ACTA PROTOZOOLOGICA

VOLUMEN I

Fasciculi: 25—30

W A R S Z A W A 1 9 6 3

INSTYTUT BIOLOGII DOŚWIADCZALNEJ IM. M. NENCKIEGO
POLSKIEJ AKADEMII NAUK
ACTA PROTOZOOLOGICA

NOTICE TO AUTHORS

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

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

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

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

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

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

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

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

Galley proofs are sent to the authors. Authors receive 100 reprints without covers, free of charge.

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

ACTA PROTOZOOLOGICA

VOL. I WARSZAWA, 30. IX. 1963 FASC. 25

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

Stanisław L. KAZUBSKI

Studies on the parasitic ciliate *Thigmocoma acuminata* Kazubski (*Thigmotricha-Thigmocomidae*)

Badania nad pasożytniczym orzęskiem *Thigmocoma acuminata* Kazubski (*Thigmotricha-Thigmocomidae*)

Thigmocoma acuminata — one of several actually known ciliates, parasites of land *Gastropoda* — had been found in autumn 1957 in the Carpathian snail *Oxychilus (Cellariopsis) orientalis* (Cless.) in the environment of Sanok (south-east Poland). The description of the species was published in 1958. Attention was called to some characters different from those in other *Thigmotricha* and, therefore the separate genus and family had been established. This publication (Kazubski 1958 b) being a preliminary note, comprised only a morphological description of the trophic form without any morphogenetic, biological or ecological data of this species.

In the following years, when studying the ciliate parasites of land snails, a more extensive material was collected for the study of morphology, biology and ecology of *Thigmocoma acuminata* and the distribution of this species in its territory was investigated. The new material gave possibility to discuss again the systematic specifity of the family *Thigmocomidae* Kazubski, 1958 in the system of *Thigmotricha*.

I wish to express my hearty gratitude to Prof. Dr. Z. Raabe for his guidance of my research and for his valuable suggestions and generous help in the course of my protozoological investigations. My thanks are also due to Prof. Dr. W. Michajłow for his constant care of my study carried out in the Department of Parasitology of the Polish Academy of Sciences. I also wish to record my thanks to Dr. Z. Zarzycki from the Institute of Botany in Kraków for his botanical and to Dr. A. Riedel for malacological consultations and for having given me access to the malacological collection of the Zoological Institute of the Polish Academy of Sciences in Warszawa.

Material and methods

By means of the method of parasitological dissection, 5451 specimens of snails from the territory of Poland were studied. The snails belonging to 92 species represented almost all the families occurring in Poland. In this number 2531 specimen belonging to 71 species [1] originated from the Carpa-

[1] In Poland 151 species are known to occur; in the Polish Carpathians and in adjacent territories — 117 species of land snails have been reported.

thian territory and 80 specimens were collected in the Transcarpathian district of Ukraina. Dissection of metanephridium and of ureter of some snail specimens of the genus *Oxychilus* Fitz. collected in the Eeastern Carpathians, in the Balkan peninsula, in the countries of the Near East and in Caucasus was executed; this last material was fixed in alcohol and stored in the Zoological Institute of the Polish Academy of Sciences.

About 300 specimens of snails belonging to the family *Zonitidae* were studied under living conditions. In this number 259 specimens belonged to the species *Oxychilus orientalis*.

Snails were collected from different sites and studied mostly on the spot, soon after collection. If transported, the material was protected against overheating, because the temperature of about 30°C involves death of some species of ciliates, among others of *Thigmocoma acuminata*.

In the laboratory, snails were placed in glass containers with some humid moss and kept in ice-chamber at the temperature of about + 7 °C. In such conditions *Oxychilus orientalis* infected with *Thigmocoma acuminata*, was kept for several months, fed with other species of snails and with *Tubifex*.

The following method of dissection was applied: the shell of the snail was removed with dissection needles and the body (whole or in parts) was slightly pressed between two glass slides. The preparation was observed under microscope (objective × 3, eyepiece × 10).

For the vital microscopic examination of the detected ciliates, an appochromatic, phase contrast and anoptral optics were used. Fixed preparations were treated with various techniques: smear preparations were fixed with the Schaudinn's fluid, and stained with iron haematoxylin of Heidenhain and with acid haemalaun of Meyer. The ciliary system was revealed after the Klein's silver nitrate impregnation.

For the study of nuclear apparatus the Zenker-Helly fixed material was stained after Feulgen method. The 6 μ sections of snail renal organ containing *Th. acuminata* were fixed in the Zenker-Helly fluid and stained with Feulgen's fuchsine and light-green. Those sections were prepared in the Laboratory of the Unicellular Organisms, of the Cytological Institute of the Academy of Sciences of USSR in Leningrad.

In all cases a possibly high number of specimens was studied in order to characterize the individual variation. The photographic method was largely applied, and gave especially good results in the study of the argentophilic system for comparison of more complex structures and for arranging them into suitable series.

From fixed snails preserved in alcohol, the ureter and "metanephridium" was taken out and fragmented with dissection needles in the material passaged in water. The ciliates found in the host were stuck to the slide with gelatin, mounted in Canada balsam and examined under phase contrast optics. Some observations concerning biology and behaviour of *Th. acuminata* were made on living ciliates in the host organism. The method of those observations (Kazubski 1961 a) consisted in illuminating the snail by a strong light beam using the low power of microscope. It may be applied only to snails with a fairly transparent shell. Moistening the shell makes the observation easier. This method gave excellent results in observation of ciliate parasites of *Oxychilus orientalis* and permitted the exact study of localization of *Th. acuminata*, of its behaviour, of timing and frequency of division etc. Infection even with

single individual of *Th. acuminata* may be detected, which is not always attainable by means of dissection.

Descriptions of field conditions and of localities, in which *Oxychilus orientalis* occurs, were performed in the course of the material collection and based on photographic records. The measurements of temperature were made with a soil thermometer 5 cm long, assuring a reading accuracy of 0.2 °C. The temperature of a spot just left by the snail was measured by putting the gauge of the thermometer into contact with it. The thermic resistivity of the snails was investigated in the Brabender o. H. (Duisburg a. Rhein) climatization chamber, type KSG. 25. B.

Morphology and morphogenesis of *Thigmocoma acuminata*

Morphology of *Th. acuminata* was discussed only in the description of the species (Kazubski 1958 b). Nevertheless this description was not full: the variability of the ciliates, their division and conjugation were not treated.

Morphology of the trophic form

Body shape

The body shape of the ciliate is very characteristic (Fig. 1, and Pl. I 1—2): like in many other *Thigmotricha* it is laterally flattened, with the mouth shifted to the ventral margin of the body. The dorsal margin is also very distinct. The anterior end of the body is round, the posterior is sharp and bent toward the dorsal side. The right of the ciliate is slightly convex, whereas the left — directed to the substrate — is slightly concave. The broadest part of the body as well as the center of the concavity located at a distance of $^1/_3$ of the body

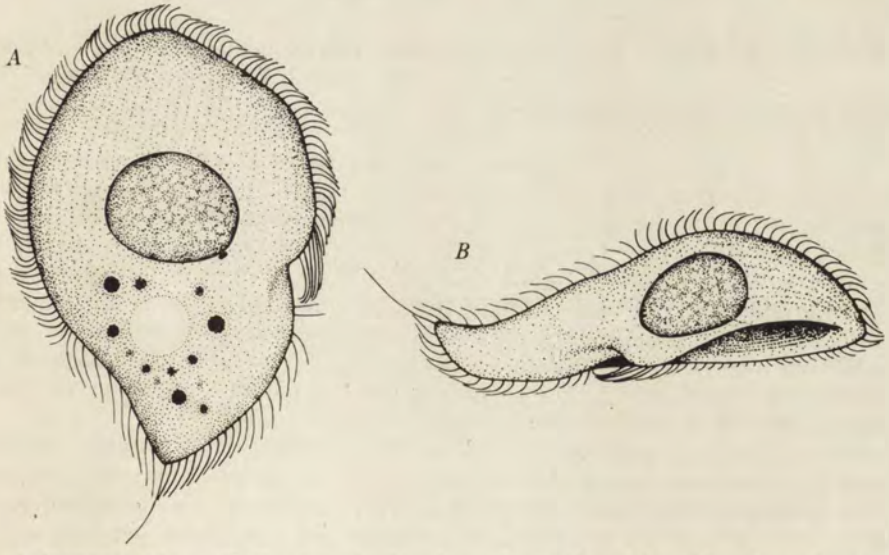


Fig. 1. General view of *Th. acuminata*; A — from the right side, B — from the ventral margin

length from the anterior end. The cytostome is disposed at the distance of $^1/_3$—$^1/_2$ of the body length from the anterior end, on the ventral margin in a slight depression which forms a shallow and rather broad buccal cavity. This region is covered from above by a small lobe of the ciliate body.

The body shape of *Th. acuminata* is usually rather constant. Yet some deviations are possible; they are caused by the state of the ciliate, e.g. in the narrow and small specimens all depressions and convexities are more distinctly marked than in the broad and big ones which may have a more rounded shape. The body shape of dividing ciliates and of very young individuals — several hours after division — differs distinctly from the typical trophic form. Those differences will be discussed later.

Dimensions of the body

The length of *Th. acuminata* amounts 45—100 μ, in most cases (about 60% of measurements) being 60—75 μ. The arithmetical mean from 190 measurements amounts 69.84 μ. The curve of the body length based on 190 measurements of specimens fixed with Schaudinn's fluid is presented in the diagram

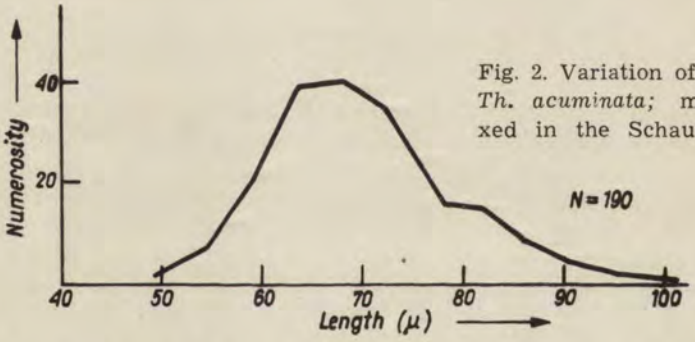


Fig. 2. Variation of length in *Th. acuminata;* material fixed in the Schaudinn fluid

(Fig. 2). The length of the dividing individuals, especially in the advanced phase of fission, may much exceed the maximal length reported above. The width of the body amounts 26—68 μ in most cases 30—50 μ; the arithmetical mean is 43.17 μ.

The more extensive study of the body dimensions of *Th. acuminata* in different phases of its life was carried out on preparations impregnated with silver nitrate. This method revealing the argentophilic system made possible the examination of a large number of individuals. On the other hand, the Klein's procedure involves some changes in the dimensions of the ciliate caused by compression and dessication of the body, and makes the results incomparable with those obtained by means of cytological fixating solutions as e.g. the Schaudinn's fluid. The data from the analysis of over 8500 ciliates are presented in the Table 1. They concern the thropic, the dividing, the post-division and the conjugating individuals.

The analysis of the trophic forms allows to classify the trophic individuals of *Th. acuminata* into 5 groups (Table 1, part 5). Since a distinct succession in the argentophilic systems of these groups, suggesting the development, is plainly observed, it may be supposed that each of the groups presents a successive stage in the growth process of the ciliate. In this sense the groups will be treated in the following considerations.

Table 1

General numeral characteristics of the studied material

	Length of individuals in μ	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	Σ
1	Size of the sample taken out of the whole material studied	4	3	27	110	253	348	410	372	272	140	45	11	—	1				2000
2	Dividing individuals:																		
	Stage I						1	—	2	1	—	1							5
	II									1	1	1							3
	III								2	1	1	1							5
	IV									3	2	1	3	2	1	—	1		13
	V											1	—	3	2	2	—	1	1	10
3	Post-division individuals	2	1	6	6	3													18
4	Conjugating individuals			3	3	4	1												11
5	Growth groups in the whole studied material:																		
	Group I			1	3	1													5
	II				4	10	12	10	2										38
	III						2	9	8	6									25
	IV							1	7	8	6								22
	V							1	—	3	9	4	1						18
	Total			1	7	11	14	21	17	17	15	4	1						108

Ciliature

Ciliature of *Th. acuminata* is not uniform over the whole surface of the body. Most cilia occur on the anterior body part; here their distribution is dense and their dimensions are much smaller than on the posterior body part. Some regions of the ciliate: an area around the centre of the right side and the concave part of the left side, are completely devoid of cilia.

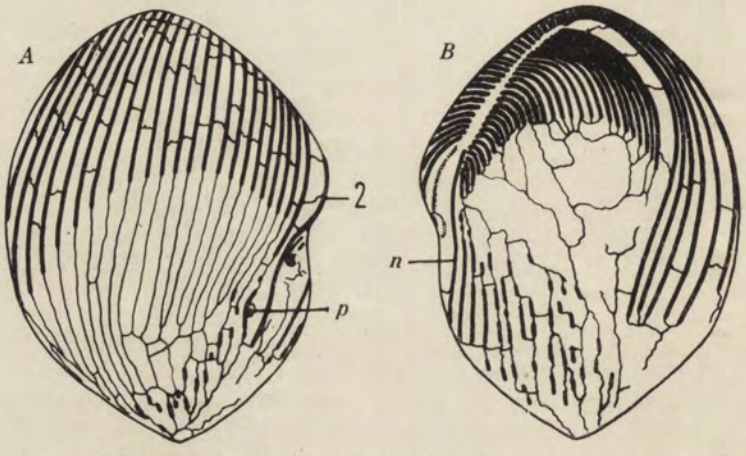


Fig. 3. Scheme of the argentophilic system of *Th. acuminata*; A — from the right side, B — from the left side

Cilia are 6—10 μ long in the anterior and 8—15 μ in the posterior part of the ciliate. The terminal region of the body bears a more rigid, curved caudal cilium 18—27 μ long.

In the ciliary system of *Th. acuminata* several complexes may be distinguished; these are: the buccal, the general, the thigmotactic ciliature and short

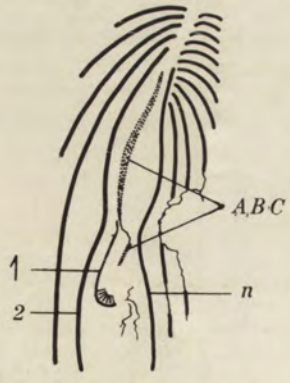


Fig. 4. Scheme of the buccal ciliature of *Th. acuminata*

rows of cilia or even single cilia in the posterior part of the body (Fig. 3 and Pl. II 5—6).

Buccal ciliature like in other *Thigmotricha* consists of a stomatogenic kinety "1"and of a polykineties zone corresponding to the elements ABC of Chatton et Lwoff (Fig. 4).

The stomatogenic kinety or "1" usually initiates at the distance of about 1/3 of the body length from the anterior end, on the ventral margin below the lobe covering the cytostome. This kinety is comparatively short, crooked at its end; it soon enters into the buccal cavity (Pl. II 7). A short fibril [2] extends upwards from the anterior end of the kinety. The stomatogenic kinety — especially on its full length — is rather rarely seen in silver impregnated preparations.

The polykinety producing the adoral zone of membranelles ABC initiates somewhat above the apex of the body [3], in the lower part of the anterior suture, on the left thigmotactic side of the ciliate and runs near the ventral margin of the body, on the left side of the lobe over the cytostome. In its further course, the polykinety continues to run on the right side of the ciliate and terminates somewhat below the beginning of the kinety "1". Two segments of the polykinety are seen: one on the left thigmotactic side of the ciliate and another, very short one, running towards the buccal cavity on the right side. A short fibril is sometimes impregnated between those two segments. Probably it should be assumed that the longer part lying on the left side of the ciliate is composed of AB elements, and the shorter part — bound to the peristome — of C elements of the polykinety.

The adoral zones of membranellae especially in young individuals are rather broad and consist of very tiny kinetosomes dispersed at random. In big individuals they seem to be more narrow, especially in the lower part of the segment AB which in some places has a character of a single row of kinetosomes (Pl. II 8). On the surface of the peristomal field, several fibrils converging in the peristomal region are seen (Pl. II 7).

General ciliature spreads over the whole right convex side of the ciliate body and also over a small part of the left side, near the dorsal margin.

The first kinety of the general ciliature — according to Chatton et Lwoff's nomenclature: the kinety "2" — is placed to the right of the buccal ciliature. It initially runs as a broad arc along the margin of the body lobe above the peristome and then becomes the right boundary of the peristomal field. This kinety terminates much below the peristome, about 2/3 of the body length from the anterior margin.

The next kinety "3" runs in its upper part as a rather small arc at a certain distance from the body margin, and in this segment it is not differing from the remaining kineties of the general ciliature. Still it is much longer than the others because its length is nearly equal to that of the kinety "2". In its posterior part, below the peristome, it slightly bends dorsally producing a small field to which the adjacent contractile vacuole is opening. In the anterior moiety of the ciliate, between the kineties "2" and "3", a small naked field is seen with 2 or 3 transverse fibrils as revealed by the silver impregnation.

[2] In this study, the term "fibril" is used exclusively in the descriptive meaning and correspond to the French "argyronème".

[3] In the previous publication (Kazubski 1958 b) only the inferior part of the polykinety ABC was described and represented in the Fig. 4, whereas its superior segment was then unknown to the author. On the other hand, the superior part of the polykinety ABC was shown in the Fig. 3 and erroneously marked as the row "1". Now — in agreement with the nomenclature introduced by Chatton et Lwoff — as the row "1" the stomatogenic kinety, belonging to the general ciliature, was marked. As a result, the determination of the general ciliature, in Fig. 2, 3 and 4 as well as in the text of the publication from 1958 are incorrect and require revision.

Then next kineties of the general ciliature have no supplementary functions. Their length amounting initially about $^1/_3$ of the body length, gradually increases so as to attain in the last kinety $^2/_3$ of the body length. Those kineties are straight, slightly converging towards the anterior part of the body, and reach at their anterior end the suture which lies along the anterio-ventral margin of the body on its left thigmotactic side. Several last kineties of the general ciliature converge and reach the suture simultaneously all together. The general number of meridional kineties of the general ciliature amounts about 28; 21—24 are on the right, and 4—7 on the left side of the body. Kinetosomes forming the rows of general ciliature are comparatively big. They are located near one another. Only in the terminal segment their pattern is more loose. This tendency is especially distinct in the last kineties of the general ciliature and in big individuals of *Th. acuminata*. Kineties are connected with one another by rows of transverse fibrils, more regular in the anterior part of the ciliate.

In the posterior part, the rows of general ciliature are prolonged as fibrils devoid of kinetosomes. Their continuation is an irregular network of fibrils of a rather constant pattern which undergoes some changes with the age of the ciliate. Near the posterior margin especially towards the ventral margin, a certain number of kinetosomes occur; they are organized in short kineties, 3—4 corpuscles in each of them, or dispersed at random (the posterior ciliature). In some individuals those corpuscles may be very scarce or they may be almost invisible.

A strong thigmotactic field is found on the left thigmotactic side of the ciliate. It is composed of the proper thigmotactic ciliature and of numerous short kineties of the posterior ciliature. A part of the general ciliature of the left side is acting together with the described system. The middle left side of the body shows no presence of any kinetosomes.

The thigmotactic ciliature is composed of 29—35 short kineties running as an arc. Their anterior ends reach as far as the anterior suture lying along the anterio-ventral body margin. The thigmotactic kineties are lying near one another; the first longest paradorsal kineties are separated by a shortest distance whereas the last, the shortest ones — by a most considerable interval. In some cases between the last kineties transverse fibrils are seen. Kinetosomes of the thigmotactic ciliary rows are somewhat smaller than those of the general ciliature.

A fairly broad un-ciliated meridional stripe is separating the thigmotactic kineties from the general ciliature. Some transverse fibrils cross the stripe free from cilia. The thigmotactic kineties terminate at about $^1/_4$ of the body length from the anterior margin. Here they pass into fibrils which join one another and this reduced number of fibrils continues as far as the posterior end of the body.

The posterior part of the left side is occupied by a fibrillar network [4] which looks as if it was formed by branching of the fibrils originating from some last thigmotactic fibrils, namely the nearest the ventral margin, and also from fibrils crossing the middle region of the body originating from the remaining thigmotactic rows. This fibrillar network comprises a considerable

[4] The origin of this part of the argentophilic system of *Th. acuminata* will be discussed after the description of division and reconstruction of the "normal" shape of the ciliate.

number of kinetosomes organized into small kineties each of them containing several kinetosomes (the posterior ciliature). Distribution of kinetosomes and the pattern of the network is — like on the left body side — more regular in young individuals than in the old and big ones.

Near the posterior end of the body, the fibrils of the left side bound to the thigmotactic ciliature, join the fibrils which are the continuation of the last rows of the general ciliature.

The last kinety of the left side is the very long non-branching „n", initiating at a higher point than the last rows of the thigmotactic ciliature. This kinety is the right boundary of the thigmotactic area. It subsequently runs along the ventral margin of the body and passes to the right side somewhat below the peristome. The kinety „n" is the left boundary of the peristomal area. The terminal segments of the last rows of the fibrillar network of the left side may also penetrate to the right aspect of the body.

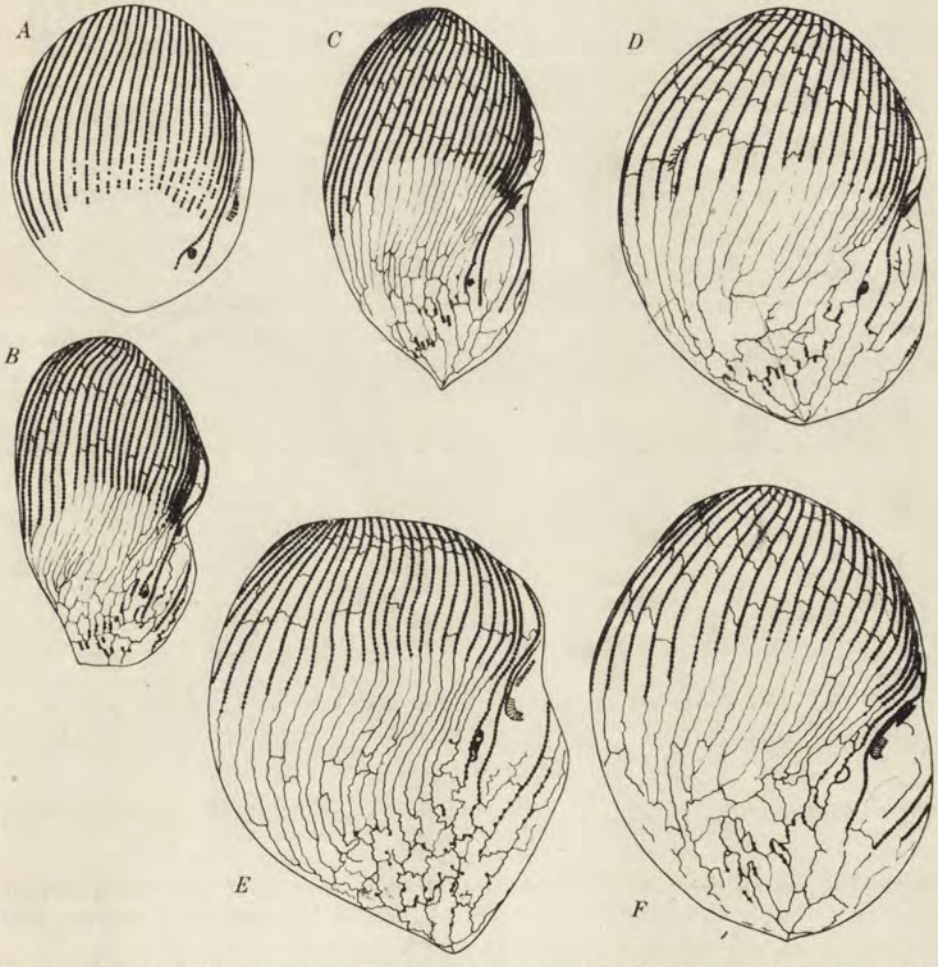


Fig. 5. Argentophilic system of *Th. acuminata* segregated into growth groups; right side. (Figures 5, 6, 8, 9 and 10 are executed in the same scale)

The argentophilic system in the growth period of the ciliate

The post-division individuals have an ovoid shape, sometimes the division margin is still marked on them. The adoral polykinety is uniform. On the left side, the general ciliature covers about $^2/_3$ of the body surface, the posterior ciliature of this body side is mostly scarcely differentiated and is still lacking near the posterior margin of the body. On the left side, the thigmotactic ciliature is normally developed and covers about $^1/_3$ of this body surface.

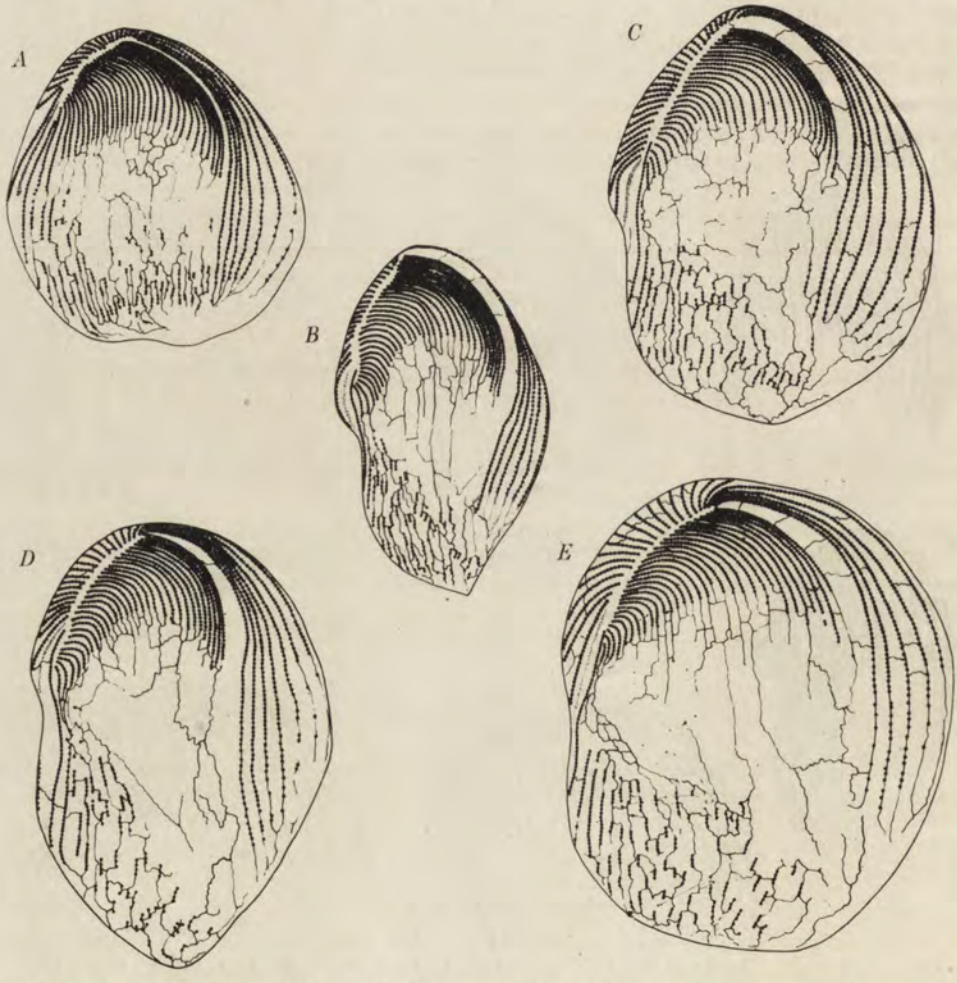


Fig. 6. Argentophilic system of *Th. acuminata* segregated into growth groups; left side

Short kineties of the posterior side are already developed but the posterior ciliature fails to assume as yet its typical feature because the posterior end of the body is not definitely shaped (Fig. 5 A and 6 A).

Group I. Ciliates in the phase of formation of the typical trophic form. In this phase the posterior body part is developing rapidly. Probably then

rupture of the polykinety ABC occurs. Short kineties of the left side take their proper position near the posterior body margin. The meshes of the fibrillar network are very elongate. At the conclusion of this phase the ciliate assumes its features typical for the trophic individual (Fig. 5 B).

Group II. The shape is elongate, the ciliates are comparatively slender. The general ciliature occupies somewhat less than $^1/_2$ and more than $^1/_3$ of the right body surface and the thigmotactic ciliature — about $^1/_4$ of the left side. The elongate meshes of the fibrillar network of the left side are regular. The short kineties of the posterior ciliature on both aspects of the ciliate are also very regular and are located near one another (Fig. 5 C and 6 B).

Group III. As to their dimensions the ciliates of this group occupy the central position in the studied material. They are less slender, more rounded than in the former group. The general ciliature occupies approx. $^1/_3$, and the thigmotactic — approx. $^1/_4$ of the body length. The fibrillar network in the posterior part of the right side is still regular but its meshes are already rather broad. Short rows of kinetosomes of the posterior ciliature, as well on the right as on the left side show no disturbances; yet they are becoming more distant from one another (Fig. 5 D and 6 C).

Group IV. The ciliates keep their shape almost unchanged. The general ciliature occupies already less than $^1/_3$, and the thigmotactic less than $^1/_4$ of the body length. In the course of the fibrillar network and in the pattern of kineties of the posterior ciliature, some disturbances begin to manifest (Fig. 5 E and 6 D).

Group V. Ciliates are very big. The characteristic shape of *Th. acuminata* begins to change: the body becomes rounded. The ratio of the ciliated part to the length of the body remains the same as in the preceding group. The disturbances in the fibrillar network of the posterior part of the ciliate are so far advanced that even breaking of single fibrils and desintegration of the short kineties occur. Kinetosomes of the posterior body end are disposed more or less irregularly (Fig. 5 F and 6 E).

The comparison of the argentophilic system of *Th. acuminata* in the growth groups just described revealed that in spite of considerable differences in the dimensions of the ciliates, the length of corresponding rows is almost the same as it was when they were formed during division. Insignificant differences which may be observed must probably be accounted for by the individual variability of the ciliates and also by the process of stretching of rows and shifting of kinetosomes which was noticed sometimes in big individuals. No increase of kinetosomes number in the short rows of the posterior body part was stated. Formation of new kinetosomes and elongation of kineties was observed only in the initial phase of division (see p. 254). As conclusion of what was stated above, it may be assumed that in the course of individual life and division of *Th. acuminata*, the length of kineties and presumably also the number of kinetosomes remains unaltered.

Internal structure

The protoplasm of *Th. acuminata* is little differentiated. The single macronucleus is shifted somewhat forwards from the center of the body. The perinuclear protoplasm is less intensely stained with haematoxylin than the

peripheral zone. Numerous food vacuoles and a single contractile vacuole are seen in the cytoplasm.

Food vacuoles occur before all in the posterior part of the ciliate. They rarely reach farther forwards than the anterior margin of macronucleus. The number of vacuoles is relatively small in young individuals, increasing up to 20—30 in the big ones. In the last case vacuoles fill up the posterior part of the body behind the macronucleus. Their average dimensions are from 2 to 6 μ. In single cases bigger vacuoles reaching 10—12 μ were observed in big individuals. Then the size of the ingested „bit" amounted mostly 2—4 μ, but food particles much bigger, up to 8 μ in diameter were also observed. They seem to have arisen by clumping together some smaller particles.

The food particles in the vacuoles of *Th. acuminata* have a fine granulated structure. The Feulgen reaction reveals the presence of fine granulated Schiff-positive substance which occurs also in small amounts in the vacuole outside the food particle, showing a light-pink colour.

The contractile vacuole is single, situated behind the macronucleus, about 1/4 of the body length from the posterior end of the ciliate. The contractile vacuole pore opens between the 2nd and 3rd kineties of the general ciliature behind the oral opening. The vacuole is acting very intensely in the ciliates immerged in water. Its activity was not noticed in the individuals remaining inside the snail ureter.

Macronucleus in very young individuals, just after completion of division, lies almost in the center of the body. In all the later stages it lies nearer the anterior end, what is connected with the increase in size of the posterior part of the body. This position shows some slight changes connected with the growth of the ciliate, and reaches in big individuals the distance of 1/3 of the body length from the anterior end (Fig. 1 and Pl. I 1, 2, 4).

The shape of macronucleus varies with the age of the ciliate. In the young individuals (up to approx. 60 μ in length) it is usually round or slightly ovoid, the long axis of the oval being oriented almost parallel to the longitudinal or to the transverse axis of the body. Both variations are equally frequent. The increase of the transverse macronucleus axis rises with the size of the ciliate and in the big specimens (>75 μ) the axes reach the ratio 1:1.7—1.9 and sometimes even much more. The longer axis is then oriented obliquely, inclined towards the cytostome. The rather regular shape of macronucleus in young individuals becomes gradually less regular in the big ones. Especially the posterior margin of nucleus becomes considerably deformed. Yet even big individuals may be found with very big round macronuclei.

The macronucleus is flattened in the direction perpendicular to the thigmotactic surface, so that in the side view it has the shape of a strongly convex lens. This shape is due to the general flattening of the body.

The dimensions of macronucleus as stated after 74 measurements of *Th. acuminata* are; the length of the longitudinal axis — 8—33 μ; arithmetical mean 18.0 μ, about 70% of measurements gave the value 13—22 μ; the length of the transverse axis — 7.5—38 μ, the mean: 21.7 μ, about 70% of measurements gave 14 — 29.5 μ.

