. (Davis 1947). Soma is not lost — its substance is used, although after being reorganized.

A very characteristic feature of organisms which underwent the somatization process, as discussed above, is that really only in their case we meet morphogenesis, even in its epigenetic meaning. In *Flagellata* or *Rhizopoda* the transmission of halfs of the reorganized organelles to the progeny occurs; the division concerns the whole organism, all the systems, and the daughter individuals receive preformed all which is their share. With the progress of somatization, some systems are differentiating and integrating simultaneously, so that they cannot divide exactly and with no remnants. Then, as it occurs in *Ciliata* (or at any rate in higher *Ciliata*) only the primordia of daughter structures are transmitted; sometimes even no organized primordia are transmitted and the progeny systems arise de novo from the undifferentiated material (cortical in the case of the kineties systems).

The processes of new-formation, i. e. the epigenetic morphogenesis become mostly pronounced where somatization is most advanced, in *Ciliata*, in the case of modified sedentary forms as *Suctoria* or *Chonotricha*. In those forms not only the arising anew of organs and systems is plainly manifested (Kormos 1957, Dobrzańska-Kaczanowska 1963), but also some other phenomena connected with the somatization, peculiar rather to *Metazoa*. This phenomenon consists in the fact that the sedentary individual is exhausting not all the substance of his body at once for the production of its buds — tomits, as it is in division, but it uses its soma gradually and at last the remnant, not transmitted to the progeny, dies (Rudzińska 1961). The last consequence of somatization — the death of soma with formation of the necron occurs. A similar phenomenon occurs also in *Myxosporidia:* death of the exhausted plasmodium with vegetative nuclei and death of the coating of the planont (envelope of the spore and the capsulae polares). Similar phenomena are found also in *Gregarinida* (reliquat somatique) and probably in other specialized groups.

The general paths of the phylogenesis of *Protozoa*

Two fundamental problems of the discussion upon the phylogenesis of *Protozoa* (as well as of all the animal world) had been extensively discussed — although not recently — and I intend merely to recall them since they are not essential to my subsequent considerations.

The first problem is how should be the hypothetical ancestor of *Protozoa* imagined: either as amoeboid being of rank of cytode similar to the — no less hypothetical — Haeckel's monerae, or as a flagellate form. The primitivity of feature speaks in favour of the first hypothesis, the second concept is supported by the fact that the flagellate stage occurs in the virtually amoeboid organisms either as a vegetative form or as a gamete. The first hypothesis

involves in consequence the concept of a much later arising of mastigium and a later fission of the organized world into the animal and plant kingdoms. The second hypothesis would interfere with the position of *Amoebina* which are, as it seems, primitive and have no sexual process.

The second problem concerns the autotrophy and heterotrophy of organisms: which of those two characters is primary or secondary. In this matter the views of primitivity of heterotrophs seem to dominate now; the living forms used at first the unorganized organic substance from which they arose. Autotrophy wauld be a secondary phenomenon connected with the formation of chloroplasts, structures of a mysterious origin, difficult to elucidate (the concept of symbiosis?).

For our further considerations it would be enough to imagine an „Archiprotozoon" — a unicellular and mononucleated organism, with the ability of forming pseudopodia and also some more stable motor organelles — undulipodia helved on the basal body. It seems adequate to imagine several developmental paths running in different directions and initiating from such hypothetical forms. Several of those development paths would certainly run towards the formation of multicellular organisms, i. e. towards the histological differentiation, producing in results *Spongiaria* and *Coelenterata* and still other *Metazoa*. Other groups after having acquired autotrophy gave origin to plants in a similar way. At last, still other paths followed the direction of cytological differentiation, modified by convergence or divergence in the complicating of the nuclear apparatus as well as of the undulipodial one and of other apparatus and systems (as the skeletal, alimentary, excretory-osmoregulating organs). This process of complicating resulted in somatization expressed in very different ways.

Those groups, although their development ran in numerous directions, may be — as already suggested — divided or systematized into three fundamental categories taking into account that the ways of their evolution present distinct „bundles".

Paths leading towards *Mastigota* are characterized by intensification of the kinetide — either by its formation of several flagella or by the multiplication of its flagella sets and formation of the polykinetide (*Polymastigina*) or — at last — by complication of this system and formation of the hyperkinetide (*Hypermastigina, Opalinata*). Only in some cases, mainly in parasitic organisms, occurs the reduction of the kinetide to metakinetide (e. g. *Leishmania*) or a complete loss of flagella (e. g. in *Sporozoa*). Complications of the nuclear apparatus consist in its multiplication, often regular, together with the multiplication of kinetides (multiplication of the karyomastigont), which occurs in *Polymastigina*, or it is effected without co-ordination with the system of undulipodia, as in *Opalinata*. In *Mastigota* a tendency to the colony formation occurs (*Phytomonadina, Choanoflagellata*) which may also be recognized as a manifestation of somatization, the more so as the colonies are mostly formed by species of a more simple structure of individuals. Elements of somatization may be found out in *Gregarinida, Opalinata*, as mentioned before, as well as in *Hypermastigina* (integration of the parabasal apparatus). In *Mastigota* paths leading towards the autotrophic *Flagellata* are distinguishing and thence — as it seems — deviations towards heterotrophy may exist (*Astasia*, many *Dinoflagellata*). In spite of those divergencies, paths leading to *Mastigota* form a rather distinct bundle, coherent and well marked off from the others.

Paths leading to *Sarcodina* are characterized by the reduction of the kinetide, by assuming its form of the metakinetide (and even akinetide in the terminology and meaning of Chatton 1925). In the more primitive groups, the ability of restitution of flagella persists, and in many groups the flagellated gametes occur. Complication of the nuclear apparatus consists in the frequent multiplication of the nucleus (polyenergide), even to a high number of nuclei and formation of plasmodia (*Pelomyxa, Foraminifera, Myxosporidia*). A great variety is found in the locomotor apparatus, pseudopodia — assuming the form of lobopodia, phylopodia, reticulopodia or axopodia. In many groups the ability occurs to produce the external and internal skeleton of the organic substance, of calcium salts, silica or strontium sulphate. The skeletal structures show a different degree of polymerization. Somatization is manifested in the differentiation of nuclei, the extending body mass (some *Foraminifera, Myxosporidia*) and even in formation of multicellular forms as the spores of *Myxosporidia* and *Actinomyxidia*. There are no links with the plant world except in the heterotrophic *Mycetozoa*. Despite those differences in the evolutionary tendencies, the groups under discussion, *Rhizopoda, Actinopoda* and the parasitic *Cnidosporidia*, show — no doubt — a phylogenetic relation and a similitude in development.

The path leading to the *Ciliata* was rather single and it was characterized by the transformation of the kinetide into the hyperkinetide and by the development and differentiation of the nuclear apparatus resulting in its form of hyperenergide. The highly advanced somatization is manifested in the processes of morphogenesis and in the specific picture of the sexual process comparable to the insemination and copulation in *Metazoa*. The element differentiating the group and its evolutionary paths is — before all — the differentiation of the cortical motor apparatus, especially of its adoral parts. It is difficult to detect in this uniform group any signs common with the ancestral form or with *Mastigota*. Despite their uniformity, *Ciliata* exhibit a great variety of forms often highly specialized.

The above review indicates — in my opinion — as well the strict differentiation of those three groups of *Protozoa* as the profoundness of the divergences characterizing their evolution. They are no less essential than those accepted as sufficient for distinguishing phyla in *Metazoa*. I realize, that my concept may evoke the objection that between the primitive *Flagellata* and *Rhizopoda*, not one but many transitional forms exist, but taking into account this fact would make impossible even a plain distinguishing of classes and orders in them. Especially clear seem to me such multiple links among *Protozoa*, which are plastic organisms, being as if in search of development paths in different manner, what is manifesting e. g. in the great variety of sexual processes and of modes of reproduction.

Much anxiety among the taxonomists attempting to reflect in their systems the natural pattern of relations, is evoked by the postulation of monophyletism of the established groups. The monophyletism of the middle and big groups (families, orders, classes) as postulated by many taxonomists, would be based on the fact of their common origin from one ancestor, from one species of the exit group. Worse than that: those taxonomists disconnect the former, sometimes well established groups if they presume that their sources are in several species, consequently that they are — in their opinion — polyphyletic.

Yet, the evolution occurred — no doubt — along numerous ways, not necessarily that one which those taxonomists imagine: only by dichotomy. The system should reflect the real course of the evolutionary development and not this as it is conceived by some authors. It could be accounted for just by a miracle that from an exit group extending over a large area and characterized by definite similar properties, only one species would be able to give another offshoot group developing radially, extending over an equally broad area and exhibiting characters being the continuation of that of the exit group. Postulation of group monophylety, if conceived in this way, means to expect miracle!

It seems reasonable to accept that the species must be in this meaning monophyletic that it originates from one exit species (species arising by crossing should be the exception). This criterion holds true also for related species and even for establishing of genera. In contrast to this, the case when groups (family, order etc.) originate not from one but from several species of the exit group, of the same or a lower rank (genus, family, order), i. e. when it arose not by the radiating but by a parallel development — is not opposed to the reasonably understood monophyletism. The true polyphyletism would be testified only by a convergent development, if the new group would arise from representatives of several exit groups of the equal or higher range. If the zoological taxonomy had to follow the rules of those „pure" monophyletists, the desintegration even of such groups (in this meaning probably polyphyletic) as amphibians, birds, mammals and arthropods, would occur.

For this reason I do not think necessary and possible to follow the rules of a strict purism as to the monophyletism of groups in the protozoological systematics, but in my opinion, the maintaining the groups clearly connected intrinsically, with no regard to the suppositions of their origin from the exit groups by the parallel evolution, is admittable and reflecting the natural relations.

The natural system of *Protozoa*

Raising the three fundamental groups of *Protozoa* to the rank of types (phylum, cladus) permits not only to accentuate the differences which distinguish them — not less essential than those which distinguish the types in *Metazoa* — but also faciliates a more adequate and clear construction of their system. As a fundamental category of a systematic rank lower than the phylum, I accept in my considerations the class (classis). It has to unite really (as it may be assumed) related organisms and developing according similar pattern. Such terms as „subphylum" or „superclassis" would be accepted only in a meaning of collective categories: groups of classes slightly differing from one another and developing in different directions, but which may be contrasted to one another with regard to some of their properties.

Consequently the taxon „classis" is considered as a more or less natural entity whereas the taxons „superclassis" or „subphylum" — only as classifying categories.

Raising the rank of groups *Mastigota, Sarcodina* and *Ciliophora* (= *Ciliata*) up to the rank of type permits in some cases to raise the rank of its subordinate groups (e. g. the rank of ordo to the rank of classis) where it is justified and, in consequence, to extend the possibilities of further classification. So — con-

tinuing — I take opportunity of presenting the system suggested by me, basing principally on the system of Cheissin and Poljansky 1963, the most fortunate, in my opinion.

The more essential problems of discussion emerging in the above considerations on the *Protozoa* system, may be presented — in my opinion — as follows:

Phylum *Mastigota*

1. The rank of taxons included to *Phytomastigina* is sometimes higher in the apprehension of botanists than in that of zoologists. In my scheme of system those groups may be treated as separate classes.

2. The division of *Zoomastigina* evoked many diverging opinions with respect to the validity of groups *Protomonadina* and *Metamonadina*, respectively *Protomonadina, Polymastigina* and *Hypermastigina* as also on account of the certainly peculiar position of *Opalinata*. In my scheme, into the animal *Flagellata* three classes: *Protomonadina, Metamonadina* (eventually with two subclasses: *Polymastigina* and *Hypermastigina*), as well as *Opalinata*, may suitably be included. The position of *Opalinata* as a separate class is stressing its distinction well.

3. The position of *Sporozpa* is — in my opinion — well established by Cheissin and Poljansky 1963; their subclasses in my system are raised to the rank of classes which accentuates their distinction. I consider the problem of the *Toxoplasmida* and *Sarcosporidia* position as unsetled although I support the arguments of the Leningrad authors.

Phylum *Sarcodina* Schmarda 1871

1. The problem of classification of *Rhizopoda* into *Lobosa, Filosa* and *Granuloreticulosa* (after Grassé 1960) seems to me not decided. Their division into *Amoebozoa* (*Amoebina*+*Testacea*) and *Foraminifera* considering them as classes in the subphylum *Rhizopoda*, seems to be more natural.

2. The problem of *Actinopoda* opposed to *Acantharia* and *Radiolaria* is also not definitely solved. In this question I incline rather to the suggestion of Tregouboff 1952, and leave them as classes: *Acantharia, Radiolaria* and *Heliozoa*, within the subphylum *Actinopoda*.

3. The problem of *Myxosporidia* had been — in my opinion — correctly settled by Cheissin and Poljansky 1963. In my system they are treated as classis, besides *Microsporidia* and *Actinomyxidia*. The problem of *Piroplasmida* well presented by the Leningrad authors is not considered by me as settled in the meaning of their position in the system.

Phylum *Ciliophora* Doflein 1901 (=*Ciliata* Perty 1852)

1. The problem of the position and of distinctness of *Peritricha* has been — in my opinion — more correctly set up by Cheissin and Poljansky 1963 than in the American suggestions. *Peritricha* are treated by me as one of the three classes of *Ciliata* (beside *Holotricha* and *Spirotricha*). I intend to return to this matter in next publication.

2. The problem of *Suctoria* seems to be more controversive. Although their well motivated connections with the more primitive *Holotricha* exist but on the other hand this group followed in its development a more specific course, distinguishing it exactly from other *Holotricha* by its structure and development.

Table 1

The proposed system of the subregnum *Protozoa*

Phylum	Subphylum		Classis	Comments or ordines
Mastigota	*Flagellata* =*Mastigophora*	*Phytomast*	7 — 10 classes	
		Zoomastigina	*Protomonadina*	
			Metamonadina	eventually two subclasses: *Polymastigina* and *Hypermastigina*
			Opalinata	
	Telosporidia =*Sporozoa* s. str.		*Coccidiomorpha*	
			Gregarinomorpha	
				addenda: *Sarcosporidia*, *Toxoplasmida*, *Haplosporidia* (?)
Sarcodina	*Rhizopoda*		*Amoebozoa*	
			Foraminifera	
			Mycetozoa (?)	
	Actinopoda		*Acantharia*	
			Radiolaria	
			Heliozoa	
	Cnidosporidia		*Myxosporidia*	
			Microsporidia	
			Actinomyxidia	
Ciliophora	*Ciliata*		*Holotricha*	*Gymnostomata*, *Apostomea*, *Suctoria*, *Trichostomata*, *Hymenostomata*, *Thigmotricha*, *Astomata*
			Spirotricha	*Heterotricha*, *Oligotricha*, *Tintinnoidea*, *Odontostomata*, *Hypotricha*, *Entodiniomorpha*
			Peritricha	*Sessilia*, *Mobilia*

3. The problem of *Chonotricha* has been — in my opinion — more correctly treated by Corliss 1960. The investigations of Guilcher 1951 and especially of Dobrzańska-Kaczanowska 1963 proved such a close relationship with *Hypostomata* that this permits to include *Chonotricha* to *Gymnostomata* as one of their suborders.

As the result of the above consideration the *Protozoa* system suggested by me would be draw up as presented by the Table 1.

In conclusion some nomenclatorical remarks:

I am not adherer of introducing new names for old taxons although they might stress better their properties. It introduces confusion and rarely gives the wished results because the new names often fail to be precise (e.g. the term *Homocaryota* is not precise since some *Plasmodroma* have differentiated nuclei).

I cannot accept the lately introduced names of type: *Euspora* Levine or *Cnidospora* (Tent. Class.) for the etymological reasons. „Spora" means a structure (in singular) and cannot mean the group characterized by this structure; *Eusporidia, Cnidosporidia* are more appropriate names. The names of groups like *Gregarina* or *Opalina* are not adequate being the names of genera; *Gregarinida* or *Opalinata* are more reasonable.

I cannot agree with introducing the ending *-ida* or *-ina* for orders resp. suborders. Those endings may be applied at most when the name of a taxon of order rank is formed from the name of genus or of family. In other cases linguistic monsters arise distorting the meaning of the primary name (e. g. *Astomatida*!).

Summary

Taking into account the great differentiation of *Protozoa* and their rank of a subregnum in the animal world, the author suggests the separation of three phyla among them, which should correspond in their ranks to the phyla distinguished in *Metazoa*. They are: *Mastigota* including *Flagellata* and *Sporozoa* s. str.; *Sarcodina* Schmarda 1871 including *Rhizopoda, Actinopoda* and *Cnidosporidia;* finally, the best differentiated, *Ciliata* Perty 1852 (= *Ciliophora* Doflein 1901).

The author discutes the main evolutionary trends characterizing the cytological differentiation of *Protozoa*. Among those evolutionary ways, many paths consist in the polymerization and, eventually, in the integration of some morphological systems. For instance, the development of the nuclear apparatus is tending from the monoenergide towards the polyenergide, and finally, to the appearance of an integrated entity, denoted by the author's term: hyperenergide (in *Ciliata).* In the development of the motor apparatus the author distinguishes, after Chatton, the monokinetide, the multiplicated polykinetide, and an integrated hyperkinetide. The resultans of these eventualities, as well as of some others, are determining the ways leading towards the different phyla and classes in *Protozoa*.

The evolutionary tendencies consisting in various modes of separation of the generative elements from the somatic ones, are denoted by the author's term: somatization. It may be manifested in *Protozoa* in an intensifying of the trophic stages, in a differentiation of nuclei into the generative and the somatic ones or in the arising of a nuclear dualism, in the appearence of differentiated multicellular entities, in the complication of sexual processes, and finally in the appearence of development phenomena bearing the character of an epigenetic morphogenesis.

Basing on the above considerations, the author presents the principles of phylogenesis in *Protozoa* and suggests an outline of their taxonomical system.

STRESZCZENIE

Ze względu na wielkie zróżnicowanie *Protozoa* i ich rangę jako subregnum królestwa zwierzęcego, autor postuluje wyodrębnienie wśród nich trzech typów, odpowiadających rangą typom wyróżnionym wśród *Metazoa*. Są to: *Mastigota* obejmujące *Flagellata* i *Sporozoa* s. str., *Sarcodina* Schmarda 1871 obejmujące *Rhizopoda*, *Actinopoda* i *Cnidosporidia* oraz dobrze wyodrębnione *Ciliata* Perty 1852 (= *Ciliophora* Doflein 1901).

Autor rozważa ogólne drogi ewolucyjne, znamionujące cytologiczne różnicowanie się *Protozoa*. Wiele z tych dróg polega na polymeryzacji i ewentualnej integracji poszczególnych układów. Np. rozwój aparatu jądrowego dąży od monoenergidy do polyenergidy i wreszcie do pojawienia się zintegrowanego układu, który autor oznacza mianem hyperenergidy (*Ciliata*). W rozwoju aparatu ruchu autor wyodrębnia za Chatton'em monokinetydę, zwielokrotnioną polykinetydę i zintegrowaną hyperkinetydę. Kombinacje tych i innych możliwości wyznaczają drogi wiodące do różnych typów i gromad *Protozoa*.

Tendencje ewolucyjne polegające na różnego typu wyodrębnianiu się elementów generatywnych od somatycznych, oznacza autor mianem somatyzacji. Może przejawiać się ona wśród pierwotniaków w rozbudowie stadiów troficznych, w różnicowaniu się jąder na generatywne i somatyczne lub powstawaniu dualizmu jądrowego, w powstawaniu zróżnicowanych układów wielokomórkowych, w komplikacji procesów płciowych, a wreszcie w pojawianiu się procesów rozwojowych o charakterze epigenetycznej morfogenezy.

Na tle tych rozważań autor przedstawia rozwój rodowy *Protozoa* i proponowany przez siebie ich system.

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The taxonomic position and rank of *Peritricha*

Stanowisko systematyczne i ranga *Peritricha*

In the course of recent years — owing mainly to the publications of Corliss 1955, 1957, 1960 and to the suggestions of the American authors expressed in their „Tentative classification of the Phylum *Protozoa* Goldfuss, 1818, emend. v. Siebold 1845" — much more attention than before was payed to the problems connected with systematics and phylogenesis of *Ciliata*. In the suggestions of Corliss as well as in the American scheme, the most essential innovations are those which concern the groups: *Suctoria, Chonotricha* and *Peritricha*, reduced to the rank of subordinate groups of *Holotricha*. This view was opposed only by Cheissin and Poljansky 1963 who left for those groups the rank of subclasses equivalent to *Holotricha* and *Spirotricha*. Nevertheless, the objections of the Russian authors concerned before all the position of *Peritricha*.

It may be agreed that the problem of position and of rank of *Chonotricha* has been ultimately solved by the study of Guilcher 1951 and more so by that of Dobrzańska-Kaczanowska 1963: they may be recognized at most as a separate suborder in the order *Gymnostomata*, besides the suborders: *Prostomata, Pleurostomata* and *Hypostomata*.

Suctoria firmly connected with the primitive *Holotricha* (Corliss 1961) may possibly be recognized within this classis as a separate order equivalent to the orders: *Gymnostomata, Apostomea, Trichostomata, Hymenostomata, Thigmotricha* and „*Astomata*".

There remains still the problem of the position and rank of *Peritricha*, a group extensively studied by me in connection with my research on *Urceolariidae* and on some *Peritricha-Sessilia*. This problem is to be developed in the present article.

Survey of views on the position of *Peritricha*

Since this group had been established by Stein 1859, *Peritricha* were treated mostly as a group equivalent to *Holotricha* Stein 1859, *Spirotricha* Bütschli 1889 and *Chonotricha* Wallengren 1895 (e.g. Kahl 1935) or as equivalent to *Holotricha, Heterotricha, Oligotricha* and *Hypotricha* (e.g. Calkins 1926). Together with those groups they were opposed usually to *Suctoria*. As a rule, *Peritricha* embraced sessile forms of a general structural type of *Vorticella, Epistylis, Scyphidia* or *Vaginicola*, with scopula as an organ attaching

to the substrate — called *Sessilia* Kahl 1933, and also forms free-swimming although parasitic, with a structural type of *Trichodina* and *Urceolaria* — called *Mobilia* Kahl 1933. *Licnophoridae* were also included to *Peritricha* as the suborder *Scaiotricha* Délage et Hérouard 1896 or as *Peritricha-Leotropa* opposed to the genuine *Peritricha-Dexiotropa* (Fabre-Domergue 1388). *Licnophoridae* were definitely excluded from *Peritricha* by Kahl 1935 and included to *Spirotricha*. Adequacy of this decision is supported by the study of Villeneuve-Brachon 1940.

Essential changes in the systematic position of *Peritricha* were tried in the recent years by Fauré-Fremiet 1950 and by Corlis 1956, 1960, 1961 who included them to *Holotricha* considering the latter as one of the two subclasses (*Holotricha* and *Spirotricha*) in *Ciliata*. In this way — according to Corliss — *Peritricha* would occupy a position equivalent to other orders in this subclassis namely: *Gymnostomata* Bütschli 1889, *Hymenostomata* Délage et Hérouard 1896, *Astomata* Schewiakoff 1896, *Apostomea* Chatton et Lwoff 1928 and *Thigmotricha* Chatton et Lwoff 1922. The position of *Peritricha* in the genealogical tree is defined at the end of the evolutionary series: *Hymenostomata* → *Thigmotricha* → *Peritricha*. A slight not much precised difference in the views of those authors consists in tracing the origin of *Thigmotricha* from *Pleuronematina* which originate from some not precisely defined *Hymenostomata*, giving also origin to *Tetrahymenina* (Fauré-Fremiet 1950, p. 120), while Corliss traces *Thigmotricha* from *Pleuronematina* and those from *Tetrahymenina* (Corliss 1956, p. 134; 1961, p. 98).

What are the arguments of both authors — before all of Fauré-Fremiet — in favour of those two phylogenetic links: *Tetrahymenina* → *Thigmotricha* and *Thigmotricha* → *Peritricha*?

Fauré-Fremiet 1950, p. 116 stated about *Thigmotricha* as follows: „On doit la connaissance de ce groupe remarquable de Ciliés épizoiques aux travaux de Chatton et Lwoff (1949); examinant d'après les indications apportées par les Hyménostomes tetrahyméniens les mécanismes compliqués de la stomatogenèse décrite par ces auteurs chez *Proboveria* par example, on remarquera, que, dans ce dernier cas, la bouche initiale est située postérieurement, sur le territoire correspondant à l'opisthe; que la cinétie 1 peut être comparée à la membrane parorale; et la polycinétie A,B,C, à le série des trois champs deltoide, trapèzoide et falciforme des *Philasteridae* et des *Lambidae* (H. Mugard)."

So as I had opportunity to state before (Raabe 1959, 1963), the problem of presumable connecting *Thigmotricha* to *Tetrahymenina* cannot be recognized as solved and — at any rate — documented by the study of Chatton et Lwoff 1949 on the division of *Proboveria*, even if the homology of its division rows A + B + C + D with the „adoral zone of membranelles" i.e. AZM, and the kinety 1 as the equivalent of UM, was recognized. The first objection concerns the number of those AZM, in *Thigmotricha* being 4 not 3 (Raabe 1963, Kazubski 1963 and also Chatton et Lwoff 1936, fig. IV); consequently there may be no question of „tetrahymenium" even in morphogenesis. Attention is to be called that I found useful and right to introduce the notion of „ambihymenium" for determining the system of one UM and of many AZM occurring in different forms in the adoral apparatus of *Holotricha* and *Spirotricha*.

Secondly, I consider not possible to trace the more primitive forms of

Hemispeiridae, as *Ancistrum* or *Ancistrumina* (Raabe 1959), with long adoral rows prolonged nearly over the whole body and poorly differentiated from the general ciliature — from forms with short and distinctly differentiated adoral rows as are *Tetrahymenina.* The exit forms for *Thigmotricha* should be looked for — in my opinion — in such forms as *Pleuronemata,* e.g. *Cyclidium* (Czapik 1963).

A more important problem is this of tracing *Peritricha.* As to this problem Fauré-Fremiet 1950 p. 117 stated:

„Toute la morphologie des Péritriches est dominée par la présence d'un organ ciliaire de fixation: la scopula (Fauré-Fremiet 1905 et 1910) et j'ai cherché le modèle initial de ce type chez les Ancistridiens et plus spécialement chez *Hemispeira* (Thigmotriches de Chatton et Lwoff). Les critiques et les interprétations nouvelles formulées à cet égard par Chatton (1936) sont intéressantes mais discutables".

„La description exacte de la ciliature buccale et de la stomatogenèse au cours de la bipartition est apportée chez *Cyclochaeta,* par Chatton et Villeneuve 1937; la comparaison des structures montre que l'haplocinétie externe peut être homologuée à la membrane parorale des Thigmotriches et de Hyménostomes tétrahyméniens, la polycinétie interne correspondant à la série adorale A, B, C, des Thigmotriches ancistridiens. Le système buccal est entièrement autonome; sa position trachement postérieure oblige à considérer le disque des Vorticellides comme une face ventrale ce qui explique la division suivant un plan perpendiculaire apparemment longitudinal".

„L'interprétation de la frange ciliaire locomotrice, située autour de la scopula, reste, dans cette hypothèse, tout à fait incertaine".

Similar theories are put forward by Corliss 1956, indicating the affinity of the adoral apparatus of *Peritricha* to that of *Thigmotricha-Stomatina* and, in consequence, to the adoral zone of membranelles (AZM) and to the undulating membrane (UM), i.e. to tetrahymenium of *Hymenostomata.*

In this matter I think just to assume the opposite position: searching for homology between the adoral spiral of *Peritricha* and the adoral rows of *Hemispeiridae* is, in my opinion, unexpected and not justified. The statement of Fauré-Fremiet is verbally based on the study of Chatton et Villeneuve 1937 who however fail to stress this homology and do not try to find in their „*Cyclochaeta astropectinis*" n. nud. the traces of tetrahymenium. On the contrary — after having given the description of the specific „division longitudinale partielle du péristome" in this species, they continue (p. 4):

„Ajoutons que, chez les Holotriches de la famille des Ancistrumidés, le péristome, formé aussi d'une polycinétie et d'une haplocinétie, est d'une structure si semblable à celui des Péritriches qu'on aurait pu penser faire dériver celui-ci de celui-là, en dépit du fait qu'ils sont enroulés l'un dans le sens anthoraire, l'autre dans le sens horaire. Mais leur comportement à la division (étudié chez les Ancistrumidés par Chatton et Lwoff) est si différent qu'une telle tentative serait tout à fait illusoire. À tout égard, les Péritriches restent très isolés dans le groupe des Ciliés".

It should be reminded that Chatton 1936 expressed a slightly different view on the direction of the adoral spiral of *Peritricha* as compared to *Hemispeiridae,* indicating that although at a certain orientation of the body

the spiral may be recognized as sinistrorsal in both cases but — except this similitude — its structure is different.

In *Hemispeiridae* — especially in *Hemispeira* and in *Boveria* — the spiral is involutive and turning to the apical point of the body tending to cytostome, and in *Peritricha* it is evolutive and evolving from the apical point. This is especially clear in such forms as *Vauchomia* (Mueller 1938) or *Campanella* (Kahl 1935) with a multiple spiral. This essential difference is — in my opinion — more important as tracing a different evolutionary trend, whereas the direction of spiralization and its interpretation becomes of minor importance.

As follows from the above considerations, there are no serious reasons for tracing the adoral spiral of *Peritricha* from that of *Hemispeiridae*, even if it is spiralized like in *Boveria*. There exists also no possibiliy of connecting the aboral girdle of *Peritricha* with the thigmotactic apparatus of *Hemispeiridae* even if concentrated on the pole opposite to the cytostome and to the adoral spiral (shifted backwards like in *Hemispeira*). The thigmotactic area of *Hemis-*

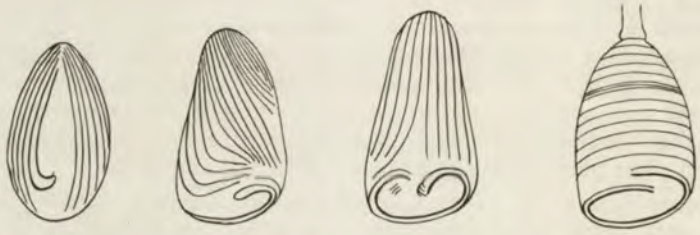

Fig. 1. Spiralization of the adoral kineties and the kinetal system in *Thigmotricha* and *Peritricha;* successively: *Ancistrumina, Cheissinia, Boveria, Peritricha-Sessilia*

peiridae remains always a system of parallel kineties whereas the aboral zone (aboral ciliary girdle) of *Peritricha* is a system of closed kineties, even fully closed, as it appears during the division processes. Besides, the cyclic system of cortical elements is in *Peritricha* still better implanted. This problem will be discussed below.

Another argument against the theory of Fauré-Fremiet and of — following him — that of Corliss, should be indicated. Tracing *Peritricha* from *Hemispeiridae* or similar forms, those authors try to reconstruct the pedigree of this extensive group (comprising in their majority organisms becoming sessile, facultatively sessile or sessile — but not parasitic) from an evolutionary branch of *Thigmotricha* very specialized and adapted to the parasitic life. Those characters on which this theory is based, fail to appear in merely primitive forms (e.g. in *Ancistrum* or *Ancistrumina*). This concept being not quite impossible, is very little probable.

In his considerations of the possible phylogenetic trends of *Peritricha*, Chatton 1936 indicated another possibility. He found an argument in finding a telotroch form of *Epistylis horizontalis* Chatton, of a character quite different from the commonly known cylindrical and axially symmetric forms. The telotroch stage of *E. horizontalis* is „horizontally polarized", flattened and elongated, which involves the loss of axial symmetry. Chatton — not without reason — tries to detect a similitude of those toletroch stage to *Chlamy-*

dodontidae, and suggests that ciliates of this kind could be the ancestors of *Peritricha*. He assumes that the free-swimming developmental forms would reflect the structure of ancestors characterized by a bilateral symmetry or a symmetry similar to it, and that the external axial symmetry of mature individuals might be a subsequent acquisition connected with the sessile mode of life.

This theory fails also to elucidate adequately the origin of *Peritricha* and, before all, the origin and evolutionary trends of their circular ciliary systems. *E. horizontalis* was found by me in *Balanus* from the Baltic Sea and it must be stated that although its body is elongated and flattened, its telotroch stages keep their full adoral spiral and their closed ciliary girdle.

There still arises a more serious objection: as it seems, the horizontal polarization of telotroch stage in *E. horizontalis* (being epibionts on *Balanus*) might not obligatorily reflect their ancestral form; on the contrary — it may be the result of a coenogenetic adaptation (sensu Sewertzoff 1931) of the free-swimming juvenile stages to the parasitic or epizoic life. This seems so more probable as the telotroch forms similar — at least concerning their external architectonic — occur also in other groups of *Ciliata*, as in *Apostomea* or in *Conidophryidae* (= *Pilisuctoridae*) — Chatton et Lwoff 1935, 1936, Raabe 1947. In this concept, the horizontal flattening of telotrochs in *Epistylis horizontalis* would be the character not of the ancestral, more primitive, state but of the secondary state of coenogenetic adaptation of juvenile forms.

