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Tetrahymena, a ciliate genus of unusual importance in modern biological research

Tetrahymena, un genre de Cilié d'une importance exceptionnelle dans la recherche biologique moderne

Among the several genera of *Protozoa* which Professor O. Jírovec has very recently considered as the most important "models in biological research" (Jírovec 1963) is the hymenostome holotrich genus *Tetrahymena* Furgason, 1940. Species of this small ciliate have been employed to great advantage in many modern research problems. It is thus worthwhile to offer a concise review of the subject here, principally because of the value of calling the attention of more workers everywhere to the virtues of these easily cultured protozoa as experimental organisms in all kinds of investigations: biochemical, cytological, morphogenetic, physiological, parasitological, genetic, or taxonomic in nature.

The writer wishes to dedicate this paper to the memory of the late Dr. Béla Párducz, of Budapest, whose contributions to the protozoological literature are well-known to readers of this journal. Deeply missed will be his active researches on the comparative morphology and, more recently, on certain physiological processes of the ciliate *Protozoa*, his critical syntheses concerning the ultrastructural bases of ciliary movement and coordination, his unusual technical skills in handling microorganisms, his vast knowledge of the literature, and his valued editorial counsel.

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General considerations

To appreciate the wide application of *Tetrahymena* as a "Testobjekt" (Jírovec 1950) one must — in essence — consider the entire sweep of the literature on species of this genus. This will be attempted, on a necessarily modest and restricted scale, in the present paper. Emphasis will be placed heavily on works appearing during the past decade, since a rather thorough review of the earlier tetrahymenological literature was published in the Journal of Protozoology 10 years ago (Corliss 1954). Analysis of data accumulated since 1953, in addition to underlining the importance of the

rapidly accumulating newer references, may be of interest from the point of view of noting past and present trends and of predicting possible fields of future significance in the uses of these ciliates. Direct citations in the present paper will be limited primarily to selected works which have been published within the past one to three years.

Through the year 1963, over 1350 papers have appeared in which species of *Tetrahymena* have been the principal or one of the principal organisms employed. Predominantly used have been strains of the type-species, *T. pyri-*

Fig. 1. Increase in the literature on *Tetrahymena*, by 10-year intervals, since 1923 (after initial lumping of papers in the relatively unproductive period 1676—1923). Note that the slope of the curve has become even steeper in the past decade

formis (Ehrenberg, 1830) Lwoff, 1947. More than 55% of these works, the earliest ones of which appeared over 150 years ago, have been published in the past 10-year period alone! Such a steady increase results in a continuing very steep slope in number of papers published against time (see Fig. 1), perhaps steeper than many workers would have predicted a decade ago.

Nearly 700 authors are involved: an increase in investigators is naturally understandable, but two facts are of interest here. Relatively many more publications are now multi-authored. And, more importantly, the number of biologists from countries other than the United States of America who have been studying *Tetrahymena* in recent years has increased disproportionately: this may be noted even among the few but representative references cited at the end of the present work. Papers have appeared in well over 100 scientific journals (including several new ones launched within the past decade) and in some eight different languages. Species of *Tetrahymena* are truly international organisms!

The five major areas of research in which *Tetrahymena* has been used are discussed briefly below, essentially under the same headings employed 10 years ago in Corliss 1954. Yet one of the most significant observations to be made in considering the output of the past decade is the greatly increased difficulty in drawing lines between these "fields" of study. Today cytology, biochemistry, and genetics — to mention three major areas once reasonably separable — overlap to a major extent, and it is important to recognize this. Investigations of a morphogenetic, systematic, or parasitological nature also often cannot be confined to a single category. Thus one prediction for the next decade is this: it may become quite meaningless to discuss the tetrahymenological literature in terms of half a dozen specific areas.

Full reviews of the work on *Tetrahymena*, since 1954, are practically non-existent. The competent account published by Elliott 1959 b in the Annual Review of Microbiology essentially represents the sole attempt, and he was hampered by space limitation. Certain facets, only, of the "*Tetrahymena* story" are covered in a still shorter work (Elliott 1959 a) published earlier in the same year; this historically important paper represented the substance of Elliott's past-presidential address delivered before the Society of Protozoologists in August 1958. Certain reviews have appeared on major areas of research in which tetrahymenids have been used; citation of these is reserved for subsequent sections of the present work. Since some 57% of all the papers ever published on *Tetrahymena* have dealt with subject matter of a biochemical or physiological nature, the reader interested in sources of references to much of the most recent and most important literature in this dominating field is advised to consult the publication by Seaman and Reifel 1963 and to watch for the chapter by Holz 1964 in the forthcoming Volume III of Biochemistry and Physiology of Protozoa edited by S. H. Hutner.

It is difficult to keep abreast of the voluminous literature on *Tetrahymena*: it is constantly appearing in a great diversity of scientific outlets. The writer has been materially aided in keeping his own records not only by the availability of unusually fine library facilities at the University of Illinois but also by receiving reprints from investigators studying these ciliates in laboratories all over the world. Continuation of such cooperation would be gratifying; in return, data from the writer's files are available, on arrangement, to any worker requesting them. In this general connection, mention should be made of the very recent communication from Holz and Nachtwey 1964 on the advisability of establishing an information exchange between workers using *Tetrahymena* as a research organism. Initial membership in the IEG

(Information Exchange Group), as proposed in this important document, would be limited to investigators actively studying processes directly related to the cell cycle in tetrahymenid species. With passage of time the group might well become enlarged. Interested readers may request more information on the subject from the coordinator, Dr. D. S. Nachtwey, Code 926, U. S. Naval Radiological Laboratory, San Francisco, California, 94135, U. S. A.

The sustained popularity of *Tetrahymena* resides primarily in its unusual ability to thrive in axenic laboratory culture, in both simple or chemically defined media. Its ready exhibition of sexuality and its ease of handling in genetic work are also important factors in its attractiveness as an organism for modern experimental research. Finally, its cosmopolitan nature, its adaptability to a variety of ecological situations, its status as an independent "animal cell" with a high rate of reproduction, and its membership in a suborder of ciliates considered pivotal from evolutionary and phylogenetic points of view make it a valuable organism in a wide range of studies, from molecular biology to protozoan taxonomy.

Cytology and systematics

Although nearly 20% of all the papers concerned with *Tetrahymena* to date fall within the broad category of "Cytology and Systematics", making it second only to "Biochemistry and Physiology", it is interesting to note that fewer than half have appeared in the period 1954—1963 and that it is now

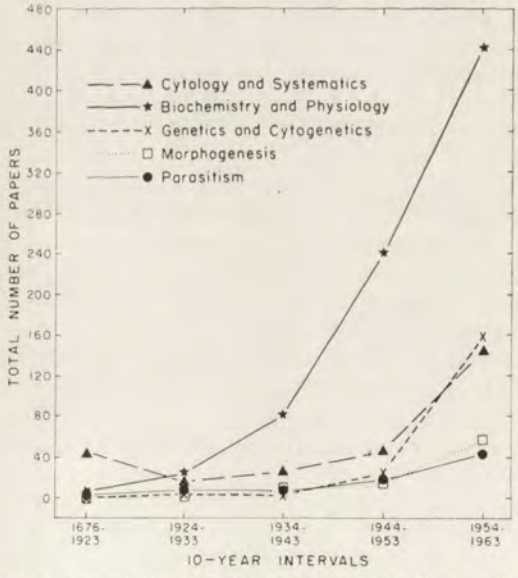

Fig. 2. Trends in the literature of the five major categories of *Tetrahymena* studies, as reflected by the increases in numbers of papers totaled for 10-years periods. Note the dominance of the "Biochemistry and Physiology" field, but also the sharp up-swing in the area labelled "Genetics and Cytogenetics"

being passed (in numbers of papers published recently) by the area labelled as "Genetics and Cytogenetics" (see Fig. 2). Many of the very earliest tetrahymenological works were naturally descriptive in nature, and most often "morphology" and "taxonomy" were closely intertwined. In recent years there has been a shift of emphasis within the field as well as a relative decline of interest in the more traditional taxonomically-oriented areas. Modern studies are often concerned with ultrastructural anatomy (e.g., Burnasheva et al. 1963, Elliott and Bak 1964a, b, Henshaw 1961, Hopkins and Watson 1963, Miller and Stone 1963, Pitelka 1961, Przybylski 1961, Roth and Minick 1961, Silvester 1964, Suhama and Yamataka 1960a, b, Watson and Hopkins 1962, Zebrun 1957; and see Pitelka 1963), much of which may have no direct systematic utility.

An up-surge in taxonomic investigations on *Tetrahymena* and other ciliates is indicated, however, in various "new approaches" to systematics (Corlis 1963), including what may be called biochemical or even molecular taxonomy. With respect to the last-mentioned field recent references of pertinence include the stimulating treatment of general biochemical possibilities by Holz et al. 1963, the comments on serological differences by Loefer and Scherbaum 1963, and the work of Sueoka 1961a, b, Schildkraut et al. 1962, and others in which use of differences in DNA base composition is explored. The well-known ability of species of *Tetrahymena* to flourish in chemically definable media is the factor which will continue to make them popular in such novel approaches to problems in protozoan systematics and phylogeny.

Although occasionally the generic name *Tetrahymena* is not used today in reference to species belonging to this genus, there is widespread agreement as to its suitability.[1] Also the type-species, *T. pyriformis*, is sometimes still referred to as *T. geleii*, but such cases are very rare. A major nomenclatural paper dealing with these problems (Corliss and Dougherty 1965) has been delayed because of the redrafting necessitated by appearance of the newly revised International Code of Zoological Nomenclature.

The number of named species in the genus now stands at 13 (Corliss 1959, 1960), listed alphabetically as follows: *T. chironomi*, *T. corlissi*, *T. faurei*, *T. glaucomaeformis*, *T. limacis*, *T. parasitica*, *T. paravorax*, *T. patula*, *T. pyriformis*, *T. rostrata*, *T. setifera*, *T. stegomyiae*, *T. vorax*. There is a critical need for a thorough review of the comparative taxonomy of these forms; and no doubt other species are awaiting description. It is also becoming increasingly difficult to keep track of the many strains isolated by dozens of investigators. And the convenient reference to sources of axenic cultures

[1] The type-species, unfortunately, is sometimes still referred to as "*Glaucoma pyriformis*." The genus *Glaucoma* remains a bonafide taxon in its own right, with *G. scintillans* as type-species. The only other species which continues to be misnamed is *Tetrahymena patula*, still rather often called "*Leucophrys patula*," not because of objection to its repaired nomenclature but generally because of unawareness that the *Leucophrys patula* of the celebrated and continuingly significant works by Maupas, 75 years ago, and by Fauré-Fremiet, 25 years ago, are members of the same species as comprises the present-day *Tetrahymena patula*. *Leucophrys*, incidentally, is not a separate genus; its name falls as a synonym of *Tetrahymena*.

published by the Society of Protozoologists (Provasoli et al. 1958) is now considerably out of date. The number of "physiological species" or syngens of *T. pyriformis*, with their included mating types, has grown rapidly, too, since the first ones were recognized some 12—13 years ago.

In spite of growing interest in ultrastructural, cytochemical, and biophysical aspects of the anatomy of tetrahymenid ciliates, work continues to be carried out — and much remains to be done — with the light microscope, using such taxonomically indispensable methods as those of silver impregnation (see Corliss 1963 and references therein). The basic tetrahymenal organization of the buccal ciliature and the mode of stomatogenesis known for these hymenostomes are still recognized as important features in phylogenetic and evolutionary considerations of ciliates in general (Corliss 1961a, Evans and Corliss 1964, Fauré-Fremiet 1950, Raabe 1964).

Biochemistry and physiology

It is not surprising that the main interest of experimental biologists in *Tetrahymena* continues to lie in the extensive subdivisions of what may loosely be called "Biochemistry and Physiology". The statistics are impressive and quite uniform: nearly 57% of all the tetrahymenological literature published through 1963 falls in this broad category; well over half of this great volume of papers appeared during the past decade; and 54% of all the works on *Tetrahymena* within the past 10 years are assignable to this area. Note the continuing steep slope of its curve in Figure 2.

Hailed some years ago as, in effect, the only animal organism capable of sustained growth under axenic conditions in a synthetic medium of known chemical composition, an exuberant but not altogether inaccurate statement, *Tetrahymena* continues to serve as one of the most ideal unicellular organisms for refined physiological investigation.

As predicted 10 years ago (Corliss 1954), a swing has occurred away from studies of a general physiological or nutritional nature; the bulk of the work is now concerned with enzyme systems and metabolic pathways (Holz 1964, Seaman and Reifel 1963). Biophysical investigations are on the increase, too, and the tie-in with areas of ultrastructure and genetics makes it difficult to assign clear-cut boundaries in a number of cases.

Only two particular sub-categories are given special mention in the present paper: the employment of tetrahymenids in assay work and the research made possible by use of synchronized cultures.

The value of *Tetrahymena* as a microbiological assay organism was first realized nearly 20 years ago. Recently there has been an important renewal of interest in such employment of this ciliate: for example, see the works by Baker et al. 1960, Celliers 1961, Foley et al. 1958, Hutner 1964a, Hutner et al. 1958, Mark et al. 1963, Reynolds 1964, Stott et al. 1963, Teunisson 1961. Along with direct assay work, *T. pyriformis* has also been used experimentally as a general pharmaceutical tool (e.g., see Aaronson 1963). Recognition of the organism as a cell is important in such considerations: one of the several reasons why the "acellularity" hypothesis is rather inappropriate when applied to such protozoa.

The tremendous success in the induction of synchronous division in controlled populations of *Tetrahymena pyriformis*, stemming from the now classical work initiated in Copenhagen by Scherbaum and Zeuthen 1954, has opened up a new and most important avenue of biochemical research. In fact, the various approaches made possible to unsolved problems long facing the cell biologist extend beyond the boundaries arbitrarily given to the present category, embracing the areas designated in this paper as "Genetics and Cytogenetics" and "Morphogenesis" as well. It is obvious that careful handling of synchronous material will result in obtaining data the proper interpretation of which may throw great light on the nature of the processes which prepare a cell and its nucleus for division or replication. But, as Scherbaum 1960 stresses in a recent excellent review, the "core of the problem resides in the effect of the synchronizing agent upon the normal metabolism in the cellular life cycle"; and he is well aware that "many unresolved questions" remain to be attacked. Since the synthetic coverages of the subject published by Zeuthen 1958 and Scherbaum 1960 a number of outstanding papers have been published, only a few of which can be cited here: Cann 1963, Chou and Scherbaum 1963, Frankel 1962, Hamburger 1962, Holz, Rasmussen, and Zeuthen 1963, Kamiya and Takahashi 1961, Klamer and Fennell 1963, Nachtwey 1963, Nishi and Scherbaum 1962, Plesner 1964, Rasmussen and Zeuthen 1962, Scherbaum 1963 a, Thormar 1962, Williams 1964, Zeuthen 1961, 1963 a; and see references in Scherbaum 1963 b and Zeuthen 1963 b.

No decrease in the rate of appearance of new publications on *Tetrahymena* in the burgeoning area of "Biochemistry and Physiology" is anticipated for some time to come. Selected citations to very recent papers of significance in representative fields (including biophysics) of this broad category, other than those already treated above, may be listed as follows; Andrus and Giese 1963, Braun et al. 1963, Burnasheva and Efremenko 1962, Child 1961, Cirillo 1962, Conner et al. 1961, Dew et al. 1962, Dewey and Kidder 1960 a-c, 1962, Dickie and Liener 1962 a, b, Dunham and Child 1961, Eichel and Rem 1962, Erwin and Bloch 1963, Frank et al. 1963, Hack et al. 1962, Heinrikson and Goldwasser 1964, Hogg and Kornberg 1963, Holz et al. 1962, Hutner 1963, Hutner and Holz 1962, Hwang et al. 1964, Jasmin 1961, Kandatsu and Horiguchi 1962, Kitching 1957, Koeler and Fennell 1964, Le Boy et al. 1964, Lindh and Christensson 1963, Loefer and Scherbaum 1963 b, Lövlie 1963, Lyttleton 1963, McCashland 1963, McCashland and Andresen 1963, Müller and Rohlich 1961, Nathan and Friedman 1962, Németh and Csík 1963, Price et al. 1962, Ray and Coleman 1963, Seaman 1961, 1963, Shorb 1963, Stephens and Kerr 1962, Sullivan and Snyder 1962, Summers 1963 a, b, Taketomi 1961, Warnock and van Eys 1963, Wells 1960, West et al. 1962.

Reviews of the "older-modern" physiological-biochemical literature, much of which remains indispensable, may be found in appropriate sections of Volumes I and II of Biochemistry and Physiology of Protozoa (Lwoff 1951, Hutner and Lwoff 1955) and in the textbook of protozoology published by Hall 1953; also see Hutner 1964 b.

Genetics and cytogenetics

The most phenomenal increase in publications in the tetrahymenological literature of the past decade has occurred in the category of "Genetics and Cytogenetics." Over 87% of all the papers ever published in this field have appeared since 1953. Although the total number lags behind those for "Cytology and Systematics" as well as "Biochemistry and Physiology" (only a little over 12% of the total literature falls here), it is significant to note that nearly 19% of all works of the past decade are assignable to this field (see Fig. 2), advancing it to second place since the count of 1954.

Included in this category are not only researches in what one might term "pure genetics" (including cytological and physiological aspects and consideration of cytoplasmic inheritance) but also investigations on the chemical basis of heredity: specifically, quantitative work on the protein and nucleic acid content of the nuclei of *Tetrahymena*. In the former group belong such recent contributions as those of Nanney and colleagues at the University of Illinois (e.g., Nanney 1963a-c, Nanney and Nagel 1964, Nanney, Nagel and Touchberry 1964, Nanney et al. 1963) and of Elliott and co-workers at the University of Michigan (e.g., Elliott 1959a, b, Elliott and Clark 1958, Elliott and Kennedy 1962). Other pertinent examples may be found in the following papers: Allen 1963, 1964b, Corliss and Dysart 1960, Kimball 1964, Orias 1963, Outka 1961, Sonneborn 1957, Wells 1961. In the latter or "DNA-RNA" subdivision of the category are assignable published works originating from many laboratories in the world. A fair sample from among recent papers should include at least the following: Cerroni 1962, Dysart 1963[2], Elliott, Kennedy, and Bak 1962, Jones and Thompson 1963, McDonald 1962, Prescott 1962, Scherbaum et al. 1959, Schildkraut et al. 1962, Stone and Prescott 1964, Sueoka 1961a, b, Szabó and Németh 1961, Wells 1962, Zeuthen 1963a.

Papers on heritable biochemical markers serve as examples of the linkage between genetics and biochemistry. The recent series of excellent investigations by Allen (see Allen 1964) on the genetic control of esterases in *Tetrahymena pyriformis* and on the role of these "esterase isozymes" signals an important break-through in this area.

Additional examples of overlapping of categories are obvious in cytological studies on chromosomes (following the already classical work by Ray 1956), ultrastructural investigations of nuclei, and allied research. With the very recent pioneering work on *Paramecium* by Sonneborn 1963 to serve as a model, the field of "cortical inheritance" offers a most rich area of study with use of species of *Tetrahymena*: see, for example, Nanney and Nagel 1964.

Once again *Tetrahymena* may be considered as an experimental organism of unusual potential importance in a wide range of studies on problems of basic significance to the developmental and molecular biologist as well as to the modern cytologist and geneticist.

[2] This important work by Dysart of the University of Illinois, published to date only in abstract form, includes the first precise, quantitative recognition of distinct classes of macronuclear ploidy (determined by dividing the macronuclear DNA amount by the haploid micronuclear DNA amount) in species of *Tetrahymena*. *T. limacis*, *T. patula*, and *T. rostrata* were used in the investigations.

Morphogenesis

In the category of "Morphogenesis," as in the preceding category, a large percentage (73%) of the works has appeared within the past decade. The trend has been from purely descriptive accounts of such morphogenetic phenomena as binary fission and stomatogenesis to highly refined experimental and physiological approaches. The area, though small in total number of papers (see Fig. 2), is destined to remain an important one. Here, too, however, an increasing overlapping with such categories as cytology, biochemistry, and even genetics renders quite artificial any attempt to establish the field as an entirely distinct and separate one.

Two principal groups of papers deserve special citation, groups which together comprise the great majority of works assignable to this category. In both instances the ability of species of *Tetrahymena* to flourish in an axenic medium and to be amenable to division synchrony through application of heat shocks has played the decisive role in making many of the studies possible at all. Nearly indispensable, too, has been the use of methods of silver impregnation which allow detailed examination of fixed organisms whose body forms have not been distorted.

Polymorphic characteristics have long been recognized in *T. patula* (see older references in Corliss 1953, 1954, and Williams 1960), and recently descriptions or redescriptions have been made of the remarkable form changes possible in other species, such as *T. vorax, T. paravorax, T. rostrata,* and *T. limacis.* But Williams and his students at the State University of Iowa have gone a major step beyond the descriptive phase, by investigating the underlying physiological factors involved in the peculiar dimorphism of *T. patula* and *T. vorax.* This work has resulted and is resulting in a continuing series of significant papers (see especially Shaw and Williams 1963, Stone 1963, Williams 1960, 1961; other investigations have been reported to date only in preliminary or "abstract" form).

At the same time Frankel, also at Iowa, members of Zeuthen's laboratory in Copenhagen, and scattered workers elsewhere have been delving into the mysteries of stomatogenesis and binary fission, particularly as these phenomena are affected by heat, cold, and various other environmental factors when synchronized cultures are used (a few of the most recent papers include Frankel 1962, 1964, Hamburger 1962, Rasmussen 1962, Thormar 1962, Williams 1963). The remarks made earlier (see paragraph on synchronous division under "Biochemistry and Physiology", above) concerning the value of such research in modern cell biology are also very appropriate here.

Parasitism

The number of papers in this category remains the smallest of all, yet over half of them have appeared within the past decade (see Fig. 2). By "parasitism" is meant, here, practically any kind of symbiotic (sensu lato) association of species of *Tetrahymena* with other organisms. The common cases may all be classified as examples of facultative endoparasitism, many perhaps resulting from purely accidental invasions. Aquatic hosts are gene-

rally involved, especially insect larvae.[3] Some interesting work has been done along experimental lines. The most up-to-date review of the subject is contained in a recent work by the writer (Corliss 1960).

Because of the cosmopolitan nature and rather non-specialized gustatory proclivities of many of the 13-odd species of *Tetrahymena* recognized to date, it is not surprising that some of them are found within the body cavity of mosquito and chironomid larvae; in the digestive tract or visceral organs of edaphic snails, slugs, and annelids; and in various tissues of wounded or poorly nourished fish or amphibia, including circulatory and central nervous systems. Yet five of the species have never been found in association with other organisms, and several have been implicated solely as accidental parasites. On the other hand, two species, *T. limacis* in slugs and snails and *T. stegomyiae* in mosquito larvae, might be considered as obligate endoparasites with a facultatively free-living phase superimposable on their normally parasitic life cycles. Such theoretical considerations, mentioned briefly in a recent abstract (Corliss 1962), deserve the future attention of workers interested in principles of general parasitology.

Since the time of the review paper cited above (Corliss 1960), which contained direct citation of the pertinent literature up to that date, relatively few publications have appeared in this field. *T. limacis* has been recovered from additional slug hosts, with changes noted in its body form and number of ciliary meridians (Corliss et al. 1962, Kozloff 1962, Windsor 1960); and *T. chironomi* has been reported from midge larvae in Germany (Barthelmes 1960) and, as a tentative identification, from a planarian in the U.S.S.R. (Jankowski 1962). Unidentified species of *Tetrahymena* have been noted in newts and carp in Holland (Stolk 1960 a, b), in earthworms in Czechoslovakia (Lom 1959), and in tropical mosquitoes from Malaya (Corliss 1961 b). Two species have been found in fresh-water clams and snails in Poland (Dobrzańska 1959, Kazubski 1958). New experimental infections have been successfully carried out, using primarily *T. pyriformis*, by Granátová 1963 in Czechoslovakia; this brief paper recalls especially to mind the classical work carried out there over a quarter-century ago by Janda and Jírovec 1937 and also the more recent investigation in America by Thompson 1958.

Miscellaneous studies

Inevitably *Tetrahymena* has been employed in research the nature of which is difficult to classify or, at least, to assign to the five major categories considered in preceding pages of the present review. As predicted 10 years ago (Corlis 1954), the group of papers arbitrarily called "miscellaneous" has grown greatly in size: over 50% of the works were published within the past decade. On the other hand, an attempt has been made (in the writer's files) to keep the group relatively small, principally by allocating a large number of "physiological-type" miscellaneous investigations, quite appropriately, to

[3] The possible employment of populations of *Tetrahymena* species capable of exhibiting facultative parasitism as "biological control agents" of such pests and/or disease-carrying insects as certain chironomids and tropical mosquitoes should be seriously investigated. Although the mode of entry into the aquatic larval forms of such potential hosts is not always known, the presence of ciliates in their haemocoel inevitably causes eventual death of the metazoan organisms.

the section on „Biochemistry and Physiology". Note that miscellaneous studies are included in the data of Figure 1 but not in those of Figure 2.

Principal papers assigned to the present category are studies mainly concerned with ecology and distribution; with techniques of isolation, general cultivation, or rendering cultures axenic; or with such unusual topics as pattern-formation. The field of ecology is a wide open one for *Tetrahymena*, as for most ciliates: much detailed work of significance remains to be done here. Distributional data of importance have been and are being collected by Elliott and colleagues (e.g., see the recent publication by Elliott, Addison, and Carey 1962). Stout 1963 (and earlier works) has found *Tetrahymena* in soils. The careful work by Mučibabić 1957 a, b on mixed protozoan populations in the laboratory (species of *Tetrahymena* plus *Chilomonas*) should be mentioned because her studies have generally been overlooked by subsequent investigators. The phenomenon of ciliates forming patterns under certain cultural conditions has attracted considerable attention to *Tetrahymena* in recent years (see Jahn and Brown 1961, Jahn et al. 1961, Jones and Baker 1946, Loefer and Mefferd 1952, Nettleton et al. 1953, Platt 1961).

But over 60% of the papers called "miscellaneous" are studies in which *Tetrahymena*, almost always *T. pyriformis*, has been employed as food for some other organism whose cytology (including ultrastructure), physiology (including aging), biochemistry, or genetics is under investigation. It could be argued that such studies are hardly "tetrahymenological": yet *Tetrahymena* performs an often indispensable, if secondary, role in them! Then, too, because the exact nutritional needs of the tetrahymenids are known, the medium for the monoxenic (formerly "zweigliedrige") culture may become quite refined. Using strains of *T. pyriformis*, considerable work of significance has been carried out on various species of hypotrichs, amoebae, and suctoria. Sonneborn 1963 has employed *Tetrahymena* as nourishment for the suctorian species (*Tokophrya infusionum*) in which he has made the important discovery of sexual mating types: his culturing technique is founded on the earlier experiences of such workers as Guilcher, Hull, Rudzińska, and their associates. In perhaps the first use of tetrahymenids as food for gymnostomes, Doroszewski 1963, in his regeneration work, has very recently reported some success in growing the carnivore *Dileptus* on *Tetrahymena*.

Summary

Among the *Protozoa* one of the most important genera from the point of view of the widespread use of its species in experimental work of great significance is the hymenostome ciliate *Tetrahymena*. Its ability to be grown axenically and abundantly in highly refined laboratory culture is the principal reason for its continuing to be a favorite organism in modern biological research. However, other very important factors in its sustained popularity include its exhibition of sexuality, its cosmopolitan nature, and its adaptability to a variety of ecological situations, including facultative parasitism.

There has been no decrease in recent years in the rate of appearance of published works concerned with research on species of *Tetrahymena*. In fact, the volume of these papers within the past decade have been considerably greater than that of the combined total of all earlier years in the long

history of observations on tetrahymenid ciliates. Over half of the tetrahymenological literature has appeared in the broad area which has been labelled "Biochemistry and Physiology", and this trend is likely to continue. But there has been an especially sharp increase, during the past decade, in investigations assignable to the category of "Genetics and Cytogenetics". There has also been no slackening of activity in the three other major areas reviewed in the present paper: "Cytology and Systematics", "Morphogenesis", and "Facultative Parasitism".

RÉSUMÉ

Parmi les Protozoaires, l'un des genres les plus importants, au point de vue de l'usage fort répandu de ses espèces dans des travaux expérimentaux de grande portée, est le Cilié hyménostome *Tetrahymena*. La raison principale pour laquelle il continue à être un organisme en faveur dans la recherche biologique moderne est la possibilité de le cultiver axeniquement et abondamment dans le laboratoire, dans des conditions extrêmement raffinées. Cependant, d'autres facteurs très importants contribuent à sa popularité prolongée: la manifestation de sa sexualité, sa nature cosmopolite, et sa possibilité d'adaptation à une variété de situations écologiques, la parasitisme facultatif y inclus.

Durant ces dernières années, il n'y a pas eu de déclin dans le nombre des ouvrages parus, rapportant des recherches sur les espèces de *Tetrahymena*. En fait, le volume de ces articles publiés pendant ces dix dernières années a été considérablement plus grand que celui du total des publications des années précédentes, dans la longue histoire des observations sur les ciliés tetrahyméniens. Plus de la moitié de la literature tetrahyménologique concerne le vaste domaine dénommé "Biochimie et Physiologie", et il est probable que cette tendance persiste dans l'avenir. Pourtant, il y a eu un progrès particulièrement important, durant ces dix dernières années, dans le volume des recherches pouvant être classifiées dans le domaine de la "Génétique et Cytogénétique". En outre, il n'y a pas eu de diminution d'activité dans les trois autres domaines examinés dans le présent article, à savoir: "Cytologie et Systématique", "Morphogenèse", et "Parasitisme facultatif".

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ACTA PROTOZOOLOGICA

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Prorodon raabei sp. n. et sa biologie

Prorodon raabei sp. n. i jego biologia

Du point de vue écologique l'espèce *Prorodon raabei* sp.n. appartient aux ciliés psammophiles littoraux, qui habitent le sable marin submergé dans les endroits plus calmes, où l'action des ondes est faible et l'accumulation de détritus s'accomplit. Je l'ai rencontré par hasard, en examinant les échantillons d'eau prises du rivage sablonneux de l'ile Bonzak à l'endroit où le bras mort de la Vistule (Wisła Śmiała) rejoint le golfe de Gdańsk. La concentration du sel est très basse ici, à savoir environ 3‰. Je n'y ai trouvé que deux individus, que j'ai pêchés, mis dans un petit recipient et commencé à cultiver. La culture s'est developpée si bien, qu'après quelques semaines j'avais à ma disposition des centaines d'exemplaires.

Au premier coup d'oeil, par son apparence, ce Cilié ressemble à l'espèce *Prorodon morgani* Kahl, mais après avoir examiné minutieusement les détails de son anatomie, j'ai constaté qu'il ne peut être identifié avec aucune des espèces décrites jusqu'ici. Les observations biologiques ont été faites sur des individus vivants cultivés dans l'eau de mer (Baltique) et la morphologie — étudiée sur les préparations argentées, faites d'après la méthode de Chatton, modifiée par Corliss.

Prorodon raabei sp.n. appartient aux grandes espèces: le corps a environ 500 μ de long. Normalement le corps est allongé, souple, vermiforme, sa partie antérieure un peu élargie et la postérieure — arrondie. Le cytoplasme est transparent, gris-pâle. Cependant il faut accentuer, que sa forme et sa couleur subissent de très distincts changements au cours de l'alimentation. L'animal bien repus prend la forme ovoïde et le cytoplasme, rempli de vacuoles digestives, devient sombre, presque noir. On a l'impression d'avoir affaire à une autre espèce. La ciliature est épaisse: les rangées des cils sont écartées de 1.7 μ. Les cils sont courts et de même longueur sur tout le corps. La vacuole pulsatile est terminale et au-dessus d'elle se trouvent des petites vacuoles satellites. Le cytostome est oval, le cytopharynx cônique a 35 μ de longueur. Tout près du cytostome commence la "brosse dorsale" („Dorsalbürste" d'après les auteurs allemands) qui court le long du corps et fait que le corps, qui à priori semble être de symetrie parfaite, est en réalité asymétrique. Elle apparaît comme une triple rangée sigmoïde de gros cils situés dans une faible dépression, d'environ 30 μ de longueur. Cette structure est encore prolongée d'une simple rangée de cils semblables, d'environ 40 μ de longueur, dont les cinétosomes sur les préparations argentées sont très bien

visibles et ressemblent à une longue échelle. L'appareil nucléaire, examiné sur les préparations colorées avec de l'hematoxyline, est constitué par un petit micronucleus rond et un volumineux macronucleus, qui se distingue par une forme très caractéristique: il est allongé, sa partie centrale est mince et les parties extrêmes élargies. Une telle forme n'a jamais été observée chez aucune espèce de ce genre.

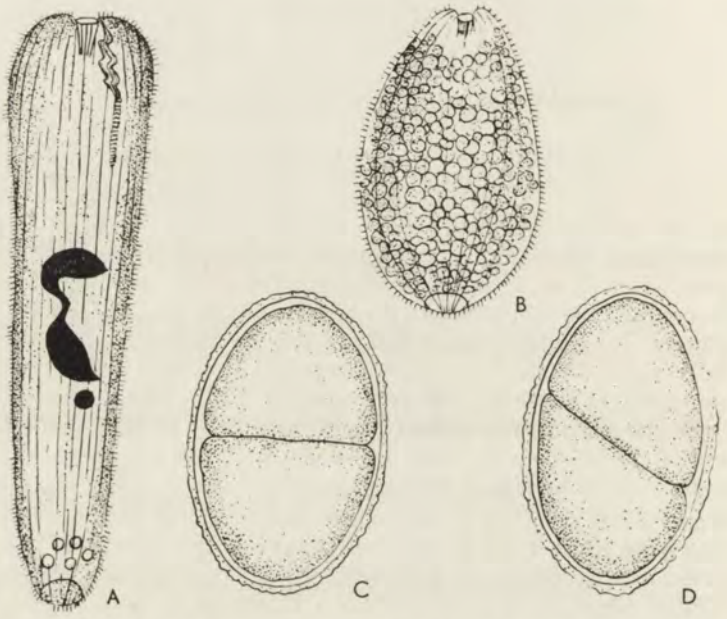

Fig. 1. *Prorodon raabei* sp. n. A. Individu typique. B. Individu repus. C et D. Kystes de division

Prorodon raabei sp.n. est une espèce carnivore mais non prédateur. Il n'attaque pas les autres Ciliés mais se nourit de tissus des animaux morts. Ainsi il appartient aux Ciliés histiophages. Dans la culture il a été nourri des *Enchytreidae* coupés en morceaux. Il passe une grande partie de sa vie dans des kystes, où il digère et se multiplie. Les kystes sont ovales et transparents. Les parois des kystes dans lesquels l'animal s'enferme pour digérer sont faibles — ils éclatent après quelques mouvements du Cilié. Au contraire les parois des kystes de division sont si solides, que l'animal travaille longtemps pour les percer. *Prorodon raabei* possède une aptitude fort intéressante de sentir la nourriture à travers le kyste. Environ trois minutes après qu'on ait jeté les morceaux d'un ver, un mouvement commence dans les kystes. Les Ciliés commencent à se tourner, d'abord lentement, puis de plus en plus vite, en frappant avec la partie antérieure du corps contre la paroi du kyste, qui éclate enfin et l'animal en sort. Les Ciliés tournent autour du ver et après avoir essayé en vain de l'attaquer à travers la peau ils s'attachent fort avec les cytostomes aux endroits blessés. Il ne peuvent pas pénétrer la dure cuticule du ver. Les animaux avalent les tissus mous en grandes portions, qui tombent

par le cytopharynx dans la vacuole digestive et passent dans la partie postérieure du corps. Ce procès s'accomplit si vite, qu'on voit l'animal changer sa forme et couleur. L'individu allongé et vermiforme devient court, ovoïde et le cytoplasme se remplit de vacuoles digestives qui lui donnent une teinte sombre, presque noire. L'animal avale même les soies du ver, qu'il excrète ensuite par le cytopyge. Après vingt minutes les tissus mous du ver sont dévorés; rien n'en reste sauf la cuticule transparente, dans laquelle fourmillent les Ciliés. Après l'absorption de la nourriture le Protozoaire ne ressemble guère la forme typique. Ses mouvements deviennent lents et lourds. L'animal cherche un endroit calme et s'y enkyste, souvent sur place, dans la cuticule vidée du ver. Il digère un ou deux jours et ensuite il est de nouveau prêt à s'alimenter. Dans la culture pourtant, la plupart des animaux ne quittent pas les kystes jusqu'a ce que des matériaux mangeables n'apparaissent. Quand les Ciliés n'étaient pas nourris ils restaient dans les kystes quelques jours, attachés au fond du récipient. Grâce à celà on pouvait non seulement changer l'eau facilement mais aussi rincer quelques fois le récipient pour écarter les restes de la nourriture et, éventuallement, d'autres espèces indésirables qui s'étaient installées dans la culture.

Comme j'ai déjà mentionné, la division a lieu aussi dans le kyste mais dans ce cas l'animal subit des changement plus profonds. L'appareil buccal et les cils sont résorbés et au bout de quelques heures un sillon transversal apparaît, qui divise le corps du Cilié en deux parties égales. Après les quelques heures suivantes, les deux moitiés se déplacent de cette façon, que la ligne de clivage parcourt le kyste obliquement. Alors, la reconstruction de l'apparéil buccal et de la ciliature suit et tous les deux individus commencent à se remuer dans le kyste, d'abord lentement, puis de plus en plus vite. Le kyste de division est beaucoup plus solide que le kyste de digestion et c'est pourquoi les animaux travaillent assez longtemps pour se faire une sortie. Quand le kyste éclate enfin, un des individus commence à se frayer un passage par l'étroite ouverture; on peut alors voir, comme le corps de ce Cilié est élastique. D'abord la partie antérieure passe par l'ouverture, fort serrée, ne contenant que de l'ectoplasme, dans laquelle l'endoplasme commence ensuite à couler. L'animal ressemble à ce moment à un clepsydre. Après plusieurs efforts laborieux, pendant lesquels le Cilié recule coup sur coup dans le kyste, il se dégage enfin. Parfois des divisions pathologiques ont lieu, donnant origine a quatre individus, qui sont plus petits, presque ronds et incapables de se nourrir. Bientôt ils périssent.

Pour une comparaison j'ai cultivé en même temps deux espèces d'eau douce, à savoir *Prorodon teres* Ehrbg. et *Prorodon cinereus* Penard. Toutes les deux étaient nourries de la même façon que *Prorodon raabei* et chez les deux j'ai observé des phénomènes semblables: le changement frappant de la forme, après que les Ciliés se sont alimentés, un „flair" excellent à travers le kyste et la division dans le kyste, avec cette différence que *Prorodon teres* se divisait en deux et *Prorodon cinereus* en quatre individus.