A distinct and rather high positive correlation may be stated between the mean dimensions of the macronucleus and the size of the ciliate (Fig. 7).

The structure of macronucleus was studied on the Zenker-Helly fixed material stained with Feulgen fuchsine. The result was an intense staining prov-

ing a high DNA (chromatine) content. Especially the round or slightly ovoid macronuclei of young ciliates stain deep. They show a fine granular structure nearly homogenous, with tiny vesicular vacuoles devoid of chromatine. With the increase of size of macronucleus its granulation becomes better visible and the ball-shaped structures with no DNA — bigger nad more distinct. With the subsequent increase of the macronucleus its structure becomes gradually loose and the outlines of the non-stainable vesicles disappear. In very big individuals, the big ovoid macronucleus shows a spongy consistence. In such big individuals, the intensity of the general staining of macronuclei is lower than in the young *Th. acuminata.*

The micronucleus of *Th. acuminata* is very small, slightly flattened. Its dimensions are: 1 — 1.5 × 2 — 2.5 μ. It mostly adheres closely to the macronucleus from the side of the peristome. In this place, on the surface of

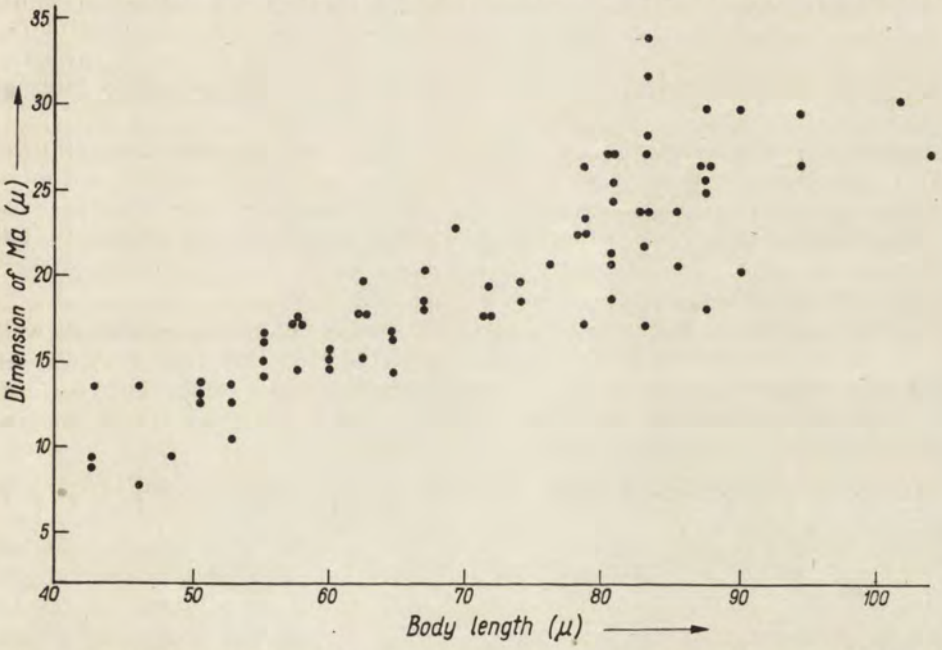


Fig. 7. Correlation between the mean dimensions of macronucleus and the body length of *Th. acuminata*

macronucleus a small concavity is seen. After haematoxylin stain, the micronuclear membrane and the deeply stained, nearly homogenous central chromatine mass are seen. After the Feulgen reaction only the nearly homogenous chromatine is stained, usually less intensely than in the macronucleus.

The above description and body dimensions concern only the *Th. acuminata* living in *O. orientalis.*

Ciliates found in *O. syriacus* (Kob.) from Lebanon and *O. duboisi* (Mouss.) from Caucasus are of the same shape and of similar pattern of ciliature but their dimensions are much smaller. The difference may probably be evoked by shrinkage after fixation with alcohol inside the host's body.

Th. acuminata from *O. syriacus* (measurements of 7 individuals) has the body 52.0 — 71.6 μ long, the arithmetical mean 63 μ. The width 27.0 — 41.6 μ, the arithmetical mean 36.2 μ. The macronucleus is ovoid, oriented obliquely; its dimensions: length of the longitudinal axis 10.4 —13.5 μ, the mean 11.6 μ; length of the transverse axis 12.5 — 16.6 μ, the mean 14.4 μ.

Th. acuminata from *Oxychilus duboisi* (measurements of 47 individuals) has the body length 41.6 — 64.7 μ, the mean 50.8 μ; the width 20.8 — 37.0 μ, the mean 29.6 μ. The macronucleus is ovoid, rarely round, its dimensions (in the same sequence as in the ciliate from *O. syriacus*): 8.3 — 11.6 μ, the mean 10.9 μ; 9.2 — 16.2 μ, the mean 12.4 μ.

*

In the structure of *Th. acuminata* some characters are noticeable which are also peculiar to other parasitic ciliates. Before all the strong flattening of the body should be pointed out. This character often occurs in parasites, especially ectoparasites and also in some species living inside the internal organs.

For most *Thigmotricha* the lateral flattening is a characteristic feature. The right side of the body remains slightly convex and on the left — a more or less distinct depression is formed. The cilia which cover this place produce a thigmotactic sucker. A similar system exists also in *Th. acuminata* (Pl. I 3) in which the depression on the left side is distinctly marked and the sucker, formed by the ciliary system of this side, is very strong.

The presence of the strong thigmotactic sucker in *Th. acuminata* as well as in other *Thigmotricha* is of a great importance in their parasitic way of life: it enables the parasite to remain on its proper spot of the host surface. Raabe 1947 b assumes the formation of clinging elements as one of the important adaptations to the parasitic way of life.

When examining the shape of *Th. acuminata*, attention should be paid to a certain elongation of its body. In this feature *Th. acuminata* exhibits a tendency peculiar to parasitic ciliates living in the lumen of organs e.g. in the intestine (Raabe 1947 b). Their elongation and stream-lined body shape surely perform an important role for those organisms living in a liquid medium, which is in permanent motion. Presence of the caudal cilium in *Th. acuminata* is a characteristic feature, recorded also in some other *Thigmotricha* e.g. in *Ancistrumina limnica* (Raabe, 1947). In this way in *Th. acuminata*, besides the adaptations to the parasitic life, some adaptations specific to rheobiontic organisms may be observed (the stream-lined outline of the body, the caudal cilium).

The peristome of *Th. acuminata* like in *Myxophyllum steenstrupi* (Stein, 1861) and in numerous *Conchophthiridae* Kahl, 1931 is protected against the direct current of the liquid and against various contaminations, by the lobe of the ventral body margin over the peristome.

The most striking feature of the ciliary system of *Th. acuminata* is the strong shortening of the kineties in relation to the body length. Among other *Thigmotricha-Stomodea*, shortening of kineties may be observed in *Hemispeirinae* König, 1894, whereas in *Conchophthiridae* Kahl, 1931 and *Protophryinae*

Cépède, 1910 only a more loose disposition of kinetosomes, proceeding backwards, may be stated. The thigmotactic kineties are most considerably shortened. A similar reduction of length of rows occurs also in numerous *Thigmotricha-Rhynchodea*, but here it is accompanied by the general reduction of the number of kineties and is connected with the sedentary way of life. Nevertheless this reduction of ciliature in the actively swimming forms is not so far advanced as it may be observed in *Th. acuminata* in which it must be compensated by migration of some segments of those rows to the posterior body end in order to form an effective sucker and probably also to gain the stability of the ciliate in the strong current of liquid. More so, an effective distribution of cilia was attained, since they are absent in some places where the intensity of their function would be low e.g. on the depressed surface of the sucker.

Another characteristic of the ciliary system of *Th. acuminata* is a considerable specialization of its regions, which is especially clear in the thigmotactic ciliature. Rows belonging to this system are very short, closely distributed, with smaller kinetosomes; they plainly differ from the general ciliature. The meridional rows of cilia in *Th. acuminata* deviate towards the ventral margin in their anterior segments. It especially concerns the rows of thigmotactic ciliature which probably express a morphological adaptation of those ciliary systems to their thigmotactic functions. Some tendencies to such deviation of ciliary rows is already observable in *Myxophyllum steenstrupi* (Stein, 1861), and in some *Conchophthiridae* e.g. *Conchophthirus discophorus* Mermod, 1914 and *C. unionis* Raabe, 1933 (according to R a a b e 1934) as well as in *Cochliophilus minor* Kozloff, 1945. However in none of those species this process is so far advanced as in *Th. acuminata*.

The buccal ciliature of *Th. acuminata* is constructed in the same manner as in the representatives of *Protophryinae* Cépède, 1910 and in the *Hemispeirinae* König, 1894. It consists of the stomatogenic kinety "1" and of membranellae ABC. The difference concerns the polykinetic zone of membranellae which in *Th. acuminata* is very long, begins somewhat below the apex of the body and ends at the upper part of the peristome, whereas the stomatogenic kinety is very short and lies slightly above the middle of the body length.

Like in many other *Thigmotricha: Ancistrum mytili* (Quennerstedt, 1867) Maupas, 1883; *Ancistrumina limnica* (Raabe, 1947) Raabe, 1959; *Eupoterion pernix* Maclennan et Connell, 1931 — in *Th. acuminata* the kineties "2" and "n" surrounding the peristomal field are outstanding, being much longer than the remaining general and thigmotactic ciliatures and fail to undergo the secondary division. In *Ancistrum mytili* (Quennerstedt, 1867), or *Proboveria loripedis* Ch. et Lw., 1936 they only slightly go beyond the end of the buccal rows or accompany them along a certain segment but in the case of *Th. acuminata* they run much further backwards than the buccal rows. It seems that their essential role — supporting the peristomal field, remains the same.

The internal structure of *Th. acuminata* does not deviate generally from the structure of other *Thigmotricha* with one round or slightly ovoid macronucleus, one micronucleus and one contractile vacuole opening near the peristomal field.

Fig. 8. Division of the argentophilic system in *Th. acuminata*; right side

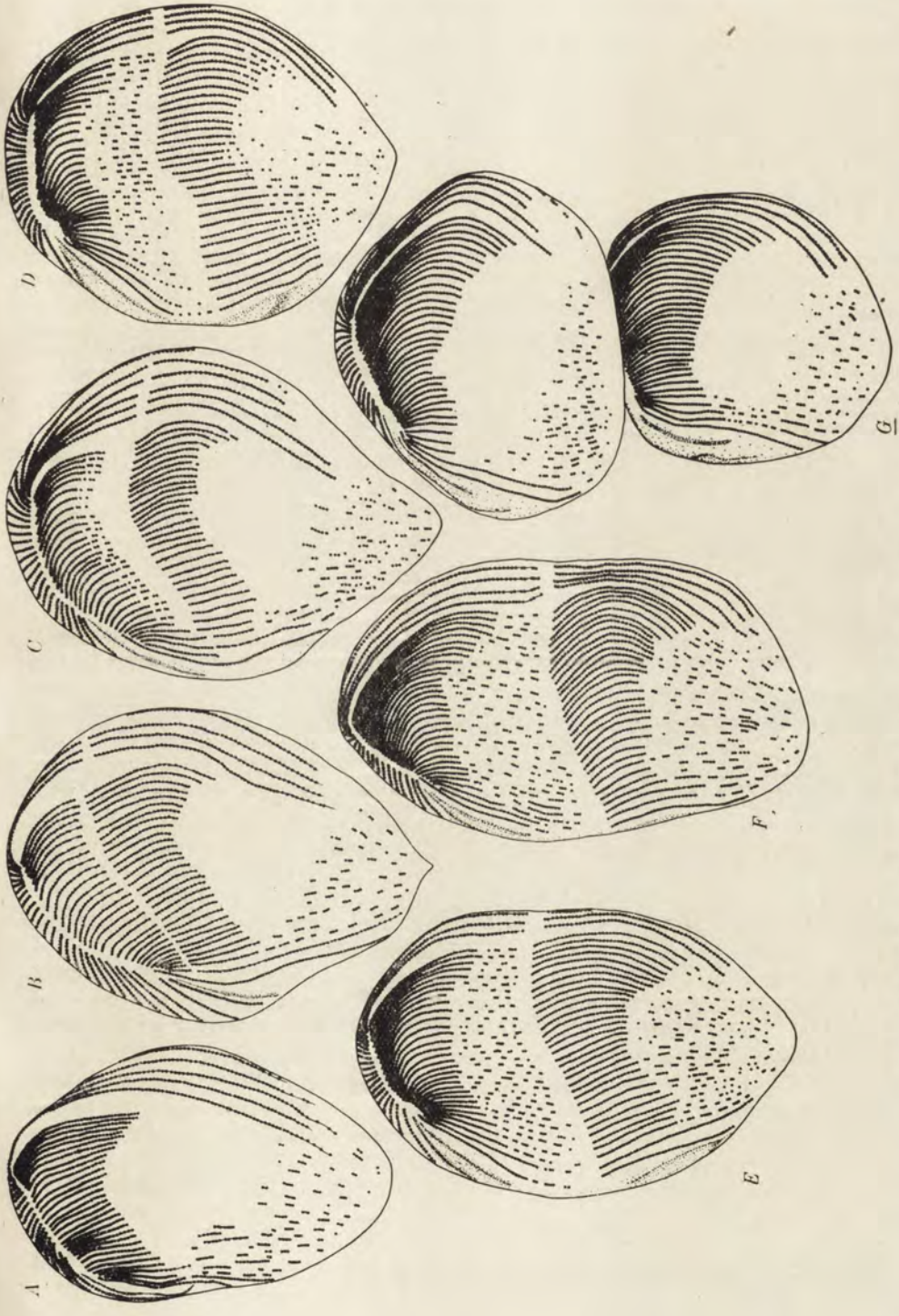


Fig. 9. Division of the argentophilic system in *Th. acuminata*; left side

Division and stomatogenesis

In *Th. acuminata* division is initiated in individuals which may be included into the III (central) and IV groups of growth on account of their body shape and structure of their argentophilic system. The size of the individuals in the initial phase of division seems to support this statement (Table 1, p. 2).

In the division process of *Th. acuminata* 5 steps were distinguished.

Stage I

Division begins by the intense growth of the body near the anterior margin, accompanied by formation of the zone where kinetosomes arise (Pl. III 9—10), similar to that as described by Frenkel 1960 in *Glaucoma chattoni*. Lengthening of kineties just in this place is also indicated by the increase of the distance between the transverse fibrils joining the anterior segments of the general ciliature rows. This process is seen in some specimens (Fig. 8 A and B). The growth of the ciliate near the anterior margin involves also the orientation of all the kineties of the general ciliature to the direction parallel to the longitudinal body axis (Fig. 8 B) as well as extension and straightening of the thigmotactic ciliature rows. They become nearly parallel to the longitudinal body axis (Fig. 9 A), being simultaneously arranged in a more loose pattern. Probably in this stage occurs also the extinction of activity of the oral opening and the stomatogenic kinety "1" straightens and occupies its meridional position.

Stage II

The rupture of the meridional kineties of the general ciliature occurs near the anterior end of the body, probably in the middle length of the rows which are stretched up to their double length. Unfortunately no material illustrating this rapid process was available. The specimens 9 B and 9 C seem to indicate that besides the principal division of kineties separating the segments which are to belong to the daughter individuals and constitute the proper fission line — several small groups (3—4 kinetosomes in each) are detaching; they will belong to the proter being the primordium of its posterior ciliature. Separation of those groups from the segments of the opisthe kineties occur later on, and is much less distinct on the right aspect of the ciliate. The fission line runs in this period obliquely being bent towards the cytostome (it is probably connected with the length and inclination of the kineties and with the positions of macronucleus) and is shifted subsequently towards the center of the body. Even after formation of the fission line, the ciliate keeps its body shape ressembling to the outline of the normal trophic individual even with its distinctly sharp posterior end (Fig. 9 B and 9 C). Gradually the ciliate assumes the ovoid shape.

The thigmotactic kineties of the proter begin already to aggregate and assume the shape of arcs whereas the disposition of the rows belonging to the opisthe is still loose and — except several kineties nearest the dorsal margin — follow a course almost meridional (Fig. 9 C).

Simultaneously the buccal ciliature of the mother individual desintegrates. Elongate fields arise consisting of very small basal bodies; the polykinety ABC undergoes some less significant transformations than the stomatogenic kinety "1", like in *Proboveria loripedis* as reported by Chatton et Lwoff 1936.

Stage III

As soon as the fission line on the ventral surface of the animal has reached approximately the middle position of the body length, the dividing ciliate assumes a nearly ovoid shape (Fig. 8 C, 8 D and 9 D). The most retarded meridional kineties are then approx. at a distance of $^1/_3$ of the body length from the anterior end of the body. Near the ends of the rows "3" of general ciliature, in both daughter individuals excretory pores of the contractile vacuole arise. In those places the kineties "3" shift somewhat aside from the kinety "2" leaving small fields between them. The old ciliature of the posterior body part, as well on the right as on the left side gradually disappears.

In this step very complex processes occur leading to formation of new peristomes in both daughter individuals, similarly as it was described by Chatton et Lwoff 1936 in *Proboveria loripedis*. As it was previously ascertained, the polykinety of the mother individual after an insignificant reconstruction remains subsequently as a whole in the proter, undergoing no considerable alterations (Fig. 9 D). In contrast to this, the stomatogenic kinety forms the organization field extending along the ventral margin of the body. Approximately in the middle of the body length this field becomes interrupted and the further organization of the new peristomes in the proter and in the opisthe proceeds independently (Fig. 8 C and D).

In the anterior part of the oral field, two bands of kinetosomes may be distinguished which give origin to a new stomatogenic kinety of the proter, as well as a few groups of bodies which probably may be considered as groups analogous to E and F after Chatton et Lwoff 1936. Those structures together with the polykinety on the left side of the ciliate, are to form the buccal ciliature of the proter.

In the stomatogenic field of the opisthe a very elongate meridional group of kinetosomes arises and gives origin to the future stomatogenic kinety. It also gives origin to the group of kinetosomes A* and to the obliquely oriented groups G, H, I (Chatton et Lwoff 1936) and also to an undefined by those authors group of granules near the stomatogenic field, named by Raabe 1963 the group K. After Chatton et Lwoff 1936, the groups G and I, together with the group A* participate in formation of the polykinety ABC of the opisthe (Pl. III 11—12).

Stage IV

The division continues further as follows. The line of division approaches along its full length the middle of the body, the kineties of the general and of the thigmotactic ciliature lengthen slightly. The excretion pores are formed. The old ciliature of the body end disappears at all. The ciliate gradually elongates and, when the division line reaches the middle region of the body, a constriction appears in the place of the future fission (Fig. 8 E and F, 9 E and F). On the oral fields the stomatogenic kineties of both daughter individuals are organized as well as the adoral polykinety ABC.

Stage V

In the last step of division the fission furrow deepens up to a complete separation of the daughter individuals. On the right side, the rows of the general ciliature assume the same form as they have in the trophic individuals. The short kineties of the posterior ciliature are not yet on their

places in the daughter individuals (Fig. 8 G). On the left side, the thigmotactic kineties aggregate and assume their specific arched shape; the short kineties of the posterior ciliature pass upon the posterior part of the body of both daughter individuals (Fig. 9 G). The buccal ciliature of both individuals is almost formed, although its final shape and pattern are not yet developed.

In the moment of fission, the daughter individuals are ovoid. Kineties have already reached their normal length. The ciliature of the right body side is not completely evolved — the posterior kineties are indeed developed, but they remain still distant from the body end. Some differences are noticeable in the number and size of such detaching kineties in both daughter individuals: in the proter they are more numerous than in the opisthe. As result of this difference between the proter and the opisthe, some adult individuals occur with few or not kinetosomes on the posterior of the right body region. In contrast to the right, the ciliature of the left body side is formed and the thigmotactic area is functioning.

In the division of the argentophilic system, the rule holds true that before division, the more complex structures are simplified (straightening of the general and thigmotactic ciliature) and after division the most functionally indispensable fragments (thigmotactic ciliature) are reconstructed earlier.

In the available material of *Th. acuminata,* the division process in the nuclear apparatus could not be followed in details. The observation of living material and some fragments of nuclei preserved after impregnation with silver nitrate indicate that in the initial phase of division, the macronucleus assumes an elongate shape, its longer axis running parallel to the longitudinal body axis. It lies initially in the anterior part of the body and then shifts gradually towards the central region. The division of macronucleus initiates when its central position is reached, which occurs more or less simultaneously with the appearence of the fission furrow. The karyoplasmatic bridge persists for some time and remains visible even in much constricted dividing individuals.

*

Division of *Th. acuminata* is essentially the same as in other related *Holotricha.* Some differences may be noticed in the division of the ciliary system. They are involved by the specificity of the argentophilic system of *Th. acuminata:* location of the meridional rows only in the anterior part of the body, and a high specialization of each part of this system. A similar course of division of the argentophilic system may be stated in the *Thigmotricha* with a similar differentiation and reduction of ciliature e.g. in *Plagiospira crinita* Issel, 1903. According to Chatton et Lwoff 1949, in this species like in *Th. acuminata* in the phase prior to division, the systematization of the kineties pattern occurs and they assume a position more related to the meridional. The fission line of *P. crinita* runs at first also obliquely to the longitudinal body axis. But in the more advanced phases, some differences in respect to *Th. acuminata* manifest: the fission furrow in *Th. acuminata* arises only when the fission line reaches approximately the middle region of the body, whereas in *P. crinita* — as represented in the figure of Chat-

ton et Lwoff 1949 (p. 219, Fig. 15, the first left) formation of this furrow takes place earlier, before the fission line has reached the middle of the longitudinal body axis. But in the more advanced phases, some differences and with a different position of its thigmotactic surface. Besides, the fission line in *Th. acuminata* appears much higher than in *P. crinita*, which is involved by the fact that the meridional rows in *Th. acuminata* take considerably less place on the body surface than the rows in *P. crinita*.

Another specific feature of the ciliary system in *Th. acuminata* is the secondary division of daughter kineties and formation of short rows in the posterior ciliature. Such a process has never been described as yet in any representative of *Thigmotricha*. The divided segments comprising several kinetosomes each, shift subsequently backwards occupying position near the posterior margin of the daughter individuals. The new posterior ciliature of the opisthe pushes somewhat the old posterior ciliature of the mother individual in the course of its shifting backwards. This process secures the gradual replacing of ciliature in the posterior daughter individual. Similar complex reconstruction processes were known till now in various *Spirotricha*.

As mentioned above, the stomatogenesis of *Th. acuminata* is almost in agreement with this process described by Chatton et Lwoff 1936 in *Proboveria loripedis* Ch. et Lw., 1936. Some rather significant differences shows the stomatogenesis in *Conchophthirus unionis* Raabe, 1933 as described by Raabe 1936. In this species, the new cytostome arises at the tip of the fibrillar thread (argyronème "s" — Raabe 1963) running backwards from the stomatogenic kinety "1". Except for this fibrillar thread, the organization area of the new cytostome has no other connections with the mother cytostome, which after some reorganization remains within the anterior individual. On the contrary the organization processes of the new cytostome in *Conchophthirus* are much similar to those occurring in stomatogenesis of *Proboveria loripedis* and *Th. acuminata*. Namely in *Conchophthirus unionis* several (four or sometimes more) transverse fields of kinetosomes arise. They subsequently become rather parallel to the longitudinal axis of the body and give features of buccal polykineties. Thus within *Thigmotricha* some common features of stomatogenesis distinctly manifest: the formation of several transverse polykinetic fields of kinetosomes, which indicates a phylogenetic unity of this group of ciliates.

In the recent publications a theory is being put forward (Corliss 1960, 1961) that the central group of *Holotricha* are the *Tetrahymenina* in the meaning of Corliss and that they gave origin to the other groups of ciliates — among others — also to *Thigmotricha*. In the light of this theory it becomes interesting to compare the morphogenesis — before all the stomatogenesis — in *Thigmotricha* with analogical processes in *Tetrahymenina*. In this group, the stomatogenesis of *Glaucoma chattoni* has been described by Frenkel 1960 and illustrated with good photographs.

When comparing the process of stomatogenesis in both groups, the following differences may be noticed: in *Glaucoma chattoni* and presumably also in other *Tetrahymenidae*, only three transverse adoral membranellae are formed, whereas in *Proboveria loripedis* and in *Thigmocoma acuminata* four of them arise, and in *Conchophthirus unionis* — according to Raabe 1963 — more than four polykinetic zones are present. There exists probably another fundamental difference: in *Glaucoma chattoni* the adoral mem-

branellae are detached as structures parallel to the stomatogenic kinety (Frenkel 1960, p. 366, Fig. 9 and 10) and in *Thigmotricha* (as shown in Fig. II and III of Chatton et Lwoff 1936) they are perpendicular to this row. The material of dividing *Th. acuminata* on which this study has been based seems also to support the opinion about formation of the polykinetic zone as a structure oriented transversally. This problem should be more investigated especially in the aspect of processes occuring in *Thigmotricha*.

The differences discussed above would indicate that in spite of certain similitudes no conclusion can be drawn in favour of a direct derivation of the adoral zone membranellae of *Thigmotricha* from tetrahymenium which is an example of the highly specialized buccal apparatus of ciliates. For that reason, the initial form should be looked for among some other ciliates groups with a less specialized oral system. For the solution of this problem a profound knowledge of morphogenesis and especially of stomatogenesis in ciliates is indispensable.

Conjugation

Conjugation occurs among very small and — judging from their ciliature — very young individuals. Dimensions of the major conjugant which after the silver impregnation technic remains not deformed, correspond approximately to the size of the first growth group (Tab. 1, p. 4).

The conjugants join by their peristomes being oriented in the same direction with their anterior ends. This may occur only when one of the ciliates is facing the substrate with its left and the other with its right side. Such pictures are seen in impregnated preparations. The observation of living material shows also that the conjugating ciliates are inclined obliquely to each other. It is caused by the position of the peristome and the shape of the ventral margin.

The ciliature of conjugating individuals shows no difference from that of the normal trophic form. The buccal ciliature remains generally unchanged and is revealed by silver impregnation. On one side of the conjugating pair, the first stomatogenic kinety of the individual, which faces the observer with his right side, is seen and closely by, the polykinety ABC of the second conjugant, seen from the right side. The stomatogenic kinety "1" begins much below the anterior suture and is rather short. The polykinety ABC showing no partition into segments begins near the apex of the ciliate and continues nearly as far as the middle of its length. Between both systems, the suture runs, joining both conjugating animals. In some cases this impregnated suture looks like a line running between the kinety "1" of one conjugant and the polykinety ABC of the other (Fig. 10).

Conjugation is a process which only casually may be observed in the ciliates which cannot be cultivated in vitro. As result of this, most informations concerning conjugation are based on the study of species which may be cultivated in laboratory; the data concerning the parasitic *Thigmotricha-Stomodea* are very scarce. Kidder 1933 a, b describes the conjugation in *Kidderia mytili* (De Morgan, 1925) Raabe, 1934 and in *Ancistrum isseli* Kahl, 1931 concentrating his study mostly on the phenomena occuring in the nuclear apparatus. Raabe 1934 presented a drawing of a presumable conjugation in *Conchophthirus discophorus* Mermod, 1914. Kaczanowski 1961 describes this process in *Protoptychostomum simplex* (André, 1915). It follows from

the above publications, that the reciprocal position of both conjugants is different in various cases. In majority of conjugating species cited above, the conjugants join by their cytostomes; then their position depends on the location of the peristomal apparatus and on the shape of the adoral body part.

Fig. 10. Conjugation of *Th. acuminata*; argentophilic system

So is also the case with *Th. acuminata*. *Protoptychostomum simplex* deviates from this scheme since its conjugants join by their opposite body poles. There is no information whether in other *Thigmotricha* like in *Th. acuminata* only young individuals are conjugating. May be it is a special case caused by the fact that in the young individuals of *Th. acuminata* the cytostome is not completely developed and that they differ in their shape from the aged forms. Those facts faciliate or even enable their conjugation.

Biology and ecology of *Th. acuminata*

The localization of the parasite in the host, the intensity and extensity of infection and the ecological environment of the host were only shortly described in the description of the species (Kazubski 1958 b). The subsequent study on biology and ecology of this ciliate was carried out in the laboratory and as field investigation. This study had to be restricted to the relation of *Th. acuminata* to a single host — *Oxychilus orientalis* (Cless.), and to this part of the ciliate area which is situated in Poland.

Localization and food requirements

Th. acuminata previously (Kazubski 1958 b) was found to parasitise "in the renal organ" of the snail. The recent precise examination of living material revealed that the part of the ureter adjacent to the nephridium of the snail, i.e. the primary or 1st order ureter, is the real localization of *Th. acuminata*.

The ciliate swims freely in the ureter rather near the wall of the organ almost touching it with concave thigmotactic side. It swims forward with its rounded end. Near the wall, the ciliates usually advance along straight lines, but sometimes — probably being at farther distance from the wall — they rotate round the transverse body axis, which is perpendicular to their thigmotactic surface. The ciliates aggregate most frequently in the proximal part of the ureter near the canal joining the metanephridium with the ureter.

When thrown aside by the motion of the liquid medium, they return to this place. They show a distinct positive rheotaxis.

A few ciliates were also observed in the mantle cavity of the snail where they penetrate from their usual place along the ureter of the second order.

The number of individuals living in one snail (intensity of infection) may amount up to several hundreds of specimens and depends — before all — on the size of the snail.

The ciliates involve no appreciable harm to their hosts. Even in a prolonged culture of infected snails no cases were observed suggesting that the death of the snail was evoked by its parasite — *Th. acuminata*. The high extensity of infection (which will be discussed later) would not speak in favour of a pathogenic action of the parasite.

Another evidence in this subject may be provided by the study of food of *Th. acuminata*. In the preceding chapter a comparatively high number of food vacuoles in *Th. acuminata* was reported and some granules of a Schiff-positive substance were described. It may be supposed that the ciliates feed on fragments of the epithelial cells lining the ureter. Yet no lesion was found in the section preparations, besides — the chromatine bodies of those cells are much bigger than the Schiff-positive granules found in the food vacuoles of the ciliate. Another possibility might be the ingestion of some cell remnants excreted from metanephridium, but this is little probable because in this case some adaptations for capturing the particles should be expected which was not ascertained.

The content of food vacuoles mostly ressembles to the Schiff-positive granules found on the surface of the epithelium lining the internal surface of the snail ureter. Probably the ciliates swimming along the wall of the ureter ingest those granules. Nothing can be said about their nature as yet. In preparations, those granules cover the ureter epithelium almost entirely producing sometimes big accumulations. In some places those granules seems to form the base of the cilia-like structures, 6 μ in length; they stain pink after the Feulgen reaction. The granules cannot be therefore neither cilia nor their basal granules since those structure should not be Schiff-positive. It might be concluded that they are some bacterial symbionts. Evidently this problem should be exactly investigated in further study.

Reproduction

The ontogenesis of *Th. acuminata* occurs entirely within the ureter of the snail. In the material from the *Oxychilus crientalis* ureter, all steps of growth may be observed as well as the division and conjugation.

As mentioned above, the allometric growth of *Th. acuminata* evokes at first the stretching of the argentophilic net in the posterior part of the ciliate and subsequently its disturbances and breaking which is visible best in the group comprising the biggest individuals. Distinct alteration bound to the growth are also occurring in the macronucleus which from a compact ball-shaped structure is transformed in big individuals into a voluminous spongy macronucleus. Those processes are irreversible and prove the advancing senility of the ciliate. Similar changes in the structure of macronucleus are also reported by Rudzinska 1961 in juvenile and old individuals of *Tokophrya infusionum* (Stein). As an additional prove of ageing in *Th. acuminata* the fact should be stressed that the division occurs only in the individuals of the III

and IV growth groups and there is no evidence of division of the oldest and biggest individuals.

Divisions occur in *Th. acuminata* very rarely. This is shown by the direct study of clones in the host (Kazubski 1961b) carried out on living material of snails infected experimentally. The frequency of division was determined by measuring the time between the first division of the single ciliate present in the host, and the subsequent divisions of the daughter individuals. Only those snails could be used for this experiment which were infected with a single specimen of the ciliate. In one case, a snail (E_2) with two parasite individuals was used because both first divisions occurred the same day. Results are summarized in the diagram (Fig. 11). Divisions occurr-

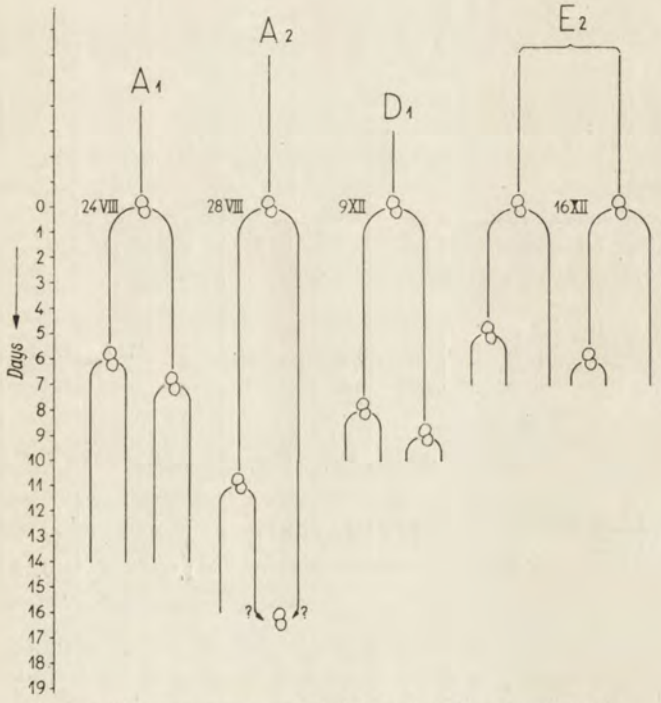


Fig. 11. Frequency of divisions in *Th. acuminata*

ed: in the clone A_1 after 6 and 7 days, in the clone D_1 after 8 and 10 days, in E_2 after 5 and days. In the clone A_2 the first division occurred after 11 days, the second division was not included into the results of experiment because the dividing individual might had originated as well from the first as from the second division. As average time of generation 7.5 days were fixed (the arithmetical mean from the experiment described above amounts 7.57 days).