Characteristic features of *Peritricha*

The axial body symmetry

Of all *Ciliata* surely the *Petritricha* show the most distinctly expressed axiality in their body structure, at least in its general architecture. However the primitive *Gymnostomata — Prostomata* have also the axial structure, but in this case the axiality is primary, connected with the strictly preserved meridional course of kineties. The generally axial structure occurs in some sessile *Spirotricha*, e.g. *Stentor*; in this case it is accompanied by the more or less meridional course of somatic kineties, except only for ambihymenium: UM and many elements of AZM. The axial structure occurs also among *Holotricha* — in *Chonotricha* — but it fails to concern the pattern of kineties, and — at last — it occurs in some *Suctoria*.

The axiality of structure in *Peritricha* is exceptionally strongly expressed and is subordinating many systems in a full and specific manner, being general in this numerous and differentiated group. The body axis is here always running through the center of the oral area which is surrounded by the complex adoral ciliary spiral and through the scopula lying on the opposite body pole, and surrounded by the parallel ciliary girdle in the telotroch forms of *Sessilia* and in *Mobilia*.

The nearly complete closure of the adoral spiral in the form of a circle (exceptions: the incomplete or multiple circuit of the spiral are — no doubt — secondary), as well as the complete and exact annular closure of the ciliary girdle — are accompanied in many forms by the parallel course of the cortical structures. They are present in very many *Peritricha-Sessilia* (as e.g. in *Scyphidia*) and in *Urceolaria patellae* Cuénot among *Mobilia* (Raabe 1961) in the form of continuous or interrupted fibers with tiny bodies ressembling

to the basal corpuscles. As it seems, the differentiation of several fibres of this kind produces basis for ciliary girdle in telotrochs. The body of *Trichodinopsis paradoxa* Clap. et Lachm. is embraced by circular furrow-like structures; *Spirochaetae* settle down in them producing the pseudociliature of those ciliates (R a a b e 1961). All that enables the comparison of the structure of *Peritricha* to pottery formed and covered with ornaments on a potter's wheel.

Axiality of the *Peritricha* body is also stressed by the structure of scopula in *Sessilia* which is rosette-shaped i.e. radial, and more so, by the structure of the skeletal apparatus of *Mobilia* (*Urceolariidae*) which is distinctly radially revolving.

A peculiar phenomenon which G e l e i 1939 indicated as well here as in *Metazoa* is connected with the axial symmetry and the sessile mode of life which involve the presence of the clinging apparatus (scopula) or of sliding organ (sucker of *Urceolariidae*). This phenomenon is shifting of the cytopyge and the outlet of the osmoregulative-excretory system as far to the oral pole (opposite to that on which the animal lies) that both apertures reach the oral

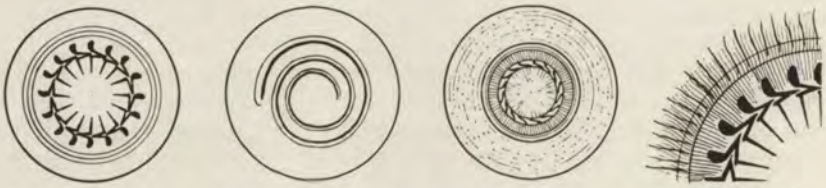

Fig. 2. Elements of the axial and rotary symmetry in *Peritricha-Mobilia;* successively: aboral disc of *Trichodina,* adoral spiral of *Vauchomia,* aboral part of *Urceolaria patellae,* sector of the aboral disc with the ciliature in *Trichodina*

opening and the „cloaca oralis" (Mundkloake) arises. Summarizing, it may be stated that the primary axial symmetry concerning the position of mouth on one — and of aperture of the contractile vacuole and cytopyge on the opposite pole of the body (which is disturbed subsequently by shifting of the mouth backwards and of the excretory outlets and cytopyge somewhat forwards) becomes in some meaning secondarily restored in those sessile organisms since both groups of organelles meet on the same body pole. The position of organelles in *Peritricha* is one of the best examples of formation of cloaca oralis, in this form unique in *Ciliata.*

The buccal apparatus

This structure was studied rather exactly by W a l l e n g r e n 1897 in the case of *Urceolaria,* then sometimes described, at last represented by C h a t t o n et V i l l e n e u v e 1937. In *Peritricha* it appears rather uniforme, not in details like the shorter or longer extension of the adoral spiral or its penetration inside the infundibulum or formation of the „operculum" or „trunk" — but in its general character. As it seems, in all cases the adoral ciliary apparatus consists of two long kineties: the external haplokinety and internal polykinety, describing a circle of about 400°. This is an evolutive circle — the inlet to cytostome lying on its centrifugal end. Both kineties encroach into infundibulum — in a manner not thoroughly and not generally elucidated — and in an altered shape, run over its walls along screwlike paths. According

to the early findings of Peschkowsky 1924 and some more recent and detailed of Lom 1962, 1963a, 1963b, the haplokinety twists spirally in the mouth while the polykinety branches into several kineties. Lom noticed some structures inside the infundibulum, called by him „peniculus". According to Lom 1963b inside the haplokinety, in the place where it encroaches into infundibulum, an additionary haplokinety appears, may be representing the branching of the principal haplokinety in the pre-division period (Chatton et Villeneuve 1937).

Presumably the action of both kineties of the adoral spiral consists in evoking a water current in the fissure between the kineties. This current forces food into the infundibulum, and later, inside it, to cytostome (Dobrzańska 1961). The back current would rinse out of the infundibulum all what is evacuated to it from cytopyge and from the contractile vacuole. So infundibulum is really acting as a cloaca oralis.

It seems little possible to equalize the adoral apparatus of *Peritricha* to any adoral apparatus of *Holotricha* or *Spirotricha*. Although the adoral apparatus of *Peritricha* is not contrasting entirely with any type of apparatus of those two groups, it differs from any of its forms and fails to represent a uniform type of structure. The differentation of the adoral ciliature in *Peritricha* (still stressed by the atrophy of the general ciliature) allows to compare it only with an adoral ciliature strongly differentiated — such as occurs in *Hymenostomata* or *Thigmotricha* as well as in *Spirotricha*. If there is anything common in the ciliature of these latter groups, it is merely the possibility to determine them as ambihymenium, the term applied by me to the system composed of one undulating membrane — UM, and of a certain number of structures constantly present or appearing in the stomatogenesis processes as the adoral zone of membranelles — AZM (Raabe 1963).

Such a homology cannot be found in regard to *Peritricha*. One haplokinety, one polykinety and additional structures ressembling slightly — as Lom thinks — the peniculus of *Paramecium*, cannot be strictly homologized with ambihymenium. As a matter of fact, in both cases structures of an undulating membrane type and of type represented by the AZM elements take part but those are possibilities occurring in all groups of *Ciliata* with a differentiated adoral ciliature. So it must be agreed that the development of the adoral ciliature in *Peritricha* found its different path, not comparable exactly to any path of other groups, at any rate different from those along which proceded *Thigmotricha* or *Hymenostomata*. Another distinctness is found in the morphogenetic processes, although here some ancestral characters might be expected, as common for groups which are akin or derive from one another.

The aboral apparatus

I use the term „aboral apparatus" for the assemblage of two seemingly independent organelles: scopula and its derivatives as well as the aboral ciliary girdle. The term is common for both elements because in the telotroch stages of *Sessilia* and in *Mobilia* they have close morphological and functional connections.

I wish not to go into the matter of homology of scopula in *Peritricha, Suctoria* or *Chonotricha*: the solution of this problem should be put off till the precise studies under electron microscope are executed. Besides, as well the solution speaking in favour of such homology or the contrary results, are of no importance for further considerations.

In *Peritricha,* the axially located scopula (its shifting occurs only in the telotrochs of *Epistylis horizontalis* — Chatton 1936) is a highly plastic structure: it may act directly as a clinging organ — as in the stalkless *Sessilia* it may produce different sort of peduncles — or may be transformed into the cup of the sucker in *Urceolariidae.* In the latter case, on its area smooth (subfam. *Urceolariinae*) or hooked and spiny (subfam. *Trichodininae*) skeletal rings arise, composed of numerous links encroaching successively one upon another in various manner. A structure of an axial rotary symmetry arises. This kind of symmetry is quite specific and almost not recurring; it was formed under the influence of the axial symmetry of the organism and of its rotary movement in one direction correlated with the direction of the adoral spiral.

The radial pattern of the skeletal ring elements is not a quite new phenomenon in *Peritricha-Mobilia*: scopula contains in *Sessilia* radial elements disposed in a rosette and — at least in some *Sessilia* — also fibrillar elements, initiating on its margin and tending towards the oral body pole (e.g. *Scyphidia* — Raabe 1952). Similar radially disposed fibers occur also in *Trichodinopsis* among *Urceolariidae.*

Scopula is embraced at a certain distance by a circular zone capable, in telotroch stages of *Sessilia,* to produce cilia disposed in circular kineties, and in *Mobilia* — endowed as a rule with such kineties. This zone is constantly marked in *Sessilia,* mostly as a densification of the circular fibers, not differing distinctly from the remaining ones. In the period of formation of the telotroch form, those fibers become more conspicuous, basal corpuscles and — at last — cilia appear on them. In some *Sessilia* those cilia exist also in the sessile stage: then the turning into the telotroch form is reduced to retracting the scopular part and to detaching the body from the substrate. So it occurs e.g. in *Ambiphrya miri* (Raabe 1952) and possibly in other *Sessilia* without stalks. Telotroch form of *A. miri* Raabe and — more so — telotrochs of epizoic *Obopercularia* sp. sp. remind the structural pattern of the family *Urceolariidae* (Lust 1950).

In *Mobilia,* the aboral ciliary ring occurs constantly and presents a complex ciliary system. The main part of it consists of the ciliary girdle as a closed polykinety; the base of it was drawn by Wallengren 1897. Outside it, a ring of more rare but stronger marginal cilia is running (possibly not in all the *Urceolariidae*), called sometimes „cirri", forming a haplokinety. Inside the ciliary girdle there is another, inner ring of cilia in the form of a haplokinety too. Those elements are distinctly seen on the electron micrographs of Fauré-Fremiet, Rouiller et Gauchery 1956.

The closure of all those ciliary rings should be stressed once again — the more so as in the externally rather similar clinging apparatus of *Licnophora* (*Heterotricha*), they are not closed.

Division and morphogenesis

The division of *Ciliata* is generally recognized as transverse, at least in this understanding that the division plain crosses the kineties (perkinetally) dividing them by half. When the kineties system becomes gradually more profoundly integrated, the elements of new formation or far reaching changes and transformations in division (morphogenesis) begin to dominate over the elements of transmission. At last, in some highly modified forms (*Suctoria, Chonotricha*) instead of the division of kineties, reconstitution of their whole

pattern occurs (Dobrzańska-Kaczanowska 1963). Nevertheless, where possible, the cross division of the kineties occurs and their parallel and more or less meridional pattern is preserved. Division (except for budding) is transverse, homotetigenic.

In *Peritricha* the division is longitudinal (in the architectural and promorphologic meaning), in some of its elements apparently symmetrigenic, although the elements of their body behave in different manners on its course. In this longitudinal fission the aboral ciliary girdle (or its primordia) divides by rupture, in this way its division is perkinetal, the division plain breaking the kineties in two sites (forming 8-shaped figure). Similar 8-pattern is seen in the division of scopula, and in *Urceolariidae* — in that of the skeletal ring as well. So, despite the cross division of the kineties, the general picture of division is quite specific. The division of the annular fibers embracing the body is similar to that described above.

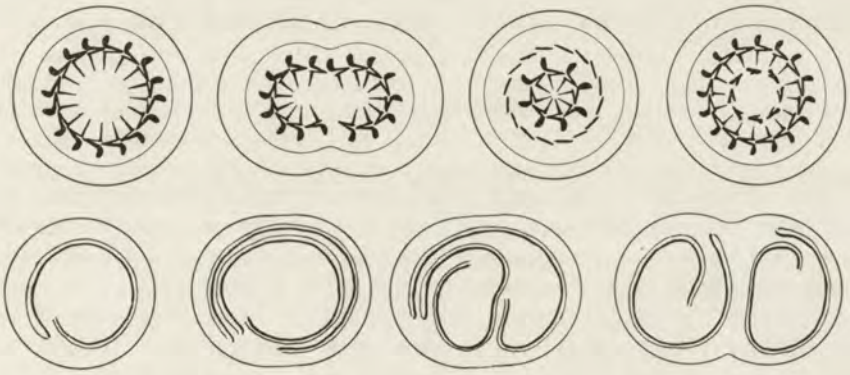

Fig. 3. Division stages of *Peritricha* — *Urceolariidae*; above — pseudosymmetrigenic division of the aboral zone; below — specific division of the adoral spiral

The division of the adoral spiral escapes this division scheme, and occurs after a different and specific pattern, as described already by Wallengren 1897 in *Urceolariidae*, but similar — as it seems — in all *Peritricha*. This division consists in the longitudinal fission of both adoral kineties and their subsequent disentangling. The details of this process are reported by Chatton et Villeneuve 1937 for their „*Cyclochaeta astropectinis*". According to these authors the haplokinety is detaching the polykinety, and inversely. As a result, a system arises (beginning from the periphery): haplo—poly—haplo—poly, i.e. two systems of haplo- and poly-, coiled one upon the other, uncoiling subsequently. The infundibular apparatus undergoes a fission during the division, but the details of this process are not exactly known.

As follows from the above considerations, the adoral kineties in *Peritricha* avoid the transverse division. Neither regression and reconstruction of the adoral system occur nor formation de novo of the adoral apparatus in a fixed place in both daughther individuals or in one of them, as it usually takes place in *Holotricha* and *Spirotricha*. Consequently such a doubling of the adoral apparatus like in *Peritricha* fails to occur in any other form.

Another character of the morphogenetic processes in *Peritricha* should be mentioned although it concerns not the whole group but only *Mobilia;* nevertheless it is involved — no doubt — by the general scheme of structure and division genuine for the *Peritricha.* This character is the transmission of the by half reduced skeletal ring with the twice reduced number of links to the progeny during division, and subsequently formation de novo in the juvenile individuals of the new ring with a normal number of links and a normal, for this species, diameter. This phenomenon is one of the best examples of a post-division reorganization of structures not belonging to the cortical ciliary system.

Sexual processes

The course of the sexual processes in *Peritricha* contains also many specific characters distinguishing this group in some degree from other *Ciliata.* Those specifities may be accounted for by the sessile mode of life in *Sessilia* and by preserving those characters also in the free-swimming *Mobilia.* Besides, they are not generally common neither in one nor in the second group.

Those specific characters consist — before all — in occurring of two types of conjugants: macroconjugants with features of a trophic individual and microconjugants which are much smaller and arise as result of divisions of the trophic individual, rapidly following each other. In sessile species, the macroconjugants usually remain upon their stalks, the microconjugants become free. Those heteroconjugants play in conjugation different roles; in extreme cases the microconjugant dies and its nuclear set and the cytoplasm are resorbed by the macroconjugant (D a v i s 1947, R a a b e 1952, and others).

Interesting findings are supplied by the study of post-conjugants executed mostly on *Urceolariidae.* D a v i s 1947 reported an interesting phenomenon of post-conjugational reorganization of organelles: a new skeletal ring is formed to replace the old one which was still quite „fit" for function. No doubt, the systems of post-conjugant should be adapted to the new genom different from the genom of pre-conjugant.

Despite its specifity, the course of conjugation in many *Peritricha* is not deviating essentially from this process in other *Ciliata.* At any rate, similar processes occur in *Suctoria* (K o r m o s 1957, 1960). Possibly the post-conjugational reorganization of the non-nuclear systems is also a more general phenomenon and in *Urceolariidae* it only finds exceptionally favorable conditions for manifestation.

Taxonomic position of *Peritricha*

Peritricha are distinguished from other *Ciliata* by several essential features; they namely are characterized by:

1. The external axial body symmetry, expressed in the circular pattern of the adoral spiral, of the aboral ciliary ring, of the pellicular fibres and of structures deriving from the scopula.
2. The complete closure of the aboral ciliary system and fibres system on the body walls.
3. The adoral spiral sinistrorsal (like in *Hemispeiridae,* in contrast with *Spirotricha*), but evolutive and abapical, with specific structures of kineties.
4. Passage of the division plain along the main body axis, but across the

aboral kineties, omitting the adoral spiral which avoids the cross division and divides by a longitudinal delamination.

All those characters, strengthened by the specificity of the morphogenetic processes, oppose *Peritricha* to other groups — *Holotricha* (with *Suctoria*) and *Spirotricha*. *Holotricha* as well as *Spirotricha* are characterized by:

1. Bilateral symmetry or secondary dissymmetry of the general body structure, except the primitive forms of a primary axial symmetry (body axis: mouth — outlet of C. V.) and meridional course of kineties.

2. Bipolarity of kineties and actually their parallel course, at least lack of circular systems fully closed around the body. Such systems fail to occur in forms of a secondary axial symmetry or with elements of such a symmetry (*Oligotricha, Tintinnoidea, Licnophora*).

3. Another type of torsion of the adoral spiral (if present) and, generally, of the pattern of adoral kineties which may be clearly reduced to the type of ambihymenium structure (UM + nAZM — R a a b e 1963).

4. Perkinetal transverse division, except for the cases of a typical budding connected with reproduction of the whole pattern of the kineties system. In cases of impossibility of division of the adoral kineties — their reduction and reorganization in both or in one of the daughter individuals.

Those differences seem not only to distinguish *Peritricha* sufficiently but even to oppose them to the both other groups in the same degree as it is the case of *Holotricha* or *Spirotricha*. It seems that *Holotricha* and *Spirotricha* have more common with each other than any of those groups or both together have with *Peritricha*.

This distinctness of *Peritricha* might be objected by arguments that their characteristic features are connected with the adaptation to the sessile mode of life and are not contradictory with their connection with *Holotricha* so as it was recognized in the case of *Chonotricha* or *Suctoria*. Nevertheless, it should be noted that although the adaptive changes in those two groups advanced farther than in *Peritricha* yet their close bond with *Holotricha* is not broken — if not in the trophic stages so at least in the morphogenetic processes and in development stages.

In *Chonotricha* the buds ressemble so much to *Hypostomata* that they might be included to them (G u i l c h e r 1951, D o b r z a ń s k a - K a c z a n o w s k a 1963). The transformation of the tomite into a sedentary and highly modified trophic form is not destroying, but only slightly modifying, the kineties system (somatic in contrast to the definition of C o r l i s s 1961). Really, the reproduction of this pattern in budding is impossible by means of division and its subsequent formation de novo as a whole in a bud — and this is the reason why *Chonotricha* might be given the rank of suborder in the order of *Gymnostomata*.

Also in the buds of many *Suctoria*, a pattern of ciliature was reported ressembling plainly to *Holotricha* or even to *Gymnostomata*, although not so exactly as in the case of *Chonotricha*. In contrast to *Chonotricha* this pattern disappears in trophonts, the body is highly modified, suctorial tentacles arise etc. In budding, the pattern of ciliature is reproduced de novo in the cortical layer of the mother individual on its spot belonging to the future bud (K o r m o s 1957, 1960). For that reasons it should be agreed that *Suctoria* went far off from their ancestors in the type of primitive *Holotricha* structure and developed radially. Their bond with *Holotricha* is of such a kind, that they

might be recognized as their equivalent group (a separate classis) as well as one of the orders of the classis *Holotricha*. It should be agreed however, that the bond *Holotricha* — *Suctoria* is, no doubt, stronger than the bond *Holotricha—Peritricha*, I think therefore possible to assume this second position and to recognize *Suctoria* as a well distinguished order in the classis *Holotricha*.

Considering the above analysis, it may be concluded that *Peritricha* occupy a quite different position than the groups just discussed: nothing — either in their morphology or morphogenesis — is inclining them towards *Holotricha* or *Spirotricha* or to any order of those classes. Such connections are neither supported by attempts of analysis and interpretation of the buccal apparatus and its morphogenesis. This what is common in *Peritricha* and *Holotricha* (or *Spirotricha*) — is the common character of all the *Ciliata*.

If the derivation of *Peritricha* from any primitive group of *Holotricha* was documented (which may be done for *Spirotricha*), even this argument should be locked upon as not essential. Any taxonomic group — despite its connections to another one — showing features of a different, specific and equally advanced evolutionary development, may be recognized as equivalent of the group to which its ancestral forms belonged (similarly as *Trematoda* and *Turbellaria*, *Arthropoda* and *Annelida*, *Aves* and *Reptilia*).

Summarizing all that was said above — I consider *Peritricha* as a group so highly distinguished from *Holotricha* and *Spirotricha* that it deserves a position equivalent of them. As to the rank which should be admitted to those three general groups of *Ciliata*, I support my view (Raabe 1948, 1964) recognizing *Holotricha* Stein 1859, *Spirotricha* Bütschli 1889 and *Peritricha* Stein 1859 as separate classes, belonging to the well distinguished phylum *Ciliata*.

Summary

Associating the discussion on the rank and taxonomic position of *Peritricha*, the author opposes to the theses of Fauré-Fremiet and Corliss who include this group to *Holotricha*. After a review of opinions on this subject, the characteristic features of *Peritricha* have been discussed, concerning the axial symmetry of their body, the adoral apparatus, the aboral apparatus, division and morphogenesis as well as the sexual processes. Basing on the clearly proved specificity of those features, the author postulates the recognition of *Peritricha* Stein 1859, as a separate classis of the phylum *Ciliata*, equivalent of two other classes: *Holotricha* Stein 1859 and *Spirotricha* Bütschli 1889.

STRESZCZENIE

Na tle toczącej się dyskusji na temat rangi i stanowiska systematycznego *Peritricha*, autor przeciwstawia się tezom Fauré-Fremiet i Corliss'a, włączającym tę grupę do *Holotricha*. Po dokonaniu przeglądu sądów na ten temat, omówione zostały cechy charakterystyczne *Peritricha*, dotyczące osiowej symetrii ich ciała, aparatu adoralnego, aparatu aboralnego, podziału i morfogenezy oraz procesów płciowych. Na podstawie stwierdzonej swoistości tych cech, autor postuluje uznanie *Peritricha* Stein 1859 za odrębną gromadę typu *Ciliata*, równorzędną dwu innym gromadom: *Holotricha* Stein 1859 i *Spirotricha* Bütschli 1889.

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Morphology and evolution of *Ciliophora*. IV. Sapropelebionts of the family *Loxocephalidae* fam. nova, their taxonomy and evolutionary history

Морфология и эволюция *Ciliophora*. IV Сапропелебионты из семейства *Loxocephalidae* fam. nova, систематика и эволюционная история

One of the tasks of the modern protozoology is the revision of the system of *Ciliophora* basing on the phylogenetical principles (Corliss 1960). The order *Hymenostomatida* attracts a special attention, since many these forms were included previously (Kahl 1926) into an unnatural, artificial family *Frontoniidae*. The main object of this investigation, genus *Loxocephalus* Eberhard 1862, was ranked among *Frontoniidae* (Kahl 1931, Gaevskaja 1949), and later among *Tetrahymenidae* (Bovee 1960, Corliss 1961). Moreover, Hall 1953 admits that *Loxocephalus* is not an independent genus but only a synonym of *Glaucoma*.

The taxonomic history of *Loxocephalus* is unusually complicated and tangled. The type-species, *L. luridus* Eberhard, 1862, was recorded only twice (Blochmann 1895, Kahl 1926) during the 100 years from its discovery. The first author has included it into the genus *Frontonia;* André 1912 also mentions *F. lurida* in a catalogue of Swiss *Ciliophora*.

Before Eberhard, different authors have repeatedly described ciliates, resembling *Loxocephalus* in their outlines, but of much smaller dimensions: *Cyclidium milium* O. F. M., 1773, *Paramecium griseolum* Perty, 1852, *Loxocephalus granulosus* Kent, 1882. Descriptions and figures of these authors are highly imperfect; only the diagnosis and perfect figures of *Paramecium milium* (O. F. M., 1773) Ehrbg., 1838, given by Fromentel 1874 gave possibility of its correct determination. Independently from this author, Stokes 1885, 1888 has described the similar, but not identical form as the type-species of his new genus *Dexiotricha* (*D. plagia* Stokes, 1885). This genus was not adopted by any following author; generic name *Dexiotricha* was commonly regarded to be a synonym of *Loxocephalus* (beginning from Bütschli 1889). Kahl 1926 has transferred *Dexiotricha plagia* into the genus *Loxocephalus;* later many authors regarded this species as synonym of *L. granulosus*. Another species studied by Stokes — *Dexiotricha centralis* Stokes, 1888, was included correspondingly into the genus *Loxocephalus* (Kahl, 1926), and later into a se-

parate genus *Dexiotrichides* Kahl, 1931. More new species of *Loxocephalus* were described by Smith 1897, Kahl 1926, 1928, 1931 and recently by Vuxanovici 1960. Kahl 1931 excludes from this genus *L. singularis* Kahl, 1926 (transferring it into *Cyclidium)* and *Loxocephalus putrinus* Kahl 1926 (synonym of *Uronema marinum*).

In the resort, 14 species are ascribed now to *Loxocephalus*. Distinction between this genus and *Dexiotrichides, Balanonema, Uronema* and some other closely allied genera is highly uncertain. It is practically impossible to make correct and reliable determination of any species, ascribed to *Loxocephalus,* except 2 well-defined ones, *L. luridus* and *L. intermedius*. E. g., the pure (clonal) line designated as L-16, cultivated during 1961, might be determined, in equal degree, as *„L. annulatus"*, *„L. lucidus"*, *„L. simplex"*, *„Dexiotricha plagia"*, so unessential and doubtful are distinctions in their diagnoses. No attempts were undertaken to use cloning, staining and impregnation methods in their classification.

The necessity of systematic revision of *Loxocephalus* and allied genera is evident.

Material and methods

This investigation was made in July—December 1961. *Loxocephalus luridus* was isolated from sapropel of the lake Možajskoe, near Leningrad; on many points along the shore-line it was a dominant species. It was never recorded in any other pond in Leningrad vicinity. Three species of *Dexiotricha,* including type-species *D. plagia,* were isolated, and successfully cultured in laboratory, from five scattered ponds and lakes in Kiriši region, 150 km far from Leningrad. *Uronema marinum,* the most common sapropelebiont, was established in cultures using material from the pond in the park of Forest-Technical Academy in Leningrad. Two species of *Cyclidium* were isolated from the Green Lake near Budogoš, in Kiriši region. Except *Loxocephalus luridus,* all the mentioned species willingly multiply in test-tubes with a few boiled hay leaves on the bottom. *L. luridus* in such culture-medium multiplies very slowly; nevertheless, this species was so abundant in fresh samples from the lake Možajskoe, that it was no need in the additional establishment of laboratory cultures.

Cyclidium glaucoma and *C. citrullus* were stained, after Bouin's fixation and adhesion following Chen 1944, with Feulgen's nuclear reaction. The same species as well as all the others were stained with Böhmer's hematoxylin. For detection of the ciliary apparatus the ciliates were impregnated after Gelei-Horvath (preferably), after Klein and, in the case of *Loxocephalus luridus,* also after Corliss wet technique.

The wet methods of Hungarian investigators (Gelei und Horvath 1931) and of American ones (Corliss 1953) were proved to be equal with respect to difficulty of manipulation and perfectness of results.

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Family *Pleuronematidae* Kent 1882

Genus *Cyclidium* O. F. M. 1773

The description of these forms is indispensable, since *Cyclidium* is in the closest relationship with *Uronema*, the most primitive genus in the new family *Loxocephalidae* (as well as *Cyclidium* itself is one of the most primitive pleuronematids). Up to the present time, several tens species of *Cyclidium* were described; nevertheless only 2 are admitted as reliable (Berger 1959).

Generic criteria of *Cyclidium* are the following: small (near 20 μ) elongate ovoid forms with small number (near 15) of ciliary somatic meridians, always with a long caudal cilium; anterior body end cilia-free; cytostome unproportionally large, exceeds one half of the ventral surface of body; internal membranellae small, dextrally shifted; external undulating membrane giant, prominent; ovoid macronucleus and spherical micronucleus (always single) are located anteriorly; contractile vacuole single, posterior. Both reliable species of this genus inhabit the polysaprobic zone of fresh-water ponds; *C. glaucoma* is one of the most common ciliates, recorded from the tens of natural sources; *C. citrullus* is of rare occurrence, it prefers the fresh-water sapropel. Only *C. glaucoma* (type-species) is to be described below.

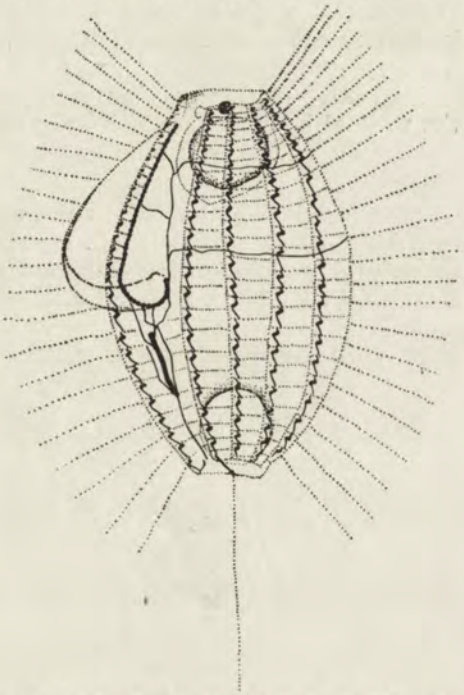

Fig. 1. *Cyclidium glaucoma* O. F. Müller, the most primitive, but true member of *Pleuronematidae*. After preparation, impregnated with modified Klein's technique. Schematized

Cyclidium glaucoma O. F. M., 1773

Morphology of *C. glaucoma* was repeatedly described in literature (Klein 1927; Párducz 1936, 1940; Gelei 1950; Berger and Thompson 1960); we have noted some details, unrecorded previously.

Body size of *C. glaucoma* is 18—20×9 μ; body ovoid, with blunt cilia-free anterior end (Fig. 1). Posterior body end is also devoid of cilia; we find here

only one large basal body of caudal cilium, connected by two fibrils (kinetodesms) with two opposite somatic ciliary meridians. Somatic cilia are gathered into 11—13 longitudinal rows (meridians); postoral meridian single, with no cilia, in this place dark argentophilic stripe (cytoproct) is revealed. In the anterior body part the somatic cilia are more dense than in the posterior one. The movement behavior of *Cyclidium* is very peculiar: sharp jumps are alternated with prolonged periods of resting.

On the dry mounts of both *C. glaucoma* and *C. citrullus*, impregnated with 5% $AgNO_3$ in diluted gelatine, washed and reduced in very weak (below 1%) methol solution, we have revealed the fine system of argentophilic pellicular rectangulars (Fig. 1), not described previously. Within each rectangular, we see 2 basal bodies of cilia closely allied to one another, shifted to the right upper angle of these structures. Such system is not peculiar for any known member of *Tetrahymenidae*, but is typical for all the members of *Loxocephalidae* (see below), and for *Uronema*.

Peristome of *C. glaucoma* is unproportionally large, exceeding one half of the ventral body surface. Thompson 1958 and de Ruiz 1959 have demonstrated within the peristome 3 elongated thin right-displaced internal membranellae.