Les observations sur la biologie de ces trois espèces acquièrent une importance plus générale si on les compare au travail de M u g a r d 1948 sur les Ciliés histiophages de l'ordre *Hymenostomatida*. Les espèces de la famille *Ophryoglenidae* passent d'après l'auteur un vrai cycle évolutif ressemblant à celui rencontré chez les formes parasites. Le Cilié abandonnant le kyste de division (théronte) est allongé, fusiforme. Après avoir trouvé une proie il

s'alimente intensivement et change de forme — on l'appele alors trophonte. Enfin, rempli de nourriture, il nage encore quelque temps, ensuite il s'enkyste et, en tant que tomonte, il se divise successivement deux fois. Les quatre tomites sé dégagent et quittent le kyste en forme de thérontes. Il y a quelques Ciliés histiophages de cet ordre comme *Glaucoma* et *Paraglaucoma* chez lesquels ce cycle est encore peu distinct et ne se manifeste que par le changement de forme; mais quant à la multiplication, ils se divisent comme la plupart des

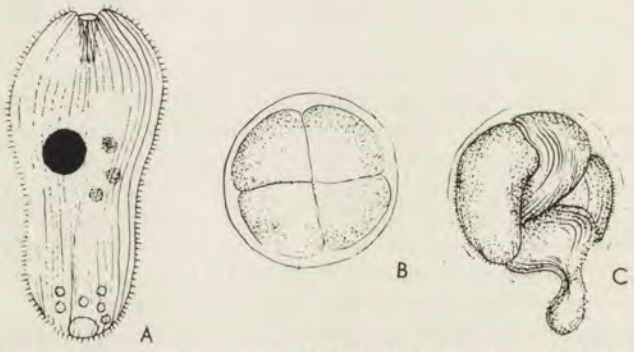

Fig. 2. *Prorodon cinereus*. A. Individu typique. B. Kyste de division. C. Sortie du kyste

Ciliés, sans enkystement. Il importe cependant de souligner, qu'à l'exception de *Paraglaucoma rostrata* ces espèces ne sont histiophages que facultativement; en principe elles s'alimentent de bactéries.

Chez *Prorodon raabei* sp.n., qui est un histiophage exclusif, le cycle physiologique est presque également distinct que chez *Ophryoglena;* il n'y manque que la palintomie — l'animal enkysté ne subit qu'une division. Mais chez *Prorodon cinereus* nous voyons déjà une double division, qui donne origine à quatre individus. D'ailleurs, le genre *Prorodon* n'est pas prêt à la multiplication dès la première alimentation; l'animal s'alimente plusieurs fois en s'enfermant chaque fois dans le kyste de digestion et ce n'est qu'après un certain temps qu'il construit le kyste de division. Donc nous avons affaire ici à un phénomène encore indécis, qui chez *Ophryoglena* devient un cycle régulier.

La conclusion qui s'impose c'est que le mode specifique d'alimentation, composée exclusivement de tissus animaux, a exercé son influence sur la biologie des Ciliés, provenant de différents groupes systématiques, leur donnant une ressemblance physiologique. L'alimentation limitée exclusivement au tissu animal mort représente une spécialisation apportant certaines désavantages parce que ce genre de nourriture ne se trouve pas toujours dans l'eau comme les bactéries, les algues ou les protozoaires. Elle y apparaît sporadiquement, quand un animal périt à cause d'une blessure ou d'une maladie et n'est pas aussitôt dévoré par un prédateur. Ainsi il est beaucoup plus difficile de se procurer une telle sorte de nourriture que celle des bacteries, des algues ou des protozoaires. C'est peut-être à cause de cela qu'une adaptation compensatrice s'est developpée: l'animal mange irrégulièrement mais, s'il en a l'occasion, il peut dévorer d'un seul coup une quantité de nourriture très substantielle, bien plus grande que les autres infusoires; il peut passer

les périodes de faim dans les kystes, grâce à quoi il ne dissipe pas son energie en vaines recherches; un excellent sens chimique chez les espèces de *Prorodon* décrites plus haut leur permet de s'orienter aussitôt, quand un matériel mangeable est apparu; enfin la palintomie apparue déjà chez *Prorodon cinereus* rend possible une multiplication plus intense dans les périodes où la nourriture est en abondance. Ce deuxième trait — l'aptitude à survivre les périodes de faim dans le kyste est surtout frappant. J'ai réussi notamment à cultiver d'une même façon, c'est à dire en les nourissant avec des vers coupés, plusières autres espèces comme *Paramecium trichium* Stokes, *Paramecium bursaria* (Ehrbg.), *Paramecium caudatum* Ehrbg, *Coleps hirtus* Nitzsch, *Saprophilus putrinus* Kahl, *Cyclidium* sp., *Glaucoma* sp. Toutes ces espèces se multipliaient intensivement. Si pourtant je ne jétais pas dans le récipient, enfermant par exemple *Paramecium trichium*, une nouvelle portion de nourriture, il n'y avait après trois jours aucun individu: ils étaient tous morts de faim. *Prorodon raabei* sp.n., par contre, pouvait attendre immobile dans les kystes sans nourriture une semaine ou encore plus longtemps et aussitôt que j'ai jeté les vers dans le récipient il venait en nombre à la proie.

Il semble alors que chez les Ciliés histiophages décrits nous avons affaire à une adaptation rationnelle, provoquée par un mode spécifique de nutrition. Ainsi des études sur la biologie des espèces histiophages appartenant à d'autres ordres seraient à souhaiter.

Résumé

L'espèce psammophile *Prorodon raabei* sp. n., trouvée sur les rivages du golfe de Gdańsk, est un histiophague s'alimentant de tissus des animaux morts. L'individu mince et allongé qui sort du kyste de division s'alimente si intensivement, s'il trouve la nourriture, que sa forme et couleur changent — il devient ovoïde et son cytoplasme, rempli de vacuoles digestives, apparaît preque noir. L'animal s'enferme alors dans du kyste et digère. La division s'accomplie également à l'intérieur du kyste. Le Cilié peut percevoir la nourriture à travers les parois du kyste; les animaux commencent à sortir des kystes 3 min. lors de l'addition de la nourriture au milieu. Une tendance à absorber des grandes quantités de la nourriture à la fois, la possibilité de survivre dans le kyste une période de faim et un sens chimique excellant qui permet de s'emparer vite de la nourriture, sont des adaptations fort avantageuses pour des histiophagues, dont la nourriture n'apparaît dans le milieu que de façon sporadique.

STRESZCZENIE

Psammofilny gatunek *Prorodon raabei* sp. n. znaleziony przy brzegu Zatoki Gdańskiej jest histiofagiem żywiącym się tkankami martwych zwierząt. Smukły i wydłużony osobnik wychodzący z cysty podziałowej żeruje po znalezieniu pożywienia tak intensywnie, że zmienia kształt i barwę — staje się jajowaty, a cytoplazma wypełniona wodniczkami pokarmowymi przybiera barwę prawie czarną. Zwierzę zamyka się wtedy w cyście i trawi. Podział również odbywa się w cyście. Orzęsek ten może wyczuwać pokarm poprzez ścianki cysty; zwierzęta zaczynają wychodzić z cyst w 3 min. po podaniu pokarmu do środowiska. Zdolność wchła-

niania dużej ilości pożywienia naraz, możliwość przetrwania okresu głodu w cyście oraz doskonały zmysł chemiczny umożliwiający szybkie odszukanie pokarmu, stanowią dla histiofagów przystosowanie bardzo korzystne, ponieważ ich pokarm tylko sporadycznie pojawia się w środowisku.

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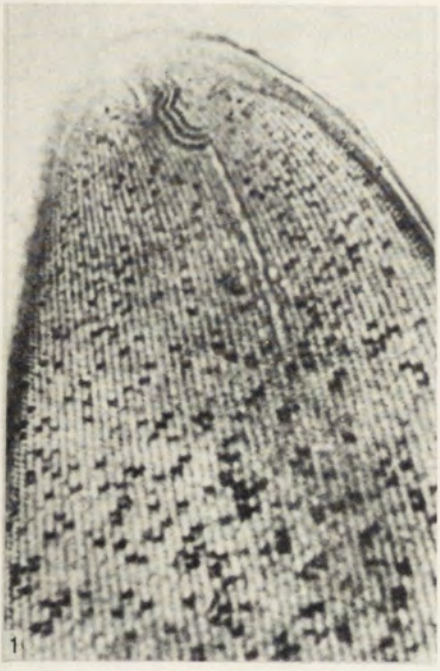
EXPLICATION DES PLANCHES I-III

1 : La partie antérieure du corps sur une préparation argentée
2 : La "brosse dorsale"
3 : Les Ciliés in vivo; la forme typique
4 : Les kystes
5 : Les Ciliés in vivo; les formes typique et ovoïde
6 : Les Ciliés autour du ver. Parmi les individus typiques on voit des formes ovoïdes, qui ont déjà absorbé la nourriture
7 : Les Ciliés attachés au ver
8-9 : L'alimentation; on voit les corps des Ciliés se remplir de nourriture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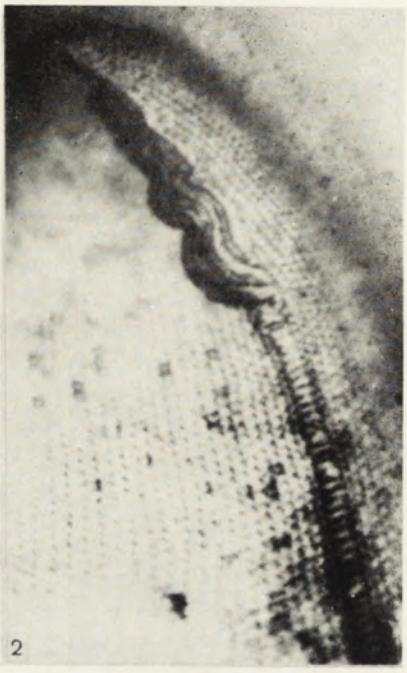
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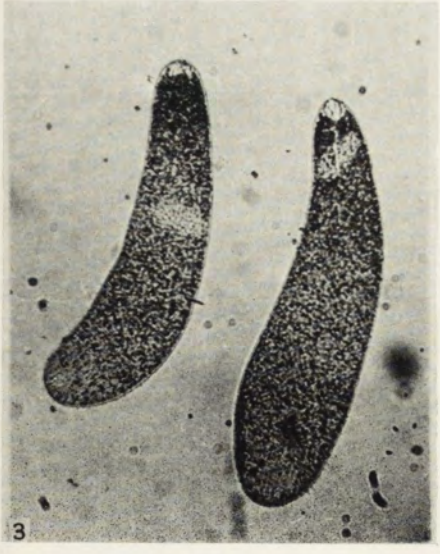
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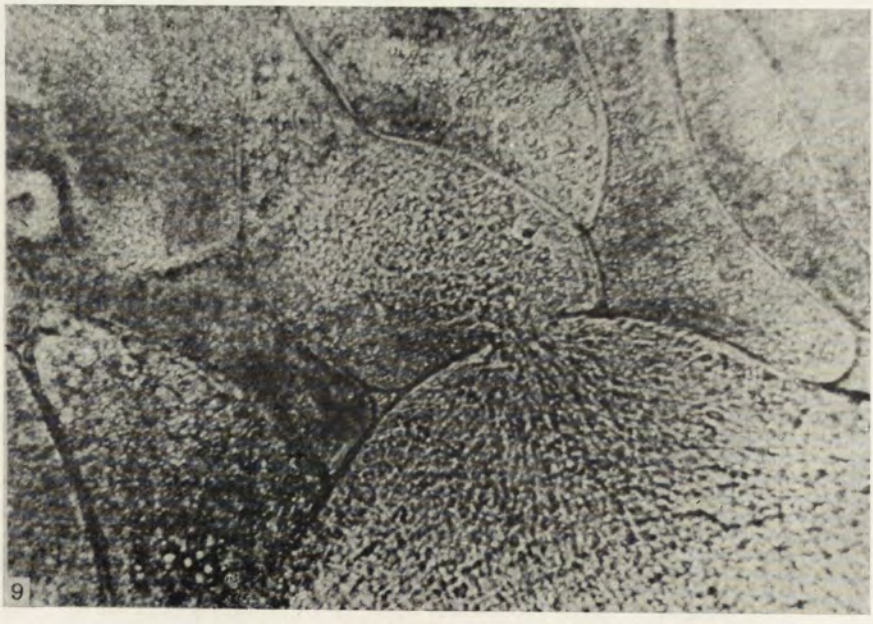
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Studies on the representatives of the family *Paraisotrichidae* Da Cunha *(Ciliata, Trichostomata).* III. Division morphogenesis in the genus *Paraisotricha* Fior. and *Rhizotricha* Wolska

Badania nad przedstawicielami rodziny *Paraisotrichidae* Da Cunha (*Ciliata, Trichostomata*). III. Morfogeneza podziałowa w rodzaju *Paraisotricha* Fior. i *Rhizotricha* Wolska

The present article is a continuation of previous publications (Wolska 1964 a, b) concerning the somatic and oral infraciliature in *Paraisotricha* Fiorentini and *Rhizotricha* Wolska.

The course of the division morphogenesis described below is based on series of single static images gained in the silver impregnated preparations (silver impregnation method — as in the previous articles — Wolska 1964 a, b). Some stages could be observed also in vivo (Fig. 1 C, D, E) in the representatives of the species *Paraisotricha colpoidea* Fiorentini. The stage corresponding to the Fig. 1 F was observed in the phase contrasting optics, in the formalin fixed material.

Paraisotricha colpoidea Fiorentini

The first division symptom observed is the appearence of 3 short kineties, each of them composed of several kinetosomes, located in the subequatorial zone, between the kineties 1 and 2 of a well grown individual (Fig. 1 and Pl. I 3). This position corresponds to the kineties triad on the right side of the peristome. This primordium of triad of the future opisthe is initially very faint and poorly impregnable. Subsequently a similar primordium (3 short kineties) appears between the kinety 1 and kinety n (primordium of the paroral kinety of the future opisthe) and the somatic kineties — beginning with the kinety 1 — start to interrupt. In most cases, at this stage, on the area of the future proter, in the two interkinetal areas just mentioned — occurrence of small, irregularly accumulated kinetosome groups may be stated (Pl. I 1—2). In different individuals they lie at different distance from the equatorial zone, sometimes near the margin of the old peristome. Two groups of migrating kinetosomes sometimes are seen in the individuals in which no divisional changes are as yet observable, as well as in the individuals at a later stage of division. They were never detected at a final stage of division and

never on the area belonging to the opisthe. The migrating kinetosomes associate distinctly with the formation of the triad primordia between the kineties 1 and 2 as well as of the paroral kinety between kineties 1 and n. The character of this association and the origin of the migrating kinetosomes has not been as yet clarified.

The migrating kinetosomes appear in different sequence left and right from the kinety 1, although in the division zone the primordium of the triad (right from the kinety 1) appears surely at first, then the primordium of the paroral kinety (left from the kinety 1). In some individuals a group of kinetosomes between kineties 1 and n is visible, whereas between kineties 1 and 2 the presence of a similar group has not been ascertained. The inconsistency in time of appearence of migrating kinetosomes and of the primordia in the equatorial zone which seem cennected with the migrating kinetosomes, might be due to some defects in the silver impregnation. The lack of a direct connection between the migrating kinetosomes and the primordium of the paroral kinety and triade might be interpreted in various manner. No doubt, the migrating kinetosomes appear in division on the area belonging to the proter, in proximity of the kinety 1, and shift along it. It may be concluded that they play a role in formation of the triad and of the paroral kinety, lying on both sides of the kinety 1.

Interruption of the somatic kineties proceeds gradually left and — with a certain delay — right from the kinety 1. Gradually appear also short interstitial kineties on the area belonging to the opisthe, between the interrupted somatic kineties (Fig. 1 B). Between the kinety n−1 and kinety n−2, and further on, pairs of the interstitial kineties arise gradually, whereas right of the kinety 2 — single insertions are formed. This process expands laterally, as long till the interstitial kineties are formed in the part of periphery reaching left from the kinety n−m (m is a value superior than 10 and depends on the number of somatic kineties in the individual given), and it expands also to the right, up to the kinety 7 or even up to 10. This process occurs during the growth of the dividing individual, of its somatic kineties and of already formed interstitial kineties. A characteristic picture is produced, in consequence, on the opisthe area (Fig. 1 and Pl. II 4). Left from the primordium of the paroral kinety an area exist, covered by parallel, dense, delicate and well grown interstitial kineties, as well as by the anteriorly prolonged delicate segments of somatic kineties of the opisthe. Right from the triad, this area is covered by single insertions which are thicker and shorter than the left ones, and by anterior segments of the somatic kineties. The insertions of the right side are shorter because they appear in the moment when the system on the left side already reach a certain level of growth. Or else, the distal extremes of the insertions of the left and right side (considering the triad and the paroral kinety as a reference point) from which the growth initiates, lie on different levels.

Despite the different time of formation of the left and right systems of insertions, the somatic kineties of the dorsal side and the lateral ones grow faster than the ventral. In consequence, the line of division rises on the dorsal side, and the plane of division is oriented somewhat obliquely to the long axis of the ciliate. About that time, the strongly elongated primordium of the triad becomes thicker and is impregnated intensely, as well as three insertions between the kinety 1 and n (primordium of the paroral kinety). From this

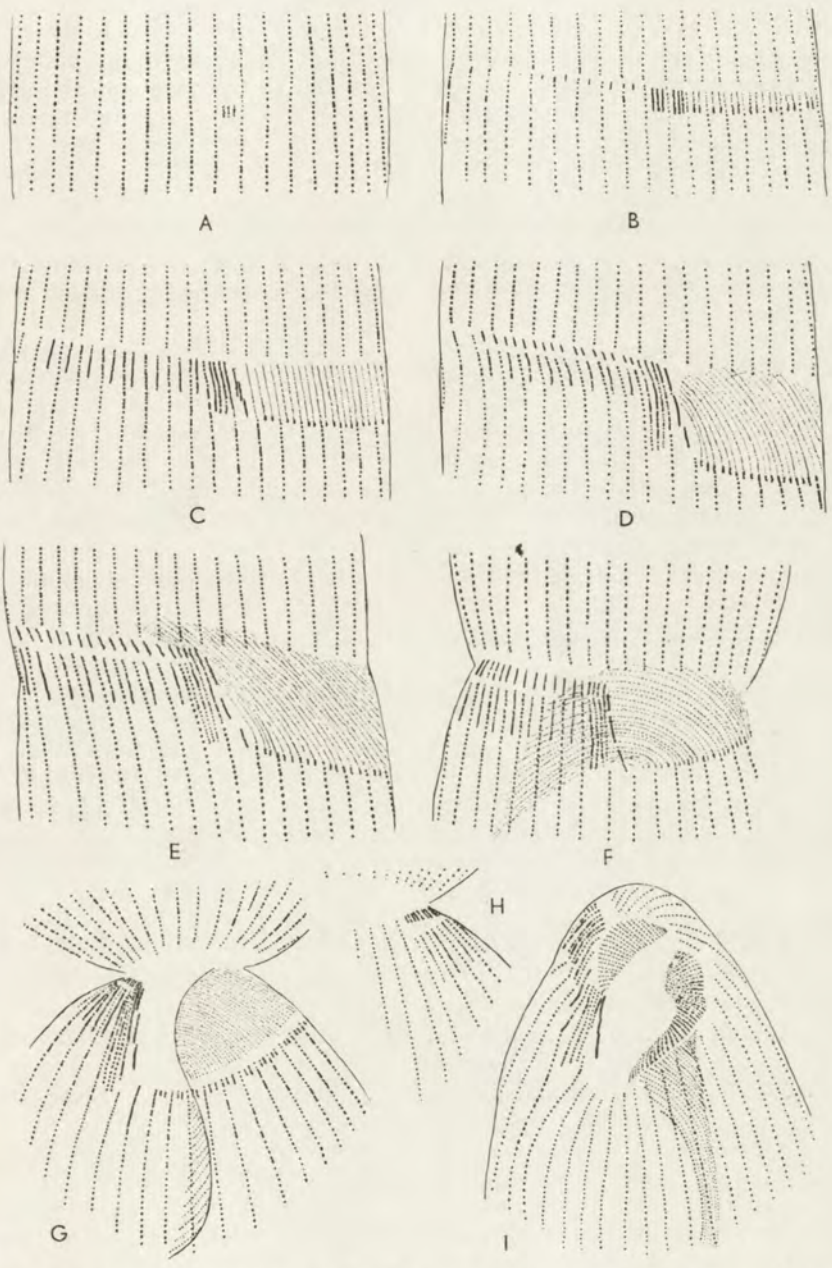

Fig. 1. *Paraisotricha colpoidea.* A—G. Successive stages of morphogenesis from the ventral side. H. Fragment of the dorsal surface on the stage corresponding to G. I. Anterior region of a fully developed specimen

moment those both elements are very sharply marked and they remain so in the fully developed individual. Three insertions, being the primordium of the paroral kinety, behave in a peculiar way: their growth is checked when compared with that of other interstitial kineties; they change only their position. It seems as if they were exposed to the mechanical influence of the more intense growth of the right side and they arrange themselves into a step-like pattern. The interstitial kineties of the left system have a characteristic form (Fig. 1 C—F, Pl. I 4, II 6), their posterior extremes are distinctly thickened. They have this feature since their origin (Fig. 1 B) and keep it till their thickened extremes are detached from the kineties of the left system. This seems to indicate that the increase of insertions occurs continuously forwards. The insertions of the right system are regularly thick on their whole length (Fig. 1 B).

The still growing kineties of the left system begin to assume an oblique position; besides, not all of them grow in the same degree. The kineties lying near the paroral kinety elongate most of all. Over the whole left area the gradation of growth is seen. The decrease of growth proceeds with the distance from the paroral kinety. The anterior extremes of the most intensely growing kineties sink underneath the proter kineties and underneath the opisthe paroral kinety (Fig. 1 D). A shallow pouch arises in which penetrate the left system kineties during their elongation.

Cilia are present on the kineties of the left system, at this stage and even on an earlier one. Cilia are sometimes revealed after silver impregnation, and at the stage corresponding to the Fig. 1 E, observed in vivo, they were very well visible in a shallow ectoplasmic pouch. Simultaneously with those transformations in the left system, probably a more rapid growth of the somatic kineties on the right and dorsal sides occurs than on the et ventral side because they lean to the right together with the insertions, then they interrupt, and detach a series of short kineties (Fig. 1 D and Pl. II 7) of their anterior segments. Somewhat later, short segments of the triad detach as well. In the Fig. 1 D a series of short kineties are seen, detached from the somatic kineties and insertions, except the insertions of the triad; in the Fig. 1 E, short kineties are yet detached of the triad. The kinety 1 fails to be interrupted at this level. Three primordial segments of the paroral kinety, in course of sinking of the kineties of the left system, and of interruption of the right side kineties with no visible changes of length — shift their position and set themselves more or less along one line.

At the stage represented in the Fig. 1 E all the primordia of ciliary system of the anterior pole, as well as of the peristome of the future opisthe, are present. The kineties triad and the paroral kinety are in their final position and form. The insertions lying right of the kinety 2 remain between the anterior segments of the general ciliature, on the right side. A series of short kineties, detached from the kineties and from the insertions of the right side is the primordium of the frontal cilia (semilunar area — W o l s k a 1964 a). The system of dense oblique kineties of the left area represents the peristomal cilia. The 2 last groups of cilia need only to assume their proper localization and further grow.

Shifting would now occur gradually with the simultaneous deepening of the division furrow which appears already, at the stage just described (Fig. 1 E). Constriction of the cell, and the quicker growth of the right and

dorsal side kineties, will promote shifting of the frontal cilia which — from their position oblique toward the somatic kineties (Fig. 1 E) — pass to the position conforming with the direction of the somatic kineties (Fig. 1 F, G) and, subsequently, to the subterminal position of the ventral side of the opisthe (Fig. 1 H). They cover also an area which shape will be determined by their irregular elongation and by the mechanical action of the growing somatic kineties of the dorsal side.

The area covered by the future peristomal kineties and that of the collar, since the stage in Fig. 1 E, has a form similar to the conclusive one. The peristomal kineties produce a system of parallel compact rows of uneven length. The marginal, the longest row, is now distinguished by bigger kinetosomes (see W o l s k a 1964 a). The length ratio of successive kineties is not yet definitive, in subsequent stages the growth of the kineties lying nearer the kinety 1 will occur.

In the further course of morphogenesis, the right side kineties (right side of the area of peristomal cilia) directed anteriorly grow, become crooked and sink underneath the paroral kinety and the triad — on the territory of the future opisthe (Fig.1 F). Finally, the marginal peristomal kinety revolves for 180° — when compared to its position in the initial stage of stomatogenesis — in the frontal plane (a transition stage between Fig. 1 F and G). A similar but slighter turn is performed together with it by the marginal neighbouring long kineties. The short kineties shift only slightly. They failed to penetrate into the pouch and remained on the surface (left side of the peristomal kineties). The just mentioned short kineties remain on the surface of the future opisthe as its collar.

The dislocation of the peristomal kineties is accompanied by their separation from the somatic ciliature. All the peristomal kineties (as well the interstitial as those which are the prolongation of the somatic kineties) interrupt closely over the limit of primordial interstitial segments and recede from the somatic ciliature (Fig. 1 H). Between the anterior segments of the somatic kineties, after their isolation from the peristomal system, short primordial segments of the insertions remain (Fig. 1 G). They disappear subsequently.

Other dislocations connected with the formation of the peristomal cavity brings the peristomal ciliature to its proper position. The long peristomal kineties, lying on the dorsal wall of the shallow pouch, are turned for 180° — as a uniform elastic plate — round the axis of the same direction as the marginal kinety. This rotation fails to concern the segments proximal the somatic ciliature. As result of this shifting, the peristomal kineties from the right side of the shallow pouch, will find themselves on its left side. The marginal kinety (with bigger kinetosomes) becomes the most right located peristomal kinety. They all are situated on the internal surface of the peristome — already deeply depressed — adhering to the ventral wall of the opisthe body (Fig. 1 J). Simultaneously, the ventral left margin of the peristome is formed by invagination (Pl. II 8). This process involves the transition of the terminal kineties system — proximal the somatic ciliature — from the pattern represented in the Fig. 1 G to that in Fig. 1 J. The marginal peristome kinety, as well as several neighbouring, shifts entirely to the inner wall of the peristome loosing connection with the body surface. Besides, the peristomal kineties system as a whole shifts left. In this way, between the

kinety n and the kinety n−2 or n−3 remains a margin of the peristome free, i.e. deprived of the ends of peristomal kineties, which should produce the collar if remaining on the surface (Fig. 1 J). Shifting left of the peristome kineties and of the collar probably exerts a mechanical action upon the somatic kineties lying left from the collar: one or sometimes two of them are interrupted. In the Fig. 1 J the kinety n−14 with the detached short anterior segment is seen. The same is represented in Fig. 1 H (the dorsal side of the stage from the Fig. 1 G).

There is no real sequence of shifts described above. The description of the process is only an enumeration of main components of the movements which are occurring simultaneously and adjust the peristomal kineties system in the proper position and shape the peristome cavity. In the initial phase of shifts, distinctly dominates the sliding of kineties in the frontal plane towards the posterior pole of the opisthe. Subsequent — more complicated shifts — are difficult to be followed. It is difficult e.g. to establish the moment when the peristomal kineties become isolated. It may be estimated rougly that it occurs at the stage of their transition to the position as in the Fig. 1 F. In many instances, however, it cannot be ascertained whether the peristomal kineties have already lost their connection with the general ciliature. They are always differentiated distinctly in the stage as in Fig. 1 G.

In the conclusive phase of division, the peristomal kineties condense, the segments of the paroral kinety approach sometimes to one another, disposing themselves into one continuous line as in Fig. 1 E, but most frequently they remain separated into two or three segments. It is difficult to ascertain whether formation of two segments of the paroral kinety is the effect of atrophy of one of its segments or of integration of two into one.

In the course of division, the mother peristome remains unchanged and passes to the proter. Reconstruction of the normal feature of the proter occurs by the growth of its body and of the somatic kineties. The behaviour of the kinety 2 shows how the characteristic system of kineties on the posterior pole of the prother (not all the kineties reach the pole) is effected. As a rule, the kinety 2 fails to reach as far as the posterior pole; sometimes however one or two kineties of undefined sequence, fail to reach it neither. During division all the kineties of the proter, concerned by the division line, terminate at the same level. In the definitely formed individual, the posterior pole bears kineties of different length. To account for this fact it may be postulated that some kineties grow backwards less intensely than the others. The behaviour of the kinety 2 suggests another mechanism. A short posterior segment is detached from the kinety 2 (as distant from the division line as the others — Fig. 1 F), and disappears after a certain time.

Paraisotricha minuta Hsiung

The course of morphogenesis of this species is quite similar to that described for *P. colpoidea* Fior. The differences concern some details connected with slight differences in the structure of both species.

Similarly as in *P. colpoidea*, there occur groups of kinetosomes migrating over the proter area, on both sides of the kinety 1. Like in *P. colpoidea* the primordium of the triad — in the form of 3 short kineties — appears first in the zone of division. Then the primordium of the paroral kinety is formed, similarly as 3 short kineties. Insertions on the left side of the ciliate are

formed subsequently, and — with a certain delay — also on the right side. Left of the kinety n — in contrast to *P. colpoidea* — 3 insertions arise between each pair of somatic kineties (Fig. 2 A and Pl. III 13); sometimes even 4 appear, between kineties situated extremely right. Left from the kinety n insertions arise on the area extending up to the kinety n-7 or n-9, depending on the number of somatic kineties which is variable in narrow limits. As result,

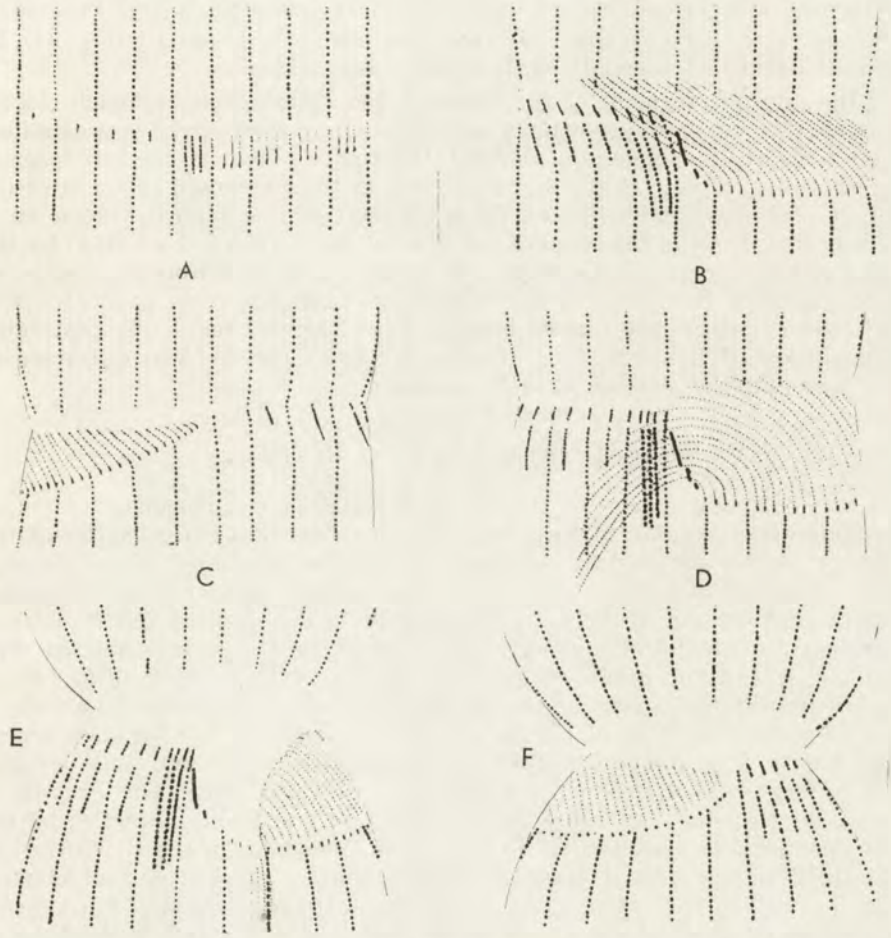

Fig. 2. *Paraisotricha minuta*, the successive stages of morphogenesis (B, C and E, F represent the same stages observed from the ventral and the dorsal sides)

the number of peristomal kineties in *P. minuta* — although embracing the periphery as far as the kinety n-7 or n-9 — approaches the number of peristomal kineties in *P. colpoidea*.

Right of the kinety 2, single insertions are formed. Sometimes between the kineties 2 and 3 two insertions are arising. Right of the kinety 2, insertions arise between all the somatic kineties on the dorsal side, except for one interkinetal area (Fig. 2 C). In the last phase of morphogenesis, this free space

is occupied by the kineties of the collar. It occurs in the process of shifting left the whole peristomal ciliature, over one interkinetal space. In *P. minuta* this shift makes free only a short segment of the peristome margin, as characteristic for this species (see W o l s k a 1964 a, b). In formation of the area of frontal cilia in *P. minuta* participate all the somatic kineties and insertions of the right and dorsal side (their isolated anterior segments — Fig. 2 F and Pl. III 11). The kinety triad of the right side retains the same pattern as in *P. colpoidea* (Fig. 2 E and Pl. II 9, III 12, 14). Only one of the 3 initial segments of the paroral kinety develops well, two other disappear gradually (Fig. 2 D, E). One well developed segment shifts slightly backwards.

In the anterior segment of the kinety 1, the kinetosomes approach closely to one another and produce a thick continuous line, after silver impregnation. Closely beneath the condensed segment, the kinety 1 loosens, the kinetosomes become faintly impregnable with silver, so that the condensed segment of the kinety 1 may easily be mistaken for a segment of the paroral kinety. So it was interpreted in the first part of the present study (W o l s k a 1964 a) by the statement that the paroral kinety in *P. minuta* is most frequently composed of two segments. In fact — as shown in stomatogenesis — the paroral kinety of *P. minuta* is definitely formed mostly of one segment (as it was postulated in description of the oral apparatus — W o l s k a 1964 b). The character of shifts is essentialy the same as in *P. colpoidea.*

Rhizotricha beckeri (H s i u n g)

In this only one species of the genus *Rhizotricha* morphogenesis develops in an essentially similar pattern as it has just been described. Shifts of the ciliary primordia are slightly different, as well as the shape of the developing peristome. Primordium of the triad and of the paroral kinety develops in the same form and in the same sequence as in *P. colpoidea* and *P. minuta.* The migrating kinetosomes behave similarly as in the former species. The insertions, left of the kinety n, arise in number of 10 or more (Fig. 3 B, C) in every interkinetal space, as far as the kinety n−4. Right of the kinety 2, in all the remaining part of periphery arise 2 insertions, but between kinety 2 and kinety 3 a higher numbers (5—7) are always formed, and between the kineties 3 and 4 three insertions may arise (Fig. 3 B). The process of isolation of the short kineties, corresponding to the frontal kineties of the genus *Paraisotricha,* is retarded. In the genus *Paraisotricha* the short kineties are formed in the initial stage of penetration of the peristomal kineties inside the wall of the ectoplasmic pouch (Fig. 1 D). In the genus *Rhizotricha,* interruption of kineties and insertions of the right and dorsal side occurs at a later stage (Fig. 3 C). This retardation is probably the result of a smaller difference in the rate of growth of somatic kineties. The insignificant allometry of the kineties growth, involves a more insignificant shift of the peristome to the ventral side, as well as a different location of the short kineties separated from the somatic ones, and of the insertions of the right and dorsal side.

The stage of stomatogenesis represented in Fig. 3 C is the last stage observed in *Rhizotricha beckeri.* This species is occurring rarely in the horses of the Łódź district. Only two samples were available for the present study in which this species occurred. In those populations advanced division

stages were not observed. The conformity of the morphogenesis process in the 3 species under study, beginning with the formation of all the elements of the ciliature of the future opisthe, allows to conclude that its subsequent course would be essentially similar. The stage of morphogenesis of *R. beckeri* represented in Fig. 3 C corresponds to the stage in Fig. 1 E of *P. colpoidea* and to the stage in Fig. 2 B of *P. minuta.*

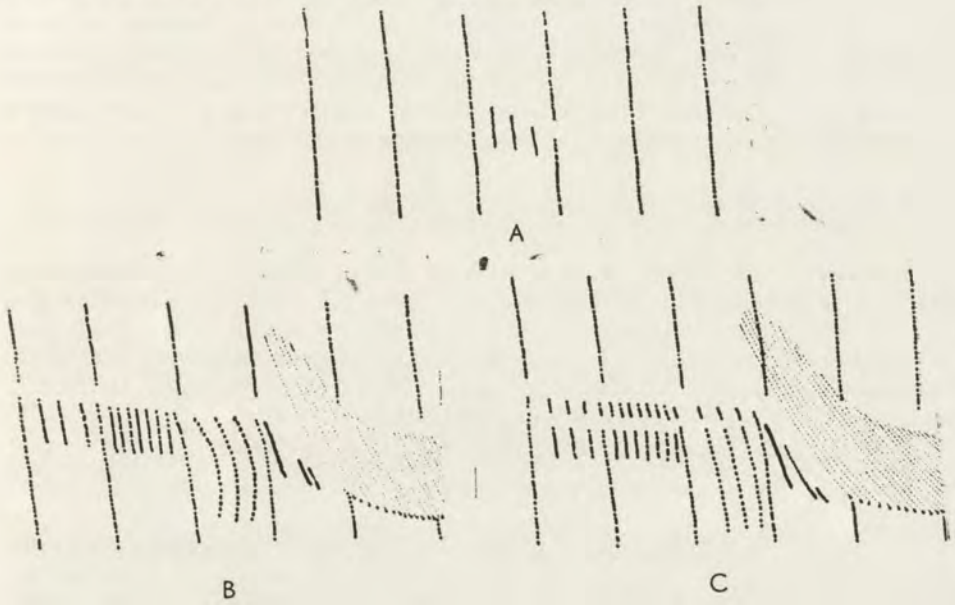

Fig. 3. *Rhizotricha beckeri.* A—C. Three stages of morphogenesis observed from the ventral side

It might be stated that in *R. beckeri* the area occupied by the peristomal kineties of the future opisthe (Pl. V 19) has another shape than in *P. colpoidea* and *P. minuta.* This shape of the ciliary area of the peristome remains in the fully developed individual. The long peristomal kineties contrast with the short ones. In the genus *Paraisotricha* the transition of the long kineties to the short ones is gradual. All the peristomal kineties in *R. beckeri* — as result of conclusive shifts — will entirely pass on the inner wall of the peristome leaving no collar on the surface.

The triad of kineties on the right side of the stage in Fig. 3 C has a definitive feature. Formation of the paroral kinety in *R. beckeri* is not clarified because of the lack of material on the final division stages. In the fully formed individuals the paroral kinety seems to be a continuous polykinety. Whether it consists of 2 or of 3 monokineties — is difficult to decide. Possibly all 3 primordial segments participate in formation of the paroral kinety. Nevertheless, the stages of morphogenesis known as yet seem to indicate the development of 2 segments and atrophy of the 3rd one (Fig. 3 C). Disappearing of the 3rd segment and the parallel disposition of the two remaining seems more plausible.