The division process is developing rather slowly. The division of an individual in E_2, from the moment when the constriction was discernable, up to the separation of daughter individuals (this period corresponds approximately to the IV and V stage) lasted 2 hrs 40 min. The time of other divisions observed was not measured precisely but it was expressed in the values of the same

range. The frequency of occurrence of dividing individuals related to the general number of the ciliates observed in silver impregnated material amounted 0.42% (Table 2).

Table 2

Frequency of dividing and conjugating individuals of *Th. acuminata*

	Months	V	VI	VII	VIII	IX	X	XI	Σ
Snails	Number of snails examined	68	89	89	27	72	86	26	457
	Number of infected snails	48	75	47	17	59	57	20	323
	%	70.5	84.2	52.8	62.9	81.9	66.3	76.8	70.7
	Number of snails with dividing ciliates	6	1	3	3	6	7	—	26
	%	12.5	1.1	6.4	17.6	10.2	12.3	—	8.05
	Number of snails with conjugating ciliates	—	—	1	2	—	2	—	5
	%	—	—	1.1	7.4	—	2.3	—	1.1
Ciliates	Number of ciliates examined	988	3057	993	603	1113	1264	514	8536
	Number of dividing ciliates	11	1	3	5	8	8	—	36
	%	1.11	0.03	0.30	0.83	0.72	0.63	—	0.42
	Number of conjugating pairs of ciliates	—	—	1	6	—	4	—	11
	%	—	—	0.10	1.00	—	0.32	—	0.13

The comparison of frequency of divisions in *Th. acuminata* in different months (Table 2) indicates that the dividing ciliates were found mostly in May, then in August and in the following autumn months up till October. The division number 36 per 7 months is to low for permitting to draw any conclusions about the connection of division in *Th. acuminata* with the annual cycle of the snail.

Regulation of the populations size

The insignificant frequency of division involves also the slow increase of population of *Th. acuminata* within the host (Fig. 11). A period of several months from the moment of infection is necessary that the "normal" level of the ciliate number in the snail was reached.

The increase of the population abundance is moderate, being regulated not only by the low rate of division but also by ejecting the superflous ciliates into the mantle cavity from the ureter and hence outside to the medium.

The observation showed the following mechanism of this process. As mentioned above, the ciliates aggregate in the proximal part of the ureter near the canal leading to metanephridium. When very numerous, they occur also in other parts of the organ. Since they freely swim in the liquid filling the ureter, the abrupt motion of the liquid in moment of evacuation drives the ciliates away to the mantle cavity. The number of parasites ejected in this way is in harmony with the number of ciliates in the distal part of ureter i.e. with the size of population. The ciliates may be also driven away when the snail withdraws its body into the shell and when its internal organs shrink.

At a low intensity of infection, e.g. in the experimental clone cultures in which the population scarcely amounts several specimens, the ciliates leaving the ureter were never observed.

The parasites driven away into the mantle cavity aggregate there for some time, and then get into the medium. This process is not connected with any definite phase of their life cycle. As shown by observation, the migration of the ciliates from the mantle cavity into the medium occurs in different circumstances. They may be driven away together with clumps of mucus. In this case they cannot move freely and remain alive as long as the medium is moist. They may also leave the host's body in water. It was often observed that they appear in water a few minutes after the infected snail has been placed into it. They were found either in mucus particles or free swimming.

In water medium the ciliates move along straight lines in various direction or sometimes they perform rotations round their transverse body axis. In water a slight swelling of the body is observed as well as an intense activity of the contractile vacuole; its contractions appear at few minutes intervals. The free life of a ciliate is not long. After about 20—30 min the vacuole slackens its work and finally its action ceases. The excretory vesicle swells unnaturally and after 30—40 min from the moment of immersion the ciliate dies. In this experiments tap water at room temperature was used.

Modes of infection

In *Th. acuminata* neither cyst formation nor a free living form are known. So the infection is possible only by more or less direct contact of the hosts. Observation show that such contact may occur rather frequently. *Oxychilus orientalis* occurs in great number and several individuals are often observed crowded one close to another. It is possible that in winter they aggregate in large groups taking shelter in various crevices. In their migrations they habitually creep over such obstacles as other molluscs met on their way. Those contacts must be considered as excellent chance of infection and of exchange of the ciliate parasites, the more so as usually they are "at hand" in the mantle cavity. A convenient moment may also be the copulation of snails: the long and close contact gives an excellent occasion for infection with ciliates. But this factor should not be over-estimated because the development of the gonad and possibility of the copulation — as comunicated by Dr. A. Riedel — take place in *O. orientalis* only when the width of the shell reaches about 7 mm. The material presented in Table 3 indicates that over 60% of snails 4.5—5.5 mm in width — beyond doubt — young and unmature, are infected with *Th. acuminata*. As consequence, the casual contacts should be looked upon as mostly responsible for the mutual transmission of infection.

The possibility of indirect contact between snails through water offers also a way of infection. As already mentioned, after immersion into water the ciliate may keep alive for some time and then the possibility of mutual infection is probable.

The above possibilites of transmitting infection were to some degree proved by experiments. The parasite-free and infected snails were put together on a Petri dish. In order to avoid the possible error, the material was selected so that both categories differed strikingly in their size. A flock of moist cotton wool and some moss were added to keep humidity, which is essential for the activeness of snails. Dishes were kept in climatization chamber at + 12 °C and at a relative humidity of 70—100%. Snails were controlled by the observation in vivo. Infection of different specimens occurred after 1, 3, 6, 7, 19, 24 and 34 days. In 4 experiments 7 specimens were infected; among them some were brought non-infected from natural medium while others were sterilized at + 30 °C in the laboratory. So in *Oxychilus orientalis* no acquired immunity against infection with *Th. acuminata* seems to exist.

Extensity of infection

In the former publication (K a z u b s k i 1958 b) as result of 49 dissections of *O. orientalis* from the Sanok environment, the extensity of infection with *Th. acuminata* amounting 69.4% was reported. In subsequent years further material concerning the infection of snails was collected. The study was carried out as well by means of dissection as of observations of living material. Since a part of those observations were executed in the laboratory in Warszawa, the snails suspected to have lost their ciliates during the transport, were excluded from the count. Finally, 711 specimens of the snails were considered. In this number, 555 snails were infected with *Th. acuminata*. The general extensity of infection amounted 78.08% of the examined material.

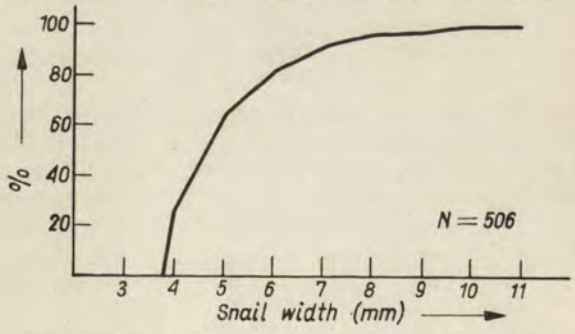


Fig. 12. Extensity of infection of *O. orientalis* with the ciliate *Th. acuminata*, in relation to the size of snails

The figures obtained for different Polish Carpathian districts were fairly various and amounted 45—84.6%. A more carefull study proved that those differences may be involved by the fact that the level of infection depends on the size of snails. In consequence the result of count is influenced by the ratio of big and small specimens in the sample studied. During 4 years the shells of 506 specimens of snails were measured. The results are summarized in the Table 3 and in diagram (Fig. 12).

The above data indicate that the juvenile individuals of *O. orientalis* fail to be infected with *Th. acuminata*. The smallest specimen of snail in which the parasite was detected measured 3.8 mm in width. Generally the ciliates occurred only in the specimens measuring over 4,5 mm in width. The absence of parasites in the very young forms may be accounted for by their narrow ureter of the 2nd order, so that the passage of ciliates through it is impossible.

Table 3

Infection extensity of *O. orientalis* with *Th. acuminata* as depending on the size of snails

Class of size (width of the shell of snail in mm)	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	Σ
Number of snails examined	1	7	20	66	72	95	125	87	29	4	506
Number of infected snails	—	—	5	42	58	87	121	85	29	4	431
%	—	—	25.0	63.6	80.6	91.6	96.7	97.7	100	100	85.2

In the classes of the snails of greater size the percentage of infection increases very rapidly, so that in the classes comprising the snails of the width over 9.5 mm all the specimens are infected. Since the size of the snails corresponds fairly well to their age, especially in young forms, it may be assumed that the rise of the infection extensity is connected with the age of the hosts and is involved by the possibility of contacts between them.

The relatively high differences of infection depending on the size of the snail indicate that the general value of extensity of infection without regard to the age characteristic of the population gives no exact information, since it may depend on the domination of mature or juvenile host individuals.

Co-parasites

Except *Th. acuminata* some other species of parasites occur in *O. orientalis*. These are: ciliates — *Semitrichodina sphaeronuclea* (Lom, 1956) (*Peritricha-Urceolariidae*) and *Tetrahymena* sp. Kazubski, 1960; the larva of the cestode *Choanotaenia crassiscolex* (Linstow, 1890) (*Cyclophyllidea-Dilepididae*); the metacercaria of a trematode *Brachylaime fulvum* Dujardin, 1843 (*Digenea-Brachylaimidae*) and nematodes [5]. Frequently snails were observed infected with *Th. acuminata* and with one or several other parasite species simultaneously. In one case the normal increase of population of *Th. acuminata* (after experimental inoculation) in a snail infected with *Brachylaime fulvum* Duj. 1843 was observed for many weeks. This snail was also infected with normally developing cysticercoids of *Choanotaenia crassiscolex* (Linstow, 1890).

The data of the simultaneous infection of *O. orientalis* with *Th. acuminata* and 4 other parasites are summarized in the Table 4. The diagram is based on informations obtained after dissection of snails with the shell width of over 5 mm. The inferior limit of the snail width 5 mm was chosen in order to eliminate the small specimens which might have been not infected with parasites on account of their age, as it occurs with *Th. acuminata*.

[5] The extensity of infection with nematodes was not registered because of the unsatisfactory knowledge of this group and of difficulty to distinguish the parasites from casualy occurring soil inhabitants and saprobiontic forms.

Figures show the infection with other parasite in the whole of the examined material and in this part of it which was infected with *Th. acuminata*. In all cases, the percentage of infection with the second parasite in the snail group with *Th. acuminata* is equally high or even higher than in the whole material representing the population of snails. This fact indicates that the simultaneous occurrence of both parasites in the same host specimen is fortuitous and proves the lack of antagonism between them.

Table 4

Simultaneous infection of *O. orientalis* with the ciliates *Th. acuminata* and some other parasite species

Number of specimens infected with other parasite	Infection with *S. sphaeronuclea*		Infection with *Tetrahymena* sp.		Infection with *Ch. crassiscolex*		Infection with *B. fulvum*	
	s	*t*	*s*	*t*	*s*	*t*	*s*	*t*
N	84	83	10	10	51	46	4	4
%	33.7	38.8	4.0	4.7	20.5	21.5	1.6	1.9

s = in total material examined, which amounted 249 anails.
t = in a part of material infected with *Th. acuminata*, which amounted 214 snails.

Another noteworthy fact is that up till now no other ciliate parasites were reported in *O. orientalis*. *Myxophyllum steenstrupi* (Stein, 1861), *Tetrahymena rostrata* (Kahl, 1926) and *T. limacis* (Warren, 1932) are all parasites of very numerous species of snails occurring sometimes in very similar ecological conditions, but were not found in *O. orientalis*. It is difficult to decide which factor involves this phenomenon, whether it is due to the antagonistic relations between those parasites and *Th. acuminata* which is the dominating parasite, or it is involved by the defense reaction of the host. Possibly the experimental study might solve this question.

Specificity of *Thigmocoma acuminata*

In the former study (Kazubski 1958 b) occurrence of *Th. acuminata* was reported as restricted to the Carpathian species *O. orientalis*. The subsequent study confirmed the lack of other hosts of this ciliate in Poland.

In 1956—62 over 5000 specimens of the snails belonging to 92 species were dissected. A half of this number belonging to 71 species were collected in the Carpathians, often in the localities of the occurrence of *Oxychilus orientalis* infected with *Th. acuminata*. In the course of those investigations the representatives of majority of species belonging to the family *Zonitidae* occurring in Poland were examined; among them 7 species occur together with *O. orientalis*. The list of investigated snails belonging to this family is presented in the Table 5.

Although an extensive faunistic research was carried out, the occurrence of *Th. acuminata* in Poland in any other snail except *O. orientalis* was not stated. However the research should not be considered as conclusive because about 60 snail species occurring in Poland had been not yet investigated, in

this number — also some species of *Zonitidae* e.g. *Oxychilus cellarius*. However, the majority of them are species existing in quite different external conditions than *O. orientalis*: living in soil, under plant roots, in warm and dry places. Small forms are dominating. To such groups less represented in the studied material belong the families: *Acmidae, Vertiginidae, Valloniidae, Enidae, Ferussaciidae* and the xerophilic representatives of *Helicidae*. For that reason finding of another hosts of *Th. acuminata* except *O. orientalis* in Poland can hardly be expected.

Table 5

List of dissected snails of the family *Zonitidae* collected in Poland

Snail species	Number of collected snails			
	from the Polish territory	from the Polish Carpathians territory	in the area of *Th. acuminata*	in localities with *Th. acuminata*
Subfamily *Zonitinae*				
Aegopinella pura (Ald.)	41	25	17	11
Ae. nitidula (Drap.)	7	—	—	—
Ae. nitens (Mich.)	19	19	—	—
Ae. minor (Stab.)	2	2	2	0
Ae. epipedostoma (Fagot)	109	104	104	63
Nesovitrea (Perpolita) radiatula (Ald.)	30	12	3	0
Oxychilus (Oxychilus) cellarius (Müll.)	0	0	—	—
O. (O.) draparnaldi (Beck)	2	—	—	—
O. (Ortizus) alliarius (Mill)	39	—	—	—
O. (O.) helveticus (Blum)	23	—	—	—
O. (Riedelius) inopinatus (Uličny)	0	0	0	—
O. (R.) depressus (Sterki)	1	1	1	0
O. (Morlina) glaber (Fer.)	51	51	21	11
O. (Cellariopsis) orientalis (Cless.)	516	516	516	504
Subfamily *Vitreinae*				
Vitrea crystallina (Müll.)	5	5	3	0
V. contracta (West.)	0	0	0	0
V. diaphana (Stud.)	124	122	113	92
V. subrimata (Reinh.)	0	0	0	0
V. transsilvanica (Cless.)	26	26	26	16
Subfamily *Daudebardiinae*				
Daudebardia (D.) rufa (Drap.)	2	2	0	0
D. (D.) brevipes (Drap.)	0	0	—	—
Carpathica calophana (West.)	6	6	6	5
Subfamily *Gastrodontinae*				
Zonitoides (Zonitoides) nitidus (Müll.)	844	17	17	0
Total	1847	908	829	702

The symbol "—" means the absence of the cited snail species in the territory, "0" means that this species occurs in this territory but is not represented in the material studied.

The dissections of the following species of *Oxychilus* Fitzinger, 1833 originating from the Balkans, Near East and Caucasus were carried out: 4 specimens of *Oxychilus (Cellariopsis) syriacus* (Kob.) from Lebanon, 6 of *Oxychilus* from the group *dalmatinus* (A. J. Wagner) = *macedonicus* Riedel from Yugoslavia, 5 of *O. (Hiramia) camelinus camelinus* (O. Bttg.) from Lebanon and Syria, 5 of *O. (Schistophallus) duboisi* (Mouss.) from Caucasus, 1 of *O. (Sch.) elegans* (O. Bttg.) from Iran, 1 of *O. (Sch.) horsti* (O. Bttg.) from Caucasus, 1 of *O. (Sch.) oscari* (Kimak.) from Bulgaria, 3 of *O. discrepans* (Ret.) from Caucasus, 1 of *O. difficilis* (O. Bttg.) from Caucasus and 2 of *O. (Oxychilus) sucinacius* (O. Bttg.) from Caucasus. All the specimens were fixed in alcohol and stored in the Zoological Institute of the Polish Academy of Sciences in Warszawa.

Dissection showed the presence of *Th. acuminata* in all examined specimens of *Oxychilus (Cellariopsis) syriacus* from Lebanon and in 2 specimens of *Oxychilus (Schistophallus) duboisi* from Caucasus. The result of investigation suggests the conclusion that *Th. acuminata* belongs to the species of a narrow specificity and that it is associated with the genus *Oxychilus* Fitzinger, 1833. Probably the occurrence of this parasite is even limited to the representatives of two rather related subgenera: *Cellariopsis* A. J. Wagner 1915 and *Schistophallus* A. J. Wagner 1914 [6]. This is also suggested by the fact that in the Polish territory, where only one representative of this group occurs — *O. orientalis* — this one is the host of *Th. acuminata* which fails to occur in the other *Zonitidae* although they live in the same conditions and even in the same habitats.

External environment

The knowledge of external conditions in which the host lives is — as a rule — of fundamental importance for elucidation of many biological and ecological phaenomena of life of the parasite. Therefore it seems advisable to analyse certain problems concerning the biology and ecology of: *Oxychilus (Cellariopsis) orientalis* (Cless.), *O. (C.) syriacus* (Kob.) and *Oxychilus (Schistophallus) duboisi* (Mouss.) which are hosts of *Th. acuminata*.

Th. acuminata was found in *O. orientalis* collected in the majority of the localities in the Carpathians investigated in the present study. It may be assumed that this ciliate always accompanies *O. orientalis* and that the conditions in which the snail occurs correspond to the parasite requirements.

The Carpathian host of *Th. acuminata* — the snail *O. orientalis* — usually occurs in leaf-woods: in *Querceto-Carpinetum medioeuropaeum* Tüxen 1936 and in *Fagetum carpatticum* Klika 1927 of the submountain region and of the lower "regiel" [7]. In the spruce forests of the upper "regiel" this snail was found only by Ložek 1949, 1951, 1957 on the Stožek and Velki K'lak (Mu-

[6] The groups *Schistophallus* A. J. Wagner 1914 and *Cellariopsis* A. J. Wagner 1915 are now included to genus *Oxychilus* Fitzinger 1833 as two separate subgenera or as one subgenus *Schistophallus*. Recently Riedel (personal communication) accepts *Cellariopsis* as a separate subgenus of the genus *Oxychilus* and includes to it: *O. (C.) orientalis* (Cless.), *O. (C.) cyprius* (L. Pfr.), *O. (C.) macedonicus* Riedel and *O. (C.) syriacus* (Kob.).

[7] Lower „regiel" and upper „regiel" are two zones of the mountain forest in Carpathians.

ransky Kras) and in the Kralowa Hol'a massif in the eastern part of the Lower Tatra.

O. orientalis occurs on the shore of small streams and in areas of resurgences, in humid and shady places grown by hydrophilic vegetation. Its habitat is the surface of soil. They are found sitting under the stones, pieces of wood, fallen leaves, or creeping on the soil. Sometimes they penetrate deeper inside the soil layer and there may be found in crevices between the stones at a depth down to 50 cm. Ložek found them under stones near the entrance to caves.

The humid banks of streams — places of *O. orientalis* occurrence — are the environment in which numerous arthropods, soil nematodes and some other small invertabrates occur. This fact is of importance for *O. orientalis* which is a predatory animal. It may also feed upon cadavers of some small animals and on plant detritus.

Usually *O. orientalis* appear collectively. In some places, in especially favourable conditions they form large agglomerations. The density of such aggregation amounts sometimes up to 30—50 specimens in 1 m^2. Sometimes under one stone over 10 snails close one to another may be found. The temperature of places in which *O. orientalis* occurs is rather low and constant, humidity is usually high. The direct observations showed that the temperature of the substrate on which the individuals of *O. orientalis* stayed, fluctuates within the limits: 5.4—16.8°C. In most cases it reaches only up to 12°C.

After having studied the main outline of the habitat conditions in which *O. orientalis* occurs (see above) it seemed reasonable to elucidate the response of *Th. acuminata* to some environmental factors. The temperature is the factor potentially the most variable and not controlled by the host organism. The action of this environment factor is permanent whereas other factors are acting directly only when the ciliate leaves the host organism.

The resistivity of ciliates to the action of temperature was studied experimentally. The snails infected with *Th. acuminata* were kept in climatization chamber and then subjected to rises and falls of temperature (depending on the series of experiment) at a relative humidity of air = 70—100%. As the start point the temperature of 7—12°C was fixed. In this temperature the snails were kept for a long time. Every one or three days the temperature was raised or lowered by 2°C. One or two snails were dissected every day. Two series of experiments with the rising and falling temperature were performed. The results of experiments conducted in different seasons: in summer and in winter, were nearly the same.

The above study indicate that the ciliates are able to support the low temperature and die only at −5°C even when the host survives this fall. In contrast to this, at − 3°C ciliates are able to live even for 3 days.

The upper limit of resistance of *Th. acuminata* is about + 28°C which is survived only by few specimens for 1 day. Temperature of + 30°C is lethal even when acting only for a few hours. This temperature was always applied with satisfactory result for sterilizing the host prior to experiment. The snails support the temperature of + 30°C and even more, acting for several hours without showing any harmful consequences.

The following experiment was executed for proving the action of abrupt changes of temperature upon *Th. acuminata*. Infected ciliates were kept for a prolonged time at the temperature of + 7°C and then transferred for one day to: 0°C, — 2°C, — 4°C, — 5°C, — 7°C, or to: + 22°C, + 24°C, + 26°C, and

+ 28°C. Then the parasitological dissection was executed. No notable differences in the survival of *Th. acuminata* when compared with the previous experiments were to be reported. Also in this experiment ciliates died only in the temperature − 5°C and + 28°C.

As it seems, the abrupt changes of temperature fail to exert any significant noxious influence upon *Th. acuminata*.

Considering the limits of temperature in which the species *Th. acuminata* lives in the Polish territory it may be assumed that the average temperature of about + 10°C up to + 11°C is the optimal for this form. This supposition is supported by observations in natural environment — occurrence of infected snails in places of a similar temperature — and also by the results of several months long observation of snail culture with normally developing ciliates in the laboratory at + 12°C.

The very high sensitivity of ciliates to the raised temperature is obvious. In spring and in autumn *O. orientalis* together with its parasites are subjected to slight frost. On the contrary in their natural environment they are seldom exposed to a higher temperature (20—28°C). So their adaptation to the last is much lower.

The ecological informations about other hosts of *Th. acuminata* are very scarce. *Oxychilus (Cellariopsis) syriacus* (Kob.) lives on shrubby rocks sheltered in crevices. Mass occurrence of those snails is also known in the caves (Riedel 1962).

Oxychilus (*Schistophallus*) *duboisi* (Mouss.) occurs in humid leaf-woods with a rich undergrowth, and also on humid lime-stone debris overgrown with vegetation. There it lives in the cool and humid spaces between the rocks.

It follows from this review that the natural conditions in which the snail host of *Th. acuminata* occurs have many common features: shade, high humidity and — involved by them — the low temperature of environment. These conditions are favourable for the rather motile mode of life. Besides all the described species occur collectively and — like other species of the genus *Oxychilus* Fitz., are either predators or omnivorous.

The facts presented above indicate a striking harmony of different ecological requirements of *Th. acuminata* and of its host. This concerns before all the temperature, shadowing and humidity. A fact most favorable for the parasite is the collective occurrence of the host and its active mode of life as a predatory animal. As result, nearly 100% of big individuals of *O. orientalis* were found infected with *Th. acuminata* irrespectively of the site of origin, which proves a high mutual adaptation of both organisms. On the other hand it also indicates a long-lasting link between them. Probably the relations of *Th. acuminata* and its other hosts are similar.

Geographical distribution

The geographical distribution of *Th. acuminata* was as yet discussed in 3 publications. In the first (Kazubski 1958 b) the description of the species and its typical locality, "locus typicus", in the environment of Sanok (south-east Poland) was reported. In the next papers (1959. 1960) with the progress of investigations, the known geographical area of the ciliate extended gradually over the large territory of the Polish Carpathians. A suggestion was also expressed (1960) that this species may be limited in its distribution to

the Carpathians only. Presently 40 localities of *Th. acuminata* occurrence are known. The distribution of this ciliate in the Polish territory is known best (29 localities). *Th. acuminata* occurs in this country in the entire Carpathians, from the mountain Luboń Wielki in the Beskid Wyspowy chain up to the eastern frontier of the country. The northern boundary of this area runs more or less along the line: Tarnów—Dębica—Rzeszów—Przemyśl. *Th. acuminata* has not been found in the Tatra Mountains, in Silesian Beskid and in environment of Ojców, biocoenotically related to some Subcarpathian regions. In the Polish territory, the boundaries of *Th. acuminata* and of *Oxychilus orientalis* areas exactly coincide. Further eastwards *Th. acuminata* was found in the snails collected in USSR on the slopes of Polonina Rovna, in the district of Hoverla (upper Prut valley); in Roumania in Putna (Radauti environment) and in the region of Sinaia Pass [8].

Probably *Th. acuminata* occurs all over the area of *O.orientalis*, which embraces a part of Western Carpathians (as far as the Beskid Wyspowy, Bělanske Tatry, the eastern part of the Lower Tatra, Slovenska Rudohora and the Bükk mountains inclusively) and the whole Eastern Carpathians.

The distribution of other hosts of *Th. acuminata* has been little studied. *Oxychilus syriacus* is known to occur in several localities in Lebanon mostly on the sea coast, an from Baalbek in the Bakaa valley. It was also reported from Tartus in Syria and from Iskanderun in Turkey (Riedel 1962). *Th. acuminata* has been stated in this host in the material collected in the Nahr el Kelb valley near Beyrouth. *O. duboisi* occurs only in Caucasus in Georgia; the snails infected with *Th. acuminata* originated from the environments of Kutaisi.

Probably *Th. acuminata* is as common parasite of those two snails as it is of the Carpathian *O. orientalis*. There is also no reason to believe that the area of *Th. acuminata* does not include — except the above mentioned regions — also the mountains of the Balkan peninsula and of Asia Minor, where another host of this ciliate might be expected since the area of its known hosts is restricted and their distribution is vicarious. The narrow specificity of *Th. acuminata* allows to anticipate that the new, not recorded hosts in those regions might be *Cellariopsis* and *Schistophallus* or some other species closely related to them.

The territory of the Black Sea basin and the North-eastern part of the Mediterranean region being the main habitat of majority of species and subspecies of the genus *Oxychilus* (including *Cellariopsis* and *Schistophallus*) is probably also the centre out of which *Th. acuminata* began to extend. In the Polish territory *Th. acuminata* appeared probably together with its host and with the beech woods after the recession of the last glaciation. According to Szafer [9] the expansion of the beech over the Carpathians took place after the refrigeration and humidification of the climate in the Polish territory in the period of 2500—500 years before our era. This expansion proceeded from the centres in the South-east Carpathians. Ložek 1957 recorded the occurrence of *O. orientalis* shells in the Muransky Kras, in the layers

[8] A detailed list of localities of *Th. acuminata* occurrence will be included in the paper discussing some biological and ecological problems and the distribution of *O. orientalis*. The paper will appear in „Fragmenta faunistica".

[9] Szafer W. 1959: Szata roślinna Polski. PWN, Warszawa (v. I, p. 586; v. II, p. 333).

between the lower and upper holocen, together with other species of snails connected with the biotop of *Abies* and *Fagus*. Nevertheless in the collection of the Zoological Institute of Polish Academy of Sciences a shell of *O. orientalis* is kept, determined by A. J. Wagner, originating from the pleistocen limetuff of Aji volgy in Hungary.

The general conclusion should be ascertained that the ciliate *Th. acuminata* of our fauna belongs to the mountain elements of the Pontic origin.

Systematic position of *Thigmocoma acuminata*

In the discussion of systematic position of *Th. acuminata* (Kazubski 1958), the conclusion was drawn that this species is distinct from all the other *Thigmotricha* and a new genus and new family were established for it. Beyond doubt, this species should be included to the *Thigmotricha* by reasons of its distinct thigmotactic field and its buccal ciliature typical for this group. The formation of a separate genus *Thigmocoma* Kazubski 1958 evoked no objection from any part. Discussion arose only because of the new separate family *Thigmocomidae* Kazubski 1958 to which this genus was included. Corliss 1961 cancelling the families which included only one genus and — according to his view — were supported by scarce records, included the family *Thigmocomidae* Kazubski 1958 into the *Hemispeiridae* König 1894, citing however its diagnosis and expressing his opinion that after a more detailed study it might be recognized as a separate taxonomic entity. In consequence, there exist a justified necessity to reconsider this problem basing on new material contained in the present paper and to discuss some consequences which are involved by establishing this new family in the system of *Thigmotricha*.

The group, suborder or order — *Thigmotricha* was established by Chatton et Lwoff and contained then 4 families: *Thigmophryidae* Ch. et Lw. 1923; *Ancistridae* Issel 1903; *Hypocomidae* Bütschli 1889 and *Sphaenophryidae* Ch. et Lw., 1921. The next years brought some new publications which introduced a huge material of facts to the knowledge of those parasitic ciliates and also some interesting taxonomical concepts. This group contains presently about 150 species belonging to 58 genera (Corliss 1961). In their monograph Chatton et Lwoff 1949, 1950 rank among *Thigmotricha* 6 families, grouped into 2 tribes: *Stomodea* Ch. et Lw., 1939, with the families *Thigmophryidae* Ch. et Lw., 1923, *Conchophthiridae* Kahl 1931 and *Hemispeiridae* König 1894; and the tribe *Rhynchodea* Ch. et Lw., 1939 with the families *Hypocomidae* Bütschli 1889 sensu Ch. et Lw. 1939; *Ancistrocomidae* Ch. et Lw. 1939, and *Sphenophryidae* Ch. et Lw. 1921. Raabe 1939, 1949 included the family *Hysterocinetidae* Diesing 1866 into *Thigmotricha*. This change was recognized by Kudo 1954 and by Corliss 1961. To *Thigmotricha-Stomodea* was included also the family *Thigmocomidae* Kazubski 1958.

The family *Thigmocomidae* Kazubski 1958 may be characterized [10] in the following diagnosis.

Thigmotricha flattened laterally, with a strong, morphologically developed thigmotactic field which is situated on the left side of the body. The meridional rows tend to shorten and fail to extend over the whole surface of the ciliate.

[10] This characteristic of the family *Thigmocomidae* and of the genus *Thigmocoma* contains some new elements when compared to the former diagnosis published in 1958.

In division, besides the general fission of rows into fragmented segments which are to belong to the proter and the opisthe, secondary fission occurs resulting in the detachment of short segments of the posterior ciliature. The buccal ciliature consists of the kinety "1" of a stomatogenic haplokinety type and of adoral membranellae ABC of the polykinety type. This ciliature tends to shorten and to migrate towards the centre of the body.

Parasites of internal organs of land snails.

The type-genus: *Thigmocoma* Kazubski 1958 comprises the ciliates with strongly flattened body sides and a differentiated ciliary system, composed of the general, the thigmotactic and the posterior ciliatures. The meridional rows of the general and thigmotactic ciliatures show a strong reduction and occupy only the anterior body part; most shortened are the thigmotactic rows. The posterior ciliature of the right body side tends to a considerable reduction or even to complete absence. Between the general and thigmotactic ciliatures, unciliated zone is present. The buccal ciliature developed in its typical form lies on the ventral margin, the cytostome — more or less in the middle of the body length.

The type-species: *Thigmocoma acuminata* Kazubski, 1958.

A detailed comparison of *Thigmocomidae* with other families leads to the following conclusions.

Thigmocomidae show few features common with *Thigmophryidae* and *Conchophthiridae*. The representatives of both families have a uniform ciliature over the whole body surface consisting of a great number of closely set meridional rows. Although the thigmotactic area is already slightly marked, but it consists — in some species e.g. *Conchophthirus discophorus* Mermod — of the anterior segments of the meridional rows of the left body side which show neither any distinct alterations nor reduction.

The structure of the buccal apparatus has been little investigated. In *Thigmophryidae*, the buccal rows were not described. They probably fail to occur in the trophic form (Raabe 1936, Chatton et Lwoff 1949). In *Conchophthirus unionis* Raabe and *C. curtus* Engelmann two buccal rows were described by Raabe 1963; one of them is considered as stomatogenetic "1" and the second — as membranellae ABC. A certain similitude to *Thigmocomidae* would follow from these statements but the differences in stomatogenesis in *Conchophthirus* and in *Thigmocoma* — as mentioned above — speak against a close relation of those two families.

The only plain similitude between *Thigmocomidae* and some representatives of the families under discussion e.g. *Myxophyllum steenstrupi* (Stein) or *Conchophthirus unionis* Raabe concern only the body shape and the general structure of the thigmotactic sucker. The similar way of life is surely responsible for those similitudes and they should be looked upon as convergent features. In conclusion, the families *Thigmophryidae* and *Conchophthiridae* should be ascertained as distant from *Thigmocomidae*.