Macronucleus and closely adjacent mirconucleus occupy the anterior body end. In the related species, *C. citrullus*, amicronucleate animals are common in natural populations, giving rise to amicronucleate lines (Berger 1959, Jankowski 1962). Such animals or lines were never established in *C. glaucoma*. The process of vegetative division is shown in Fig. 2; division rate in fresh cultures is near three fissions a day. Micronucleus of *C. glaucoma* is

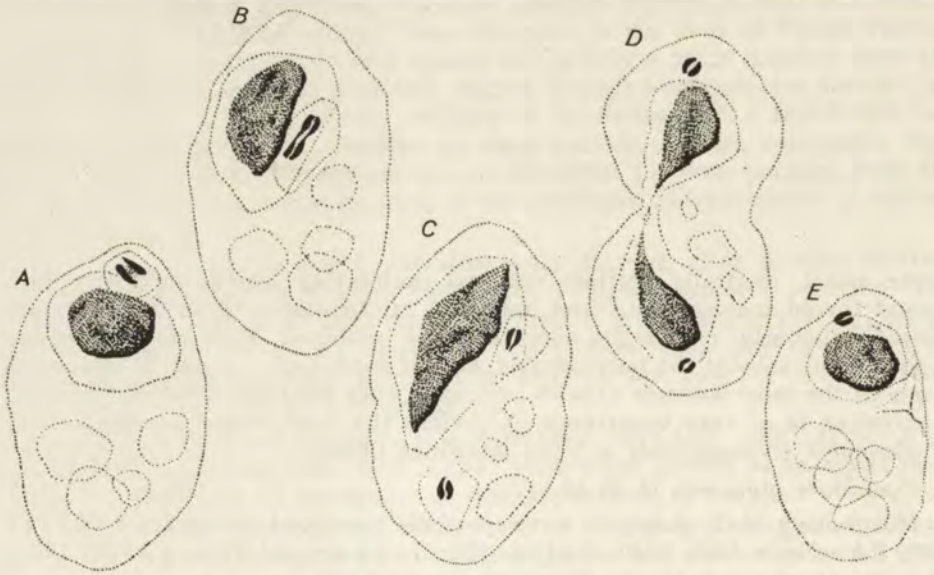

Fig. 2. *Cyclidium glaucoma* O. F. Müller, after Feulgen-stained preparations; vegetative division process

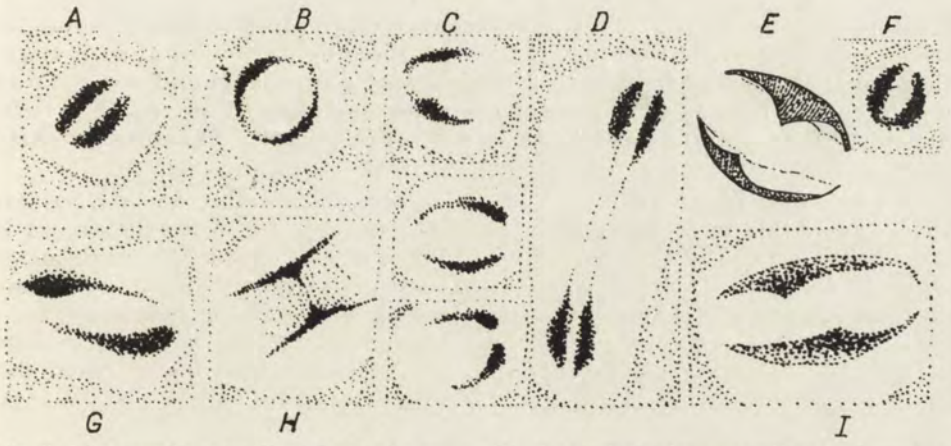

Fig. 3. Micronucleus in *Cyclidium glaucoma* (Feulgen-stained preparations); A — frequent shape of micronucleus in interphase, B — ring-like micronucleus in the prophase, C — its three optical sections, D — division of the micronucleus, E, F, I — hypothetical structure of micronucleus in the interphase, G, H — late prophase just before division

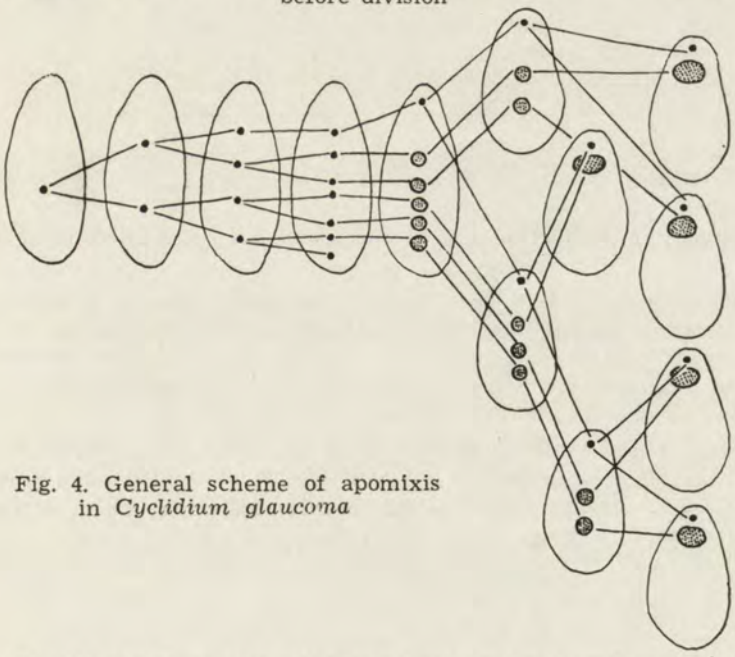

Fig. 4. General scheme of apomixis in *Cyclidium glaucoma*

of highly peculiar and uncertain structure: it is always composed of 2 separate Feulgen-positive bodies, that never fuse together or separate entirely from one another. They are not chromosomes, since during fission both are dividing into two equal parts due to tranversal constriction and breaking, but not to duplication of each part. Micronuclei of vegetative animals are very large (near 3,5 μ); soon after division (Fig. 2 E) they are twice smaller. This fact supports our hypothesis that micronucleus of *C. glaucoma* is constantly tetraploid. Fig. 3 A, presents Mi of freshly-divided cell, Fig. 3 G, H — that of

dividing animals, visible from two different sides. In some animals dividing Mi seems ring-like (Fig. 3 B); Fig. 3 C presents three optical levels of such stage, showing that we deal here with 2 independent components and not with an uninterrupted ring as it may seem from the first sight. Figs. 3 E, I present hypothetical organization of Mi of *C. glaucoma* in interphase or early prophase stage.

We did never observe conjugation of *C. glaucoma* in nature nor in laboratory cultures; this is probably secondarily agamic species. Nevertheless, as it was shown previously (Jankowski 1962), in cultures of this form about a half or sometimes even more, animals undergo apomictic nuclear reorganization process (Fig. 4). This process is going on with striking frequency, both in well-fed and starving cultures. During apomixis, micronucleus divides without the following cell-division (Fig. 5), both daughter nuclei reach the

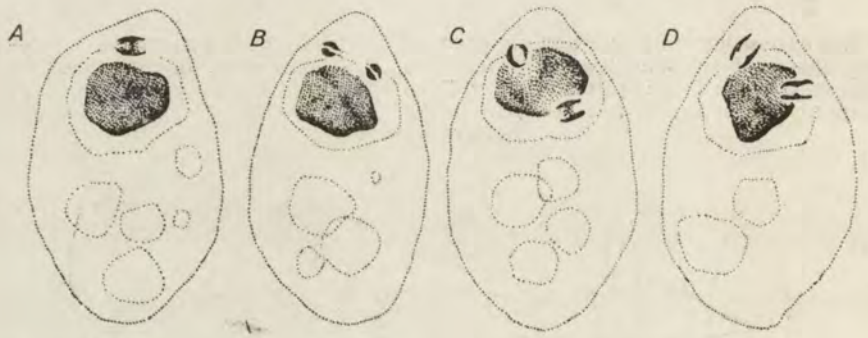

Fig. 5. First micronuclear division in apomixonts of *Cyclidium glaucoma*

volume of maternal nuclei and divide once more. Out of 4 small resulting nuclei 3 are growing later and undergo the 3 rd division, but one of them not divides (Fig. 6 A-F). Seven small nuclei thus produced never divide further, one of them becomes new Mi, six other — new macronuclei, distributed among the progeny of apomixont (Fig. 6 F-Q).

Encysting stages were never recorded for *C. glaucoma*, though wide distribution of this species in nature, e. g. in tree lichens (Thompson 1960) or in foam of cicade larvae indicates that resting cysts may exist in this species.

Family *Cohnilembidae* Kahl 1939

Genus *Uronema* Dujardin 1841

Small (near 30 μ) elongate-ovoid ciliates with asymmetrically curved anterior end; somatic cilia gathered into 15—16 meridians; caudal cilium always present; movement not jumping (unlike *Cyclidium*); anterior body end devoid of cilia; peristome not so large as in *Cyclidium* or any other pleuronematid: it is located centrally or somewhat anteriorly, and includes small, weakly developed undulating membrane and three small internal membranellae; pellicle always with argentophilic rectangular structures each with a pair of right-

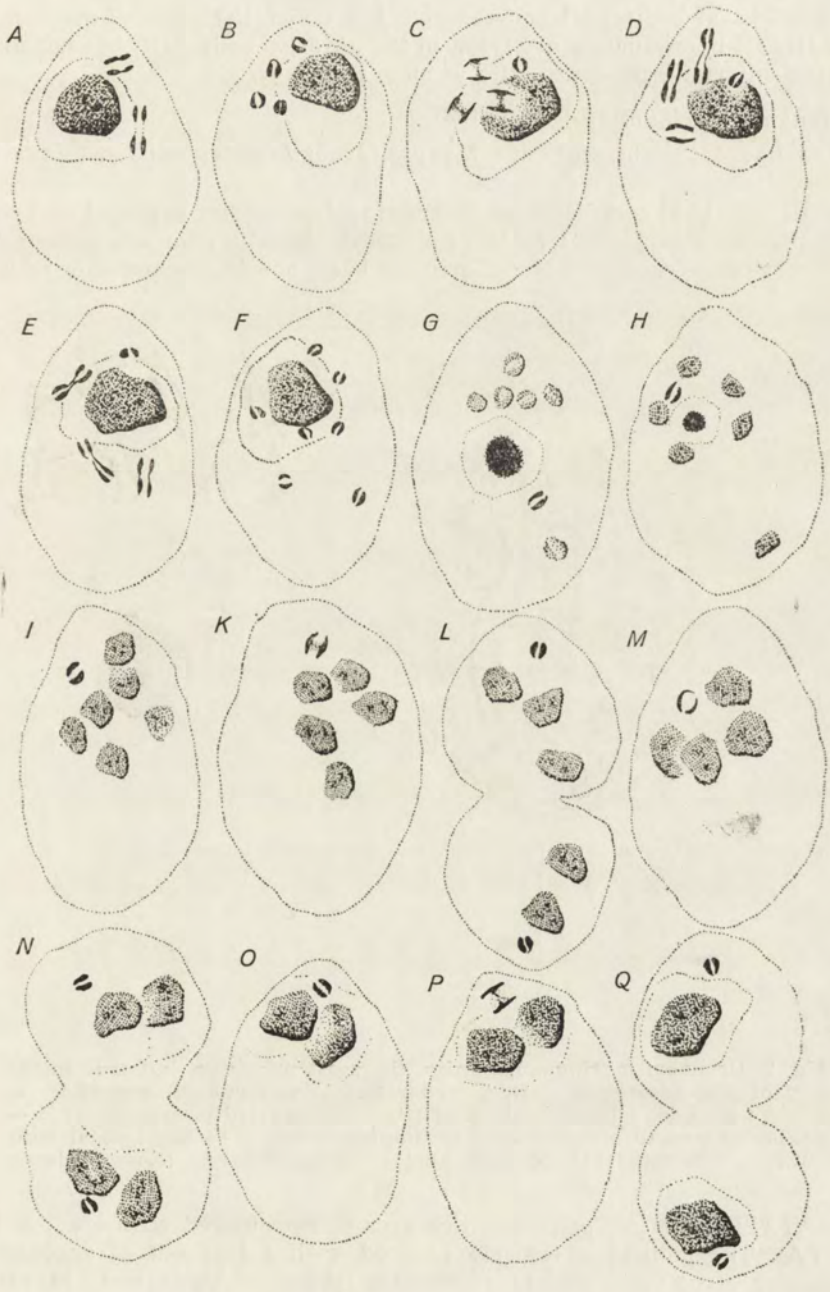

Fig. 6. *Cyclidium glaucoma* O. F. Müller, after Feulgen-stained preparations; details of apomixis; A—B — the second micronuclear fission; E—F — the third micronuclear fission; G—K — differentation of micronucleus and macronuclear anlagen; L—Q — restoration of the normal nuclear apparatus

-displaced basal bodies; trichocysts zone lies under the pellicle; macronucleus and adjacent micronucleus are lying in the anterior body half, but not directly near the anterior end; contractile vacuole posterior.

Uronema marinum Dujardin, 1841

syn.: *Cryptochilum nigricans* Maupas, 1883; *Loxocephalus putrinus* Kahl, 1926.

Small (28×16 μ) ovoid ciliates, common in fresh-water sapropel and polysaprobic marine biotopes (T u c o l e s c o 1962). Somatic cilia are gathered into 14—15 meridians, each with 16—17 pairs of basal bodies. The anterior body pole

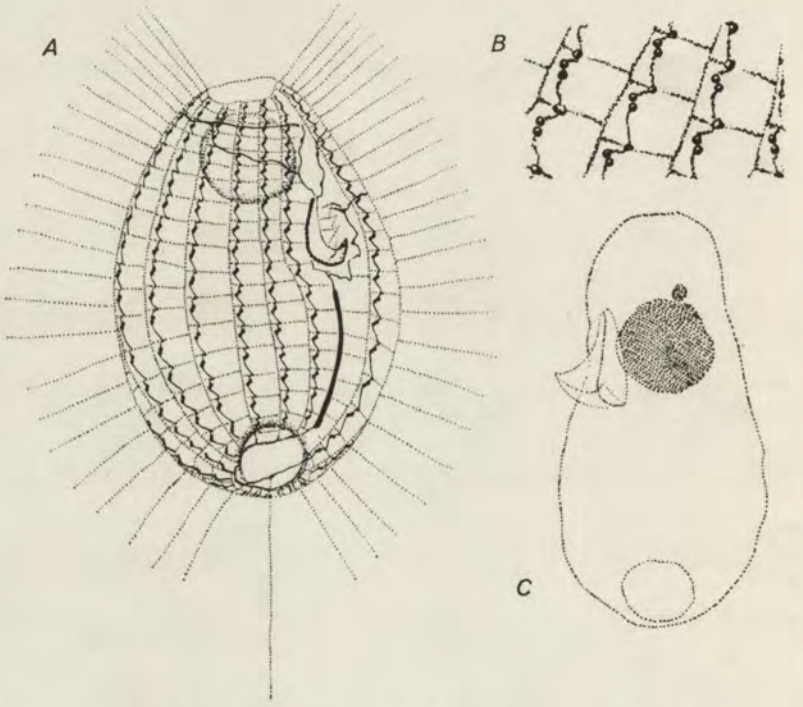

Fig. 7. *Uronema marinum* Dujardin, the most primitive, but true member of *Cohnilembidae*, after preparations impregnated according to Klein's method; schematized; A — after impregnated preparations; B — pellicular structure as revealed by impregnation; C — the typical body outline, cytostomal shape and nuclear disposition in living animals

is free of cilia, like in *Cyclidium;* this area is marginated with circular fibril (Fig. 7 A). The pellicle is entirely covered with a fine net of argentophilic rectangular structures, closely resembling those of *Cyclidium.* In each of these structures we may distinguish (Fig. 7 B) a pair of basal granules, shifted into the upper right angle, a pair of kinetodesms, connecting these granules with those of neighbouring rectangulars, and a single pore of trichocyst between the neighbouring basal bodies (the trichocysts are absent in *Cyclidium glaucoma).*

The oral apparatus of *Uronema marinum* includes (Fig. 7 A) the external unlulating membrane (very weakly developed), and three internal membranes, that may be distinguished in living conditions. Peristome, of triangular shape, is located in the anterior body half, subcentrally, but not directly near the anterior end, like in *Tetrahymena* or *Dexiotricha*. It is only 5—6 μ long (near $^1/_6$ of the body length), while the peristome of *Cyclidium* reach no less than $^1/_2$ of body length, the peristome of *Pleuronema* — near $^3/_4$ of the body length. Nevertheless, many small-mouthed *Uronema*-like ciliates were ascribed by K a h l 1931 to *Cyclidium*.

Cytoproct is revealed on the ventral body surface; it is large readily darkening stripe, occupying the place of postoral ciliary meridian. The upper end of cytoproct gives rise to thin branching fibril, that encircles the anterior body half like in *Cyclidium*. Somatic cilia are more dense in the anterior body surface than in the posterior one. Posterior body pole bears the caudal cilium, connected with two opposite ciliary rows.

The life-cycle of *Uronema* is complicated. Division occurs only in free-swimming animals; cysts of division („Vermehrungszysten" of *Colpoda*-type) are never formed. Large percent of animals, nevertheless, encysts in starved cultures; the ability of *Uronema* to form resting cysts differ it from the loxocephalids as well as from *Cyclidium*. In mass-cultures, started from a single specimen, conjugation stages were commonly observed; both partners unite like *Colpidium* or *Dexiotricha*, i.e. with their anterior ends, but not with their ventral sides, as *Frontonia* or *Paramecium* do. At last, polymorphism was noted in clones of *Uronema marinum*; amidst plenty of small (20 μ, typical size) specimens swim scattered giant (50 × 23 μ) animals, whose dimensions are conserved in many generations, when we establish subclones from these forms. This polymorphism is of another kind, than in *Tetrahymena* (W i l l i a m s 1960), since giant *Uronema* possess proportionally developed cytostome (in *Tetrahymena* giants are macrostomes). Giant *Uronema* are capable of conjugation; after several weeks the gigantism is lost and the culture return to the normal body size, except several specimens, that again yield cultures of „giants". We cannot say whether this polymorphism is a peculiarity of our stock or a feature of a whole species.

Macronucleus of *Uronema marinum* is located slightly above the peristome level, but not directly under the anterior body end, micronucleus is not compound (unlike *Cyclidium*). Apomictic nuclear reorganization, so common in *C. glaucoma*, is absent in *Uronema marinum*.

Uronema halophila (Kahl, 1931) comb. nov.

syn.: *Loxocephalus halophilus* Kahl, 1931; *Lox. ellipticus* Kahl, 1931.

Body elongated, with blunt anterior end; body size near 25 × 12 μ. *U. halophila* inhabit the lower layers of sapropel, penetrating in it deeper, than *U. marinum*. Unlike *Dexiotricha, U. halophila* never occur in mass population; in sapropel from different sources we can find only a few scattered specimens of this species.

In its general outline and colour *U. halophila* resembles *Dexiotricha plagia*: both species possess dark, not transparent cytoplasm filled with numerous refractile bodies (Fig. 8 A). In living condition (Fig. 8 A) we may distinguish: the cytostome near the anterior body end, spherical macronucleus located centrally or somewhat shifted into the posterior body half, grayish food-

vacuoles in the anterior body half, and, finally, a single contractile vacuole located in the posterior body part, but not directly near the posterior pole. Cilia are long; posterior body end bears a long prominent caudal cilium. *Uronema*, as well as the loxocephalids, never ingests purple bacteria, the preferred food of many other sapropelebionts (*Metopus*, *Brachonella*).

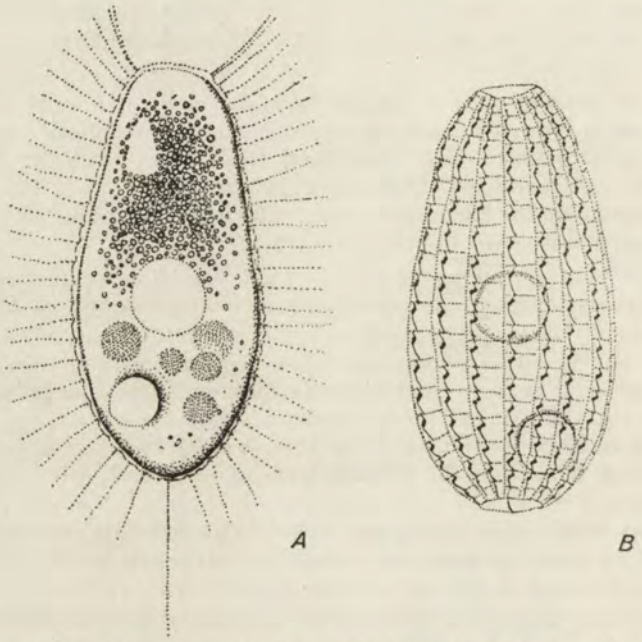

Fig. 8. *Uronema halophila* (Kahl) Jankowski; A — in living conditions; B — after impregnation with dry Klein's technique, dorsal side; schematized

In specimens impregnated after Klein (Fig. 8 B) there become visible 13—14 longitudinal ciliary meridians and the net of argentophilic pellicular structures; small (like in *U. marinum*) medial cytostome on the ventral side, and a single postoral ciliary meridian, bearing no cilia and occupied by cytoproct.

U. halophila divides in free-living condition; conjugation or cyst-formation were never observed.

Family *Loxocephalidae* fam. nova

Genus *Dexiotricha* Stokes 1885

Ciliates of moderate size (50—70 μ); body elongated, not ovoid, always dark and not-transparent due to numerous refractile mineral bodies in the cytoplasm; the cilia gathered into 25—35 longitudinal meridians; anterior body end free of cilia, posterior end with a long caudal cilium; 7 circular or semicircular (unclosed) ciliary rows are coming on the cytostome level; out of 3 postoral ciliary meridians two possess complete ciliation, one — only several

cilia, its main part is occupied by stripe-like cytoproct; trichocysts are commonly present; contractile vacuole in impregnated specimens looks like an ampulla; body surface is covered with fine net of argentophilic rectangulars, each containing the basal bodies in right upper angle. Cytostome is subanterior, not subcentral, relatively small, with small external membrane and three internal membranelles; its external upper edge bears also an additive membrane-like structure. Macro- and micronucleus are localized in central body part or near the posterior end, but not near the anterior one. Contractile vacuole is localized in the same region.

Type-species: *D. plagia* Stokes, 1885.

Dexiotricha plagia Stokes, 1885

syn.: *Loxocephalus plagius* (Stokes) Kahl, 1931; *Loxocephalus lucidus* Smith, 1897; *Loxocephalus annulatus* Kahl, 1926; *Uronema simplex* Penard, 1922; *Loxocephalus simplex* (Penard) Kahl, 1926; *Colpidium pannonicum* Gelei, 1932

Frequent in fresh-water sapropel, but never reported in literature from any sea biotope (unlike *Uronema*). Body size 61 × 27 μ (the largest known species of *Dexiotricha*). Body elongated, with somewhat rounded anterior and posterior ends; anterior body part (above the cytostome) in living specimens is asymmetrically sinistrally-curved, when observed from the ventral side (Fig. 9).

Under small power of the microscope the animals looks brownish or even black, but one may easily state, using immersion-objectives, that this „staining" is not due to pigmentation of the cytoplasm but to numerous spherical scattered bodies, that are colourless but highly refractile. These bodies are not preserved in animals fixed with Bouin's fluid, they dissolve very easily in diluted acids or alkalies, and cannot be stained with mercuric-bromphenolblue solution, reagent for proteins; they may be electively demonstrated with van Cossa method, using $AgNO_3$. This analysis shows that we deal with typical mineral inclusions, common in ciliates (e.g., in *Paramecium*) in form of crystals or concretions. Fauré-Fremiet 1957 has demonstrated mineral nature of these bodies in „*Loxocephalus granulosus*" (*Dexiotricha* sp.) by direct cytochemical analysis. The presence of these inclusions in *Dexiotricha* permits easy distinguishing of this genus from *Colpidium*, in living condition, since all the known species of *Colpidium* are colourless. Mineral inclusions in cytoplasm of *Dexiotricha* are markedly decreasing in starving clonal cultures, up to their entire loss in depressive animals.

Macro- and micronucleus are located in the midst of the body; the contractile vacuole, looking like a small ampulla (after wet impregnation), is also located on macronuclear level, below the ventral body surface.

Cilia are gathered into 38 meridians, that come parallel each to another on the dorsal surface and meet each other at the angle, on the ventral surface. Thus the kinetome of the left and right body sides is not symmetrical: on the left (in Fig. 9 — right side) side of animal ciliary meridians are lying along the longitudinal body axis; on the right, they come at the angle to this axis and only some of them reach the posterior body end (Fig. 9, Pl. I 5). There are 3 postoral meridians, that not reach the anterior body pole; out of them, two extremal meridians reach the posterior body end, but the middle one bears only 3 pairs of basal bodies just below the cytostome; in the posterior body part it is continued as a long thick argentophilic cytoproct. Posterior

body end bears rounded flat area, almost free of cilia (except the single basal granule of the caudal cilium). Like in *Cyclidium* and *Uronema,* this granule is connected with two opposite ciliary meridians.

The peristome is subanterior; it forms a small traingular concavity with 3 internal membranellae and a large marginating external membrane. Large bag-like cytopharynx proceeds from the cytostome into the body. On the right upper angle of the cytostome, just above the undulating membrane, a stripe composed with 5—6 closely placed basal bodies is seen; it very resembles the basal stripe of an undulating membrane, but is shorter. I was

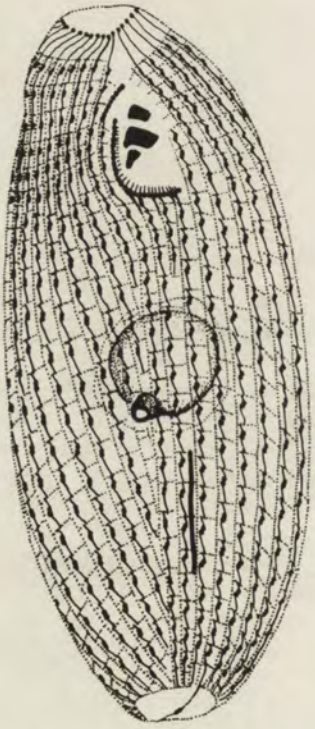

Fig. 9. *Dexiotricha plagia* Stokes; after preparation, impregnated following wet technique of Gelei and Horvath; schematized

not able to trace, whether this stripe bears a membrane (fused cilia) or separate cilia. In any case, it intensifies the function of the undulating membrane. Purely conventionally, for the simplicity of referring, I designate this structure as „additional membrane" of the ciliary buccal complex — M_5. If it would be proved that this structure is a separate membrane, then the buccal apparatus of *Dexiotricha* and *Loxocephalus* was pentahymenous, not tetrahymenous one. This detail is by no means significant in our decision to establish the new family.

The anterior body half, on the cytostome level, is encircled with 7 distinct transverse ciliary rows, especially clear on the right side of the animal (Fig. 9). The rows are formed by the upper basal bodies of all the somatic meridians. It is evident that these circular rows serve for intensification of the feeding activity of the animal: they produce an additional water current, carrying the

food particles into the cytostome. In the preceeding paper (Jankowski 1963) was demonstrated the differentiation of the kinetome of various sapropelebionts into three separate zones: somatic (movement), buccal (ingestion) and perizonal (intensifying of water current in the buccal area). The seven, mentioned above, ciliary rows on the cytostome level are analogous to the perizonal stripe of *Metopus*, perizonal spiral of *Caenomorpha*, perizonal ciliary field of *Stegochilum*, and may be also called „perizonal" rows. Together with the „additional membrane M_5" these rows form the so-called „perizonal complex" modification of somatic ciliature in the service of the cytostome.

The entire pellicle of *Dexiotricha plagia* is covered with a net of numerous argentophilic rectangular pellicular structures (near 870, in *Uronema marinum* near 240), each with 2 basal bodies, shifted into the upper rigth angle of these structures. Such rectangulars were noted previously in *Dexiotricha* sp. by Klein 1928 and Gelei 1940, 1950.

D. plagia may be easily cultivated on hay infusion, giving flourishing cultures of unusual density. The animal divides twice a day. Encysting was never observed in cultures, both fresh and starved; in many lines selfing-conjugation was noted, but no less than 60—70% of clones were stable in this respect. It may seem probable thus that mating-types may exist in *Dexiotricha*, like in *Tetrahymena* (Elliott 1959), *Paramecium* (various authors) and possibly in some other hymenostomatids. Cytological picture of conjugation process in *Dexiotricha plagia* will be presented separately; it exhibits far-coming resemblance with that of *Tetrahymena pyriformis* (Ray 1956) or *Colpidium*.

Dexiotricha raikovi sp. nov.

Species name is given in honour of Dr. Igor B. Raikov (Cytological Institute, Leningrad).

In living condition this species is indistinguishable from *D. plagia*: both forms possess the coinciding body shape and tint; macronucleus, micronucleus and contractile vacuole are in both species central. *D. raikovi* is somewhat smaller, than *D. plagia* ($50 \times 23\,\mu$ versus $61 \times 27\,\mu$), but they cannot be determined using only this characteristic. On the other hand, *D. raikovi* may be easily distinguished from *D. plagia* on impregnated mounts (Figs. 9, 10).

D. raikovi possesses only 20—22 ciliary meridians, each with 20—23 pairs of basal bodies. Anterior body part is encircled with 7 unclosed semicircular perizonal ciliary rows, i.e. the transversal ciliary rows on the cytostome level are seen ventrally on the right side and on the dorsal side, but not ventrally on the left side. In this respect *D. raikovi* resembles *D. milium*, but not *D. plagia*. *D. raikovi* differs from *D. milium* in another position of its nuclei: in *D. milium* both macro- and micronucleus are posterior — in *D. raikovi* both are central, like in *D. plagia*.

The ciliary complex of the peristome includes the external membrane M_1, three internal membranellae M_2, M_3 and M_4, and the so-called additional membrane M_5, on the right outer margin. Contractile vacuole in impregnated specimens looks like a long ampulla (Fig. 10 B). Cytoproct, like in other *Dexiotricha*, looks like highly argentophilic, prolonged stripe, that runs along the body axis on the ventral body surface. Like in *D. plagia*, kinetome is asymmetrical on the right and left sides of the ventral surface.

Pellicle bears near 480 argentophilic rectangular structures, each with the

shifting of the basal bodies into the right upper angle, so characteristic for *Dexiotricha*. A zone of trichocysts is present under the pellicle.

Selfing-lines are common in *D. raikovi*; nuclear processes in maiting animals follow the same scheme, as in *D. plagia*. Exconjugant of *D. raikovi* possesses 1 micronucleus and 3 developing macronuclei, thus giving rise to 3 caryonides (Pl. II 15—17). No doubt, description of the conjugation process in „*Loxocephalus granulosus*" (*Dexiotricha* sp.) by Behrend 1916 is mistakable; Behrend states no pronucleogenesis, nuclear exchange nor syngamy in this process. Just these stages are common in our preparations.

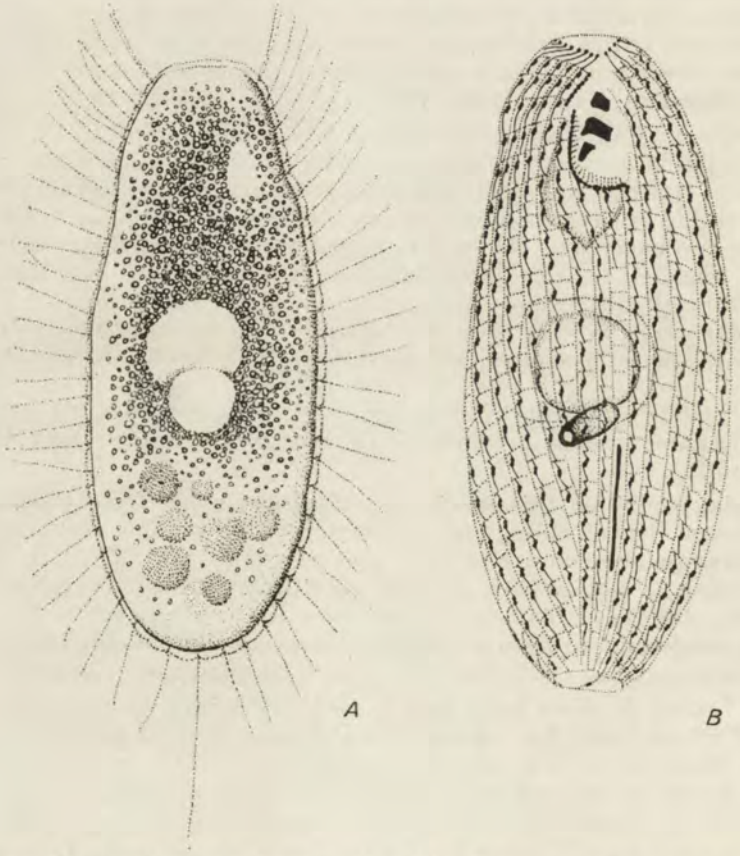

Fig. 10. *Dexiotricha raikovi* Jankowski, after preparation, impregnated after Gelei-Horvath (B) and in living conditions (A)

Cytological knowledge of the conjugation processes in *Ciliophora* is of great importance for their correct determination; e.g., freshly-disjoined exconjugant of *Loxocephalus* contains 3—7 micronuclei and a single growing macronucleus; in *Dexiotricha* it contains 1 micro- and 3 macronuclei; in *Colpidium* — 2 micro- and 2 macronuclei. Morphologically closely related species of *Paramecium* (e.g., *P. woodruffi* and *P. polycaryum*) may be easily distinguished basing on their conjugation processes (in *P. woodruffi* macronuclear fragmenta-

tion begins from the actual beginning of conjugation, while in *P. polycaryum* it takes place only after disjoining of a pair).

Only *Loxocephalus moniligranulatus* Kahl, 1926, resembles *Dexiotricha raikovi*. The first species, nevertheless, possesses as few as only 12—14 ciliary meridians, while their number in *D. raikovi* was estimated as 20 to 22.

Dexiotricha colpidiopsis (Kahl, 1926) comb. nov.

syn.: „*Paramecium milium* (Müll.) Ehr., 1836" in Fromentel 1874, not in Ehrenberg 1836; *Loxocephalus colpidiopsis* Kahl, 1926; *Loxocephalus enigmaticus* Vuxanovici, 1960.