*

There are no reliable data as to formation of the peristomal fibres in the species under study. Possibly those fibres arise in the last moment prior the separation of the proter and opisthe, when the peristomal kineties are disposed in their proper position. The connection between the frontal and dorsal kineties by means of several fibres is probably established also in the last stage of morphogenesis in the genus *Paraisotricha*.

The specific and complex character of stomatogenesis in the representatives of genus *Paraisotricha* Fiorentini and *Rhizotricha* Wolska involves reflexions on the adequacy of leaving those genera within the order *Trichostomata*. An attempt of evaluation of the morphological and morphogenetic characters, on the base of the infraciliature investigated, will be the content of the last, conclusive, part of the present cycle of articles.

Summary

The author describes the successive stages of division in *Paraisotricha colpoidea*, considering in details the arising of the interstitial kineties and the differentiation of the peristomal kineties. The features of morphogenesis in *Paraisotricha minuta* and *Rhizotricha beckeri* are comparatively presented. The complex character of stomatogenesis evokes doubts as to the taxonomic position of these species in the order *Trichostomata*.

STRESZCZENIE

Autorka opisuje etapy podziału *Paraisotricha colpoidea* szczegółowo analizując proces wytwarzania się kinet wstawkowych i wyróżnicowywania się kinet peristomalnych. Porównawczo przedstawione są właściwości morfogenezy *Paraisotricha minuta* oraz *Rhizotricha beckeri*. Złożony charakter stomatogenezy zbadanych gatunków pozwala wątpić w słuszność zaliczania ich do rzędu *Trichostomata*.

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EXPLANATION OF PLATES I—VI

Paraisotricha colpoidea

1: Group of migrating kinetosomes between the kinety 1 and n (×1650)

2: Group of migrating kinetosomes between the kinety 1 and 2 (×1650)

3: A triade primordium (×1650)

4: A triade primordium, on the left the primordium of the paroral kinety. Note the characteristic form of the left side insertions, the insertions of the right side are still short (×1650)

5: The peristomal kineties of the future opisthe during their sinking underneath the somatic kineties of the proter (×900)

6: The peristomal kineties of the opisthe, clearly sinking and assuming an oblique disposition. A surface view (×900)

7: View of the right side. Short kineties after separation (×900)

8: A late stage of morphogenesis. Surface view (8a) and optical section (8) (×900)

Paraisotricha minuta

9: Sinking of the peristomal kineties in the future opisthe (×900)

10: The same (×1650)

11: Short frontal kineties of the future opisthe, after their separation. Surface view of the right dorsal side (×900)

12: Sinking peristomal kineties of the opisthe. A surface view (×900)

13: The same. Right surface view (a), optical section (b) and left dorsal surface view (c) (×1650)

14: The same stage as 13 (×900)

Rhizotricha beckeri

15: Migrating kinetosomes between the kinety 1 and 2 (×1650)

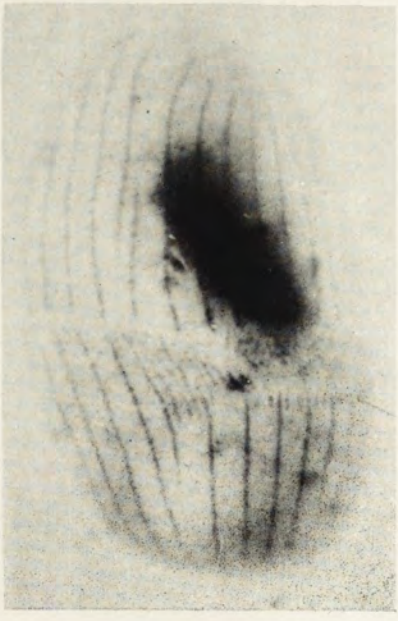
16: Sinking peristomal kineties of the opisthe. An optical section (×900)

17: The same (×1650)

18: A stage similar as 16. Right side (a) and left side (b) views (×900)

19: The same stage. Note the characteristic pattern of the area of the peristomal kineties in the opisthe (×900)

20: A similar stage. Optical section (×1650)



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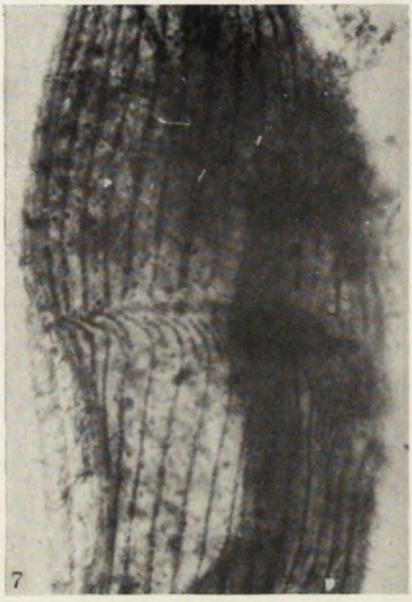

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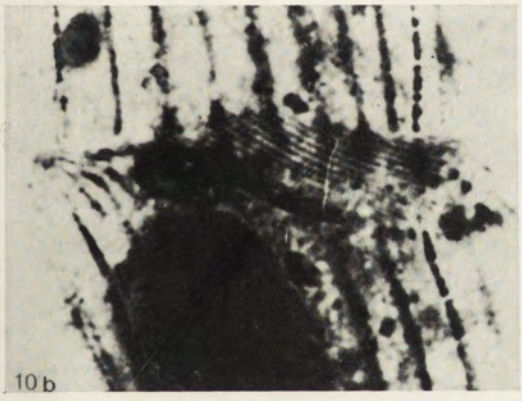
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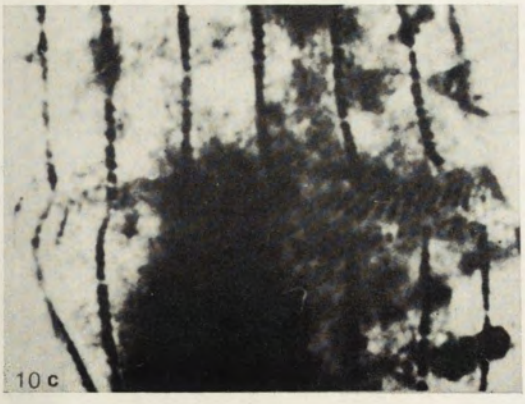
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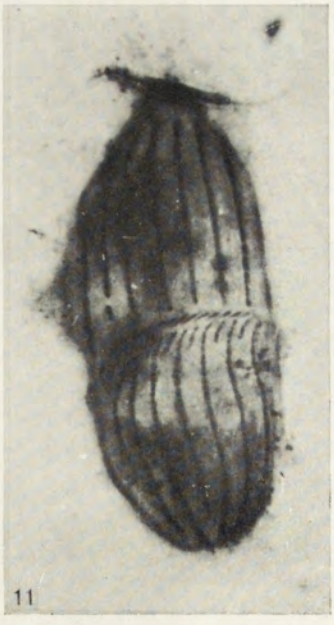
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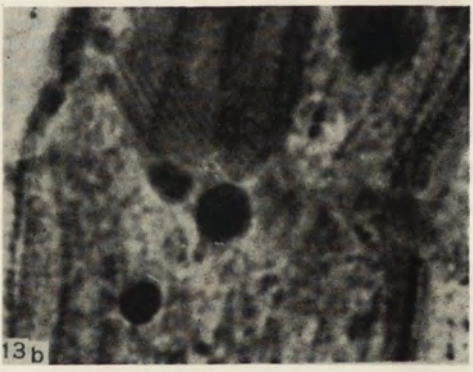
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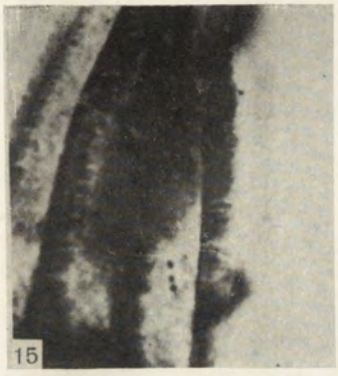
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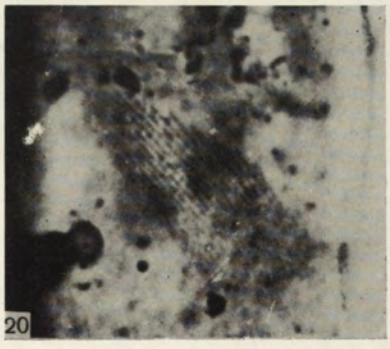

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Harold SANDON

Some species of *Trichodina* from South Africa[1]

Quelques espèces de *Trichodina* de l'Afrique du Sud

Trichodina xenopodos Fantham from the bladder of the clawed toad, *Xenopus laevis*

The only published account of this ciliate is the original one of Fantham 1924 who described it as follows: "These organisms were vase-shaped or urn-shaped, each had a large adhesive disc, a relatively uniform, horse-shoe-shaped macronucleus (with only a few forms showing slight lobulations), small dense micronucleus and from 48 to 64 uncini or hooklets on the disc. The maximum diameter of various specimens was from 75 μ to 92 μ and the depth about 60 μ. For purposes of reference it may be named *Trichodina xenopodos*, the species being new". As Lom 1958 has pointed out, this description is inadequate, only one of the characters mentioned being of systematic value, namely the number of denticles which is very exceptionally high for the genus. In view of this I have recently re-examined notes and preparations made by myself at Cape Town between the years 1937 and 1940 from which a more satisfactory account of this organism is possible.

Methods

A number of different histological techniques were used, principally fixation in either Bouin's or Schaudinn's fluids followed by a variety of haematoxylin or carmine stains, or by Feulgen's or Mallory's stains. None of these was satisfactory for showing either the skeletal or ciliary structures. For these purposes by far the best results were obtained with Gelei's osmic-toluidine blue technique. These preparations have mostly faded somewhat by now but, even so, still permit (especially with the aid of phase contrast) confirmation of drawings made from them when they were fresh. Other techniques used included relief staining with opal blue and with nigrosin and a variety of methods intended to elucidate the chemical nature of the skeleton. On this matter my results have been superseded by more recent investigators using more refined techniques (notably Fauré-Fremiet et Thaureaux 1944) and these preparations are only mentioned here because, although they yielded no new anatomical information, they did provide some additional confirmation of observations made by more conventional methods.

[1] With an Appendix on the abuse of simile in morphological descriptions.

It is unfortunate that, at the time, the importance of silver impregnation was not appreciated. An attempt to use Klein's dry silver method was so unsatisfactory (mainly owing to the presence of mucus) that it was abandoned. Fortunately one such preparation was kept in which a single individual shows the denticles reasonably well.

Shape

F a n t h a m's description of the organisms as "vase- or urn-shaped" proved very misleading, especially as, in a later description of the species from the bladder of *Bufo regularis* he says "it is less vase-shaped than that from *Xenopus*, being more bowl-like, with a shorter neck." The comparison to a vase seemed quite inappropriate for the Cape Town specimens, none of which had anything that I would call a neck. For this reason I was for long in doubt as to whether I was dealing with the same species. As Cape Town is some 1600 kilometers from the type locality, Johannesburg, the possibility of varietal or even specific differences between the trichodinas from *Xenopus* at the two places had to be considered. I was unable to trace any preparations made by F a n t h a m but, by the kindness of Dr. N. Patterson of the University of the Witwatersrand, Johannesburg, I was able to consult an unpublished thesis by Miss W e i n b r e n n 1925 which contained some brief notes and freehand sketches of *T. xenopodos*. Seeing that her work was done under the supervision of Prof. F a n t h a m at about the time when he published his account of this species, we may assume that her identification, description and drawings were accepted by him. The appearence of her organism was so similar to my own as to leave no doubt as to their identity.

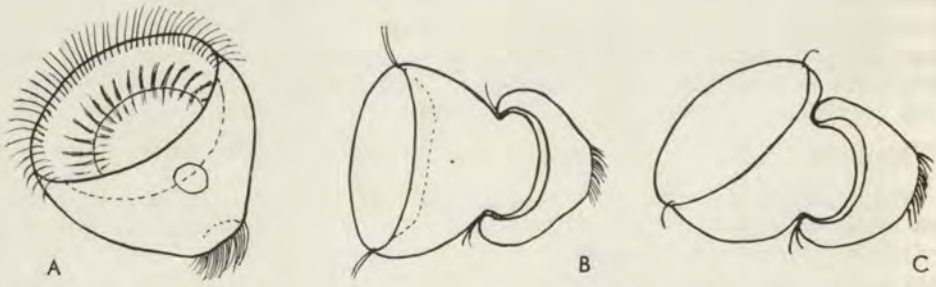

Fig. 1. *T. xenopodos*. Freehand sketches from life. A. Normal appearance when swimming. B—C. Pairs in "pseudoconjugation"

Living individuals swimming freely (Fig. 1) usually have the form of a truncated cone with slightly convex sides, the greatest diameter being slightly anterior to the adhesive disc. The cone is roughly symmetrical about its adoral-aboral axis and its length varies between 0.55 and 0.8 of the greatest width. Sections of the frog's bladder wall often contain many very asymmetrical individuals (Fig. 2) which give the impression that they had been stretching out actively to one side to reach down into the crevices of the epithelium, but most probably these had simply been passively distorted by the violent contraction of the bladder wall when this was removed from the body and fixed.

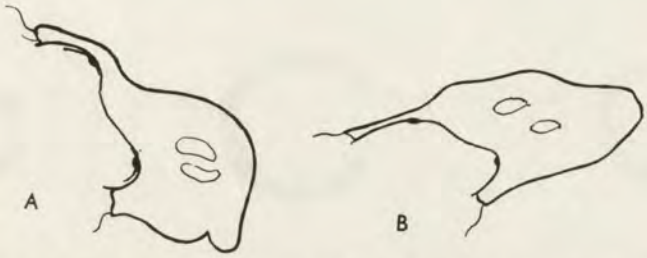

Fig. 2. *T. xenopodos*. Strongly distorted forms as often found in sections of bladder wall

Size

No satisfactory measurements were made of living individuals and the permanent preparations show considerable differences according to the method of fixation. As the preparations were made from many different infections at different times there seems no doubt that the differences found must be attributed to the techniques used. For greatest width (measured perpendicular to the adoral-aboral axis), 20 specimens fixed in Bouin's fluid gave a range of 49 μ to 75 μ (mean 60.4 μ); 71 fixed in Schaudinn's fluid gave 39 μ to 94 μ (mean 67.9 μ); 59 fixed in osmic gave 54 μ to 104 μ (mean 76.8 μ) and 10 fixed dry for relief staining or silver impregnation, 69 μ to 111 μ (mean 91.2 μ). The Bouin and Schaudinn preparations show other signs of shrinkage. Fantham's figures of 75 μ to 92 μ may therefore be taken as typical though larger or smaller specimens are not uncommon. Weinbrenn 1925 confirms Fantham's measurements but states that larger specimens with diameters up to 108 μ were observed though she suggests that these larger measurements may have been due to flattening of the specimens.

Similar variations were found in the diameters of the adhesive disc. After Bouin's fixative this ranged from 39 μ to 69 μ (mean 50 μ); after Schaudinn's, from 32 μ to 74 μ (mean 49 μ); after osmic, from 36 μ to 83 μ (mean 61 μ) and after dry fixation, from 56 μ to 78 μ (mean 68 μ).

The skeletal ring is less subject to shrinkage, its diameter ranging from 29 μ to 47 μ (mean 37.5 μ) after either Bouin's or Schaudinn's fluid and from 31 μ to 60 μ (mean 45 μ) after osmic or dry fixation. Reynoldson 1955 recommends that determinations of size in urceolarians should be based on the skeletal ring owing to its freedom from shrinkage when treated with reagents.

Nucleus

The macronucleus, as in most urceolarians, is horseshoe-shaped and lies in a plane approximately parallel to that of the skeletal ring. Its size and shape are rather variable (Fig. 3). Its overall diameter (measured at its widest point, perpendicular to the main axis of the horse-shoe) is commonly between 0.4 and 0.6 of the maximum diameter of the animal. Smaller values, down to 0.3, are not uncommon but, owing to the difficulty of recognising the early stages of reproduction, these may be due to the beginning of the pre-divisional condensation of the nucleus. The width of the gap between the two ends is also variable. Sometimes they are very close together, the

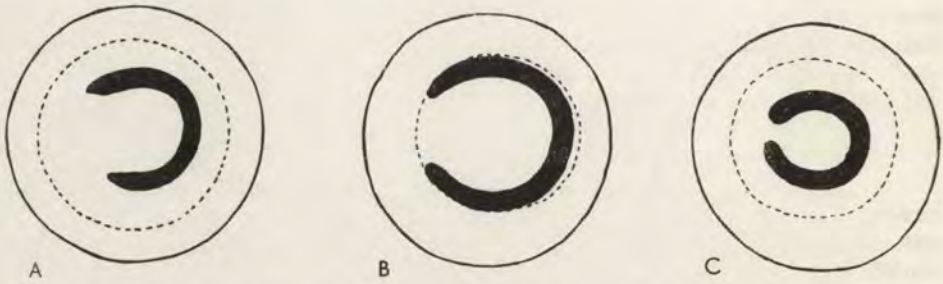

Fig. 3. *T. xenopodos.* Varying appearances of macronucleus

distance between them being only about 0.19 of the greatest separation of the two arms (measured from their centres) but in other cases the arms are almost parallel and the corresponding figure may be nearly 0.8.

Fantham mentions that a small dense micronucleus is present and this is shown in Weinbrenn's drawing near the middle of the concavity of the macronucleus. No trace of a micronucleus can be found in any of my specimens. Dr. J. P. Thurston has kindly given me some preparations of trichodinas from the bladder of *Xenopus laevis* from Kampala, Uganda and these too have no micronucleus. The existence of both micronucleate and amicronucleate races of the same species is not impossible, but reluctance to accept the existence of micronuclei in the Johannesburg specimens without further confirmation is justified by the fact that, when only low magnifications are used, it is often impossible to ascertain whether a contaminating particle, such as a frog's erythrocyte, is inside the ciliate's body or merely lying against its surface. The position of the micronucleus in Weinbrenn's drawing is very unusual for organisms of this group (though it has been described in *T. fariai* Cunha et Pinto) but is a very probable one for an erythrocyte entangled in the adoral groove.

In spite of the absence of micronuclei, pairs apparently in conjugation were seen on a number of occasions in both living and fixed preparations (Fig. 1). At first it was assumed that the association was accidental, the upper partner merely using the other as a substrate in place of the cells of its host in the manner described by Colwin 1944 in *Urceolaria.* Nevertheless, the fact that the upper partner in *T. xenopodos* is invariably smaller than the other, thus resembling a microconjugant, may be significant. The further fact that in one pair a double skeletal ring (indicating recent division) is clearly visible in the "microconjugant", would accord with Colwin's observation that, in any population, conjugation is commonly associated with binary fission. On the other hand, the macronuclei in both partners are always normal whereas, according to Diller 1963, conjugation between amicronucleate Paramecia is associated with macronuclear changes comparable, though not identical, to those occurring in normal conjugation.

Adoral zone

This is very inconspicuous and no mention is made of it by either Fantham or Weinbrenn. It is best seen in the toluidine blue preparations (Fig. 4A). It is situated at the apex of the conical body lying in a plane roughly parallel to that of the adhesive disc. Its maximum diameter is only about one

third to one half of the overall diameter of the animal and, in specimens fixed when adhering to the glass slide, it makes only about three quarters of a complete circuit. It is thus intermediate between the condition found in the genus *Trichodina* as defined by Lom 1958 in which "the adoral region performs a spiral of at least one complete turn" and that in *Trichodinella* in which "it is in the form of an arch up to 180°". It should be noted, however, that the peristomial apparatus has a certain elasticity. Adherent individuals

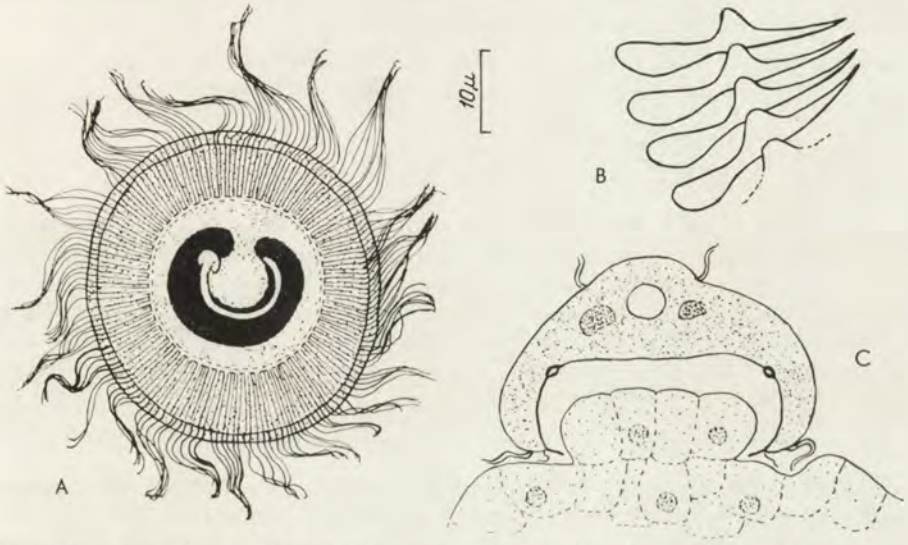

Fig. 4. *T. xenopodos*. A. Viewed from aboral end, showing position of adoral zone (osmic, toluidine blue). B. A group of denticles (Klein's silver method). C. Optical section of individual attached to a group of cells; denticular blades strongly arched and border membrane in "gripping" position (osmic, toluidine blue)

are short and wide and in this condition the ends of the adoral zone are pulled somewhat apart. In live, freely-swimming individuals or in those fixed while lying on their side, the adoral end of the animal is narrower and the ends of the zone come closer together, more nearly completing the circuit. In all other respects this species very clearly belongs to the genus *Trichodina* and the fact that the adoral zone does not fully comply with the generic definition indicates the need for a minor amendment to this definition and not the relegation of this species to another genus.

Denticles

These are clearly visible in the osmic-toluidine blue preparations but their detailed form can only be revealed by silver impregnation. They resemble closely those of *T. urinicola* as figured by Lom 1958. The blades are approximately 7 µ long by 2.7 µ broad and the rays about 9 µ by 1.2 µ (Fig. 4 B).

Among the 63 specimens from which reliable counts were made, the number ranges from 39 to 53 with a mean of 47, but in all but 8 of these it lies between 44 and 50. Out of 8 recently divided specimens in which the old half-ring is sufficiently clear, this has 22 denticles in 5 cases and 21, 23 and

24 in one each of the others, corresponding to a range of from 42 to 48 in the complete ring. Weinbrenn, presumably repeating Fantham's statement, says that the number „appears to be" between 48 and 64 but adds her own observation that sometimes only 45 can be seen and none of her drawings shows more than that number. In view of the difficulty of making accurate counts except in suitably prepared specimens and of the absence of any information about the methods used by Fantham or the numbers counted by him, we are justified in doubting whether the figures given by him are typical of the species and indicate a significant difference between the Johannesburg and Cape Town populations.

Radial pins

These, too, are clearly visible in the osmic preparations. There are from 6 to 8 to each pair of denticles.

Other structures

The velum is only moderately developed and the border membrane and main ciliary ring are typical of the genus. A posterior ring of rather feeble cilia arising near the base of the border membrane can be seen in suitably oriented specimens (Fig. 4 C). No anterior circlet of cilia or bristles is present.

The contractile vacuole, when fully formed, fills the whole of the space surrounded by the macronucleus. The precise arrangement of its discharge channel could not be ascertained.

Relations to host

These ciliates are commonly found gripping the epithelium of the host's bladder by means of the sharply inturned border membrane. The grip is so effective that when they are removed e.g. by rubbing the inside of the bladder on a glass slide or cover glass, the cavity of the adhesive organelle is often completely filled with a mass of torn-off cells. Whether or not any destruction of tissue takes place when the trichodinas are not disturbed could not be established conclusively though some preparations of bladder wall show thin patches which may be due to this. The species which parasitise the gills of fish certainly cause very extensive destruction and Davis 1947 states that epithelial cells in various stages of digestion can be found in their food vacuoles. He considered that these cells were the principal, if not the only, food of these ciliates. In my preparations of *T. xenopodos* no particulate matter of any kind was ever found in the food vacuoles and it seems that their nourishment must be taken exclusively in the liquid form. A similar observation was made by Breitschneider 1935 on *T. entzi* from the bladders of *Rana* spp. The frog's epithelial cells show no sign of digestion by exo-enzymes so presumably the nourishment is derived from the urine.

Mechanism of adhesion

In view of the uniqueness of the structures forming the adhesive organelle of the urceolarians and of the amount of study that has been devoted to its anatomy, it is strange how little attention has been given to the precise way in which this organelle works. Zick 1928 is the only author who has attempted to give a comprehensive functional interpretation of the anatomy of these ciliates. He noted how, in a sagittal section of *T. pediculus* fixed in situ on

the surface of a hydra, the host's epithelial cells were raised as if sucked up to form a mound filling the cavity of the adhesive organelle. This he regarded as conclusive evidence that the organelle acts by suction and he assumed that this suction is brought about by a deepening of the cup. He was, however, unable to explain how this deepening is effected. He realised that, as the skeleton is the most rigid structure in the body, it must act as origin and not as insertion of any contractile fibrils attached to it so that any tension produced by their contraction would have no effect on it. The best suggestion he could make was that the increased concavity of the disc was due to a general contraction of the endoplasm.

There is general agreement as to the reality of a sucking action. Fauré-Fremiet et Thaureaux 1944 for urceolarians in general and Reynoldson 1950 for *U. mitra* have noted the great difficulty of removing these ciliates from a glass slide by means of a pipette; they can be easily displaced but not detached. At such times, however, the "sucking disc", so far from being deeply concave, is flattened against the substratum. In my own preparations of individuals fixed while adhering to the surface of a cover glass, as one focusses the objective down into the cup, the central part of the adhesive disc comes into view almost at the same time as the periphery or sometimes even slightly sooner, showing that the disc is as flat as possible or even very slightly convex. Various authors, including Doflein in his "Lehrbuch", have given pictures showing the ciliates on the bodies of their hosts and all show them in this flattened condition. Diagrams of Reynoldson 1950 showing *U. mitra* in various activities (swimming freely, gliding over

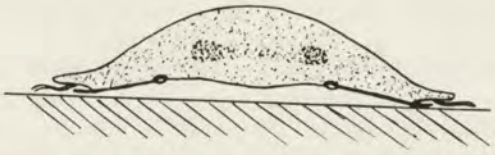

Fig. 5. *Trichodina* sp. Diagrammatic section showing form of body and attitude of skeletal element when gliding over a smooth surface: "sucking" position

the surface of the host and feeding while attached to the host) are especially instructive for they show that it is during swimming that the adhesive organ is most contracted and during gliding that it is most spread out. The ratio of length to width is shown as about 1.25 during swimming, 1.5 during feeding and 0.3 during gliding. Fauré-Fremiet et Gauchery 1956 have demonstrated the presence of longitudinal fibrils which they name "myoides rétracteurs de la cupule adhésive". Following Zick, it is difficult to understand how such fibrils could move the skeleton of the disc. On the contrary, it seems likely that their contraction pulls the adoral end of the animal down towards the disc and in doing so increases the hydrostatic pressure inside the animal causing the disc to spread out i.e. they make it flatter instead of deeper.

The gripping action observed by Zick and highly developed in *T. xenopodos* and in the fish gill parasites requires a different mechanism. Here

certainly the adhesive organelle is strongly concave and, in addition, its border membrane is turned sharply inwards so as to bite, like sharp teeth, into the host's tissue. (Fig. 4 C). How is this condition brought about? We have concluded that the longitudinal cytoplasmic fibrils are not competent to deform the skeleton in this way but no other contractile elements suitable for the purpose were found by Favard, Carasso et Fauré-Fremiet 1963 in their exhaustive electron-microscope studies of urecolarians. On the other hand the ciliary mechanism of the disc appears to be much more elaborate than would be required for ordinary locomotion. Indeed MacLennan 1939 considered that in *C. domerguei* the principal ring of cilia (which he regarded as membranellae) plays little part in swimming, but comes into action when the ciliate is in contact with its host and then, owing to the diagonal setting of their bases (an arrangement described by Davis 1947 as being like the teeth of a worm gear wheel) their thrust both turns the body in a counter-clockwise sense (as viewed from the adoral end) and holds it against the skin. By similar reasoning we can believe that, on a soft internal epithelial surface, this action would not merely hold the animal against the substratum but would press the margin of the disc down among the cells. Furthermore, the arrangement of the cilia suggests that their action would have a centripetal component which would tend to constrict the margin of the disc bringing it into the attitude required for gripping. If we are correct in concluding that the arching of the peripheral parts of the skeleton of the disc is brought about by action of the cilia, it may be that the intimate association between the posterior ring of cilia and the radial pins, shown by Favard, Carasso et Fauré-Fremiet 1963, contributes to this action.

It is therefore suggested that the "neutral" condition of the adhesive organelle, in which its skeleton is unstressed, is that assumed during swimming; that when the organelle comes into contact with a suitable surface either the pressure caused by the shortening of the body pushes the margin of the organelle outwards causing the disc to become very flat or alternatively the action of the powerful cilia pushes this margin down into the substratum and at the same time inwards towards the main axis of the ciliate causing the disc to become concave and cup-like.

The border membrane by which the ciliate becomes anchored firmly to its host is almost entirely skeletal. Its cytoplasmic covering, revealed by the electron microscope studies of Favard, Carasso et Fauré-Fremiet 1963, is too fine to be visible with the light microscope and contains no contractile elements. This membrane is therefore incapable of any independent movements. Normally it appears as a continuation of the radial pins and is hinged to their distal ends. If now the curvature of the radial pins is such that the border membrane is inclined slightly inwards, then the pressure against the substratum will force it inwards still more so that it appears, in section, like a recurrent barb, hooked into the flesh of the host (Fig. 4 C). If, on the contrary, it is initially turned slightly outwards, then the pressure against the substratum will turn it outwards still more so that it lies flat against the surface, as it does during gliding (Fig. 5).

If the views expressed here are correct, they should help towards an understanding of the functional significance of the structure of the skeleton. In the sub-family *Urceolariinae* this consists, apart from the radial pins,

simply of a ring of imbricated units devoid of radial processes. Zick considered that these units can become, to some extent, more or less closely packed together giving the ring greater elasticity than a simple undivided ring would have. It is not clear how such compressibility of the ring would be of advantage to the animal. On the contrary, the effectiveness of the disc, depending, according to the present view, on forces acting at its periphery, is likely to be enhanced if its more central part is supported by an incompressible ring. The value of the articulated structure of this ring would come, not from its compressibility in its own plane, but from its flexibility perpendicular to this plane through which the adhesive organelle can become moulded to the contours of the substratum so as to have the uniformly close contact required for adhesion. The further role of the skeletal ring as origin of the contractile fibrils does not concern us here.

In the sub-family *Trichodininae* each unit of the skeletal ring has two long processes, a centripetal ray and a centrifugal blade. The former is narrow and, as shown by Lom 1958, has thickened margins which give it a girder-like form. It is consequently relatively inflexible and doubtless enhances the action of the ring in maintaining constancy of shape of the central part of the organelle. The blade is, on the contrary, broad, thin and very flexible, and this flexibility is increased by its narrow, hinge-like junction to the centre piece. Favard, Carasso et Fauré-Fremiet 1963 have shown that it is bound to the radial pins by fibrillar, ligament-like material. The resultant structure would be very elastic; it would become strongly arched under pressure from the marginal cilia but spring back into the neutral position when the marginal forces are relaxed. It is this arching of the blade-plus-radial-pin complex which brings the border membrane into the inturned position from which it can be forced into the gripping position.

Evolution and adaptation in the *Urceolariidae*

Raabe 1963 in his recent review of the systematics of this family, suggests that it originated from a permanently telotrochoid peritrich having a scopula surrounded by a ring of cilia and that the sub-families *Urceolariinae* and *Trichodininae* diverged at a very early stage in their evolutionary histories. We may believe that the common ancestral form was unspecialised in relation to its animate or inanimate substratum, and that evolution has involved an obligate dependence upon a particular type of host (though not necessarily a detailed host-specificity). Reynoldson 1955, for instance, has shown the close dependence of *U. mitra* on its planarian host and how it moves about over the surface of its host's body to the position most favourable for catching the minute organisms on which it feeds. Such a life requires ability to glide about easily over the substratum and at the same time to resist being swept away by the water currents upon which its feeding depends. These requirements are admirably met by the "sucking" mechanism described above, combining adhesion with mobility and depending for its effectiveness on the presence of the flexible but incompressible imbricated ring and on a suitable arrangement of the cilia.

These ciliates are harmless commensals, using their hosts merely as supports but not as direct sources of food. The *Trichodininae*, on the other hand, through the presence of denticles with rays and blades, have acquired the

ability to grip the host's tissues by means of the border membrane and have thus become, potentially at any rate, tissue-destroying parasites.

Raabe notes further that in this group endoparasitism is generally associated with an increase in the number of units in the skeletal ring and that this "is, no doubt, in connection with the plasticity of the sucker which may cover more efficiently areas of the intestinal wall of the host". This accords with the suggestion made above since the internal surfaces are more irregular than the smooth outer ones and close contact with them accordingly requires a more flexible skeletal ring.

Again Raabe points out that, except in the genus *Semitrichodina*, reduction of the adoral spiral is always associated with a simplification of the skeleton. *T. xenopodos* is an exception to this rule, but this is a moderately large species whereas, according to Raabe, the simplification of the skeleton is probably connected primarily with reduced body size, the correlation with the length of the adoral spiral being secondary. Evolutionary adaptation of animals to extreme conditions not infrequently affects a particular organ in two opposite ways. In some species the organ may become bigger in order to maintain its old function under more difficult conditions, while in others it may become smaller as a new function replaces the old. So, among the urceolarians, adaptation to endoparasitic life has led in some genera to an extension of the food gathering mechanism to compensate for the sparser food supplies while in others the acquisition of new feeding habits, such as the substitution of liquid for solid food, has led to the reduction of this mechanism. It has been suggested that in some of the endoparasitic members of the family food may be taken through the surface of the adhesive disc, but I can find no evidence of either food vacuoles or pinocytotic vesicles in this region and it seems more probable that the liquid food is taken in by the normal route i.e. by the cytostome. For this purpose a long adoral zone would be unnecessary.

Life history

Multiplication of *T. xenopodos* takes place freely in the bladder of the host, for division stages have often been found. No indications of encystment have been found and nothing is known of the route by which infection takes place. A plausible assumption is that the ciliates first infect the gills of the tadpoles and that when the tadpoles metamorphose, the ciliates pass down the alimentary canal to the bladder in the same way as the trematode *Polystomum* which is often associated with them there. An alternate route is suggested by the observation of Fantham 1930 that, in fish, species of *Trichodina* found normally on the gills are sometimes present in the heart blood. Rather surprisingly, although *Xenopus* tadpoles in various stages of development were examined on a number of occasions, no trichodinas were ever found on their gills. It is unfortunate that other parts of these tadpoles were not examined, for Dr. Thurston has recently shown me a preparation of the tail of a *Xenopus* larva from Uganda which is heavily infected with trichodinas The species cannot be determined but it is tempting to assume that this probably represents the early stage of infection by *T. xenopodos.*

Trichodina sp.
from the gills of *Rana grayi* tadpoles

Probably the commonest of all amphibians in the neighbourhood of Cape Town is *Rana grayi*. Ecologically it differs from *Xenopus* in being associated with temporary rain pools rather than with more permanent bodies of water. During the search for trichodinas on the gills of *Xenopus* tadpoles, a parallel examination was made of adults and tadpoles of this frog, the tadpoles being taken at various dates between the end of August and mid-December and being in various stages of development from the first appearance of the fore legs to the beginning of metamorphosis. No ciliates were found in the bladders of any of the adults but the gills of the tadpoles were commonly heavily infected by a small species of *Trichodina*. When the *Rana* and *Xenopus* tadpoles were kept together in the same vessel, no transfer of the ciliates took place and it was noticed that, unlike *T. xenopodos*, the species from the *Rana* tadpoles died very quickly when removed from their host. Doubtless for this reason my preparations of isolated individuals all show some degree of maceration, sometimes only the naked skeleton remaining. Sections of infected gills were more successful from this point of view but, unfortunately, owing to the small size of the ciliates and the amount of mucus surrounding them, their structural details can be seen only very imperfectly. No valid description of this species can therefore be given but the following points may be recorded.

Overall diameter of body (22 specimens); 9 μ to 23 μ, average 18 μ.

Diameter of adhesive disc (17 specimens); 8 μ to 22 μ, average 16.4 μ.

Diameter of denticulate ring (17 specimens) 5 μ to 11 μ, average 8.8 μ.

Number of denticles; (25 specimens); 20 to 24, average 22.

Number of radial pins to each pair of denticles; about 6.

The detailed form of the denticles could not be determined. Even when the blades and middle pieces are strongly stained, the rays are usually completely invisible and even at the best they can only be vaguely discerned rather than clearly seen. There is, however, no doubt that they are present thus eliminating the possibility that the species might belong to either of the genera *Dogielina* or *Trichodinella* from which they are absent. Feeble development of the rays in such a small species is in accordance with the views of R a a b e mentioned above.

The shape of the body is more or less hemispherical, the sides and anterior end being rather uniformly rounded and the length never exceeding 0.8 of the maximum width.

No reliable observations could be made of the adoral zone or cytopharynx.

The macronucleus is relatively large and there is no micronucleus.

Apart from its smaller size, there is little in the foregoing description to distinguish this species from the amicronucleate members of the one found by D i l l e r 1928 on the tadpoles of various species of *Anura* in the neighbourhood of Philadelphia, U.S.A. In view of the great climatic differences between Philadelphia and Cape Town differences in size may be of no systematic significance.

D i l l e r describes the macronucleus of his species as having a heterogeneous structure, perhaps best described as pocketed; after Schaudinn fixation and Heidenhein staining numerous black granules of varying sizes and staining capacities appeared suspended in little clear pockets in a more faintly

staining, finely granular matrix. The macronucleus of the Cape Town species is very similar to this except that the granules are confined to the extreme periphery of the pockets or vacuoles which otherwise appear empty. The granules are especially numerous against the main nuclear membrane.

In view of the inadequacy of the morphological data, it seems best to follow the wise example of Diller and to refrain from naming this species.

Of the many hundreds of individuals seen in the gill sections, none are gripping the epithelium after the manner of *T. xenopodos* in its host's bladder. A few have their adhesive discs closely applied to the host's tissue but the majority are lying freely in the interstices of the gills, often surrounded by strands of mucus.