The *Thigmocomidae* show much more of common with *Hemispeiridae* sensu Chatton et Lwoff 1949. Their similitudes concern before all the differentiation of the general and the thigmotactic ciliatures and also the reduction of the number of meridional rows and of kinetosomes in each row. Also the elements of the buccal ciliature are the same and the course of stomatogenesis is fully similar.

The family *Hemispeiridae* has been studied in details by Chatton et Lwoff 1949. They distinguished two subfamilies in it: *Protophryinae* Cépède 1910 and *Hemispeirinae* König 1894 — both with distinct evolutionary tendencies. *Protophryinae* are characterized by the origin of their thigmotactic field from the anterior segments of the meridional rows on the left body side. Those rows keep their normal length and reach as far as the suture on the posterior body pole. In *Hemispeirinae,* the thigmotactic field is formed by the dorsal kineties, which are much reduced and closed, as if in parantheses, by the nearest kineties of the general ciliature. In both subfamilies, the peristomal kineties migrate backwards and twist round the posterior body pole.

In *Thigmocomidae* some properties may be observed which are characteristic for one subfamily and simultaneously excluding them from the other. *Thigmocomidae* as well as *Protophryinae* are flattened laterally; their thigmotactic area lies on the left body side but it is formed by the whole thigmotactic rows and not only by their anterior segments as it is in *Protophryinae.* As in *Hemispeirinae* the rows are much shortened, especially the thigmotactic rows are strongly reduced; they are highly differentiated morphologically and owing to their lateral position they are not confined by the system of "parantheses" by the general ciliature. For that reason, the *Thigmocomidae* should rather be treated as at least a taxonomical unity equivalent to *Protophryinae* and to *Hemispeirinae.*

There are yet some features essentially different in *Thigmocomidae* and in both subfamilies discussed. Those differences concern the ability to secondary fissions of kineties, formation of the posterior ciliature, the tendency of the buccal ciliature to migrate towards the center of the body and the domination of the membranellae ABC over the stomatogenic kinety. The known representatives of *Protophryinae* and *Hemispeirinae* are parasites of aquatic animals: molluscs, holothurians; they live on walls of various organs washed by water (e.g. the mantle cavity and gills of molluscs and water lungs of holothurians).

In contrast to the former, the only species of *Thigmocomidae* is the endoparasite of land snails. It shows a profound biological and ecological adaptation to the parasitism in this group of land invertabrates. Its close and prolonged connection with the host is proved by its high specificity.

All those differences induce to support the theory put forward in 1958: *Thigmocomidae* occupy an independent position among *Thigmotricha* being distinct from *Hemispeiridae* König 1894 sensu Chatton et Lwoff 1949.

The comparison of *Thigmocomidae* to other families of *Thigmotricha* presents no special difficulty. They differ from *Hysterocinetidae* by the lack of the characteristic naked sucker; from other *Thigmotricha-Rhynchodea* they are distinguished by the presence of the peristomal apparatus, although in *Ancistrocomidae* a similar reduction of the kineties in length and number is observed.

It seems to follow from the above discussion that the analysis of the mutual relations of the subfamilies: *Protophryinae* Cépède 1910 and *Hemispeirinae* König 1894, included presently into the family *Hemispeiridae* König 1894 sensu Chatton et Lwoff, 1949 would be of great importance. Both subfamilies show some essential differences concerning — before all — the position of the thigmotactic area (on the left side in *Protophryinae* and on the dorsal in *Hemi-*

speirinae) and also the mode of the reduction of ciliature. In *Protophryinae* the rows keep their length similar to the body length and the reduction of ciliature is expressed in the more scarce distribution of kinetosomes in the posterior segments of rows, whereas in *Hemispeirinae*, reduction concerns the length of rows, especially of the thigmotactic rows. It seems obvious that both groups were developing independently from one another.

As a most important feature, common to both subfamilies in the family *Hemispeiridae*, Chatton et Lwoff 1949 put forward the structure of the adoral rows. However the presence of a similar construction of the buccal ciliature can scarcely be looked upon as an essential link of those two subfamilies, since the buccal ciliature of *Thigmocomidae* and *Conchophthiridae* is formed also on the same principle, as well as probably that of other groups not belonging to *Thigmotricha*. As conclusion, it seems justified to support the concept of distinction of those two subfamilies as separate families. As the present discussion is not exhausting the problem which should be solved in relation to the entire system of *Thigmotricha*, the decisive conclusion must be left to the future revision of *Thigmotricha*.

Some concepts arise concerning the position of the family *Thigmocomidae* in the system of *Thigmotricha*. The presented material indicates that *Thigmocomidae* should in no case be bound with the group of the families: *Thigmophryidae*, *Conchophthiridae* and *Hysterocinetidae* characterized by their dense, non reduced ciliature. In contrast to this the relationship is distinctly perceptible in the case of *Protophryinae*, *Hemispeirinae* and *Ancistrocomidae*, in which the ciliature is never so dense and even shows the tendency to reduction as well of the meridional rows as of the kinetosomes in each row. The *Protophryinae* and *Hemispeirinae* are related to *Thigmocomidae* by the common type of buccal ciliature and by the similar course of stomatogenesis — whereas *Hemispeirinae* and *Ancistrocomidae* are linked to *Thigmocomidae* by the reduction of their ciliature and before all — by the distinction of short rows of the thigmotactic ciliature.

Summary

Thigmocoma acuminata Kazubski, 1958 (*Thigmotricha-Thigmocomidae*) is one of the several presently known ciliates parasites of the land *Gastropoda*. The present study is a monographic account of investigations treating of different problems of morphology, morphogenesis as well as biology and ecology of this species. The morphology of the trophic form, its division and conjugation, the argentophilic system and its changes in the growth process, the morphogenetic and stomatogenetic reorganization in division — have been analyzed in details.

The biological and ecological research concerned the following problems: localization of the parasite inside the host ureter, intensity of infection of snails, harmfulness of the parasite to the host, the presence of some senile individuals incapable to division, the low frequency of division involving the low rate of the population increase in the host, the mechanism of regulation of numbers in the population, the ways of infection, the extensity of infection in the Carpathian snail *Oxychilus orientalis* (Cless), relation of *Th. acuminata*

to other parasites of *O. orientalis*, specificity of *Th. acuminata* as a parasite and the description of the environment of the host occurrence.

The ciliate occurs in the Carpathians (in Poland, USSR and in Roumania) as a parasite of *Oxychilus (Cellariopsis) orientalis* (Cless.), in Lebanon in *O. (C.) syriacus* (Kob.) and in Caucasus in *O. (Schistophallus) duboisi* (Mouss.).

Supplementary diagnoses of the genus *Thigmocoma* Kazubski 1958, and of the family *Thigmocomidae* Kazubski, 1958 were precised and the distinctness of *Thigmocomidae* as a separate family among *Thigmotricha* was reconsidered.

In favour of diversity of the family *Thigmocomidae* from the subfamily *Protophryinae* Cépède 1910 the reduction of their ciliature, from the subfamily *Hemispeirinae* König 1894 localization of their thigmotactic area, and from the whole family *Hemispeiridae* König 1894 sensu Chatton et Lwoff 1949 — their ability of development of the posterior ciliature and the tendency of their peristome to shift backwards, were put forward.

STRESZCZENIE

Thigmocoma acuminata Kazubski, 1958 (*Thigmotricha-Thigmocomidae*) jest jednym z kilkunastu, znanych obecnie, orzęsków pasożytujących u lądowych *Gastropoda*. Przedstawiona praca jest monograficznym opracowaniem tego gatunku.

W pracy szczegółowo omówiono morfologię postaci troficznej *Th. acuminata* oraz morfologię podziału i konjugacji. Szczególną uwagę zwrócono na struktury argentofilne. Omówiono między innymi zmiany zachodzące w układzie argentofilnym w czasie wzrostu oraz procesy morfogenetyczne i stomatogenetyczne w czasie podziału orzęska.

W części poświęconej biologii i ekologii *Th. acuminata* rozpatrzono między innymi: lokalizację orzęska w moczowodzie ślimaka, intensywność zarażenia ślimaków oraz kwestię szkodliwości tego orzęska dla żywiciela. Przy omawianiu rozrodu *Th. acuminata* zwrócono uwagę na obecność osobników, które uznano za starcze, i które prawdopodobnie nie przystępują już do podziałów. U *Th. acuminata* występuje niska częstotliwość podziałów, wiąże się z tym także niskie tempo wzrostu populacji tego orzęska w żywicielu. Omówiono również istniejący mechanizm regulacji populacji *Th. acuminata*, sposoby zarażania nowych osobników ślimaków: ekstensywność zarażenia karpackiego ślimaka *Oxychilus orientalis* (Cless.), stosunki *Th. acuminata* do innych pasożytów występujących u *O. orientalis*, specyficzność *Th. acuminata* oraz środowisko zewnętrzne, w jakim występują żywiciele tego pasożyta. *Th. acuminata* występuje w Karpatach (w Polsce, ZSRR i Rumunii) u *Oxychilus (Cellariopsis) orientalis* (Cless.), w Libanie u *O. (C.) syriacus* (Kob.) i na Kaukazie u *O. (Schistophallus) duboisi* (Mouss.).

Podano uzupełnione diagnozy: rodzaju *Thigmocoma* Kazubski 1958 i rodziny *Thigmocomidae* Kazubski 1958 oraz rozpatrzono samodzielność rodziny *Thigmocomidae* w obrębie *Thigmotricha*. *Thigmocomidae* różnią się od podrodziny *Protophryinae* Cépède 1910 redukcją urzęsienia, od podrodziny *Hemispeirinae* König 1894, odmiennym umiejscowieniem powierzchni tigmotaktycznej, a od całej rodziny *Heimispeiridae* König 1894, sensu Chatton et Lwoff 1949 zdolnością do wytwarzania urzęsienia tylnego oraz brakiem tendencji do przemieszczania się gęby na tylny koniec ciała orzęska.

REFERENCES

Chatton E. et Lwoff A. 1936: Les remaniements et la continuité du cinétome au cours de la scission chez les Thigmotriches Ancistrumidés. Arch. Zool. exp. gén. 78, 84—91.

Chatton E. et Lwoff A. 1949: Recherches sur les ciliés Thigmotriches. I. Arch. Zool. exp. gén. 86, 169—253.

Chatton E. et Lwoff A. 1950: Recherches sur les ciliés Thigmotriches. II. Arch. Zool. exp. gén. 86, 393—485.

Corliss J. O. 1960: Comments on the systematics and phylogeny of the *Protozoa*. Syst. Zool. 8 (yr. 1959), 169—190.

Corliss J. O. 1961: The ciliated *Protozoa*; characterization, classification, and guide to the literature. Pergamon Press, Oxford.

Frenkel J. 1960: Morphogenesis in *Glaucoma chattoni*. J. Protozool. 7, 362—376.

Kaczanowski A. 1961: Studies on *Protoptychostomum simplex* (André, 1915), *Ciliata-Thigmotricha*. Acta Parasitol. Polon. 9, 257—272.

Kazubski S. L. 1958 a: *Semitrichodina* gen. nov. *sphaeronuclea* (Lom, 1956) *(Peritricha-Urceolariidae)* in *Schistophallus orientalis* Cless. *(Pulmonata-Zonitidae)* in Poland. Bull. Acad. Pol. Sci., Cl. II, 6, 109—112.

Kazubski S. L. 1958 b: *Thigmocoma acuminata* gen. nov., sp. nov., *(Thigmotricha-Thigmocomidae* fam. nov.) a parasite of the renal organ of *Schistophallus orientalis* Cless. *(Pulmonata-Zonitidae)*. Bull. Acad. Pol. Sci., Cl. II. 6, 167—172.

Kazubski S. L. 1958 c: *Trichia lubomirskii* Slos. *(Helicidae)*, a new host of *Tetrahymena limacis* (Warren, 1932) Kozloff, 1946 *(Ciliata)* and *Zonitoides nitidus* Müll. *(Zonitidae)* a new host of *T. rostrata* (Kahl, 1926) Corliss, 1952 in Poland. Bull. Acad. Pol. Sci., Cl. II. 6, 247—252.

Kazubski S. L. 1958 d: Wyniki dotychczasowych badań nad *Ciliata* i niektórymi innymi pasożytami ślimaków lądowych. Wiadom. Parazytol. 4, 665—666.

Kazubski S. L. 1959: Studies on the Ciliate Parasites of the land snails in Poland in the years 1956—58. J. Protozool. 6 (suppl.), 102.

Kazubski S. L. 1960: Materialy k poznaniju fauny parazitičeskih infuzorij nazemnych moljuskov Karpat. Flora i fauna Karpat (sbornik rabot). Izd. AN SSSR. Moskva 220—223.

Kazubski S. L. 1961 a: Zastosowanie metody wielokrotnych obserwacji przyżyciowych do badania pasożytów ślimaków lądowych. Wiadom. Parazytol. 7, (suppl.) 159—161.

Kazubski S. L. 1961 b: Vital observations on the fission rate in *Thigmocoma acuminata*. First International Conference of Protozoologists. Praha, August 1961.

Kidder G. W. 1933 a: On the genus *Ancistruma* Strand *(Ancistrum* Maupas). I. The structure and division of *A. mytili* Quenn. and *A. isseli* Kahl. Biol. Bull. 64, 1—20.

Kidder G. W. 1933 b: Studies on *Conchophthirus mytili* De Morgan. I. Morphology and division. Arch. Protistenk. 79, 1—24.

Kozloff E. N. 1945: *Cochliophilus depressus* gen. nov., sp. nov. and *Cochliophilus minor* sp. nov., holotrichous ciliates from the mantle cavity of *Phytia setifer* (Cooper). Biol. Bull. 89, 95—102.

Kudo R. R. 1954: Protozoology. 4. ed. Charles C. Thomas, Springfield. Illinois.

Ložek V. 1949: Makkýše Muranskeho Krasu. Přirodovedný Sbornik 4, 119—158.

Ložek V. 1951: Plž *Schistophallus orientalis* Cl. v Muráňském a Jihoslovenském Krasu. Československý Kras 4, 219—222.

Ložek V. 1957: Malakozoologické výzkumu na Hornim Hronu. Biologia 12, 44—62.

Raabe Z. 1934: Weitere Untersuchungen an einigen Arten des Genus *Conchophthirus* Stein. Mem. Acad. Pol. Sci. Lettr. Cracovie B (II) 211—235.

Raabe Z. 1936: Weitere Untersuchungen an parasitischen Ciliaten aus dem polnischen Teil der Ostsee. Ann. Mus. Zool. Pol. 11, 419—442.

Raabe Z. 1939: Z badań nad rodziną *Hysterocinetidae* Diesing *(Ciliata-Holotricha)*. Spraw. Tow. Nauk. Warsz. 32, 29—57.

Raabe Z. 1947 a: Recherches sur les ciliés Thigmotriches (*Thigmotricha* Ch. & Lw.). II. Espèce nouvelle d'eau douce du genre *Ancistrina* Cheissin. Ann. Univ. M. Curie Skłodowska, Sect. C 2, 111—120.

Raabe Z. 1947 b: Drogi przystosowań morfologicznych do życia pasożytniczego wśród wymoczków. Les voies des adaptations morphologiques à la vie parasitique chez les ciliés. Ann. Univ. M. Curie-Skłodowska, Sect. C, 2, 299—411.
Raabe Z. 1949: Studies of the family *Hysterocinetidae* Diesing. Ann. Mus. Zool. Pol. 14, 21—68.
Raabe Z. 1963: La stomatogenèse chez *Conchophthirus (Ciliata-Thigmotricha)*. Acta Protozool. 1, 139—146.
Riedel A. 1957: Revision der Zonitiden Polens (*Gastropoda*). Ann. Zool. Warszawa 16, 361—464.
Riedel A. 1962: Materialien zur Kenntnis der *Zonitidae (Gastropoda)* des Nahen Ostens, nebst Besprechung der Gattung *Eopolita* Poll. in breiteren geographischen Rahmen. Ann. Zool. Warszawa 20, 261—298.
Rudzinska M. A. 1961: The use of a protozan for studies on aging. II. The macronucleus in young and old organisms of *Tokophrya infusionum*, light and electron miscroscope observations. J. Gerontol. 16, 326—334.

EXPLANATION OF PLATES I—III

1—2: *Thigmocoma acuminata* Kazubski, 1958, living, lens × 20, anoptral contrast system, Reichert

3: *Th. acuminata*, ciliary waves on the thigmotactic area, Zenker-Helly fixation, Feulgen, immersion, phase contrast optics, Reichert.

4: *Th. acuminata*, living, the thigmotactic rows and the granular structure of macronucleus are seen, immersion, phase contrast, Reichert.

5: *Th. acuminata*, argentophilic system, right side, $AgNO_3$, immersion.

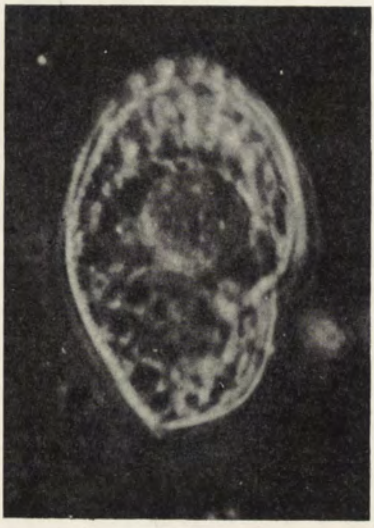
6: *Th. acuminata*, argentophilic system, left side, $AgNO_3$, immersion.

7: *Th. acuminata*, buccal ciliature, right side of the ciliate, stomatogenic row "1" and the proximal segment of the adoral polykinety are seen, $AgNO_3$, immersion.

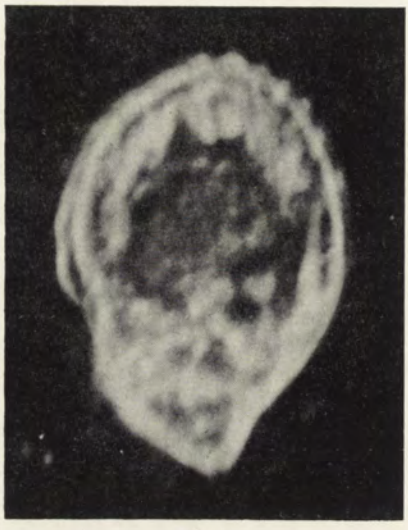
8: *Th. acuminata*, buccal ciliature, left side of the ciliate, the anterior segment of the adoral polykinety is seen, $AgNO_3$, immersion.

9—10: *Th.acuminata*, initial phase of division, zone of rise of the kinetosomes number, $AgNO_3$, immersion.

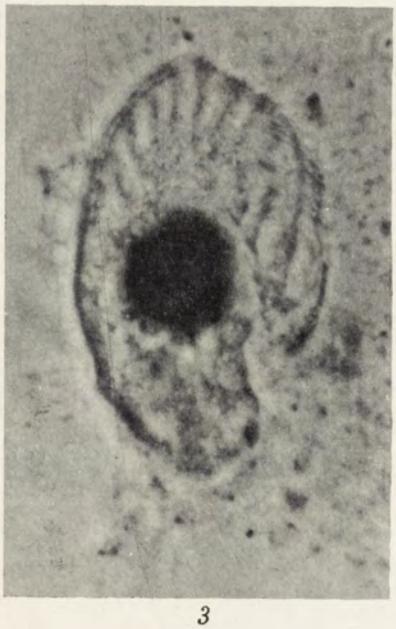
11—12: *Th. acuminata*, division, stomatogenesis, occurrence of transverse groups of kinetosomes, $AgNO_3$, immersion.



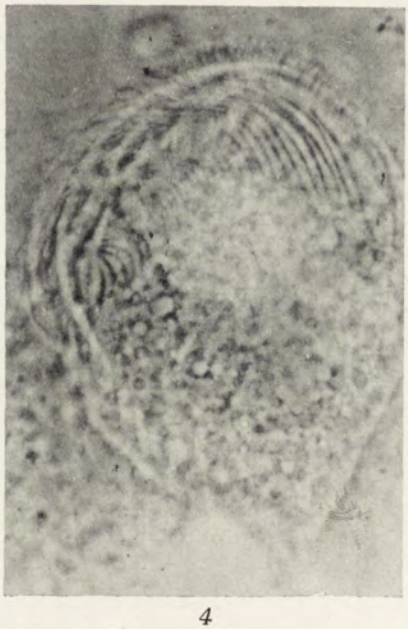
1



2



3

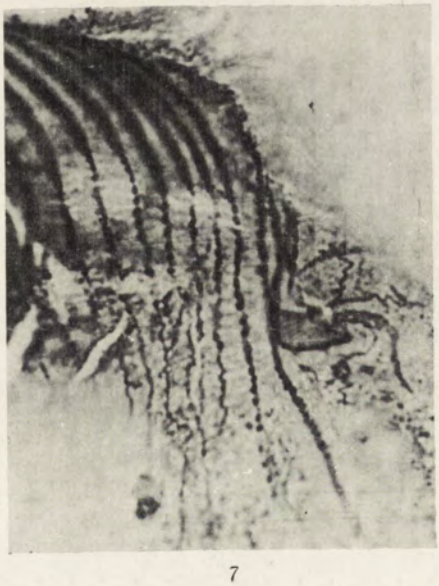


4

S. L. Kazubski auctor phot.

5

6



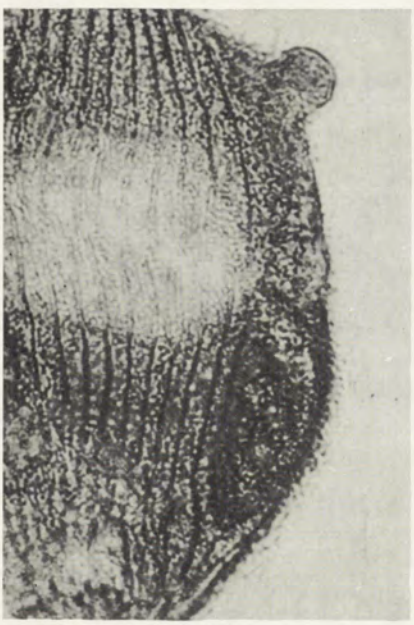
7

8

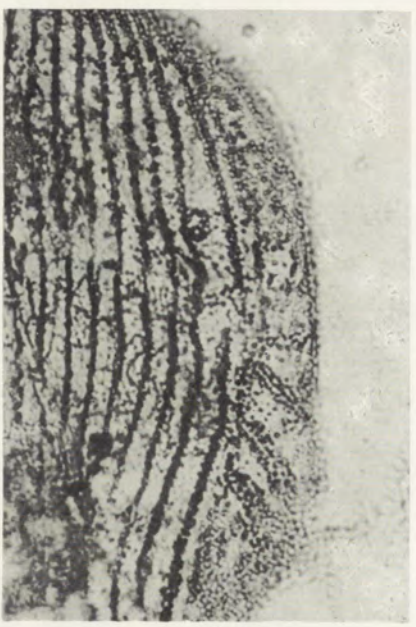
S. L. Kazubski auctor phot.

9

10



11



12

S. L. Kazubski auctor phot.

ACTA PROTOZOOLOGICA

VOL. I WARSZAWA, 30. IX. 1963 FASC. 26

Department of Protozoology, Institute of Parasitology, Czechoslovak Academy of Sciences, Praha 2, Viničná 7

Jiří LOM and Jiří VÁVRA

Fine morphology of the spore in *Microsporidia*

Ultrastruktura spor mikrosporidií

Microsporidian spores are known to be a notoriously difficult object for observations in light-microscopy. Their small dimensions and thick spore cases caused the lack of exact knowledge of their inner morphology until the first electron microscope investigations of Weiser 1959 and Huger 1960. The most peculiar and till recent time also the most enigmatic structure of the spore is the polar filament, which can be extruded out of the spore in response to a given stimulus. The explication of this process was dependent on the knowledge of the structure of the filament, so that this question has attracted the attention of Krieg 1953 before the two papers mentioned above. We have been able to observe the process of extrusion in detail (Lom and Vávra 1963) and it was necessary to complete it with electron optic observations presented here.

Methods

The following species of *Microsporidia* were used in our study: *Plistophora hyphessobryconis* Schäperclaus from *Carassius auratus* f. *bicaudatus*, *Bacillidium cyclopis* Vávra from *Acanthocyclops americanus*, *Bacillidium* sp. from tubificid Oligochaetes, *Glugea (Pyroteca)* sp. from *Eucyclops serrulatus*. The spores, freshly removed from their hosts, were fixed in Pallade's and Wohlfarth-Bottermann 1957 fluid and embedded into Vestopal W.; 1% phospho-tungstic acid in 75% acetone was used for staining during dehydration. Thin sections were cut on Reichert OMU ultramicrotome and examined with Elmiskop I Siemens and Tesla BS 242 electron microscopes. [1]

In order to obtain extruded filaments in *Plistophora hyphessobryconis*, we applied the hydrogen peroxide in approximatively 5% concentration. Since the remnants of the H_2O_2 reacts strongly with osmic acid in fixing fluids, producing innumerable bubbles, which prevent the formation of a pellet suitable for further dehydration, we employed the potassium chromic sulphate solution for washing the extruded spores before fixation. For details in artificial extrusion of microsporidian spores see Lom and Vávra 1963.

[1] We are greatly indebted to Dr. J. Rozsíval, director of the Institute of the Physics of Solid Materials of ČSAV and to Drs. M. Ryšavá and V. Matěna from the Institute of Physics of ČSAV for allowing us generously to use their electron microscopes.

Results

The spore case is of compound structure, consisting of three different layers. A very thin inner membrane adheres to the sporoplasm and a very thick middle layer forms the proper solid envelope of the spore visible as light in electron micrographs because of its transparency in electron beam. The third outer layer is finely wrinkled, and is evidently composed of two to three very thin additional layers. The total spore case width on the whole spore surface is about 0.1 μ (in *P. hyphessobryconis)* with the exception of the anterior pole, where the membrane becomes considerably thin (about one fifth of its thickness on the remaining surface) and as a fine layer covers a structure of electron dense material identified with "McManus positive cap" (Vávra 1959 and Huger 1960).

The content of the spore shell is composed of four main constituents: the polaroplast, the sporoplasm itself, the coiled filament and the hinder vacuole.

The polaroplast — this term introduced by Huger 1960 is identical with the often described "anterior vacuole". It occupies the anterior third of the spore in spores of "*Nosema* type", in spores of *Bacillidium* it extends more deeply. The polaroplast is a little defined structure which is difficult to preserve in intact state during fixation because of its enormous swelling ability. The swelled polaroplast may be found to protrude into the posterior vacuole (Pl. I 2, P_1). In relatively intact spores, fixed in isotonic solutions, it is formed by minute tubuli and granules. After fixation in the usual Palade's fixing fluid (i.e. hypotonic) it is demonstrated as consisting of irregular lamellae (in *P. hyphessobryconis* — Pl. I 1—2, P) or vesicles in *Glugea (Pyroteca)* sp. The polaroplast causes by its swelling the extrusion of the polar filament (Lom and Vávra 1963).

The polar filament is the most conspicuous organelle of the whole spore. It is evident from longitudinal sections through the spore that the filament is coiled in one to several layers around the walls in the hinder half of the spore, the threads forming the limits of the posterior vacuole (Pl. I 1—2, F). The most anterior thread of the filament deflects into the middle of the spore, extending in the direction of the longitudinal axis, penetrates the polaroplast (Pl. I 2) and is attached to the apex of the spore (Pl. I 6).

In all *Microsporidia* of the "*Nosema* type", we found the same arrangement of polar filament within the spore. In spores with a shorter polar filament it is coiled in a single layer around the walls of the spore. In *P. hyphessobryconis* where the fully extruded polar filament reaches 500 μ, there are up to four layers (Pl. I 1—2, F). The filament is a complex structure consisting of several interposed laminar concentric layers making in cross section the impression of a central fibre and surrounding rings (Pl. I 1—3, F). We suppose that it is due to a considerable compression of the filament within the spore. The true tube-like nature of the filament can be clearly seen only in sections of extruded filaments, the diameter of which is far more greater with the walls consisting of about five couples of laminae (Pl. I 4—5, L) surrounding the central hollow tube. The width of the polar filament within the spore varies only insignificantly along its whole length. Only the short stretch beneath the apex of the spore is of wider diameter, perhaps because of a great strain, exerted upon this part of filament during the first stage of filament extrusion.

In spores of the genus *Bacillidium,* their special organelle — manubrium — reveals a structure comparable to that of the polar filament. Manubrium is

a tube; in cross sections it is composed of several concentric structures whose appearance and number changes according to the position of the cross section (Pl. II 8, M; 8a). The whole diameter of the manubrium decreases with growing distance from the anterior end of the spore. Our observations do not supply yet a complete idea of the construction of the manubrium, however, the concentric structure remembering that of polar filament as well as the ability to be everted in the same manner as the filament, suggest that they are quite homologous. For the time being we can distinguish at least five layers in the manubrium: a thick outer wall consisting of two not distinct layers; its inner limit is represented by a double membrane. Inside the circle formed by this membrane there appears a thick fibre which seems to be absent in cross sections taken at some levels (Pl. II 8, M). In such cases the axial part is occupied by four very inconspicuous fibres (Pl. II 8a).

The sporoplasm was badly preserved in our material and no organelles such as mitochondria or Golgi apparatus could be detected. In some preparations an imperfectly preserved nucleus could be observed (Pl. I 1—2, S).

The posterior vacuole, a typical character of microsporidian spores, shows no regular structure in electron microscope. It is most probably due to the fixation that the originally thin fluid of this vacuole is irregularly floculated (Pl. I 1—2, V).

Discussion

The comparison of results achieved in our study of different microsporidian spores with data of Huger 1960 indicate that there is a unique plan in their structure. They all possess a spirally coiled polar filament which in the family *Mrazekiidae* is modified, being straight, relatively short and thick.

Although our present knowledge still contains some gaps in explanation of the exact structure of the polar filament in intact spores (e.g. the presence of the central black dot — "fibre" — in the cross sectioned filament) it is quite clear that the filament is a hollow tube which is proved by its function (Lom and Vávra 1963) as well as by electron micrographs of extruded filaments presented here (Pl. I 4—5). Thus our results do not agree with Krieg's 1963 opinion that the filament is a solid structure. Recently Puytorac 1962 gave a preliminary report on the ultrastructure of *Mrazekia lumbriculi*, the manubrium of which is composed of three concentric layers and of a polar filament passing whole the length through. His pictures remind our observations on the spores of *Bacillidium*.

Being a pipe-like organella, the microsporidian polar filament can be homologized with the filament of myxosporidia. Nowadays the exact function of myxosporidian polar filament is rather enigmatic, but from optical observations it has been known since long time that its mode of extrusion is also the eversion. Our preliminary observations on the ultrastructure of the spores of *Henneguya psorospermica* confirm the fact that the polar capsule contains a coiled, inverted, hollow filament. The fine structure of myxosporidian filament differs at first sight from that of microsporidia by the lack of concentric laminar structures in intact state. Moreover the filament appears to be spirally twisted longitudinaly (Pl. II 9). Cheissin et al. 1961 investigating the spores of *Myxobolus uniporus* and *Myxobolus carassii* described the filament as consisting of two filaments closely coiled together. Our observations in

H. psorospermica whose dimensions are far more greater than that of two *Myxobolus* species mentioned above, reveal clearly that there is a single filament twisted into spiral folds within the polar capsule. Having these facts in mind we can compare the fine structure of myxosporidian filaments with the ultrastructure of stenoteles of *Hydra* as representative of coelenterate nematocysts. The latter are principally of the same structure as the polar capsules of myxosporidia (Chapman and Tilney 1959). The coelenterate filament reminds in its twisted folded appearance (no circular sections as in *Microsporidia*) much more the situation in myxosporidian polar capsules than that of *Microsporidia*.

A common feature of all structures containing polar filaments capable of extrusion is a massive outer envelope, present in the spore case in *Microsporidia*, in the envelope of the polar capsule in *Myxosporidia* and in the nematocysts in *Coelenterata*. This is evidently necessary to prevent the rupture of the formation during the extrusion which is supposed to be evoked by swelling. E.g. our data (Lom and Vávra 1963) indicate that the intrasporal pressure causing extrusion in microsporidian spores can reach values comparable with the osmotic tension as high as 60 atmospheres. The spore membrane is thick and unelastic and all force originating from the swelling structures within the spore is directed to evert the filament.

Summary

Electron microscopic examination of ultrathin sections of spores, representatives of several microsporidian species and genera, reveal that all types of spores are principally of the same structure comprising five chief constituents.

The spore case has a composite fine structure consisting of three different layers. The polaroplast is a tubular or granular formation capable of enormous swelling and supplying the energy for the extrusion of polar filament. Polar filament is a tube-like formation, consisting of thin lamellae as shown on sectioned filaments extruded artificially by hydrogen peroxide. The manubrium of the representatives of family *Mrazekiidae* is however a modified polar filament. On the apex of the spore there is the polar cap consisting of electron dense material to which the beginning of the filament adheres. It is supposed to be the sensitive spot, through which the stimulus for extrusion is accepted. The sporoplasm is situated between the polaroplast and the fluid-filled posterior vacuole.

The structural relations between microsporidian, myxosporidian and coelenterate extruding filaments are discussed.

SOUHRN

Elektronová mikroskopie ultratenkých řezů sporami různých druhů a rodů mikrosporidií ukazuje, že všechny typy spor mají v zásadě stejnou stavbu. Sestávají z pěti hlavních komponent.