Müller 1773 and 1786 has described 10 species of *Cyclidium*, among them *C. milium*. His description and figures exclude any possibility of correct determination of *C. milium*; probably it was a species of *Colpidium*. Ehrenberg 1838, who had right to reject this name, has transferred *C. milium* into *Paramecium* and described and figured under the name *Paramecium milium* (Müll.) Ehrbg. a species of *Pleuronema*. I see no sense in transferring *Paramecium milium* into the genus *Pleuronema* Duj. 1841, since it will be only new synonym of the type-species, *Pleuronema crassum* Duj., 1841. In any case it is clear now, that Fromentel 1874, who decribed and figured under the name „*Paramecium milium*" a species of *Dexiotricha*, has mistaken in such designation, since *Paramecium milium* of Ehrenberg is giant-mouthed *Pleuronema*. The species of *Dexiotricha*, figured by Fromentel, must have been designated thus not as „*Dexiotricha milium* (Ehr.) comb. nov.", but as „*Dexiotricha colpidiopsis* (Kahl) comb. nov."

D. colpidiopsis is frequent in the sapropel; its body shape and size (51 × × 24 μ) resemble those of *D. raikovi*, but its Ma, Mi and contractile vacuole are lying near the posterior body end (Fig. 11), on the level of the cytoproct. Cytoplasm is filled with a number of mineral bodies, that give the grayish tint to the animal; these inclusions darken readily when employing dry impregnation techniques, but not during wet treatment (following Gelei—Horvath or Corliss). Somatic cilia are gathered into 24 ciliary rows (if counted along the equator); they are running parallel to each other or to the body axis that differs *D. colpidiopsis* from *D. plagia* or *D. raikovi*. Three typical for *Dexiotricha* postoral ciliary meridians are present, one of them (middle one) is reduced to 3 pairs of cilia lying behind the cytostome.

Triangular subanterior peristome has the typical structure: it contains 3 internal membranellae (Pl. II 9), whose shape is much resembling that of membranellae in *Tetrahymena* (Corliss 1959 b) and *Sathrophilus* (Stout 1956; Thompson and Cone 1961, 1963). The right outer margin of the cytostome is occupied with an undulating membrane; a series of short fibrils is directed inward the cytostome from the basis of the membrane (Pl. II 13, indicated by an arrow). The additional membrane-like structure, M_5, contains in its basis several closely placed basal bodies of the somatic meridian neighbouring with the cytostome (Pl. II 9). The spacial distribution of internal membranellae is seen in the Plate I 3.

Anterior body part bears 7 semicircular unclosed perizonal rows. The anterior extremity of the body looks like flat, cutted area; it bears no cilia. Posterior body end bears a long caudal cilium. Contractile vacuole in *D. milium* is located near the posterior body end; in all the impregnated specimens it looks like a long thick-walled ampulla (Pl. I 6), though animals were fixed at

various stages of its functioning. No trace of such ampulla is visible in living specimens, where contractile vacuole is rounded, spherical. Probably this vacuole has constant walls; it becomes squeezed in fixed animals and then darkens in silver nitrate solution. The outer excretory pore of this vacuole is always single, while *Tetrahymena* possesses 2 to 4 such pores. As I know, such peculiar ampullae were not still discovered in any other ciliophoran; e. g. in *Tetrahymena* we may impregnate (using wet methods) the outer pores, but not the vacuole itself. Gelei 1925, 1935 and Gelei jun. 1939 have achieved almost elective impregnation of the contractile vacuoles in *Paramecium;* nevertheless in this case they revealed not a constant in shape ampulla but vacuoles and channels in such shape, in what they were catched by the fixative.

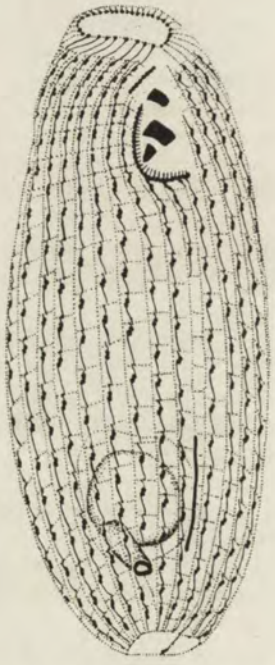

Fig. 11. *Dexiotricha colpidiopsis* (Kahl) Jankowski; after preparation. impregnated with Gelei-Horvath's technique; schematized

In cultures of *D. colpidiopsis,* like in *D. plagia* and *D. raikovi,* there occurs very frequently conjugation process. Nuclear processes in *D. colpidiopsis* are differing in some respects from those in *D. raikovi*; these data will be presented separately. Encystation process was never recorded in laboratory clones of *D. colpidiopsis.*

Genus *Loxocephalus* Eberhard, 1862

Large sapropelebiontic ciliates, resembling *Frontonia* in their body contour; body size among 120—160 μ; cytoplasm is blackish due to numerous mineral refractile bodies; somatic ciliary meridians are very numerous; anterior body end bears no cilia-free area, posterior one — with a tuft of the long caudal cilia; a trichocyst zone is seen under the pellicle. Cytostome small,

triangular, lying in the anterior body half; its ciliary apparatus is identic to that in *Dexiotricha*. Anterior body half is encircled with 9 semicircular transverse ciliary rows. Macronucleus single, spherical, centrally located; the number of micronuclei varies from 2 up to 8. *Loxocephalus* may be regarded to as a polymerized and complicated *Dexiotricha*.

Type-species: *L. luridus* Eberh., 1862 (The only other, more or less reliable species, is *L. intermedius* Kahl, 1928, known from salt water.)

Loxocephalus luridus Eberhard, 1862

syn.: *Frontonia lurida* (Eberh.) Blochmann, 1895.

Large (160×63 μ) ciliates of the ovoid body shape, closely resembling in the outlines the much common species of *Frontonia (F. leucas, F. microstoma)*. Ciliates, if freshly collected, looks black, (Pl. V 25), that permits easy distinguishing of *L. luridus* from grayish, greenish or clear *Frontonia*. *Frontonia* feeds, preferably with green and blue-green algae or diatoms; food vacuoles of *Loxocephalus* are colorless. Resemblence of *Frontonia* and *Loxocephalus* is only superficial, as it will be seen below.

The black body tint of *L. luridus* is due to the numerous highly refractile mineral bodies within the cytoplasm; in the dark field *Loxocephalus* looks entirely bright, while in *Paramecium* or *Frontonia* only mineral crystals are luminescent. In aged cultures *Loxocephalus* lose refractile bodies almost completely and looks clear (Pl. V 27).

Somatic cilia are gathered into 120 ciliary meridians; kinetome of the left and right body parts is not symmetrical: on the left (Fig. 12 A), the somatic meridians are running along the longitudinal body axis; on the right (Fig. 12 A; Pl. IV 24) they run at the angle to this axis; on the dorsal body surface (Pl. IV 22, V 26) they run again along the body axis. Kinetome of the anterior body half is highly complicated. Meridians located on the left side of the cytostome are turning above it on the right (Fig. 12 A, B), and run transversally to the body axis, passing then on the dorsal side (Pl. III 20). On the cytostome level, on the right (ventrally, Fig. 12 A) and on the dorsal body surface are seen 9 unclosed semicircular perizonal rows (Pl. V 26). Our preparations show quite identical structures in *Frontonia vernalis*, what may be considered only as a case of convergence, since *Loxocephalus* and *Frontonia* belong to quite different evolutionary lines. *Ophryoglena*, a ciliate of the same body shape, as *Loxocephalus*, has no transversal perizonal rows (Savoie 1961; Canella-Rocchi e Trincas 1961).

All the body surface of *L. luridus* is covered with a fine net of pellicular argentophilic structures, strinkingly resembling those of *Dexiotricha* and *Uronema*, since the pairs of basal bodies are shifted in each mesh to its right upper angle. The total number of these structures was estimated to amount about 5300; in the primitive *Uronema marinum* their number is near 240. On the body surface of *Loxocephalus luridus* the meshes are hexagonal; in the perizonal area they are quite rectangular (Pl. III 18—20, IV 22; Fig. 12).

The outer pore of the contractile vacuole lies on the ventral body side, slightly beyond the level of macronucleus (Pl. IV 24), in the area, where the somatic meridians are coming together. Pl. III 19, shows the interruption of somatic meridian in this area; no kinetodesms are emerging from the last pair of basal bodies of interrupted meridian. The contractile vacuole is impregna-

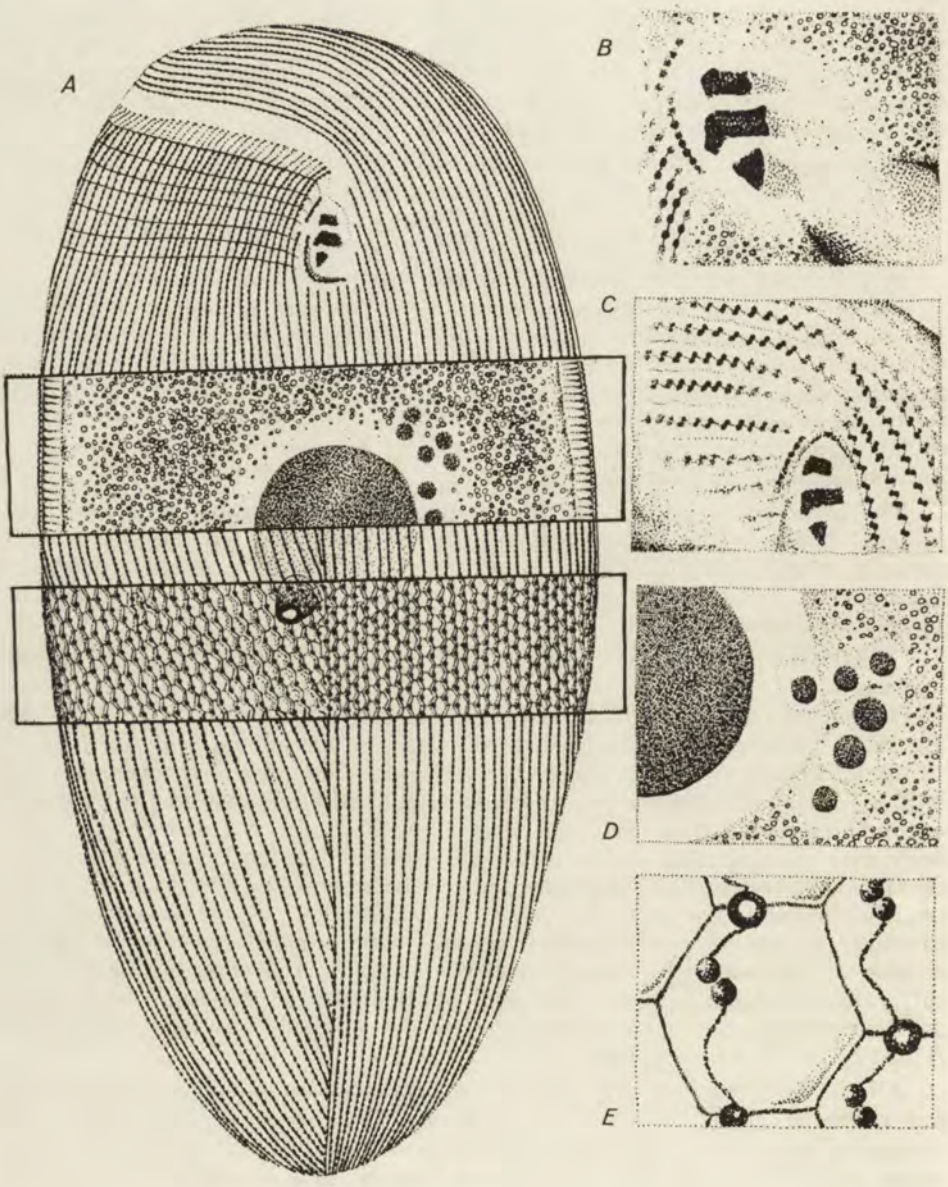

Fig. 12. *Loxocephalus luridus* Eberhard; A — generalized scheme of its organization: somatic ciliary meridians, nuclei, pellicular structures, buccal apparatus and contractile ampulla; B — the buccal apparatus, after photomicrograph; C — kinetome of the left body side above the cytostome, after photomicrograph; D — unusual variation of micronuclear shape within a single specimen; E — a part of argyrome, under high magnification, after photomicrograph

ted as large ampulla (Pl. V 28); in some specimens we see two vacuoles with two independent outer pores (Pl. V 30).

Small (11 × 6,5 μ) triangular peristome is located subanteriorly (Fig. 12 A); it gives rise to thick bag-like cytopharynx (Fig. 12 B). On its right external side the cytostome is marginated with the complex of M_1 and M_5 (Pl. IV 23; Fig. 12 B). Thus, the cytostomal ciliary apparatus not resemble this of *Frontonia*, that possesses, within its split-like peristome, 3 longitudinal ciliary stripes (Thompson 1959; Small and Profant 1960). Cytoproct of *L. luridus* looks like an argentophilic stripe, located on the ventral body surface in the posterior body half.

Nuclear apparatus of *L. luridus* consists in a large (23 μ) spherical macronucleus and 4—8 adjacent micronuclei (3 μ); in some specimens are seen both normal-sized micronuclei and those of twice smaller dimensions amounting 1,6 μ (Fig. 12 C).

Loxocephalus luridus divides in free-swimming condition, with a low frequency (once a day). In spring 1961, the samples taken from the lake Možajskoe contained abundant conjugating pairs; the conjugants are joining with their anterior ends, that is like *Dexiotricha*, but not like *Frontonia*. In fresh-disjoined exconjugants 3 to 7 micronuclei and a single growing macronucleus are visible (Pl. V. 31); the ancient macronucleus dissolves without fragmentation (caryorrhexy), and become substituted by the new one.

Discussion

Generic independence in loxocephalids

All the preceding authors considered the generic name *Dexiotricha* as a synonym of *Loxocephalus; L. luridus* and primitive *Dexiotricha* species were artificially united within one genus. None investigator had argumented this opinion; in the best case, they refer to their predecessors, as far as to Bütschli (Vuxanovici 1961, 1962; Corliss 1961; Fauré-Fremiet 1957; Gelei 1950; Gaevskaja 1949; Bhatia 1936; Lepsi 1926; Kent 1926, 1931; Behrend 1916; Kent 1882; Bütschli 1889). Moreover, some „tradition" was established to designate various species of this genus as „*Loxocephalus granulosus* Kent, 1882". Fauré-Fremiet 1957 designated thus a species of *Dexiotricha* with central position of this nuclei; Gelei 1950 — a species with terminal (posterior) position of the nuclei. But in the original description of *Loxocephalus granulosus* macronucleus was shown in the anterior body half, near the anterior end; contractile vacuole — in posterior body half, but not directly near the posterior pole. Such position of these organelles is not at all peculiar for *Dexiotricha*; in its species macro-, micronucleus and contractile vacuole occupy the same position, central or posterior. Probably Kent figured a species of *Dexiotricha*, but showed nucleus or vacuole on a wrong place. This mistake excludes any possibility of correct determination of *Loxocephalus granulosus*; it seems better do not cite this name as a possible synonym of *Dexiotricha plagia* or *D. colpidiopsis*, but regard it as nomen nudum.

There is no doubt as to generic independence of three related genera: *Uronema, Dexiotricha* and *Loxocephalus*. Type-species of these genera differ not only in their dimensions; we have seen above far-coming differencies in their nuclear apparatus, kinetome, argentophilic pellicular structures, buccal

and vacuolar apparatus and so on. We clearly see three successive levels of organization: first step is represented by the primitive *Uronema*; the second by several species almost identical but differing in some details which are belonging to the very sharply bordered genus of *Dexiotricha*; the third step is the highly-organized giant *Loxocephalus*.

Kahl 1931 regarded his genus *Cardiostoma* Kahl 1928 (now *Cardiostomatella* Corliss 1960) as closely related to *Loxocephalus*. Bearing in mind the recent description of *Cardiostomatella* by Dragesco 1960, it should be related to *Physalophrya*, not to *Loxocephalus*. We cannot, further, maintain the genus *Balanonema* Kahl 1931, created for two species ascribed originally by Penard to the genus *Uronema* (*U. dubium*). These forms too closely resemble *Dexiotricha*, especially *D. colpidiopsis*; interruption of ciliary rows in *B. biceps*, figured by Kahl 1931, seems to be highly improbable.

Polymerization of organelles in loxocephalids

Morphological comparison of the 4 above described genera leads to conclusion, that the closest phylogenetic relations exist between them. *Uronema*, the most primitive cohnilembid, closely resembles *Dexiotricha* on impregnated mounts (Pl. VI 33, 34); *Uronema halophila* resembles *Dexiotricha* also in living condition. Both genera present the same type of organization, but realized on different levels: *Uronema* has no perizonal rows, small size, small number of somatic meridians, no additional membrane-like structure in peristome; its peristome is subcentral, like in *Cyclidium*, what is among the hymenostomatids a primitive character. Like in *Cyclidium*, the macronucleus of *Uronema* is subanterior, and the contractile vacuole posterior; the number of somatic rows in both genera more or less coincides. Thus, *Uronema* is related to *Dexiotricha* as well as to *Cyclidium;* its closest phylogenetic relations with *Cyclidium* were proved also by preceding authors (Párducz 1934, 1936, 1940; Gelei 1950). In spite of this affinity, *Cyclidium* is a true member of *Pleuronematidae*, since it possesses a giant, unproportionally developed peristome: *Uronema* is a true member of *Tetrahymenina* (a suborder) since it possesses a small, proportionally developed peristome. Both genera may have been derived from a common ancestor, but they diverged giving rise to two independent evolutionary lines: 1. from an unknown ancestral form to *Cyclidium* and then to highly-organized pleuronematids (*Pleuronema, Cristigera, Histiobalantium*); 2. from the same ancestral form to *Uronema* and then to highly-organized cohnilembids (*Anophrys, Lemboides*). Thus the inclusion of *Cyclidium* and *Uronema* into two mentioned families is not artificial, but natural; *Pleuronematidae* and *Cohnilembidae* are different evolutionary lines, arosen from the common ancestors.

It seems desirable for the future to maintain the separation of the genera *Uronema* and *Loxocephalus* in different families (up to now *Loxocephalus* was included to *Frontoniidae* by Kahl 1931, to *Tetrahymenidae* by Corliss 1961, and *Uronema* — to *Pleuronematidae* by Hoare 1927, to *Frontoniidae* by Kahl 1931, to *Philasteridae* by Mugard 1949, and to *Cohnilembidae* tentatively by Corliss 1961). *Uronema* is a representative of *Cohnilembidae* since it is the beginning link in their evolution.

In the morphological line *Dexiotricha raikovi—D. colpidiopsis—D. plagia—Loxocephalus intermedius—L. luridus* one can see a distinct tendency to

polymerization of practically all the organelles; this process is accompanied by an enlargement of the body, from 50 μ to nearly 160 μ. Polymerization involves the ciliary meridians, cilia, trichocysts, perizonal transversal rows, argentophilic pellicular structures, caudal cilia, sometimes even contractile vacuoles and so on. Number of micronuclei increases from 1 to 8. The cellular center — macronucleus — also increases in its size, achieving, with no doubt, the higher levels of polyploidization. This process is, according to Poljansky i Rajkov 1960 „... one of the forms of polymerization, that is ... a principle of the progressive evolution of *Protozoa*". Evolution in *Loxocephalidae* does not consists only in simple polymerization of organelles; it involves also their complication, transition into another, higher level of organization (appearence of perizonal rows; of contractile ampulla; of pellicular hexagonals instead of rectangulars; of asymmetry in kinetome, etc). These facts perfectly confirm the opinion of Poljansky (in: Dogiel, Poljansky i Cheissin 1962, p. 466): „... polymerization of chromosomal complexes in macronucleus ... resulted in general intensification of functions and increase of the level of morphological differentiation".

The need for establishment of the new family

The correct systematic position of *Dexiotricha—Loxocephalus*, as a separate evolutionary line, is to be precised. The order *Hymenostomatida* is subdivided now into 3 suborders: *Tetrahymenina, Peniculina, Pleuronematina* (possibly *Pseudomicrothorax* will be separated into a fourth suborder — Corliss 1958 a, b). Loxocephalids satisfy to characterization of the first mentioned suborder.

This suborder includes 4 families: *Ophryoglenidae* (4 genera), *Cohnilembidae* (6 genera), *Philasteridae* (6 genera), *Tetrahymenidae* (4 reliable genera *Tetrahymena, Glaucoma, Colpidium, Stegochilum*). But near 33 genera (!) are still of indefinite systematic position (Corliss 1961).

Ophryoglenidae include large forms with simple somatic ciliature, with outstanding organization of the buccal apparatus, dividing in cysts, with peculiar feeding activities. *Philasteridae* include elongated small forms with long but shallow buccal cavity and autonomous stomatogenesis; they are also particular in their buccal organization. *Cohnilembidae* are slightly different from *Philasteridae* and in many points resemble loxocephalids. The last family, *Tetrahymenidae*, needs a serious elucidation.

According to Corliss 1952, 1957, 1960 c, 1961, and other investigators, the following characterization must be given to *Tetrahymenidae:* small free-living or parasitic (Corliss 1960 d, Jankowski 1962 c) ciliates of pyriform shape, sometimes with caudal cilium; the arrangement of ciliary organelles is simple; absence of pellicular rectangular argentophilic structures; trichocysts as a rule are absent; in many species there are „secondary meridians" running along the ciliary ones; contractile vacuole is usually posterior and never looks like an ampulla; macronucleus is central; all the ciliary meridians are running parallel to each other and thus reach the posterior body end; postoral meridians are ciliary ones, unlike *Uronema* (where they bear only the cytoproct) or *Dexiotricha* (cytoproct plus 3 pairs of cilia); no perizonal rows nor „additional membrane"; division sometimes in cysts, conjugation in many species unknown (e. g. in *Tetrahymena rostrata*).

In the case of *Loxocephalidae* fam. nov. and *Pleuronematidae*, two evolution-

ary lines arose from a common ancestor. *Tetrahymenidae* are not directly connected with them; they form an independent, third line; an artificial uniting them with loxcephalids would be unnatural, contradictory with the demands of a modern systematics, that should be based on phylogenetical principles. Moreover, if we unite loxocephalids and tetrahymenids in one family, we must change then all the characterization of *Tetrahymenidae,* because the characters of both groups do not coincide practically in any point. It may be supposed that *Tetrahymenidae* have originated from an ancestor, resembling, but not identical with form, that gave rise to loxocephalids and pleuronematids. The ancestor of tetrahymenids was a small ciliate with subanterior cytostome and simple ciliature, without pellicular hexa- or tetragones; it has given rise to three genera, remaining still on more or less equal level of complexity — *Tetrahymena, Glaucoma* and *Colpidium;* they do not exhibit any tendency of complication and polymerization of cellular organelles leading to the next evolutionary step. It is even possible that the form, resembling the hypothetical ancestor of *Tetrahymenidae,* will be found among living ciliates.

The monophyletic origin of *Cohnilembidae* and *Pleuronematidae* is evident since the simplest genera (*Uronema* and *Cyclidium*) differ practically only in size of the peristome. In all other points they are more or less coinciding, and the resemblance of pellicular structures and kinetome is striking. But these primarily insignificant differencies determined the following highly significant evolutionary divergence of the removed progeny: in the phylogenetic progeny of *Cyclidium* evolution involves practically only the buccal apparatus, while in that of *Uronema* — it involves the somatic structures. *Tetrahymenidae,* not related directly to neither *Cyclidium* nor *Uronema,* exhibit no tendency for the predominant hyperdevelopment of the buccal or somatic structures.

As a consequence it becomes a natural necessity to establish a separate family for loxocephalid ciliates, that differ from *Tetrahymenidae* in three main points: a different origin; highly complicated ciliature and pellicle; tendency to polymerization and complication of all the cellular organelles.

I give the following characterization to the new family: exclusively free-living forms, prefering polysaprobic or sapropelic environment; macronucleus spherical, subcentral or posterior; cytoplasm is usually dark due to the refractile mineral bodies; micronuclear number varies from 1 to 8. Contractile vacuole in *Dexiotricha* and *Loxocephalus* looks like ampulla and has only one outer pore (2—4 in *Tetrahymenidae*); it is frequently located in the same positions as the macronucleus, that is centrally or (rarely) posteriorly; distinct trichocyst zone under the pellicle; pellicle itself covered with fine net of tetra- or hexagons with basal bodies in their right upper angles; secondary cilia-free meridians are absent; somatic cilia are gathered in rows of progressively complicated configuration; *Dexiotricha* and *Loxocephalus* possess transversal perizonal ciliary rows on the cytostome level; anterior body half (above the cytostome) asymmetrical; kinetome of right and left ventral body sides also asymmetrical (*Colpidium striatum* among tetrahymenids exhibits the same type convergence); cytoproct large, band-like, commonly occupies the place of one of the postoral ciliary meridians; peristome is triangular, subanterior, with tetrahymenous complex and the additional membrane-like structure; division only in free-swimming condition, without cysts; resting cysts were not recorded; conjugation is common.

Summary

The author gives the diagnoses, the synonyms, and data concerning the life-cycles of the following ciliates: *Cyclidium glaucoma* (*Pleuronematidae*), *Uronema marinum, U. halophila* (*Cohnilembidae*), *Dexiotricha plagia, D. colpidiopsis, D. raikovi, Loxocephalus luridus* (*Loxocephalidae* fam. nov.). The existence of a distinct morphologic line *Dexiotricha—Loxocephalus* is reflecting the evolutionary history of this group. On the other hand, *Uronema* is closely related to *Cyclidium* and *Dexiotricha*.

The most primitive pleuronematid, *Cyclidium*, initiates a trend characterizing with a predominant hyperdevelopment of the buccal apparatus; yet, the loxocephalids present a clear tendency to polymerization and complication of their somatic organelles; the ciliary rows, the trichocysts, the argentophilic pellicular structures, the caudal cilia, the transversal perizonal rows on the cytostome level, the micronuclei. This trend was evidently stimulated by the progressive polymerization of the macronucleus, resulting, first of all, in a considerable increasing of the body size.

Detailed characterizations of the families *Tetrahymenidae* and *Loxocephalidae* are given; the both characterizations are nearly in all different.

РЕЗЮМЕ

Представлены диагнозы, синонимика и данные по жизненному циклу инфузорий *Cyclidium glaucoma* (*Pleuronematidae*), *Uronema marinum, U. halophila*, (*Cohnilembidae*) *Dexiotricha plagia, D. colpodiopsis, D. raikovi, Loxocephalus luridus* (*Loxocephalidae* fam. nov.). Существует четкий морфологический ряд *Dexiotricha—Loxocephalus*, отражающий эволюционную историю этой группы. В свою очередь, *Uronema* близкородственна *Cyclidium* и *Dexiotricha*.

Наиболее примитивная плейронематида *Cyclidium* дает начало линии с преимущественным гиперразвитием буккального аппарата; локсоцефалиды же проявляют ярко выраженную тенденцию к полимеризации и усложнению соматических органелл — ресничных рядов, трихоцист, аргентофильных пелликулярных структур, хвостовых ресничек, перизональных кольцевых рядов на уровне цитостома, микронуклеусов; стимулом такого пути явилась, очевидно, возрастающая полимеризация макронуклеуса, вызывавшая прежде всего значительное увеличение размеров тела.

Приведена подробная характеристика семейства *Tetrahymenidae* и *Loxocephalidae*; обе характеристики почти полностью не совпадают.

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EXPLANATION OF PLATES I—VI

Different species of the genus *Dexiotricha*

1—4: Anterior body end in *D. colpidiopsis;* impregnation after Gelei-Horvath
5: The outer pore of the contractile vacuole in *D. plagia*, in the region of fusion of meridians; impregnation after Klein
6: The contractile ampulla of *D. colpidiopsis* (Gelei-Horvath technique)
8: Postoral meridians of *D. colpidiopsis* — the middle one is shortened (Gelei-Horvath technique)
9: The buccal apparatus of *D. colpidiopsis* (Gelei-Horvath)
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Loxocephalus luridus Eberhard

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32: *Tetrahymena pyriformis*, after Corliss 1958 b
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34: *Dexiotricha* sp., after Klein 1928

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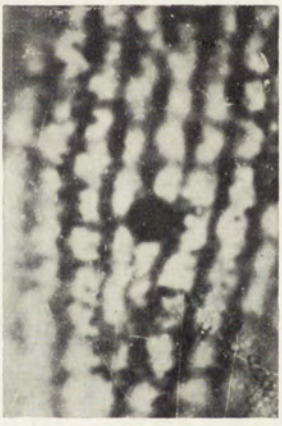

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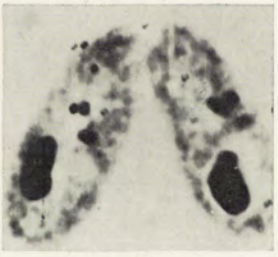

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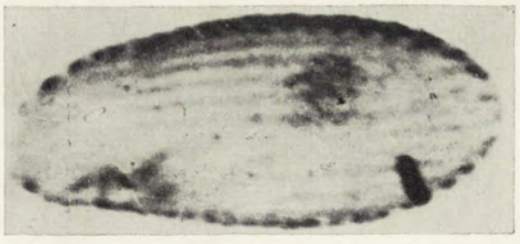

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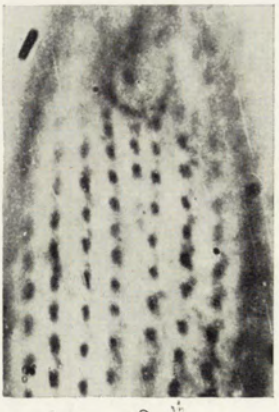

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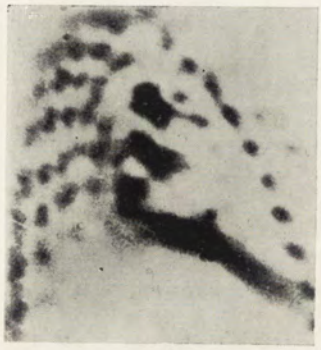

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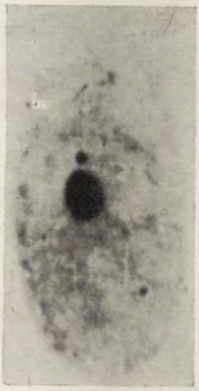

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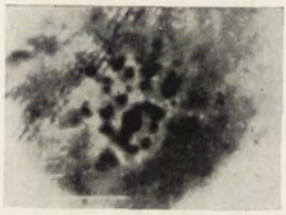

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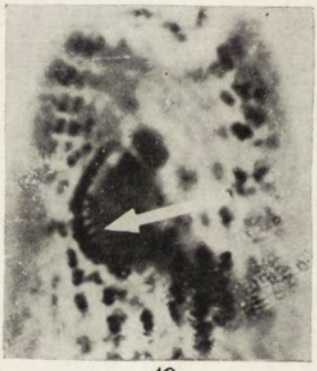

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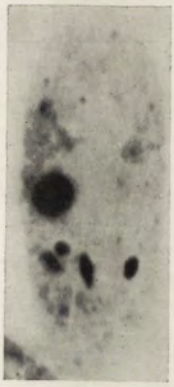

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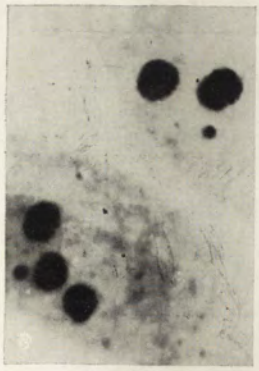

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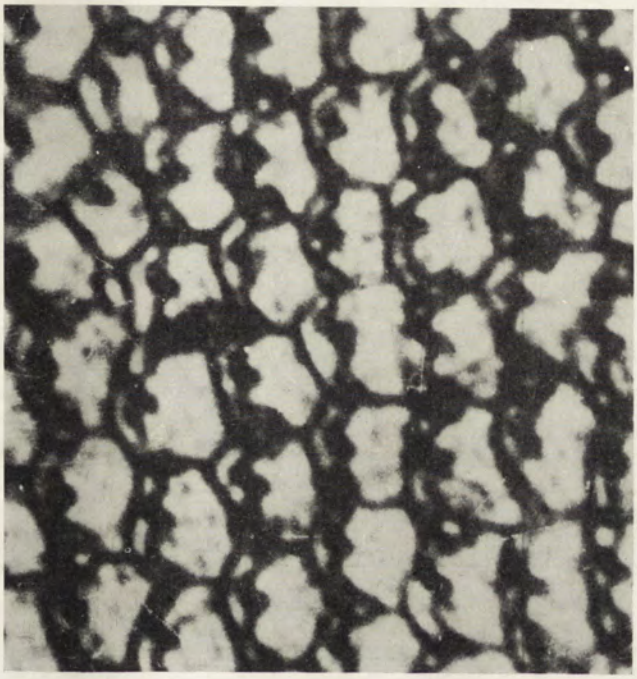