Trichodina oxystelis sp. n. from marine molluscs

Among the other species of urceolarians recorded but not named by Fantham 1930 is one from the foot and mantle of the littoral marine gastropod, *Oxystele impervia,* collected at St. James' near Cape Town. During my years there, I examined individuals of several species of *Oxystele* from this locality and found a trichodina commonly present on the species known to the Cape Town workers (e.g. Bokenham and Neugebauer 1938) as *O. variegata* Anton. Mr. N. Tebble of the British Museum (Natural History) has kindly informed me that the correct name of Fantham's *O. impervia* is *Diloma* (*Oxystele*) *sagittifera* (Lamarck) and that *O. variegata* is a variety of the same species. There is no indication that the St. James' population comprises more than one variety of this species and consequently it seems certain that Fantham and I were examining the same host. Nevertheless, whereas he describes the trichodina found by him as "a large *Trichodina*... its maximum diameter being from 50 μ to 67 μ with a disc about 40 μ in diameter", my own specimens are all much smaller, the greatest diameter, after fixation, ranging from 25 μ to 48 μ with a mean value of 38.3 μ. The adhesive disc is of the same relative size in the two cases, being, in my specimens, between 0.64 and 0.88 (mean 0.76) of the overall diameter of the ciliate though in one case it is as low as 0.44. In fixed specimens the length of the body varies between 0.40 and 0.83 of the maximum width, but among living, freely-swimming individuals some extremely short ones were seen in which, from a freehand sketch made at the time, the length could not have exceeded one eight of the width. A similar very short individual of *T. domerguei* is figured by Precht 1935.

The difference in size between Fantham's specimens and mine cannot be attributed to season for his were collected during January and February 1930 whereas my own preparations, from which these measurements were made, are dated 5th March, 1940. On that occasion 7 of the 11 host individuals examined were infected. Similar infections were observed at other times but large individuals such as those described by Fantham were never seen. A species of *Scyphidia* was usually also present.

A similar, possibly identical, trichodina was found less frequently on *O. sinensis* (Gmelin) but never on *O. tigrina* (Dillwyn). This choice of hosts merits further investigation in view of the fact that ecologically *O. tigrina* is intermediate between the other two species, being confined to the middle

region of the inter-tidal zone whereas *O. sinensis* is limited to the lower region of this zone and *O. variegata* to the middle and upper regions. A larger urceolarian (diameter 38 μ to 67 μ) was found on the amphineurans, *Dinoplax* and *Cryptoplax*, but in such small numbers that no adequate observations could be made.

The following notes refer to specimens from *O. variegata* (Fig. 6).

Denticulate ring

The diameter ranges from 10 μ to 28 μ (average 16.25 μ) and the number of denticles from 20 to 24 (modal number 21, average number 21.7). The shape of the individual denticles could not be ascertained clearly but the blades appear to be moderately broad and hooks can be seen in some cases.

Radial pins

These are about 6 to 7 times as numerous as the denticles.

Nuclei

The macronucleus is of the usual horse-shoe shape, the gap between the two ends being always ventral. A compact micronucleus is situated near its left-hand end (as viewed from the adoral end of the animal). The system adopted by Lom 1958, following Dogiel, for defining the position of the micronucleus is only applicable if the two nuclei lie in more or less the same

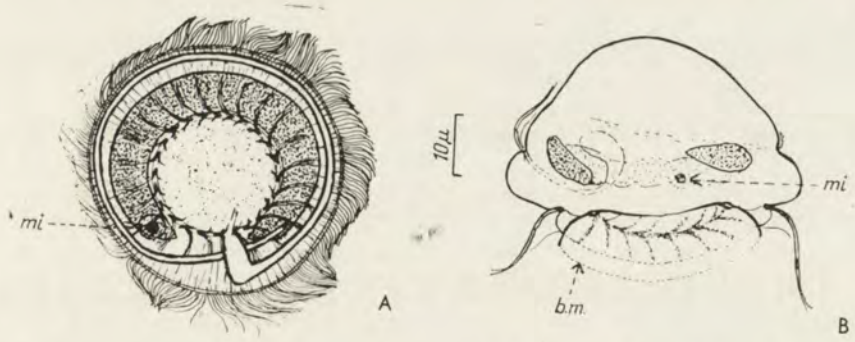

Fig. 6. *T. oxystelis* sp. n. A. Aboral view. Adoral groove shown but not its ciliature (Bouin, Cole's haematoxylin). B. Optical section with part of the denticulate ring shown on bottom focus (Bouin, haematoxylin)

plane. This is not the case here as the micronucleus is situated posterior to the macronucleus and when viewed from above or below may appear either directly underneath its left arm or internal or external to this. Division stages are not uncommon.

Adoral groove

This is usually clearly visible, making rather more than one complete turn (about 450°) and having a diameter only slightly less than that of the animal. The terminal part curves closely round the right end of the macronucleus to form the cytopharynx.

Movements

In contrast to *T. xenopodos* whose swimming is slow, these ciliates dart about rapidly when removed from their host, so much so, indeed, that they recall the jumps of an *Halteria*. This suggested that they might belong to the genus *Cyclochaeta* in which an additional circle of especially strong cilia, reminiscent of the bristles of that genus, is present, but no such cilia could be found here.

Of the species previously recorded from marine molluscan hosts, *T. patellae* Cuénot has simple denticles without blades or hooks and has therefore been transferred to the genus *Urceolaria,* while *T. tegula* Hirshfield has a ring of aboral cilia and therefore belongs to the genus *Cyclochaeta*. Both of these also differ in size and in other particulars from the present species. The nearest resemblance is to *T. branchicola* Tripathy 1948 from the gills of marine fish from the English and French sides of the English Channel but, in view of the great differences in localities and hosts it seems most unlikely that the two species can be the same. The name *T. oxystelis* sp.n. is therefore proposed.

Deposition of types

The preparations on which the foregoing descriptions are based have been deposited at the British Museum (Natural History), South Kensington, London. Fantham gave the name *T. xenopodos* "for purposes of reference only" and, in view of the absence of any of his material or of any valid description based on it, it is recommended that the Cape Town specimens, described here, shall be considered typical of the species and that if further study should reveal constant differences between the Johannesburg and Cape Town populations, the former should be considered as of varietal status.

As no single specimen or preparation can show all the details of systematic importance, none is designated as the type of the species but the whole collection is to be regarded as constituting the syntype collections of the two species here described.

Acknowledgments

My cordial thanks are due to Dr. N. Patterson and Miss C. Weinbrenn of the University of the Witwatersrand for enabling me to consult and to quote from an unpublished thesis written by the latter; to Dr. J. P. Thurston of Makerere College, Kampala, Uganda for specimens of the trichodina from *Xenopus laevis* in that country; to Mr. N. Tebble of the British Museum for claryfying the names of the species of *Oxystele* and to Dr. E. Fauré-Fremiet for his valuable criticisms of my earlier and fallaceous ideas concerning the function of the urceolarian skeleton. I would also take this opportunity of expressing my thanks to Dr. N. A. H. Millard of the University of Cape Town not only for preparing the excellent sections used in this work but for much other assistance generously given during my years at that University.

Appendix

On the abuse of simile in morphological descriptions

In the foregoing paper mention has been made of the difficulty caused by Fantham's description of *T. xenopodos* as "vase or urn shaped". This case is not unique and, indeed, the urceolarians, perhaps as a result of their unusual aesthetic appeal, have evoked a remarkable number of imaginative but ambiguous and therefore meaningless comparisons. The following have come to my notice.

a shallow bowl or pot — *T. mugilis* — Fantham 1930;
a rather deep elegant vase — *T. blennii* — Fantham 1930;

a cup — *T. okajimae* — Ibara 1931;
a plate — *T. cardiorum* — Raabe. Z. and J. 1959;
a saucer — *C. domerguei* — Mac Lennan 1939;
saucers or bells — *Trichodinidae* — Davis 1947;
bell-shaped to discoidal — *T. myicola* — Uzmann and Stickney 1954;
a barrel — *T. urinicola* — Fulton 1923;
hemispheric to hat-shaped to flat — *T. urinicola* forma *taeniatus* — Lom 1958;
a flat hat — *T. unionis* — Hampl 1955;
a derby hat or turban; in the rarer expanded condition an hour-glas — *T. tegula* — Hirschfield 1949;
a turban — *T. bulbosa* — Davis, 1947; *T. spheroidesi* — Padnos and Nigrelli 1942;
an old-fashioned sun-bonnet — *T. bursiformis* — Davis 1947;
a loaf of bread — *T. sphaeronuclea* — Lom 1956;
a butterfly net — *U. synaptae* — Colwin 1944.

Descriptions intended to serve for the diagnosis of new species (or of other new taxa) differ from most scientific publications in that they cannot become obsolete. They must be unambiguous for all times and to workers in all countries. Most of the examples quoted above are valueless for two reasons and as a result any use that could have been made of body form as a diagnostic character is lost. In the first place they are not precise. Is there any real difference, and if so what is it between species described respectively as like a plate, a saucer or a flat hat or those likened to a bowl, a pot and a cup? In the second place few human artefacts have the necessary quality of invariability to convey the same meaning to people in all places and at all times. Indeed, the more universal an object (such as hats and loaves of bread) the more likely they are to differ widely in shape from place to place and from time to time. Can we imagine the difficulties of a student in, say, Africa or Asia who needs to know the shape of an old-fashioned sun-bonnet as worn in a particular part of the U.S.A. or of one in the 21st century who tries to know the particular type of hat (presumably men's, not ladies') worn in a particular country in the mid 20th century?

The need for precision becomes even more important in view of Jahn's 1962 reminder that the time is approaching when systematic data will be handled by computers. Computers can do many things but we can hardly expect them to use imagination in interpreting similes.

We rarely notice that even the most commonly used similes are only valid in so far as the conventions associated with them are recognised. For instance we often speak of things as being "bell-shaped" or "dome-shaped" but in fact there are many different shapes of bells and of domes. Indeed some bells are "dome--shaped" and some domes are "bell-shaped". Fortunately in these cases we all know the conventions. But what about "saddle-shaped" which appears in many books on Zoology? Already a large proportion of zoologists have never seen a horse's saddle at close quarters and would think more readily of the saddle of a motor cycle. Natural objects are more consistent than human artefacts and in this case we could say "like the articular surface of a bird's cervical vertebra". That would be unambiguous, but is not quite the kind of statement that could be fed conveniently into a computer.

Ideally, therefore, all definitions of form should be given in mathematical terms, but here we have the opposite danger of implying too great precision. Thus the information given by Thompson 1917 that in *T. pediculus*, when the upper surface is a plane the equatorial groove is a catenoid surface of rotation and when it is depressed this tends to a nodoidal form would be inapproriate in a definition of this species because, even if we could assume that protozoologists all know the characteristics of catenoids and nodoids and that their specimens permitted the making of the relevant measurements, it is almost certain that no given specimen would in fact conform exactly to the definition.

It is suggested that the following vocabulary of simple geometrical terms should be adequate for the description of the the body form of any species of urceolarian and that its use would permit meaningful comparison between the descriptions of different authors. For use with other groups of ciliates a few additional terms would be required:

sphere, hemisphere, cylinder, cone;

elongated, shortened, compressed, depressed, truncated;
base, apex, margin;
straight, concave, convex, sigmoid, parallel, spiral, oblique;
out-turned, in-turned.

Two further points may be noted here. The first is that, while for some purposes independent sets of measurements of the various dimensions or structures are useful, for defining form it is the ratios of these measurements to some standard dimensions which are most important. Separate data concerning lengths and widths tell us little when we really need to know the limiting and modal values of the ratio of length:width.

Finally, it is not only in morphological descriptions that the motto "for all places and for all times" is important. Few experiences are more frustrating than the attempt to follow an author's technique when, in his account of it, he refers to articles or materials by names which may be household words in his own community but are unknown elsewhere and which cannot be found in dictionaries or other standard reference books. If the use of trade names is necessary they should always be accompanied by explanatory notes or by the address of their manufacturers.

As lectures we are trained to make our words audible to the people at the remotest part of the hall. As writers we have the corresponding obligation to make our writing intelligible to readers remote from us in space, time, language and cultural traditions. The custom of publishing research papers in the same form as that in which they were originally presented to one's immediate colleagues often leads to a neglect of this obligation and to unexpected confusions.

Summary

The species of *Trichodina* common in the bladder of *Xenopus laevis* in the region of Cape Town is described. It differs from *T. xenopodos* as originally described by Fantham in having rather fewer denticles and no micronucleus, but reasons are given for considering the species as identical and, in view of the inadequate and provisional nature of the original description of *T. xenopodos*, it is recommended that the material here described shall be considered as type material of this species.

Division stages are found frequently in the bladders of the hosts, but no other reproductive stages have been found and the mode of infection is unknown. None of the ciliates were found on the gills of *Xenopus* tadpoles, but more recently some have been found on the tails of these tadpoles in Uganda.

The method by which *T. xenopodos* adheres to its host is described and suggestions are made as to the functional significance of the various components of the urceolarian skeleton.

No trichodinas were found in the bladders of *Rana grayi* but a species was abundant on the gills of its tadpoles which, except for their smaller size, resembled closely the amicronucleate members of the species described by Diller from tadpoles from Philadelphia.

A new species, *T. oxystelis* sp.n., is described from the marine littoral mollusc *Diloma* (*Oxystele*) *sagittifera* (Lamarck).

In the appendix attention is drawn to the impossibility of comparing the body forms of the different species of urceolarians owing to the common habit of defining these by comparison with objects which are themselves usually very variable in form. The need for taxonomic units to be defined in terms which are, and will always remain, unambiguous to workers in all parts of the world is stressed and a basic vocabulary for the purpose is suggested.

RÉSUMÉ

L'espèce de *Trichodina* commune dans la vessie de *Xenopus laevis* dans la région du Cap est décrite. Elle diffère de *T. xenopodos* décrite par Fantham en ayant plutôt moins de denticules et pas de micronucleus, mais des raisons sont données pour considérer les deux espèces comme identiques et, en vue de l'insuffisante et provisoire nature de la description originelle de *T. xenopodos*, il est recommandé que le matériel décrit içi soit considéré comme le type de cette espèce.

Des individus en cours de division ont été trouvés assez fréquemment dans la vessie des hôtes, mais on n'a jamais trouvé d'autre phase réproductive et le mode d'infection est inconnu.

On n'a pas trouvé ces Ciliés sur les branchies des têtards de *Xenopus*, mais récemment quelques-uns ont été trouvés sur la queue des ces têtards en Uganda.

La methode par laquelle *T. xenopodos* adhère à son hôte est décrite et quelques suggestions sont faites par rapport à la significance fonctionnelle des parties varieés du squellette urcéolarien.

On n'a pas trouvé des trichodines dans la vessie des *Rana grayi* mais une espèce très abondante fut trouvée sur les branchies de leurs têtards qui, à part ses petites dimensions, ressemble aux membres sans micronoyaux de l'espèce décrite par Diller des têtards de Philadelphia.

Une nouvelle espèce, *Trichodina oxystelis* sp.n., est décrite, provenant du mollusque littoral marin, *Diloma (Oxystele) sagittifera* (Lamarck).

Dans un appendice l'auteur attire l'attention sur l'impossibilité de comparer les formes du corps des differentes espèces des urcéolariens à cause de l'habitude courante de les définir par comparaison avec des objets de forme variable. It souligne la necessité des définitions des unités taxonomiques en termes qui sont, et devront toujours rester, unambigues aux travailleurs du monde entier et suggère un vocabulaire élementaire servant dans ce but.

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Notes on the morphogenesis of the polar filament in *Henneguya* (*Protozoa, Cnidosporidia*)

Poznámky k morfogenesi polárního vlákna u *Henneguya* (*Protozoa, Cnidosporidia*)

In the previous paper (Lom and Vávra 1963) the structure of the polar filament was briefly mentioned in a ripe polar capsule of *Henneguya psorospermica* Thélohan. The aim of the present communication is to describe stages of development of this interesting formation. Whereas the electron microscopic observations corroborated the findings of first students of this protozoan group who, already in the past century, had observed the polar filament to be spirally coiled within the polar capsule, no detailed data were supplied by the light microscopy concerning the stages of development of this organelle. The only certain fact known, has been the occurrence, within the capsulogenic cells of the sporoblast, of a sphaerical formation — with or without tube-like projection — out of which the capsule with the threads evidently arises.

Material and methods

Developing plasmodia of *Henneguya psorospermica* Thélohan have been found repeatedly in the gills of perches (*Perca fluviatilis*) during the spring months. Pieces of heavily infected gill plates were fixed in Pallade's buffered fluid for 15—45 minutes, dehydrated in an acetone series, stained during dehydration by phosphotungstic acid and embedded into methacrylate (8 parts of butyl- to 2 parts of methylmethacrylate), and polymerized by UV light. Thin sections were cut on a Reichert's ultramicrotome OMU and examined in TESLA BS 242 and BS 413 electron microscopes.

Observations

The first stages of developing polar capsules present in our material appear within the capsulogenic cells of the sporoblast in the form of almost sphaerical formations, identical with the "vacuoles" observable in the light microscope. These ball-like bodies are provided with thick, electron dense walls, and they contain a mass of small electron dense granules scattered through an amorphous, electron transparent matrix.

After this sphaerical body has increased, so much that it fills up almost the entire volume of the capsulogenic cell, leaving but a thin sheath of plasm

around, the first coils of the polar filament appear within the capsular body. There is a strong evidence, that the formation of the filament begins at first in the tubular structure (to be described below) from which place it passes into the ball-like precapsular body (Pl. X 10). In the forming threads of the filament, the fine double membranes of its walls are clearly visible. Nine to eleven windings are formed within the ball-like body, and situate regularly beneath its walls.

Then the whole formation assumes the shape of a ripe polar capsule, on elongating. The inner electron transparent matrix turns into a heavily electron dense material. In a ripe capsule, the double membrane of the filament walls can never be so clearly resolved as in the beginning of its formation. The substance filling the lumen of the threads of a ripe capsule is no more so electron dense as in the first stages of the polar filament development.

In contrast to microsporidian filament, in myxosporidia it is not circular in cross section. Since the formation of its first rudiments myxosporidian filament is spirally twisted along its longitudinal axis; in cross section it strongly resembles the figure 8. In some cases, this twisting looks like two separate, mutually coiled filaments (see also Cheissin et al. 1961), since in the bridge of the figure 8 the filament walls are pressed together very closely, or are indistinct. This mode of twisting can be easily reproduced and understood by a model made by twisting an empty rubber tube. The significance of this peculiar structure of the inverted coiled filament within the polar capsule can be explained only by its function: the extruded filaments (it is commonly known that the myxosporidian filaments evert on extruding like a finger of a glove) can be straightened only if they are in the coiled state twisted as described above. Would they be, in their inverted state, circular in outline and not twisted, they would maintain their windings even after extrusion. The intracapsular pressure, however high it may be, would not be sufficient enough to straighten the relatively thick filament completely. In microsporidia, the extruding filaments often preserve the zig zag path, though they are much finer and their walls are extremely elastic (Lom and Vávra 1963 a).

The plasmogenic cells contain two kinds of mitochondria — one with typical tubuli mitochondriales and the other with atypical internal structure. Furthermore they contain peculiar tubular formations, irregularly coiled within the plasm. Judging from the number of cross and oblique sections within one capsulogenic cell it is of considerable length. It has a thick electron dense envelope, in some cases it reveals a sort of fine striation. Beneath this envelope a much finer membrane can be observed. The core consists of coarse granules, situated in what resembles to a tube like arrangement which, in cross sectioned tube, may give the false impression of a similar fibrillar arrangement as seen in cilia. This tube is passing over into the ball-like rudiments of the polar capsule. We are inclined to look for the origin of the balls just in these tubules for the following reasons: we see it to be connected with young, still small capsular rudiments, but not with those which have formed already the threads; further, the structure of the tubes is principally the same as that of the balls; and finally, the tubular formation disappears in ripening capsulogenic cells and cannot be seen around ripe polar capsules. It remains still an open question, how these tubules originate during the process of the differentiation of sporoblast within the capsulogenic cell.

Conclusive remarks

These data make possible a very interesting comparison. Slautterback and Fawcett 1959 confirmed by electron microscope observations the existence of a tubular formation participating in the development of a nematocyst of *Hydra*. This tubular formation has been observed already towards the end of the past century by light microscopists (Jeckeli 1882). Slautterback and Fawcett could prove that this tube originated from the Golgi apparatus, a question which in our material has not yet been solved. Slautterback 1963 observed slender microtubuli in nematocysts of *Hydra*; structures which can be compared with his finding can be observed in our material around the capsulogenic tube. Another case in which formation of extrudible organs of myxosporidia and coelenterates converge are the observations of Chapman and Tilney 1959 showing, that the polar filament in certain types of nematocysts of *Hydra* originates also from the intracapsular matrix.

Recently, proposals have been made to treat cnidosporidia and especially myxosporidia as belonging to *Mesozoa* (Grell 1956, Gottschalk 1959 etc.) rather than to the phylum *Protozoa*. Our observations seem to support such a view, but we must keep in mind that it may be a case of simple convergence, both functional and morphological, found so often in the animal kingdom. A thorough study of the whole cycle of vegetative stages must be done, before any more profound conclusions concerning the phylogenetic relations between coelenterates and myxosporidians could be drawn.

Summary

Electron microscope examination enables to follow the arising of ball-like formations — rudiments of the polar capsules, inside the capsulogenic cells of the sporoblast. The proper polar filament is formed within these rudiments and their tubular projections. The successive windings of the filament develope step by step. The ball-like rudiments of the polar capsules probably originate from a long tubular structure lying in the cytoplasm of the capsulogenic cells. The developmeent of the polar filament of *Henneguya* resembles strikingly its development in coelenterates.

SHRNUTÍ

V elektronovém mikroskopu můžeme pozorovat jak uvnitř kapsulogenních buněk sporoblastu vznikají kulovité útvary — "zárodky" polárních kapsulí. Shlukováním elektron-opakních granul roztrušených v jejich obsahu se tvoří vlastní polární vlákno. Jednotlivé závity vlákna vznikají postupně. Kulovité "zárodky" polárních kapsulí vznikají pravděpodobně zduřením dlouhého trubicovitého útvaru ležícího v plasmě kapsulogenních buněk. Vývoj polárního vlákna nápadně připomíná vývoj polárního vlákna u láčkovců.

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EXPLANATION OF PLATES I—X

1: Tubular formations within the capsulogenic cell, in oblique sections. In the lower left corner the ball-like body. W — wall of the tubular formation, M — matrix, G — coarse granules in the center. Notice the structural similarity of tubular formations and ball-like body

2: Tubular formations under high magnification in oblique section. Above them a badly preserved mitochondrium. I — inner layer of the wall of tubule

3: One end of the tubular structure, beginning its transformation into the ball-like precapsular body. C — the cell membrane of the capsulogenic cell

4: A section through the whole capsulogenic cell with a grown up ball-like precapsular body. T — cross sections of still persisting tubular formation, A — mitochondria of an uncommon, B — of a common type, S — future ridge of a half of the shell

5: A longitudinal section through the sporoblast in which both capsulogenic cells, and behind them the plasm of the germ, can be seen

6: The ball-like formation within which the first threads of the twisted fillament (F) are appearing. The shape of this precapsulogenic formation begins to elongate. The double membranes forming the wall of the filament are well visible in the enlarged inset of this photograph

7: A longitudinal section through two ripening capsules. In the left one, the filament is already formed in its whole length. The matrix is homogenous and clear, the content of the filament is electron dense. In the right one, only the first winding of the filament is formed, with well visible membranes of its walls

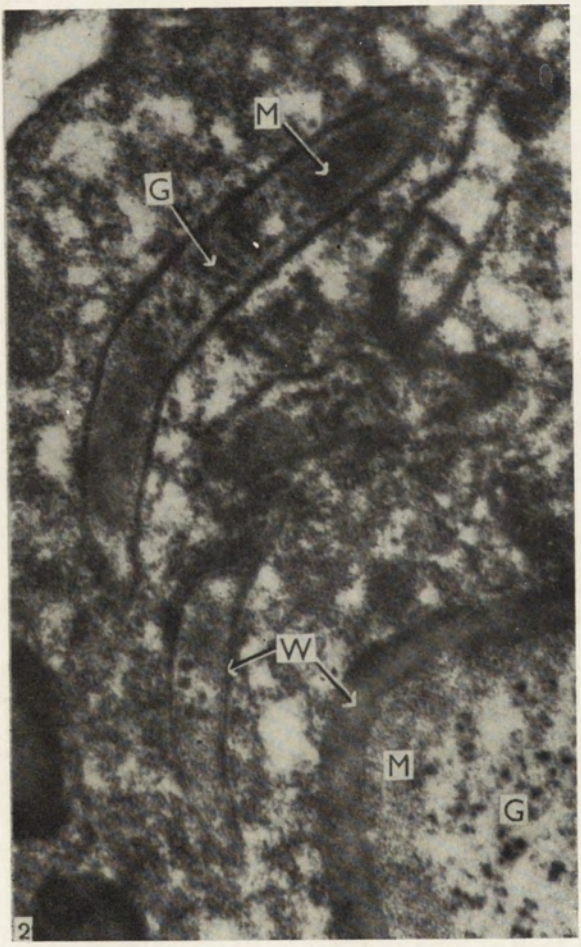
8: A longitudinal section of a ripe polar capsule. The matrix (M) is electron dense, much more than the content of the filament which become relatively electron transparent

9: A cross section through the tip of a ripe spore, still situated in the plasmodium in the gills. Note the clearly visible shell valves (S), and the relative thickness of the wall (W) of the capsules

10: Section through the capsulogenic cell of the sporoblast. A rather obliquely cut developing capsule is seen, joined with the tubular formation, of which the remaining part is out of section. The developing filament enters inside the capsule (F). Inside the capsule are still coarse granules G (35 0000×)

11: The developing filament (F) can be seen in two cross-sections of the tubular formation. The other part of this tube (T) is still without any filament, as well as the part of the tube, joining the capsule and the capsule itself (21 000 ×)

J. Lom et J. Vávra auctores phot.



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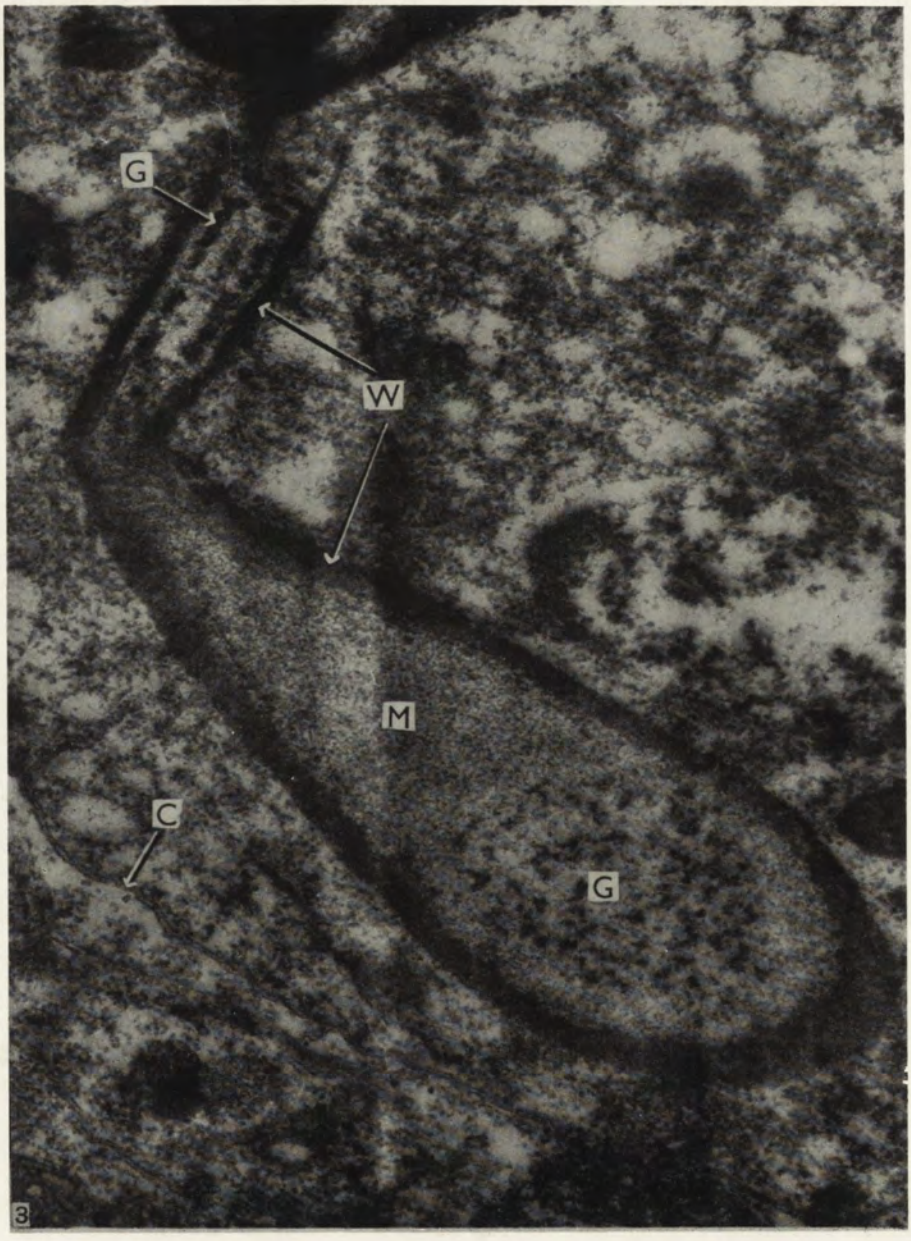

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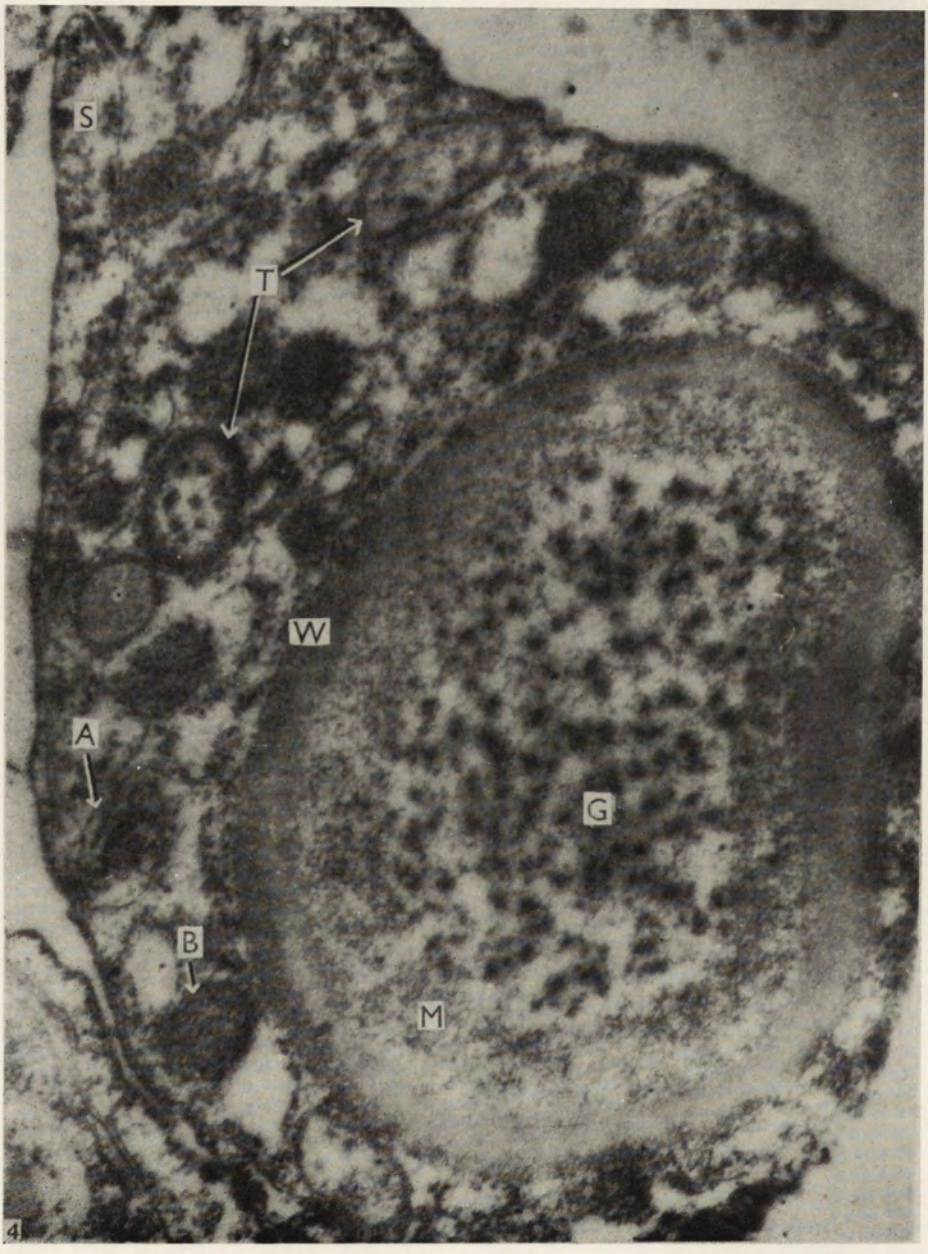

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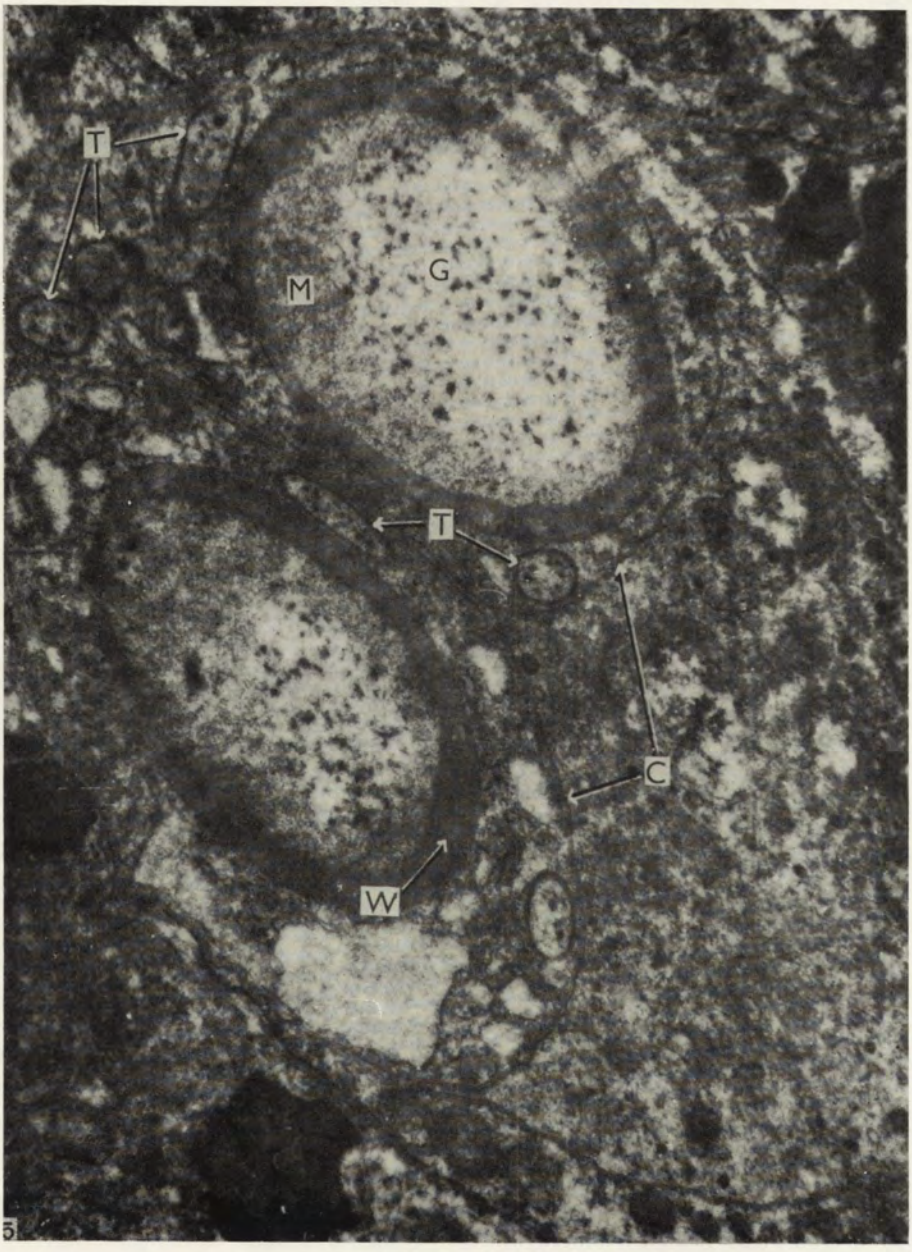

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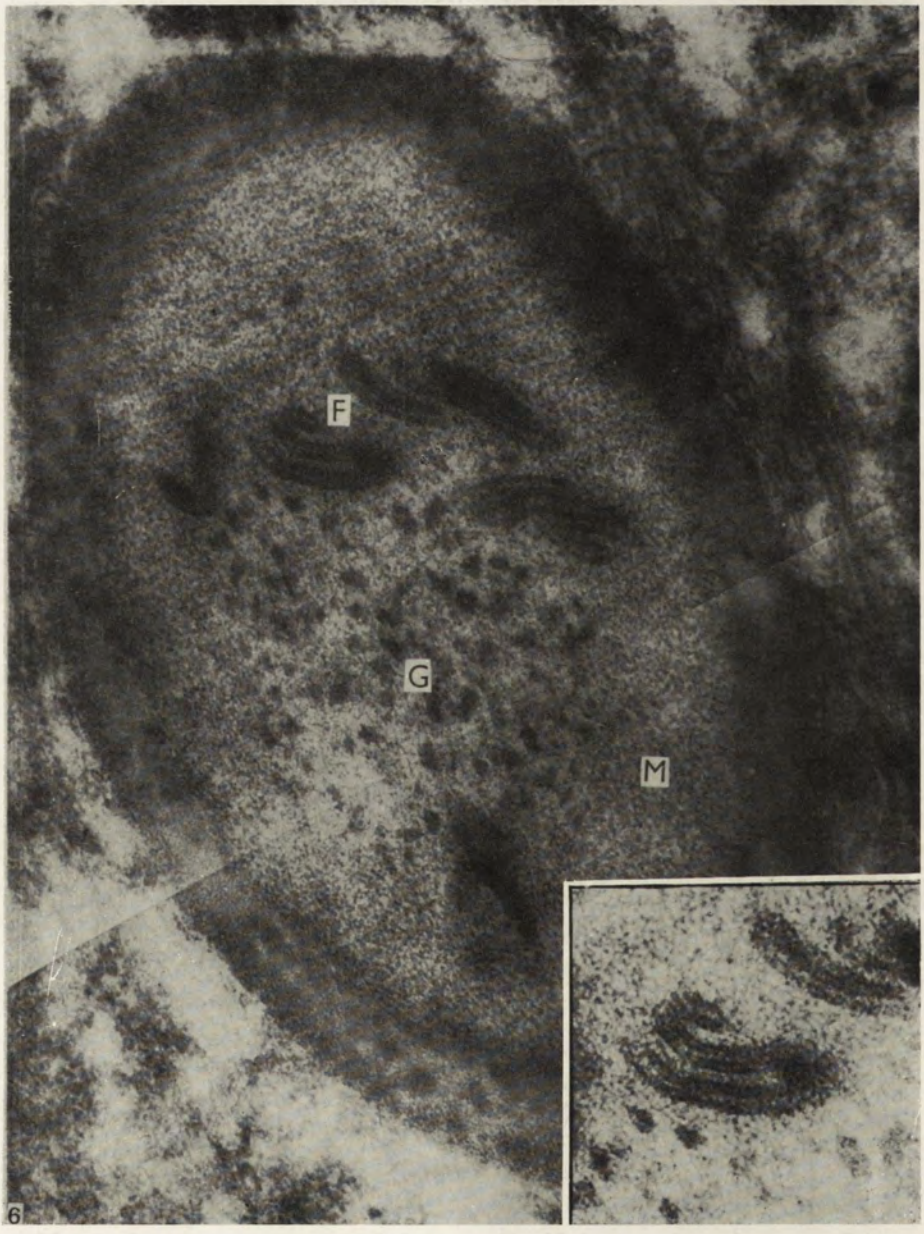