Obal spory má složitou strukturu ze 3 dalších vrstev. Polaroplast má ultrastrukturu z jemných tubulů nebo granul, která je schopna enormního bobtnání čímž je dodávána energie pro vystřelení spory. Polární vlákno je trubicovité

a jak ukazují řezy vlákny umělě vystřelenými vlivem peroxydu vodíku, sestává z jemných lamel. Manubrium zástupců čeledi *Mrazekiidae* je pouzu modifikované polární vlákno. Začátek vlákna je připevněn k t.zv. polární čepičce — vrstvičce hmoty málo prostupné pro elektrony, která je umístěna na špičce spory. To je zřejmě citlivé místo, kterým prochází podněty vedouci k vystřeleni spory. Sporoplasma je položena mezi polaroplastem a zadní vakuolou která je naplněna tekutinou.

Jsou diskutovány strukturální vztahy mezi polárními vlákny mikrosporidií, myxosporidií a láčkovců.

REFERENCES

Chapman G. B. and Tilney L. G. 1959: Cytological studies of the nematocyst of *Hydra*. I. Desmonemes, isorhizas, cnidocils and supporting structures. II. The stenoteles. J. Biophys. Biochem. Cytol. 5, 69—85.

Cheissin E. M., Schulman S. S. i Vinnitchenko L. P. 1961: Stroenie spor *Myxobolus*. Citologija 3, 662—667.

Huger A. 1960: Electron microscope study on the cytology of a microsporidian spore by means of ultrathin sectionnning. J. Insect. Pathol. 2, 84—105.

Krieg A. 1955: Über Infektionskrankheiten bei Engerlingen von *Melolontha* spec. unter besondere Berücksichtigung einer Mikrosporidien-Erkrankung. Zbl. Bakt., 2 Abt. 108, 535—538.

Lom J. and Vávra J. 1963: The mode of sporoplasm extrusion in microsporidian spores. Acta Protozool. 1, 81—90.

Puytorac P. de 1962: Observation sur l'ultrastructure de la microsporidie *Mrázekia lumbriculi* Jírovec. J. de Microscopie 1, 39—46.

Vávra J. 1959: Beitrag zur Cytologie einiger Mikrosporidien. Acta Soc. Zool. Bohemoslov. 23, 347—349.

Weiser J. 1958: *Nosema laphygmae* n.sp. and the internal structure of microsporidian spore. J. Insect. Pathol. 1, 52—59.

Wohlfarth-Bottermann K. E. 1957: Die Kontrastierung tierischer Zellen und Gewebe im Rahmen ihrer elektronmikroskopischen Untersuchung an ultradünnen Schnitten. Naturwiss. 44, 287—288.

Note added in proof

The following papers referring to the fine structure of microsporidian spores appeared after this paper has been submitted to publication: Kudo R. R. and Daniels E. W. 1963: An electron microscope study of a microsporidian *Thelohania californica*. J. Protozool. 10, 112—120; Puytorac P. de 1962: Observations sur l'ultrastructure de la microsporidie *Mrazekia lumbriculi*, Jírovec. J. de Microscopie 1, 39—46; Scholtyseck E. und Danneel R. 1962: Über die Feinstruktur der Spore von *Nosema apis*. Deutsche Entomol. Zeitschr. 9, 472—476. Their findings corroborate in main outlines our observations. A detailed discussion is beyond the scope of this note.

EXPLANATION OF PLATES I—II

1: Longitudinal section through the spore of *Plistophora hyphessobryconis*; in the right upper corner a part of a tangentially sectioned spore; W — the spore wall; L_1, L_2, L_3 — the inner, the middle and outer layer of the spore wall; F — cross-sectioned coiled filament; F_1 — the filament in longitudinal section; V — the space of the posterior vacuole; P — the swelled polaroplast; S — the sporoplasm.

2: Longitudinal section of a spore in which the swelled polaroplast is shown (P) penetrating into the space of the posterior vacuole P_1.

3: A cross section of polar filament (F) coiled about the spore wall. Note its inner concentric structure.

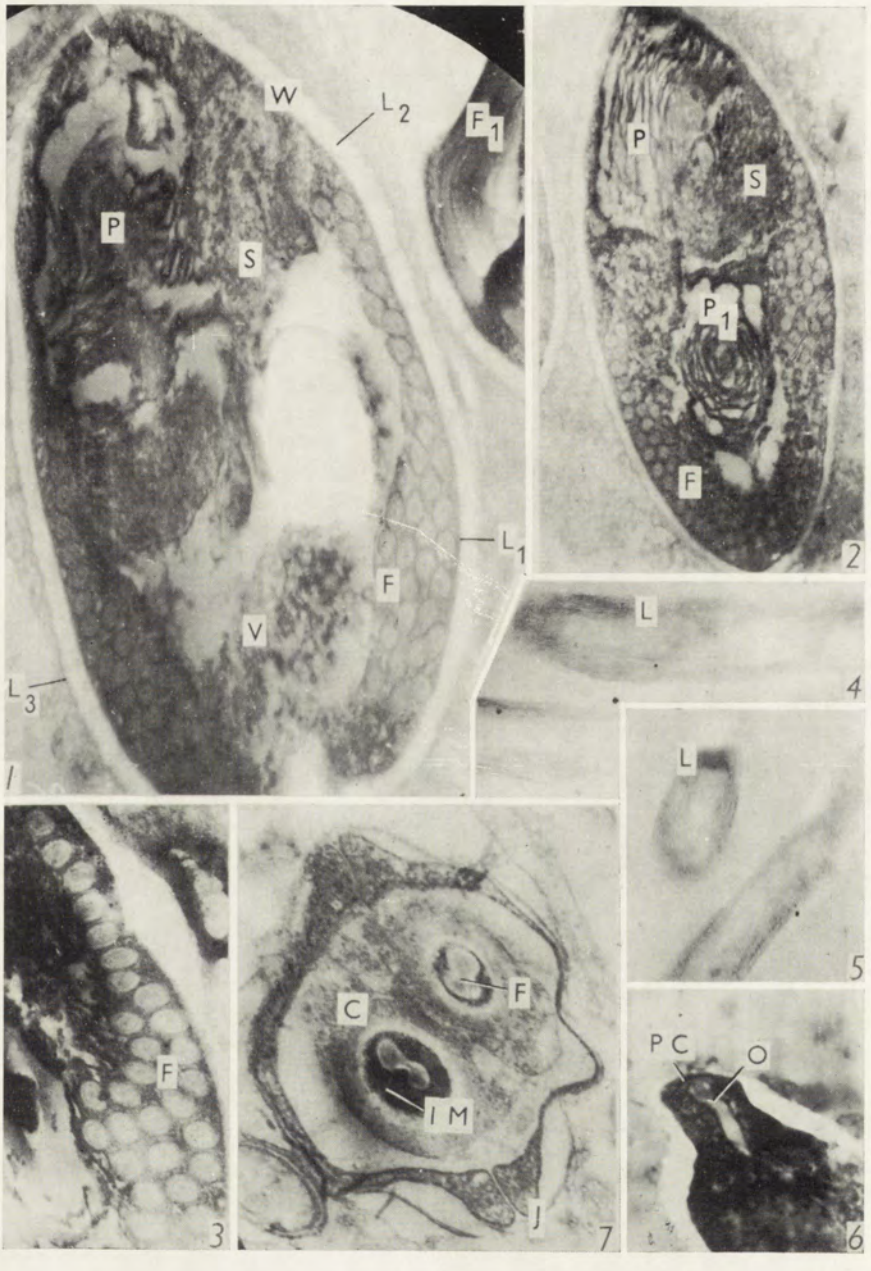
4—5: Longitudinal and oblique section through the tube of the extruded filament. L — double layers of fine lamellae, composing the wall of the filament.

6: Longitudinal section through the apex of the spore, with the origin of the polar filament (O), attached to the polar cap structure (PC).

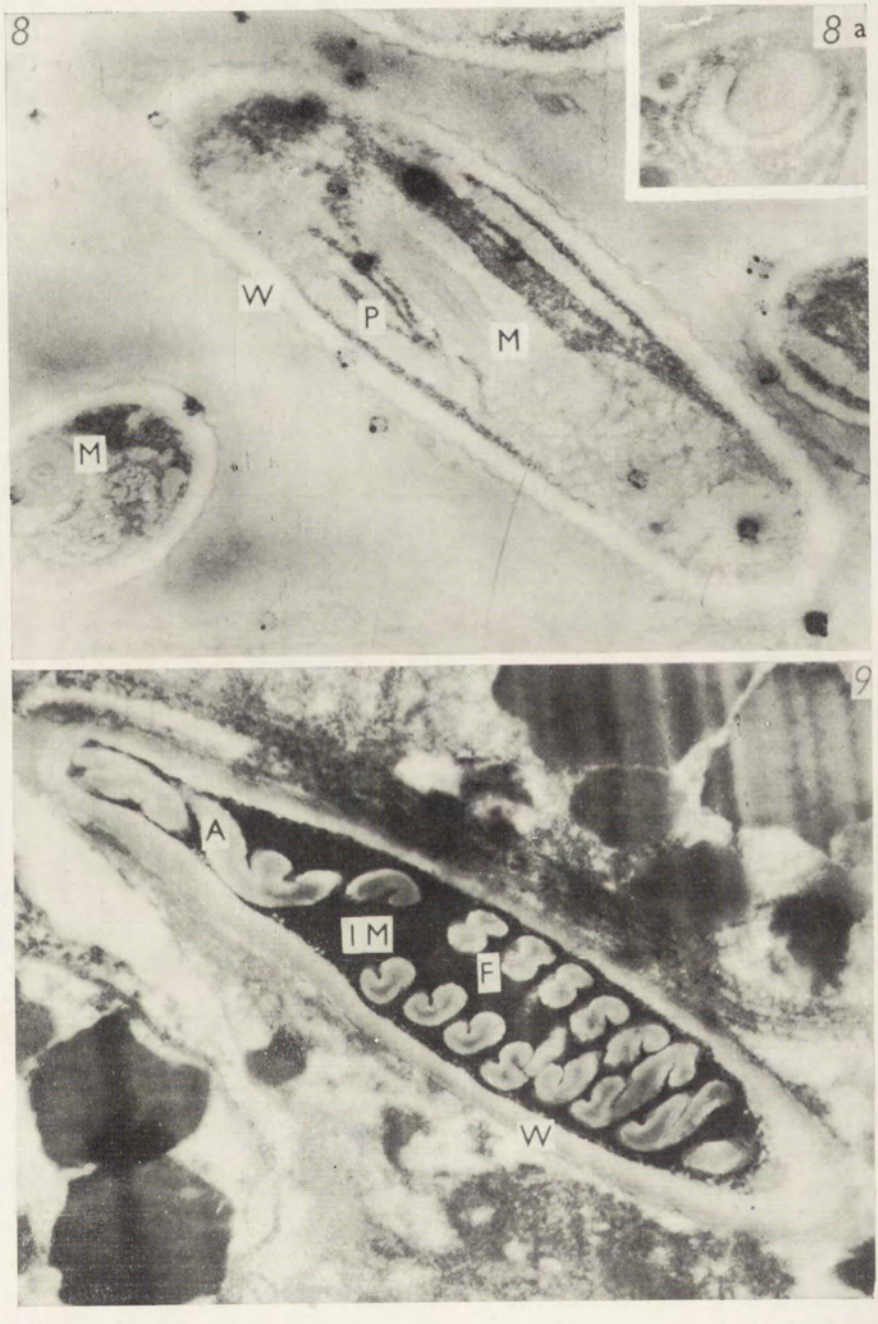
7: A section through the special part of the myxosporidian, *Henneguya psorospermica*: J — the place of the junction of the two shell valves; C — polar capsules with intracapsular matter (I M) and polar filament itself (F).

8: An oblique section through the spore of *Bacillidium cyclopis*; at rigth a cross section; W — wall of the spore; M — manubrium; P — polaroplast, partly swelled. Inset 8 a: another cross section of manubrium.

9: Longitudinal section through the polar capsule of *Henneguya psorospermica*. F — windings of the coiled polar filament; A — the part of the polar filament, ascending to the apex of the capsule; IM — intracapsular matter; W — wall of the polar capsule.



J. Lom et J. Vávra auctores phot.



J. Lom et J. Vávra auctores phot.

ACTA PROTOZOOLOGICA

VOL. I WARSZAWA, 30. IX. 1963 FASC. 27

Institute of Cytology, Academy of Sciences of the USSR
Leningrad F-121, Prospekt Maklina 32

I. B. RAIKOV, E. M. CHEISSIN and E. G. BUZE

A photometric study of DNA content of macro- and micronuclei in *Paramecium caudatum*, *Nassula ornata* and *Loxodes magnus*

фотометрическое изучение содержания ДНК в макро- и микронуклеусах *Paramecium caudatum*, *Nassula ornata* и *Loxodes magnus*

Recent research has shown, that the macronucleus of the majority of ciliates is a highly polyploid nucleus. Arguments for this conclusion were obtained by means of various methods. The macronucleus divides by simple constriction, being capable also to multiple division and even to regeneration from a small fragment. Nevertheless, Sonneborn 1947 found the macronucleus to be genetically active, its genotype remaining unaltered during clonal mutiplication. These properties of the macronucleus can be explained by admitting its polyploid nature (Sonneborn 1947; Grell 1950, 1953; Fauré-Fremiet 1953).

The polyploidy of the macronucleus is confirmed also by studies of its structure. In developing macronuclei, endomitotic duplication of chromosomes was observed repeatedly (Poljansky 1934; Peschkovskaya 1936, 1948; Grell 1949; Saito and Sato 1961 b). In adult macronuclei of a number of species, a vast number of thread-like chromosomes was recorded (Grell 1952; Schwartz 1958; Ruthmann und Heckmann 1961, Ruthmann 1963). Sometimes it was possible to follow their endomitotic duplication when macronuclei are preparing to division (Sato and Saito 1959; Saito and Sato 1961 a; Saito 1961; Kaneda 1961; Raikov 1962).

Another proof of the theory of macronuclear polyploidy can be found in quantitative cytochemical data, mainly in comparison of DNA contents in macro- and micronuclei. These findings show that the macronucleus contains many times as much DNA than the micronucleus of the same species, thus indicating polyploid nature of the former (Moses 1950, and others — see Table 5).

Quantitative cytochemical and autoradiographic investigations allowed also to ascertain, that DNA synthesis (replication) occurs usually during interphase in macronuclei as well as in micronuclei (Seshachar 1950 a; Walker and Mitchison 1957; Kimball and Barka 1959; Kimball and Perdue 1962; Guttes and Guttes 1960; Prescott 1960; Woodard et al. 1961; McDonald 1962; Cheissin et al. 1963). Interphase macro- and micronuclei can have two levels of DNA content — re-

spectively a lower or presynthetic one, and a postsynthetic one, the latter being twice as high as the former. Thus ciliate nuclei behave like nuclei of the majority of metazoan cells. However, in *Euplotes* macronuclear DNA replicates during interphase, while micronuclear DNA performs replication during anaphase and telophase of mitosis (Prescott et al. 1962).

Along with ciliates with polyploid macronuclei, an appreciable number of lower holotrichs was recently found, which have comparatively chromatin-poor macronuclei (genera *Trachelocerca, Tracheloraphis, Trachelonema, Loxodes, Remanella, Geleia* and others). Cytological studies of these forms (Fauré-Fremiet 1954; Raikov 1958 a, b, 1959 a, b, 1963 a, b) allowed us to suppose the diploid character of their macronuclei (see Raikov 1958 a, 1963 c). This assumption was based on low DNA content of these nuclei and on the absence of signs of endomitosis during their development. According to this view, macronuclei of the above-mentioned forms are phylogenetically more primitive than polyploid macronuclei of the higher ciliates. But the supposition of macronuclear diploidy in these lower ciliates has not yet been checked by means of quantitative methods.

The task of the present work is to compare the DNA quantities in the two types of nuclei in ciliates with polyploid macronuclei (*Paramecium caudatum, Nassula ornata*) as well as in a representative of the previously mentioned lower ciliates — *Loxodes magnus*.

Material and techniques

A clone of *Paramecium caudatum* grown at 22 °C in the mineral salt medium of Losina-Losinsky 1931 was used. The ciliates were fed periodically with yeast cell suspension.

The specimens of *Nassula ornata* belonged also to one clone cultured in Peters' salt medium at 12—18°C and fed with blue-green algae *Oscillatoria* sp. (see Raikov 1962). Up to now, nobody succeeded in obtaining clonal cultures of *Loxodes magnus*; therefore we had to use specimens from a natural population of a pond near Leningrad.

Nuclear DNA was measured on whole Feulgen stained mounts by the photographic method (for theory see Brodsky 1956). The ciliates were fixed with Nissenbaum's 1953 sublimate mixture, which sticks them to the slides, and stained according to the standard Feulgen technique (hydrolysis time 6 min.). Then they were photographed on a special microscope MUF-6 in monochromatic green light (546 mμ line of the mercury arc lamp SVD-120A). An optical wedge with fields of known optical density was photographed simultaneously on the same plate.

The densities of the negatives were measured with a scanning microphotometer MF-4 at 10-fold magnification. The mean elevation of each curve over the background was determined planimetrically and compared with the curve obtained from the corresponding optical wedge. This permitted to find the mean extinction of each nucleus (E). For the macronuclei, this procedure was repeated twice, and the average value of E was taken as the final figure.

The DNA content of a nucleus was calculated in arbitrary units by multiplication of its mean extinction by its area ($Q = ES$). For determination of nuclear areas (S) the negative image was projected by means of a photomagni-

fier on paper, the outlines of nuclei were traced and measured with a planimeter (in square centimeters).

Investigation of macronuclei of *Loxodes* presented a special technical difficulty. They have a large central nucleolus, which is Feulgen negative, but, nevertheless, gives a picture of "false absorption" because of its strong refringence resulting in non-specific light scattering. DNA is localized in these nuclei only in a thin peripheric layer.

For excluding this false absorption and obtaining more precise data on DNA content of macronuclei, the following procedure was used. Only the peripheric part of the macronucleus, projected on the photographic plate as an absorbing "ring" (see Pl. II 3, Ma), was measured during negative scanning. The extinction of this "ring" was multiplied by its area (i.e. nuclear area minus nucleolar area); thus the quantity of DNA (Q_r) was determined in this "ring". However, the full DNA content of the macronucleus (Q) is greater than the value obtained, for this method does not take into account the DNA located over and under the nucleolus.

Assuming that the DNA might be evenly distributed under the nuclear membrane, a correcting coefficient (c) was found, which allows the calculation of DNA content in the whole nucleus, provided the quantity of DNA in the "ring" is known. This coefficient depends on the relation between the nuclear radius (R) and the nucleolar one (r):

$$c = \frac{R}{\sqrt{R^2 - r^2}}$$

To determine the DNA content of the whole macronucleus of *Loxodes*, the quantity of DNA calculated for the "ring" was multiplied by this coefficient:

$$Q = Q_r \cdot \frac{R}{\sqrt{R^2 - r^2}}$$

The nuclear area (S), measured planimetrically on enlarged drawings, is a^2 times greater than the true nuclear area on the preparation, a being the total linear magnification at drawing the nucleus. Therefore comparable data on DNA content in nuclei drawn at different magnifications could be obtained only after dividing the DNA quantity (Q) by the square of magnification $\left(Q_0 = \frac{Q}{a^2}\right)$ or, which is the same, after multiplying the former by a coefficient inverse to the square of magnification ($Q_0 = Q \cdot k$, where $k = 1/a^2$). The total magnification was obtained by multiplying the magnifications of the objective lens, the ocular and the photomagnifier.

Paramecium caudatum and *Nassula ornata* were photographed with a 50 × (n.a. 0.65) objective, *Loxodes magnus* — with a 90 × (n.a. 1.25) oil immersion lens. The magnification of the ocular was in all cases 3 ×, that of the photomagnifier — 15 ×. Thus, the coefficient (k) was $1.97 \cdot 10^{-7}$ for *P. caudatum* and *N. ornata*, and $0.6097 \cdot 10^{-7}$ for *L. magnus*. All final results are given in $X \cdot 10^{-7}$ arbitrary units od DNA.

The authors wish to express their gratitude to G. V. Selivanova for her valuable advices concerning photometric techniques, and to G. I. Sergejeva for help in the mathematical processing.

Results

Paramecium caudatum

The nuclear apparatus of *P. caudatum* consists of an oval macronucleus and one micronucleus close to it. The content of nuclear DNA was measured in 25 individuals, the results being represented in Table 1.

Table 1

DNA content of *Paramecium caudatum* nuclei (arbitrary units · 10^{-7})

Category of individuals	Number of individuals	DNA content of Ma		DNA content of Mi		Relation between DNA contents of Ma and Mi		Average polyploidy degree of Ma
		Extremes	Mean	Extremes	Mean	Extremes	Mean	
I. Presynthetic Ma and Mi	10	11.8—17.4	14.71	0.13—0.22	0.19	58—103 / 1	79.5 / 1	159 n
II. Presynthetic Ma and postsynthetic Mi	4	12.5—17.6	15.05	0.24—0.36	0.32	44—53 / 1	47.0 / 1	—
III. Synthetic Ma and presynthetic Mi	5	17.7—21.7	20.10	0.18—0.22	0.19	100—109 / 1	104 / 1	—
IV. Synthetic Ma and postsynthetic Mi	6	19.3—22.2	20.40	0.26—0.38	0.33	52—77 / 1	61.8 / 1	124 n

It has been established earlier for the same strain, that DNA synthesis in the macronucleus takes place during the second half of the interdivision interval (Cheissin et al. 1963). During the first 8—10 hours after division, the DNA content of this nucleus remains on a constant low level (presynthetic period). During the second half of the interval, the quantity of macronuclear DNA grows gradually, being doubled only during plasmotomy [1]. This period of time (from 10—12 to 18—19 hours) is characterized by a higher DNA content, which approaches the postsynthetic one.

DNA measurements were carried out on paramecia at random stages of the interphase, which resulted in obtaining variable figures corresponding to the presynthetic as well as to the synthetic periods.

According to DNA content of the macronucleus, all individuals can be divided into two groups. Macronuclei of the first group are in the presynthetic period, those of the second group — in the synthetic one. Macronuclei of the 2nd group were not found to contain a double amount of DNA as compared with those of the first group, since we did not measure their DNA content just

[1] The process of DNA synthesis in the macronucleus of *P. aurelia*, according to Kimball and Barka 1959 and Kimball and Perdue 1962, is also gradual and is completed only just before division. The graduality of macronuclear DNA synthesis was recorded also for *Stentor* (Guttes and Guttes 1960). It is most clearly expressed in *Euplotes*, where DNA replication takes place in the so-called reorganization band, moving slowly along the macronucleus (Gall 1959; Kimball and Prescott 1962; Prescott, Kimball and Carrier 1962).

before and during division, when the DNA quantity is known to reach its maximum and when replication of DNA is being completed in all genomes of the polyploid macronucleus. Into the group of synthetic macronuclei we included nuclei at different stages of DNA synthesis, having from 17.7 to $22.2 \cdot 10^{-7}$ arbitrary units of DNA. In presynthetic macronuclei, the DNA content varies from 11.8 to 17.6 units; this variability apparently depends on initial differences of DNA quantity among individuals belonging to one clone (Ovchinnikova et al. 1963).

In the micronuclei, the DNA content is clearly represented by two levels — a presynthetic and a postsynthetic one. DNA synthesis in the micronucleus goes on rather rapidly, and either nuclei with low DNA content (less than $0.22 \cdot 10^{-7}$ units), or those with greater, practically doubled content ($0.24—0.38 \cdot 10^{-7}$ units) are met.

Comparing DNA levels in macro- and micronuclei, 4 categories of individuals can be distinguished (Table 1).

The first category is characterized by low DNA levels in both types of nuclei. These are individuals, in which DNA synthesis has not yet begun after division (presynthetic condition). The second category is composed of specimens having macronuclei with presynthetic DNA content, and postsynthetic micronuclei. The existence of this category shows, that micronuclear DNA synthesis may begin and end earlier, than the macronuclear one. The animals of the third category have synthetic macronuclei and presynthetic micronuclei. Thus, contrary to the category II, DNA synthesis begins here in some macronuclear genomes earlier than in the micronucleus. Finally, specimens of the fourth category have synthetic macronuclei and postsynthetic micronuclei, which corresponds to the second half of the interdivision interval.

Strictly speaking, only individuals of the first category must be taken into account while determining the degree of macronuclear polyploidy, for only they have the most characteristic DNA content, unchanged by synthetic processes and comparable in both nuclei. We may consider also the category IV, but the quantity of DNA in these animals is not yet fully doubled in the macronucleus, while in the micronucleus it has already reached the postsynthetic level. Therefore the polyploidy degree obtained by comparison of macro- and micronuclei of individuals of the category IV turns out somewhat underestimated.

The comparison of nuclei of the first category of animals shows, that the average DNA content of the macronucleus surpasses that of the micronucleus 80 times, the relation varying in some individuals from 58 to 103 times. This fact may be explained by the instability of DNA quantity in macronuclei among individuals of one clone (Ovchinnikova et al. 1963). In the category IV, the mean relation of DNA quantities in macro- and micronuclei is 62. Taking the micronucleus for a diploid nucleus, we can determine the average degree of macronuclear polyploidy as 160 n (from 116 to 206 n in individual cases).

Nassula ornata

The nuclear apparatus of *N. ornata* is represented by a large spherical or oval macronucleus (diameter ca. 40 μ), and by several micronuclei adjacent to it (Pl. I 1). The number of the latter varies from 2 to 14; most individuals have from 4 to 8 micronuclei (Raikov 1962).

Table 2
DNA content of *Nassula ornata* nuclei (arbitrary units · 10^{-7})

Category of individuals	Specimen No.	DNA content of Ma	Micronuclei			Relation between DNA contents of Ma and Mi	Polyploidy degree of Ma
			Number	DNA content			
				Extremes	Mean		
I. Presynthetic Ma and Mi	4	40	2	0.2—0.32	0.28	143 : 1	286 n
	8	46	3	0.34—0.40	0.38	121 : 1	242 n
II. Postsynthetic Ma and presynthetic Mi	1	55	3	0.22—0.38	0.31	177 : 1	—
	2	52	1	0.36	0.36	144 : 1	—
	6	57	5	0.24—0.46	0.37	154 : 1	—
	9	57	2	0.24—0.30	0.27	203 : 1	—
	11	56	2	0.32—0.34	0.33	170 : 1	—
III. Postsynthetic Ma and Mi	3	63	2	0.59—0.61	0.60	105 : 1	210 n
	5	56	2	0.53—0.63	0.58	96.5 : 1	193 n
	7	55	2	0.42—0.57	0.50	110 : 1	220 n
	10	58	3	0.47—0.59	0.51	114 : 1	228 n
Average polyploidy degree							230 n

The DNA content of macro- and micronuclei was measured in 11 specimens of *N. ornata*. Only these micronuclei, which were not overshadowed by the macronucleus, could be measured. The results of DNA determination are given in Table 2.

The analysis of the data shows, that according to their DNA content the macronuclei fall into 2 groups — with a lower presynthetic (Nos. 4 and 8) and a higher postsynthetic (other animals) DNA quantity. The former have 40—46·10^{-7} arbitrary DNA units, the latter — more than 52 (up to 63) units. The presence of nuclei with intermediate DNA content indicates the graduality of DNA replication in macronuclei of this species also. However, while investigated microscopically, endomitotic chromosomes in the macronucleus of *Nassula* split synchronously (Raikov 1962). Obviously, elementary DNA fibrils might replicate in different genomes of this nucleus at different time; this process becomes completed in all macronuclear genomes just before division, and only after that microscopically visible synchronous chromosome spiralization and splitting (endomitosis) begins.

According to DNA content of their micronuclei, the animals can be divided also in two groups. The individuals of the first one (Nos. 1, 2, 4, 6, 8, 9 and 11) have micronuclei with little DNA quantity (0.22—0.46, the means from 0.27 to 0.38·10^{-7} arbitrary units). Specimens of the second group (Nos. 3, 5, 7 and 10) contain considerably more DNA in micronuclei — from 0.42 to 0.63, the means from 0.50 to 0.60·10^{-7} arbitrary units. DNA levels found in the micronuclei obviously correspond to the periods before and after DNA replication.

The synthesis of DNA takes place more or less synchronously in different micronuclei of one animal. This is confirmed by the fact that all micronuclei of a single animal usually appear to be either presynthetic or postsynthetic. This

observation corresponds well with the synchrony of micronuclear mitoses at division in this species (R a i k o v 1962).

The comparison of DNA levels in macro- and micronuclei allows to distinguish 3 categories of individuals of *N. ornata* (but not 4, as in the case of *P. caudatum*). The first category (Nos. 4 and 8, Table 2) is characterized by a low presynthetic DNA level in the macronucleus as well as in the micronuclei. Apparently, these are recently divided animals. Macronuclei with a postsynthetic or intermediate (No. 2) DNA content and presynthetic micronuclei are characteristic for the ciliates of the category II (Nos. 1, 2, 6, 9 and 11). The existence of this category shows that in *Nassula* macronuclear DNA synthesis precedes the micronuclear one. Animals of the third category (Nos. 3, 5, 7 and 10) have postsynthetic macronuclei and micronuclei. These are obviously predivision stages. Thus individuals of *Nassula* belonging to the categories I, II and III correspond to the categories I, III and IV of *Paramecium* respectively. No cases of micronuclear DNA synthesis preceding the macronuclear one (as in the category II of *Paramecium*) were found in *Nassula*.

The comparison of DNA content of *Nassula* macronuclei with that of the micronuclei gives the lowest figures in the category III, and the highest — in the II (see Table 2). Obviously, the degree of macronuclear polyploidy should be determined by comparing presynthetic micronuclei with presynthetic macronuclei, and postsynthetic micronuclei — with postsynthetic macronuclei. Individuals of the category II should not be taken into account at all, since their nuclei represent different stages of DNA replication and cannot be compared directly.

Admitting the micronucleus to be diploid, the degree of polyploidy of presynthetic macronuclei proves to be 242—286 n, and that of the postsynthetic ones — 193—228 n. At average, the macronucleus of *N. ornata* is consequently 230-ploid.

These results are in sharp disagreement with our previous data. Morphological studies of *N. ornata* macronuclei revealed chromosomes therein, which splitted longitudinally shortly before cell division, i.e. became duplicated by endomitosis (R a i k o v 1962). Chromosome numbers were counted in macro- and micronuclei (although very imprecisely, because of their great number in both types of nuclei and due to the fact that counts were carried out on sections). The chromosome number in the macronucleus was tentatively estimated at 400—800, whereas in the diploid micronucleus it is ca. 40. Thus the haploid number (n) of this species may be about 20. Assuming that macronuclear chromosomes are not complex "Sammelchromosomen" (i.e. chain-like aggregates of true chromosomes), a conclusion was drawn, that the macronucleus may be ca. 20—40-ploid (most probably 32-ploid).

According to the theoretical considerations of G r e l l 1953, 1962 the existence of "Sammelchromosomen" in the macronucleus of ciliates is most probable. Recently, this opinion found some confirmation in studies carried out on *Bursaria truncatella* by R u t h m a n n und H e c k m a n n 1961 and on *Loxophyllum meleagris* by R u t h m a n n 1963. However, in *Nassula* any morphological signs of a complex nature of macronuclear chromosomes failed to be found.

According to photometric data, the macronucleus of *N. ornata* is at average 230-ploid. Consequently, if it contained separate chromosomes, the number of

the latter should exceed 4000 (230·20 = 4600), i.e. it should be 5 to 10 times greater than the counted number. Such a big error of chromosome counting is highly improbable, the more so that the process of counting on sections could rather result in a tendency to overestimate their number (a cut chromosome is being held for two or three), than to underestimate it.

The presence in the macronucleus of *N. ornata* of DNA quantities 7—8 times greater than it could be expected from chromosome counts may be explained in two ways.

Firstly, the macronuclear chromosomes of this species may be still complex ("Sammelchromosomen"), though morphological boundaries of true chromosomes in the chain are indistinguishable. It is difficult to determine the exact chromosome length on sections, where they were counted. Therefore it is possible, that macronuclear chromosomes are longer than previously admitted, and represent complex aggregates.

If it is the case, what should be the number of "Sammelchromosomen" in the *Nassula* macronucleus? It is most probable, that complex chromosomes are formed by a linear aggregation of all the chromosomes of each haploid genome. Only in this case the whole genomes ("Sammelchromosomen") could become segregation units at macronuclear division, the latter condition being necessary to avoid increasing aneuploidy in daughter nuclei (G r e l l 1953). Taking this opinion as a working hypothesis, we should expect that the number of complex chromosomes in the *Nassula* macronucleus would be equal to its polyploidy degree, i.e. approximately 230. This figure is 2—3 times smaller than those obtained by chromosome counting. However, because counting on sections tends to yield overestimated figures, we believe this interpretation to be acceptable.

Secondly, the discrepancy between morphological and photometrical data could be explained by polyteny of macronuclear chromosomes. Its degree should be in this case approximately 8 (3 successive reduplications of chromonemata). Up to now polyteny is described only for micronuclear chromosomes of *Tetrahymena* (A l f e r t and B a l a m u t h 1957). The solution of this question might be apparently obtained through electron microscopic studies of *Nassula* nuclei.