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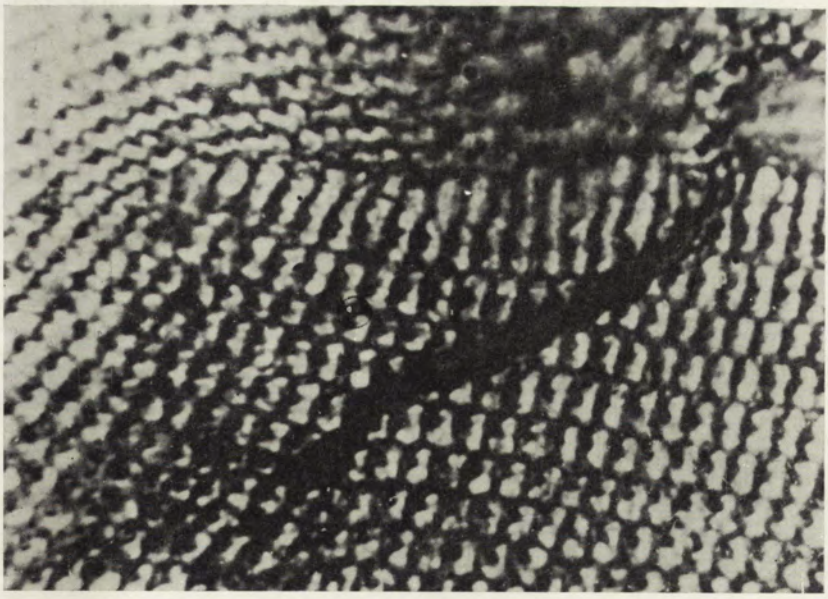

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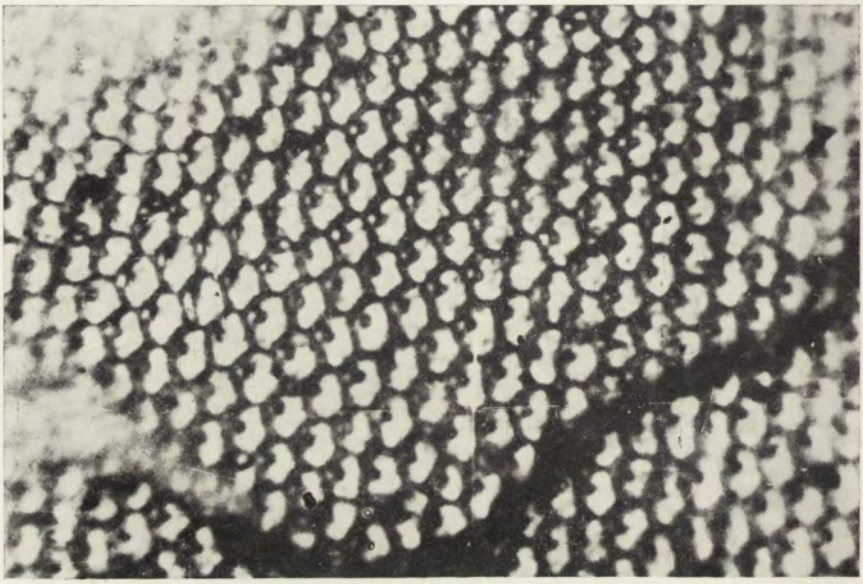

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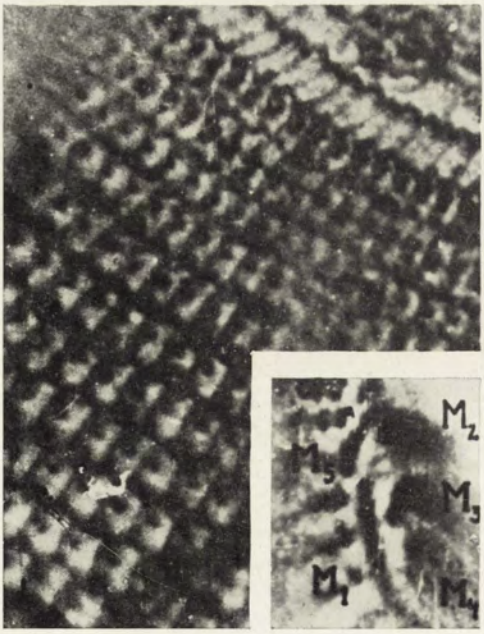

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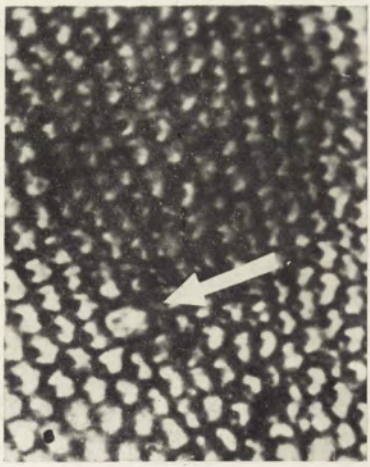

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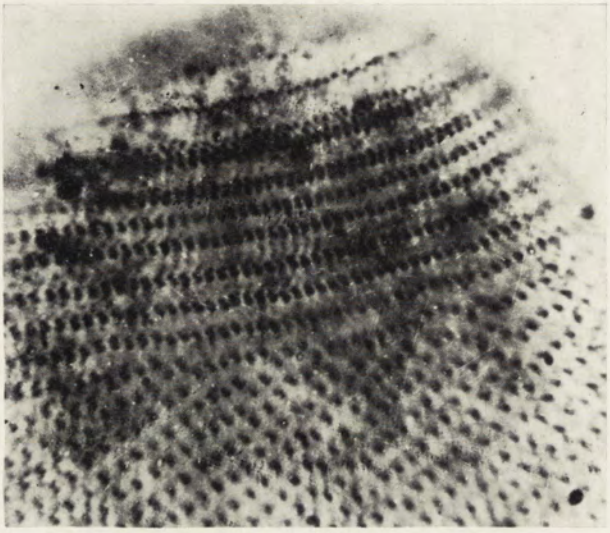

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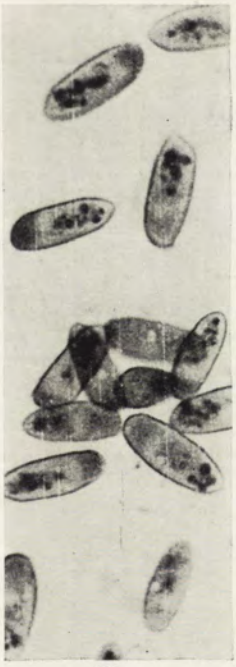

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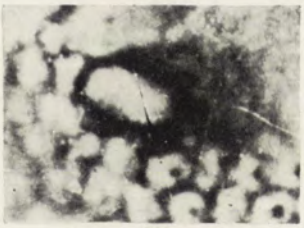

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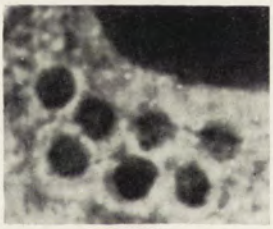

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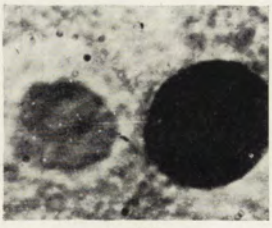

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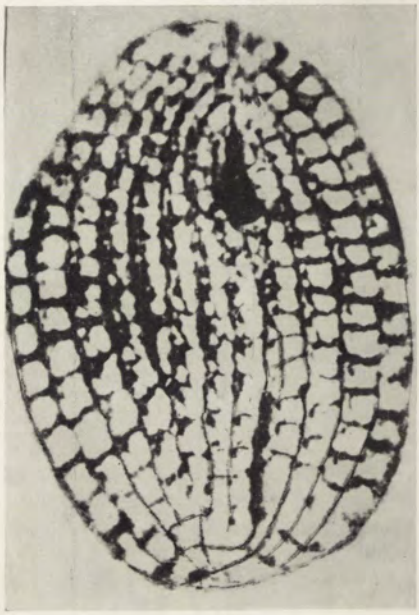

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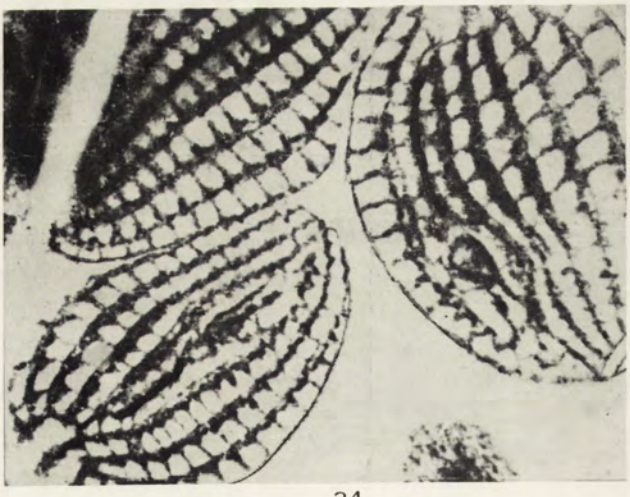

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A. W. Jankowski ex Klein et Corliss

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The cell shape of *Dileptus* in the course of division and regeneration

Kształt komórki *Dileptus* w trakcie podziału i regeneracji

A comparison of cell division and regeneration in *Dileptus* is the aim of the investigation carried out by the authors. The description of the changes in the external pattern of the ciliate cell in the course of these two processes is, of necessity, the first part of this work. The process of formation of two individuals out of one by way of fission was compared with a process resulting in the same effect i.e. the artificial bisection. Observations were concerned with changes in the general patterns of cell structure and the contour of the fission line. The formation of the anterior part of the opisthe was compared to the process of regulation and regeneration in the posterior fragment after bisection.

The problem of the relation between the mechanisms underlying the morphogenesis in the normal life cycle and those in control of regeneration in ciliates is as now still more concrete than it was 50 years ago and a new, more modern approach seems desirable. The classical material used by authors in this domain are the *Hypotricha* where the accentuated differentiation of the motor apparatus makes a detailed study of morphogenesis possible (Dembowska 1925, Jerka-Dziadosz 1963). The study in *Holotricha* is more difficult because of the small dimensions of the individual units of the motor apparatus and their uniformity. The most detailed data were obtained by Frankel 1960 a, b in *Tetrahymena*.

Dileptus, a ciliate with a high capability of regeneration is also the suitable object for comparative studies however some details of its fine structure still remain obscure. The morphology of *Dileptus* was investigated by several authors — Vissher 1927, Peschkowsky 1927, and Jones 1961, its ultrastructure — by Fauré-Frémiet 1961 and Dumont 1961. According to Dumont, a fiber with the branches on both sides of it connected with feeding cilia is situated along the ventral side of the proboscis. The convex band with the trichocysts grains lies on the ventral surface of the proboscis. The complex of feeding cilia and this ridge can easily be observed in the course of the morphogenesis and in the further descriptions will be refered to as the proboscis band. A distinction between its components is however not in every case possible.

The process of division in *Dileptus* was investigated by Vissher 1927, Studitsky 1930, Hayes 1938 and Peschkowsky 1927. Their main purpose was to trace the behavior of the nuclear apparatus.

Regeneration in *Dileptus* was studied by Sokoloff 1924, and Vapenik 1927 who investigated the conditions necessary for the regeneration as also the factors exerting their influence upon it. Morphogenesis during regeneration was not however studied by these authors. The tracing of the general patterns of morphogenesis in division and regeneration is the aim of the present paper.

The authors wish to express their sincere thanks to Prof. Z. Raabe for his valuable suggestions.

Material and methods

Two species of *Dileptus* provisionally determined by the authors as *D. anser* O. F. M. and *D. cygnus* Clap. et Lachm. were used for the investigations.

The cultures originated from individuals collected from ponds in the neighbourhood of Warsaw. The Pringsheim solution was used as the culture medium and the rations of *Colpidia* were added as a food.

Observations on the living material were carried out on the single ciliates placed in oil chambers of de Fonbrune. The bisection was either performed with a sharpened needle before placing in the chamber or inside the chambers using a micromanipulator. The bisections were regulated as to fall in the middle of the body length, proboscis not counted. The stained preparations were performed by the means of nigrosin negative staining as well as by the hematoxylin methods. Some single stained ciliates were placed in the oil chambers for more thorough observation from all sides. Such ciliates or regenerating fragments were dissected by means of a micromanipulator. The cut of apical surface of the fragment could be observed from above. For the study of the division stages the preparations of ciliates from cultures rich in dividing individuals were made. The investigations of large quantities of individuals were also performed in the course of study of regeneration. The mass culture was sectioned as a whole and the portions of the ciliates were fixed in the 10 minutes intervals after sectioning. More detailed studies of the behavior of kinetosomes are now under way.

Results

The cell division

In both species the first easily observed sign of division are the changes upon the dorsal side of the body occurring in the middle of the cell body. The rudiment of proboscis band becames visible in this area and a kind of ciliary tuft (Pl. I 1) can be observed on the dorsal margin of the ciliate. The band removes along the left side in the direction of the point where the new cytopharyngeal complex is in the state of development. The cytopharyngeal complex primordium is formed upon the ventral side of the ciliate, below the place where upon the dorsal margin the rudimental proboscis band may be visible. The primordium of the cytostomal complex is situated near the same meridian as the cytostome of the future proter (Fig. 1). This primordium de-

velops independently from the old cytostomal complex as also from the dorsal primordium of the proboscis band. The future cytopharyngeal complex looks like a cone with the slightly convex base upon the surface of the ciliate. (Pl. I 2). This circular area upon the ventral side of the animal is deprived of cilia. In the later stages of division the connection is established between the proboscis

Fig. 1. The dividing individual in the late stage III

Fig. 2. The regenerating posterior fragment in the corresponding stage

band and the cytopharyngeal complex. It seems as if the proboscis band surrounded the cytostome forming the labial ribs. At this time the formation of the two cones of trichits of the pharyngeal complex is completed. The newly formed cytopharyngeal complex removes now toward the left side, about 90^{c} from the proters cytostome. In the last stages of division the rear part of the proter

becomes narrow, the connection between the future individuals grows finer and finer. Immediately before the separation the proter is fixed to the opisthe by the left side of the proboscis. This place of the last connection can be situated near the base of the future proboscis of the opisthe or further towards its apex.

The shape of the fission line is a little oblique in relation to the long axis of the cell (Fig. 1). In the course of division the part of the ciliary meridians upon the dorsal and left side of the future opisthe becomes deflected towards the median dorsal area. At this stage rows are directed towards the place of origin of the future proboscis band. In this region the ciliary rows have tendency to parallel orientation in relation to the fission line (Fig. 1, Pl. I 3).

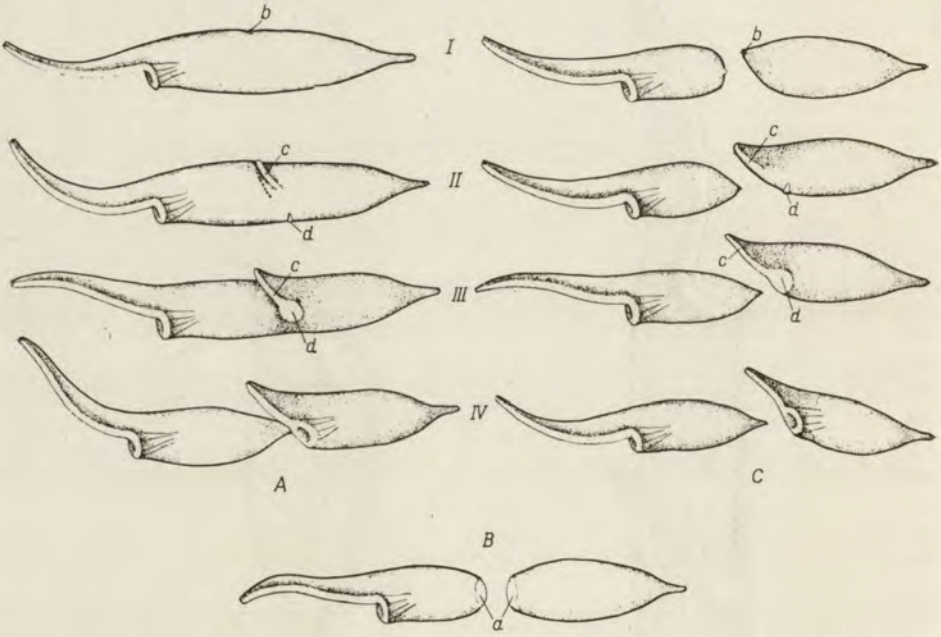

Fig. 3. The division and regeneration in *D. anser;* A — division stages I, II, III, IV; B — cicatrization of the cut surface; C — regeneration stages I, II, III, IV (a — the surface of the bisection cut, b — ciliary tuft, c — rudimental proboscis band, d— primordium of cytopharyngeal complex)

Upon the right side of an animal in the second stage (Fig. 3) of division the fine oblique line becomes visible. This line joins the primordium of the cytopharyngeal complex with the rudiment of the proboscis band situated on the opposite side of the cell. The line crosses the ciliary meridians without disturbing their direction (Pl. I 4). When the fission is completed this line approaches the proboscis band and becomes situated on the left of the proboscis.

Differences between the proter and opisthe are especially remarkable in the case of *Dileptus.* Immediately after the rupture of the connection between the two individuals the proboscis of the opisthe is much shorter than that of the proter. The posterior sprout of the proter is not yet normally developed being not of the normal length as in the case of the opisthe. So in the both

species under consideration we have to do with some kind of differentiating division. The regulation of the shape must occur in both individuals. This process is however quick and in about 30 minutes after the fission one cannot distinguish between the proter and the opisthe. The behavior of dividing individuals is rather complex and hardly susceptible to an adequate description yet one remarkable effect there can be observed in *D. anser*. At the very moment of the rupture of connection between the individuals the opisthe exhibits the ciliary reversion and swims backwards with its posterior end directed in the opposite direction to the proter. This occurred regulary in all the cases observed.

In the observed cases in *D. anser* the mean duration of the division stages from the beginning of stage II (Fig. 3) till the separation of ciliates is 46 minutes in the oil chambers in 20° C.

Regeneration

Investigations were carried out upon anterior and posterior fragments after bisecting transversally in the middle of the body length (proboscis not counted). Although special attention was paid to the posterior fragment, the anterior one was also observed. In the anterior fragments in both species of *Dileptus* there is no visible change in the form of the cytopharyngeal complex and in the proboscis after the operation. The length of the proboscis is not regulated in spite of its changed proportions in relation to the whole body. The feeding of the ciliate and the killing of the prey can go on in the anterior fragment during the cicatrization of the wound and the regeneration of the posterior sprout. The normal proportions of an individual are subsequently restored.

The process of the cicatrization of the wound is similar in the anterior and the posterior fragment although it is different in the both investigated species. In *D. anser* a convex area, deprived of cilia, becomes visible upon the surface of the cut immediately after the operation (Pl. II 8). The surface of the cut becomes contracted. The ciliated margins of the wound move toward each other until they meet in the middle. The ciliary meridians however do not increase their length during this process. In *D. cygnus* the contraction of the wound is immediate. The outflows of cytoplasm occur after the bisection and after the pellicular margins meet each other. The proper bisection of *D. cygnus* is more difficult than that of *D. anser* because the former ciliate can be more easily destroyed by the crushing. This may be due to the more liquid consistency of cytoplasm. Upon the fragments of *D. cygnus* after the transection the longitudinal ribs become visible due to the striated pellicle which is banded after the outflow of the cytoplasm. The further stages of regeneration upon the rear fragment are similar in the both species.

The first stage of regeneration is marked by appearance (after 30—50 min.) of the tuft of cilia upon the apex of the fragment. This tuft is situated outside the wound near its margin, upon the dorsal side. Later it encroaches upon the surface of the cicatrizated spot. Simultaneously the side of the fragment with the primary tuft increases its length in the anterior direction. As the result, the shape of the anterior part of the fragment becomes oblique and flattened resembling the fission line during the cell division (Figs. 1, 2, 3).

In the second stage of regeneration (Fig. 2) the proboscis band descends towards the primordium of the cytopharyngeal complex which yet becomes visi-

ble. The proboscis band is not as much developed as in the corresponding stage of the division. Its normal thickness is achieved at the third stage (Figs. 2, 3) when it is already connected with the future cytostomal complex. It seems as if the proboscis band surrounded the cytopharyngeal complex, and formed the labial ribs. At the IV stage of regeneration (Fig. 3) the opening of the cytostome appears upon the cytopharyngeal complex and the proboscis becomes elongated. This stage being completed, the normal shape of the animal is restored.

So the proter and the anterior fragments bear some of the marks of the old individual while the opisthe and the posterior fragment have the newer developmental features.

Discussion

In comparing the morphogenetic processes engaged in division on the one hand and in regeneration on the other, the essential similarities as well as the marked differences can be found. As to the general discussion of the subject the reader may be referred to Fauré-Fremiet 1948, Summers 1941 and Balamuth 1940. As to *Dileptus* the analogies concern, in the first place, the shape of the line of the fission furrow which is copied exactly in the course of regeneration by the pattern of the anterior border line of the posterior fragment. The behavior of the old cytopharyngeal complex as well as of the proboscis is also similar in the proter and the anterior fragment. In both cases the normal functioning of all organelles uninterrupted by fission or fragmentation can be observed and no visible marks of reorganization can be seen in the first period. The differences between the proter and opisthe are similar in *Lacrymaria* (Bovee 1957). This is not the case in the higher ciliates where we have to do with greater equivalence of morphogenetical events in both individuals. May be, in the case of *Dileptus* such an equivalence could be found by the study of the fine structures however the functional criteria do not speak in the favour of it.

The observed occurrence of the ciliary reversion immediately after the separation of the daughter individual can be compared to the reversion observed upon the posterior fragment after the bisection (Doroszewski 1962).

Localization of the places of development of the proboscis as also of the cytopharyngeal complex, is rather complicated. Upon the posterior fragments the cytopharyngeal complex takes its origin upon the same meridian as is situated the cytostome in the future proter. In spite of this there is as yet no reason to accept the existence of a stomatogenic kinety. During the process of division the location of the future cytopharyngeal complex is determined as to the position on the anteroposterior axis. This is not the case in the posterior fragment. In this instance the cytopharyngeal primordium and the one of proboscis band are located near the surface of the bisection, independently from the length of the produced fragment. It is formed on the undamaged side of the section. So here the localization is perhaps determined by some anteroposterior gradient of the fragment as a whole, after escaping from the inhibiting influence of the existing organelles of anterior fragment.

As to the other *Holotricha*, according to Frankel 1960, in *Glaucoma* the location of the new cytostome after the damage of the old one is similar.

In later fission stages the morphogenetic events in the opisthe resembles the changes in the posterior fragment more and more. After the post-divisional separation the opisthe becomes hardly distinguishable from the regenerating posterior fragment. So the two processes under consideration, approach closer and closer to finally converge in the same and result in the formation of the complete organism.

Throughout these considerations the question of terminology constantly arises. If we say: „the regeneration of the anterior fragment" it is not clear whether this refers to the anterior part of the hind fragment or to the rear part of the anterior fragment. The word „fragment" is not itself the best term, some authors use the word „piece". Some general term woud be usefull so as the old term of Balbiani 1893 for the fragments „Merozoa" is still in the use (Fauré-Fremiet 1948). Refering to this traditional term and to the terms „proter" and „opisthe" we can propose the name „promer" for the anterior fragment and „opimer" for the posterior fragment.

At the end let us consider some peculiarities of the division in *Dileptus*. Localization of the places of developing of the rudimental proboscis band and the primordium of the cytopharyngeal complex is different at the beginning, later the contact becomes established. It is not surprising if we take in account the different composition of these two elements. The cytopharyngeal cone is formed of the rows of trichits situated around the future cytostome opening. The specific ciliature of proboscis and the trichocyst grains are formed near the fission line. The process of its formations begins upon the other side of the cell.

As has been said, the fission line in *Dileptus* is oblique in relation to the long axis of the body and is situated more towards the anterior in the dorsal side and more posteriorly on the ventral side. The occurrence of the oblique fission among *Holotricha* was already reported by Pénard 1922 for *Lacrymaria olor*. In this species however, oblique division is not the rule. More essential may by the relation of the fission line to the kineties. The pictures obtained in *Dileptus* resemble these obtained by Fauré-Fremiet 1955 as also by Kraschenninnikow and Wenrich 1958 in *Balantidium*.

Summary

The comparative observations concerning the cellular patterns in the process of division and regeneration in *Dileptus* were carried out. The bisection of the ciliate evokes a set of regulative changes upon the posterior fragment subsequently followed by the intervention of the mechanism of division. The posterior fragment repeates the morphogenetic sequence of division in opisthe until the normal form of the cell is restored. In regeneration the cytopharyngeal complex as well as proboscis band are formed upon the same meridians as in the division. The location of this rudiments as to the long axis of the cell, depends on the location of the bisection cut. The elements in formation encroach upon the cicatrized spot.

The anterior margin of the regenerating fragment resembles the pattern of the fission furrow and is similarly flattened.

The terms „promer" and „opimer" are proposed by the authors to indicate the anterior and the posterior fragments after bisection.

STRESZCZENIE

Przeprowadzono porównawcze obserwacje nad cytoarchitektoniką podziału i regeneracji u *Dileptus*. Stwierdzono, że w pierwszej fazie po przecięciu osobnika zachodzą na tylnym fragmencie zmiany o charakterze regulacyjnym, następnie zostaje włączony mechanizm morfogenezy podziałowej i fragment ten podlega zmianom analogicznym do procesów zachodzących na opistorze przy podziale, aż do odtworzenia normalnej formy wymoczka. Regeneracja zawiązków kompleksu cytofaryngalnego wentralnej strony proboscis następuje na określonym południku, podobnie jak jego powstawanie przy podziale. Lokalizacja zawiązka na długiej osi ciała zależy przy regeneracji od miejsca przecięcia i występuje obok miejsca zranienia. Powstające elementy nasuwają się na zabliźnioną powierzchnię. Zarys przedniej krawędzi regenerującego fragmentu przybiera kształt bruzdy podziałowej i wykazuje analogiczne spłaszczenie.

Autorzy proponują wprowadzenie terminów „promer" i „opimer" na oznaczenie przedniego i tylnego fragmentu otrzymanego w następstwie przecięcia.

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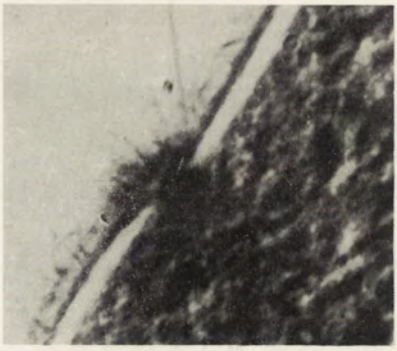
EXPLANATION OF PLATES I—II

The division of *D. anser*

1: The first rudiment of the proboscis band
2: The primordium of cytopharyngeal complex
3: The ciliary meridians and the rudimental proboscis band in late stage II of the division
4: The fission line upon the left side of the cell in the same stage of the division.
5: The II stage of the division; the rudimental proboscis band and the primordium of cytopharyngeal complex can be seen upon the opposite sides of the cell
6: The next stage of the division
7: The individuals just before the separation

The regeneration of *D. anser*

8: The posterior fragment after the bisection; the surface of the bisection cut
9: The beginning of regeneration; the primary ciliary tuft on the apex of the fragment
10: The rudiments of the proboscis band and of cytopharyngeal complex
11: The cytostom in the later stage of regeneration.



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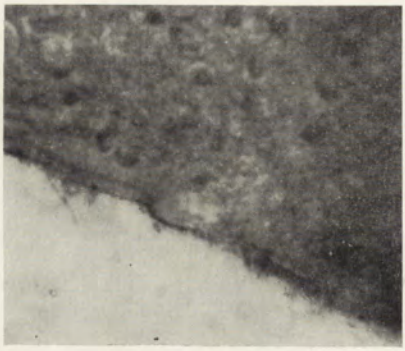

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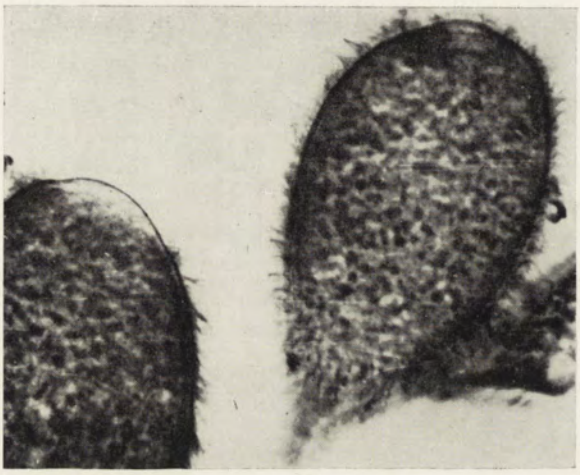

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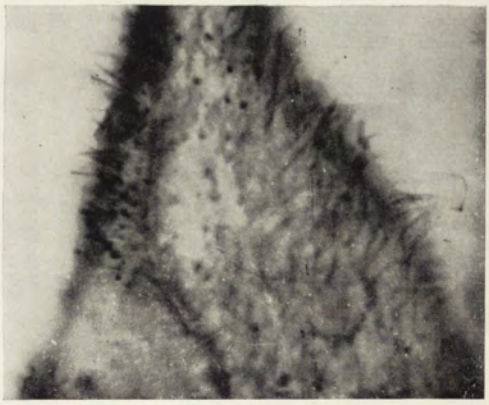

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Rôle des ions K^+ et Ca^{2+} dans l'excitabilité de la cellule protozoaire. I. Equilibrement des ions antagonistes

Znaczenie jonów K^+ i Ca^{2+} w pobudliwości komórki pierwotniaczej. I. Zrównoważenie jonów antagonistycznych

Les effets antagonistes du potassium extérieur et du calcium sur l'excitation et les réponses locomotrices des Protistes sont connus depuis longtemps. Dans un travail précédent (Grębecki 1963), ils ont été comparés aux effets antagonistes des stimulants électriques, étant donné qu'un cathélectrotonus et le potassium inversent le battement des cils, tandis qu'un anélectrotonus et le calcium renormalisent leur travail ou l'activent remarquablement. De plus, dans le même article, il a été démontré que la relation entre l'effet rebroussant de K extérieur et l'effet renormalisant de l'anode, est exactement linéaire. La tâche de la série de recherches commencée par le travail présent est de fournir des renseignements exacts sur les rapports entre les deux paires de stimulants antagonistes, chimiques et électriques.

Le rôle du potassium dans la génération du potentiel intracellulaire et dans l'excitation est généralement reconnu dans la physiologie cellulaire. D'autre part, l'influence du calcium sur le comportement des Protistes et sur leurs propriétés électrophysiologiques est si suggéstive qu'elle incline les chercheurs à abandonner les théories classiques; ou bien on suppose l'existence de deux composantes du biopotentiel des cellules protozoaires, l'une dépendant de K et l'autre de Ca (Kinosita 1954), ou même on n'attribue de rôle effectif qu'à la quantité de Ca dans la membrane (Jahn 1962). Donc les recherches entreprises semblent être d'autant plus importantes qu'elles peuvent contribuer à une reévaluation de ces théories, en developpant notre connaissance des principes ioniques de l'excitation chimique et électrique.

Il semble nécessaire, au début, de préciser exactement, de façon quantitative, le rapport entre le K et le Ca dans le milieu, c'est-à-dire de trouver quelle proportion entre les concentrations de ces deux ions doit être gardée, en vue que leur effet antagoniste soit balancé toujours sur le même niveau.

Une telle tentative, entreprise très soigneusement par Kamada and Kinosita 1940, n'a pas réussi; il a été seulement constaté que le quotient

de $[K^+]$ et $[Ca^{2+}]$ ne donne pas la proportion cherchée.[1] Le nouvel essai de préciser le rapport entre K et Ca dans le milieu est devenu d'autant plus intéressant qu'au cours de sa réalisation a paru le travail théorique de Jahn 1962 recalculant les données de Kamada and Kinosita 1940 pour démontrer que la relation entre les deux ions considérés obéit aux principes de l'équilibre de Donnan.

Méthodique

On se servit du *Paramecium caudatum*, provenant des cultures maintenues plusieures années dans notre Laboratoire. Avant l'expérience les Paramécies étaient soigneusement lavées maintes fois, d'abord dans de l'eau redistillée et ensuite dans une solution exigée de $CaCl_2$. Tout le procédé donnait une dilution du milieu initial égale à 1 : 10 000, ce qui reduisait l'erreur de la teneur en Ca dans le milieu expérimental à des limites 0.4—0.006‰ à peu près. Le milieu était privé d'autres ions que Ca^{2+} et Cl^-.

Les Paramécies étaient incubées dans des solutions définies de $CaCl_2$ durant 24 heures, en vue de bien stabiliser les rapports entre le calcium extérieur et celui contenu dans la membrane, ainsi qu'entre le potassium restant à l'intérieur de la cellule et celui qui probablement sort dans le milieu privé de cet ion. Les Paramécies supportaient très bien cette „période d'adaptation" à des solutions de $CaCl_2$ employées, qui variaient de 0.25 mM jusqu'à 16 mM.

Après 24 heures on étudiait l'influence du K^+, en melangeant l'échantillon en proportion 1 : 1 avec les solutions de KCl (contenant la même quantité de $CaCl_2$ que le milieu des Paramécies). On se servait de deux critères différents caractérisant la réponse des Protistes: 1. on mesurait la durée du rebroussement ciliaire, 2. on determinait la vitesse et le caractère du mouvement dans un temps défini après l'addition du KCl.

Il était important de choisir une méthode convenable de la mesure de la durée du rebroussement ciliaire. Étant donné que la renormalisation du travail de la ciliature n'est pas instantanée, mais qu'elle s'effectue par la suite d'une période du mouvement „culbutant", il est impossible de determiner la durée du rebroussement subi par un seul individu. Pourtant, on peut le faire pour un groupe de Paramécies. En tant que fin du rebroussement on considérait le moment où la plupart des individus „culbute", mais où il reste encore quelques Paramécies nageant à reculons, tandis que quelques autres commencent à nager normalement. La réponse d'un groupe est tellement uniforme que ce moment peut être determiné avec une précision de quelques secondes. Chaque mesure était répétée 20 fois au minimum.

Pour mesurer la vitesse de la nage et comparer le caractère des trajectoires parcourues, on employait la téchnique d'enregistrement photographique du mouvement, d'après Dryl 1958.

Toutes les expériences étaient effectuées avec $t = 20 \pm 1^{\circ}C$.

[1] D'ailleurs, Kamada and Kinosita 1940 répètent la vieille erreur de Mast and Nadler 1926 en admettant qu'il y a une concentration de K produisant une durée maximale du rebroussement ciliaire. C'est déjà Oliphant 1938 qui a clairement démontré que la durée du rebroussement ne peut qu'augmenter en fonction de $[K^+]$ introduit dans le milieu. En reproduisant les expériences de Kamada and Kinosita 1940 on voit que dans des milieux très riches en K le rebroussement en effet devient abrévié, mais il n'est pas suivi déjà d'une pleine renormalisation du comportement; il s'agit donc d'un effet toxique qui commence à intervenir.

Durée du rebroussement ciliaire en fonction de $[K^+]$ et $[Ca^{2+}]$

Dans la première série d'expériences on mesurait la durée du rebroussement ciliaire évoqué par de différentes quantités de K^+, agissant en présence des différentes quantités de Ca^{2+} (0.25, 0.5, 1, 2, 4, 8 et 16 mM $CaCl_2$). Les concentrations du KCl étaient employées en partant des valeurs minimes, à peine suffisantes pour produire un rebroussement, jusqu'aux valeurs relativement élévées inversant les battements des cils pour quelques minutes. Pourtant, on ne depassait jamais cette teneur en K qui apporte des premiers effets toxiques, en évitant de cette manière l'artéfact de Mast and Nadler 1926 (l'abréviation apparente du rebroussement dans les concentrations trop élévées).

Cette précaution gardée, le temps du rebroussement augmente régulièrement avec l'augmentation de $[K^+]$ ou avec la diminution de $[Ca^{2+}]$. La Fig. 1 montre le rapport entre la durée des battements inversés et la concentration du $CaCl_2$. Chaque courbe correspond à une autre teneur en K. La Fig. 2 présente les mêmes mesures du temps du rebroussement, en fonction de la concentration du KCl. On y voit que cette relation peut être considérée en tant que linéaire[2], quelle que soit la quantité de calcium présent dans le milieu.

Donc, la relation entre la durée du rebroussement ciliaire (t_r) et la teneur en K, peut être exprimée par une équation du type suivant:

$$t_r = a[K^+] + b \tag{1}$$

On peut préciser cette équation pour toutes les sept concentrations de $CaCl_2$ présent dans les expériments, en calculant les paramètres réels des courbes montrées par la Fig. 2. On en obtient:

$CaCl_2$ 0.25 mM	$t_r = 0.32[K^+] - 0.94$	(2)
$CaCl_2$ 0.5 mM	$t_r = 0.23[K^+] - 1.09$	(3)
$CaCl_2$ 1 mM	$t_r = 0.17[K^+] - 0.94$	(4)
$CaCl_2$ 2 mM	$t_r = 0.13[K^+] - 1.18$	(5)
$CaCl_2$ 4 mM	$t_r = 0.10[K^+] - 1.07$	(6)
$CaCl_2$ 8 mM	$t_r = 0.07[K^+] - 1.28$	(7)
$CaCl_2$ 16 mM	$t_r = 0.05[K^+] - 1.18$	(8)

Il semble admettable que la constante b ne change que dans des limites de l'erreur d'expérience et on peut estimer sa valeur moyenne en tant que -1.1. En introduisant cette valeur dans l'équation générale du temps du rebroussement (1) on obtient:

$$t_r = a[K^+] - 1.1 \tag{9}$$

Il ne reste donc qu'à définir le sens du coéfficient a qui exprime l'angle d'inclinaison des courbes par rapport à l'axe des ordonnées. On conclut aisément

[2] Le caractère linéaire de cette relation conteste l'existence d'un „maximum" du rebroussement (Mast and Nadler 1926 et Kamada and Kinosita 1940) et, d'autre part, il reste entièrement en accord avec les résultats d'Oliphant 1938.

que cet angle dépend de la teneur en Ca. En effet, le calcul prouve que la relation entre la valeur du coéfficient a et la teneur en Ca est bien définie:

$$\lg a = -0.43 \lg [Ca^{2+}] - 0.76 \tag{10}$$

Autrement dit:

$$a = \frac{1}{\frac{2.3}{5.83 \sqrt{[Ca^{2+}]}}} \tag{11}$$

Après l'introduction de ce dernier résultat à l'équation (9) on obtient enfin la formule cherchée qui caractérise la relation entre la durée du rebroussement ciliaire et la teneur en K et en Ca dans le milieu ambiant:

$$t_r = \frac{[K^+]}{\frac{2.3}{5.83 \sqrt{[Ca^{2+}]}}} - 1.1 \tag{12}$$

Ou bien, ce qui semble plus pratique:

$$\lg [K^+] = \lg (t_r + 1.1) + 0.765 + 0.43 \lg [Ca^{2+}] \tag{13}$$

Les valeurs t_r y sont exprimées en minutes, $[K^+]$ et $[Ca^{2+}]$ en mM KCl ou en mM $CaCl_2$ respectivement.

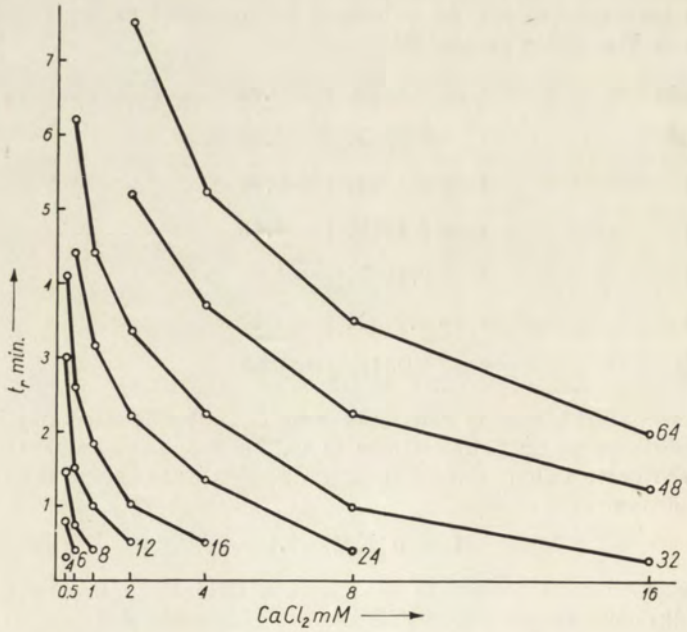

Fig. 1. Durée du rebroussement ciliaire présentée en fonction de $[Ca^{2+}]$; les chiffres inscrits à côté des courbes indiquent les concentrations de KCl employées pour produire la réponse

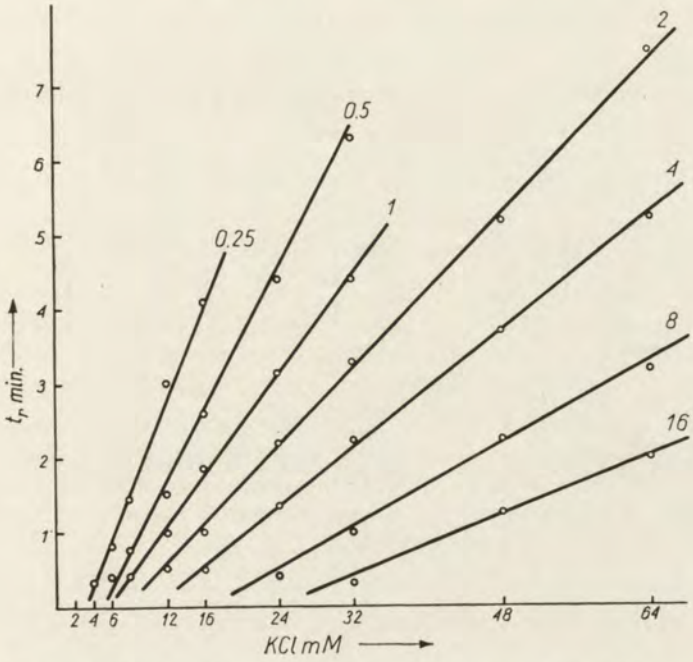

Fig. 2. Durée du rebroussement ciliaire présentée en fonction de [K+]; les chiffres inscrits à côté des courbes indiquent les concentrations de $CaCl_2$ présentes dans les milieux experimentaux

Cette équation générale permet de prévoir la durée des battements ciliaires rebroussés pour toutes les concentrations données de KCl et de $CaCl_2$, ou bien, par contre, de calculer les concentrations nécessaires pour obtenir un rebroussement voulu. Si on n'a besoin d'évaluer ces paramètres qu'approximativement il est plus facile de se servir des données contenues dans la Table 1. Les résultats obtenus sont donc d'une importance pratique pour la suite des recherches entreprises, leurs implications théoriques seront discutées à la suite de ce travail. D'abord, il semble pourtant utile de vérifier cette équation par d'autres procédés expérimentaux.

Relation entre $[K^+]$ et $[Ca^{2+}]$ assurant une durée constante du rebroussement ciliaire

On a repris les expériences en partant de principes différents des précédents. Au lieu de mesurer la durée du rebroussement ciliaire pour les concentrations de KCl choisies préalablement, on a cherché à établir les teneurs en K qui produisent des battements rebroussés pour 0.5, 1, 2 et 4 minutes. On l'a répété dans toutes les sept concentrations de $CaCl_2$, employées auparavant.

L'idée essentielle de cette expérience consiste donc à trouver directement un équilibre entre K et Ca. Autrement dit, il s'agit de définir une telle proportion entre les deux ions en question pour laquelle l'effet rebroussant serait constant sans égard à leur concentration totale.

Table 1

Concentrations de KCl (en mM) produisant une durée définie du rebroussement ciliaire (t_r), en présence de différentes quantités de $CaCl_2$ dans le milieu

$CaCl_2$ mM / t_r	0.25	0.5	1	2	4	8	16
0.5′	5.0	6.7	9.5	12.8	16.5	22.5	30.0
1′	6.7	9.0	13.0	17.0	22.5	31.0	40.5
2′	9.5	12.8	17.2	24.0	30.0	42.0	—
4′	15.0	20.5	25.5	37.0	—	—	—

La Table 1 contient les chiffres informant quelles concentrations de KCl (en mM) rebroussent les cils pour 0.5, 1, 2 et 4 minutes, en agissant avec des différentes quantités de Ca. Le diagramme d'équilibre entre K et Ca prouve que la relation entre les deux ions est exactement linéaire pour chaque durée constante du rebroussement ciliaire (Fig. 3).

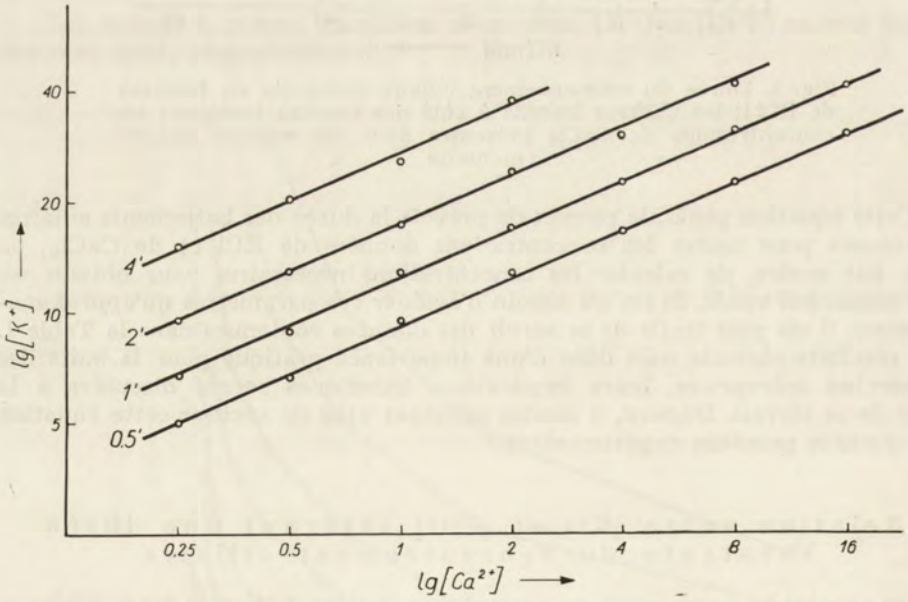

Fig. 3. Rapport entre la teneur en K et la teneur en Ca dans le milieu équilibrées en vue d'obtenir une durée constante du rebroussement ciliaire; les chiffres inscrits à côté des courbes indiquent les durées choisies pour l'équilibrement

Après le calcul des paramètres, on constate que le rebroussement ciliaire dure 0.5 min., quelle que soit la concentration totale, si:

$$\lg [K^+] = 0.96 + 0.43 \lg [Ca^{2+}] \qquad (14)$$

D'ailleurs, en introduisant $t_r = 0.5$ à l'équation (13) on obtiendrait:

$$\lg [K^+] = 0.969 + 0.43 \lg [Ca^{2+}] \quad (14')$$

Pour un rebroussement durant 1 min. il a été trouvé que:

$$\lg [K^+] = 1.09 + 0.43 \lg [Ca^{2+}] \quad (15)$$

tandis que l'équation (13) exige que:

$$\lg [K^+] = 1.087 + 0.43 \lg [Ca^{2+}] \quad (15')$$

De même, pour une inversion du travail ciliaire durant 2 min. l'expérience a démontré que:

$$\lg [K^+] = 1.24 + 0.43 \lg [Ca^{2+}] \quad (16)$$

et le calcul d'après l'équation (13) donne:

$$\lg [K^+] = 1.256 + 0.43 \lg [Ca^{2+}] \quad (16')$$

Enfin, les cils se trouvent rebroussés pendant 4 min., si:

$$\lg [K^+] = 1.43 + 0.43 \lg [Ca^{2+}] \quad (17)$$

et l'équation (13) suggère que:

$$\lg [K^+] = 1.473 + 0.43 \lg [Ca^{2+}] \quad (17')$$

Il est évident que la série d'équations (14)—(17), dérivée directement des expériences, est presque entièrement identique à la série (14')—(17'), calculée d'après l'équation (13) qui a été établie auparavant. Il s'ensuit de là que les rapports entre la teneur en K, la teneur en Ca et l'effet de rebroussement ciliaire, ont été établis correctement et estimés avec une précision suffisante.

D'autre part, les équations (14)—(17), ainsi que (13), peuvent être généralisées, ce qui permet de conclure quelle proportion entre le potassium et le calcium assure un effet rebroussant constant; $t_r = \text{const.}$, si:

$$\lg [K^+] - 0.43 \lg [Ca^{2+}] = \text{const.} \quad (18)$$

Si on garde les proportions indiquées par la formule (18), on peut changer les concentrations totales des ions dans le milieu, sans influencer durée du rebroussement ciliaire.

Equilibrement de $[K^+]$ et $[Ca^{2+}]$ et le caractère du mouvement des Paramécies

Il semble utile de concevoir, si la formule (18) définissant l'équilibre des ions antagonistes, K^+ et Ca^{2+}, ne concerne que la durée du rebroussement ciliaire, ou bien, si elle reste également valable pour les autres caractéristiques du comportement de la Paramécie.

On a repris les expériences dans les milieux contenant 0.5, 1, 2, 4 et 8 mM $CaCl_2$, en exposant les Paramécies à l'action du KCl dans des concentrations différentes calculées de façon à ce que la valeur constante de $\lg [K^+] - 0.43 [\lg Ca^{2+}]$ soit égale à 0.5, 1 ou 1.5. Entre la 60° et la 65° seconde après l'addition du KCl le mouvement des Paramécies était enregistré sur une pellicule photo-

graphique, ce qui permettait de calculer ensuite la vitesse du mouvement et de comparer l'allure des trajectoires parcourues.

La Table 2 indique en tant que conditions d'expérience: les valeurs de $[Ca^{2+}]$ et de $[K^+]$, ainsi que les proportions entre ces deux ions caractérisant les trois séries des épreuves. En tant que résultats, la Table 2 apporte les vitesses de la nage (chaque valeur constituant une moyenne de 50 mesures) et les notes renvoyant à la Planche I qui contient les photographies des trajets.

Il semble évident que, si la proportion entre $[K^+]$ et $[Ca^{2+}]$ est maintenue constante conformement à la formule (18), la vitesse de la nage des Paramécies reste égale, quelle que soit la concentration totale des ions étudiés. De même, le caractère du mouvement, c'est-à-dire la sinusoïté des trajectoires, reste intacte, si on garde un niveau d'équilibre entre le K et le Ca calculé d'après la formule (18), ce qui est clairement démontré par la Planche I.

Il est donc possible d'admettre que les formules trouvées, exprimant l'équilibre entre le calcium et le potassium contenus dans le milieu, portent un caractère suffisamment général pour les supposer valables en ce qui concerne l'excitabilité et les réponses locomotrices des Paramécies.

Table 2

Comportément des Paramécies dans les milieux caracterisés par des proportions constantes entre le potassium et le calcium

$CaCl_2$ mM	0.5	1	2	4	8	Rapport entre K et Ca
KCl mM	2.35	3.16	4.26	5.73	7.71	
Vitesse du mouvement en μ/sec.	846 ±225	830 ±266	894 ±253	866 ±288	878 ±237	$\lg[K^+] - 0.43 \lg[Ca^{2+}] = 0.5$
Caractère des trajets	Pl. I 1	—	Pl. I 2	—	Pl. I 3	
KCl mM	7.43	10.00	13.50	18.10	24.41	
Vitesse du mouvement en μ/sec.	453 ±149	483 ±194	446 ±121	478 ±194	426 ±140	$\lg[K^+] - 0.43 \lg[Ca^{2+}] = 1.0$
Caractère des trajets	Pl. I 4	—	Pl. I 5	—	Pl. I 6	
KCl mM	23.50	31.60	42.60	57.30	77.10	
Vitesse du mouvement en μ/sec.*	−886 ±128	−805 ±66	−844 ±71	−865 ±83	−857 ±112	$\lg[K^+] - 0.43 \lg[Ca^{2+}] = 1.5$
Caractère des trajets	Pl. I 7	—	Pl. I 8	—	Pl. I 9	

* Les valeurs négatives de la vitesse indiquent qu'il s'agit d'une nage à reculons.

Discussion

Le résultat essentiel des recherches présentées ci-dessus est renfermé par la formule constatant que le comportement des Paramécies ne dépend pas de la teneur absolue en K et en Ca, si:

$$\lg [K^+] - 0.43 \lg [Ca^{2+}] = \text{const.} \qquad (13)$$

Par contre, le comportement se trouve altéré, si on change cette proportion entre les deux ions en question.

Il est interessant de suivre l'idée de Jahn 1962 et de confronter la formule (18) avec cette relation entre les ions univalents et bivalents qui soit conforme aux principes de l'équilibre de Donnan. Le calcul de Jahn, basé sur les résultats de Kamada and Kinosita 1940, postule que l'effet de K et de Ca reste constant, si:

$$\frac{[K^+]}{\sqrt{[Ca^{2+}]}} = \text{const.} \qquad (19)$$

Autrement dit:

$$\lg [K^+] - 0.5 \lg [Ca^{2+}] = \text{const} \qquad (20)$$

Sans doute la formule obtenue ci-dessus (18) et la formule de Jahn 1962 conforme à l'équilibre de Donnan (20) sont presque identiques. L'unique différence entre le coéfficient expérimental (0.43) et le théorique (0.5) aurait pu être considérée comme négligeable.

Or, même cette petite divergence n'est point fortuite. La formule (18), ainsi que (20), sont simplifiées ne comprenant que les concentrations des ions en question, sans tenir compte de leur activité. Donc, il faudrait exprimer la formule théorique exacte autrement:

$$\lg \alpha_K [K] - 0.5 \lg \alpha_{Ca} [Ca^{2+}] = \text{const.} \qquad (20')$$

Dans cette dernière équation les valeurs α_K et α_{Ca} correspondent à des coéfficients d'activité des deux ions, changeant toujours avec leurs concentrations. Si on recalcule les résultats expérimentaux compris dans le travail présent, en introduisant ces coéfficients, on arrive en moyenne à corriger la formule (18) de la manière suivante:

$$\lg \alpha_K [K^+] - 0.498 \lg \alpha_{Ca} [Ca^{2+}] = \text{const.} \qquad (18')$$

L'accord entre la formule expérimentale (18') et la théorique (20') devient alors complèt et idéal. Il ne reste donc qu'à confirmer que les effets antagonistes exercés par le potassium et le calcium sur le comportement des Paramécies obéissent entièrement aux principes de l'équilibre de Donnan.

En acceptant cette conclusion, il faut souligner quand-même qu'elle ne peut servir d'argument en faveur de la deuxième thèse de Jahn 1962, qui postule que le potassium n'agit qu'en supplantant le calcium dans la membrane cellulaire. La relation constante entre la concentration de l'ion univalent et le radical carré de la concentration de l'ion bivalent est si générale, en tout ce qui concerne l'équilibre des solutions, qu'elle ne peut nous donner presque aucun renseignement sur le rôle exacte du K et du Ca dans la génération des

biopotentiels et dans l'excitabilité; autrement dit, quelle que soit la théorie, elle restera en accord avec cette relation.

D'ailleurs, une étude précise du rapport de ces deux ions était indispensable justement pour entamer une série de recherches concernant le fond ionique de l'excitabilité de la cellule protozoaire. En revenant alors aux questions méthodiques il faut noter que les formules (18′) et (20′) comprenant les coéfficients d'activité des ions, n'ont été introduites que pour prouver leur identité. Au point de vue pratique le calcul de l'activité, changeant avec chaque concentration employée, semble peut-être peu convenable, et la formule se limitant à la teneur en K et le radical carré de la teneur en Ca semble suffisamment précise pour qu'on puisse stabiliser le comportement des Paramécies.

Résumé

Le comportement de *Paramecium caudatum* reste constant, sans égard à la teneur absolue du milieu en K et en Ca, si on garde une proportion constante de concentration des deux ions, exprimée par la formule:

$$\lg [K^+] - 0.43 \lg [Ca^{2+}] = \text{const.}$$

Si on introduit à cette formule les coéfficients d'activité des ions, elle devient entièrement conforme aux principes de l'équilibre de Donnan. On ne peut en tirer encore aucune conclusion concernant le rôle de K et de Ca dans la génération des biopotentiels et dans l'excitabilité de la cellule protozoaire, mais on gagne une base méthodique pour l'étude exacte de ce sujet.

STRESZCZENIE

Zachowanie się *Paramecium caudatum* pozostaje niezmienne niezależnie od bezwzględnej zawartości K i Ca w środowisku, jeśli przestrzega się stałego stosunku stężeń obu jonów, zgodnie ze wzorem:

$$\lg [K^+] - 0.43 \lg [Ca^{2+}] = \text{const.}$$

Po wprowadzeniu do tego wyrażenia współczynników aktywności jonów uzyskuje się jego pełną zgodność z zasadami równowagi Donnana. Nie pozwala to jeszcze na wyciągnięcie żadnych wniosków dotyczących roli K i Ca w generacji biopotencjałów i w pobudliwości komórki pierwotniaczej, zyskuje się jednak podstawy metodyczne dla ścisłych badań w tym kierunku.

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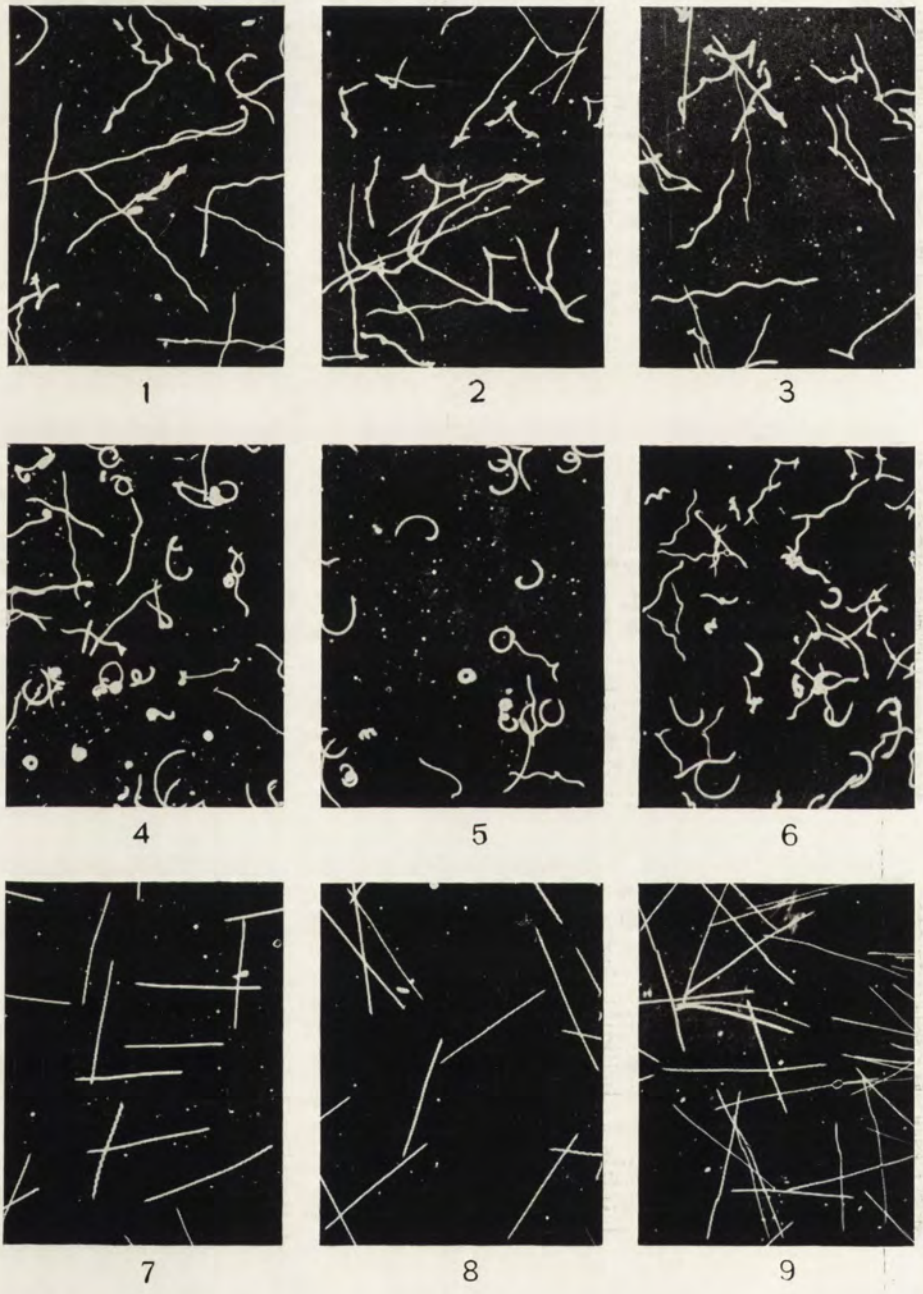
EXPLICATION DE LA PLANCHE I

Trajets parcourus par les Paramécies pendant 5″ avec des différentes proportions entre $[K^+]$ et $[Ca^{2+}]$. Le comportement reste identique sans égard à la concentration des ions, si $\lg [K^+] - 0.43 \lg [Ca^{2+}]$ reste constant, étant égal soit à 0.5 (phot. 1, 2, 3), soit à 1.0 (phot. 4, 5, 6), soit à 1.5 (phot. 7, 8, 9). Les concentrations correspondantes du KCl et du $CaCl_2$ sont présentées dans la Table 2.



A. Grębecki auctor phot.

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Effect of chemical agents on the motor responses in *Balantidium coli* (Malmsten)

Wpływ czynników chemicznych na reakcje ruchowe *Balantidium coli* (Malmsten)

In the preceding article (Rostkowska 1963) the toxic action of some chemical compounds upon *Balantidium coli* was investigated. The present study is a complement to those findings.

The problem of chemotaxis in *B. coli* may be of importance for practical reason, especially so for administration of medicals. The negative chemotaxis would be a factor inhibiting the effect of drugs, whereas the positive one may be favorable for the application of protozoacidal compounds. The chemotaxis in free-living protozoa has been studied extensively and thoroughly but the chemotactic reactions of parasitic protozoa remain not investigated.

I express my most vivid thanks to Prof. Dr. Zdzisław Raabe for his valuable suggestions in the course of this research.

Methods

In the study of chemotaxis in free-living protozoa, methods worked out by Massart 1889, Jennings 1897, 1899, 1906 and Barratt 1905 have been widely applied. The most general method of the chemotactic study in protozoa, on which all the present interpretations of those phenomena are based, is the method of Jennings 1906. This method has been recently modified by Dryl 1952 and supplemented by the photographic registration of movement (Dryl 1958). Nevertheless the slow and twisted movement of balantidia makes this method inapplicable.

The positive chemotactic reactions in *B. coli* were studied by an original method (Kadłubowski and Rostkowska 1961) which is a modification of that of Massart 1889. Our procedure was as follows: in a layer of paraffine on a slide two round areas of a diameter of 8 mm are cut out. The length of the canal joining those two areas amounts 3 mm. This connection could be interrupted by a coverslip adjusted vertically. In one of the two areas 0.05 ml of a rinced and densified protozoa culture — in the invariably used buffer solution — was placed. The number of experimented balantidia fluctuated from 61 to 83. In the second area 0.05 ml of the studied compound diluted in the same medium was placed. The coverslip was removed and the

slide was placed in a humid chamber at 37°. After 10 min., the connection between the two areas was interrupted, protozoa were killed by addition of $HgCl_2$ to the medium, and count in the both areas was excuted. Experiments were made using various concentrations of the compounds studied, repeating 3 times the experiment with every solution. The mean value was calculated.

In the method described above the positive chemotactic response proceeds as follows. When increasing the concentration of an experimented solution, the number of balantidia passing into another area gradually increases and reach a certain maximum (Pl. I 1—2); when the even higher concentrations of the compound are applied, the number of balantidia found in the experimental medium falls (Pl. I 3). The question arises which concentrations should be choiced as characterizing the intensity of the response. It seems the most convenient to determine in each case the initial concentration resulting in an accumulation of 50% of balantidia in 10 min. (ED_{50} min.) and the final concentration at which in this delay no animal pass into the experimental solution (ED_0 max.). These both concentrations offer the critheria of the response the best comparable with those furnished by the classical methods.

In the standard methods of research on chemotaxis (Jennings, Dryl), in the case of the positive chemotactic reaction there exists some treshold concentration producing an increased numerosity of protozoa in the drop of the substance studied, when compared with the exit solution. Another concentration essential in the experiment is that at which the ring appears i.e. the ciliates fail to enter the studied drop. In the method applied in the present study, the transition of 50% of balantidia to the second area (concentration ED_{50} min.) corresponds to the threshold of chemotaxis. The concentration being (in the Jennings method) the upper limit of the chemotactic accumulation, corresponds to ED_0 max., i.e. this concentration at which balantidia cease to pass to another area.

The ranges of the chemotactic effect of the compounds studied presented in the diagrams are the result of all the experimented concentrations of the given compound. In the tables only the concentrations ED_{50} min. and ED_0 max. are presented. ED_{50} min. was calculated like LD_{50} using the transmuted Fischer's formula (Rostkowska 1963) and ED_0 max was evaluated by extrapolation.

The methodics applied in this study involves much more difficulties in evaluation of the occurrence of an eventual negative chemotaxis. If to a medium containing the substance studied, at any of its concentration 50% of individuals fail to enter — this fact may be due as well to the negative chemotaxis as to the lack of the chemotactic reaction at all. For that reason the reverse procedure was applied: balantidia were introduced to the studied solution at once and, 10 min. later, the percentage of individuals which passed to the control medium was counted. Then ED_{50} min. and ED_0 max. were calculated. In one of the earlier articles (Kadłubowski and Rostkowska 1961) the ED_{50} min. and ED_0 max. calculated in this way were accepted as the measure of negative chemotaxis of the compound studied, which was not quite precise. In fact it is a positive chemotaxis in respect to the control medium as shown by ciliates exposed to the experimented solution. As example the reaction of swimming out of *B. coli* from the saccharose solution to the control medium is represented (Pl. I 4). In the reaction of this type the characteristic radial spreading of ciliates occurs, after they passed to the control medium. In contrast to this, in the medium containing a toxic com-

pound, even in a non-toxic concentration, balantidia tend to aggregate. It should be stressed that the upper limit (ED_0 max.) of the chemotactic outrun could hardly be determined because the simultaneous toxic action of the compound inhibited the movement of balantidia.

The solutions of compounds non-soluble or precipitating in the phosphate buffer were prepared in 0.85% solution of NaCl. Those compounds were: $MgCl_2$, $CaCl_2$, Na_2SO_4, NH_4Cl, aureomycin (aureomycin hydrochloride Leclerle), sulphathiazole.

Results

The first experiments concerned chlorides of ammonium, potassium, sodium, calcium, magnesium and mercurium (Table 1). *Balantidium coli* shows no positive chemotaxis to the experimented chlorides. But when placed at once in the solutions of those chlorides, the ciliates react by a positive chemotaxis to the control medium, in the case of NH_4Cl, $MgCl_2$ and $CaCl_2$. Fig. 1 shows that swimming out of balantidia from the solutions of magnesium chloride as well as of calcium and ammonium chloride begins at a concentration of low toxicity and increases with the rise of concentration. The highest number of reacting balantidia occurs at the concentration in which about 20% of individuals die. Subsequently the number of reacting ciliates falls and extincts

Table 1

Chemotaxis of *B.coli* to substances under investigation (concentration in mM)

Substance		Type of chemotaxis	ED_{50} min.	ED_0 max.
Chlorides	NH_4Cl	swimming out	0.04	197.0
	NaCl	no reaction	—	—
	KCl	no reaction	—	—
	$MgCl_2$	swimming out	273.6	2068
	$CaCl_2$	swimming out	405.3	894.3
	$HgCl_2$	no reaction	—	—
Sodium salts	Na_2SO_4	swimming out	215.5	1992M*
	Na_2CO_3	no reaction	—	—
	Na_2NO_3	no reaction	—	—
	NaJ	swimming out	27.4	362M*
	Na_2HPO_4	no reaction	—	—
	NaCN	swimming out	42.1	583.9M*
Carbohydrates and amincacids	glucose	swimming out	157.0	872.6
	saccharose	swimming out	0.017	95000
	glycine	swimming out	521.0	1329M*

* Values calculated by extrapolation — practically not attainable.

in the concentration LD_{50}. (The concentrations at which the reaction of outrun could not be stated on account of a high mortality, are marked on the diagram by crosses.)

The results of the above experiments indicate that cations Na^+, K^+ and Hg^{2+} fail to evoke any chemotactic reaction in *B. coli*. The reaction to the ion NH_4^+ begins at a concentration much lower than the toxic.

In the subsequent experiments the chemotactic reaction of *B. coli* to some sodium salts: sulphate, carbonate, nitrate, iodide, phosphate and cyanide was investigated (Table 1). *B. coli* fails to show a positive chemotaxis to any of those salts. It reacts only to the ion SO_4^{2-}, J^- and CN^- swimming out of the experimental to the control solution. In sodium sulphate the outflow of ciliates increases with the rise of concentration. Inhibition of the reaction occurs only when solution becomes toxic. The curve of sodium cyanide and sodium iodide indicate a similar increase of intensity of the negative chemotactic reaction with the increase of concentration and its fall with the subsequent increase of concentration (Fig. 2).

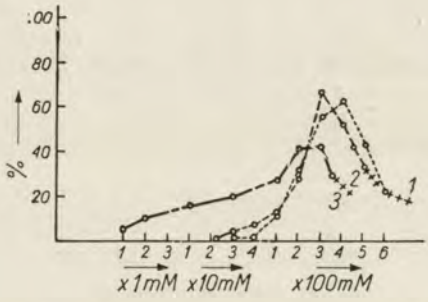

Fig. 1. Range of concentration of chlorides evoking the swimming out in *B. coli;* 1. $MgCl_2$, 2. $CaCl_2$, 3. NH_4Cl

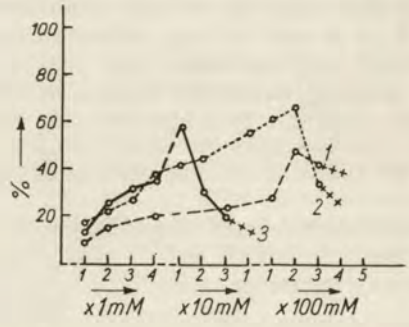

Fig. 2. Range of concentration of sodium salts evoking the swimming out in *B. coli;* 1. Na_2SO_4, 2. NaJ, 3. NaCN

Since in its natural medium *B. coli* often lives in presence of carbohydrates and aminoacids, its reaction to glucose, saccharose and glycine was experimented. As follows from the Table 1, the ciliate shows a positive chemotaxis to the control medium swimming out from the solutions of those compounds. A defensive role might be plaid by these reactions since the mean concentrations at which the outflow of balantidia occurs are lower than the toxic concentrations. This is especially clearly marked in the case of saccharose.

In the experiments of Chejfec 1935, *P. caudatum* produced a geotactic accumulation in the 0.8—1.5% concentration of glucose. Dryl 1961 stated the positive chemotaxis in *P. caudatum* in the glucose solution.

The course of the *B. coli* reaction to the studied carbohydrates and aminoacid is shown in the Fig. 3. It indicates a rather broad range of concentrations. In 100 mM glucose, about 50% of balantidia react by swimming out; the number of reacting individuals falls with the rise of concentration up to the toxic concentration. Also in the non-toxic solutions the number of reacting individuals diminishes with the fall of concentration of solution. The result is similar in the case of saccharose and glycine.

The chemotactic reaction of *B. coli* to following organic acids was investigated: formic, acetic, oxalic, lactic, citric, ascorbic and desoxycholic acids. All the above acids belong to those compounds which balantidia meet in their natural conditions.

Table 2

Chemotaxis of *B. coli* in respect to acids
(concentrations in mM)

Substance	Type of chemotaxis	ED_{50} min	ED_0 max.
Formic acid	swimming in	2.3	17.4
Acetic acid	swimming out	0.00006	0.0003
Oxalic acid	swimming in	0.3	76.0
Lactic acid	swimming in	0.009	6.8
Citric acid	swimming in	0.5	5.9
Ascorbic acid	no reaction	—	—
Desoxycholic acid	no reaction	—	—

It follows from the Table 2 that balantidia show a positive chemotactic reaction to the formic, oxalic, lactic and citric acids. They swim out to the control medium from the solutions of the acetic acid and fail to react chemotactically to the ascorbic and desoxycholic acids. The positive chemotaxis of *B. coli* to numerous acids is in agreement with the reactions of *P. caudatum*

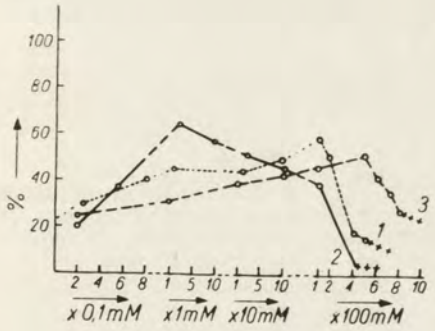

Fig. 3. Range of concentration of carbohydrates and aminoacids evoking the swimming out in *B. coli;* 1. glucose, 2. saccharose, 3. glycine

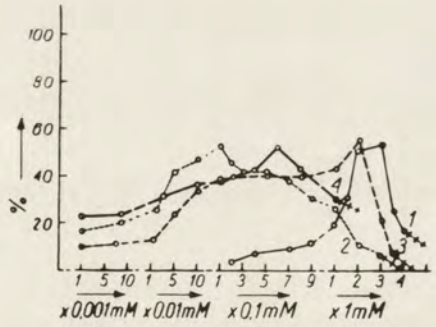

Fig. 4. Range of concentration of some acids evoking the positive chemotaxis in *B. coli;* 1. formic acid, 2. oxalic acid, 3. lactic acid 4. citric acid

(K a g a n 1939 and others report the positive chemotactic reaction as a typical response of *P. caudatum* even in respect to the acetic acid). Fig. 4 shows the range of the acids solutions evoking a positive chemotaxis. The smallest range is that of formic acid whereas those of other acids (acting positively in lower concentrations) are similar.

In the case of bile and antibiotics used in experiments (penicillin, streptomycin and aureomycin) balantidia swim out of the studied solution; for no concentration 50% of chemotactically reacting protozoa was found.

The following protozoacidal drugs were experimented: atebrin, chinosol, sulphathiazole, neosalvarsan, phenol, potassium permanganate and ethanol The positive chemotactic action upon balantidia (Table 3) was stated for:

Table 3

Chemotactic reactions of *B. coli* in respect to drugs (concentrations in mM)

Substance	Type of chemotaxis	ED_{50} min.	ED_0 max.
Atebrin	swimming in	4.2	14.1
Chinosol	swimming in	1.6	21.7
Neosalvarsan	swimming in	4.9	13.8
Sulphathiazole	no reaction	—	—
$KMnO_4$	no reaction	—	—
Phenol	swimming out	0.17	0.3
Ethyl alcohol	swimming in	2.479	9915

atebrin, chinosol, neosalvarsan and ethyl alcohol, while the positive chemotactic reaction towards the control solution was evoked by phenol, and potassium permanganate. Sulphathiazole failed to evoke any chemotactic effect. The mean concentrations at which protozoa react chemotactically are rather high. The high concentration of ethyl alcohol evoking the positive chemotactic effect is especially noteworthy.

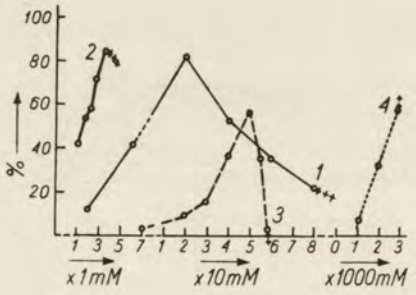

Fig. 5. Range of concentration of some drugs evoking the positive chemotaxis in *B. coli;* 1. atebrin, 2. chinosol, 3. neosalvarsan, 4. ethyl alcohol

It follows from the Fig. 5 that the range of the positive chemotactic effect of atebrin upon *B. coli* is the widest of all. Balantidia react still positively in the concentration which kills about 72% of them (10 and 20 mM), then no reacting protozoa are found. The positive reaction occurs also in the non-toxic solution, rising initially with the rise of concentration (up to 80%). The range of the positive reaction to neosalvarsan includes also the concentration killing about 90% of individuals. For chinosol the range of concentrations causing the positive chemotaxy embraces the toxic concentrations evoking about 80%

of mortality. At this concentration the positive chemotactic reaction concerns about 86% of protozoa; it falls subsequently and in the concentration 8 mM (killing 50%) the reaction of balantidia extincts. The concentration of ethyl alcohol evoking the positive chemotaxy in 50% of individuals coincides with the concentration causing 50% of mortality ($ED_{50} = LD_{50}$).

Discussion

Kagan 1939 studied two groups of salts. In one of them the anion remained constant, cation was changed, in the other was the reverse. Kagan's study allowed to conclude that the chemotactic reaction in *P. caudatum* depends mostly on cation, since salts with the same cation and different anions failed to differ in the character of the evoked reaction nor in its intensity. The same results on the same object were reported by Dryl 1952. The conclusions of the present study are rather inconsistent with the above findings because balantidia showed a different chemotaxis not only in the case of salts with different cations but also with different anions.

The conclusions of the present study are not supporting the view of Johnson 1929 and of Dryl 1952 that the chemotactic reaction depends more upon pH than on the chemical structure of the substance. As far as *B. coli* is concerned, the opposite view seems rather true.

The discussion of the interdependence between toxicity (Rostkowska 1963) and chemotaxis seems to be essential. In the case of compounds out of which balantidia swim away towards the control medium, the number of reacting ciliates increases with the rise of concentration ($MgCl_2$, $CaCl_2$, NH_4Cl, NaJ, glycine). The mean concentrations of those compounds evoking swimming out of balantidia equaled the concentrations LD_{50} or higher which excludes the possibility of a defensive role of chemotaxy. In the concentrations lower than the lethal, this reaction appeared only in the case of sodium cyanide, glucose, saccharose, acetic acid and phenol, but even then the number of reacting individuals increased merely with the rise of concentration.

In the case of compounds of a positive chemotactic action upon *B. coli*, their concentrations evoking the reaction in 50% of ciliates (ED_{50}) equaled approximately the concentrations LD_{50} killing the same number of ciliates (formic and citric acids, chinosol and ethyl alcohol). The positive chemotactic concentration of neosalvarsan was even higher than those of the above compounds. It should be stressed that the reaction to all compounds appeared in concentrations higher than the lethal and the number of reacting individuals diminished only because of their toxicity. For some compounds like oxalic and lactic acids, atebrin, the concentrations ED_{50} were lower than LD_{50}. In all cases the number of reacting ciliates increased with the rise of concentrations of the experimented compound and diminished only in the lethal concentration as result of mortality.

The problem of interdependence between toxicity and chemotaxis in free-living protozoa is not a new question. Jennings 1899 basing on his study on *Paramecium aurelia* found no interdependence between those two phenomena since many substances evoking the negative chemotaxis are slightly or not toxic at all, whereas substances involving the positive chemotaxis may

be very toxic. Dryl 1959 stated that there exists a correlation between chemotaxis and toxic properties of compounds of a similar chemical structure. Dryl's conclusion is based on his experiments with alcohols. In the sequence of alcohols (primary, secondary, tertiary) toxicity increased and chemotaxis diminished with the rise of the molecular weight. The experiments of the present study support rather the suggestion of Jennings than that of Dryl.

The positive reaction of *B. coli* to the protozoacidal drugs is a striking fact. It may be supposed that the protozoacidal drugs chemotactically positive exert their killing effect already in small amounts, since protozoa accumulate in their presence. Possibly the occurrence of this positive chemotactic reaction may be of importance not only in applying the protozoacidal drugs against balantidium but against other protozoa living in the alimentary tract as well. Protozoa exposed to the action of drugs may approach the medium in which they soon die.

Summary

The chemotactic reaction of *Balantidium coli* to formic, oxalic, lactic, and citric acids, atebrin, chinosol, neosalvarsan and ethyl alcohol is positive. *B. coli* swims out towards the control medium from solutions of NH_4Cl_2, $MgCl_2$, $CaCl_2$, Na_2SO_4, NaJ, NaCN, acetic acid, glucose, saccharose, glycine, phenol and potassium permanganate. It fails to react chemotactically to NaCl, KCl, $HgCl_2$, Na_2CO_3, Na_2NO_3, Na_2HPO_4, ascorbic and desoxycholic acids, and sulphathiazole. Balantidia show a different chomotaxis not only to salts of different cations but also of different anions. The experiments indicate the lack of an essential dependence between toxicity and chemotaxis in *Balantidium coli* since the reaction of swimming out from the experimental solution to the control medium occurs only in concentrations higher than the toxic ones. More so, sometimes the positive chemotaxis to compounds of a high toxicity occurs.

STRESZCZENIE

Spośród zbadanych związków *B. coli* reaguje chemotaktycznie dodatnio na kwas mrówkowy, szczawiowy, mlekowy, cytrynowy, atebrynę, chinozol, neosalwarsan i alkohol etylowy. Wypływanie *B. coli* do środowiska kontrolnego występuje z roztworów NH_4Cl, $MgCl_2$, $CaCl_2$, Na_2SO_4, NaJ, NaCN, kwasu octowego, glikozy, sacharozy, glicyny, fenolu, nadmanganianu potasu. Balantidium nie reaguje chemotaktycznie na NaCl, KCl, $HgCl_2$ Na_2CO_3, Na_2HPO_4, kwas askorbinowy, dezoksycholowy i sulfatiazol. Balantidia wykazują różną chemotaksję nie tylko wobec soli o różnych kationach ale i przy różnych anionach. Doświadczenia wskazują na brak istotnej zależności między toksycznością a chemotaksją, skoro reakcja wypływania ze środowiska eksperymentalnego do kontrolnego pojawia się dopiero w stężeniach przekraczających granicę toksyczności, a — co ważniejsze — zdarza się nawet chemotaksja dodatnia w stosunku do substancji silnie toksycznych.

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EXPLANATION OF THE PLATE I

1: Positive chemotactic response in respect to the solution of the lactic acid 0.02 mM; in 10 min. 54% of balantidia pass into the experimented solution

2: The same with 3.5 mM lactic acid; only 11.7% of balantidia enter the solution in 10 min.

3: The same with 4 mM lactic acid; in 10 min. 6.6% of individuals are found in the solution

4: Balantidia swimming out from the solution of saccharose 2 mM; in 10 min. 65.5% of individuals pass into the control medium

J. Rostkowska auctor phot.

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Effect of chemical agents on some vital functions of *Balantidium coli* (Malmsten)

Wpływ czynników chemicznych na niektóre czynności życiowe *Balantidium coli* (Malmsten)

The present article is a continuation of the study (Rostkowska 1963, 1964) concerning the toxic and chemotactic action of some compounds upon *B. coli* and reports the results of the experiments on the effect of some chemical compounds upon such vital functions as nutrition, reproduction and encystation.

Those problems have not been studied in parasitic protozoa although they might be of importance in administring medicines. Even small amounts of chemical compouds in the medium may evoke disturbances involving the gradual extermination of the parasite population.

I express my hearty thanks to Prof. Dr. Zdzisław Raabe for his valuable informations in the course of this research.

Nutrition

Only scarce informations concerning the nutrition of *B. coli* are found in the literature. It is known that it feeds on carbohydrates from different food remnants and presumably engulfs red and white blood cells, fragments of tissues and also microorganisms. In culture it is mostly fed on starch.

In this study the observations are limited only to experiments with erythrocytes. For experiments human blood was used diluted 1 : 25 000, rinced twice in 0.85% NaCl solution and centrifuged at about 200 rotations per min. Test tubes with 2 ml of culture rinced in 0.85% NaCl solution, mixed with 0.5 ml of erythrocytes suspension were placed in thermostate at 37°. After 10, 20, and 30 min. samples of balantidia were killed with $HgCl_2$ on a slide. Balantidia with engulfed erythrocytes were counted as well as the number of erythrocytes per one individual (Table 1).

In the experiments concerning the effect of chemical compounds upon erythrocytophagy, samples (0.25 ml) of balantidia were placed in 1.75 ml of the studied solution with addition of 0.5 ml of erythrocytes suspension. Compounds were used in solutions killing 50% of individuals as well as in higher and lower concentrations. Experiments were carried out with atebrin (evoking the positive chemotaxis) and with magnesium chloride (balantidia placed in $MgCl_2$ react positively to the control medium).

Table 1

Effect of chemicals on erythrocytophagy in *B. coli* (N% — percent of individual ingesting erythrocytes, n — mean number of erythrocytes absorbed per individual)

Substance	Atebrin 0.00001 mM		$MgCl_2$ 0.2 mM		Control medium	
Exposure time	N%	n	N%	n	N%	n
10′	27.8 ± 0.8	0.44	10.3 ± 1.8	0.12	67.8 ± 9.9	1.99
20′	32.9 ± 0.5	0.80	11.1 ± 1.4	0.15	83.4 ± 2.1	3.13
30′	44.2 ± 1.6	1.12	14.2 ± 2.7	0.19	91.4 ± 0.9	3.37

Table 1 indicates that the number of balantidia with absorbed erythrocytes falls 6 times under the influence of magnesium chloride and more than twice after the action of atebrin. The mean number of erythrocytes engulfed by one individual diminishes in a similar proportion. The result is represented in diagram in the Fig. 1, where the thickness of the line indicates the number of the absorbed erythrocytes. As well atebrin as magnesium chloride in toxic concentrations (LD_{50} and higher) contribute to the complete inhibition of erythrocytophagy. Concentrations of atebrin and $MgCl_2$ in the Table 1 are much lower than LD_{50}.

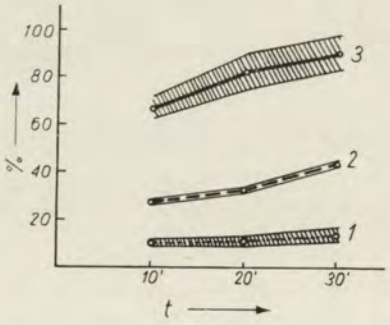

Fig. 1. Mean numbers of balantidia which ingest the erythrocytes as depending on the time of exposure to different media; 1. $MgCl_2$,

The above observations indicate that even small doses of the drug involve disturbances in the nutrition of balantidia. However the problem whether the drugs evoke only inhibition of erythrocytophagy or of ingestion of food in general, remains not elucidated.

Similar results were obtained by Seravin 1957 in his study of the influence of $MgCl_2$ and Dogiel und Isakova-Keo 1928 on the effect of $CaCl_2$ and $MgCl_2$ upon feeding in *Paramecium*.

The study on nutrition in *B. coli* cannot be looked upon as complete. Yet it speaks in favour of the view that *B. coli* is able to ingest erythrocytes — which was reported in the literature only as a supposition — and indicate a distinct influence of chemical compounds on inhibition of erythrocytophagy.

Reproduction

Reproduction of *B. coli* has been extensively described in the literature. Jameson 1927, Nelson 1934, 1935, Knauff 1936, Lamy et Lamy 1951 reported the dependence of the division rate on the character of the nutrient, on the frequency of inoculation of strains, on the development of microflora in culture and on the value of the medium pH. There are no informations about the influence of chemical compounds on division.

Experiments of this series were executed using the solution of atebrin, of formic acid and magnesium chloride in concentrations killing 50% of ciliates and also some lower. One series of solutions was prepared diluting the chemical compounds with the nutrient, the other — using the buffered 0.85% solution of NaCl. In each test tube with 3 ml of solution 1, 3, 5, 7, 9, 11, 15 balantidia were placed. Both series of experiments were carried out simultaneously in thermostate at 37°. After 24 hrs. samples of single tubes were collected, killed with $HgCl_2$ and the experimental and control ciliates were counted. Results are shown in the diagram (Fig. 2).

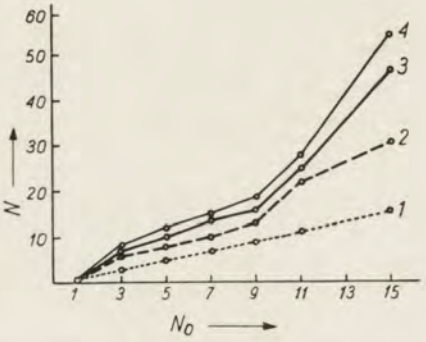

Fig. 2. Effect of chemicals on the reproduction in *Balantidium coli;* 1. atebrin, 2. $MgCl_2$, 3. formic acid, 4. control medium

The curves indicate that in the control balantidia cultures in average about 2 divisions in 24 hrs. occurred. No division occurred under the action of atebrin 0.01 mM; the exit number of protozoa was found still after 24 hrs. Reproduction is slightly inhibited under the action of $MgCl_2$ 0.1 mM. In the medium with formic acid 0.01 mM, the number of balantidia after 24 hrs. corresponds in some samples to the control number or is near it.

It should be pointed out that in the tests where more individuals were put together (over 8 individuals) their division rate was higher. The experimented compounds applied in concentration LD_{50} inhibited the ciliates division completely.

Encystation

The literature concerning the encystation in parasitic protozoa is rather extensive. Rees 1927, Suhanova 1953, 1955, 1959, 1960, Lom 1956, Mihalčenko 1958 have discussed the process od cysts formation mostly under the influence of temperature, changes of pH, phosphorus salts and sodium chloride. The resistibility of cysts to changes in the medium conditions is also discussed. The results are often contradictory and deserve revision. The

least profound is the study of encystation in *B. coli*. Most informations on this process concern the statement of cysts in feces, the morphological structure of cysts, their resistibility against the medium factors and desinfection. In the literature the opinion may be found that *B. coli* fails to produce cysts in the laboratory conditions. In the course of the present study over 100 inoculations in normal conditions (23.III.1961—1.I.1962) were followed and no cysts were found in any culture. Nevertheless, the cyst formation might be obtained after a chemical treatment.

For the study of cysts formation under the influence of chemical compounds NaCl, atebrin, formic acid and $MgCl_2$ were applied. The compound under study (2.5 ml) was placed in a test tube with 0.5 ml of medium containing 53—68 balantidia. After mixing both amounts a concentration killing 50% of ciliates or higher and lower were obtained. The experimental material was kept in thermostate at 37°. Ten experiments for every compound were carried out. After 10, 30, 60 min. and after 12 and 24 hrs. two samples of every tube were examined; living individuals and cysts were counted. Cysts were transferred into the normal nutrient for excystation and observed after 10, 30, 60 min. and 6, 12, 24 hrs. at the temperature of 37°. However even after 48 and 72 hrs. a part of cysts kept their normal shape and character (the majority of them became desintegrated after this period of time). In another series of experiments bile in dilution 1 : 10, 1 : 50, 1 : 100, 1 : 300 was added to the nutrient. In those solutions all the cysts desintegrated already after 4 hrs.

In the next series of experiments HCl was added to the nutrient containing cysts considering that in stomach the cysts are exposed for some time to the action of this acid. HCl was added to the nutrient in such amounts that pH 0.8, 1.2, 1.4, 1.63, 1.92 and 2.11 was obtained. To another portions trypsin and pepsin were added. Several tenth of experimental series were studied and in none of them excystation was observed. Staining cysts with safranin and eosin was tried after fixation in the Schaudinn's fluid; only in some cysts nuclei were stained and no visible vacuoles were detected.

Suhanova 1959 studied the action of temperature upon the viability of cysts in *Opalina ranarum, Balantidium elongatum* and *Balantidium duodenale* infecting young frogs with those ciliates. Excystation was stated 6—8 hrs. or 18—24 hrs. after infection, depending on the length of action of the temperature applied. Excystation of *Opalina ranarum* in laboratory conditions was also observed by Suhanova; cysts were kept in the Ringer's solution with addition of some bile; the movement of cytoplasm was seen but the process of excystation till its completion i. e. till leaving the cyst by the ciliate — escaped observation. Lom 1956 in his study of excystation in *Nycthotherus cordiformis* added pepsin and trypsin to the nutrient but the expected effect failed to appear.

Mihalčenko 1958 and Suhanova 1959 stated the existence of pre-cystal forms in *B. entozoon, B. elongatum, B. duodenale*. Only those forms are capable to encyst and to excystation. In this respect the above authors return to the former view of Stein 1867 that after division the posterior individual characterized by some morphological peculiarities is the pre-cystal form.

The experiments and observations presented in this article hardly permit to draw far reaching conclusion concerning encystation and excystation. Formation of two forms of cysts might eventually be suggested. One form would

be represented by normal cysts appearing as invasion forms developed from the pre-cystal individuals. Only those cysts would be able to excyst. The second type of cysts probably occur in extreme conditions (e. g. in the above experiments) as result of action of a poisonous compounds applied in a non-toxic or slightly toxic concentration. They are rather spurious cysts with no possibility of life, invasion and excystation.

Summary

Balantidium coli shows the ability of absorbing erythrocytes. This ability diminishes 6 times under the action of magnesium chloride, and twice after addition of atebrin. About 2 divisions in 24 hrs. occur in *B. coli*. In more dense cultures the rate of division is higher. Under the influence of sodium chloride, magnesium chloride, formic acid and atebrin (in concentrations lower than LD_{50}) cysts are formed. Presumably those cysts are not normal stages of ontogenesis but rather some spurious forms with no capacity of excystation and of invading.

STRESZCZENIE

Balantidium coli wykązuje zdolność pobierania erytrocytów. Zdolność ta zmniejsza się pod wpływem działania chlorku magnezu 6-krotnie, pod wpływem działania atebryny 2-krotnie. U *B. coli* występuje około 2 podziałów na dobę. W próbach gęstszych częstotliwość podziałów była większa. Pod wpływem działania atebryny, kwasu mrówkowego i chlorku magnezu zmniejsza się liczba podziałów. *B. coli* pod wpływem działania chlorku sodu, chlorku magnezu, kwasu mrówkowego i atebryny (w stężeniach niższych niż LD_{50}) tworzy cysty. Wydaje się, że nie są to cysty normalne wynikające z ontogenezy, lecz raczej cysty poronne nie mające zdolności ekscystacji i inwazji.

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Exoerythrocytal development stages of *Piroplasma bigeminum*

Внеэритроцитарные формы развития *Piroplasma bigeminum*

The knowledge of all stages of the parasite life cycle is essential for the adequate understanding of its taxonomic position as well as of the problems of immunity, pathogenesis and therapy. Yet the informations concerning the stages of development of piroplasmae in their vertebrate hosts remain till recent time utmost scarce and not consistent. The development stages of piroplasmae in erythrocytes are investigated best. The trophozoits in erythrocytes mostly multiply by the simple binary division. In some species of piroplasmae multiple division within erythrocytes has been described. Kinoshita 1907 reported the erythrocytal schizogony in *Babesia canis,* and Cerruti 1962 in *Babesia perroncitoi.*

Till recently the question concerning the existence of exoerythrocytal development stage in piroplasmae and its precedence of the erythrocytal stages is solved in different ways. According to some authors (Reusse 1954, Sudačenkov 1960, Hoyte 1961) piroplasma reproduces only within erythrocytes; others (Paraense 1949, Boero 1954) find the existence of exoerythrocytal stages possible — without giving any convincing evidences; some others (Ivanič 1936—37, Trofimow 1952, Kolabskij, Gajdukov i Tarverdjan 1961) report different forms of exoerythrocytal reproduction of *Piroplasmida.* Kolabskij and co-workers studying the exoerythrocytal stages of piroplasmae used for infection the blood forms of *P. canis* and of *P. bigeminum,* previously passaged several times through the warm-blooded host. The authors detected inclusions which they consider as intracellular development stages of piroplasmae in the endothelial cells of dog and in liver, brain and lymphatic nods of cattle. Ivanič also described some intracellular forms of piroplasmae. It seems however not clear in which cells different development stages of the investigated piroplasmae are parasitizing. The author reports the development of *Babesiella bovis* in the leukocytes with a big nucleus. In the liver cells Ivanič observed the development of the multinuclear giant plasmodia, infecting sometimes whole cell groups at once. All those investigations were executed on a not extensive and rather casual pathological material. Trofimov 1952 studied the pathological anatomy and pathogenesis of nuttalliosis in horse. He found inclusions in the cytoplasm of endothelial cells of liver, kidney, lungs and lymphatic nods.

Those inclusions are considered by the author to be reticulo-endothelial stages of nuttallia development.

In sum, the gathered data on the exoerythrocytal stages of *Piroplasmida* fail to give a clear and complete information about the development cycle of the parasite in the vertebrate host and require an exact revision by means of other methods.

In the attempt to contribute to this question, the author of the present study tried to state the presence of piroplasma in the blood of the vertebrate host during the incubation period (K r y l o v 1962). This problem was solved by transfusion of a considerable quantity of blood (300—350 ml), from the bull-donor infected with piroplasma through the tick *Boophilus calcaratus*, to the steril bull-acceptors in different moments of the incubation period. It was stated that piroplasmae may be detected in blood by means of a biological test, already 4 days after contacting the tick (at the 16 days long incubation period).

*

Nevertheless, a special experiments had to be performed in order to ascertain whether some development stages of piroplasma outside the erythrocytes exist in the incubation period or in time of manifestation of the clinical disease symptoms. For this purpose 6 individuals of cattle, originating from a zone healthy as to the blood-parasite diseases, were inoculated by placing a great number (20.000—60.000) of *B. calcaratus* larvae infected with *P. bigeminum*. Examination of different organs and tissues was performed on the lst, 4th and 7th day after stating the presence of parasites in the peripheral blood of the animals under experiment (by means of microscopic method). Those time intervals were chosen for following reasons. If we presume that the metagenetic development of the parasite, connected with different type of reproduction and different localization, occurs — the highest number of exoerythrocytal stages could be expected either in the final stage of the incubation period or at the peak of parasitemy.

Another procedure was performed for elucidating the way of penetration and for detecting the primary forms of piroplasmae inoculated to the vertebrate host by the intermediate host. Every day of the incubation period punctate of lymphatic nods of the patella fold was examined as well as the biopsy was made of the skin and of the subcutaneous peritoneum from the places of the mass aggregation of ticks. Two specimens of bulls were examined. The smears and imprints of organs and tissues were fixed with methyl alcohol and stained after Romanovski.

In the punctates taken during the incubation period from the lymphatic nods piroplasmae were never detected microscopically although the area from which lymph collects into those nods was entirely coated by the adhering ticks infected with *Piroplasma bigeminum*.

Examination of skin fragments and of subcutaneous tissue sampled together with the adherent ticks revealed only in one case single amoeboid mononucleated forms of piroplasmae freely lying in the lumen of the capillary (Fig. 1 A—C). Those stages of development resemble to the reproduction forms of piroplasmae found in the salivary glands of tick *B. calcaratus* (M u r a t o v i C h e i s s i n 1959). Those forms occurring rather rarely, were found during the incubation period on the 10th day after placing ticks, a few days prior to appearence of parasites in the erythrocytes.

V a s i n a i D e m i n a 1949 studying the development stages of *Plasmodium gallinaceum* in the skin of hens, evoked experimentally a local aseptic inflammation in order to retain the sporozoits in the centre of inflammation. This method faciliated considerably the search of sporozoits. In the present investigations this method had to be abandoned because a rather acute local inflammation of the subcutaneous tissue was evoked although by the clinging ticks transmitting piroplasmae. Nevertheless the author failed to detect any form of piroplasma, morphologically different from those found in the salivary glands of tick. The observations reported above indicate that the piroplasma forms introduced by the tick into the vertebrate host fail to remain and to reproduce in skin but evidently migrate with the flow of blood and possibly also of lymph into the internal organs.

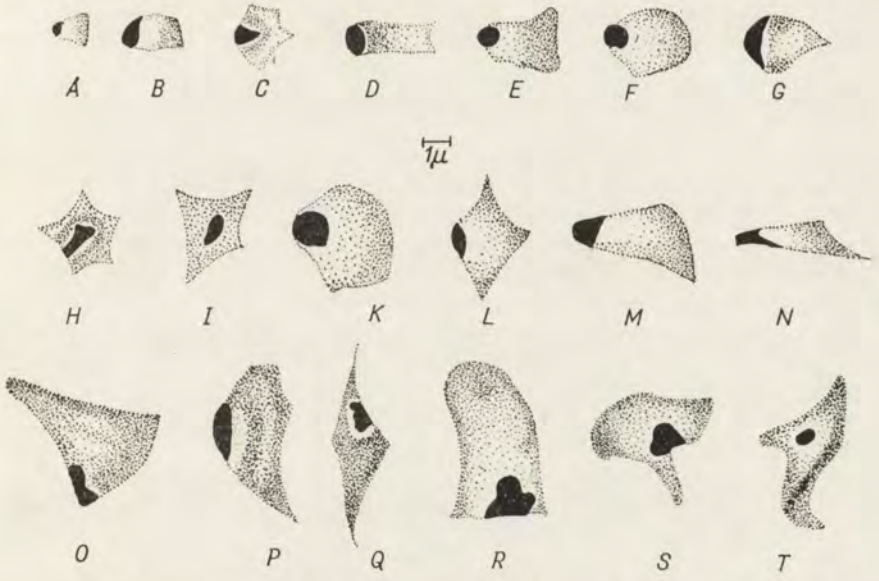

Fig. 1. Mononuclear exoerythrocytal stages of the development of *Piroplasma bigeminum*

The extraerythrocytal forms were found in the internal organs of 3 out of 6 animals under experiment (in one of them the 1st day, in the second the 4th and in the third the 7th day after detection of parasites in the erythrocytes under microscope). The highest number of the exoerythrocytal forms occurred in the animal killed the 7th day after appearence of parasites in the peripheral blood which coincided with the period of the most acute clinical symptoms and parasitary reaction.

The exoerythrocytal forms of development were stated in lungs, kidney, liver, heart muscle, spinal cord and in spleen. In all cases the exoerythrocytal forms of parasites were found lying out of cells, in blood plasma. Most frequently round or irregular bodies occurred, measuring from $1.2 \times 1.2\,\mu$ to $3.0 \times 4.8\,\mu$, with nuclei of various shape and size (Fig. 1 D—T). In some others 2—3 nuclei were present with a connection between them (Fig. 2 A—C), rearly 5—15-nuclear stages were found measuring up to $7.7 \times 9.9\,\mu$ (Fig. 2 E—J).

Between the small mononuclear and the big forms all the intermediate occur, suggesting their growth and transformation into the big ones. The multinucleate structures arise by the multiple division of nucleus which may be often observed in preparations.

In the multinucleate structures, the process of separation and budding of one or several amoeboid bodies is observed (Fig. 2 K). The process of segmentation of the multinucleate forms is asynchronic. On the periphery or on one of the sides of the multinucleate schizont, mononucleate or sometimes binucleate irregular bodies are detaching, whereas the central part or the opposite side remains unsegmented. This multiple division reminds the asynchronic „schizogony" or even rather the nucleogony which this parasite undergoes in the tick transmitter (Muratov i Cheissin 1959).

Fig. 2. Multinuclear exoerythrocytal stages of the development of *Piroplasma bigeminum*

Except the multiple division of the big schizonts, the single division into two individuals in the amoeboid stages may be observed.

All those stages reproduce in the blood plasma of the internal organs capillaries. The highest number of them occur in the organs well supplied with oxygen, especially in the capillaries of lungs[1]. It was observed that the number of exoerythrocytal stages of piroplasmae accrews concurrently with the erythrocytal forms. Detection of the erythrocytal stages of piroplasma development in the first day of the clinical symptoms and also 7 days later, speaks in favour of the possibility that the multiple division of piroplasmae

[1] I express my thanks to Prof. E. M. Cheissin for consulting this group of my preparations.

outside the erythrocytes may be repeated several times and possibly may also alternate with the monotomic reproduction in the erythrocytes. The study of the liver cells and other organs failed to reveal the intracellular forms of piroplasmae.

In this way, at the conclusion of the incubation period exoerythrocytal stages of multiple division are found in the internal organs, besides the erythrocytal forms of the piroplasma development. Are they preceding the erythrocytal stage of the parasite or are both forms occurring simultaneously, remains as yet unknown, because the multiplying exoerythrocytal forms failed to occur in the incubation period. In the course of the clinical disease both forms of reproduction occur concurrently but their alternating is also possible.

In contrast to the development of the malaria plasmodium in the organism of the vertebrate host, in piroplasmosis is observed no disappearing of parasites from blood in the course of the incubation period. This was shown by experiments of infecting new acceptors with the blood on the 4th day of incubation period (K r y l o v 1962). It may be assumed therefore that the stages of development of piroplasma are present in blood since the moment of introducing them with saliva of the tick.

Summary

The development cycle of piroplasma in the vertebrate host was studied. Exoerythrocytal forms of the parasite were found in the lumen of the capillaries of lungs, kidney, liver, heart muscle, spinal cord and spleen. Exoerythrocytal forms were detected in the incubation period and on the 1st, 4th and 7th day of manifestation of the disease symptoms. Exoerythrocytal forms reproduce by multiple nucleogonic division. The number of nuclei in the dividing parasite fluctuates from 2 to 15. The most intense reproduction occurs in the lumen of capillaries in internal organis rich in oxygen.

РЕЗЮМЕ

Изучался цикл развития пироплазм в позвоночном хозяине. Внеэритроцитарные формы паразита были найдены в просвете капилляров легких, почек, печени, сердечной мышцы, спинного мозга и селезенки. Внеэритроцитарные формы обнаружены в инкубационный период и при проявлении клинических признаков заболевания на 1-й, 4-й и 7-й день. Внеэритроцитарные формы размножаются множественным делением путем нуклогонии. Количество ядер в делящемся паразите колеблется от 2-х до 15-и. Наиболее интенсивно размножение идет в просвете капилляров внутренних органов, богатых кислородом.

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Testacea du seston des riviéres Wkra et Narew

Testacea sestonu Wkry i Narwi

Durant les années 1959—1960, en été, le Laboratoire de la Protection des Eaux a mené des recherches sur le degré de pollution des rivières Wkra et Narew. Pendant ces recherches des échantillons du seston ont été prélevés dans ces rivières et les *Testacea* y ont été déterminés.

Le but de ce travail était de compléter les données concernant le présence des *Testacea* en Pologne, étant donné que ce groupe y a été très peu étudié. En raison du caractère du milieu que représente le cours d'une rivière et du matériel peu abondant qu'on a pu recueillir il n'a pas été possible de tirer de ces recherches des conclusions écologiques de caractère plus général.

Rivières étudiées

Wkra

La Wkra est un affluent d'importance secondaire de la rive droite du Bug. Sa longueur est de 232.2 km et son bassin occupe un territoire de 5402.1 km^2. C'est une rivière de plaine au cours lent, son débit moyen à l'embouchure est de Q = 17 m^3/sec.

D'après Biliński et autres 1963, durant l'année 1959 sur 13 postes d'observation échelonnés le long de la rivière, 1 poste démontrait une pollution de l'eau très élevée, 5 — une pollution importante, et à 7 postes l'eau était relativement peu polluée. Dans ces deux cas les pollutions provenaient des réseaux urbains (postes 2 et 4), dans le reste elles provenaient de l'industrie agraire.

L'été 1959 le seston de la Wkra en majeure partie se composait d'algues. *Diatomea* et *Flagellata* y dominaient. Le zooplancton était peu nombreux et représenté par des *Ciliata* et *Rotatoria*. *Testacea* s'y trouvaient en quantités infimes, inférieures à 20 individus/l d'eau, de 0 à 4 espèces par poste d'observation. Les échantillons étaient prélevés en faisant passer 30 l d'eau par un filet planctonique. Les espèces étaient déterminées et comptées dans la chambre de Sedwick-Rafter. Les échantillons ont été prélevés en été 1959 à 13 postes situés le long de la rivière.

Narew

La Narew est l'affluent le plus important de la rive droite du Bug et en même temps l'une des plus grandes rivières du bassin de la rive droite de la

Vistule. Son cours supérieur d'une dizaine et quelques kilomètres passe par le territoire de l'URSS. La longueur de son cours en Pologne est de 412 km. Elle reçoit en Pologne 61 affluents dont une partie draine les lacs et les tourbières de Mazurie. La Narew se jette dans le Bug à Serock et forme avec cette rivière le lac artificiel de Zegrze achevé en 1962. La connaissance de la composition des *Testacea* de la Narew rendrait possible d'établir l'origine des espèces formant la population du nouveau bassin.

Toute la partie étudiée de la Narew passe par une basse plaine. Aux 3 postes supérieurs le fond de la rivière était vaseux, aux postes suivants jusqu'à l'embouchure il était sablonneux. Des près humides, inondés pendant les crues, s'étendent le long de la majeure partie du cours de la rivière. Les rives sablonneuses de la rivière, surtout dans son cours inférieur, étaient couvertes d'asiers. La végétation aquatique y était très pauvre et était représentée en majeure partie par *Typha*, *Acorus* et *Potamogeton*.

D'après Gajewski 1961 la Narew fait part des rivières les plus propres de Pologne. Ce n'est qu'à deux postes, aux environs de milieux urbains et à un poste situé en aval d'une fabrique de cellulose et de papier, que son degré de pollution devenait assez élevé.

Le seston, dans lequel les espèces des *Testacea* étaient déterminées, se caractérisait par la domination des *Diatomea*. Le zooplancton y était pauvre, ce n'est que dans la partie finale que *Rotatoria* dominaient nettement. Les espèces des *Testacea* étaient déterminées dans des échantillons prélevés en avril et en août. En avril 5 espèces ont été trouvées, tandis qu'en août on en a trouvé 20. Dans les échantillons d'hiver et d'automne on ne trouvait, que des coquilles vides d'une façon sporadique.

Revue des espèces

Arcella vulgaris Ehrenberg

C'est une espèce eurytopique. Dans la Wkra elle a été trouvée au poste 1, où la rivière avait 1.5 m de largeur et 20 cm de profondeur, un fond sablonneux couvert de vase et d'une végétation de l'*Elodea canadensis* (pH 8.2, O_2 — 7.6 mg/l, taux de saturation en oxygène — 84.3%, Ca — 64.4 mg/l, Fe — 1.3 mg/l). Dans la Narew on l'a trouvée au printemps à deux postes du cours moyen de la rivière où le pH était aux deux postes égal à 8.0, la saturation en oxygène 86.4 et 84.3%. L'eau contenait 10.0 et 9.8 mg/l de O_2, 57.3 et 56.5 mg/l de Ca, 0.6 et 0.5 mg/l de Fe.

**Arcella vulgaris* Ehrenberg f. *undulata* Deflandre

Cette espèce est nouvelle pour la Pologne[1]. Bartoš 1954 note qu'elle se trouve parmi les plantes aquatiques et le sapropel. Dans la Narew elle a été trouvée avec *A. gibbosa* var. *loevis*.

**Arcella conica* (Playfair) Deflandre

C'est une espèce probablement cosmopolite. Hofker 1942 et Grospietsch 1954 l'ont trouvé dans les régions polaires. Beaucoup d'auteurs l'ont noté en Europe. Sa présence n'avait pas été constatée jusqu'à présent en Pologne. Cette espèce se trouve le plus souvent parmi des plantes aquatiques. Deflandre 1927 la trouve dans un petit bassin, Bartoš 1954

[1] Le signe * indique les espèces nouvelles pour la Pologne.

Table 1

Index des espèces trouvées dans les rivières étudiées

L'espèce	Wkra	Narew
Arcellidae		
Arcella vulgaris Ehrenberg	+	+
**A. vulgaris* Ehr. f. *undulata* Defl.	–	+
A. gibbosa Penard	+	+
**A. gibbosa* Penard var. *mitriformis* Defl.	+	–
A. gibbosa Pen. var. *loevis* Deflandre	–	+
A. discoides Ehrenberg	+	+
**A. conica* (Play.) Deflandre	+	–
A. megastoma Penard	+	+
A. haemisphaerica Perty	+	+
A. rotundata Play. var. *aplana* Deflandre	+	–
Centropyxidae		
Centropyxis aculeata Stein	+	+
C. constricta (Ehr.) Penard	–	+
C. discoides (Pen.) Deflandre	–	+
C. ecornis (Ehr.) Leidy	–	+
C. arcelloides Penard	–	+
Difflugiidae		
Difflugia acuminata Ehrenberg	+	–
D. corona Walich	–	+
**D. fallax* Penard	+	+
D. globulosa Dujardin	+	–
D. hydrostatica Zacharias	–	+
**D. lebes* Penard	–	+
D. lobostoma Leidy	+	–
**D. oviformis* Cash	+	–
D. scalpellum Penard	+	–
Nebelidae		
Leusquereusia spiralis (Ehr.) Bütschli	–	+
Nebela colaris (Ehr.) Leidy	–	+
Cyphoderiidae		
**Cyphoderia trochus* Penard	+	+
**C. calceolus* Penard	–	+
Euglyphidae		
**Pareuglypha reticulata* Penard	+	–
Trinema lineare Penard	–	+

dans une rivière, Decloître 1959, Grospietsch 1954 parmi des mousses hydrophiles, Ertl 1955 dans des tourbières sur des sphaignes et dans la plancton. Elle a été notée à deux postes du cours moyen de la Wkra. Le pH de l'eau à ces postes était 7.5 et 7.8. L'eau contenait 6.9 et 7.7 mg/l de O_2 et sa saturation en oxygène était de 69.5 et 79.1%. Sa teneur en Ca 67.3 et 65.1 mg/l, en Fe — 0.4 et 0.3 mg/l.

Arcella discoides Ehrenberg

Espèce aquatique ubiquiste, très commune, cosmopolite. Elle a été trouvée en Pologne par Harnisch 1924 dans des tourbières des Sudètes et par Moraczewski 1961 dans le littoral du lac Kisajno. Dans les eaux de la Wkra elle a été trouvée à 3 postes. A ces postes la rivière était largement répandue, peu profonde et traversait des prés tourbeux et des champs cultivés. Le pH était de 7.5, 7.5 et 7.4, la teneur en O_2 — 4.4, 6.9 et 8.1 mg/l, la saturation en oxygène 44.0, 69.5 et 84.7%, la teneur en Ca 71.6, 67.3 et 65.8 mg/l, en Fe 0.4, 0.6 et 0.3 mg/l. Cette espèce a été également constatée en été dans le seston du cours supérieur de la Narew. Les conditions y étaient les suivantes: pH 7.7 et 8.5, saturation en oxygéne 98.3 et 73.1%, 8.7 et 6.7 mg/l de O_2, 53.0 et 61.6 mg/l de Ca, 1.6 et 0.4 mg/l de Fe.

Arcella gibbosa Penard

Espèce aquatique se trouvant dans différent types de bassins. Elle a été notée dans le cours supérieur de la Wkra à des pH 7.75 et 7.55, O_2 — 6.72 et 4.38 mg/l, saturation en oxygène 71.6%, Fe — 0.8 et 0.6 mg/l. Elle a été également trouvée en été à deux postes du cours inférieur de la Narew aux pH 7.85 et 8.40, saturation en oxygène 89.9 et 112.1%, teneur en oxygène 7.92 et 9.92 mg/l, en Ca 63.0 et 55.8 mg/l, en fer aux deux postes 0.8 mg/l.

Arcella haemisphaerica Perty

Cette variété a été constatée en Pologne par Moraczewski 1961. On la trouve parmi les plantes aquatiques (Bartoš 1954), dans le littoral peu profond (Moraczewski 1961) et dans des tourbières sur des mousses humides ou sèches (Ertl 1955). Deflandre 1928 la note comme une espèce des eaux acides. La présence de cette espèce coïncide probablement avec celle de l'*Arcella gibbosa*. Elle a été trouvée en été dans le seston d'un poste du cours moyen de la Narew où le pH était 7.85, la saturation en oxygène 73.1%, la teneur en O_2 — 7.31 mg/l, en Ca — 61.6 mg/l et en Fe — 0.4 mg/l.

**Arcella gibbosa* Penard var. *mitriformis* Deflandre

Espèce nouvelle pour la Pologne. Decloître 1958 l'a trouvée parmi les mousses d'un marécage, Thomas 1954 — dans le plancton d'un petit bassin. Elle a été trouvée en été à deux postes de la Wkra. Á l'endroit du prélevement des échantillons la rivière était peu profonde, au fond vaseux, aux rives couvertes d'acores et de roseaux, pH 7.5, O_2 — 4.4 et 6.9 mg/l, saturation en oxygène 44 et 69,5%, Ca — 71.6 et 67.3 mg/l, Fe — 0.4 et 0.6 mg/l.

Arcella haemisphaerica Perty.

Espèce cosmopolite presente dans toutes les zones climatiques. Constatée en Pologne par Bugayski 1930 et Moraczewski 1961. C'est une espèce eurytopique, très répandue dans différents types de bassins. Elle a été trouvée dans le cours moyen de la Wkra à des pH 7.5 et 7.9, oxygène — 6.9 et 7.8 mg/l, saturation en oxygène 69,5 et 78,6%, calcium — 67.3 et 65.5 mg/l, fer — 0.4 et 0.3 mg/l. Elle était présente en été dans le cours moyen et supérieur de la Narew (pH 7.8 et 7.9, saturation en oxygène 73.1 et 91.8% , teneur en O_2 6.7 et 8.3 mg/l, Ca — 60.1 et 61.6 mg/l, Fe — 0.4 et 1.0 mg/l.

Arcella megastoma Penard

Espèce aquatique non constatée dans les tourbières. Trouvée en Pologne par Moraczewski 1961 dans le lac Kisajno. Elle a été trouvée dans le

seston du cours moyen et supérieur de la Wkra. Le pH de l'eau aux postes où elle fut trouvée était 7.5, 8.1 et 8.1, la saturation de l'eau en oxygène y était 69.5, 86.8 et 99.0% et elle contenait 6.9, 8.3, 9.5 mg/l de O_2, 67.3, 64.4 et 70.1 mg/l de Ca et 0.4 mg/l de Fe. En été cette espèce a été observée dans le seston de la Narew à presque tous les postes à des valeurs des estimations chimiques allant de 7.7 à 8.4 pour le pH, de 73.1 à 112,1% pour la saturation en oxygène, de 51.5 à 61.6 mg/l de Ca, de 6.7 à 9.9 mg/l de O_2, de 0.4 à 1.0 mg/l de Fe. Cette espèce y a été également trouvée au printemps à un poste au pH 7.85, à la saturation en oxygène 86.7%, 9.62 mg/l de O_2, 55.4 mg/l de Ca, 0.5 mg/l de Fe.

Arcella rotundata Playfair var. *aplana* Deflandre

Espèce hydrophile, présente parmi les plantes aquatiques et d'après Ertl 1955 très nombreuse dans les tourbières. Constatée en Pologne par Moraczewski 1961. Dans la Wkra elle a été trouvée près de l'embouchure. Le pH était 8.3, la saturation en oxygène 110.1%, les teneurs en O_2 — 10.6 mg/l, en Ca — 70.9 mg/l, en Fe — 0.3 mg/l.

Centropyxis aculeata (Ehrenberg) Stein

Espèce ubiquiste et cosmopolite, très fréquente en Pologne. Trouvée dans le cours moyen de la Wkra à pH 7.9, oxygène 7.7 mg/l, 78.6% de saturation en oxygène, teneur en Ca 65.5 mg/l, en fer 0.3 mg/l. Dans la Narew elle a été observée le long de tout le cours de la rivière. Les valeurs des estimations chimiques variaient dans les limites décrites pour *A. haemisphaerica.*

Centropyxis constricta (Ehrenberg) Penard

C'est une espèce ubiquiste et cosmopolite. Trouvée de nombreuses fois en Pologne. Dans le seston de la Narew elle ne fut trouvée qu'à un poste où le pH de l'eau était 7.85, la saturation en oxygène 73.1%, l'eau contenait 6.7 mg/l de O_2, 61.6 mg/l de Ca et 0.4 mg/l de Fe.

Centropyxis discoides (Penard) Deflandre

C'est une espèce aquatique cosmopolite et probablement ubiquiste. Elle a été constatée en Pologne par Moraczewski 1961. Elle a été trouvée dans le seston de la Narew en été dans le cours inférieur à un pH 7.75, O_2 — 8.24 mg/l, Ca — 63.0 mg/l, Fe — 0.6 mg/l.

Centropyxis ecornis (Ehrenberg) Leidy

Espèce cosmopolite et ubiquiste. Trouvée en Pologne par Moraczewski 1961. Présente dans tout le cours étudié de la Narew.

Centropyxis arcelloides Penard

C'est une espèce cosmopolite et ubiquiste. Constatée en Pologne par Moraczewski 1961. Elle a été trouvée dans le seston de la Narew à un poste au pH 8.2, O_2 — 9.3 mg/l, Ca — 70.1 mg/l et Fe — 0.6 mg/l.

Difflugia acuminata Ehrenberg

Espèce cosmopolite et ubiquiste, présente surtout au fond des bassins. Trouvée en Pologne dans les lacs des Tatras par Minkiewicz 1914, dans la lagune Zalew Wiślany par Biernacka 1956 et dans le littoral du lac Kisajno par Moraczewski 1961. Cette espèce a été trouvée à un poste du cours inférieur de la Wkra. Le pH y était égal à 7.3, l'eau contenait 8.1 mg/l

de O_2, 65.8 mg/l de Ca, 0.3 mg/l de Fe. Son degré de saturation en oxygène était 84.7%. L'espèce a été constatée également à un poste du cours moyen de la Narew (pH 7.9, O_2 — 7.7 mg/l, Ca — 60.1 mg/l, Fe — 1.0 mg/l).

Difflugia corona Wallich

Espèce cosmopolite, le plus souvent présente dans les petits bassins. Trouvée quelques fois en Pologne. Minkiewicz 1914 l'a constatée dans les lacs des Tatras, Bugayski 1930 dans les prés humides et les étangs (dans le tube digestif des grenouilles), Moraczewski 1961 dans le littoral du lac Kisajno. Elle fut trouvée dans le seston du cours supérieur de la Narew. Les analyses de l'eau établirent les valeurs suivantes: pH — 7.75, O_2 — 7.3 mg/l, Ca — 58.7 mg/l, Fe — 0.4 mg/l.

**Difflugia fallax* Penard

Espèce assez rare présente dans des petits bassins (Penard 1902, Kourov 1925) et dans des tourbières (Opravilova 1960). Trouvée la première fois en Pologne dans le cours inférieur de la Wkra. Le pH de l'eau égalait 8.3, la saturation en oxygène — 110.4%, l'eau contenait 8.3 mg/l de O_2, 70.9 mg/l de Ca, 0.27 mg/l de Fe.

Difflugia globulosa Dujardin

C'est une espèce cosmopolite. Constatée en Pologne par Minkiewicz 1914 dans les lacs des Tatras, par Steinecke 1936 dans les tourbières de la partie nord de la Mazurie et par Moraczewski 1961 dans le littoral du lac Kisajno. On la trouve aussi bien dans les petits bassins que dans les lacs et dans d'autres milieux, elle peut donc être considérée en tant qu'espèce ubiquiste. Elle a été trouvée dans la Wkra à un pH 8.1 et à une saturation en oxygène égale à 86.3%. L'eau contenait 8.3 mg/l de O_2, 64.4 mg/l de Ca, et 0.4 mg/l de Fe. Elle a été observée dans le seston de la Narew tout le long de cette rivière.

Difflugia hydrostatica Zacharias

Cette espèce fut trouvée en Pologne par Zacharias 1911 aux environs de Wrocław. D'après Penard 1902 et Bartoš 1954 elle est présente dans la zone pelagique des lacs, d'aprés Kourov 1925 dans le plancton des étangs. Deflandre 1927, par contre, l'a trouvé dans la vase du fond d'un lac. Elle a été trouvée dans le cours inférieur de la Narew au même poste que *C. arcelloides.*

**Difflugia lebes* Penard

C'est une espèce rare, jusqu'à là non constatée en Pologne, présente dans le littoral des lacs sur des plantes aquatiques. Elle fut trouvée dans le seston de la Narew en été à deux postes du cours supérieur et inférieur de la rivière. Le pH y était respectivement 7.8 et 8.4, la saturation en oxygène 91.8 et 112.1%. L'eau contenait 8.3 et 9.9 mg/l de O_2, 60.1 et 55.8 mg/l de Ca, 1.6 et 0.8 mg/l de Fe. Aux deux postes les rives étaient couvertes d'une végétation aquatique peu abondante.

Difflugia lobostoma Leidy

Espèce cosmopolite. Trouvée en Pologne dans les lacs des Tatras par Minkiewicz 1914 et dans le littoral du lac Kisajno par Moraczewski

1961. C'est une espèce typique des petits bassins, fréquemment présente dans le littoral des lacs. Elle fut trouvée à un poste du cours inférieur de la Wkra. Le pH de l'eau égalait 8.25, la saturation en oxygène 95%, l'eau contenait 9.1 mg/l de O_2, 70.9 mg/l de Ca, et 0.3 mg/l de Fe.

**Difflugia oviformis* Cash

Espèce nouvelle pour la Pologne. On la trouve surtout dans les petits bassins (Cash 1909, Deflandre 1927), sur les plantes aquatiques (Bartoš 1954 et Chardez 1961) ou dans des étangs et tourbières (Oye 1953 et Grospietsch 1958). C'est donc une espèce hydrophile, de petits bassins, se trouvant parmi les plantes submergées. Dans le seston de la Wkra elle ne fut trouvée qu'à un poste à un pH 7.85, une saturation en oxygène 78.6% et une teneur en O_2 — 7.72 mg/l, en Ca — 65.5 mg/l et en Fe — 0.27 mg/l.

Difflugia scalpellum Penard

C'est une espèce assez rare. En Pologne elle fut rencontrée par Biernacka 1957 dans le seston du lac Rożnowskie. D'après Penard 1902 et Averintzev 1906 c'est une espèce caractéristique pour le profondal des lacs. Bartoš 1954 la compte au nombre des espèces des lacs présentes dans le littoral. On peut présumer que c'est une espèce vivant dans des lacs, qui ne se trouve dans d'autres bassins que par hazard. Elle fut trouvée dans la Wkra dans le milieu suivant: pH 7.35, saturation en oxygène 84.7%, 8.1 mg/l de O_2, 65.8 mg/l de Ca, 0.3 mg/l de Fe.

Lesquereusia spiralis (Ehrenberg) Bütschli

Cette espèce n'a été constatée en Pologne qu'une seule fois par Minkiewicz 1914 dans les lacs des Tatras. C'est une espèce cosmopolite, très répandue dans différents milieux. D'après Thomas 1959 elle se trouve dans les petits bassins, les lagunes, les tourbières, les étangs, les lacs de montagne et les sources, vivant au fond ou sur des plantes, au pH 7—8. C'est donc une expèce aquatique, ubiquiste. Elle a été trouvée en été dans le seston d'un seul poste de la Narew, dans son cours supérieur. Le pH y était 7.75, O_2 — 7.3 mg/l, Ca — 61.1mg/l, Fe — 0.4 mg/l.

Nebela collaris (Ehrenberg) Leidy

Espèce cosmopolite, constatée en Pologne par Harnisch 1924 dans les tourbières des Sudètes et par Steinecke 1934 dans les tourbières de la Mazurie. Beaucoup d'autres mentionnent qu'elle se trouve surtout dans les tourbières et les petits bassins. Bartoš 1954 l'a trouvé dans les mousses aérophites, Chardez 1960 sur des sphaignes à un pH 5.0, Schönborn 1962 dans des prairies submergées d'un lac oligotrophique. Elle peut donc être considérée en tant qu'espèce ubiquiste. Elle a été trouvée au printemps à un poste du cours moyen de la Narew (pH 8.0, 9.8 mg/l de O_2, 56.6 mg/l de Ca, 0.6 mg/l de Fe).

**Cyphoderia calceolus* Penard

Cette espèce est nouvelle pour la Pologne. Trouvée par Penard 1902, Bartoš 1954 et Grospietsch 1957 dans le sapropel des lacs, avant tout dans le profondal. Elle ne fut constatée qu'à un poste du cours supérieur de la Narew, en été, avec *L. spiralis*. C'est sans aucun doute une espèce de lacs, caractéristique pour les parties profondes des grands lacs, qui s'est trouvée dans la rivière de facon fortuite.

**Cyphoderia trochus* Penard

C'est encore espèce nouvelle pour la Pologne. Elle a été constatée dans la vase du profondal de lac par Penard 1902, Cash 1915, Grospietsch 1957, Štěpanek 1959 et Schönborn 1962 ainsi que dans des tourbières, par Deflandre 1927. Cette espèce est aussi certainement une espèce de lacs et ce n'est que par hasard qu'elle se trouve dans d'autres milieux. Dans la Wkra elle fut constatée au pH 8.2, à une saturation en oxygène égale à 84,3%, à une teneur en Ca 64.4 mg/l, en O_2 7.6 mg/l, en Fe 1.3 mg/l. Elle fut également trouvée en été dans le seston de la Narew, dans son cours moyen (pH 7.9, 8.2 mg/l de O_2, 60.1 mg/l de Ca et 0.4 mg/l de Fe.

Trinema lineare Penard

Espèce cosmopolite et ubiquiste. Constatée en Pologne dans des tourbières des Sudètes par Harnisch 1924. Trouvée dans le seston du cours supérieur de la Narew.

**Pareuglypha reticulata* Penard

C'est une espèce assez rare présente surtout au fond des bassins. Constatée par Thomas 1959 et Penard 1902 dans des petits bassins, elle fut également trouvée par Penard 1902 et Kourov 1925 dans le litoral des lacs. En Pologne elle n'avait pas été encore constatée. Cette espèce était présente à un poste du cours moyen de la Wkra. Le fond y était sablonneux, privé de végétation. Le pH y égalait 7.35, la saturation en oxygène 84.7%, la teneur en oxygène 8.1 mg/, en calcium 65.8 mg/l, en fer 0.3 mg/l.

Résumé

Le matériel recueilli concerne la faune des *Testacea* portée par le courant de deux affluents du cours inférieur du Bug. Ces deux rivières ne se discernent que très faiblement par la composition chimique de l'eau. Toutes les deux peuvent être considérées en tant que rivières mésotrophiques, la Wkra étant une rivière alfa-mésotrophique tandis que la Narew — beta-mésotrophique.

Testacea sont des organismes vivant surtout au fond des bassins ou sur des plantes, ne se trouvant que très rarement dans le plancton. Leur présence dans le seston de rivière est accidentelle, ces organismes étant passivement portés par le courant après avoir été enlevés des plantes et du fond.

Dans le seston des deux rivières étudiées 30 espèces des *Testacea* on été constatées:

Arcellidae	10	*Nebelidae*	2
Centropyxidae	5	*Euglyphidae*	2
Difflugiidae	9	*Cyphoderiidae*	2

Parmi ces 30 espèces 11 se sont avérées nouvelles pour la faune de la Pologne.

STRESZCZENIE

Zebrane materiały obejmują faunę *Testacea* niesioną przez nurt dwóch dopływów dolnego biegu rzeki Bug. Rzeki te różniły się bardzo nieznacznie składem chemicznym wody. Obie można uznać za rzeki mezotroficzne: Wkrę za alfa-mezotroficzną a Narew za beta-mezotroficzną.

Testacea są organizmami występującymi głównie na dnie zbiorników, lub na roślinach, natomiast w planktonie występują bardzo rzadko. Obecność ich w sestonie rzecznym jest przypadkowa, są bowiem, jak większość organizmów w tym środowisku, biernie unoszone przez prąd, porywane z roślin i z dna.

W sestonie obu badanych rzek spotkano 30 gatunków *Testacea*, w tym:

Arcellidae	10	*Nebelidae*	2
Centropyxidae	5	*Euglyphidae*	2
Difflugiidae	9	*Cyphoderiidae*	2

Wśród 30 gatunków spotkanych we Wkrze i Narwi 11 było nowych dla fauny Polski.

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