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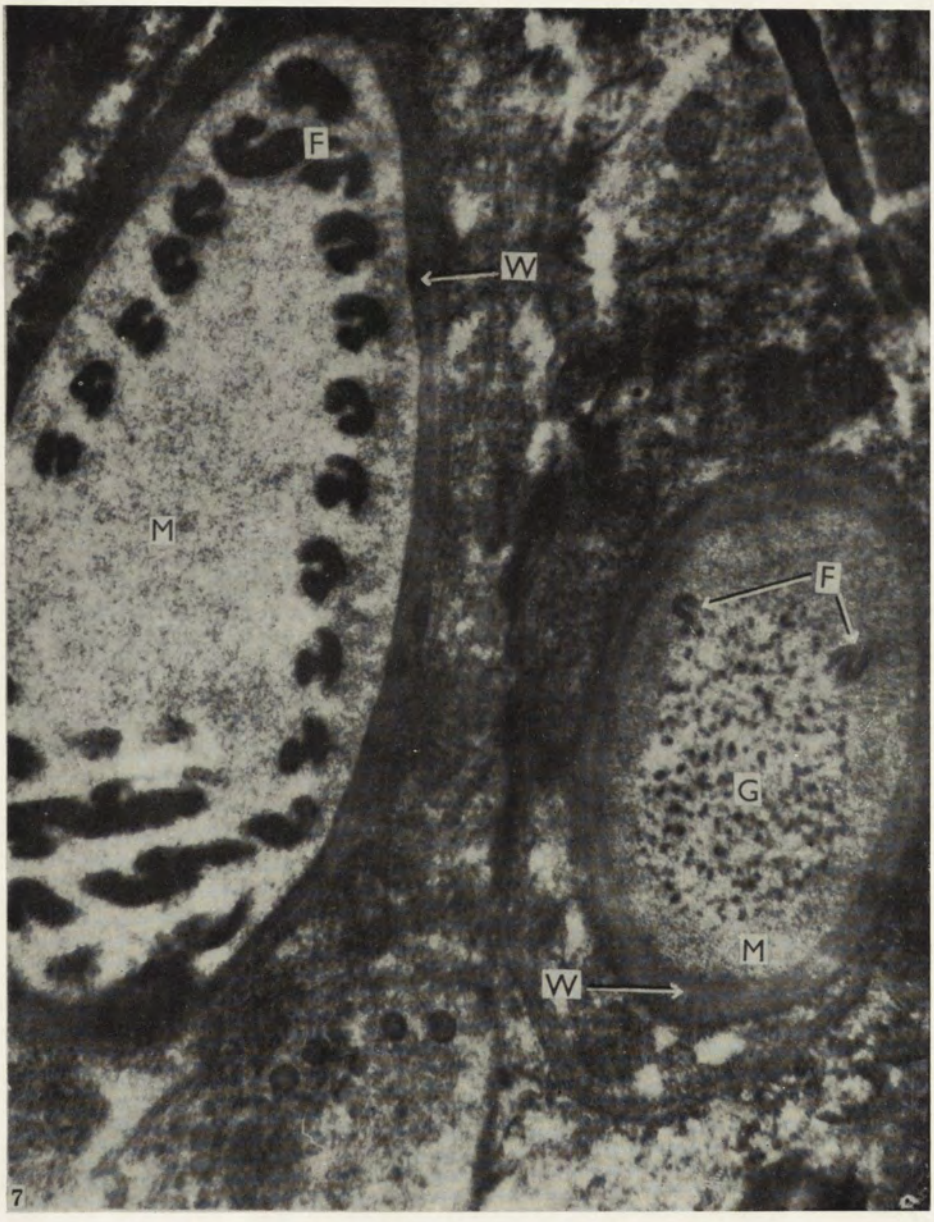

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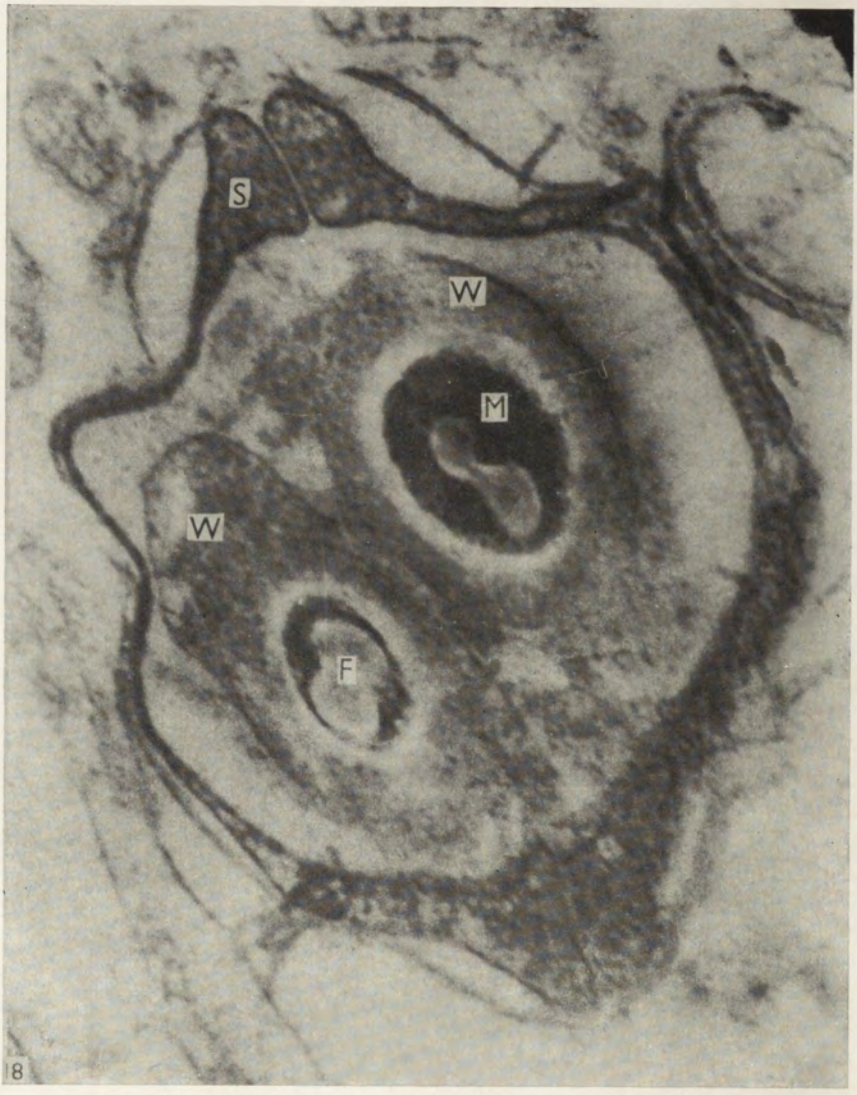

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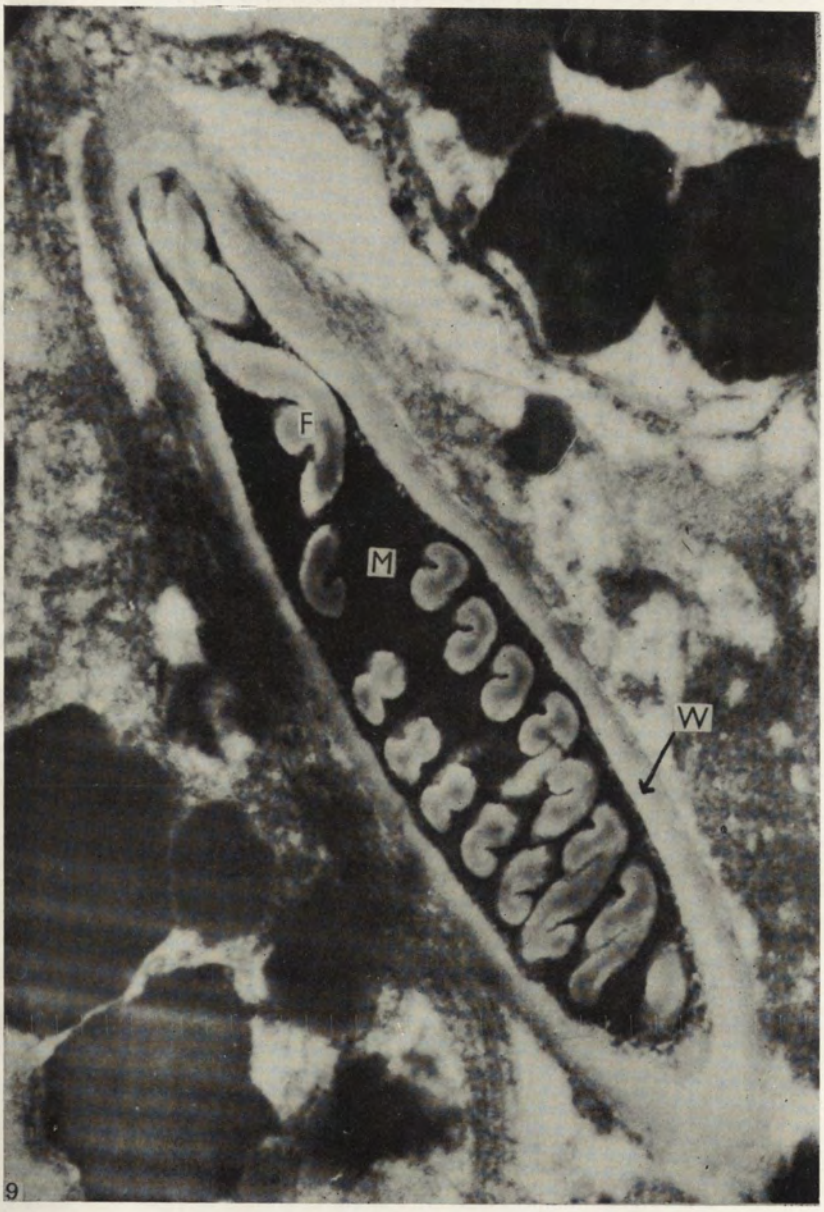

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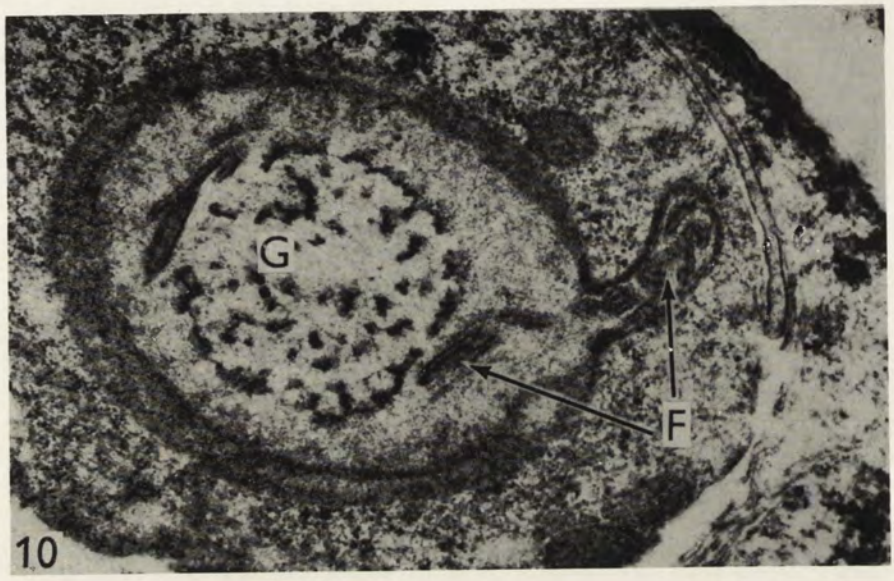

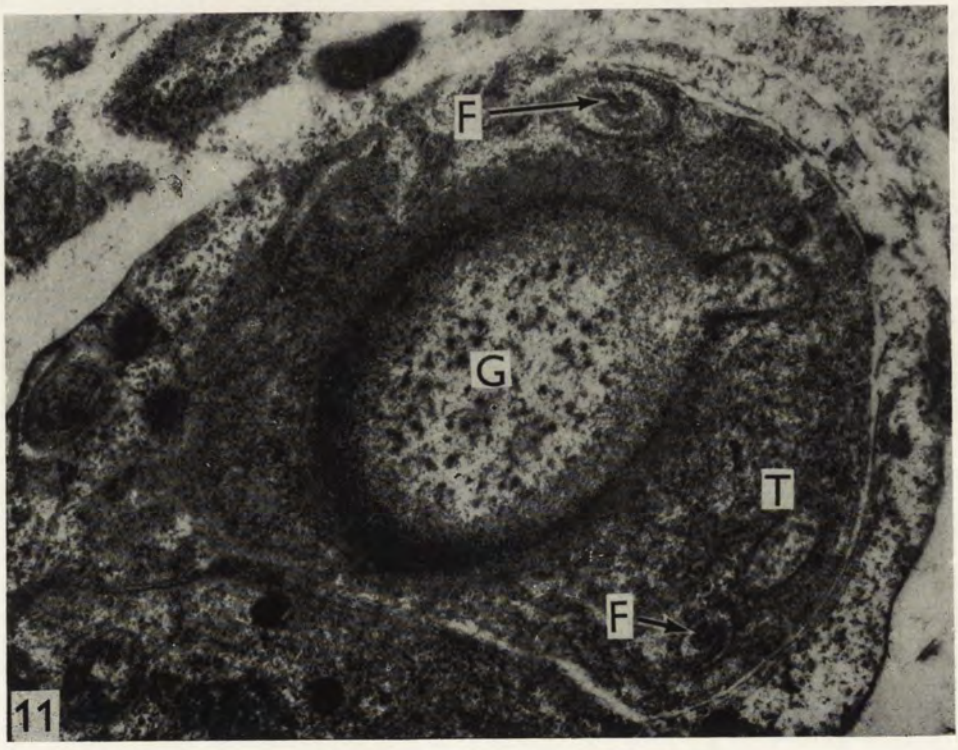

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Electron microscopic study of the structure of the contractile vacuole in the ciliate *Ichtyophthirius multifiliis* (Fouquet)

Электронномикроскопическое изучение строения сократительной вакуоли инфузории *Ichtyophthirius multifiliis* (Fouquet)

The comparative electron microscopic examination of different ciliates is one of the ways of elucidating some common regularities in the structure of their organelles in connection with the function performed by them. In this regard the study of the structure of the contractile vacuole — as the organelle sharing in the process of secretion and osmoregulation — is of great interest.

The ultrathin structure of the contractile vacuole has been investigated by different authors in various ciliates (Grassé 1952, Rudzińska 1958, Fauré-Fremiet et Rouiller 1959, Puytorac 1960, Schneider 1960). As result of those investigations some common features were stated as well of the structure of the vacuole itself, as of its discharge and injection canals, and also of the cytoplasm surrounding the vacuole. On the ground of data gained by means of electron microscopic method different views were expressed concerning the mechanism of the contraction of the vacuole and the role of its canals in the water transport. Nevertheless those statements are based on a scarce material because the electron microscopic study of the contractile vacuole embraced only a small number of representatives of ciliates. Among them, mostly the free-living fresh-water ciliates and some representatives of *Astomata*, parasites of the *Oligochaeta* intestine, were studied. It seems interesting to investigate the contractile vacuole in others, not yet examined ciliates, to gain more facts for elucidating the mechanism of their function.

For this purpose the ciliate *Ichthyophthirius multifiliis* has been chosen, a parasite of different species of fishes, characterized by a great number of slowly pulsating contractile vacuoles.

Material and methods

The adult form of the ciliate *Ichthyophthirius multifiliis*, shortly before encystment, was the object of study. For the electron microscopy the material was fixed in 2% solution of OsO_4 in the veronal-acetate buffer at pH 7.4. When passing the material through alcohols, contrasting the objects in 2% solution of phosphowolframic acid was performed. Ultrathin sections were

prepared on ultramicrotome UMT-2 and studied in the electron microscope JEM-5g at the acceleration voltage 80 kV and the 30 μ diameter of the diaphragme aperture of the lens.

For the light microscope study whole mount preparations were executed applying the method of Chatton and Lwoff, modified by Corliss 1953 for silver impregnation of the surface structures of the ciliate.

Results

The trophont of *Ichthyophthirius multifiliis* has a great number of contractile vacuoles scattered over the whole body, closely beneath the pellicle. In a silver impregnated whole mount, examined in the light miscroscope, the round excretory pores of the contractile vacuoles between the longitudinal rows of basal granules of cilia are distinctly seen (Pl. I 1). The pore is round, 0.8 — 1 μ in diameter. No details of its structures can be discerned in the light optics.

The electron micrographs of tangential sections of the ciliate reveal that the wall of contractile vacuole pores has a fibrillar structure (Pl. II 3). The longitudinal sections show that the pore is the outlet of the discharge canal connecting the contracting vacuole with the external medium. The discharge canal is a permanent structure of the contractile vacuole, produced by the invagination of the inner layer of the pellicle (Pl. I 2). The length of the discharge canal is about 1 μ. Its wall consists of tubular ring fibrils (Pl. III 4—5) disposed in one row. It can hardly be stated whether the wall of each canal consists of numerous ring fibrils or the cross sections of fibrils seen in longitudinal section of the canal are really one fibril twisted as a spiral. Their diameter is about 200 Å. Radial fibrils initiate at the wall of the proximal segment of the discharge canal, run in the surrounding cytoplasm and terminate closely at the wall of the contractile vacuole (Pl. III 4). Their structure is the same as that of the fibrils of the canal.

A double membrane separates the vacuole cavity from that of the discharge canal (Pl. I 2, III 4). The wall of the central receptacle of the contractile vacuole is coated by a membrane. On the outer surface of the membrane lies a row of tubular fibrils disposed in rows and oriented downwards (Pl. II 3, IV 6). Those fibrils, as well as the fibrils of the discharge canal, show a tubular structure with a light core and a dense outer layer. Their diameter is 160—200 Å.

In diastole condition the contractile vacuole assumes the shape of a round vesicle with the diameter approx. 3.5 μ with an even smooth wall, and in systole — of a multilobular figure with a great number of processes issuing into the cytoplasm (Pl. IV 6). This feature of the vacuole in systole suggests the supposition that, in the process of discharging the liquid, the wall collapses keeping the same thickeness as in diastole. In course of filling with liquid, the vacuole wall gradually assumes its normal shape but it fails to distend.

In the direct contact with the contractile vacuole, in the cytoplasm of *Ichthyophthirius multifiliis*, numerous very thin injection canals were detected, especially well distinguishing in condition of systole (Pl. IV 7, V 8). The diameter of these canals amounts about 800 Å. They are seen in the light microscope. These canals adhere with their one end to the wall of the

contractile vacuole (Pl. V 9). Although in the material under study the communication between the lumen of the canals and the vacuole cavity was never stated, there is no reason to doubt that the content of canals is poured out into the vacuole cavity. The canal wall is constructed of tubular fibrils of the same kind as those which form the wall of the receptacle of the contractile vacuole (Pl. IV 7). Around the injection canals a great number of tortuous tubules of the endoplasmic reticulum are disposed with multitude of Pallade's granules on the external side of their membranes. Those tubules of the endoplasmic reticulum distinguish this region of cytoplasm as a differentiated zone. Among the multitude of tubules, vesicles are located; osmophilic granules are demonstrable on their membranes. Some micrographs show that those tubules of the endoplasmic reticulum open directly to the injection canals (Pl. V 8). A great number of mitochondria are present in cytoplasm around the injection canals and around the receptacle of the contractile vacuole. In some cases, the contact of mitochondria with the wall of the contractile vacuole is observed.

Discussion

After having compared the ultrathin structure of the contractile vacuole of the ciliate *Ichthyophthirius multifiliis* with the vacuoles in other ciliates, it was revealed that despite a similar general pattern of the structure, some specific organization characters of the contractile vacuole exist in this species.

As pointed out by Pitelka 1963, one of the common features of organization of the ciliates with contractile vacuole, is the presence of a cytoplasm zone around the vacuole, called the canalicular-vesicular zone. This zone is especially well developed in some *Peritricha*, e.g. in *Campanella* and *Ophrydium* (Fauré-Fremiet et Rouiller 1958) and in some *Astomata* (de Puytorac 1961). The specific structural elements of this zone are tubules and vesicles. This zone formes a 20 — 200 μ thick layer around the contractile vacuole. It has been stated in all cases that the vesicles and tubules of the zone described open into the contractile vacuole or into the injection canals. The degree of development of the canalicular-vesicular zone varies considerably in different species which were examined with this respect. In *Tokophrya infusionum* (Rudzińska 1958) the number of vesicles and tubules is not very high. When describing the canalicular-vesicular cytoplasm zone of ciliates, the authors consider its structural elements — vesicles and canalicles — as specific structures different from the elements of the endoplasmic reticulum. Simultaneously the direct connection of vesicles and canalicles of the zone with the canals of the endoplasmic reticulum has been stated (Pitelka 1963).

In examination of the ciliate *Ichthyophthirius multifiliis*, a dense network of tubules 150—200 Å in diameter (Pl. IV 7) was demonstrated in cytoplasm near the contractile vacuole and injection canals. In this region of cytoplasm rounded vesicles 0.1 — 0.16 μ in diameter were found as well, yet their number being much lower when compared with the number of tubules. The presence of those structural elements in the cytoplasm of *Ichthyophthirius multifiliis* near the vacuole proves the existence of the canalicular-vesicular zone in this ciliate. Nevertheless, the analysis of micrographs failed to reveal any difference between the tubules and vesicles of this zone and the elements

of the endoplasmic reticulum. The membranes of those vesicles and tubules are rough, i.e. they bear the Pallade's granules. Detection of any tubules and vesicles deprived of those granules was impossible. Consequently, there is reason to assume that in *Ichthyophthirius multifiliis* the canalicular-vesicular zone presents a special perivacuolar cytoplasm zone, distinguished by a high development of tubules of the endoplasmic reticulum.

As to its function, this zone is a draining system promoting the inflow of liquid into the injection canals of the contractile vacuole (Pl. IV 7, V 8). Evidently this canalicular-vesicular zone corresponds to the "Nephridialplasma" described by Gelei 1925 a and b, in his study of protozoa in light microscope. He ascribed to it great importance in the process of excretion. Nassonov 1924 applying the osmification method in the study of several ciliates (*Campanella, Lionotus, Nassula, Paramecium*) demonstrated an osmiophilic structure around the contractile vacuole, which he considered homologous to the Golgi apparatus of *Metazoa*. In such forms in which the injection canals exist (e.g. in *Paramecium*) this osmiophilic "muff" surrounds them entirely. The data from electron microscopy concerning the structure of the perivacuolar cytoplasm zone — including the findings of the present study — allow to postulate that the osmiophilic "muff" around the contractile vacuole and its injection canals, described by Nassonov and Gelei is identical with the canalicular-vesicular zone. Osmification of the cytoplasm region around the contractile vacuole is evoked by the concentration of membranous structures in this zone which reduces the osmium tetroxide. Nevertheless, these membranous structures are not identical with the ultrastructures specific for the Golgi complex but essentially different from them.

As pointed out above, the wall of the contractile vacuole in *Ichthyophthirius multifiliis* consists of a membrane with fibrils adhering to it. Each of the fibrils has a complex structure and — according the results of Rudzińska 1958 represents a periodic protein structure. Their functional role is a problem of great interest, connected with the attempts to elucidate the mechanism of discharging the vacuole. Schneider 1960 suggests that the system of tubular fibrils in the wall of the central receptacle of the contractile vacuole of *Paramecium caudatum* and *P. aurelia* induces the contraction of its wall in systole. Consequently, he postulates the contractile function of these fibrils. Nevertheless, the solution of the problem cannot be accepted definitely since the tubular fibrils are often characteristic of the ultrastructure of such organelles in which the contractile function has not been documented (e.g. the medial body of *Lamblia* — Cheissin 1964, rods of the pharynx in *Nassula* — Rouiller, Fauré-Fremiet and Gauchery 1956, and others). So it is difficult to decide whether those fibrils are supporting elements or are able to contract. An indirect evidence that in *Ichthyophthirius multifiliis* they are not contractile, is the fact of collapse of the contractile vacuole wall in systole and formation of numerous folds. If the fibrils were apt to contraction it should be presumed that the contractile vacuole wall in systole would be a vesicle with a small diameter and a more thick wall. It seems more probable that the fibrils of the contractile vacuole wall in *Ichthyophthirius multifiliis* have a certain elasticity and promote the expansion of the vacuole in the moment of diastole. Evidently, the continuous filling the vacuole with liquid, by means of the injection canals, continues till the distension of the vacuole wall is possible. The limit

of this distension is determined by the elasticity of fibrils. The subsequent passing of the liquid into the vacuole — meeting a considerable resistence of the wall — evokes evacuation of the content through the discharge canal.

As to the mechanism of contraction of the vacuole and of the role of the surrounding cytoplasm in this process, another view was expresed by Bairati and Lehmann 1956, who demonstrated a dense network of fibrils in the cytoplasm of *Amoeba proteus* around the contractile vacuole. The authors suggested that the contraction of these fibrils results in the systole of the vacuole. No fibrils similar to those described by Bairati and Lehmann were found in *Ichthyophthirius multifiliis* in the course of the present study.

The tubular fibrils in the discharge canal wall are possibly also supporting elements, being identical in their structure with the fibrils of the wall of the contractile vacuole and of the injection canals. As to the radial fibrils of the injection canals, their contractile function is suggested by their disposition. However, the structure of those fibrils is fully consistent with this of other tubular fibrils, contractile function of which is rather disputable; for that reason it seems to be too early to speak with conviction of the contractility of the radial fibrils.

In contrast to *Paramecium caudatum* and *P. aurelia*, the injection canals of the vacuole in *Ichthyophthirius mulifiliis* have also tubular fibrils as well as the walls of the vacuole receptacle. These canals join the receptacle without the presence of ampulla which is distinctly seen in *Paramecium* (Schneider 1960). In the wall of ampulla tubular fibrils are present; Schneider ascribes to them the contractile function. In *Ichthyophthirius multifiliis* the injection canals keep their constant form and fail to shrink when filled with the liquid from the canals of the endoplasmic reticulum. This fact makes the contractile function of these fibrils, forming their wall, doubtful.

The essential difficulty in explanation of the discharge mechanism of the contractile vacuole in *Ichthyophthirius multifiliis* consists in the presence of a double membrane separating the cavity of the contractile vacuole from the lumen of the discharge canal. This membrane is possibly the continuation of the cellular membrane. Any disruption of this membrane was in no case observed although a satisfactory number of sections of the vacuole were observed as well in diastole as in systole. A membrane of the same character, separating the cavity of the contractile vacuole from the lumen of the discharge canal, was found by Schneider 1960 in *Paramecium caudatum* and in *P. aurelia*. Schneider expressed the view that in systole this membrane breaks under the pressure of the liquid and between two contractions, is every time reconstructed. The absence of any disruption of the membrane breaks under the pressure of the liquid, and between two conthe hypothesis of Schneider. The question of evacuation of the contractile vacuole content in *Ichthyophthirius multifiliis* remains open, for the time being. However, since of the present study failed to demonstrate any structure which might be responsible for evacuation of the vacuole content, and elucidate its mechanism, the concept of Schneider seems to be the unique. Nevertheless, it seems difficult to postulate that the reconstruction of the membranous wall could occur in such a short time as that of the vacuolar cycle. In our opinion this problem requires further investigation.

Summary

The electron microscopic examination of the ciliate *Ichthyophthirius multifiliis* demonstrated that the contractile vacuole of this ciliate consists of a receptacle, of very thin injection canals and a discharge canal. The last one opens outside by a pore. Tubular fibrils, 200 — 250 Å in diameter, are present in the wall of the vacuole receptacle. In systole the receptacle shrinks forming finger-shaped processes. A great number of tubules and vesicles of the endoplasmic reticulum are located around the injection canals. The membranes of the tubules and vesicles are rough. The mechanism of the vacuole contraction is discussed. The possibility of a supporting function of the tubular fibrils of the receptacle and of injection canals is put forward.

РЕЗЮМЕ

Приводятся данные электронномикроскопического исследования инфузории *Ichtyophthirius multifiliis*. Показано, что сократительная вакуоль состоит из резервуара, очень тонких приводящих каналов и выводного канала, открывающегося порой. В стенке резервуара вакуоли и в стенке приводящих каналов имеются трубчатые фибриллы 200—250 Å в диаметре. Во время систолы резервуар спадается, образуя пальцевидные выросты. Вокруг приводящих каналов располагается большое количество трубочек и пузырьков эндоплазматической сети. Момбраны трубочек и пузырьков являются шероховатыми.. Обсуждается вопрос о механизме сокращения вакуоли. Высказывается предположение об опорном характере трубчатых фибрилл, составляющих стенку резервуара вакуоли и приводящих каналов.

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EXPLANATION OF PLATES I—V

1: Whole mount, silver impregnated preparation of the ciliate *Ichthyophthirius multifiliis* (×1500)

2: Longitudinal section of the contractile vacuole. DC — discharge canal, CV — contractile vacuole, M — mitochondria. Arrow indicates the membrane separating the discharge canal from the cavity of the contractile vacuole (×30 000)

3: Tangential section of the ciliate on the level of the pore. Ring fibrils forming the wall of the pore are distinctly seen. P — apperture of the pore, M — mitochondria (×50 000)

4: Longitudinal section of the discharge canal of the contractile vacuole. F — ring fibrils in the wall of the discharge canal, RF — radial fibrils initiating at the wall of the discharge canal, CV — contractile vacuole. Arrow indicates the membrane separating the vacuole from the discharge canal (×30 000)

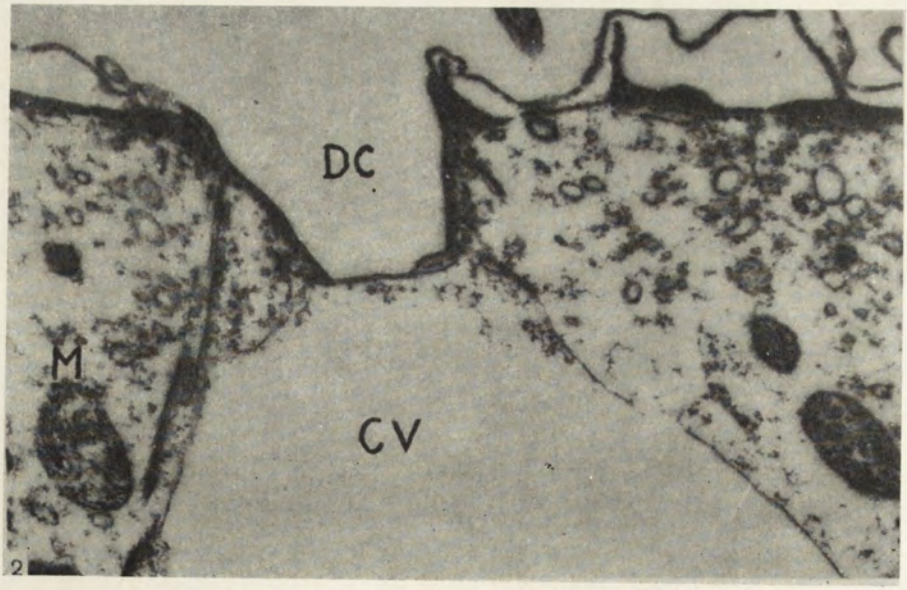
5: Section of the wall of the discharge canal in high magnification. In the canal wall, ring fibrils are distinctly seen (×120 000)

6: Tangential section of the contractile vacuole in systole. The shrunk wall of the vacuole, with fibrillar structure is distinctly seen. DC — discharge canal, CV — contractile vacuole (×71 000)

7: Injection canals (IC) of the contractile vacuole. ER — endoplasmic reticulum (×50 000)

8: Section of the injection canal of the vacuole. The outlet of the endoplasmic reticulum tubules (ER) into the injection canal (IC) is seen (× 87 000)

9: Section of the ciliate body in the region of the injection canals. IC — injection canals, ER — endoplasmic reticulum, CV — contractile vacuole (×25 000).