Loxodes magnus

Loxodes magnus is a multinucleate ciliate belonging to the lower *Holotricha* and having from 8 to 31 macronuclei and from 5 to 32 micronuclei (Pl. I 2). Recently, the nuclear apparatus of this species was studied in detail by F a u r é-F r e m i e t 1954 and R a i k o v 1958 b, 1959 a. The macronuclei (Pl. II 3, Ma) have a spherical form, with the diameter of ca. 7 μ only, and contain a very large central nucleolus. Unlike macronuclei of the majority of ciliates, the macronuclei of *L. magnus* are stained faintly by the Feulgen technique; only small chromatin elements in their peripheric layer give a positive reaction (Pl. II 3). The micronuclei (Pl. II 3, Mi) are, on the contrary, compact and sharply Feulgen positive. It is very remarkable, that macronuclei of *Loxodes* are incapable to divide: at cell division they are passively distributed between the daughter individuals. The micronuclei divide mitotically, the mitoses occurring asynchronously. A number of micronuclei becomes transformed from time to time into macronuclear anlagen (Pl. II 3, MaA), which grow later into adult macronuclei. The anlagen differ from the latter by the absence of a lar-

Table 3

DNA content of *Loxodes magnus* nuclei (arbitrary units · 10^{-7})

Specimen No.	Presynthetic Mi			Postsynthetic Mi			Ma-anlagen			Adult Ma		
	Number of Mi	DNA content		Number of Mi	DNA content		Number	DNA content		Number of Ma	DNA content	
		Extremes	Mean		Extremes	Mean		Extremes	Mean		Extremes	Mean
1	1	0.21	0.21	5	0.24—0.33	0.28	3	0.35—0.40	0.375	6	0.35—0.47	0.43
2	—	—	—	6	0.30—0.45	0.34	—	—	—	8	0.15—0.43	0.30
3	2	0.15—0.21	0.18	5	0.24—0.31	0.28	3	0.21—0.25	0.24	4	0.30—0.36	0.335
4	3	0.16—0.22	0.195	2	0.24	0.24	2	0.23—0.32	0.27	5	0.30—0.36	0.34
5	3	0.15—0.21	0.17	2	0.23—0.27	0.25	3	0.20—0.33	0.25	3	0.23—0.34	0.28
6	5	0.15—0.21	0.18	—	—	—	2	0.26—0.36	0.31	6	0.23—0.36	0.305
7	4	0.15—0.22	0.17	1	0.27	0.27	1	0.29	0.29	4	0.27—0.36	0.31
8	4	0.17—0.20	0.18	—	—	—	—	—	—	5	0.12—0.25	0.195
9	6	0.14—0.21	0.18	2	0.24—0.26	0.25	1	0.305	0.305	5	0.28—0.39	0.33
10	—	—	—	7	0.25—0.32	0.28	3	0.31—0.38	0.34	6	0.30—0.41	0.34
11	1	0.20	0.20	2	0.23—0.24	0.24	1	0.24	0.24	5	0.28—0.40	0.34
12	1	0.18	0.18	4	0.23—0.34	0.28	3	0.19—0.29	0.23	6	0.21—0.30	0.24
Total	80	0.14—0.22	0.181 ± 0.005	36	0.23—0.45	0.283 ± 0.009	22	0.19—0.40	0.288 ± 0.013	63	0.12—0.47	0.314 ± 0.010
Relative DNA content		0.64			1.00			1.02			1.11	

ge central nucleolus; they have either no nucleoli at all, or contain 1—3 small nucleoli.

As mentioned above, it was suggested previously (Raikov 1959 a) that the macronuclei of *Loxodes* remain diploid; thus they differ from the macronuclei of higher ciliates, which become polyploid. In the present study we tried to check whether *Loxodes* macronuclei are really diploid, by means of photometric determination of their DNA content.

The results of DNA measurement in the nuclei of 12 individuals of *L. magnus* are represented in Table 3; in all 66 micronuclei, 22 macronuclear anlagen and 63 adult macronuclei were measured.

Like in both species discussed above, the micronuclei of *L. magnus* fall into 2 classes of DNA content. Presynthetic micronuclei contain from 0.14 to $0.22 \cdot 10^{-7}$ arbitrary DNA units, the mean being $0.181 \cdot 10^{-7}$. Postsynthetic micronuclei are characterized by a higher DNA content — from 0.23 to $0.45 \cdot 10^{-7}$ units (mean — $0.283 \cdot 10^{-7}$). Between these two levels, some transitory values (0.20—0.24) can be stated, which correspond to micronuclei fixed during DNA replication. This resulted in the fact that the mean DNA

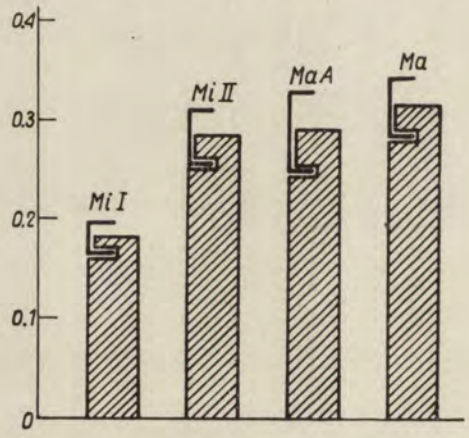


Fig. 1. Mean DNA content of presynthetic micronuclei (Mi I), postsynthetic micronuclei (Mi II), macronuclear anlagen (MaA) and adult macronuclei (Ma) of *Loxodes magnus* (in arbitrary units $\cdot 10^{-7}$)

Lines on the tops of columns represent 3 standard errors in each direction of the mean value (M ± 3m), thus corresponding to 99.7% confidence limits.

values of presynthetic and postsynthetic micronuclei show the relation less than twofold (the DNA content of the former being at average 64% from that of the latter; see Table 3 and Fig. 1).

Unlike *N. ornata*, the majority of individuals of *L. magnus* contain a mixture of presynthetic and postsynthetic micronuclei in different proportions (see Table 3). This is just what could be expected, because in *L. magnus* micronuclear mitoses are asynchronous (Raikov 1959 a).

The mean DNA content of developing macronuclear anlagen ($0.288 \cdot 10^{-7}$) proved to be very close to the DNA level of the postsynthetic micronuclei (0.283), the difference being only 2% (Table 3). Table 4 shows that this difference is statistically unsignificant ($t = 0.315$, $P > 0.5$). This fact proves that macronuclear anlagen originate in the majority of cases from postsynthetic micronuclei, and do not undergo any endomitotic polyploidization during their development. At average, they retain the DNA content corresponding to the diploid postsynthetic level (Fig. 1).

The mean DNA content of adult macronuclei is only by 11% higher than that of postsynthetic micronuclei (Table 3, Fig. 1). This difference is

statistically significant ($0.05 > P > 0.01$; see Table 4). The difference between DNA quantities of developing anlagen and adult macronuclei is 9%, but due to a greater standard error of the mean DNA content of the anlagen it proved to be of a little significance ($0.2 > P > 0.1$). In any case, there exist no manifold (even twofold) DNA changes during the development of macronuclei in *Loxodes*; therefore any polyploidization of developing and adult macronuclei is here out of question. The macronuclei contain a mean quantity of DNA practically equal to that found in diploid postsynthetic micronuclei (Fig. 1).

Table 4

Significance of differences between mean DNA quantities in *Loxodes magnus* nuclei

	Postsynthetic Mi, $M \pm m =$ 0.283 ± 0.009	Ma-anlagen $M \pm m =$ 0.288 ± 0.013	Adult Ma $M \pm m =$ 0.314 ± 0.010
Presynthetic Mi, $M \pm m =$ 0.181 ± 0.005	$t = 10.35$ $0.001 > P$	$t = 7.65$ $0.001 > P$	$t = 12.1$ $0.001 > P$
Postsynthetic Mi, $M \pm m =$ 0.283 ± 0.009	———	$t = 0.315$ $P > 0.5$	$t = 2.32$ $0.05 > P > 0.01$
Ma-anlagen $M \pm m =$ 0.288 ± 0.013	———	———	$t = 1.56$ $0.2 > P > 0.1$

The little increase of DNA content in adult macronuclei in relation to the developing ones might be perhaps connected with an accumulation of the so-called metabolic DNA of the heterochromatin as a result of gene activation, the latter undoubtelly occurring at transformation of micronuclei into nuclei that are biochemically active and rich in RNA. Analogous phenomena were found in metazoan nuclei, for instance, in the "Balbiani rings" of dipteran salivary gland chromosomes (Breuer and Pavan 1956; Ficq and Pavan 1957).

A detailed analysis of Table 3 shows, however, that macronuclear anlagen can sometimes arise from presynthetic micronuclei also. In this case, the macronuclei retain a lower (diploid presynthetic) DNA content. All the measured macronuclei [2] of the specimen No. 8 can be given as an example (Table 3). In a number of other individuals, the populations of both developing and adult macronuclei are heterogenous, some nuclei showing postsynthetic, others — presynthetic DNA levels (Nos. 2, 12). This is easily understandable, since the macronuclei of *Loxodes* are always of unequal age, some of them being formed recently, others being received from the mother individual (Fauré-Fremiet 1954; Raikov 1959a). Nevertheless, macronuclei with postsynthetic DNA content strongly predominate.

[2] As the measurements were done by the photographic method, not all the nuclei of an individual were measured, but only those which appeared in focus on the negative plate.

The data confirm, consequently, our previous supposition about the diploidy of *Loxodes* macronuclei and the absence of any significant DNA synthesis during their development.

Discussion

A considerable body of evidence concerning DNA content of ciliate nuclei, obtained mainly from photometric studies of Feulgen stained preparations, has now accumulated in the literature. If the micronucleus is considered to be diploid in all cases[3] a comparison of DNA contents of the micro- and the macronucleus allows to draw a conclusion about the polyploidy degree of the latter. Literature data of this kind, together with the results presented above, are brought together in Table 5.

Table 5

DNA content of macro- and micronuclei and polyploidy degree of macronuclei in different ciliates

	Species	Relation of DNA contents in Ma and Mi	Degree of polyploidy of Ma	References
Polyploid macronuclei	*Paramecium caudatum*	40:1	80 n	Moses 1950
	The same	25—30:1	50—60 n	Walker and Mitchison 1957
	The same	~75:1	~150 n	Blanc 1962
	The same	58—103:1	~160 n	This paper
	Paramecium aurelia	430:1	860 n	Woodard, Gelber and Swift 1961
	Chilodonella uncinata	~35:1	~70n	Seshachar 1950b
	Tetrahymena limacis	10—50:1	20—100 n	Dysart, Corliss and De la Torre 1962
	Tetrahymena patula	100—400:1	200—800 n	
	Nassula ornata	115:1	230 n	This paper
	Epistylis articulata	~700:1	~1400 n	Seshachar and Dass, 1954
	Eursaria truncatella	2500:1	5000 n	Ruthmann und Heckmann 1961
Diploid Ma	*Loxodes magnus*	1.11:1	2 n	This paper

[3] It is by far not always the case. In particular, micronuclear heteroploidy has been described in *Paramecium bursaria* and in some stocks of *P. caudatum* (Chen 1940), tetraploidy — in a clone of *Tetrahymena pyriformis* (Ray 1958), and polyteny of micronuclear chromosomes — in another stock of *T. pyriformis* (Alfert and Balamuth 1957).

As it is seen in this table, the macronuclei of the majority of ciliates are highly polyploid, the degree of their ploidy varying widely in different species as well as within one species (*Tetrahymena limacis, T. patula*). The variability of data reported in four separate papers on *Paramecium caudatum* could be probably explained by stock differences. The stocks might have different absolute DNA quantities not only in the macronuclei, but also in the micronuclei. The latter might be connected with micronuclear heteroploidy known for this species (Chen 1940).

A sharp difference exists between the degree of macronuclear polyploidy of two closely related species — *P. caudatum* and *P. aurelia* (Table 5). Nevertheless, the dimensions of the macronuclei of these species are more or less alike; probably, their absolute DNA contents differ not very much from each other. Alike are also their body dimensions. In this case, the difference of ploidy degrees might depend not on the final DNA content of the macronucleus, but on the difference of initial DNA contents in the micronuclei. The micronucleus of *P. aurelia* is in fact much smaller and contains less DNA than does that in *P. caudatum*. Numerous observations show that no correlation exists between DNA content and the dimensions of micronuclei, on one hand, and the body size of a ciliate, on the other. Thus e.g., large-sized *Spirostomum* has exceedingly small micronuclei. On the contrary, macronuclear dimensions do correlate clearly with the body size, the absolute DNA content of the macronucleus being probably determined primarily by the amount of cytoplasm. This content is reached by endomitotic polyploidization of the micronucleus, the number of endomitoses necessary for it being the greather, the smaller and DNA-poorer the micronuclei of the species are. Therefore a higher degree of polyploidy is needed to reach the necessary DNA content in the macronucleus of *P. aurelia* than in that of *P. caudatum*.

A conclusion can be drawn of the above-said, that the degree of macronuclear polyploidy represents only a comparatively poor correlation with the systematic position of the given ciliate (leaving aside the lower ciliates with their diploid macronuclei). It depends much stronger on the relation of body size to micronuclear size. According to this, the highest degrees of macronuclear polyploidy are met in large ciliates having small micronuclei (*Bursaria truncatella*). *Spirostomum ambiguum* is expected to give even a greater figure, but this species has not yet been studied photometrically.

Quite apart from the others is the group of lower *Holotricha* having comparatively DNA-poor macronuclei. In all these forms, including *Loxodes*, these somatic nuclei are incapable to undergo division. The present investigation has shown, that the macronuclei of *Loxodes* are really not polyploid, but retain a diploid character, just as we supposed previously. A study of other forms of this kind (*Trachelocercidae, Geleia, Remanella*) would probably give analogous results, since morphological and cytochemical peculiarities of their macronuclei resemble extremely those in *Loxodes*. Consequently, the results obtained confirm the hypothesis postulating that the evolution of nuclear dualism in ciliates has passed two phylogenetic stages: that of primary differentiation of diploid macronuclei and that of their polyploidization (see Raikov 1963 a, c).

Conclusions

1. Two levels of DNA content can be distinguished in the macro- and micronuclei of *Paramecium caudatum* and *Nassula ornata* — a presynthetic and a postsynthetic one.

2. A presynthetic macronucleus of the strain of *P. caudatum* under study contains at average 80 times more DNA than a presynthetic micronucleus. The degree of macronuclear polyploidy in this strain is consequently about 160 n.

3. In some cases, DNA synthesis in the micronuclei of *P. caudatum* can begin earlier, than that in the macronucleus, while in others — it initiates later. Thus, the time of DNA synthesis in both types of nuclei is not strictly correlated.

4. In the macronucleus of *Nassula ornata* DNA replication begins earlier than in the micronuclei.

5. The macronucleus of *N. ornata* contains 96—143 times more DNA than a single micronucleus of the same specimen. The macronucleus of this species is at average 230-ploid.

6. The discrepancy between the degrees of macronuclear polyploidy in *N. ornata* determined by chromosome counting (about 32 n) and by DNA measurement (230 n) could be explained by admitting either a complex nature of macronuclear chromosomes ("Sammelchromosomen"), or their polyteny.

7. The micronuclei of a given specimen of *Loxodes magnus* are usually a mixture of presynthetic and postsynthetic nuclei; this is connected with asynchrony of micronuclear mitoses.

8. The mean DNA contents of presynthetic and postsynthetic micronuclei, macronuclear anlagen and adult macronuclei of *L. magnus* are related as 0.64 : 1 : 1.02 : 1.11 respectively. Thus the macronuclei of this species originate usually from postsynthetic micronuclei and retain a diploid character. A little (11%) increase of DNA content of adult macronuclei might be explained by heterochromatin accumulation. These data completely exclude a possibility of endomitotic polyploidization during development of macronuclei in *Loxodes*.

РЕЗЮМЕ

Количество ДНК в ядрах *Paramecium caudatum, Nassula ornata* и *Loxodes magnus* определялось путем фотометрирования окрашенных по Фельгену тотальных препаратов. Пресинтетический макронуклеус *P. caudatum* содержит в среднем в 80 раз больше ДНК, чем пресинтетический микронуклеус, и является приблизительно 160-плоидным. Количество ДНК в макронуклеусе *N. ornata* превосходит таковое в микронуклеусе в среднем в 115 раз. Несоответствие между степенью полиплоидности макронуклеуса этого вида, определенной по количеству ДНК (230 n) и путем подсчета хромосом (около 32 n), может быть объяснено либо сборным характером хромосом макронуклеуса, либо их политенией.

В связи с несинхронностью митозов у *Loxodes magnus* микронуклеусы в пределах одной особи обычно представлены смесью пресинтетических и постсинтетических ядер. Среднее содержание ДНК в пресинтетических и постсинтетических микронуклеусах, зачатках макронуклеусов и взрослых макронуклеусах этого вида относится как 0.64:1:1.02:1.11. Макронуклеусы *L. magnus*, следовательно, образуются обычно из постсинтетических микронуклеусов и сохраняют диплоидный характер, что подтверждает высказанное нами ранее предположение об отсутствии эндомитотической полиплоидизации макронуклеусов *Loxodes*.

REFERENCES

Alfert M. and Balamuth W. 1957: Differential micronuclear polyteny in a population of the ciliate *Tetrahymena pyriformis*. Chromosoma 8, 371—379.

Blanc J. 1962: Observations sur la teneur en acide désoxyribonucléique de l'appareil nucléaire du Cilié. C. R. Acad. Sci. 254, 2822—2824.

Breuer M. E. and Pavan C. 1955: Behaviour of polytene chromosomes of *Rhynchosciara angelae* at different stages of larval development. Chromosoma 7, 371—386.

Brodsky V. J. 1956: Citofotometrija. Uspehi sovrem. biol. 42, 87—107.

Cheissin E. M., Ovchinnikova L. P., Selivanova G. V. i Buze E. G. 1963: Izmenenie količestva DNK v makronukleuse *Paramecium caudatum* v period od delenija do delenija. Acta Protozool. 1, 63—69.

Chen T. 1940: Polyploidy and its origin in *Paramecium*. J. Hered. 31, 175—184.

Dysart M. P., Corliss J.O. and De la Torre L. 1962: Comparative DNA measurements in two species of *Tetrahymena*. J. Protozool. 9 (Suppl.), 17.

Fauré-Fremiet E. 1953: L'hypothèse de la sénescence et les cycles de réorganisation nucléaire chez les Ciliés. Rev. Suisse Zool. 60, 426—438.

Fauré-Fremiet E. 1954: Réorganisation du type endomixique chez les *Loxodidae* et chez les *Centrophorella*. J. Protozool. 1, 20—27.

Ficq A. and Pavan C. 1957: Autoradiography of polytene chromosomes of *Rhynchosciara angelae*... Nature 180, 983—984.

Gall J. H. 1959: Macronuclear duplication in the ciliated protozoan *Euplotes*. J. Biophys. Biochem. Cytol. 5, 295—308.

Grell K. G. 1949: Die Entwicklung der Makronucleusanlage im Exkonjuganten von *Ephelota gemmipara* R. Hertwig. Biol. Zentralbl. 68, 289—312.

Grell K. G. 1950: Der Kerndualismus der Ciliaten und Suctorien. Naturwissenschaften 37, 347—356.

Grell K. G. 1952: Die Struktur des Makronucleus von *Tokophrya*. Arch. Protistenk. 98, 466—468.

Grell K. G. 1953: Der Stand unserer Kenntnisse über den Bau der Protistenkerne. Verhandl. Deutsch. Zool. Gesell. Freiburg 1952, 212—251.

Grell K. G. 1962: Morphologie und Fortpflanzung der Protozoen (einschliesslich Entwicklungsphysiologie und Genetik). Fortschr. Zool. 14, 1—85.

Guttes E. and Guttes S. 1960: Incorporation of tritium-labelled thymidine into the macronucleus of *Stentor coeruleus*. Exp. Cell Res. 19, 626—628.

Kaneda M. 1961: Fine structure of the macronucleus of the gymnostome ciliate, *Chlamydodon pedarius*. Jap. J. Genet. 36, 223—234.

Kimball R. F. and Barka T. 1959: Quantitative cytochemical studies on *Paramecium aurelia*. II. Exp. Cell. Res. 17, 173—182.

Kimball R. F. and Perdue S. W. 1962: Quantitative cytochemical studies on *Paramecium*. V. Exp. Cell Res. 27, 405—415.

Kimball R. F. and Prescott D. M. 1962: Desoxyribonucleic acid synthesis and distribution during growth and amitosis of the macronucleus of *Euplotes*. J. Protozool. 9, 88—92.

Losina-Losinsky L. 1931: Zur Ernährungsphysiologie der Infusorien. Arch. Protistenk. 74, 18—120.

McDonald B. B. 1962: Synthesis of desoxyribonucleic acid by micro- and macronuclei of *Tetrahymena pyriformis*. J. Cell Biol. 13, 193—203.

Moses M. J. 1950: Nucleic acids and proteins of the nuclei of *Paramecium*. J. Morphol. 87, 493—536.

Nissenbaum G. 1953: A combined method for the rapid fixation and adhesion of Ciliates and Flagellates. Science 118, 31—32.

Ovchinnikova L. P., Selivanova G. V. i Cheissin E. M., 1963: Issledovanie metodom ultrafioletovoj citofotometrii vlijanija golodanija na količestvo DNK i RNK u *Paramecium caudatum*. Morfologija i fiziologija prostejših (sbornik rabot), 44—53.

Peschkovskaya L. S. 1936: Izmenenija jadernogo apparata *Climacostomum virens* pri konjugacii. Biol. Žurn. 5, 207—220.

Peschkovskaya L. S. 1948: Metamorfoz jadernogo apparata pri polovom processe dvuh vidov brjuhoresničnych infuzorij. Tr. Inst. citol. gistol. embriol. 3, 201—208.

Poljansky G. 1934: Geschlechtsprozesse bei *Bursaria truncatella* O. F. Müll. Arch. Protistenk. 81, 420—546.

Prescott D. M. 1960: Relation between cell growth and cell division. IV. Exp. Cell Res. 19, 228—238.

Prescott D. M., Kimball R. F. and Carrier R. F. 1962: Comparison between the timing of micronuclear and macronuclear DNA synthesis in *Euplotes eurystomus*. J. Cell Biol. 13, 175—176.

Raikov I. B. 1958 a: Der Formwechsel des Kernapparates einiger niederer Ciliaten. I. Die Gattung *Trachelocerca*. Arch. Protistenk. 103, 129—192.

Raikov I. B. 1958 b: Citohimičeskie osobennosti jadernogo apparata ravnoresničnych infuzorij *Loxodes striatus* i *L. magnus*. Dokl. AN SSSR 121, 1079—1082.

Raikov I. B. 1959 a: Der Formwechsel des Kernapparates einiger niederer Ciliaten. II. Die Gattung *Loxodes*. Arch. Protistenk. 104, 1—42.

Raikov I. B. 1959 b: Citologičeskie i citohimičeskie osobennosti jadernogo apparata i delenija ravnoresničnoj infuzorii *Geleia nigriceps* Kahl. Citologija 1, 566—579.

Raikov I. B. 1962: Der Kernapparat von *Nassula ornata* Ehrbg. (*Ciliata, Holotricha*). Zur Frage über den Chromosomenaufbau des Makronucleus. Arch. Protistenk. 105, 463—488.

Raikov I. B. 1963 a: Jadernyj apparat i delenie *Remanella granulosa* Kahl i *R. rugosa* Kahl. (*Holotricha, Gymnostomatida*). Morfologija i fiziologija prostejšin (sbornik rabot), 20—34.

Raikov I. B. 1963 b: Jadernyj apparat ravnoresničnych infuzorij *Geleia orbis* Fauré-Fremiet i *G. murmanica* Raikov. Acta Protozool. 1, 21—30.

Raikov I. B. 1963 c: On the origin of nuclear dualism in ciliates. Proceed. I Internat. Conf. Protozool., in press.

Ray C. 1958: Tetraploidy in *Tetrahymena pyriformis*. Proceed. X Internat. Congr. Genet. 2, 229—230.

Ruthmann A. 1963: Die Struktur des Chromatins und die Verteilung der Ribonucleinsäure im Makronucleus von *Loxophyllum meleagris*. Arch. Protistenk. 106, 422—436.

Ruthmann A. und Heckmann K. 1961: Formwechsel und Struktur des Makronucleus von *Bursaria truncatella*. Arch. Protistenk. 105, 313—340.

Saito M. 1961: A note on the duplication process of macronuclear chromosomes in a peritrichous ciliate, *Vorticella campanulla*. Jap. J. Genet. 36, 184—186.

Saito M. and Sato H. 1961 a: Morphological studies on the macronuclear structure of *Paramecium caudatum*. II. Zool. Mag. 70, 73—80.

Saito M. and Sato H. 1961 b: Morphological studies on the macronuclear structure of *Paramecium caudatum*. III. Zool. Mag. 70, 81—88.

Sato H. and Saito M. 1959: Morphological study on the macronuclear structure in interphase and prefission stage of *Tetrahymena geleii* W. Zool. Mag. 68, 209—214.

Schwartz V. 1958: Chromosomen im Makronucleus von *Paramecium bursaria*. Biol. Zentralbl. 77, 347—364.

Seshachar B. R. 1950 a: Desoxyribonucleic acid content of the ciliate micronucleus. Nature 165, 848.

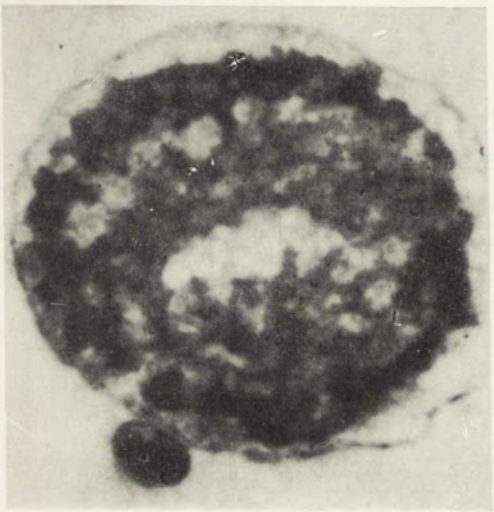
Seshachar B. R. 1950 b: The nucleus and nucleic acids of *Chilodonella uncinatus* Ehrbg. J. Exp. Zool. 114, 517—514.

Seshachar B. R. and Dass C. M. S. 1954: Photometric study of desoxyribonucleic acid (DNA) synthesis in regenerating macronucleus of *Epistylis articulata* From. Proc. Nat. Inst. Sci. India 20, 656—659.

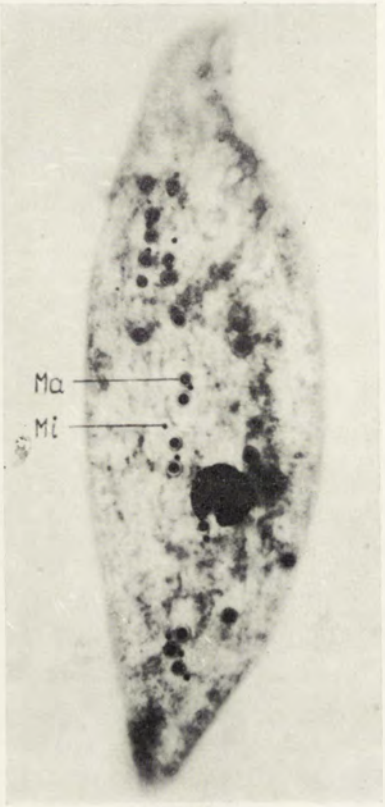
Sonneborn T. 1947: Recent advances in the genetics of *Paramecium* and *Euplotes*. Advances in Genetics 1, 263—358.

Walker P. and Mitchisson J. 1957: DNA synthesis in two ciliates. Exp. Cell Res. 13, 167—170.

Woodard J., Gelber B. and Swift H. 1961: Nucleoprotein changes during the mitotic cycle in *Paramecium aurelia*. Exp. Cell. Res. 23, 258—264.



1



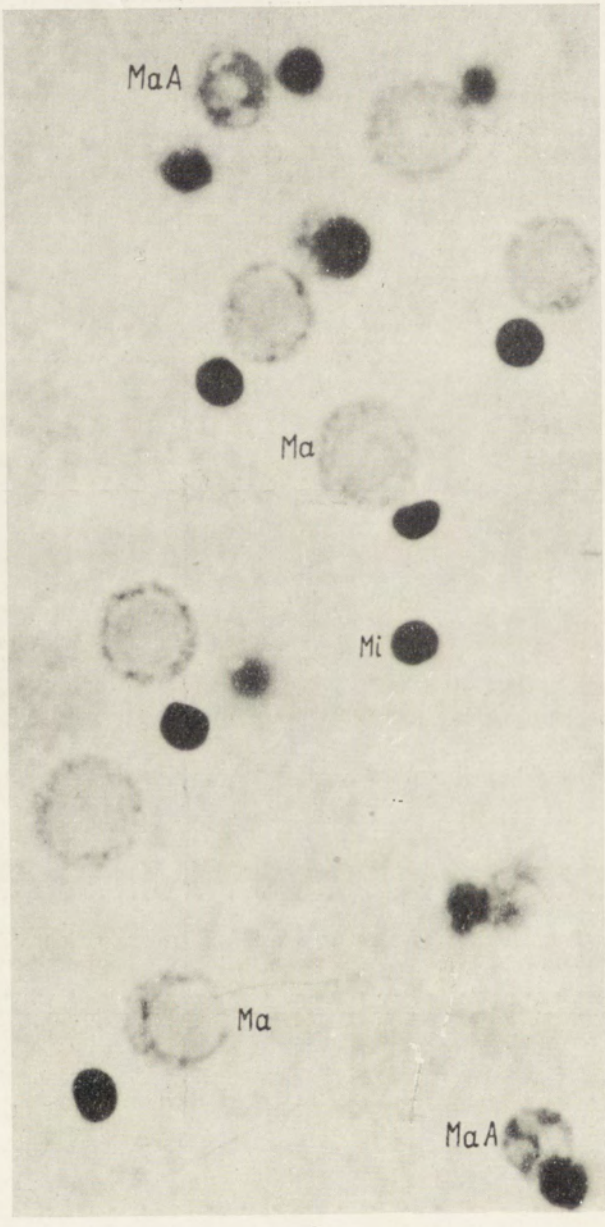
2

1: Section through the macronucleus and one micronucleus of *Nassula ornata*. Feulgen reaction, photomicrograph, 1800 × (from R a i k o v 1962).

2: General view of *Loxodes magnus*. Hemalum stained whole mount, photomicrograph, 290 × (from R a i k o v 1958 b). Ma — macronuclei, Mi — micronuclei.

3: Nuclei of *Loxodes magnus*. Whole mount, Feulgen reaction, photomicrograph, 2000 ×. Ma — macronuclei, Mi — micronuclei, MaA — macronuclear anlagen.

I. B. Raikov et al. auctores phot.



3

I. B. Raikov et al. auctores phot.

ACTA PROTOZOOLOGICA
VOL. I WARSZAWA, 30. IX. 1963 FASC. 28

Department of General Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa 22, Pasteura 3

Leszek KUŹNICKI

Reversible immobilization of *Paramecium caudatum* evoked by nickel ions

Badania nad odwracalną immobilizacją *Paramecium caudatum* wywołaną przez jony niklawe

The problem of slowing the movement of *Protozoa* or their complete immobilization is interesting for protozoologist not only for its methodological aspect. It also offers possibilites to analyse the activity of the ciliary apparatus and other cell functions.

Gelei 1935 ascertained the immobilizing action of Ni^{2+} salts ($NiCl_2$, $NiSO_4$, $Ni(NO_3)_2$) upon *Paramecium caudatum*. He also described the influence of Ni^{2+} on the action of the contractile vacuoles and their injection canals, on the cyclosis of endoplasm, on ingestion, on excretion of food vacuoles, on the silverline system and on the co-ordination of the ciliary apparatus. Those primary informations on the biological action of Ni^{2+} ions failed to evoke a special interest of scientists and the method of immobilization of ciliates by means of Ni^{2+} ions was not recorded in the protozoological monographs except for the publications of Tartar 1950, 1961. Only Tchakhotine 1938 and later Thomas 1953 tried to analyse — more fully than Gelei — the anesthetic (immobilizing) action of $NiSO_4$ upon *Paramecium*.

Just recently the influence of nickel salts on protozoa began to evoke a vivid interest. This factor is being applied and analysed in different ways (Bovee 1958, Seravin 1961, 1962 a, 1962 b, Párducz 1962, Pitelka and Párducz 1962, Puytorac, Andrivan et Serre 1962).

In light of the recent study, nickel salts might be recognized as one of the most effective and universal immobilizatory agents for protozoa. Despite of the progress in this field, some problems connected with the immobilization of *Paramecium caudatum* — the ciliate which has been most investigated — remain obscur, controversive or altogether open.

1. The high differences of concentration at which in similar time immobilization was atteined by different authors are confusing and involve difficulty in exact determination of optimal concentrations. In this question the most firm position is assumed by Gelei 1935. According to this author it is impossible to determine the optimal concentration for single species because — expect the clonal differences — the resistibility of ciliates against the immobilizing action of nickel ions shows considerable fluctuations connected with the character of medium (kind of the nutrient, degree of oxygen supply etc.).

2. There is a great divergence in the opinions on factors evoking immobilization. The view of Gelei 1935 was in this subject not determined. He expressed the opinion that the nickel ions act upon the co-ordinating system, or that they rather make impossible the effective movement of cilia which normally advance the ciliate forwards.

Thomas 1953 obtained the best immobilization effects with the concentration 0.02% $NiSO_4$ in the culture medium. In those conditions, the movement of the ciliate is completely inhibited after 2 hrs. This state is called by the author "the period of sleep" and is characterized by the immobilization of cilia in some regions of the body, spreading gradually over the remaining regions, although the immobilized cilia may resume their work for short moments. Only a prolonged stay of immobilized ciliates in the solution of Ni SO_4 evokes a full immobilization of all the cilia — including the peristomal — although without their impairment.

Seravin 1961 reported that $NiSO_4$ in concentration 0.01—0.001% stops the movement of *Spirostomum*, *Euplotes*, and *Paramecium* without inhibiting the activity of their cilia for 1—3 hrs. According to Seravin the immobilizing action of this factor consists in suppressing the spontanic metachronal impulses which co-ordinate the whole activity of the motor system in ciliates.

Those discrepancies are also reflected in the terminology which is not well established. Some authors refer to narcosis occurring in *Protozoa*, other to anaesthesis or ciliary anaesthesis, some others — to paralysis.

In the present paper the term immobilization will be used as the most descriptive and general, without deciding about the nature of the phenomenon.

3. The return to norm after immobilization with nickel salts remains an open question. Only Thomas 1953 reported that paramecia may return to norm when transferred to the culture medium 30 min. after the onset of $NiSO_4$ action i.e. when the slowing of motion, according to the authors observations, just begins. Yet in his opinion, the case of $NiSO_4$ action may be considered as a reversible narcosis of *Paramecium caudatum*.

The present paper is an attempt to answer the 3 above questions.

Methods

Paramecium caudatum was used for experiments in several clones originating from cultures grown for many years on the milk nutrient. Concurrently cultures on lettuce infusion innoculated with *Aerobacter aerogenes* were run. The temperature of rooms in which the cultures were kept was 18 ± 1 °C.

For experiments ciliates were taken from the thigmotactic rings, after removal of suspended detritus. For attaining the suitable density which amounted ca 100 indiv./ml, the calculated quantity of the filtered medium of the same cultures on lettuce infusion inoculated with *Aerobacter aerogenes* were run (tap water, distilled water or buffer), ciliates were collected during the rinsing from their geotactic aggregations.

All solutions were prepared on bidistilled water. The solution being experimented was added to the ciliate sample in the volume ratio 1 : 1 and stirred after mixing. Each concentration was tested in 20 samples.

The preliminary observations concerned the immobilizing action of nickel sulphate and chloride and revealed no essential differences in the effects of

those two compounds. Consequently, the immobilization is evoked exclusively by the action of the nickel ions; in subsequent experiments only nickel chloride was used. Its toxic concentration was determined previously (Grębecki and Kuźnicki 1955, 1956). For *Paramecium caudatum* from milk culture it amounted for 24 hrs LD_{50} = 0.008 mM which was supported by recent experiments in the same medium conditions.

One of the causes of diverse opinions upon the problem of immobilization by action of chemical agents introduced into the medium is the lack of comparable criteria of immobilization. Such fixed criteria — if even arbitrary — seem to be absolutely necessary for the quantitative comparison of results.

The following criteria for analysis of the immobilizing action of $NiCl_2$ on *Paramecium caudatum* were set up:

1 As the state of immobilization such a limitation of the ciliate movement will be considered, in which it cannot swim (in any direction) along a distance exceeding its body length and its rotary movement is limited to 180°.

2. In a sample containing about 100 individual, at least 90% of ciliates should show immobilization simultaneously.

3. Ciliates should remain immobilized for no less than 30 min. and the general percentage of immobilized individuals should not diminish.

4. The factor evoking immobilization should not cause perceptible pathological changes typical for paranecrosis (Makarov 1935, 1938) and, before all, should not deform the shape of the cell.

5. After the transfer to the initial medium or after rinsing, the normalization of movements and of other functions should concern at least 50% of the initial number of ciliates. The experiment set in this way allows to ascertain in which conditions (concentration, time of action) depending also on the character of medium, nickel ions may evoke a reversible cutoff of the motory apparatus, without impairing the cell itself.

Another problem analysed in the present study — the behaviour of the motor apparatus during immobilization — was studied in living material in the dark field and phase contrast optics.

The influence of the ion composition of medium upon immobilization

The preliminary observations carried out on a clone grown on milk nutrient and on lettuce infusion gave strikingly high differences of concentrations in which nickel chloride produced similar intensity of immobilization. These results confirm the observations of Gelei 1935 about the influence of the nature of medium upon the immobilizing action of the nickel ions. The behaviour of different clones grown on the same nutrient showed less essential differences. Subsequent experiments had in view to detect the cause of this phenomenon.

Ciliates originating from the same milk nutrient were divided into 3 groups. The first group was rinsed with distilled water, the second — with tap water, the third — with phosphate-citric buffer applied by Dryl 1959. Then the range of the nickel chloride concentrations was determined which give similar immobilization effects in the same period of time in all three groups. The results (buffer: 0.4—2 mM, tap water 0.02—0.08 mM, distilled water 0.008—

0.03 mM) show that the dynamic of the immobilizating action of nickel ions upon protozoa depends on the presence and concentration of other ions in the medium.

The culture medium with protozoa was diluted 200 times with distilled water in order to ascertain the influence of the biologically important ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) upon the dynamic of immobilization processes. Then,

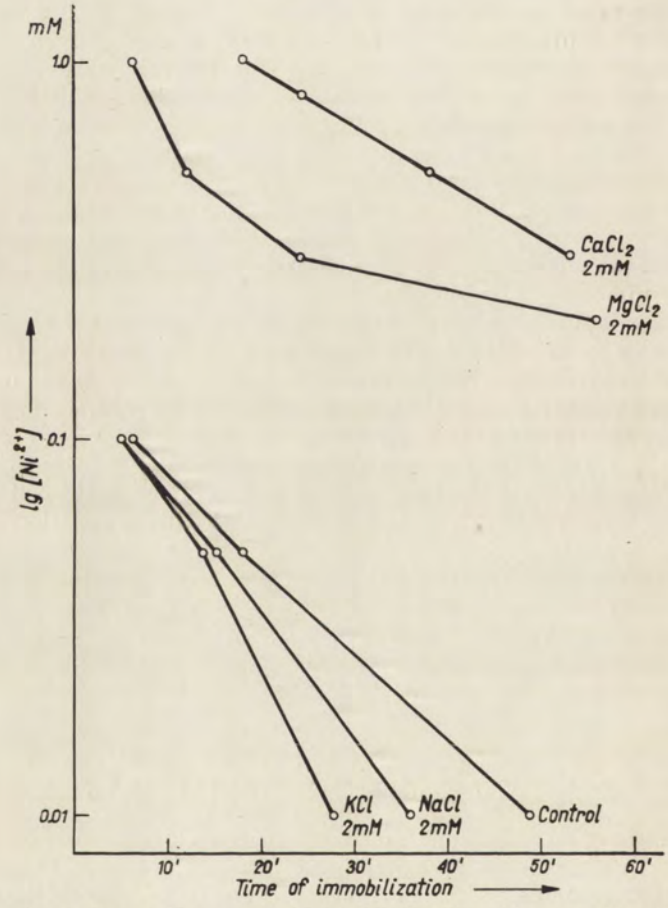


Fig. 1. The influence of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} on the dynamics of the immobilizing action of Ni^{2+} on *Paramecium caudatum*

5 hrs later the whole material was distributed into 5 samples. In one of them the ciliates were left in distilled water while the others were rinsed in following solutions: 2 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$.

After 1 h, solutions of different concentrations of nickel chloride were added and the time was measured when immobilization of more than 90% of individuals in the sample occurred. In this way it was possible to establish which of the cations plays the decisive protecting role against the immobilizing action of nickel ions. The results are represented in the diagram (Fig. 1).

When compared with the 200 times with distilled water diluted culture medium, the experiment indicates that the Na and K ions slightly accelerate the immobilization, whereas the Mg and Ca ions play a protective role. The differences are tremendous. For instance, 0.1 mM nickel chloride solution evokes the immobilization of all the individuals within 5 min. in presence of KCl, but it fails to act in presence of $CaCl_2$ at all. In this case, 50% of ciliates survive 24 hrs.

In the subsequent experiments the course of immobilization depending on Ca concentration in the medium was to be stated. A series of experiments was carried out in which the ciliates were rinsed in distilled water (according the former procedure) and placed in the solution of $CaCl_2$ of different concentrations. Then the time of the onset of immobilization evoked by 0.5 mM $NiCl_2$ was determined.

The protective action of Ca was stated already in the concentration 0.05 mM $CaCl_2$ in the cultures diluted 200 times with distilled water. It was also stated that for the protective influence of the calcium ions is not essential whether this factor has been added to the culture before the action of the nickel salts or together with them.

The above experiments allow to conclude that the decisive factor of different dynamics in the immobilization activity of the nickel ions upon *Paramecium caudatum* is surely the calcium ions concentration in the medium (the magnesium ions concentration being of a minor importance). Other factors play a rather secondary role.

Behaviour of the motor apparatus in the course of immobilization

The immobilization of ciliates evoked by the nickel ions is a characteristic phenomenon uniform for all the individuals of a sample except some individual differences in resistibility of single ciliates. This uniformity manifest the more distinctly the more rapid is the action of the nickel ion. This is illustrated by the case described below, when all the individuals were immobilized within 32 min.

In the course of 10—12 min. from the beginning of the experiment, the velocity of swimming falls, simultaneously the spiralization diminishes and the rotary movement round the long body axis becomes slower. In this phase the ciliates collect in the lower layer of the medium as result of their passive fall to the bottom of the vessel. For the next 5—6 min. ciliates do not swim but their movement becomes a short of crawling. Their rotation is nearly stopped. The ciliate stops more and more frequently, performs short reversions and at last its progressive movement becomes thoroughly inhibited. Frequency of short reversions increases distinctly but they gradually last shorter. Up from this moment it is possible to consider the immobilization as accomplished although the characteristic "winces" of the ciliate body still occur. The course of this process suggests that the immobilization evoked by nickel chloride proceeds gradualllly as result of the fall of effective action of cilia.

The ciliates immobilized by nickel chloride perform short reversions at intervals of several seconds. Those reversions involve quick withdrawals of the ciliates $^1/_2$—$^2/_3$ of their body length, and then a quick return to their exit position. This characteristic phenomenon has not been described as yet by

any author. The rotation movement is thoroughly suppressed. The behaviour of the ciliate produces impression as if it tried in vain to overcome an invisible obstacle. In this case the definition "immobilization" is in harmony with the criteria of immobilization phenomenon as established in the introduction.

The observations in the phase contrasting optics and in the dark field seem to indicate that any of the theories of the immobilization mechanism put forward till present time is not satisfactionary (Gelei 1935, Thomas 1953, Seravin' 1961).

Prior to complete immobilization the metachronism and the co-ordination of the ciliary movement persist.

In the "immobilization phase", cilia of the whole body keep working till perceptible pre-mortal changes of the ciliate appear. However the work of cilia is not normal since they are not able to perform an effective stroke and their movements occur only on a limited way which produces an impression as if they were stiff and adjusted at a constant angle to the body surface.

In this situation no metachronism is observed. It does not prove a full suppression of co-ordination because short reversions still occur as result of a synchronic stroke of all the cilia from the posterior end forwards. The ciliary movement is limited and the synchronic stroke is followed by return of cilia to their initial position and — in consequence — by return of the ciliate to its formed place. Fig. 2 illustrates the behaviour of *P. caudatum* and its locomotory apparatus after immobilization with nickel chloride.

The frequency of reversions which appear after immobilization every 4—10 sec. gradually falls when the ciliates remain in the nickel chloride solut-

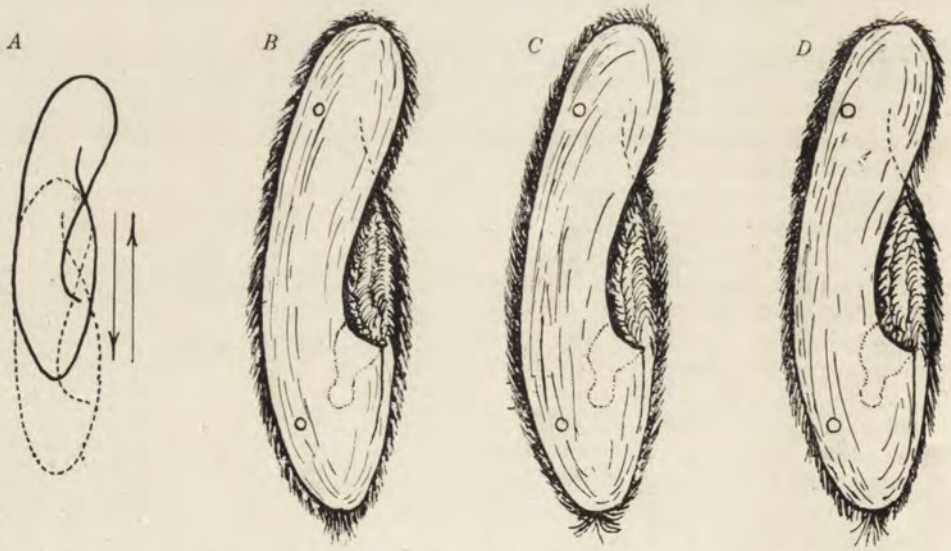


Fig. 2. Diagram illustrating the behaviour of *P. caudatum* and its ciliary apparatus after immobilization with Ni^{2+}

A — character of movements which may be performed by the immobilized ciliate, B — ciliary apparatus after a full immobilization of the ciliate, C — ciliary apparatus during the short-lasting reversion, D — return of cilia to their exit position involving the shift of the ciliate to its former place.

ion for a prolonged time. The disappearence of this reaction indicates the beginning of typical pre-mortal changes. The macronucleus becomes distinctly visible, the body is being deformed and large areas with immobile cilia appear. The movement of all the other cilia is chaotic. The contractile vacuoles enlarge unnaturally. The peristomal cilia keep their movement longer than the somatic ones so that their partial immobilization may be observed only in those individuals which are distinctly deformed. The peristomal apparatus — like in the case of chloral hydrate (Grębecki and Kuźnicki 1961, Kuźnicki 1963) — shows a much stronger resistibility against the action of immobilizing factors.

Conditions of renormalization

The nickel salts may evoke the immobilization of ciliates or may fail to evoke it, depending on the calcium and magnesium ions concentration in the medium. It may also be stated that the time of action of the nickel salts solution before the deformation of the cell sets on, depends on the time when immobilization occurred. It was stated that the most convenient for practical reasons are such concentrations of nickel salts which evoke immobilization within 30—60 min. In those cases almost 100% of individuals become immobilized simultanously and show no deformation of the body for over 30 min. irrespectively of the concentration applied.

The individual differences of resistibility are considerable. Single ciliates may persist in the state of immobilization up to 4 hrs without signs of pre-mortal changes. If they are to be used for subsequent experiments, they should not stay in the nickel salts solutions longer than 45—60 min. from the moment of immobilization, because the sensibilization to strong light and to mechanical stimuli gradually develops in all the ciliates even in those which are not deformed. In order to decide whether the nickel salts may be considered as a fully effective immobilizer for *P. caudatum*, it seems essential to ascertain in which degree the immobilized individuals may attain the renormalization. Therefore a series of experiments was executed on the ciliates of one clone kept in different media: in milk nutrient, lettuce infusion, after rinsing with the phosphate-citric buffer, with tap water or with distilled water. In each case, such a concentration of nickel chloride was adjusted that the immobilization of 90% of individuals occurred within 40—50 min. Then, 30 min. after immobilization, ciliates were rinsed, every series in its primary medium. Rinsing was repeated 4 times diluting the medium 1 : 10 every time which resulted in 10 000 dilution of immobilization medium. After 12 hrs the percentage of individuals which recovered their normal movement was calculated in the sample. After rinsing with distilled water all the ciliates die and the intoxication occurs within the same period of time as in the samples where the ciliates still remained in the nickel chloride solution. After rinsing with tap water, milk nutrient, lettuce infusion or phosphate-citric buffer, 20—45% of individuals recovered their normal conditions.

Quite different results were obtained after rinsing the ciliates with 5 mM calcium chloride solution in distilled water. In all cases irrespectively of the character of medium, 95—100% of ciliates recovered their movement. The action of calcium ions involves a full restitution of vital functions even in those ciliates in which some paranecrotic changes appeared. When solution of

calcium chloride is applied, a mass renormalization way be evoked even in the ciliates which remained in immobilizied conditions for 60 min. or more.

In several series of experiments were applied various concentrations of nickel chloride for immobilization and different media for the subsequent rinsing which resulted in different percentage of renormalization. For this experiment, ciliates from a milk culture were used. Their milk medium was diluted 200 times with distilled water prior to addition of nickel chloride solution.

The above experiments indicate that the Ca ions perform not only a protective role against the toxic and immobilizing action of nickel chloride salts, but that they exert a decisive influence in renormalization of inhibited vital functions in the immobilizied ciliates. Consequently, for a mass recovery of immobilized ciliates, 2—5 mM solution of calcium chloride should be always used. Higher concentrations of calcium salts fail to increase essentially the stimulating action of this factor which seems to depend on the presence of sodium and potassium ions in the medium. This accounts for the fact that calcium chloride is the most effective antidote against nickel salts when traces content of sodium and potassium is present.

In the course of renormalization of movement in ciliates, processes develop mostly in a reverse sequence to those which occur in immobilization. The characteristic "stiffness" of cilia gradually disappears and the amplitude of their stroke increases. Initially the ciliate movements show an increased frequency and lengthening of paths of their short-lasting reversions. Together with the onset of foreward swimming, the rotary movement appears although it is not fluent. The next step is the recovery of spiralization of movement but the velocity of swimming is still lowered.

The time of onset of the progressive movement and its full renormalization depends on the time which is necessary for immobilization and also on the length of the period when they were immobilized. In the case when immobilization occured within 40—50 min. and ciliates were rinsed in 5 mM solution of calcium chloride 30 min. after the onset of immobilization, the progressive movements of 50% of individuals in a sample appear after 75—90 min. The full normalization of the movement rate in all the ciliates appears only after 8—11 hrs. Divisions of immobilized ciliates are observed only 72—85 hrs after the moment of rinsing.

Similar ion relationships as for *P. caudatum* were also stated in immobilizing action of Ni^{2+} upon some other ciliates (*P. aurelia, P. bursaria, Tetrahymena pyriformis, Colpidium colpoda* and *Spirostomum ambiguum*). In all cases the renormalization of immobilized ciliates shows a mass character only after rinsing them with calcium chloride.

Discussion

The above results indicate that in suitably adjusted conditions the action of Ni^{2+} ions on *Paramecium caudatum* may: 1. evoke the immobilization of the ciliate as a mass phenomenon, 2. keep the ciliates in the state of immobilization for more than 30 min. without involving perceptible pathological changes, 3. allow after rinsing a full recovery of movement and of other vital functions of immobilized ciliates. Consequently the nickel ions fulfill all criteria of effective immobilizer.

The ionic composition of medium plays the most important role as well in the immobilization phenomena as in those of renormalization. The decisive factor is here the calcium ions concentration. The absolute concentration of Ca ions is the principal factor evoking different dynamics of immobilizating action of Ni^{2+} in the same concentration. The concentration of magnesium ions may also be of some importance but their concentration in the media mostly applied and their protective influence are much inferior to that of the Ca^{2+} ions. In contrast to this the concentration of univalent ions as Na^{+} or K^{+} when similar to that of natural media, has no essential influence upon the dynamics of immobilizing action of nickel ions.

In the literature some cases of the protective role of calcium ions against different noxious agents in ciliates were reported (Chalkley 1930, Eisenberg-Hamburg 1932, Losina-Losinsky 1948, Seravin 1958).

All those informations seem to support the general cytological regularity concerning the fall of the membrane permeability influenced by calcium (or magnesium) ions and the antagonistic action of the sodium and potassium ions. This regularity has been illustrated on the protozoa material by Tartar 1957 in his study on shedding the pigment by the action of different factors in *Stentor coeruleus*.

The protective role of calcium ions against the immobilizing action of Ni^{2+} as well as in the renormalization process — as stated in the present study — is a new argument in favour of the great importance of this factor in the processes occurring in the cortical structures and in the cytoplasmic membranes of ciliates.

The experiments concerning the influence of the ion composition of medium upon the dynamics of immobilization process seem to indicate plainly that the protective action of calcium and magnesium ions is the result of changes of the permeability of the cytoplasmic membranes in ciliates. This action is so strong that it cannot be accounted for by the changes in the ions Ni^{2+} activeness only. Some slight differences between the phenomena occurring in the KCl and NaCl solutions and in the 200 times diluted culture medium respectively, might be the results of interference of two opposite factors: changes of permeability and of the number of thermodynamically active Ni^{2+} ions. Similar results were found by Puytorac, Andrivan et Serre 1962, as follows from their concise abstract.

For the stimulating action of calcium ions in the renormalization process (following the immobilization with nickel salts) no satisfactory explanation was found. It may be assumed that in the superfluous calcium content in the medium, a more rapid and effective elimination of Ni^{2+} from the cortical layer of the immobilized ciliates occurs. It should be also remembered that magnesium and calcium ions activate the pyrophosphatase in cilia (Child 1961).

The most disputable problem connected with the action of Ni^{2+} is the mechanism of immobilization. Some controversies in the views on this subject may be due to the different experimental conditions. Some differences in the behaviour of ciliates and of their motor apparatus may be observed in connection with the different period of time between the addition of Ni^{2+} and the onset of immobilization. In the case of high concentrations when the immobilization occurs at once or within a few minutes in most individuals, ciliates behave in very different manners: some of them die at once, others

stop and discharge their trichocysts, shedding also their cilia in some body regions, some are immobilized with no perceptible pathological changes. But in the last of the mentioned groups, the behaviour of the ciliary apparatus is not uniform. Some keep their ciliary movement over the whole body surface while in the others some regions of immobilized cilia appear.

A similar variety of behaviour may be observed in very low concentrations, when immobilization occurs later than after 60 min., or fails to concern a part of individuals. One of the manifestations of those differences is the inversion of the left spiraling to the right, as described recently by Párducz 1962 in *Paramecium multimicronucleatum*. The most uniform is the behaviour of ciliates in those experiments when the immobilization as a mass phenomenon occurs within 20—60 min. Neither death of ciliates nor pathological changes nor discharging trichocysts are then observed. In the paramecia immobilized in this manner, the movement of cilia persists for a long time. In the case just described as well Seravin 1961, 1962 a as Párducz 1962 are inclined to consider the immobilization of the ciliates as result of suppression of conductivity and of disturbances in the co-ordination of ciliary movement. This view is based on the absence of metachronal waves and on the non-coordinated activity of cilia in the immobilized individuals. The absence of metachronal waves is a fact but it might be also the consequence of a limited possibility of effective ciliary work and not the direct cause of immobilization. The constant occurrence of short reversions in immobilized individuals disagrees with the postulation of lack of generation and propagation of metachronal impulses. The attempt to explain the origin of short-lasting retreats by some other co-ordination mechanism in reversion (Seravin: personal communication) seems not to be satisfactory because the reversion is followed by a co-ordinated stroke of cilia beating backwards, and — as result of it — the ciliate returns to its exit position. So in the course of nickel immobilization, the conductibility remains, as well as the capacity of co-ordinated action of cilia in the forward movement and in the reversion.

Makarov 1935, 1938 claimed that the immobilization of ciliates must always be the result of far advanced paranecrotic changes. In the light of informations supplied by the study of immobilization with nickel salts, this view seems to be unjustified since in suitable conditions Ni^{2+} induce a reversible paralysis of movement in *Paramecium* without any perceptible pathological changes in the cell.

It remains to discuss the question whether the reversible immobilization of ciliates by action of Ni^{2+} may be considered as narcosis. This is not only the matter of terminology since the acceptation of existence of narcosis phenomena serves as an argument in favour of functional analogy between the conductive system in *Protozoa* and the nervous system of higher organisms.

The narcosis in ciliates would be real only in this case if the immobilization induced by suppression of propagation of impulses by a selective action of a substance upon the conductive system, could be successfully ascertained. In the case of immobilization evoked by nickel salts such phenomena are not observed. Immobilization is rather the result of a reduced possibility of performing the effective work by cilia. In the immobilized individuals, during their temporary reversion and return to their exit position, the conduction of impulses occurs along the whole body. The movement of all the cilia — as

well in the forward as in the backward stroke — seems to be co-ordinated. Owing to this fact, despite the considerable limitation of the effective work of cilia, the shifting of the body is possible.

All that facts seem to justify the view that immobilization evoked by Ni^{2+} ions cannot be included to the phenomena of narcosis.

The immobilization evoked by Ni^{2+} shows a certain analogy to that involved by the homological antiserum treatment in *Paramecium*. It seems adequate to apply the term "immobilization" to all the phenomena discussed above and to the case analysed in the present study — the "nickel immobilization".

Summary

The dynamics of immobilizing Ni^{2+} action upon *Paramecium caudatum* depends chiefly on the Ca^{2+} concentration in the medium. The influence of Mg^{2+} is much weaker and that of the univalent cations (Na^{+}, K^{+}) is of a secondary importance. The increase of Ca^{2+} concentration requires a much higher raise of Ni^{2+} concentration for producing a similar immobilization effect. The strong protective action of Ca^{2+} may be accounted for by the fall of permeability of cytoplasmic membranes for the Ni^{2+}. Calcium ions play also a decisive role in the renormalization of the immobilized ciliates. The mass return to norm in *P. caudatum* occurs only after rinsing them in 2—5 mM solution of Ca^{2+} in the presence of trace quantity of Na^{+} or K^{+} ions, if they remained immobilized longer than 30 min. The immobilization of *P. caudatum* by nickel ions (occurring as a mass process after 30—60 min.) takes place as result of limitation of the capacity of performing the effective work of cilia. The ciliary movement itself and the possibility to perform the co-ordinated work as also to conduct impulses — remain. Considering the above facts, the immobilization of ciliates by the action of Ni^{2+} ions cannot be qualified as narcosis phenomenon.

STRESZCZENIE

Dynamika immobilizującego działania jonów Ni^{2+} na *Paramecium caudatum* zależy w głównej mierze od stężenia Ca^{2+} w środowisku. Wpływ jonów Mg^{2+} jest znacznie mniejszy, zaś koncentracja kationów jednowartościowych (Na^{+}, K^{+}) ma znaczenie drugorzędne. Wzrost stężenia Ca^{2+} wymaga dla uzyskania podobnych efektów immobilizacyjnych znacznego podwyższenia koncentracji Ni^{2+}. Wybitnie osłaniające działania Ca^{2+} daje się wyjaśnić zmniejszeniem przepuszczalności błon cytoplazmatycznych wymoczków wobec Ni^{2+}. Jony wapnia odgrywają również decydującą rolę w renormalizacji immobilizowanych wymoczków. Masowy powrót do normy *P. caudatum* przebywających w stanie unieruchomienia dłużej niż 30 min. następuje jedynie po przepłukaniu wymoczków 2—5 mM roztworami chlorku wapnia, przy śladowych obecnościach jonów Na^{+} lub K^{+}. Immobilizacja *P. caudatum* pod wpływem jonów niklu (występująca jako zjawisko masowe po 30—60 min.) zachodzi w wyniku ograniczenia możliwości wykonania efektywnej pracy przez rzęski. Ruch jednak samych rzęsek i możliwość wykonania przez nie skoordynowanych ruchów, jak i przewodnictwo impulsów, pozostają nadal zachowane. W związku z tym unieruchomienie wymoczków pod wpływem jonów Ni^{2+} nie może być zakwalifikowane jako zjawisko narkozy.

REFERENCES

Bovee E. 1958: Nickel sulfate as an anesthetic for protozoa. Turtox News 36, 78.

Chalkley H. W. 1930: Resistance of *Paramecium* to heat as affected by changes in hydrogen-ion concentration and in inorganic salt balance in surrounding medium. USA Pub. Health Service, Pub. Health Depts. 45, 481—489.

Child F. M. 1961: Some aspects of the chemistry of cilia and flagella. Exp. Cell. Research 8 (Suppl.), 47—53.

Dryl S. 1959: Antigenic transformation in *Paramecium aurelia* after homologue antiserum treatment during autogamy and conjugation. J. Protozool. 6 (Suppl.), Abstr. 96.

Eisenberg-Hamburg E. 1932: Einfluss der Sr-Salze auf die Bewegung von *Paramecium caudatum*. Die Rolle des Ca und der Konzentration der Wasserstoffionen. Arch. Protistenk. 77, 108—124

Gelei J. 1935: Ni-Infusorien im Dienste der Forschung und des Unterrichtes. Biol. Zbl. 55, 57—74.

Grębecki A. i Kuźnicki L. 1955: Stosunek *Paramecium caudatum* do chemizmu środowiska i ochronny wpływ skupienia wobec substancji nieorganicznych. Folia Biol. 3, 127—157.

Grębecki A. and Kuźnicki L. 1956: Autoprotection in *Paramecium caudatum* by influencing the chemical properties of its medium. Acta Biol. Exp. 17, 71—107.

Grębecki A. and Kuźnicki L. 1961: Immobilization of *Paramecium caudatum* in the chloral hydrate solutions. Bull. Acad. Pol. Sci., S. Sci. Biol. 9, 459—462.

Kuźnicki L. 1963: Recovery in *Paramecium caudatum* immobilized by chloral hydrate treatment. Acta Protozool. 1, 177—185.

Losina-Losinsky L. K. 1948: Vlijanie solej na holodoustojčivost infuzorij i pričiny smerti pri ohlaždenii. Žurn. Obšč. Biol. 9, 441—453.

Makarov P. 1935: Experimentelle Untersuchungen an Protozoen mit Bezug auf des Narkose-Problem. Protoplasma 24, 593—606.

Makarov P. V. 1938: Problema obščego i kletočnego narkoza. Arh. Anat. Gistol. Embriol. 19, 5—104.

Párducz B. 1962: Studies on reactions in Ciliates. IX. Ciliary coordination of right spiralling *Paramecia*. Annal. Mus. Hist. Nat. Nation. Hung., Pars Zool. 54, 221—229.

Pitelka D. R. and Párducz B. 1962: Electron-microscope observations on paralyzed *Paramecium*. J. Protozool. 9 (Suppl.), Abstr. 2.

Puytorac P. de, Andrivon C. et Serre F. 1962: Sur l'action cytonarcotique des sels de nickel chez *Paramecium caudatum* Ehrb. J. Protozool. 9 (Suppl.), Abstr. 23.

Seravin L. N. 1958: Vlijanie na vyživaemost *Paramecium caudatum* dvuh odnovremenno dejstvujuščih solej. Vest. Leningr. Univ. 21, 90—101.

Seravin L. N. 1961: Rol adenozintrifosfata v bienii resniček infuzorij. Biohimija 26, 160—164.

Seravin L. N. 1962 a: Osobennosti razvitija obščego narkoza u *Spirostomum ambiguum*. Citologija 4, 52—58.

Seravin L. N. 1962 b: Fiziologičeskie gradienty infuzorii *Spirostomum ambiguum*. Citologija 4, 545—554.

Seravin L. N.: Personal communication.

Tartar V. 1950: Methods for the study and cultivation of protozoa. In: Studies Honoring Trevor Kincaid. (Edited by M. E. Hatch), University of Washington Press, Seattle.

Tartar V. 1957: Reactions of *Stentor coeruleus* to certain substances added to the medium. Exp. Cell Research 13, 317—332.

Tartar V. 1961: The biology of *Stentor*. Pergamon Press, Oxford, London, New York, Paris.

Tchakhotine S. 1938: Études physiologiques et embriologiques au moyen de la methode des microgouttes. Trav. Stat. Zool. Vimereux 13, 647—663.

Thomas R. 1953: Action anesthésique du sulfate de nickel sur *Paramecium caudatum*. Bull. Microsc. Appl., 2e série 3, 73—76.

ACTA PROTOZOOLOGICA

VOL. I WARSZAWA, 30. IX. 1963 FASC. 29

Department of General Biology, M. Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa 22, Pasteura 3

Marek DOROSZEWSKI

The response of *Dileptus* and its fragments to the puncture

Reakcja *Dileptus* i jego fragmentów na ukłucie

Experiments dealing with the reactions of *Dileptus anser* O.F.M. to various kinds of mechanical stimulation were described in the previous papers (Doroszewski 1961, 1962, 1963). All these researches were carried out upon highly mobile ciliates; most of them was performed upon swimming individuals. Stimulation by the puncture was especially difficult to perform and the ciliary movement difficult to observe. Complementary experiments reported in the present paper were carried out when a more suitable material was available. On partly different material some previous experiments could be repeated in a modified version. The purpose of this study is to find the areas in which ciliates and their fragments are sensible to puncture.

The author wishes to express his gratitude to Docent Dr. S. Dryl for reading the manuscript and to Miss K. Golińska for the technical assistance and performing the figures.

Material and methods

Experiments were performed upon the species of *Dileptus* provisionally determined as *Dileptus gigas grojecensis* Wrześn., 1870, identified by the authors (cf. Jones and Beers 1953) with *Dileptus cygnus* Clap. et. Lachm., 1859. The final determination is postponed until the results of the revision of the genus now carried out by Dragesco will be published.

The natural station of this ciliate was found in the pond near Sadyba, Warszawa. In spite of *D. anser* this species is rather sedentary and the different structure of Ma gives also some experimental advantages. The ciliates were cultivated by the means of the methods already described (Doroszewski 1962). The preparation of material for the treatment was also similar to that used in the mentioned work.