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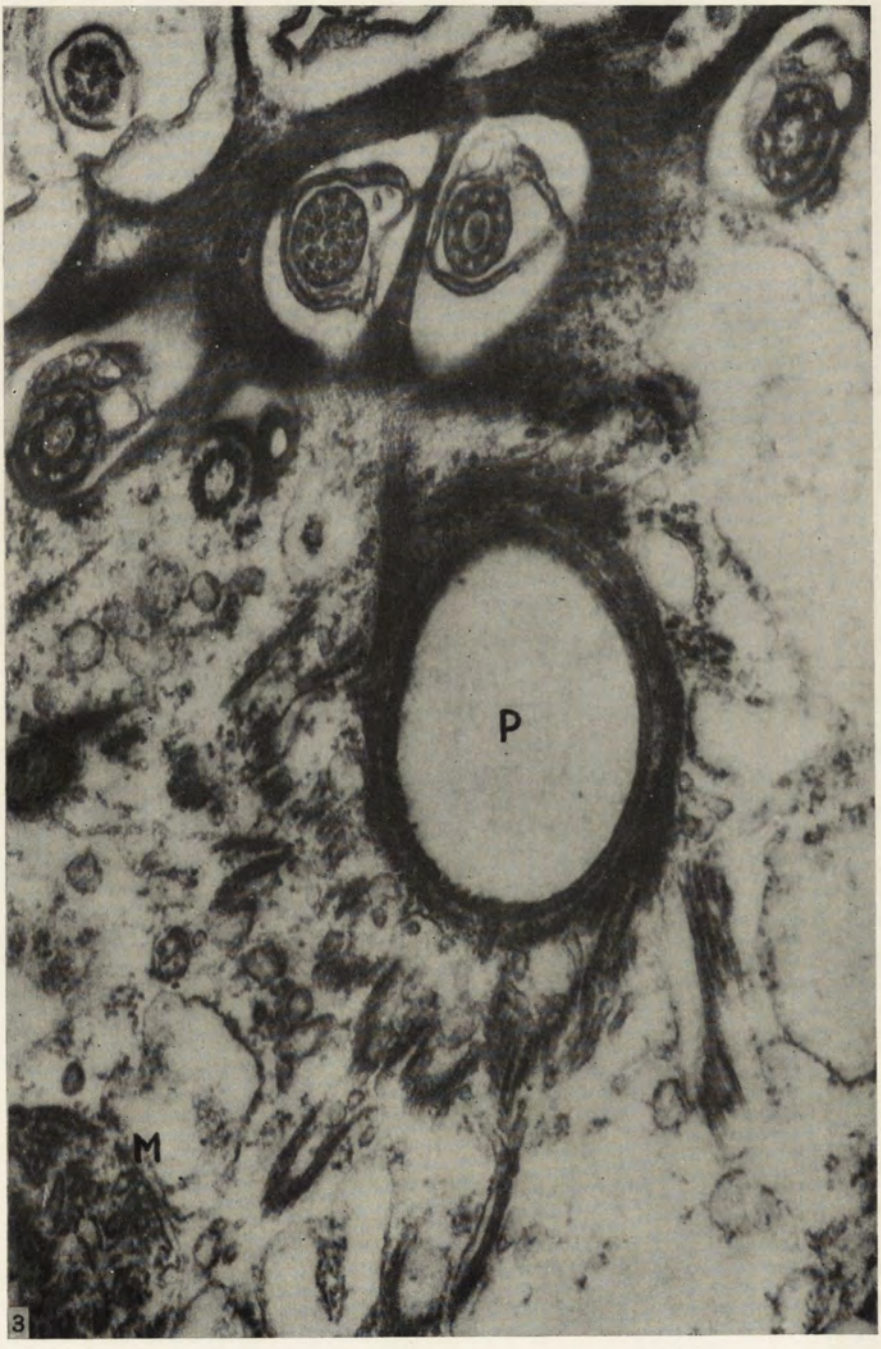

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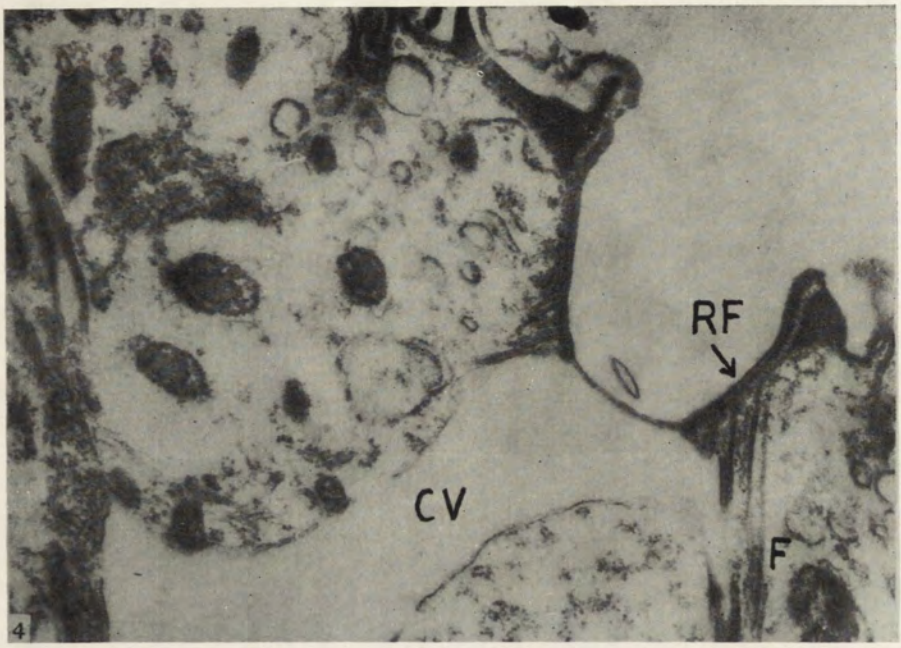

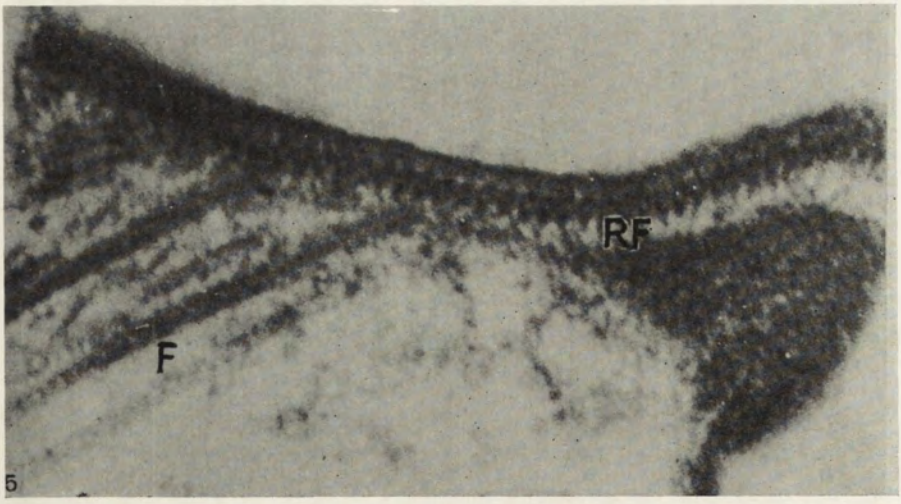

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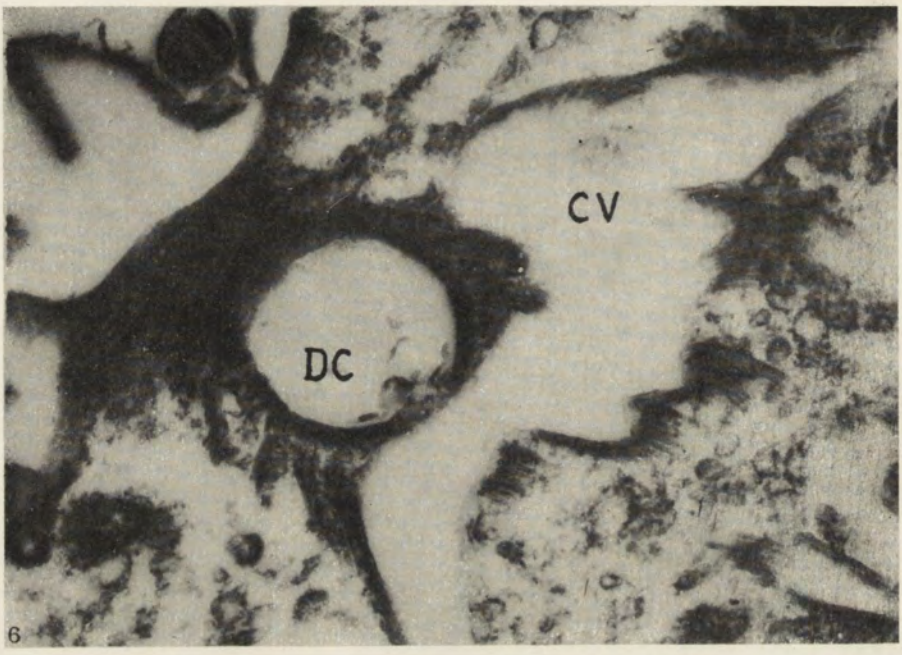

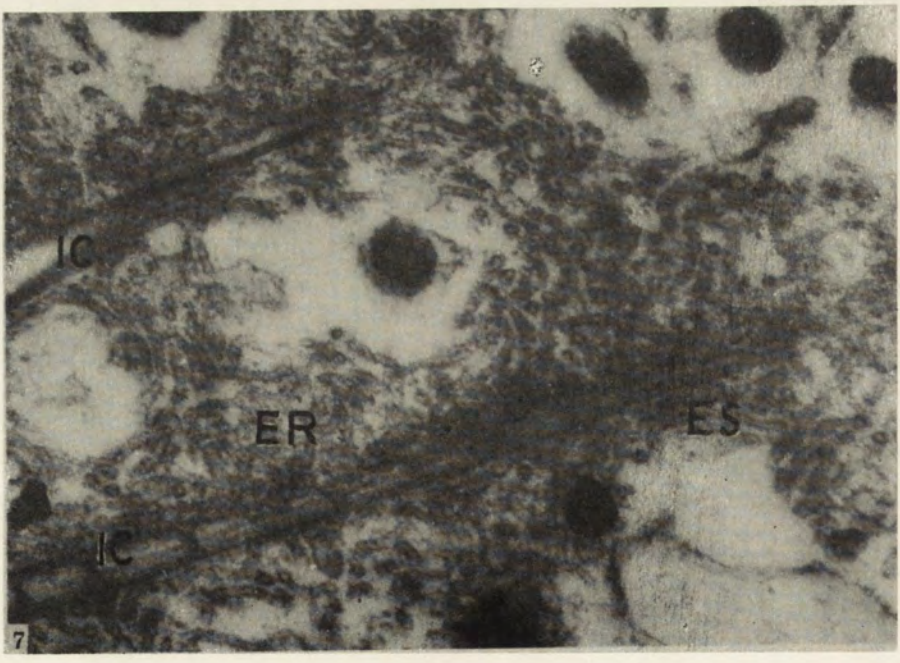

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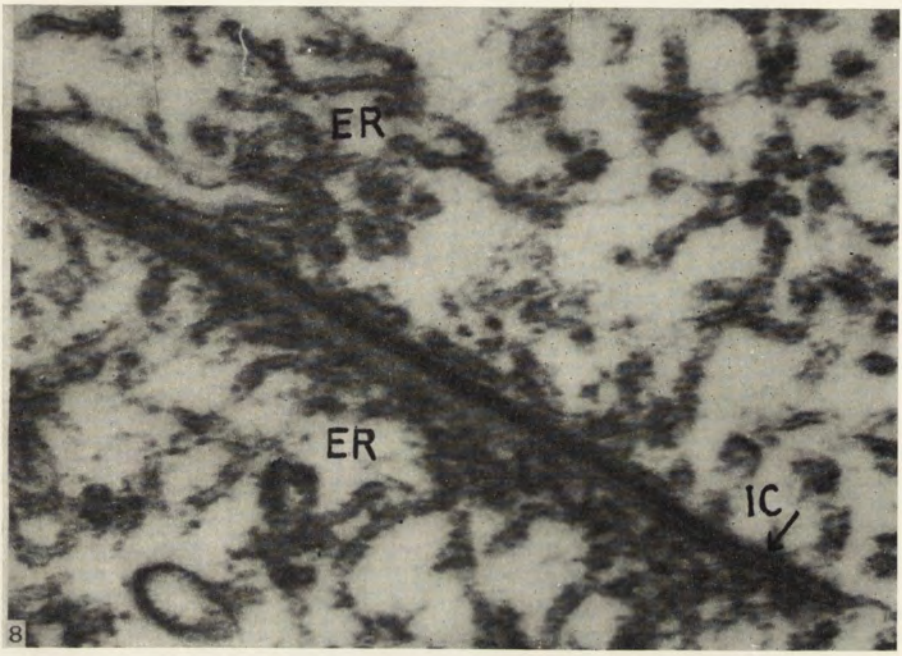

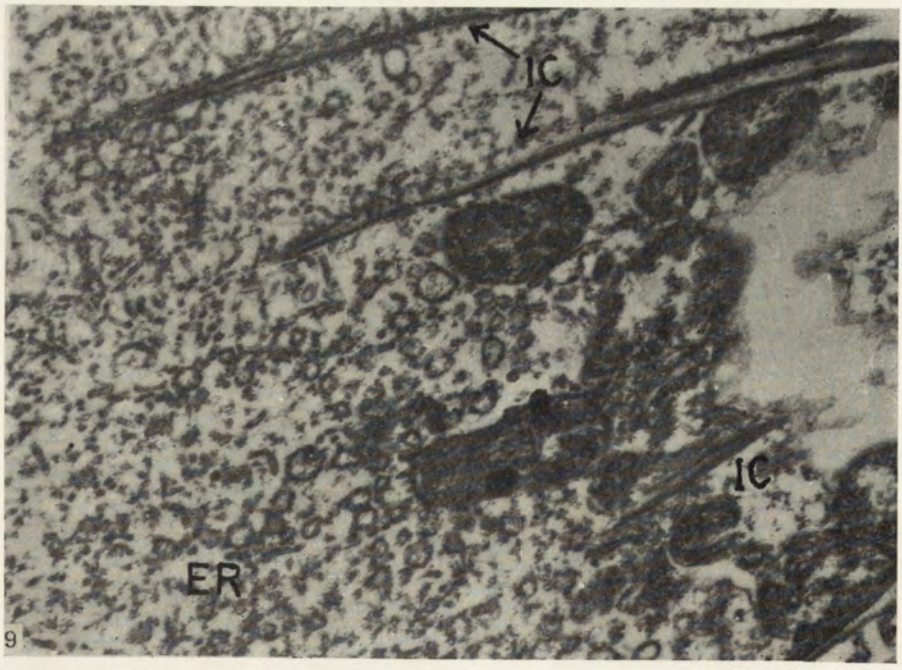

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Photometric study of the DNA content in the nuclei of *Spirostomum ambiguum* (*Ciliata, Heterotricha*)

Фотометрическое исследование содержания ДНК в ядрах *Spirostomum ambiguum (Ciliata, Heterotricha)*

Macronucleus (Ma) of the great majority of ciliates is polyploidal. The degree of polyploidy of Ma — as stated by many authors (Moses 1950, Walker and Mitchison 1957, Seshachar 1950, Seshachar and Dass 1954, Woodard, Gelber and Swift 1961, Ruthmann und Heckmann 1961, Blanc 1962, Dysart 1963, Raikov, Cheissin, Buze 1963, Cheissin and Ovchinnikova 1964, Cheissin, Ovchinnikova, Kudriavtsev 1964) — fluctuates in different species from several tens up to several thousands.

In one of our preceding articles (Raikov and oth. 1963) the view was expressed that the degree of Ma polyploidy depends to some extent on the ratio of the body dimensions to the size of micronuclei (Mi). Consequently, the highest polyploidy should be expected in big ciliates with small Mi. Indeed, *Bursaria truncatella* — as reported by Ruthmann und Heckmann 1961 — with rather small Mi and a very voluminous body, has a big band-shaped Ma, its polyploidy ranging up to 5000 n. On the other hand, different *Paramecium* species with much smaller body dimensions than *Bursaria* and with a comparatively big Mi — have a polyploidy not exceeding 1000 n. It seemed interesting to determine the degree of polyploidy in some other big ciliates with small Mi, as e.g. *Spirostomum*. The body length of this ciliate reaches 1.5 — 2 mm., its Ma is slightly shorter and moniliform. Dimensions of the numerous Mi are not exceeding 1.6 μ^2.

For determination of the polyploidy degree of Ma it was essential to establish the period of interphase in which the reduplication of DNA in the Ma of *Spirostomum* occurs. This is complicated by the fact that in the phase preceding the division, the moniliform macronucleus condenses, becomes ovoid and subsequently, just before division, it stretches out and assumes a ribbon-like form. In the moment of the ciliate division, fission of the macronucleus into two daughter nuclei occurs (Bishop 1923, Seshachar and Padmavathi 1959, Padmavathi 1956, Eberhardt 1962).

The study of the DNA content dynamics in the Ma of some ciliates (*Paramecium caudatum, P. aurelia, Tetrahymena pyriformis* and others) in different periods between two divisions revealed that the lowest quantity of DNA in the nucleus is present immediately after division (the pre-synthetic

nucleus). Later on in the interphase period, a gradual increase of DNA content occurs reaching the double value before division (post-synthetic Ma), when compared with the DNA content in the pre-synthetic nucleus. In some ciliates, DNA synthesis sets on already in the first half or in the beginning of interphase (in *Tetrahymena pyriformis* — Prescott 1960) in the others (*Paramecium caudatum, P. aurelia, Euplotes*) this process proceeds intensely in the second half of interphase and even in its very conclusion (Walker and Mitchison 1957, Kimball and Barca 1959, Kimball and oth. 1960, 1961, Gall 1959, Cheissin and oth. 1963). After the information of Mc Donald 1958, 1962, in *Tetrahymena pyriformis* the DNA synthesis occurs in the course of the whole interphase but is most intense in its middle period. Guttes and Guttes 1960 observed a continuous DNA synthesis over nearly the whole interphase period in *Stentor coeruleus*, the time between the two divisions being 30 hrs.

The interphase period in *Spirostomum ambiguum* is still more prolonged than that in *Stentor*. It would be interesting to elucidate: 1 in which degree this fact may influence the DNA synthesis dynamics, 2. at what time between two divisions the DNA synthesis begins, 3. when it is most intense, and 4. when it ceases. Some data concerning the terms of interphase DNA synthesis in ciliates allowed to presume that with the prolongation of the interphase period, DNA synthesis extends in time as well, and really occurs in the second half of the interphase — (Cheissin and oth. 1963). In *Spirostomum* — like in *Stentor* — the interphase period is not only prolonged but Ma is condensed shortly before division. The problem of the connection between the intensity of DNA synthesis and those alterations in form and size which occur in Ma before division — remains not elucidated.

Material and methods

S. ambiguum was found in a small pond near Peterhof (environments of Leningrad) and cultivated in the artificial medium prepared after Bishop 1923, in high narrow containers, at the temperature 22°. At this temperature division occurs each 72 — 74 hrs. For the study of DNA synthesis dynamics in the interphase period, the time between two successive divisions was divided into 7 stages (Fig. 1) corresponding to the following intervals:

Stage 1 — immediately after division, before the separation of the daughter individuals or after their fission, when the ribbon-shaped Ma is no longer joined by a bridge (Fig. 1 A).

Stage 2 — 1—2 hrs. after division (Fig. 1 B); Ma is transformed from a ribbon-like into a moniliform one but the single nods still are not clearly expressed.

Stage 3 — 24 hrs. and Stage 4 — 48 hrs. after division. At the stages 3 and 4 the nucleus becomes distinctly moniliform (Fig. 1 C and 1 D). The number of nods fluctuates from 25 to 34.

Stage 5 — 62—65 hrs. after division, when shrinking and condensation of Ma occurrs. This is the so called stage of condensation after Seshachar and Padmavathi 1959 (Fig. 1 E).

Stage 6 — is characterized by the stretching of Ma (post-condensation stage). It becomes ribbon-shaped or saussage-shaped but is not yet constricted. This stage follows in 68—70 hrs. (Fig. 1 F).

Stage 7 — onset of division; a constriction appears in the body, Ma is much stretched in length and at the level of the body constriction it becomes slightly narrowed but no two daughter Ma are still formed (Fig. 1 G). This stage corresponds to the 72th—73th hour of the process. After 74 hrs. the fission of ciliate is fully accomplished.

For determination of the polyploidy degree, the ciliates of the 1st and 2nd stage were used, i.e. those in which Ma is in the pre-synthetic period (G_1). At this time Mi is also in pre-synthetic stage. As stated by the authors previously (Raikov and oth. 1963, Cheissin and oth. 1963, Ovchinnikova and oth. 1963) to gain most exact coefficients of polyploidy it is necessary to compare the DNA content of both presynthetic Ma and Mi.

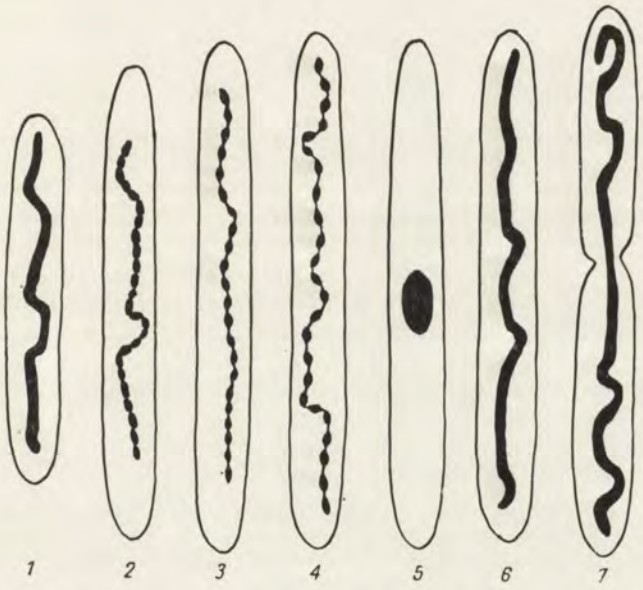

Fig. 1. Development stages in *Spirostomum ambiguum*

Determination of the DNA content in Ma in different periods between divisions was based on its specific absorption at the wave length 265 mμ. For this purpose the ciliates were photographed on the cytospectrophotometer MUF-4 with the lens 10 × 0.20 and the photoeyepiece 3 ×. Ciliates were fixed with absolute alcohol and acetic acid (9 : 1) after Mc Donald 1958. RNA was extracted from nuclei and cytoplasm by the ribonuclease solution. Negatives were measured on the microphotometer MF-4. The degree of blackening obtained was expressed in the value of optic density, the calculation basing on the standard curve of photoplates used, obtained with graduated reducer. The area of Ma was also measured on photomicrographs. Quantity of DNA ($\times 10^{-5}$) was calculated in arbitrary units after the formula $Q = D \cdot S$, i.e. as result of the optic density (D) on the area (S). The number of the ciliates investigated was 135.

Since Mi in *Spirostomum* is very small, it could not be used for the photometric measurements in UV rays for determination of the polyploidy

degree. Mi could not be discerned by visual examination of preparations in the fluorescence screen of the microscope. For that reason only the whole mount preparations stained after the Feulgen's procedure (hydrolized in HCl for 6 min.) were used for determination of polyploidy. Preparations were photographed at the wave length 546 mμ. Methods are discussed in details in our former articles. The figures determining the DNA content in Ma and in Mi are represented also in arbitrary units. It should be mentioned that the figures obtained by photometry in the visible light and that by the photometry in the ultraviolet cannot be compaired with each other as to their absolute coefficients because in the ultraviolet absorption the real quantity of DNA is directly measured, whilst the light absorption photometry records only the quantity of dye bound with DNA, proportional to its quantity.

Results

Change of the DNA quantity in Ma in the interphase period

These investigations concerned the determination of the specific Ma absorption in the ultraviolet spectrum at the wave length 265 mμ.

Immediately after division (stage 1) Ma has the shape of a ribbon and contains the minimal DNA quantity (Table 1). In different individuals, its quantity fluctuates in the limits from 7 up to 20×10^{-5} arbitrary units. Most frequently, however, Ma with a DNA content of 10—14 units occur; consequently the average DNA content in Ma of the stage 1 amounts $12.5 \times \times 10^{-5}$. This value may be considered characteristic for the DNA content in the presynthetic (G_1) Ma. The area of those Ma varies from 7 to $14 \times \times 10^{-5}$ cm², i.e. in some individuals double dimensions of Ma are observed. The average area of Ma amounts 10.46×10^{-5} cm².

Table 1

Changes of the DNA content in Ma of *Spirostomum ambiguum* following the successive stages of the interdivisional period (based on data from UV-photometry at the wave length 265 mμ)

Stage	$Q \times 10^{-5}$ arb. un.		$S \times 10^{-5}$ cm.²
	Extremes	Mean	
1	7 — 20	12,58	10.5
2	8 — 17	11.88	10.2
3	10.5 — 21	15.30	11.6
4	12.9 — 34	21.70	17.6
5	11 — 30	19.35	11.0
6	11 — 29	19.68	15.7
7	17 — 43	29.70	23.9

In the stage 2, two hours after division, no deviations either in DNA content or in Ma dimensions are observed (Table 1). Ma is not yet becoming moniliform but single constrictions of the nucleus are already observable.

After 24 hours (stage 3), the nucleus becomes moniliform and some individuals appear in which the DNA content in Ma is slightly higher than it was

observed in the stages 1 and 2, when individuals were found with 7—8 $\times 10^{-5}$ arbitrary units of DNA content. In the stage 3, ciliates with a minimal DNA content 10.5 $\times 10^{-5}$ arbitrary units occurred. The nuclear area remains unaltered. In average it amounts 11.58 $\times 10^{-5}$ cm^2, but presence of single nuclei with the area about 16 $\times 10^{-5}$ cm. was observed. At this stage evidently the process of DNA reduplication in single genoms of Ma is just beginning.

A still more considerable tendency to rise the DNA content is observable on the stage 4 (48 hrs. after division). Usually 50% of individuals contain a Ma with the DNA quantity nearly double, when compared with the stage 1. Considering that the presynthetic DNA content in Ma fluctuates from 7—8 to 18—20 $\times 10^{-5}$ arb. units, the DNA content in the 4th stage — being 21—33 — may be considered as proximate the postsynthetic one (G_2). Since in the exit stage the DNA content in the pre-synthetic Ma shows a considerable variation, it also remains on the stage 4. Consequently, it may be expected that the reduplication of DNA has been fully accomplished in a number of Ma whereas in the others it embraced only a part of the genoms. Since the lowest coefficient of DNA content in Ma on the stage 4 rose from 7 (stage 1) up to 12.9 — consequently the DNA synthesis set on in all the genoms. In average the DNA content rose up to 21.7 and the nuclei area increased to 17.6 $\times 10^{-5}$ cm.^{2}.

Subsequently, a short time before division the condensation process follows in Ma which becomes ovoid (stage 5). Then the DNA content is not changed when compared to the preceding stage although a slight diminution of the mean size — down to 19.35 — is observed. This shift seems insignificant since this value is very near the DNA coefficient on the preceding stage.

The same DNA quantity remains on the following stage 6, when the nucleus — shortly before division — stretches longitudinally and becomes constricted. At the stage 6, the DNA content shows the mean value 19.68 $\times$ $\times 10^{-5}$ arb. units, the extremes ranging from 11 up to 29.10^{-5} arb. units. When compared with the stage 4, the area of the ovoid nucleus is reduced to 11—10^{-5} cm.^{2} from 17.6 $\times 10^{-5}$ cm.^{2} in the preceding stage.

When the nucleus elongates again, its area increases much up to 15.7 $\times$ $\times 10^{-5}$ cm^2. Before the onset of the body constriction it highly elongates and assumes the form of a twisted ribbon. Its area enlarges up to 23.9 $\times 10^{-5}$ cm^2. On this stage (7) the quantity of DNA in the Ma reaches its maximal values. In some ciliates Ma contains up to 43 $\times 10^{-5}$ arb. units, the minimal content being 16.5 $\times 10^{-5}$ arb. units. The mean quantity of DNA is 29.7 $\times 10^{-5}$ arb. units. In this way Ma of this stage is a post-synthetic one, i.e. in all its genoms occurred the duplication of DNA.

The mean value of DNA content in the post-synthetic Ma is slightly higher (29.7) than it could be expected (25—26 $\times 10^{-5}$ arb. units) but this fact does not interfere with the regularity of duplication of the DNA content in Ma during the interphase period. It should be remembered that in the pre-synthetic Ma the DNA content fluctuates from 8 to 20 $\times 10^{-5}$ arb. units and in the post-synthetic ones — from 16.5 to 43 $\times 10^{-5}$ arb. units, i. e. practically the full duplication of DNA content takes place as well in the pre-synthetic Ma with a small content of DNA (8 $\times 10^{-5}$ arb. un.) as in the big ones (20 $\times$ $\times 10^{-5}$ arb. un.). Variation of the numeric coefficients of the DNA content in Ma may probably be accounted for by the fact that usually the distribution of DNA in daughter nuclei occurs not regularly in 50% of individuals. Some

individuals before the complete fission were observed in which the daugther Ma were already not joined by the thin bridge; their DNA content coefficients were found: 20—14, 16—13, 13—9, 15—11, 13—8 $\times 10^{-5}$ arb. units. In consequence, considerable differences in DNA content arise in the pre-synthetic Ma.

Degree of the Ma polyploidy

Results of the investigation of the Ma polyploidy degree are based on the Feulgen stained mounts.

The degree of polyploidy was determined by using the data established for the Ma of the first and second stage. The micronuclei in pre-synthetic conditions — immediately after division and fission of the daughter ciliates — were also measured. The micronucleus of *Spirostomum* is very small, its area fluctuates within the limits from 0.02 to 0.019 $\times 10^{-6}$ cm^2. The DNA content of Mi in pre-synthetic stages fluctuates very insignificantly from 0.005 to 0.009 $\times 10^{-6}$ arbitrary units. Nevertheless, most frequently Mi occur containing 0.005—0.007 $\times 10^{-6}$ arb. un. Ma contains from 34 to 57 $\times$ $\times 10^{-6}$, in average 43.4 $\times 10^{-6}$ arb. un. The DNA content in Ma exceeds that in Mi average 6575 times, and in single individuals this ratio of DNA content in Ma and in Mi may vary from 5700 to 11400 times. If we consider the Mi as diploidal, consequently, Ma would be of a very high polyploidy, in average amounting 13150 n, and reaching up to 22800 n.

Discussion

For *Spirostomum ambiguum* the prolonged interphase period is characteristic, in which the transformation of the Ma shape occurs. Divisions occur, at the temperature 22°, after 72—74 hrs. The presynthetic period (G_1) lasts evidently about 24 hrs., since already in the ciliates of the 3rd stage (24 hrs.) a slight increase of the DNA content in Ma was observed in some individuals. The synthetic period (S), when the reduplication of single genoms occurs, lasts at least 44 hrs.

The peculiarity of the interphase period in *Spirostomum* is the fact that the DNA synthesis process is not continuous, as in *Paramecium* or *Tetrahymena*, but proceeds in two periods. The first period lasts till the formation of the ovoid nucleus from the moniliform one (stage 3—4). About that time, in the course of 35—50 hrs., an increase of DNA content in Ma occurs, but its whole quantity fails to reach the double value when compared with that of the pre-synthetic Ma. If in the pre-synthetic Ma the average content was 12 $\times 10^{-5}$ arb. un. — this value rises up to 19—21 $\times 10^{-5}$ arb. un. towards the end of formation of the ovoid nucleus (stage 5). Subsequently, a pause in synthesis is observed when the increase of the DNA quantity fails to occur. The ovoid nucleus and the ribbon-shaped one (stages 5 and 6) contain the same DNA quantity. Finally the second conclusive period of the DNA synthesis follows just before division of the ciliate. At that time (stage 7) Ma is ribbon-shaped and on the ciliate body a slight, scarcely perceptible, constriction appears. The DNA content in such a postsynthetic (G_2) Ma proves to be already doubled when compared with the pre-synthetic Ma.

In this way, the first period of DNA synthesis initiates in the first half of interphase and interrupts in its second half. The second, rather short period of synthesis occurs already in the very conclusion of the interphase

period (Fig. 2). Consequently, in *Spirostomum ambiguum* — although the interphase period is long (72—74 hrs.) — the DNA synthesis embraces only the $^2/_3$ of this time, i.e. like in *Stentor*, whereas in *Paramecium caudatum* the DNA synthesis in Ma occurs only in the second half of the interphase which lasts 18 hrs. in all (C h e i s s i n and oth. 1963).

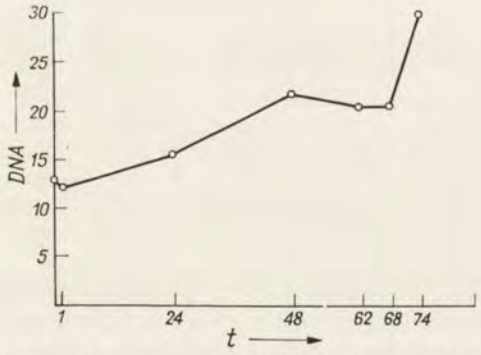

Fig. 2. Changes of the DNA content in Ma of *Spirostomum ambiguum* during interphase (data from UV-photometry at the wave length 265 mμ). Abscissae: time in hrs., coordinates: DNA content in the arbitrary units (Q×10—5)

The considerable prolongation in time of the synthetic period in *S. ambiguum* is presumably connected with the fact that Ma is being condensed towards the completion of the second half of interphase period and then stretches out again before division. It could be postulated that in time of condensation of Ma the process of endomitosis occurs in most of the genoms. Although this was not observed in microscope but it could be presumed basing on some indirect comparative evidences. In the article on *Nassulopsis elegans* R a i k o v 1964 reported an interesting fact of completion of DNA reduplication in all the Ma genoms of this ciliate a long time before the onset of the visible morphological endomitoses in this ciliate. In endometaphase and endoanaphase Ma is already in the phase G_2, i.e. had the post-synthetic quantity of DNA. The reduplication of DNA occurred in the endointerphase, i.e. like in the mitosis of *Metazoa* cells in the interphase period. The interruption in the DNA synthesis during Ma condensation in *S. ambiguum* may be considered as a transition to the endomitosis process in the majority of genoms. As follows from the Table 1, towards the moment of Ma condensation, its DNA content approaches the double value. This fact proves that in the preceding period, during the presumed endointerphase, in the moniliform Ma, the reduplication process embraced a considerable number of genoms. Conclusion of the DNA synthesis occurs already in the ribbon-shaped Ma, after its condensation and prior its fission into two sister nuclei. So in contrast to *Nassulopsis elegans*, in *S. ambiguum* the interruption of DNA reduplication in the condensed Ma does not prove a complete passage to the phase G_2,

since some of the genoms reduplicate already after the period of Ma condensation.

The voluminous Ma in *S. ambiguum* has a high degree of polyploidy. This is connected with the fact that Mi in this ciliate is very small, when compared with Ma. Its area is 5000 times smaller than that of Ma. In *P. woodruffi* and *P. calkinsi* (Cheissin and oth. 1964), of a much lower polyploidy than that of *S. ambiguum*, the area of Ma exceeds that of Mi only 60—130 times. It could be presumed that *Bursaria truncatella*, having big body dimensions (volume 0.3 mm.^{3}) and a long ribbon-like Ma, should have a higher polyploidy than *S. ambiguum*. Nevertheless the results of investigations were just inverse: although the absolute dimensions of *S. ambiguum* (volume 0.15 mm^3.) are smaller than that of *B. truncatella* — the high polyploidy of Ma in *Spirostomum* is evoked by the very small dimensions of Mi and by its extremely low DNA content. The area of the Mi in *B. truncatella* is 10 times larger than in *S. ambiguum*.

The high degree of Ma polyploidy in *S. ambiguum* (13150 n in average) is the result of 12—13 endomitotic cycles in the development of the diploid synkaryon. For comparison *P. woodruffi* may be mentioned with its polyploidy amounting 880 n. In this species only 8—9 endomitotic cycles occur. Evidently their number — conditioning the formation of the highly polyploidal Ma — is more or less restricted in the ciliates, and the 12—13 cycles observed in *S. ambiguum* are near the limit. In other ciliates investigated in this line (Cheissin and oth. 1964) the number of endomitotic cycles is also constant and determines, in respect to the diploid exit quantity of DNA, the degree of Ma polyploidy.

Summary

The photometric investigation proved that in *S. ambiguum* the DNA synthesis in Ma occurs between two divisions and embraces the $^2/_3$ of this period. This process initiates approx. 24 hrs. after division, the interdivision period being 72—74 hrs. The DNA reduplication takes place not simultaneously in all the genoms. As consequence of this, during the synthesis, individuals are found with different degree of increase of the DNA content in Ma, when compared with its pre-synthetic quantity. In the moment of condensation of Ma, in the major part of genoms the reduplication of DNA is accomplished but the entire quantity of DNA in Ma still fails to reach its double value. During the condensation of Ma and later, in course of its stretchig, no synthesis of DNA takes place. It sets on again in a part of genoms just before the fission of Ma. It may be assumed that in the condensed Ma the endomitoses occur in most genoms. The reduplication of DNA occurs in the endointerphase preceding the condensation of Ma. The considerable difference in the DNA content in the pre-synthetic Ma may be in some degree due to the irregularity of DNA distribution during the division of sister Ma.

The polyploidy of Ma in *S. ambiguum* is high, amounting in average 13150 n. This polyploidy is accomplished in 12—13 endomitotic cycles in the development of the diploid synkaryon. The high polyploidy of Ma is conditioned by the comparatively very small dimensions of Mi and its scarce content of DNA.

РЕЗЮМЕ

Фотометрическое исследование показало, что у *S. ambiguum* синтез ДНК в Ма происходит в период между двумя делениями и охватывает около $^2/_3$ времени всего этого периода. Этот процесс начинается примерно через 24 часа после деления, при 72—74 часовом периоде между двумя делениями. Редупликация ДНК происходит не во всех геномах одновременно, вследствие чего во время синтетической фазы встречаются инфузории, Ма которых имеет различную степень увеличения содержания ДНК по сравнению с пресинтетическим его количеством. К моменту конденсации Ма большая часть геномов уже закончила редупликацию ДНК, но общее количество ДНК в Ма еще не достигает полного удвоения. При конденсации Ма и после при его растягивании, синтеза ДНК не происходит. Он возобновляется у части геномов перед самым разделением Ма. Можно предположить, что в конденсированном Ма происходят эндомитозы в большинстве геномов, а редупликация ДНК осуществляется в эндоинтерфазе в период предшествующий конденсации Ма. Значительное различие в содержании ДНК в пресинтетическом Ма может быть отчасти обусловлено неравномерностью распределения ДНК при делении сестринских Ма.

Ма *S. ambiguum* обладает высокой плоидностью, равной в среднем 13 150 n. Такая плоидность достигается 12—13 эндомитотическими циклами при развитии из диплоидного синкариона. Высокая плоидность Ма обусловлена относительно очень малыми размерами Ми и малым содержанием в них ДНК.

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Gradient stomato-caudal d'excitabilité des Ciliés

Gębowo-ogonowy gradient pobudliwości orzęsków

Toutes les généralisations concernant le comportement des Ciliés doivent tenir compte du gradient d'excitabilité existant sans doute dans la ciliature. Cette nécessité a été soulignée auparavant (Grębecki 1962) dans le cas de la théorie électrotonique de la galvanotaxie des Paramécies.

L'excitation de la Paramécie par le courant constant se développe le mieux avec sa position homodrome; on aperçoit cette regularité sur les dessins de Kamada 1931, elle a été minutieusement étudiée par Kinosita 1936, enfin Viaud et Bonaventure 1956, l'ont appellée "la loi de maximum de l'excitation" (l'animal prend le position qui rend la stimulation la plus efficace). D'après Kinosita il s'agit d'un gradient de l'excitabilité cathodique, maximale dans la région antérieure du corps et diminuant vers l'arrière, et d'un gradient de l'excitabilité anodique disposé à l'inverse. En introduisant les notions de la théorie électrotonique de la galvanotaxie (Jahn 1961) on pouvait conclure qu'il existe un gradient antério-postérieur d'excitabilité électrotonique de la Paramécie. L'idée du gradient antério-postérieur est aussi soutenue par Kitching 1961.

L'idée du gradient antério-postérieur semble rester en accord avec de nombreuses données concernant d'autres aspects de l'activité ciliaire. Párducz 1954a démontré que chez les *Paramecium*, *Colpidium* et quelques autres Ciliés les ondes metachroniques originent normalement au bout postérieur du corps et avancent vers les régions antérieures, tandis qu'au cours du mouvement rebroussé ils sont propagés du devant vers l'arrière. Seravin 1962a, b introduit la notion de deux gradients antério-postérieurs opposés, également pour les *Spirostomum*, en postulant l'existence d'un mécanisme coordinant le mouvement normal — localisé postérieurement, et d'un autre mécanisme antagoniste déclenchant et contrôlant le rebroussement — localisé dans la région antérieure. Doroszewski 1963 constate que les *Dileptus* répondent par une poussée en avant s'ils sont mécaniquement stimulés de l'arrière et par un recul si le stimulant parvient du devant. On peut inférer des resultats obtenus plus tôt par le même auteur (Doroszewski 1961) que pendant une locomotion à reculons cette polarité de réponse est inverse. Le même type de comportement fut observé par Jensen 1959 chez les Paramécies stimulées avec un faisceau des rayons UV. Chez les *Opalina* la répartition des ondes métachroniques s'avère extrêmement variable, néan-

moins la direction de leur propagation semble aussi changer avec chaque inversion du battement ciliaire (Okajima 1953).

Cependant, des difficultés surgissent devant l'idée d'un tel simple gradient antério-postérieur, s'il s'agit du fonctionnement des cils péristomaux. En ce qui concerne l'orientation et le mouvement dans des champs électriques, des asymétries restent inexpliquées (c'est-à-dire la différence de l'excitabilité du côté oral et dorsal de la Paramécie et les altérations de sa nage en hélice). Párducz 1954 ne tire pas de conclusions claires sur la propagation des impulses à la surface orale et Seravin 1962 b restreint même la technique de son étude seulement à la face dorsale. La ciliature du péristome semble échapper aussi au gradient antério-postérieur dans le cas de différents processus d'immobilisation ou de destruction de la ciliature, ainsi que de sa réactivation ou régéneration (Kuźnicki 1963 a, b).

Il ne reste qu'à accepter la théorie du gradient antério-postérieur telle qu'elle est, en proclamant cependant l'autonomie des cils péristomaux, ou bien à essayer de modifier seulement l'idée de ce gradient unique. La première voie a été dernièrement choisie par Jensen 1959 postulant l'existence des trois centres coordinateurs chez la Paramécie: le postérieur, l'antérieur et le cytostomal, ce qui efface l'image de l'antagonisme bipolaire de l'excitation. L'étude présente est basée — au contraire — sur la convinction qu'il existe un gradient unique, et ce n'est que sa direction antério-postérieure qui, correcte en général, est pourtant simplifiée en negligeant l'asymétrie dorso-ventrale du Cilié.

Mouvement progressif

On a repris l'étude sur la locomotion normale du *Paramecium caudatum* en employant des Ciliés restant dans le milieu de culture, bien aéré, ou lavés avec des solutions contenant quelques mM du $CaCl_2$. Dans ces conditions le mouvement progressif est très bien prononcé, rapide et sans reculs. Pour analyser la répartition des ondes métachroniques, ainsi que l'orientation des battements ciliaires, on se servait de la fixation rapide avec du OsO_4 introduite dans ce but par Párducz 1952. Le matériel fixé était ensuite décalcifié et alors — coloré avec de l'hematoxyline en présence du $NiCl_2$ (Grębecki 1964 b), ce qui fournit des resultats beaucoup plus uniformes que la coloration classique d'après Heidenhein, employée par Párducz.

Les resultats démontrent que, pour un mouvement progressif bien prononcé, le travail de toute la ciliature est exactement uniforme et entièrement subordonné à des ondes métachroniques originant dans la région caudale. Les ondes très regulières disposées de façon typique (direction à peu près NW—SE) couvrent aussi la face orale du corps et même la dépression péristomale (Pl. II 6—7). Elles se courbent légèrement dans cette dépression, ayant passé au-delà de la bouche; l'impression se produit que les ondes encerclent le cytostome par devant; on caractérise bien cet image en parlant des ondes "concentriques" dans le péristome. L'uniformité de la coloration obtenue en presence du Ni^{2+} (Grębecki 1964 b) permet de constater que l'allure concentrique des ondes metachroniques de la ciliature péristomale est absoluement typique pour tous les individus provenant d'un échantillon qui manifeste un mouvement progressif bien prononcé. Sur quelques photographies publiées par Párducz 1956 a et 1959 on peut trouver la même image, mais elle n'a pas été décrite et reconnue comme règle, par cet auteur.

L'analyse des positions des cils fixés permet de confirmer entièrement la découverte importante de P á r d u c z 1954 révélant que le battement effectif du cil est parallèle à la direction de l'onde. Donc, les forces exercées par les cils pendant une nage rapide peuvent être représentées, comme le montre la Fig. 1. On peut en conclure que dans ces conditions le mouvement progressif est le plus efficace, en hélice très allongée, ce qui reste accord avec toutes les données précédentes. Cependant, le battement des cils péristomaux arrangés dans des ondes "concentriques" produit un effet complètement inattendu. La ciliature péristomale, en travaillant de cette façon, collabore dans la locomotion, or, elle ne peut pas amener de l'eau vers le cytostome, mais elle doit produire des courants surpassant la bouche. Il ne reste qu'à postuler que la Paramécie ne peut pas prendre la nourriture pendant une rapide nage progressive. Pourtant, d'après J e n n i n g s 1906, l'opinion contraire est acceptée. Les expériences suivantes avaient donc pour but de clarifier cette question.

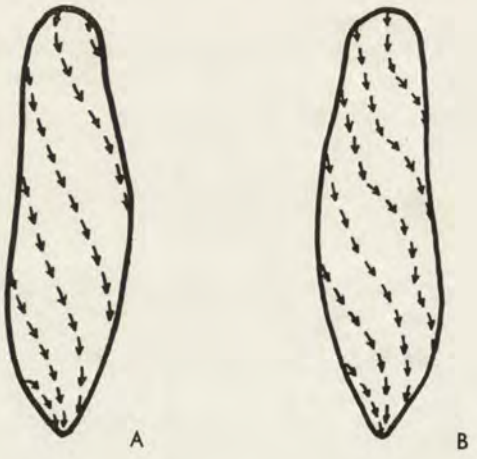

Fig. 1. Diagramme de la dislocation des ondes métachroniques et de la direction des battements ciliaires efectifs pendant le mouvement progressif bien prononcé. A. Côté dorsal. B. Côté oral; l'allure "concentrique" des ondes dans le péristome montre que les cils n'amènent pas d'eau vers la bouche

Stimulation mécanique

Il a été constaté que les Paramécies, manifestant une nage progressive fortement accentuée par l'addition du $CaCl_2$ (jusqu'à 10 mM), forment toujours des vacuoles alimentaires si le milieu contient une suspension (de la lévure, du lait, de l'encre de Chine, du ver pilé, etc.). Tout récemment B r u t k o w s k a (communication personnelle) a également établi que le calcium reduit la phagocytose des Paramécies mais ne la supprime pas entièrement.

Pour l'expérience suivante les Paramécies étaient soumises préalablement à l'action de 5 mM de l'hydrate de chloral, pour 48 h., ce qui détruit la ciliature du corps sans toucher les cils péristomaux (G r ę b e c k i and K u ź n i c k i 1961). Cette technique rend possible l'observation des courants d'eau dans le péristome en évitant l'interférence du battement ciliaire du corps entièr. Les Paramécies déciliées de cette manière étaient placées, après un lavage soigné, dans des solutions riches en calcium et contenant une suspension de la graisse du lait. L'agitation du milieu par les cils péristomaux était enregistrée sur la pellicule photographique, d'après la technique décrite autre

part (Grębecki 1961). La Planche I 1 démontre que — malgré la teneur élevée en Ca^{2+}, ce qui devrait produire des ondes "concentriques" dans le péristome — un courant alimentaire se produit, amenant des particules suspendues vers la bouche.

Les expériences effectuées en présence des suspensions sont donc, à première vue, contradictoires par rapport aux résultats obtenus auparavant — en fixant les Paramécies nageant vite dans des mêmes milieux sans suspensions. On peut supposer alors, que c'est la présence même des particules suspendues qui agit en tant que stimulant mécanique déclenchant un courant alimentaire dans le péristome.

Pour vérifier cette supposition on fixait les Paramécies nageant dans des solutions qui étaient riches en Ca^{2+} et contenaient, à la fois, une suspension de l'encre de Chine. La photographie presentée par la Planche I 4 montre qu'en effet le présence des particules change l'allure des ondes metachroniques dans ces ondes prouve u'ils provoquent un courant alimentaire dirigé vers forment un faisceau débutant de la bouche. L'analyse des positions des cils dans ces ondes prouve qu'ils provoquent un courant alimentaire dirigé vers le cytostome; le travail de la ciliature du corps n'est pas touché et elle retient son allure typique pour la locomotion progressive (Fig. 2).

Fig. 2. Diagramme de la dislocation des ondes métachroniques et de la direction des battements ciliaires effectifs pendant le mouvement progressif dans une suspension de l'encre de Chine; côté oral; les ondes dans le péristome sont disposées en éventail, ce qui correspond à un courant alimentaire

Cependant, une légère altération de la sinusoïté des trajectoires parcourues par les Paramécies est à présumer, dans le cas des suspensions, en conséquence du changement de l'activité ciliaire dans le péristome même. La Planche I 5 démontre les trajets enregistrés d'après la technique de Dryl 1958; le compartiment central apporte des trajets parcourus dans le milieu contenant l'encre de Chine, tandis que l'encadrement est découpé d'un enregistrement contrôle, effectué dans la même solution sans particules solides. On y voit nettement que les hélices décrites par les Paramécies en présence de la suspension deviennent moins allongées, c'est-à-dire le pas d'hélice est un peu raccourci et l'angle de la déviation augmente. En effet, la Paramécie avance moins vite et elle se met à balancer un peu plus que dans de l'eau pur. Des pareilles altérations du mouvement dans des suspensions furent observées également par Jensen 1959.

Ces modifications du caractère de la nage progressive s'accordent bien à la conclusion précédente, à savoir que les cils péristomaux n'agissent plus en ondes "concentriques" subordonnées à la coordination de la ciliature du corps entier, mais qu'ils battent autrement, en produisant le courant alimentaire et en déviant la Paramécie de l'axe de sa trajectoire. Toutes les expériences mentionnées dans ce chapître prouvent que les particules suspendues agissent en tant que stimulant mécanique qui produit une altération du travail ciliaire. Cette altération commence évidemment de la bouche et englobe la ciliature de la dépression péristomale. Ainsi les cils péristomaux échappent à la contrôle des ondes métachroniques originant dans la région caudale et ils se trouvent coordinés d'autre façon, probablement par des ondes provenant du cytostome.

Stimulation chimique

Rebroussement ciliaire

Pour analyser le travail ciliare typique pour l'état du rebroussement (le mouvement à reculons) les Paramécies étaient passagées à des solutions riches en potassium et pauvres en calcium (c'est-à-dire à des milieux caractérisés par des valeurs élévées de la proportion $[K^+]/\sqrt{[Ca^{2+}]}$ — d'après les conclusions de Jahn 1962 et Grębecki 1964 a). Quelques instants après le passage le matériel était fixé d'après Párducz 1952 et coloré d'après Grębecki 1964 b.

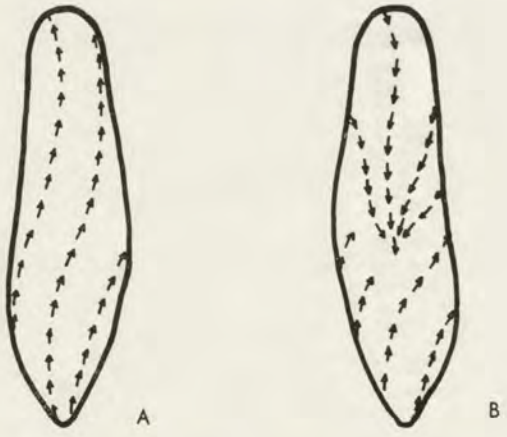

Fig. 3. Diagramme de la dislocation des ondes métachroniques et de la direction des battements ciliaires effectifs pendant le mouvement à reculons. A. Côté dorsal. B. Côté oral; les ondes "radiales" dans le péristome dirigent le battement effectif des cils péristomaux directement vers la bouche

En ce qui concerne le travail de la ciliature du corps, on ne peut que confirmer les données de Párducz 1954, à savoir qu'à la surface dorsale les cils sont coordinés en ondes disposées à peu près dans la direction NE—SW, propagés du devant vers l'arrière. Étant donné que les cils battent effectivement en parallèle à la lame de l'onde, on peut construire un diagramme du travail de la ciliature du corps (Fig. 3 A), entièrement conforme à des traits essentiels du mouvement à reculons.

Cependant, pour la surface orale, une autre répartition des ondes s'avère également régulière. Les ondes commencent de la bouche et continuent, en

éventail, jusqu'au bord antérieur du corps (Pl. II 8 et III 11). Cet arrangement des cils péristomaux, demontré déjà par P á r d u c z 1956a et 1959 sur quelques photographies, sans le décrire, est reconnu dans l'étude présente en tant que règle générale pour la nage à reculons: au cours du rebroussement ciliaire la dislocation "concentrique" des ondes metachroniques dans le péristome, change pour une dislocation "radiale".

Le diagramme du travail de la ciliature péristomale pendant le rebroussement ciliaire (Fig. 3 B) montre que les cils situés dans la dépression ne battent pas effectivement vers le bord antérieur du corps, mais vers le cytostome (Pl. II 9 et III 12). Leur comportement est donc identique à celui qui a été décrit pendant la stimulation par les particules des suspensions. Par conséquent, on peut s'attendre à ce que les effets soient également analogues; autrement dit, il est à présumer que les Paramécies, même pendant le maximum du rebroussement ciliaire, sont déviées de leurs axes en manifestant des trajects helicoïdaux et qu'elles produisent des courants alimentaires.

L'impression que la Paramécie, dans le stade initial d'un fort rebroussement ciliaire, nage en ligne droite (P á r d u c z 1959) n'est qu'illusoire. Si on enregistre le mouvement en aggrandissant considérablement le trajet on voit que le Cilié balance nettement (Pl. II 10). La Fig. 4 A montre que celà doit se produire, car les cils péristomaux, en battant vers le cytostome, s'opposent

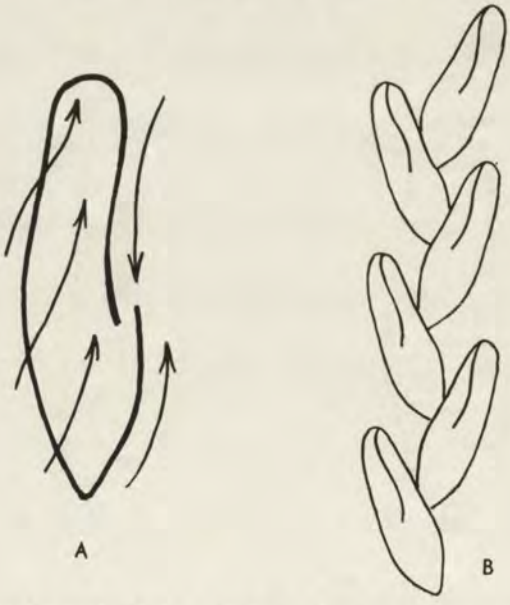

Fig. 4. Origine du balancement de la partie antérieure de la Paramécie pendant le mouvement à reculons. A. Les flèches indiquent que les cils péristomaux battant vers la bouche s'opposent au battement de la ciliature générale. B. Diagramme dressé d'après un enregistrement cinématographique, prouvant qu'en effet de cette asymétrie motrice le péristome balance en se déviant toujours de l'axe du mouvement

au travail de la ciliature du corps qui est dirigé vers le front de l'animal. Pendant le pivotement résultant d'une telle asymétrie de la force motrice, le péristome doit se tourner toujours vers l'axe de l'hélice, et c'est justement ce qui a été prouvé par une analyse cinématographique (Fig. 4 B).

Il en résulte de plus, que pendant le rebroussement ciliaire la Paramécie produit toujours un courant alimentaire. En effet, B r u t k o w s k a (communication personnelle) a conclu que la phagocytose des Paramécies est complètement insensible ou même augmentée par un excès du K^+ dans le milieu.

L'auteur observait également à maintes reprises des Paramécies formant des vacuoles remplies de différentes suspesions, durant la nage à reculons. Le courant alimentaire chez la Paramécie nageant à l'inverse dans une suspension est très spectaculaire — le Cilié, tout en reculant, entraîne une longue queue de particules derrière lui (Fig. 5).

Fig. 5. Paramécie nageant à reculons dans une suspension; elle entraîne des particules derrière elle grâce au courant alimentaire manifesté par les cils péristomaux

Le courant alimentaire manifesté dans des solutions qui évoquent le rebroussement ciliaire était enregistré chez les Paramécies depourvues de la ciliature du corps par l'hydrate de chloral (Pl. I 2—3). Même des plus hautes concentrations du K^+ ne peuvent pas forcer les cils péristomaux à battre vers l'antérieur du corps — ils ne frappent que vers le cytostome, en démontrant la dislocation "radiale" des ondes métachroniques.

Gradient stomato-caudal

Il ne reste qu'à accepter que les ondes "radiales", avec le battement ciliaire dirigé vers la bouche, présentent justement le symptôme du rebroussement, typique pour la région péristomale.

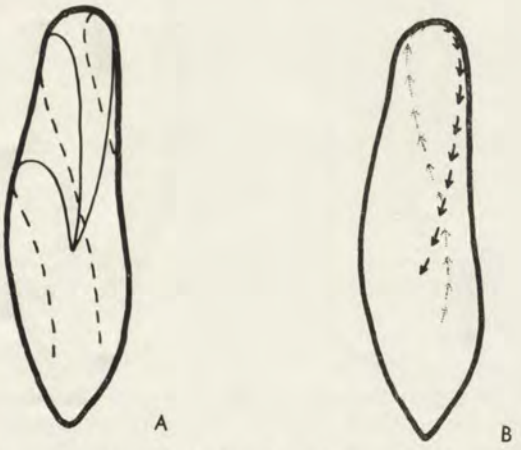

Fig. 6. Diagramme démontrant que les ondes "radiales" du péristome, ayant pénetré à la surface dorsale, forment des ondes "rebroussées" du corps (A) et que le battement effectif, dirigé dans le péristome vers la bouche, doit se transformer, à la face opposée, en battement inverse (B)

Les transformations du caractère des ondes métachroniques confirment cette conclusion: dans la ciliature du corps la direction des ondes typique pour le mouvement progressif (NW—SE) change pour une autre direction, sécante (NE—SW); également dans le prérístome les ondes "concentriques" sont remplacées par des ondes sécantes — "radiales".

Une observation microscopique très détaillée des Paramécies fixées durant leur rebroussement ciliaire prouve que les ondes "radiales" dans le péristome ne forment pas de système séparé, mais qu'elles se prolongent jusqu'au bord antérieur, traversent le champ frontal, et alors, en gagnant la face dorsale, elles forment les ondes de la ciliature du corps. L'unité des ondes "radiales" du péristome et des ondes "rebroussées" du corps entier est dressée en diagramme (Fig. 6 A). La Fig. 6 B montre qu'en effet le battement dirigé vers la bouche dans le champ péristomal ne peut se transformer à la face opposée du corps qu'en battement rebroussé typique, si les cils sont coordinés par un système unique des ondes continues.[1]

L'unité des ondes "radiales" dans le péristome qui ramènent l'eau vers la bouche, et des ondes "rebroussées" dans la ciliature du corps qui causent le mouvement à reculons, permet d'abandonner l'hypothèse des trois centres coordinateurs, érigée par Jensen 1959. Il suffit de conclure que le travail de toute la ciliature est subordonné à un simple gradient bipolaire. Tenant compte que le potassium (et les autres ions inversant le battement ciliaire) agissent comme un cathélectrotonus chimique et le calcium — comme un anélectrotonus chimique (Grębecki 1963 b), on peut formuler la théorie du gradient unique de la manière suivante.

Le gradient d'excitabilité cathélectrotonique (c'est-à-dire le gradient de la sensibilité des cils au rebroussement) qui apparaît à la surface dorsale de la Paramécie en forme de gradient antério-postérieur, est en effet le gradient stomato-caudal; l'excitabilité cathélectrotonique est maximale à la région buccale et elle decroît graduellement vers la région caudale, en encerclant

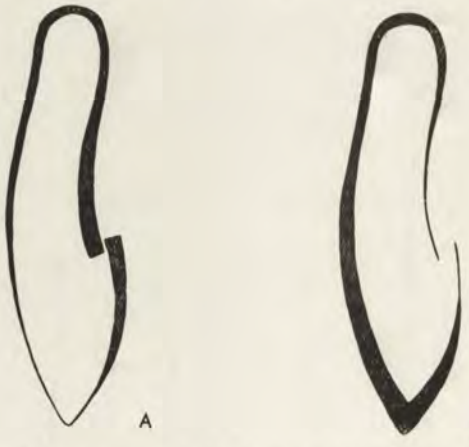

Fig. 7. Représentation schématique du gradient d'excitabilité de la Paramécie marqué par l'épaisseur du contour. A. Gradient de la tendance au rebroussement ciliaire (excitabilité cathélectrotonique). B. Gradient de la tendance à reproduire le travail normal de la ciliature (excitabilité anélectrotonique)

seulement le champ frontal (Fig. 7 A). Le gradient d'excitabilité anélectrotonique (c'est-à-dire le gradient de la tendance à produire le battement normal de la ciliature) qui donne l'impression d'un gradient postério-antérieur à la surface dorsale, est en réalité caudo-stomal; l'excitabilité anélectrotonique est maximale en arrière du corps et elle tombe vers le cytostome, en surpassant également la région frontale (Fig. 7 B).

[1] La jonction des ondes péristomales et somatiques est impossible à démontrer sur une photographie, car elles n'apparaissent qu'à de différentes positions de réglage du microscope.

Par conséquent, on peut aussi accepter la théorie des gradients bipolaires dressée pour les *Spirostomum* par Seravin 1962 a et b, avec une légère modification. Il faut dire que le mécanisme coordinant le travail normal de la ciliature est localisé en effet dans la région postérieure du corps, mais celui qui contrôle le battement rebrousseé, agit à partir de la région buccale.

Autrement dit, la théorie du gradient stomato-caudal unique peut renfermer également les découvertes de Párducz 1954, concernant la propagation des ondes métachroniques. Les ondes du battement normal obéissent au gradient d'excitabilité anélectrotonique, donc elles originent à la region caudale et elles contrôlent toute la ciliature en progressant vers l'antérieur. Les ondes du battement rebroussé obéissent au gradient d'excitabilité cathélectrotonique, donc elles commencent de la bouche et, pour gagner la région caudale, elles encerclent le champ frontal d'un côté; si elles n'atteignent que le bord antérieur du corps, un courant alimentaire dans le péristome est la seule réponse; quand elles pénètrent à la surface dorsale, elles se dispersent vers l'arrière (comme Párducz 1954 l'a vu le premier), saisissent toute la ciliature du corps et alors, le mouvement à reculons apparaît.

Propagation et disparition du rebroussement

L'idée du gradient stomato-caudal semble applicable dans le cas de la propagation du rebroussement le long de la surface du Cilié, quand il se met à reculer, ainsi qu'à la propagation de la renormalisation à travers la ciliature, quand il vient de reprendre la direction habituelle de son mouvement. Prenant en considération toutes les données sur la polarité d'action rebroussante et renormalisante du champ électrique (Kamada 1931, Kinosita 1936, Grębecki 1963 b) on peut s'attendre à ce que le rebroussement ciliaire commence du cytostome, traverse le champ frontal et finisse par saisir les cils postérieurs, et que la renormalisation — par contre — apparaisse d'abord dans la région caudale.

Quand la Paramécie se met à nager à reculons sous l'influence d'une concentration élévée du K^+ extérieur, il semble que le rebroussement apparaît dans toute la ciliature sur-le-champ. L'impression se produit que le prémier frappement des cils à l'inverse soit synchrone, ce qui a été déjà décrit par Jennings 1906 et Párducz 1956 b. La reponse se déclenche de façon également instantanée dans le cas d'un recul bref ("avoiding reaction") ainsi qu'au debut d'une retraite à longue distance ("ciliary reversal"). Sans doute, le synchronisme apparent ne peut resulter que d'une considérable vitesse de la conduction du premier impulse initiant le rebroussement ciliaire; néanmoins, cette vitesse du phénomène rend impossible d'observer si le rebroussement commence de la bouche, comme il est supposé.

La renormalisation du travail ciliaire semble plus facile à suivre. Après une période de nage à reculons, la Paramécie restant dans la même solution du KCl, ne reprend la direction normale de sa locomotion que graduellement. Le travail ciliaire à ce stade intermédiaire a été étudié par Párducz 1959. Il arrive à une conclusion inverse à notre supposition — à savoir que les cils antérieurs sont rénormalisés les prémiers. Cette opinion est également contradictoire à tous les resultats précédents qui indiquent que c'est la partie postérieure des Ciliés qui est la plus sensible à des stimulants renormalisant le battement ciliaire (Kamada 1931, Kinosita 1936, Jensen 1959, Seravin 1962 a et b. Doroszewski 1963, Grębecki 1963 b).

La conclusion de Párducz 1959 est basée sur deux arguments principaux: 1. il assert que les cils péristomaux recommencent les premiers à frapper vers l'arrière, et 2. il présente des photographies des individus portant des ondes normales dans la région antérieure de leur corps, et rebroussées de l'arrière. Le premier argument tombe, car il a été déjà prouvé dans l'étude présente que dans la péristome le battement vers l'arrière (c'est-à-dire vers la bouche) existe dès le debut du mouvement à reculons et il ne signale pas la renormalisation, mais — par contre — il est justement le symptôme du rebroussement des cils péristomaux. Pour vérifier le deuxième argument de Párducz, on a repris l'étude des ondes chez les individus fixés, en profitant du perfectionnement de la technique de coloration (Grębecki 1964 b). Basant sur l'étude de la ciliature de plusieurs milliers d'individus, il ne reste qu'à refuser la valeur conclusive à la série des photographies publiée par Párducz 1959 — on ne peut arranger une série analogue qu'en choisissant certains individus et en rejetant la plupart d'autres. Cela démontre qu'on ne peut pas tirer des conclusions basant sur des exceptions — chez presque tous les individus, étant au cours de la renormalisation, les ondes métachroniques manquent tout à fait, bien qu'on les voit toujours pendant le mouvement normal et rebroussé. Leur absence est probablement liée justement à la réorganisation de l'activité motrice. Par conséquent, l'analyse des ondes métachroniques ne peut dans ce cas ni motiver la conclusion tirée par Párducz 1959, ni fournir de preuves en faveur de l'opinion opposée, developpée par l'auteur présent.

Des données indirectes sur ce sujet furent obtenues en se servant d'une technique différente. On essayait d'évoquer le rebroussement ciliaire chez les Paramécies nageant dans une microgoutte en y opérant avec de très minces pipettes contenant des solutions différentes du KCl. Par analogie, on tendait à renormaliser les Paramécies reculantes, en se servant des pipettes remplies de $CaCl_2$. Il a été mis en évidence qu'il est beaucoup plus facile de rebrousser la ciliature si les ions K^+ atteignent le Cilié du devant, et de la renormaliser, si le Ca^{2+} intervient du derrière. C'est ce qui était attendu, et qui est entièrement en accord — d'autre part — avec les effets de la stimulation mécanique des *Dileptus* (Doroszewski 1963), et de la stimulation des Paramécies avec des rayons UV (Jensen 1959).

En résumant, on peut constater que l'hypothèse déduite de la théorie du gradient stomato-caudal, postulant que le rebroussement commence de la bouche et la renormalisation — de la région caudale, n'est pas encore bien prouvée mais elle semble la plus probable. En tout cas, elle s'accorde bien avec des plusieures études précédentes (Kamada 1931, Kinosita 1936, Párducz 1954, Jensen 1959, Seravin 1962a et b, Doroszewski 1963, Grębecki 1963 b), ne contradisant que l'opinion de Párducz 1959 qui était mise en question.

Stimulation électrique

Sinusoïté du mouvement galvanotactique

En ce qui concerne la galvanotaxie, l'idée d'un gradient antério-postérieur suffit pour bien expliquer les changements de la vitesse de la nage en fonction de l'intensité du courant (Kamada 1931, Viaud et Bonaventure 1956, Grębecki 1962 et 1963 b) et les différences entre l'excitabilité ma-

nifestée dans la position homodrome et antidrome, notées par plusieurs chercheurs. Cependant, elle nous ne donne pas de renseignements sur la nature des changements de la sinusoïté des trajectoires parcourues par les Paramécies avançant vers la cathode, dans les champs faibles et intenses (stades de Statkewitsch 1904, observés ultérieurement par Viaud et Bonaventure 1956 et Grębecki 1962). Or, l'origine des stades de Statkewitsch devient évidente si on admet justement que le gradient est stomato-caudal.

Les Planches IV—V montrent ces altérations de l'allure hélicoïdale des trajets, enregistrées avéc la téchnique de Dryl 1958.[2] Chaque photographie présente, sur sa partie marginale, le caractère du mouvement dans l'échantillon avant la fermeture du circuit, tandis que le compartiment central — le mouvement manifesté par les mêmes individus dans le champ électrique d'une densité définie. On y voit que la sinusoïté des trajets reste intacte au dessous du seuil de la reponse galvanotactique (0.075 mA/cm² — Pl. IV 13) et ne change que très peu au seuil même (0.150 mA/cm² — Pl. IV 14). Dans les courants modérés (0.300, 0.600 et 1.200 mA/cm²) la déviation hélicoïdale du mouvement augmente fortement (Pl. IV 15—V 17). Avec des courants plus forts (2.400 mA/cm² — Pl. V 18) le phénomène s'efface de nouveau, car le ralentissement de la nage progressive réduit considérablement le pas de l'hélice.

Cette altération de l'hélice devient aisément compréhensible si on tient compte que la théorie du gradient stomato-caudal exige que les cils péristomaux, sous l'action rebroussante de la cathode, battent directement vers la bouche, en s'opposant à la ciliature du corps qui bat vers le bout antérieur, sur le champ excité.

L'observation des courants d'eau produits par les cils péristomaux, chez les individus dépourvus du reste de la ciliature par l'hydrate de chloral (technique de Grębecki and Kuźnicki 1961), prouve que cette explication est correcte. La stimulation cathélectrotonique, même la plus intense, ne peut pas forcer les cils du péristome à battre en avant; ils frappent toujours vers la bouche en produisant les même courants alimentaires qui étaient vues avec la stimulation mécanique et chimique. Cela est conforme au résultat récent de Brutkowska et Dryl (communication personnelle) démontrant que la stimulation électrique des Paramécies n'empêche la phagocytose qu'à des intensités très élevées, dépassant plusieurs fois celles qui suffisent à obtenir le rebroussement cathodique.

Il en resulte une asymétrie de l'effet de Ludloff 1895, c'est-à-dire sur le champ exposé à l'action de la cathode seuls les cils du corps battent en effet vers le front du Cilié, tandis que la ciliature du péristome s'en oppose. On a reussi à observer et à enregistrer cette asymétrie. Dans ce but on incubait d'abord les Paramécies dans l'hydrate de chloral, mais seulement durant 24 h., ce qui affaiblissait seulement la ciliature. Après, on les passageait à une suspension de la graisse du lait avec de la methylcellulose. Dans ces conditions la locomotion galvanotactique vers la cathode est tellement ralentie qu'on peut même employer la technique d'enregistrement microphotographique des courants d'eau autour du Cilié (Grębecki 1961).

[2] Le montage employé pour produire la galvanotaxie était le même que dans les études précédentes (Grębecki 1962, 1963 a et b).

L'asymétrie du rebroussement cathodique n'est pas facile à apercevoir, parce que son apparence dépend de la position du Cilié par rapport à l'observateur. Presque toujours on ne voit, sur des contours de l'animal, que des cils somatiques, et leur rebroussement cathélectrotonique semble entièrement symétrique (Fig. 8 A). Cependant, quand sur le contour de l'animal roulant autour son axe, apparaissent les cils péristomaux, on aperçoit une nette asymétrie motrice (Fig. 8 B). En dépit du battement inversé de la ciliature générale, les cils du péristome battent toujours vers l'arrière (vers la bouche). On peut employer des courants extrêmement forts, mais on ne reussit jamais à diriger le travail ciliaire du péristome vers le bout antérieur du corps.

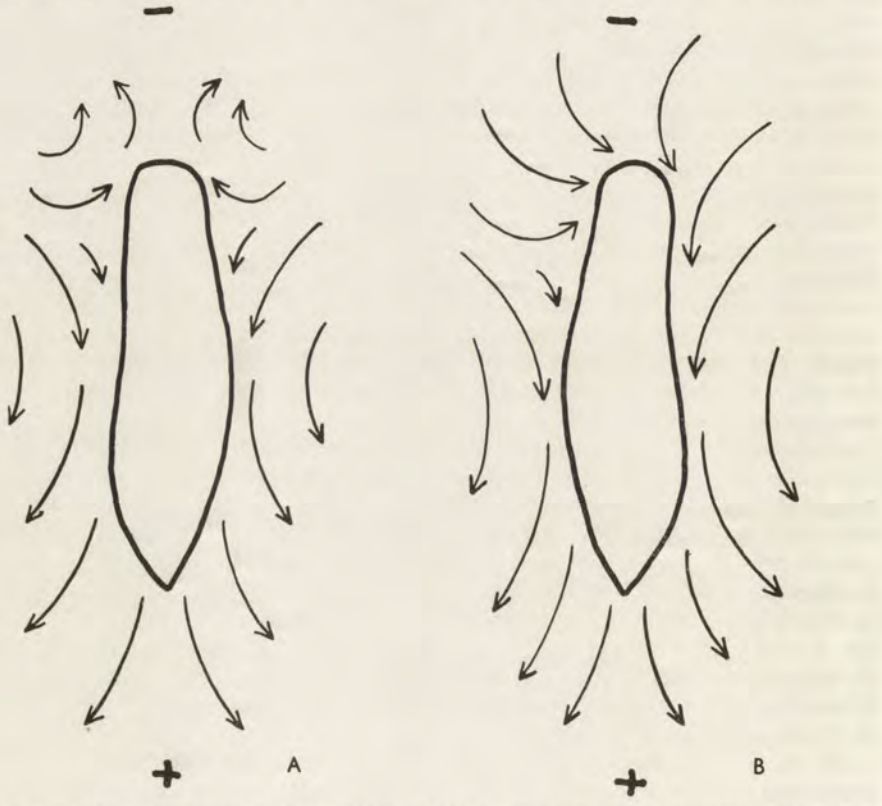

Fig. 8. Diagramme démontrant l'asymétrie motrice pendant un rebroussement cathodique (effet de Ludloff). A. Les courants symétriques entourant une Paramécie vue du côté dorsal. B. L'asymétrie des courants quand la Paramécie est tournée vers l'observateur par son côté "latéral"

La Planche VI 19 apporte un enregistrement du rebroussement cathodique typique, dressé de façon schématique par plusieurs chercheurs depuis sa découverte par L u d l o f f 1895. Il est entièrement symétrique, car la Paramécie — dans ce cas — est tournée vers l'observateur presque exactement de son côté dorsal. Les photographies suivantes (Pl. VI 20—VII 22) démontrent que, quand la Paramécie tourne, l'asymétrie dorso-ventrale devient de plus en plus visible, jusqu'au degré maximal schématisé par la Fig. 8 B.

L'asymétrie du rebroussement cathodique, demontrée ci-dessus, doit provoquer des déviations de l'axe du mouvement avançant vers la cathode. Si la Paramécie nage librement dans son milieu, la rotation du corps prévient un changement général de la direction du mouvement qui reste donc toujours cathodique. Cependant, l'asymétrie motrice se manifeste en tant que deformation de l'allure hélicoïdale de la trajectoire parcourue, le Cilié même tournant toujours son côté oral un peu vers la cathode (Fig. 9). Étant donné que les battements des cils péristomaux ne se trouvent jamais dirigés vers

Fig. 9. Diagramme dressé d'après un enregistrement cinématographique, prouvant qu'en effet de cette asymétrie motrice, le péristome balance restant exposé toujours vers la cathode

le front, l'augmentation de l'intensité du stimulant ne peut que renforcer l'asymétrie de la force motrice. En conséquence, la sinusoïté des trajets devient de plus en plus prononcée (Pl. IV 15—V 17). Il est évident que ces changements ne sont autre chose que les stades de Statkewitsch 1904, inexpliqués jusqu'à présent, qui deviennent donc aisément compréhensibles en tant qu'effet d'une asymétrie motrice resultant de la disposition stomato-caudale du gradient de l'excitabilité.

Orientation dans le champ

Les effets de l'asymétrie de l'effet de Ludloff sont différents quand le Cilié soumis à l'action d'un champ électrique ne tourne pas autour de son axe. Au premier coup d'oeuil il semble qu'il doit s'orienter obliquement ou même perpendiculairement par rapport au champ, avec son côté oral tourné vers la cathode et le côté dorsal — vers l'anode. Cela arrive en effet si la Paramécie nage près du fond de la cuve sans rotations. Ce phénomène est connu depuis Pütter 1900, pour les Paramécies et surtout pour des nombreuses hypotriches, bien qu'il était expliqué par l'interférence de la thigmotaxie. En effet il est expliqué par le caractère stomato-caudal du gradient de l'excitation.

D'autre part, en force de l'asymétrie considerée, la Paramécie devrait être plus capable à se réorienter si elle se trouvait tournée vers la cathode par son côté dorsal, et moins capable — si elle exposait à cette électrode son côté oral. En effet, une telle différence de la réponse a été rapportée déjà par Roesle 1903. En conséquence, les détours de la Paramécie, s'orientant dans le champ lors de la fermeture du circuit, devraient s'effectuer le plus souvent par un pivotement vers le côté dorsal, ce qui a été également noté à maintes reprises (Jennings 1906, Viaud et Bonaventure 1956, Grębecki 1962).

En résumant, on peut constater que l'asymétrie motrice de la réponse à l'excitation électrotonique explique quelques aspects du comportement

galvanotactique, inexplicables par le simple gradient antério-postérieur, à savoir: 1. la nature de la sinusoïté du mouvement dirigé vers la cathode et ses changements, 2. les différences de reponse du côté oral et dorsal, 3. l'orientation oblique des Ciliés restant sans rotations sur le substrate.

Remarques générales

Rapports structurels

La théorie du gradient stomato-caudal trouve un appui dans les études sur les gradients morphogénétiques. Il est généralement admis que le champ oral est un centre organisant la morphogenèse chez les Ciliés, tandis que le bout antérieur ne joue aucun rôle dans ces processus. L'exemple peut-être le plus frappant de l'accord des deux gradients est fourni par l'étude de Totwen--Nowakowska 1963 sur le régénération du segment caudal de la Paramécie. Il y fut démontré que le primordium d'un nouveau faisceau de cils caudaux se forme au bord dorsal de la blessure, c'est-à-dire asymétriquement et justement — à l'endroit le plus éloigné de la bouche.

Cependant, il est impossible d'indentifier le cours du gradient stomato-caudal avec le cours des cinéties ou des fibrilles soupelliculaires. L'opinion de Párducz 1958, indiquant que la propagation des ondes n'est pas étroitement liée au système fibrillaire, semble bien justifiée.

Prenant en consideration toutes les données contemporaines sur l'electrophysiologie des Protistes, il faut admettre que les ondes métachroniques parcourant la surface ciliée résultent des impulses électriques, probablement analogues (ou plutôt homologues) au caractère des impulses nerveux parcourant l'axon. De ce point de vue, il semble le plus raisonnable de supposer qu'ils sont conduits par le membrane cellulaire même, comme c'est le cas chez toutes les cellules excitables. Probablement donc, le gradient stomato-caudal d'excitabilité des Ciliés est basé sur un gradient parallèle des propriétés bioélectriques de la membrane. L'existence chez les Ciliés de gradients qui peuvent être liés aux paramètres bioélectriques résulte même du travail classique de Child 1914. La dislocation stomato-caudale du gradient de la perméabilité et du potentiel de surface chez la Paramécie a été supposeé par l'auteur déjà plus tôt (Grębecki 1963 c).

A première vue, l'idée de la direction stomato-caudale du gradient d'excitabilité semble réactiver l'hypothèse du "neuromotorium" de Rees 1922, Gelei 1936 et Klein 1942. En effet, il semble intéressant de concevoir s'il s'agit seulement de deux gradients bipolaires opposés, ou bien — de deux centres coordinateurs définis, agissant en concurrence l'un à l'autre.

Il est à présumer qu'après la destruction de la région d'origine des impulses, leur génération est reprise par le champ voisin, si on descend seulement le cours d'un gradient, ou bien qu'elle est complètement interrompue, si la région initiante est un centre différencié. Partant de ce principe on essayait d'évoquer le rebroussement ciliaire avec du KCl chez les fragments des *Paramecium* et des *Prorodon* sp., découpés à différents niveaux. Toujours un rebroussement ciliaire se produit; chez tous les fragments des *Prorodon* et presque tous des *Paramecium* il en résulte une nage à reculons; seulement chez les morceaux antérieurs des Paramécies le rebroussement, grâce à l'asymétrie motrice évoquée par les cils péristomaux, se manifeste en forme de rotations. Les résultats sont donc conformes aux données ultérieures (Wor-

ley 1934, Milicer 1935, Doroszewski 1958), en prouvant que l'activité ciliaire de la Paramécie n'est pas contrôlée par un "neuromotorium" ni par d'autres centres différenciés. Pour les *Paramecium* et les *Prorodon* le gradient stomato-caudal d'excitabilité est un gradient proprement dit.

Cependant, chez les *Dileptus* la situation semble différente. Doroszewski 1963 prouve qu'après la coupure, la partie antérieure ne peut répondre au stimulant que par un recul, et la postérieure — par contre — manifeste seulement des poussées en avant. Ces défauts d'excitabilité disparaissent avec la régéneration des structures découpées. Les *Spirostomum*, après l'amputation de la région antérieure, sont également incapables de nager à reculons (Seravin 1962 b).

En comparant les résultats concernant les *Prorodon* et *Paramecium* d'une part, et les *Dileptus* et *Spirostomum* de l'autre, on ne peut qu'accepter la vue de Seravin 1962 a, à savoir que dans les différentes lignes phylogénétiques des Ciliés, soit l'évolution préserve la plasticité régulatrice en laissant aux deux pôles le caractère de deux extrémités du gradient, soit elle transforme les deux pôles du gradient en centres différenciés. Or, dans les deux cas la polarité conserve son caractère stomato-caudal.

Phylogenèse du gradient

Il semble généralement admis, d'après Fauré-Fremiet 1950 et Corliss 1956, que le type primitif des Ciliés est représenté par des formes radialement symétriques, avec la cavité buccale disposée apicalement, telles comme le sont certaines *Rhabdophorina* modernes. Considérons le cours possible du gradient d'excitabilité dans la ciliature d'un tel Cilié.

Il est évident que dans ce cas la notion du simple gradient antério-postérieur est l'unique idée qu'on peut s'imaginer. Si on admet (ce qui est le plus

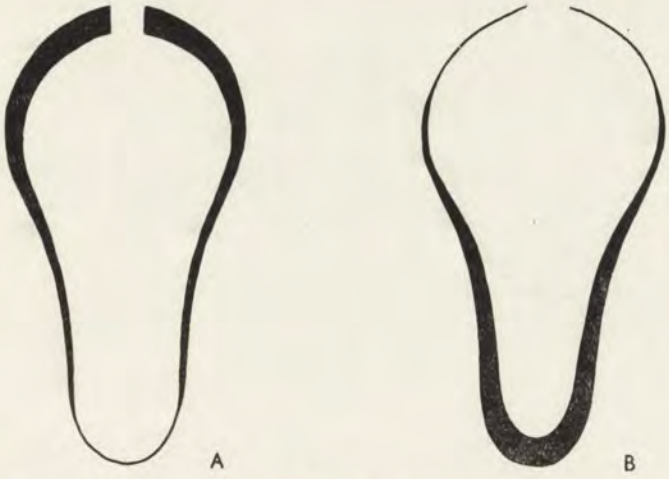

Fig. 10. Direction probable du gradient d'excitabilité à la surface d'un Cilié primitif, entièrement symétrique. A. Gradient de sensibilité cathélectrotonique. B. Gradient de sensibilité anélectrotonique. (Contour dressé en prenant pour modèle la forme de *Prorodon viridis*, d'après Fauré-Fremiet 1924)

probable) que l'origine caudale des ondes métachroniques normales et l'origine antérieure des ondes rebroussées, établie par Párducz 1954 (*Paramecium, Colpidium*), Seravin 1962 b (*Spirostomum*) et Párducz 1964 (*Ophryoglena*), est le type principal, sinon général, de la coordination ciliaire — on peut présumer l'existence d'une bipolarité symétrique de l'excitation chez un Cilié primitif. On peut s'attendre à ce que la tendance au rebroussement ciliaire (l'excitabilité cathélectrotonique) tombe symétriquement vers l'arrière du corps, tandis que la tendance à reproduire le battement normal (l'excitabilité anélectrotonique) augmente régulièrement dans cette direction (Fig. 10 A—B).

Or, il faut se rendre compte que le gradient antério-postérieur, à la surface d'un tel Cilié primitif, est de même stomato-caudal, car l'axe apex—extrémité distale est identique à l'axe os—cauda. Cependant, au cours d'évolution des Ciliés le cytostome est graduellement repoussé à côté du corps; en effet ce côté s'avère oral, c'est-à-dire la symétrie radiale s'efface en consequence d'une différentiation dorso-ventrale. Autrement dit, la direction antério-postérieure n'est plus synonyme à la direction stomato-caudale.

Quelle direction s'avère alors plus importante quand les deux axes bifurquent, ce qui est le cas de la plupart des Ciliés spécialisés? Toute notre connaissance de la morphologie des Ciliés semble indiquer que l'axe stomato-caudale est une ligne principale organisant le corps, profondement engendrée du point de vue phylogénétique, tandis que l'orientation de l'axe antério-postérieure ne constitue qu'un caractère secondaire portant des traits d'une

Fig. 11. Courants d'eau entourant un individu du *Prorodon* sp. pendant son mouvement à reculons, provoqué par le KCl

adaptation fonctionnelle. Pour cette raison, le gradient d'excitabilité chez la Paramécie reste stomato-caudal, ce qui produit des asymétries motrices par rapport à l'axe antério-postérieur.

En vue de vérifier ces considérations, on observa le comportement des *Prorodon* sp. (*Rhabdophorina*). Malheureusement, l'espèce employée n'était

pas entièrement symétrique, sa bouche étant située d'un côté d'une convexité renforcée des trichites. Pourtant on a constaté que chez ces Ciliés: 1) l'allure hélicoïdale de la trajectoire parcourue est très peu accentuée, car c'est seulement la convexité apicale qui balance en se déviant de l'axe de la locomotion; 2) le même fait on observe pendant une nage à reculons dans des solutions du KCl; 3) le courant alimentaire produit près de la bouche est très faible (Fig. 11), la capture des particules semble donc, dans ce cas, encore principalement liée à la nage progressive; 4) les altérations de la sinusoïté du mouvement pendant la galvanotaxie ne sont qu'à peine visibles; 5) l'image des ondes métachroniques est encore identique aux deux surfaces du corps — ils se propagent partout en avant pendant le mouvement normal, et partout en arrière au cours du rebroussement ciliaire (Fig. 12).

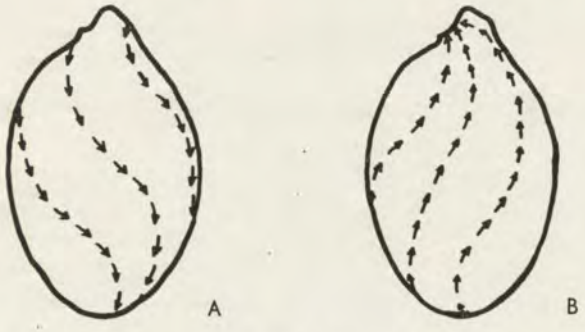

Fig .12. Diagramme de la dislocation des ondes métachroniques et de la direction des battements ciliaires effectifs chez les *Prorodon* sp. pendant la locomotion normale (A) et le mouvement à reculons (B)

L'étude du comportement des *Prorodon* confirme donc la thèse que le gradient stomato-caudal d'excitabilité des Ciliés est d'un caractère primaire au point de vue phylogénétique. Cette conclusion s'accorde bien également avec la dernière étude de P á r d u c z 1964, démontrant que chez les *Ophryoglena* (*Tetrahymenina*) l'hélice semble souvent plus allongée que chez les Paramécies, et qu'elles peuvent parfois nager même en ligne droite (en rotant toujours), ce qui n'arrive jamais chez les Paramécies. La différentiation dorso-ventrale chez les *Ophryoglenidae* est déjà marquée, néanmoins la cavité buccale des *Ophryoglena* n'est pas repoussée sur la côté si fortement que chez les *Paramecium*.

Caractère adaptatif du gradient

La disposition stomato-caudale du gradient d'excitabilité des Paramécies explique certaines asymétries de leur comportement dans le champ électrique. Il faut pourtant admettre que les stimulants électriques, de tel genre qu'on emploit pour provoquer la galvanotaxie, n'ont pas d'importance biologique. La stimulation électrique n'est plutôt qu'un moyen extrêmement favorable pour l'étude des régularités fondamentales de l'excitation.

Or, les stimulants mécaniques et chimiques sont des signaux de nourriture ou de danger dans des conditions naturelles. La théorie du gradient stomato-caudal permet de dresser une image conséquente du comportement des Ciliés par rapport à ces stimulants.

La ciliature des Paramécies nageant dans de l'eau pure, sans suspensions, bien aérée, est entièrement subordonnée à des ondes provenant de la région caudale, c'est-à-dire des ondes NW—SE sur le corps et des ondes "concentriques" dans péristome. En effet, le Cilié avance vite et ne produit pas de courant alimentaire. C'est justement ce qui est le plus opportun dans un endroit aride.

Quand la présence d'une suspension, ou des certains ions dans le milieu, signale la proximité de la nourriture, l'excitation commence à se développer à partir du cytostome. Si le stimulant est faible, elle ne saisit que les cils péristomaux qui forment alors des ondes "radiales" et battent vers la bouche. En conséquence, le Cilié avance toujours, mais avec un courant alimentaire qui se produit dans le péristome; c'est la situation montrée sur le fameux

Fig. 13. Paramécie manifestant le mouvement progressif avec un courant alimentaire (d'après Jennings 1906 — simplifié)

dessin de Jennings 1906 (Fig. 13), indiquant que la Paramécie peut entraîner vers la bouche des particules flottant devant elle. La transformation du battement ciliaire dans le péristome ralentit aussi le mouvement progressif et augmente le balancement du péristome, les deux phénomènes étant avantageux, à la proximité de la nourriture.

Quand le stimulant devient plus fort, l'excitation dépasse le péristome, traverse le champ frontal et produit des battements rebroussés à la région

Fig. 14. Paramécie décrivant de grands cercles avec le péristome et manifestant un fort courant alimentaire, sans avancer ni reculer (d'après Jennings 1906 — simplifié)

antérieure de la face dorsale. En effet, la locomotion devient extrêmement ralentie, entièrement entravée, ou légèrement inversée. Le courant alimentaire persiste, en amenant la suspension vers la bouche et en faisant le péristome décrire des larges cercles. C'est le phénomène connu depuis Jennings 1906 sous le nom des "mouvements de recherche" (Fig. 14). Tout le

comportement à ce degré de l'excitation contribue à l'absorption maximale de la nourriture[3].

Si le stimulant est encore plus intense, l'excitation progresse encore plus loin le long du gradient stomato-caudal, c'est-à-dire le rebroussement ciliaire saisit toute, ou presque toute, la ciliature du corps. En conséquence, le Cilié effectue un bref recul, la réponse de fuite bien connue ("avoiding reaction" de Jennings 1906). C'est justement ce qui est nécessaire, car le stimulant d'une intensité hypernormale peut être nocif. D'ailleurs, la chimiotaxie est basée sur cette réponse.

Il arrive parfois que le stimulant soit extrêmement fort. Dans ce cas l'excitation, débutant de la bouche, produit aussi l'inversion du travail de toute la ciliature du corps, mais au lieu d'un recul bref, le mouvement à reculons se produit durant plusieures secondes ou même minutes. C'est le phénomène connu depuis Mast and Nadler 1926, sous le nom du rebroussement ciliaire ("ciliary reversal"). Sa durée dépend de l'intensité du stimulant, ce qui est aussi avantageux, car en effet le Cilié se retire à une distance plus longue si l'excitation est plus intense.

Après s'être retirée de la zone nocive, la Paramécie reprend la direction normale de son mouvement; c'est le milieu normal qui joue maintenant le rôle de stimulant de nature anélectrotonique, renormalisant le battement ciliaire. La renormalisation progresse donc probablement le long du gradient stomato-caudal à l'inverse: de la région postérieure jusqu'à la bouche. Il en résulte le fait couramment visible que la Paramécie en renormalisant sa locomotion passe à rebours par tous les stades de l'excitation énumérés ci-dessus. Elle nage à reculons de plus en plus lentement; elle s'arrête en décrivant de larges cercles par le péristome; elle se met à avancer d'abord lentement, en balançant et en manifestant le courant alimentaire; enfin elle peut reprendre le mouvement progressif bien prononcé.

En résumant, on peut constater que le mouvement normal, la progression ralentie avec un courant alimentaire, l'arrêt et les mouvements de recherche durant la capture de la proie, le recul bref de fuite, et le mouvement à reculons, basent sur un principe commun. Chaque réponse ne correspond qu'à un autre degré de l'excitation progressant le long du gradient stomato-caudal. Autrement dit, chaque niveau de l'excitation provoque une telle réponse qui est justement requise dans la situation rencontrée, ce qui met en évidence le sens adaptatif du gradient.

Résumé

Chez les Ciliés primitifs, conservant la symétrie radiale, le gradient d'excitabilité est antério-postérieur et stomato-caudal à la fois. Quand le cytostome est repoussé une différentiation dorso-ventrale apparaît, et le gradient d'excitabilité reste stomato-caudal, ce qui produit alors des asymétries motrices par rapport à l'axe antério-postérieure.

Chez la Paramécie, la sensibilité des cils au rebroussement (excitabilité cathélectrotonique) est maximale à la région buccale et elle decroît graduellement vers la région caudale, en encerclant seulement le champ frontal.

[3] Il arrive souvent, durant la capture de la proie, que la Paramécie tombe au fond du récipient et y reste presque entièrement immobile ne manifestant que le courant alimentaire. Dans ce cas donc, intervient un nouveau facteur, très peu connu — la thigmotaxie, qui n'est pas prise en considération dans l'étude présente

Le battement ciliaire dirigé directement vers la bouche est donc justement le symptôme du rebroussement dans le peristome. La tendance à produire le battement normal (excitabilité anélectrotonique) suit un gradient exactement inverse. En effet ce n'est qu'à la surface dorsale que les gradients coïncident avec l'axe antério-postérieur. Les ondes métachroniques du battement normal et rebroussé sont propagés le long de ces gradients, à partir de la région caudale ou buccale, respectivement.

La disposition stomato-caudale des gradients d'excitabilité explique les asymétries de la réponse et les altérations du caractère hélicoïdal du mouvement dans les champs électriques. De plus, elle offre une explication commune du mécanisme du mouvement normal, de la locomotion ralentie avec des courants alimentaires, de l'arrêt et des mouvements de recherche près de la nourriture, des reculs brefs de fuite, et du mouvement à reculons. Toutes ces réposes, avantageuses du point de vue écologique, ne sont évoquées que par différents degrés de l'excitation progressant le long du gradient stomato-caudal.

STRESZCZENIE

U orzęsków prymitywnych, które zachowują symetrię promienistą, gradient pobudliwości jest zarazem przodo-tylny i gębowo-ogonowy. Gdy w toku ewolucji cytostom zostaje przesunięty na bok ciała i pojawia się zróżnicowanie grzbietobrzuszne, gradient pobudliwości pozostaje gębowo-ogonowy, co wywołuje asymetrie motoryczne w stosunku do osi przód-tył.

U *Paramecium* wrażliwość rzęsek na rewersję (pobudliwość katelektrotoniczna) jest najwyższa w okolicy gębowej i stopniowo spada ku okolicy ogonowej, okalając jedynie powierzchnię przednią. Uderzenia rzęsek skierowane wprost ku gębie są więc właśnie przejawem rewersji w peristomie. Tendencja do odtwarzania normalnej pracy rzęsek (pobudliwość anelektrotoniczna) określona jest gradientem ściśle przeciwstawnym. W wyniku tego, tylko na powierzchni grzbietowej gradienty przebiegają zgodnie z osią przód-tył. Fale metachroniczne ruchu normalnego i rewersyjnego rozchodzą się wzdłuż tych gradientów, odpowiednio poczynając od okolicy ogonowej albo od gębowej.

Gębowo-ogonowy rozkład gradientów pobudliwości tłumaczy asymetrie reakcji i zakłócenia śrubowego charakteru ruchu w polach elektrycznych. Co więcej, daje on jednolite objaśnienie mechanizmu ruchu normalnego, ruchu zwolnionego z jednoczesnymi prądami pokarmowymi, zatrzymania się i ruchów poszukiwawczych w sąsiedztwie pokarmu, krótkich reakcji ucieczki, oraz ruchu wstecznego. Wszystkie te reakcje, korzystne z ekologicznego punktu widzenia, odpowiadają tylko różnym poziomom pobudzenia, które rozprzestrzenia się wzdłuż gradientu gębowo-ogonowego.

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EXPLICATION DES PLANCHES I—VII

1: Enregistrement microphotographique du courant alimentaire manifesté par la Paramécie avec 10 mM $CaCl_2$

2: Enregistrement microphotographique prouvant que le courant alimentaire est fortement accentué dans les conditions du rebroussement ciliaire (32 mM KCl + 1 mM $CaCl_2$); vue du côté dorsal

3: Le même, vue du côté "latéral"

4: Disposition „radiale" des ondes métachroniques dans le péristome pendant le mouvement progressif en présence d'une suspension de l'encre de Chine. C — le cytostome

5: Comparaison de l'allure hélicoïdale des trajets parcourus par les mêmes individus dans une suspension de l'encre de Chine (le compartiment central) et avant l'addition de la suspension; la présence des particules altère la sinusoïté des trajectoires

6: Disposition "concentrique" des ondes métachroniques dans le péristome du *Paramecium caudatum* pendant le mouvement progressif fortement accentué par la présence de 10 mM $CaCl_2$ dans le milieu

7: Préparation analogue, fortement aggrandie pour démontrer que dans les ondes "concentriques" les cils ne battent pas vers le cytostome, mais l'encerclent

8: La disposition "radiale" des ondes metachroniques dans le péristome pendant le mouvement à reculons provoqué par 32 mM KCl (+ 1 mM $CaCl_2$)

9: Une préparation analogue, fortement aggrandie pour démontrer que dans les ondes "radiales" les cils battent vers le cytostome en amenant un courant alimentaire à la bouche

10: Enregistrement macrophotographique des premières 5 sec. d'un mouvement à reculons, très bien prononcé, évoqué par 32 mM KCl (+ 1 mM $CaCl_2$); trajets fortement aggrandis pour démontrer que le péristome balance en se déviant de l'axe du mouvement

11: Disposition "radiale" des ondes visualisée sur toute la région antérieure de la Paramécie manifestant un rebroussement chimique. Monté des trois poses pour faire tout le plan également net

12: Ciliature péristomale pendant le rebroussement chimique, vue du profil. Les battements effectifs dirigés vers la bouche, en bas (E), et les cils dans le stade de retour, en haut (R)

13: Comparaison de l'allure hélicoïdale des trajets parcourus par les mêmes individus dans un champ électrique (le compartiment central) et avant la fermeture du circuit (l'encadrement); au dessous du seuil de la galvanotaxie (0.075 mA/cm^2) la sinusoïté des trajets reste encore intacte

14: Le même avec 0.150 mA/cm^2 — au seuil de la réponse galvanotactique; la sinusoïté n'est pas visiblement altérée

15: Le même avec 0.300 mA/cm^2; la sinusoïté commence à changer

16: Le même avec 0.600 mA/cm^2; sinusoïté fortement altérée

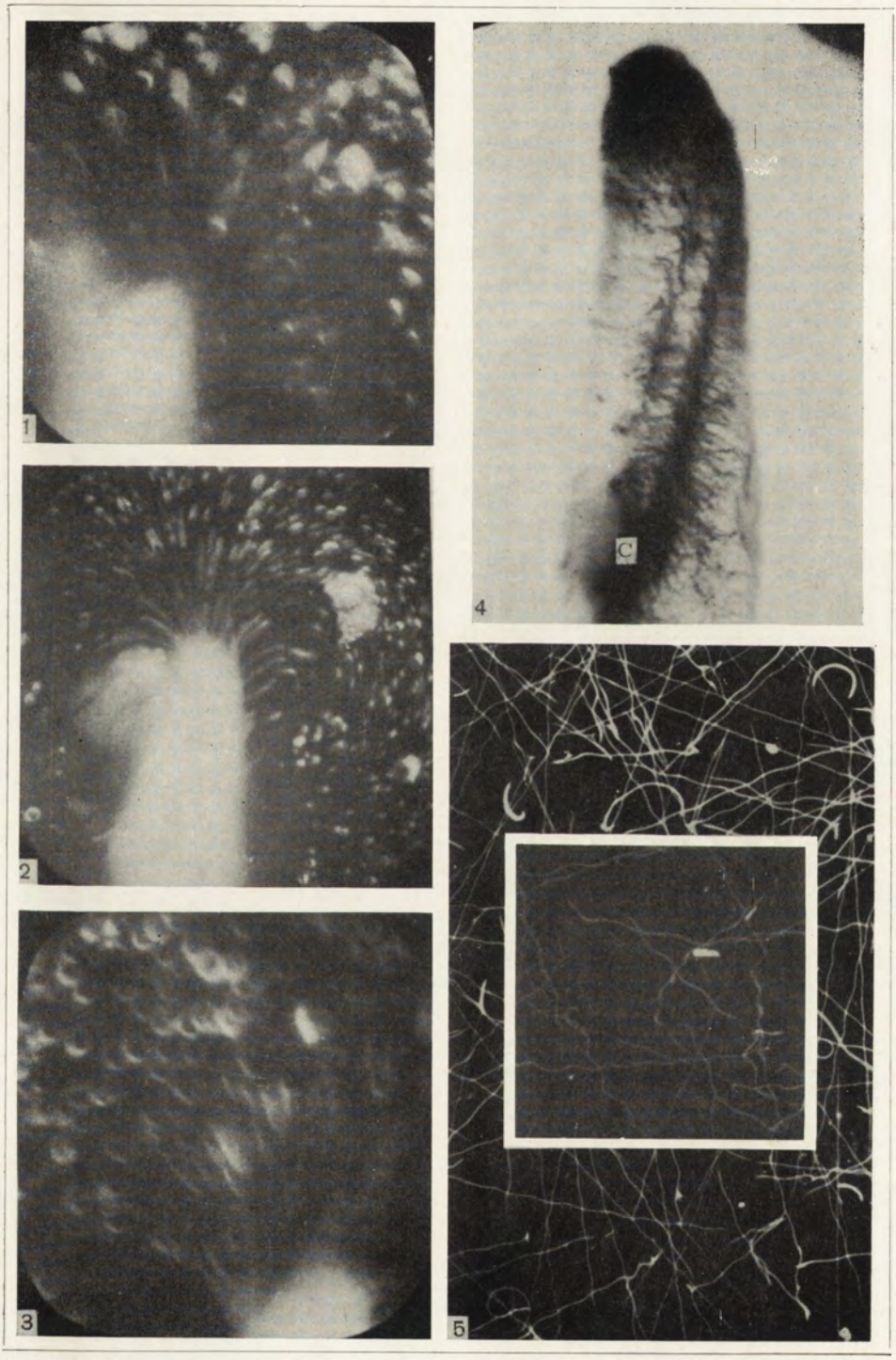
17: Le même avec 1.200 mA/cm^2; une considérable altération de la sinusoïté persiste

18: Le même avec 2.400 mA/cm^2; la sinusoïté change son caractère encore une fois, car le ralentissement du mouvement galvanotactique raccourcit le pas de l'hélice

19: Enregistrement microphotographique des courants d'eau entourant le Cilié pendant le mouvement galvanotactique; les courants correspondent au rebroussement cathodique des cils (effet de Ludloff classique); il est symétrique car la Paramécie est vue exactement du côté dorsal

20: Le même chez la Paramécie tournée un peu sur un côté; la symétrie des courants s'efface un peu

21—22: Deux enregistrements démontrant une nette asymétrie des courants quand la Paramécie, en nageant vers la cathode, expose à l'observateur son côté "latéral"



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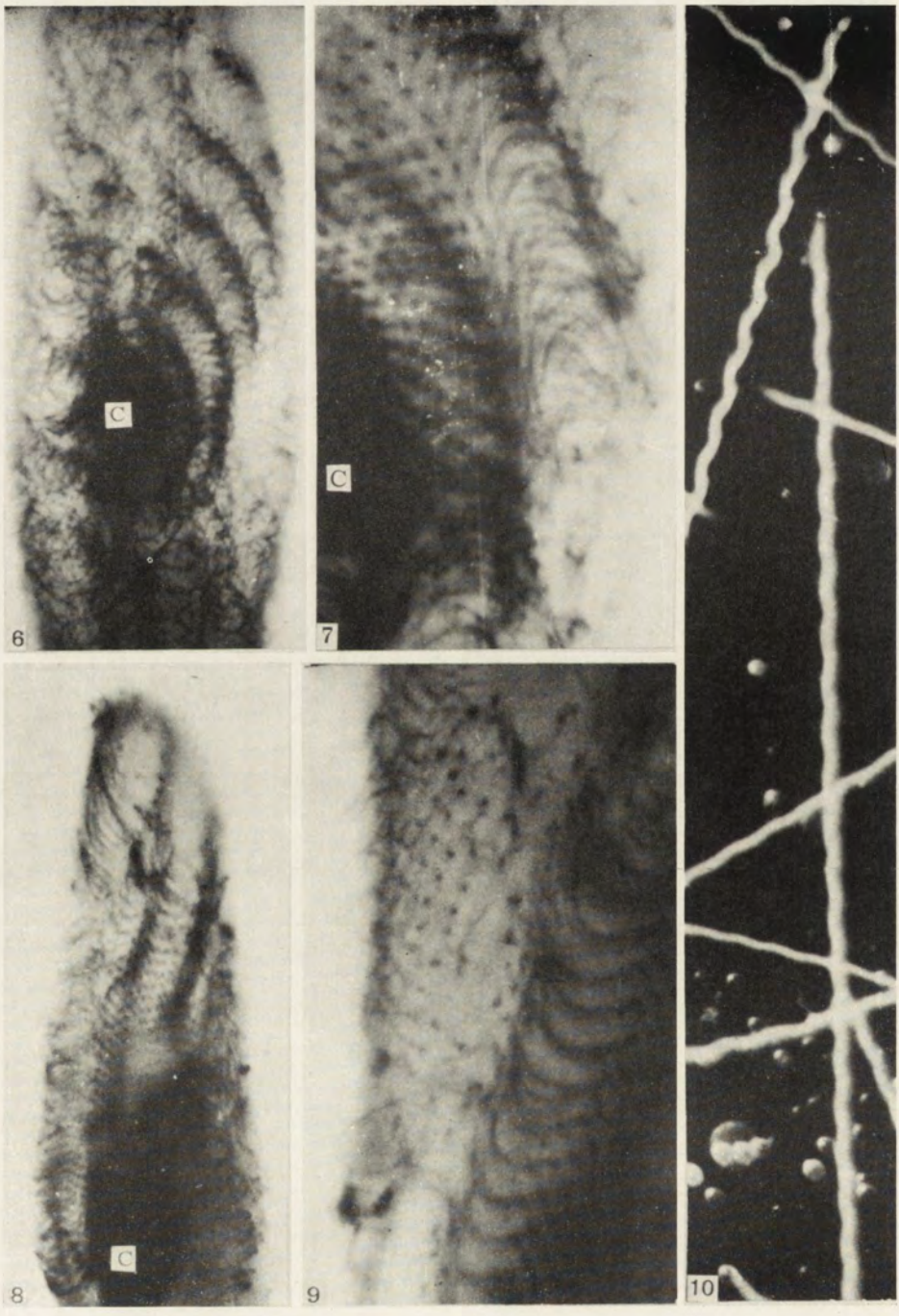

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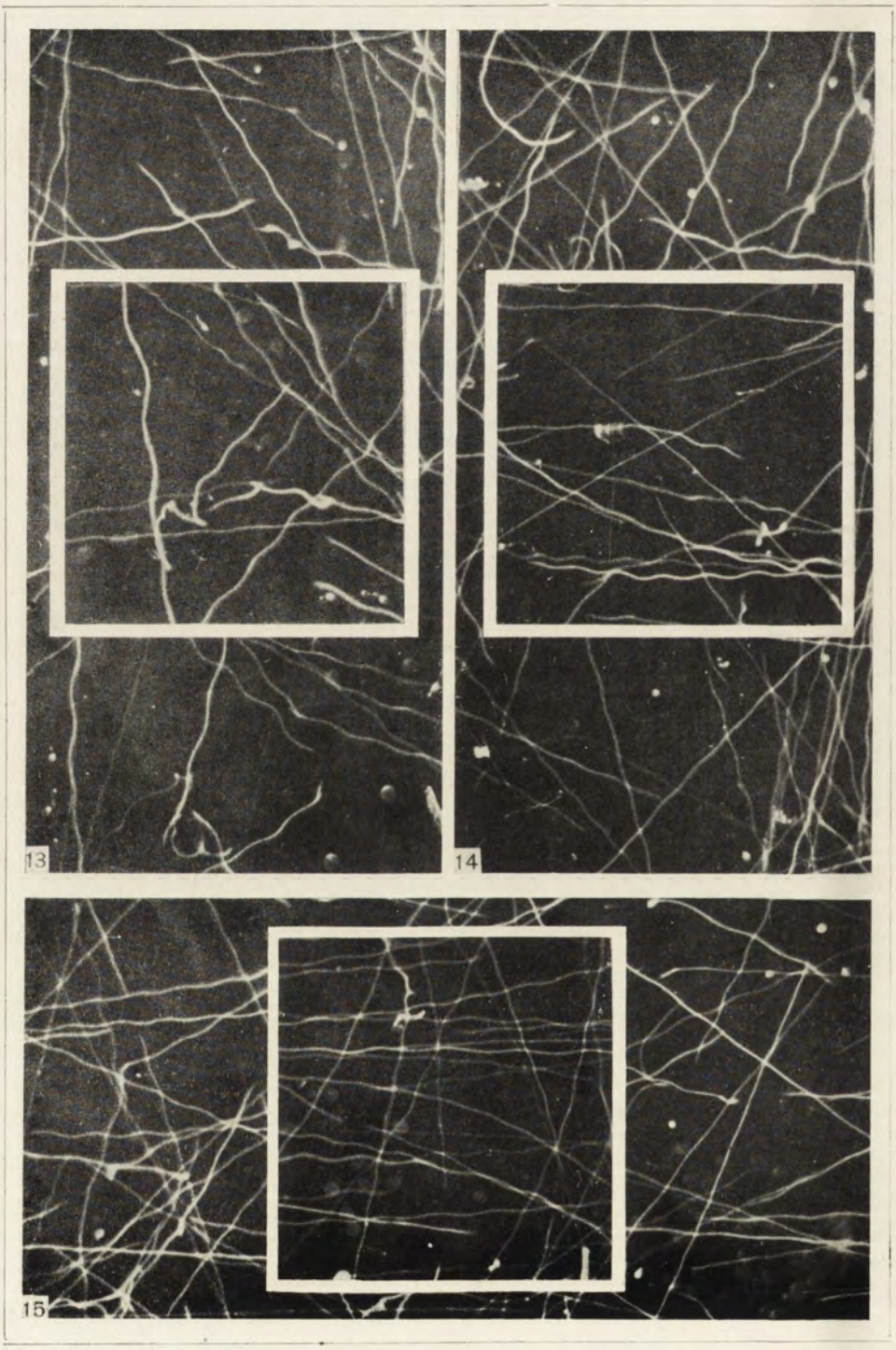

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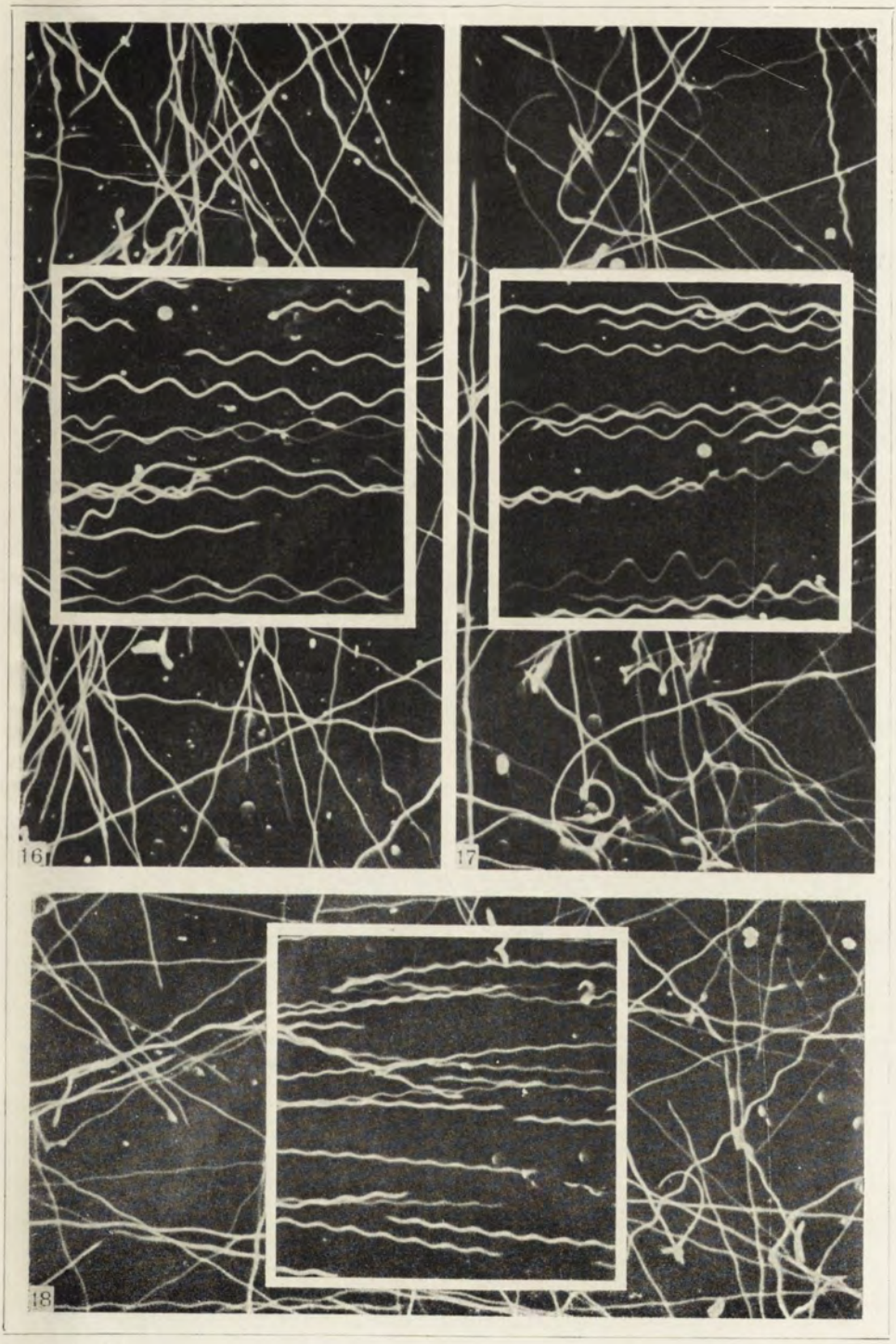

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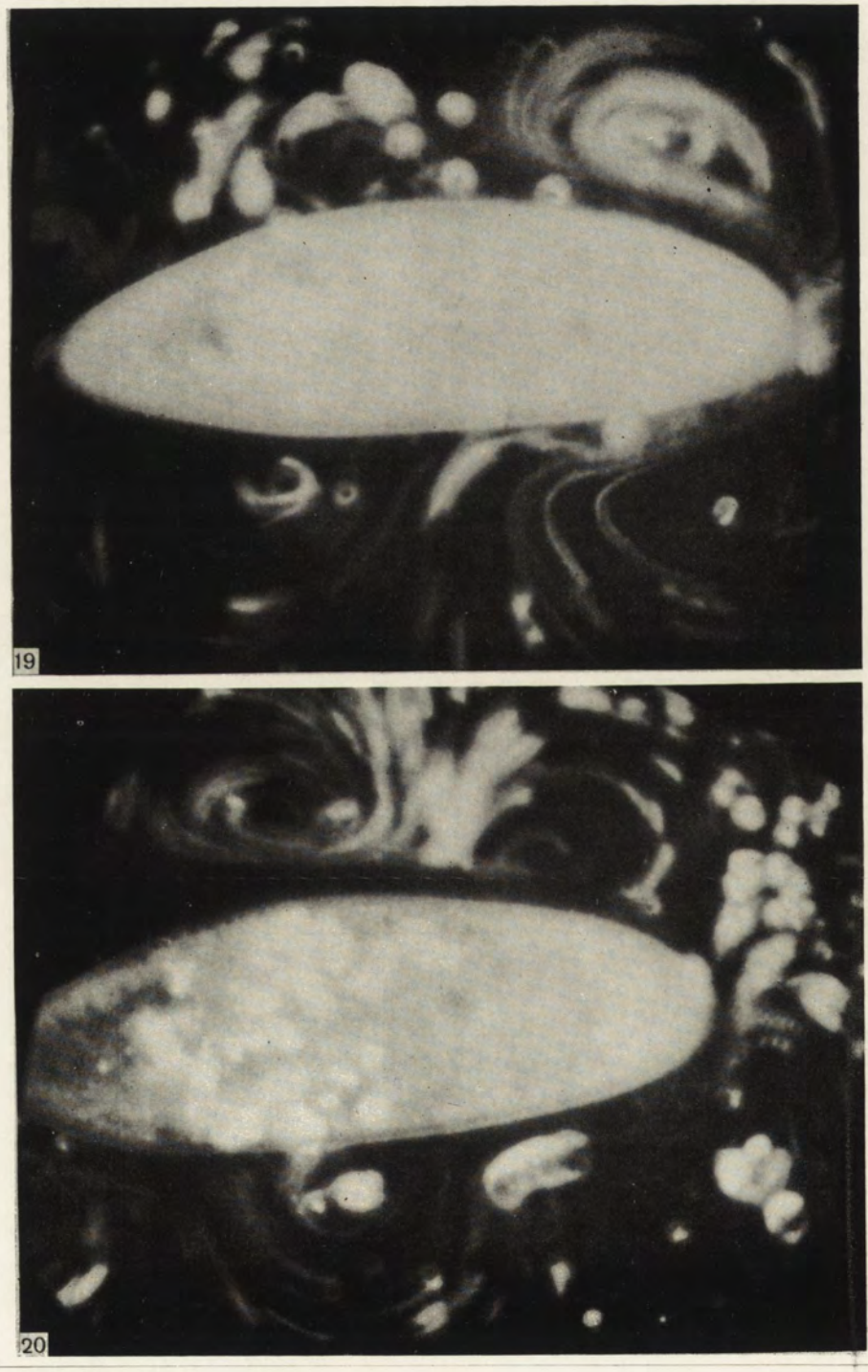

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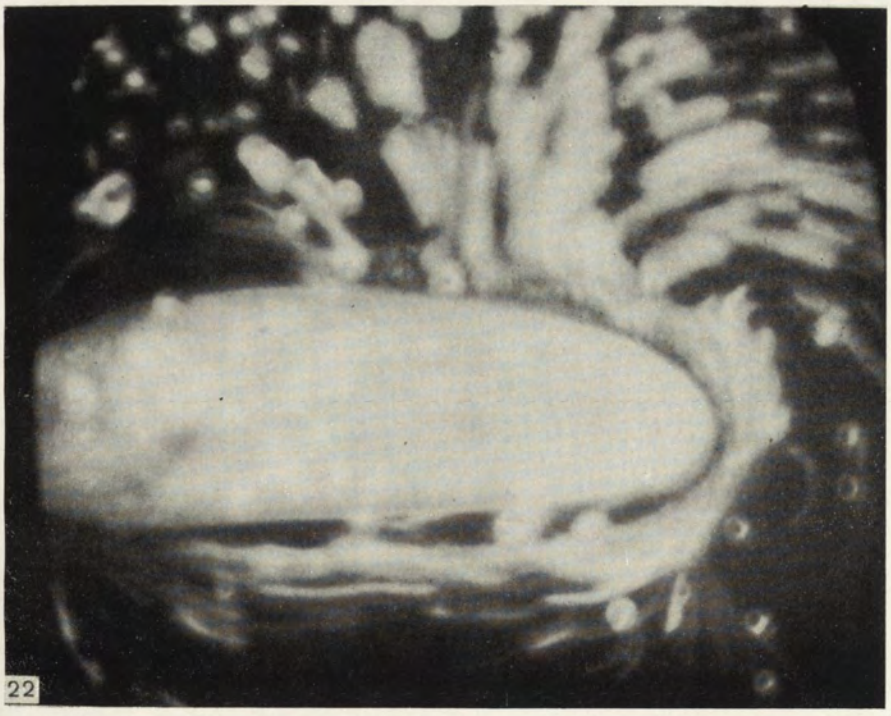
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