The experiments were performed upon individuals placed in de Fonbrune's oil chambers. When it was necessary to obtain a larger quantity of ciliate fragments in the shortest possible time, the ciliates were bisected free-hand by means of a spear-point needle and placed later in the chambers for experiments. Sharply pointed glass needles of the standard shape and dimensions, made by means of de Fonbrune's microforge, were used for the stimulation. The location of punctures could be registered by photomicrographs. The moving

pictures were taken and analized (Pl. I 5). The depth of the drop of water was so adjusted as to secure the freedom of the movement for the ciliate under investigation.

Experimental results

The stimulation of the whole individual

Reactions to touching

The intact individual placed in the de Fonbrune's camera was tested as to the reactions to touching by means of the glass needle. While the ciliate was resting and the cilia in various parts of the body were touched, a distinct reaction was observed when the cytostomal area or the proboscis was stimulated. In this case the rapid backward withdrawal was executed by the ciliate, accompanied by a contraction of the proboscis. The distinct ciliary waves were appearing on the proboscis. If the larger surface of the ciliary coat was touched the reaction was easier to evoke.

The following experiment was performed to state whether the reaction depends on the direction of the stimulus applied or upon location of the stimulated place in the cell. The touching of the ciliary surface was executed from the end of the posterior sprout in the direction of the anterior pole of the

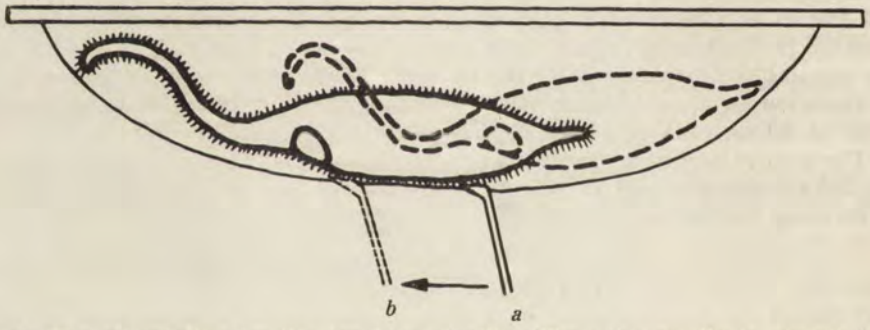


Fig. 1. The stimulation by the touching of cilia. When the needle is moved from position (a) till (b) the withdrawal reaction is evoked

body. In the moment when the needle reached the cytostomal area a rapid withdrawal of the animal was observed. The location of the reception area is shown upon the Fig. 1. If the posterior end of the cell was touched together with the cilia a rapid starting in the forward direction could be observed in some cases.

Reactions to puncture

The punctures were performed by means of an upward movement of the glass needle perpendicularly to the length of the body in the direction of the cover glass (Fig. 2, 3). The reactions of the ciliate to the punctures applied in various points were investigated. All the experiments begun while the ciliate was resting, executing only the typical searching movements of the proboscis. When the puncture was performed across the posterior sprout the rapid starting of the animal in the forward direction was evoked. The similar effect followed the puncture of the whole posterior part of the cell. It could occur that the forward movement was so rapid that the animal rushed forward with the

rupture of the pellicle by the needle near the puncture. The anterior border of the forward response area is shown upon the drawing (Fig. 4). In the middle part of the cell the responses are rather weak, and it is not clear whether the

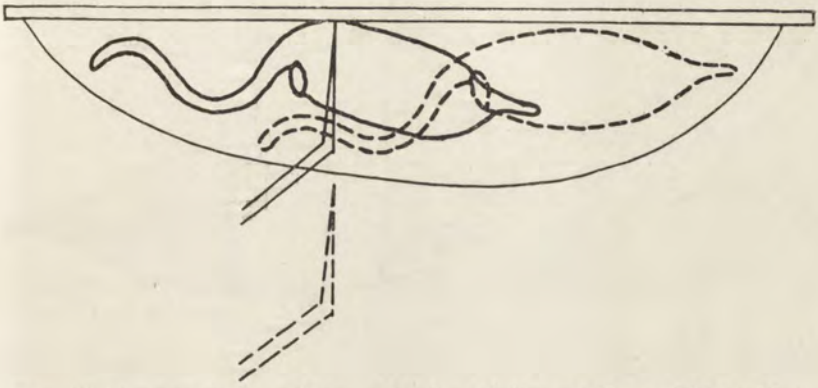


Fig. 2. The puncture and the releasing of the needle is followed by the withdrawal

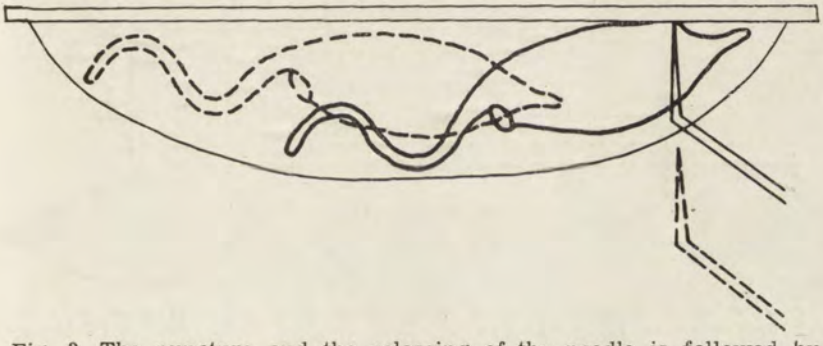


Fig. 3. The puncture and the releasing of the needle is followed by starting forward

unsusceptible band exists or not. The punctures executed in the anterior part of the body evoked the withdrawal, beginning the cycle of avoiding reaction if there was room enough. The rupture of the pellicle could also occur. The area near the cytostome was the most susceptible. The punctures through proboscis itself were not executed due to the technical difficulties. It was also possible to fix the animal on the spot by the puncture and, after some seconds, to obtain the backward movement when the needle was released (Fig. 2, 3). While the animal was held the cilia were moving as in the state of reversion.

The stimulation of the fragments

Reactions to bisection

In response to the transversal bisection of the individual post-operational ciliary reversion upon the posterior fragment was observed as in the case of *Dileptus anser*. The results were similar to those previously reported (D o r o s z e w s k i 1962). The differences concerned the position of the border line between the posterior part of the cell reacting with reversion to the bisection.

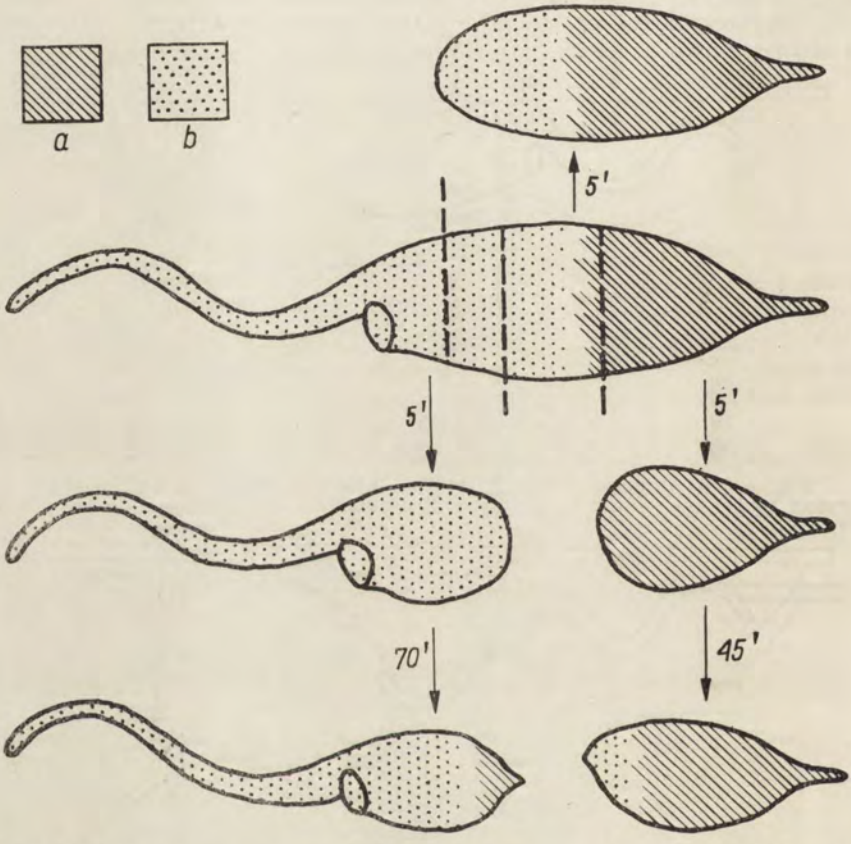


Fig. 4. The reception areas sensitive to puncture in the whole ciliate and its fragments after the bisection
a— forward response area, b— backward response area. The numbers indicate the time after the bisection.

This line is situated in the case of other species more anteriorly (Fig. 4), where the posterior fragment not capable of response is proportionally much larger.

Reactions to puncture

Immediately after the transection of the body by the means of the micromanipulation the reactions to puncture of the fragment were investigated. The special attention was paid to the posterior fragment. When the level of cutting was situated in the front part of the cell (Fig. 4) the stimulation of the anterior part of the fragment evoked the reversion and the withdrawal of the animal. In other cases, however, this effect was not obtained. On the contrary, the puncture executed in any one point of the fragment caused the forward motion.

The shape of fragment responding only with the starting forward is shown on the Fig. 4. In the cases here reported only the reactions of the posterior fragments containing the nuclear material and hence capable of regeneration

were investigated. The length of the fragments not reacting with withdrawal is below one half of the intact individual (proboscis not included). In the further course of experiments the puncture was applied to the series of fragments of the shape shown in Fig. 4. As a rule each fragment was punctured only once to avoid the additional effects of the previous punctures. After 35 minutes the first fragment when stimulated in its anterior area reacted with the withdrawal. The mean duration of time after the bisection, sufficient to the recovery of this differential response, was 45 minutes. At this time the regeneration of the anterior part of the fragment was already on its course (Fig. 4, Pl. I 4).

The stimulation of the most anterior tip of the fragment may, however, remain without response.

As to the anterior fragment, its general behaviour is more complex due to the presence of the proboscis and its locomotory role, and in consequence all the observations are less conclusive. After the bisection, however, while dealing with the fragment shown upon the drawing the stimulation of the posterior area evokes only withdrawal. The recovery of the forward starting response in anterior fragment to the stimulation of its posterior part lasted longer than the restitution of the backward response area in the posterior fragment (Fig. 4). In the middle fragment (containing the border line of the areas) the differential reactivity was observed. In the course of experiments 200 posterior fragments were investigated.

Discussion

The reaction to touching has a rather high threshold so that only some experiments of this kind were possible, revealing the specific receptive role of the cytostomal area and the proboscis. As to the reactions to puncture we have to do with the effect of starting forward in the response, to the stimulation of the posterior part of the body and the one of withdrawal following the stimulation of the frontal area. Both effects were already reported by Clark 1946 for *Spirostomum* and by Seravin 1962 for several ciliates including *Dileptus anser*.

As compared to the results obtained with the reactions of *Dileptus* to touching we have here to do with the greater stability of the reception areas location.

The observation of sensitivity of the fragment to puncture in the first 30—40 minutes after the operation resembles rather the results of Clark on *Spirostomum* then the previous results of the author (Doroszewski 1961) obtained with the tactile stimulation. In the case of the fragments described in the present report the reactivity to puncture is localized in respect to the position in the whole individual. The differentiation of response due to the place of stimulation occurs when the regeneration is already in its full course. The recovery of reactivity occurs earlier than in the case of *Spirostomum* but this difference corresponds well to the different speed of regeneration in both ciliates. The location of the susceptibility to puncture may be more stable than in the case of the tactile stimulation. The responses to cutting correspond to that found by the author previously in *Dileptus anser* and resembles the finding of Holmes 1907 in *Loxophyllum*.

The larger dimensions of the posterior fragment showing no response to puncture of anterior area correspond here to the larger dimensions of the fragments not reacting to the bisection. The similar traumatic nature of the both stimuli becomes here evident.

The difference between the conclusions of Jennigs and Jamieson 1902 and Holmes 1907 on the one hand and that of Clark 1946 on the other consists in the duration of the recovery of reactivity in the ciliate fragments. According to Jennings it is immediate while according to Clark it takes place when the morphological regulation is accomplished. As to the results of stimulation of the dividing ciliates the paper of Seravin 1962 can be consulted. In the experiments described above dealing with reactions of *Dileptus* to puncture it was found that it occurs while process of regeneration is still far from being finished. During the division of ciliate the normal behaviour of opisthe is observed immediately after the separation of daughter individuals, while its size and shape are still far from normal and the proboscis is compartively small.

In the case here described the deliverance from the dominance of anterior pole after the bisection is not by itself the sufficient condition of the recovery of reactivity yet the differential reactivity appears before the complete regeneration of the cellular structures. Under the natural conditions the reaction to touching can be treated as the negative thigmotaxis connected with the avoiding of the noxious stimulus. Such interpretation is however complicated by the lack of the direct connection between the direction of stimulation and reaction.

Summary

The reactions of the ciliate *Dileptus* to the local puncture were investigated by the use of de Fonbrune's micromanipulator and the movie camera. In the resting individual the stimulation of the anterior part of the ciliate evokes a backward withdrawal, while the stimulation of posterior part results in starting of the movement in the forward direction.

These responses depend on the location of the stimulated part on the cell, not upon the direction of the stimulus. Immediately after the bisection the posterior fragment when stimulated in any place by the puncture, can react only with the forward movement. When the regeneration is already in its course, the differentiated reactivity appears and the stimulation of anterior and posterior parts evokes the normal reactions.

The results are compared with the analogous findings of Clark and of Seravin.

STRESZCZENIE

Badano reakcje wymoczka *Dileptus* na ukłucie igłą szklaną przy użyciu mikromanipulatora de Fonbrune'a i zastosowaniu zdjęć kinematograficznych. U osobnika znajdującego się w stanie spoczynku podrażnienie przedniej części komórki powoduje cofnięcie się, a podrażnienie części tylnej ruch naprzód.

Reakcja nie zależy od kierunku działania bodźca, a od miejsca podrażnienia komórki. Po przecięciu na odpowiednim poziomie osobnika fragment tylny przy

podrażnieniu wykazuje jedynie reakcje ruchu naprzód, podobnie jak część tylna całego osobnika. Zróżnicowanie reakcji przy drażnieniu przodu lub tyłu fragmentu pojawia się wraz z postępującą regeneracją.

W dyskusji przeprowadzono porównanie z analogicznymi wynikami Clarka i Seravina.

REFERENCES

Clark A. M. 1946: The reactions of isolated parts of *Spirostomum*. J. Exp. Biol. 22, 88—94.

Doroszewski M. 1961: Reception areas and polarization of ciliary movement in ciliate *Dileptus*. Acta Biol. Exp. 21, 15—34.

Doroszewski M. 1962: The occurrence of the ciliary reversion in the *Dileptus* fragments. Acta Biol. Exp. 22, 3—9.

Doroszewski M. 1963: The response of the ciliate *Dileptus* and its fragments to the water shake. Acta Biol. Exp. 23, 3—10.

Holmes S. J. 1907: The behaviour of *Loxophyllum* and its relation to regeneration. J. Exp. Zool. 4, 399—418.

Jennings H. S. and Jamieson C. 1902: Studies on reactions to stimuli. The movements and actions of pieces of ciliate infusoria. Biol. Bull. Woods Hole 3, 229—234.

Jones E. E. and Beers C. D. 1953: Some observations on structure and behaviour of the ciliate *Dileptus monilatus*. J. Elisch. Mitch. Sci. Soc. 69, 42—48.

Seravin L. N. 1962: Mehanizm reversii bienia resnic u infuzorii *Spirostomum ambiguum*. Citologija 4, 652—660.

Wrześniowski A. 1870: Beobachtungen über Infusorien aus der Umgebung von Warschau. Zeit. Wiss. Zool. 20, 467—511.

EXPLANATION OF THE PLATE I

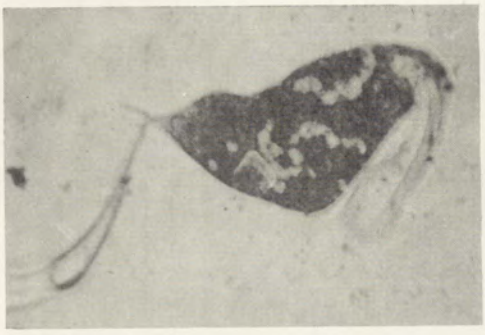
1: The whole ciliate. The posterior sprout is touched by the needle (magn. ca 100 ×).

2: Touching of the proboscis.

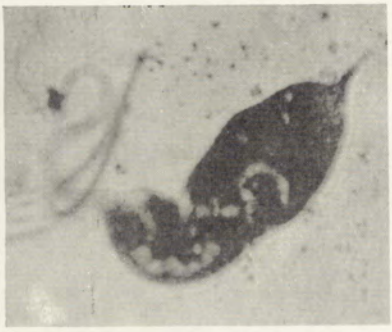
3: The puncture executed through the fragment (magn. ca 200 ×).

4: The shape of the posterior fragment 45 min. after the bisection (magn. ca 200 ×).

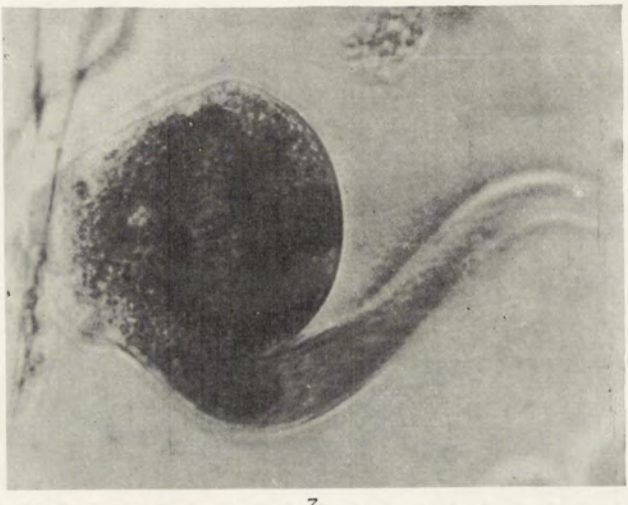
5: An example of the moving picture registration of experiments. The proboscis is cut off (the speed of the camera 16/sec. — each second shot is presented).



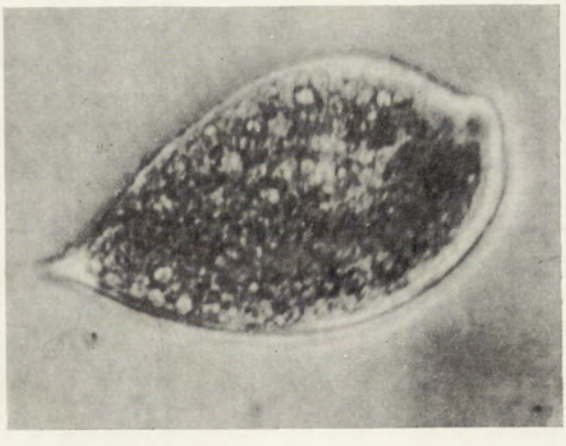
1



2



3



4

5

M. Doroszewski auctor phot.

ACTA PROTOZOOLOGICA

VOL. I WARSZAWA, 30. IX. 1963 FASC. 30

Institute of Cytology, Academy of Sciences of the USSR,
Leningrad F 121, Prospekt Maklina 32

A. E. KARAPETJAN

Determination of growth rate of *Lamblia intestinalis* cultivated in vitro

Определение темпа роста *Lamblia intestinalis* в культуре

Till recent time, determination of growth rate [1] of *Lamblia* was impossible because culture methods of this parasite were not developed. Now the determination of growth rate of microorganisms and of freely suspended protozoa in monobacterial cultures presents no special difficulty.

Botero 1961 for determination of growth rate of the culture of *Entamoeba histolytica*, sampled material for count from the same test tubes at definite intervals. The resulting figures were placed in curves.

In contrast to *Amoeba*, the *Lamblia* culture is successful only in presence of symbionts: yeast and yeast-like fungi (Karapetjan 1960, 1962). Division of *Lamblia* in culture occurs only when the protozoa adhere (with sucker disks) to the inner surface of flask. Under those conditions, the usual methods of determination of the growth rate proved to be inapplicable. It should be pointed out that the simultaneous growth of two different microorganisms cultures in the same flask involves a rather rapid exhaustion of the nutrient medium and its acidification with the metabolism products. The everyday replacing of the nutrient by the fresh one and removal of the yeast deposit proved to be indispensable for the satisfactory growth and division of *Lamblia*. All the facts mentioned above involve a rather slow growth of *Lamblia* in the culture and make their count in the same flask practically impossible the more so as shaking delays in some way the division of parasites. Considering all stated above it becomes obvious that some other better applicable methods should be found.

*

For experiments, cultures of *Lamblia intestinalis* together with *Candida guilliermondi* were used, passaged approx. 20 times within 7 months. The parasites spread continuously over the wall of the glass flask of 5 ml of capacity ($4 \cdot 10^5$—$5 \cdot 10^5$ of *Lamblia* individuals/ml of medium). For determination of growth rate two media of different composition were applied. The first — Li-2 medium consisted of: filtrated human serum 40%, chick embryo extract 5%, Henks' salts solution 55%.

[1] In this case "growth rate" means the time necessary for the *Lamblia* culture to double the number of individuals.

The second — Li-3 medium contained: filtrated human serum 25%, triptic brew of Hattinger 20%, chick embryo extract 5%, Henks' salt solution 50%.

In each series of experiments, the growth rate of *Lamblia* in culture was determined simultaneously in 16—20 flasks. Before inoculation, *Lamblia* individuals were sampled from the content of several flasks, strongly shaken prior to sampling. Medium was changed previously. After the count of *Lamblia* and of *Candida* in 1 ml of medium using the chamber of Gorjaev, each flask was inoculated with exactly 3 ml of diluted culture. So e.g. the count revealed that in the first series of experiments the number of inoculated *Lamblia* was 8000/ml and in the second series — 37500/ml. After inoculation *Lamblia* were incubated in flasks at 37°. Subsequently from each series of flasks containing the same medium, one flask was taken every day for the count of *Lamblia* and of yeast-like fungi. 24 h later, after a strong shaking (till the change of medium), the number of parasites in the first series of flasks was determined. The next day the count was executed in the second series of flasks, the third day in the third series and so on. In the remaining flasks, the everyday change of medium and removal of *Candida* from the flask bottom was done.

*

In the Table 1, results of the everyday count of *Lamblia* in 1 ml of culture medium (medium Li-2 and Li-3) from different flasks are presented. Fig. 1 and 2 show logarithmic curves of growth and division of *Lamblia* and yeast-like fungi in the two media: Li-2 and Li-3. As clearly seen in the diagram,

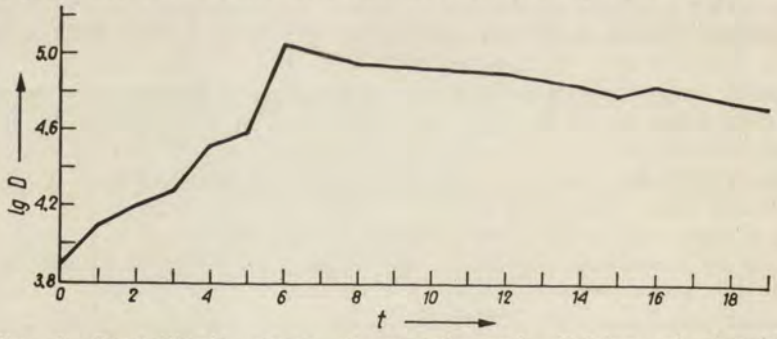


Fig. 1. Logarithmic curve of growth and division of *Lamblia intestinalis* in medium Li-2
t — time in days, *D* — number of *Lamblia*/ml.

the curves of growth and division of *Lamblia* are rather similar to those of other protozoa and microorganisms. Comparison of the presented diagrams shows that the rate of division of *Lamblia* is changing with the composition of medium used. So in the medium Li-3 which is more rich in nutritive substances, the culture of *Lamblia* is more effective and its growth is quicker than in the medium Li-2. [2]

[2] Those differences might also depend on the fact that the medium Li-3 was initially inoculated by a higher number of individuals. This factor may be the reason why the cultures in Li-3 reach sooner the maximum of growth, whereas the more abundant store of food in the medium Li-3 is probably responsible for the maximum of growth being reached at a higher level of density.

Since the everyday change of the nutrient medium is indispensable, the delay of growth and division of *Lamblia* occurs not only as result of accumulation of metabolism products and of exhaustion of medium — but mainly in consequence of gradual increase of number of *Candida* in the flask. The experiments prove that the number of yeast-like fungi adhering to the walls of the flask and not removed after the everyday change of medium, increases

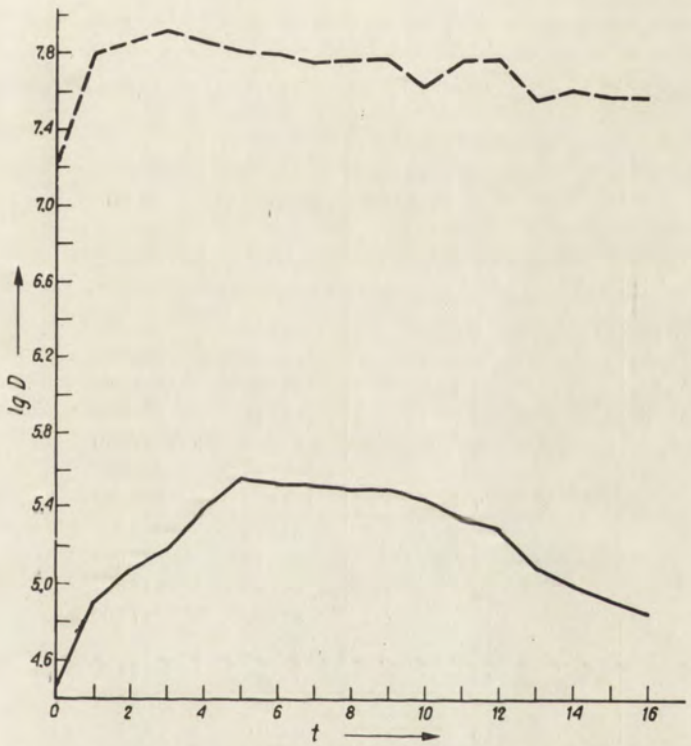


Fig. 2. Logarithmic curve of growth and division of *L. intestinalis* and *Candida guillermondi* in medium Li-3

Full line — curve of division of *Lamblia*, dotted line — curve of division of yearst-like fungi, other explanations — as in Fig. 1

every day. In this way the curve of division of the yeast-like fungi (Fig. 2) shows only the approximate number of *Candida* individuals in the medium with no regard to the individuals adhering to the wall of the flask.

For determination of the growth rate of *Lamblia* in the logarithmic phase, the following formula (F r e y t a g und F r e y t a g 1956) was applied:

$$X = \frac{\lg b - \lg a}{t \cdot \lg 2}$$

(a — number of *Lamblia* ind./ml in the beginning of experiment,
b — number of *Lamblia* ind. at the end of experiment,

t — duration of experiment,
X — number of divisions in 1 h.)

Counts executed with the aid of this formula show that in the mixed cultures of *Lamblia* and *Candida* in the medium Li-2, when the chick embryo extract kept for 1 month in — 20° is used, the growth rate of *Lamblia* in the logarithmic phase is approx. 38 h, and in the medium Li-3 — 36 h.

Table 1

Growth of population of *Lamblia intestinalis* in cultures (ind./ml.)

Nutrient medium: / Days	Li-2	Li-3
Initial number	8 000	37 500
1	12 500	80 000
2	15 000	115 000
3	20 000	150 000
4	30 000	270 000
5	45 000	387 000
6	107 000	360 000
7	100 000	365 000
8	95 000	340 000
9	90 000	320 000
10	85 000	277 000
11	82 500	237 000
12	80 000	217 000
13	75 090	147 500
14	65 000	135 000
15	62 000	87 500
16	65 000	82 500
17	62 500	—
18	56 000	—
19	56 000	—

A prolonged observation of the *Lamblia* culture shows that the growth rate of *Lamblia* may sometimes deviate from the above results, depending also on the freshness of the chick embryo extract, age of the culture, composition of the nutrient, time of inoculation, number of *Candida* individuals in the flask and, possibly on some other non-precised factors. Besides the direct count of parasites each day of incubation, some other tests determining the conditions of the culture more exactly seem to be expedient for the evaluation of the condition of the culture and also for the comparison of growth in several *Lamblia* cultures. So e.g. for comparing the rate of division in *Lamblia*

of different cultures, the potency of growth was determined and expressed as the ratio of the final number of *Lamblia* to the number of inoculated parasites, in the same period of time.

In the above experiments when the medium Li-2 was applied, the potency of growth was 1.6 (24 h after inoculation) and in the medium more rich in the nutritive substances (Li-3) it reached the value 2.1 in the same period of time.

A study of the mitotic activeness was advisable for determination of the physiological viability and intensity of division in *Lamblia*. The mitotic activeness could be easily determined by the count of dividing parasites out of 1000 individuals in the culture. So in the second series of experiments (using the Li-3 medium) in the logarithmic phase this ratio was expressed by the numbers 180—200/1000, in the stationary phase — 100—120/1000, and in the phase of decline — 60—80/1000.

No doubt, this count may reveal only the individuals in their final stage of division while the initial stages practically cannot be detected in the low magnification. Nevertheless in some cases this test seems to be useful for evaluation of the viability of the *Lamblia* culture. In single cases it seems necessary to count the dead individuals. In present experiments (Fig. 2) the intense death of *Lamblia* is observed in the final period of the stationary phase (200—400/1000). In contrast to this result, in the beginning of the stationary phase the number of dead individuals is insignificant (10—50/1000).

Summary

The growth rate of *Lamblia* culture was determined by means of a special method using two media of different composition. For inoculation a 7-month old culture of *Lamblia intestinalis* mixed with *Candida guilliermondi* was applied. In the mixed culture of *L. intestinalis* and *C. guilliermondi* the growth rate is 36—38 hrs. In the medium Li-3 which was more rich in nutritive substances the growth is more intense than in the medium Li-2. The growth rate depends also on the presence of chick embryo extract and on the age of the culture.

РЕЗЮМЕ

При помощи специально разработанной методики был определен темп роста лямблий в культуре при использовании двух различных составов питательных сред. Для опытов использовалась 7-ми месячная культура *Lamblia intestinalis* совместно с *Candida guilliermondi*. В совместной культуре *L. intestinalis* и *C. guilliermondi* темп роста лямблий равняется 36—38 часам. На более богатой питательными веществами среде Li-3 рост интенсивнее, чем на среде Li-2. Темп роста зависит также и от присутствия в культуре куриного эмбрионального экстракта и от возраста культуры.

REFERENCES

Botero R. D. 1961: Rate of growth of *Ent. histolytica* in different culture media. Trans. Roy. Soc. Trop. Med. Hyg. 55, 539—546.

Freytag K. und Freytag K. 1956: Experimentelle Bestimmung des durchschnittlichen zeitlichen Abstandes zwischen den Teilungen in einen Bakterienkultur. Mathem. Naturwiss. Unterr. 9, 222—223.

Karapetjan A. E. 1960: Metodika kultivirovanija ljamblij. Citologija 2, 379—384.

Karapetjan A. E. 1962: In vitro cultivation of *Giardia duodenalis*. J. Parasitol. 48, 337—340.

Fasciculi praeparati:

E. M. Cheissin and G. I. Poljansky: On the taxonomic system of *Protozoa* [О системе простейших] — J. Dobrzańska-Kaczanowska: Comparaison de la morphogenèse des Ciliés: *Chilodonella unicinata* (Ehrbg., 1838), *Allosphuerium paraconvexa* sp. n. et *Heliochona scheuteni* (Stein, 1854) [Porównanie morfogenezy u orzęsków: *Chilodonella uncinata* (Ehrbg., 1838), *Allosphaerium paraconvexa* sp. n. oraz *Heliochona scheuteni* (Stein, 1854)] — A. Kaczanowski: Morphology and morphogenesis of the sucker in *Hysterocineta paludinarum* Diesing (*Ciliata, Thigmotricha*) as observed in living material [Badania przyżyciowe nad morfologią i morfogenezą przyssawki u *Hysterocineta paludinarum* Diesing (*Ciliata, Thigmotricha*)] — V. F. Mashansky, L. N. Seravin and L. N. Vinnichenko: Ultrastructure of the ciliate *Loxodes rostrum* (O. F. M.) in relation to the mode of its water transport [Ультратонкое строение инфузорий *Loxodes rostrum* (O. F. M.) в связи с особенностями водообмена] — B. Skoczylas, H. Panusz and M. Gross: Isolation of macronuclei from *Paramecium caudatum*. The role of calcium ions [Izolowanie makronukleusów *Paramecium caudatum*. Rola jonów wapniowych] — J. Rostkowska: Effect of chemical agents on the survival of *Balantidium coli* (Malmsten) [Wpływ czynników chemicznych na przeżywalność *Balantidium coli* (Malmsten)] — J. Moraczewski: *Testacea* du seston des rivières Wkra et Narew [*Testacea* sestonu Wkry i Narwi]

Państwowe Wydawnictwo Naukowe
(PWN — Polish Scientific Publishers)
Warszawa

Cena zł 26.—

Fasciculi: