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Study on the family *Blepharocorythidae* Hsiung. II. *Charonina ventriculi* (Jameson)

Badania nad rodziną *Blepharocorythidae* Hsiung. II. *Charonina ventriculi* (Jameson)

The smallest protozoon of the family *Blepharocorythidae* was described from cattle rumen by Jameson 1925 under the name *Charon ventriculi*. Almost simultaneously this protozoon was described by Dogiel 1926 as *Blepharocorys bovis*. Strand 1928 changed the name *Charon* — as a nomen praeoccupatum — for *Charonina*.

Two other species were subsequently included into this genus: *Charonina equi* (Hsiung, 1930) from the colon of horse and *Charonina nuda* Hsiung, 1932 from the cattle rumen.

The description of Jameson 1925 differed slightly from that of Dogiel 1926. Jameson stated the presence of two bundles of cilia at the posterior pole of the protozoon; Dogiel described one semicircular zone at the posterior pole. Dogiel 1934 remained the species of Jameson in the genus *Blepharocorys* Bundle, 1895 as *Blepharocorys ventriculi* (Jameson) finding in it all the characters of the genus *Blepharocorys*. Jirovec 1933 stated in this species the presence of two bundles of cilia which are especially distinctly visible in living protozoa but stuck together and are indistinct in stained preparations. He used generic name *Blepharocorys* for this species as first.

Strelkov 1939 fully supported the view of Dogiel, seeing no reason of formation a separate genus for the ciliate in question. This author expressed the view that the two ciliary bundles at the posterior pole of *B. ventriculi* arise presumably as result of splitting of the main zone which occurs — according to the author — in the other species of *Blepharocorys* as well. This occurs — according to Strelkov — in *B. curvigula* f. *cirrata* in which a supplementary bundle of cilia — cirrus — arises.

According to my observations based on the silver impregnated material of ciliates from the cattle rumen and from the horse intestine, the problem appears different. In the species of the genus *Blepharocorys* which are known to me (except "*Blepharocorys*" *ventriculi*) only one not divided ciliary zone exists at the posterior pole. In *B. curvigula* f. *cirrata*, the cirrus is not a differentiated structure in the infraciliature. It is only a part of the cilia of the posterior zone, which cling together to form a bundle. This phenomenon resembles those occurring in the ciliary zones of the representatives of the order *Entodiniomorpha*, where syncilia (Noirot-Timothée 1960) have no

reflect in infraciliature. In "*Blepharocorys*" *ventriculi* instead two separate caudal zones exist.

On account of the above statements, and of another property of the ciliature which is of a taxonomic importance, I consider as necesary to describe the somatic and buccal infraciliature of "*Blepharocorys*" *ventriculi*, omitting the general description of the ciliate structure which was reported by the above authors.

Material and methods

The content of cow rumen brought in a thermos flask from the slaughter-house of Łódź, was preserver in 10% formalin. After elimination of thick vegetal particles protozoa were impregnated following the method applied by me previously (Wolska 1966 a).

Results

Dimensions of "*Blepharocorys*" *ventriculi* in the silver preparations: length — 27—36 μ, width — 11—13 μ.

The somatic ciliature of "*Blepharocorys*" *ventriculi* consists of two zones in the anterior and two in the posteror part of the body. The anterior zones have in general the same position as in the other species of the genus *Blepharocorys*.

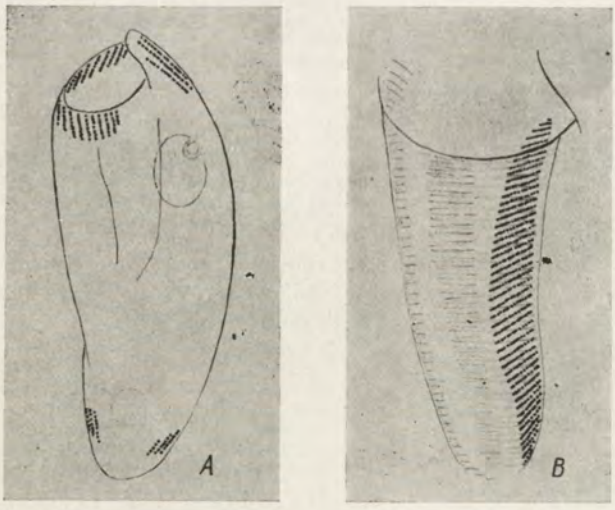

Fig. 1. *Charonina ventriculi* (Jameson). A. Somatic infraciliature (view of the left side). B. Buccali infraciliature (view of the ventral side)

One of the zones is located on the dorsal side of the small frontal process (dôme frontal of Dogiel) which enclose the buccal overture (not the cytostome) on the dorsal and partially on the left side. This zone is composed of a few kineties only. Another narrow zone (on the ventral lip) composed of short oblique kineties, runs around the right ventral and partially the left margin

of the overture which is pushed to the left side (Fig. 1 A). At a short distance of the posterior pole lie two small zones, one on the ventral, another on the dorsal side (Fig. 1 A, Pl. I 1—3). The disposition of the posterior zones reminds that of the caudal bundles in *Cycloposthium* sp.

The buccal concavity which leads to cytostome is funnel-shaped and reaches approximately as far as half of the body length. The right dorsal wall of the funnel is coated by a zone of short oblique kineties. This zone has the shape of a band narrowing towards the ends (Fig. 1 B, Pl. I 4). Any connection of this zone with the somatic ones has not been stated. From that margin of the zone which is nearer the left wall of the funnel, run semicircular fibers describing nearly the whole left wall of the funnel. Over the right ventral wall run short parallel fibers producing a ladder-like system, which is broader in its anterior part and gradually becomes more narrow. Besides the above fiber systems there seems to exist a third one composed of short parallel fibers which produce a dark streak on the left wall of the funnel after the silver impregnation method. Those fibers lie presumably in another plane than the semicircular ones. When observed in whole preparations, they coincide with the free extremes of the semicircular fibers. Those structures are faintly discernable bacause of the small dimentions of the protozoon. However the images found in well impregnated preparations seem to speak in favour of three groups of independent fibers running in the same direction on the wall of the funnel (Pl. I 5).

On the right side of the protozoon, nearer the ventral margin, under the zone of kineties of the ventral lip, there is a group of big kinetosomes, loosely dispersed or sometimes grouped into short rows (Pl. I 6). Possibly it is a group homologous to the group of kinetosemes on the vacuole described by me in the genus *Blepharocorys* (W o l s k a 1966 b).

Some of the division stages were observed (Pl. II 7—11). Individuals in division were found rarely and failed to provide a sufficient material for a full description of morphogenesis. However some significant facts of a general importance (for the *Blepharocorythidae*), and some others — for the characteristic of the species under study deserve being reported.

The primordia of the ciliature of the opisthe are formed in the subcuticular vacuoles as sygnalized already for "*Blepharocorys*" *ventriculi* by D o g i e l 1926. The buccal ciliature arises independently of the somatic one, as decribed in general outline by me for the genus *Blepharocorys* (W o l s k a 1966 b). The primordia of every one of the ciliary zones arise separately. The two caudal zones of "*Blepharocorys*" *ventriculi* are formed independently of each other, consequently splitting of the single zone into two fails to occur. All the ciliary primordia of the opisthe arise without connection with the ciliature of the prother. The buccal apparatus of the opisthe together with its fibers is fully organized prior the separation of opisthe and prother (Pl. II 10, 11).

The course of morphogenesis of "*Blepharocorys*" *ventriculi,* although not followed in all its details, coincides generally with the description of morphogenesis in the genus *Blepharocorys* (W o l s k a 1966 b).

A similar type of morphogenesis occurs in the ciliates living in the same medium which are on a higher degree of evolution — in *Entodiniomorpha.* Such a distinct similitude of morphogenesis in *Blepharocorythidae* and *Entodiniomorpha* seems to prove a close relation of these two groups.

Discussion

"*Blepharocorys*" *ventriculi* has two distincly separated caudal zones. In division of the ciliate those zones are formed independently of each other. Consequently it is not a secondary partition of an essential single zone but a primary phenomenon. This feature distinguishes "*Blepharocorys*" *ventriculi* from the other species of the genus *Blepharocorys*.

In the buccal funnel of "*Blepharocorys*" *ventriculi* only one ciliary zone exists. It is composed of short oblique kineties and resembles the somatic zones. In the species of the genus *Blepharocorys*, besides the zone of this character, exists another zone of long kineties (W o l s k a 1966 b). The lack of differentiation of the buccal ciliature is another character distinguishing this species from those of the genus *Blepharocorys* Bundle.

Considering the above facts it should be assumed that J a m e s o n was right when establishing a separate genus for this species. According to the rules of the zoological nomenclature this genus should be named *Charonina* Strand, 1928 [synonim: *Charon* Jameson, 1926, non *Charon* Karsch, 1879 *(Arachn.)*], and its type species is *Charonina ventriculi* (Jameson, 1926) Strand, 1928 [syn.: *Blepharocorys ventriculi* (Jameson, 1925) Jirovec, 1933, *B. ventriculi* (Jameson, 1925) Dogiel, 1934 and *B. bovis* Dogiel, 1926] — the species discussed in the present study.

The problem of the other two species which had been included by H s i u n g into the genus *Charonina*, remains insolved till the moment of precise examination of ciliature, the more so as S t r e l k o v 1939 created for one of them — *Charonina equi* (Hsiung, 1930) a new genus *Charonnautes*. The position of *Charonina nuda* is still more uncertained because of the lack of ciliature on the posterior end of its body.

On account of the existence of two caudal zones as well as of lack of differentiation of the buccal ciliature, the genus *Charonina* should be recognized as more primitive than the genus *Blepharocorys*.

Summary

The infraciliature of the species *Charonina ventriculi* (Jameson) is described and its morphogenesis is generaly characterized. The view is put forward that this species cannot be included into the genus *Blepharocorys* Bundle.

STRESZCZENIE

Autorka opisuje infraciliaturę gatunku *Charonina ventriculi* (Jameson) i charakteryzuje ogólnie jego morfogenezę. Autorka stwierdza, że gatunek ten nie może być włączany do rodzaju *Blepharocorys* Bundle.

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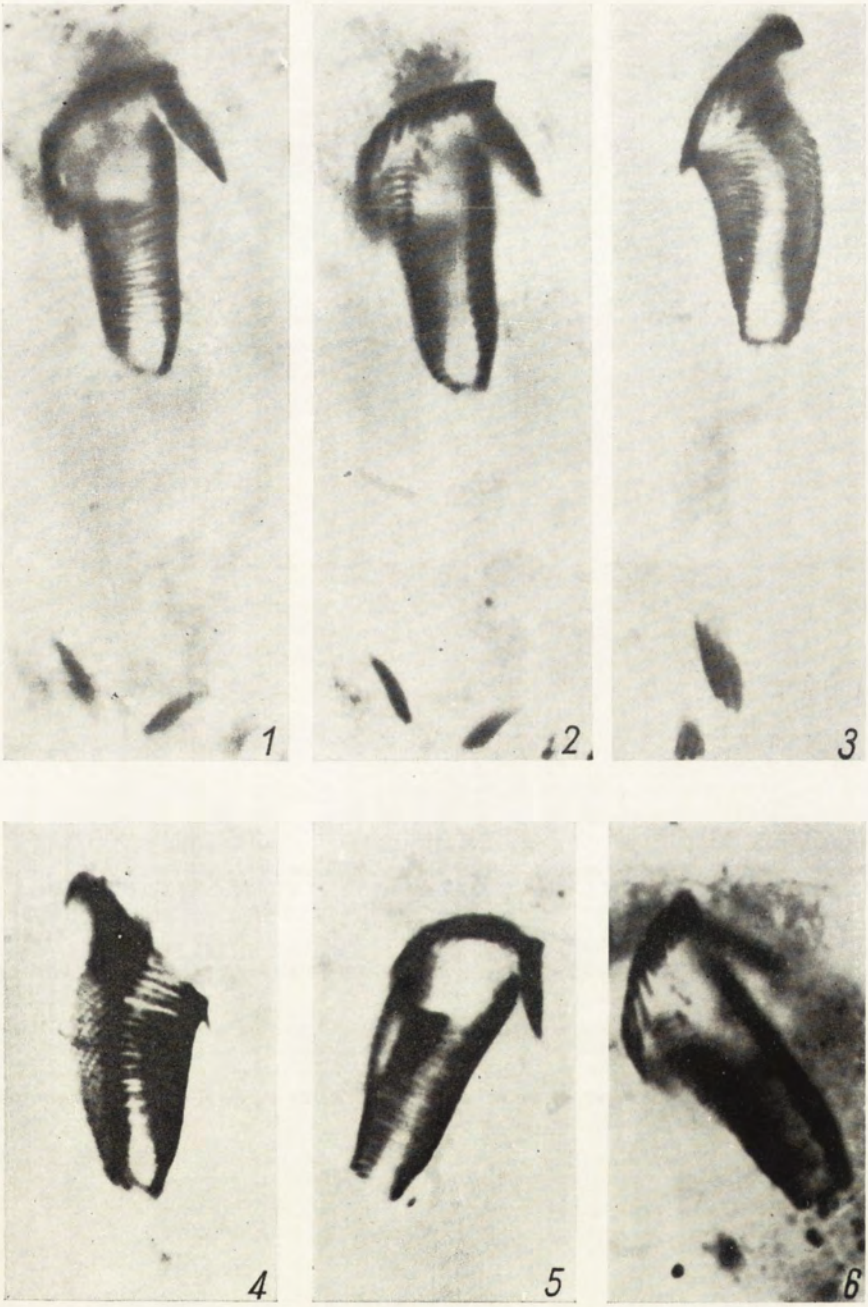
EXPLANATION OF PLATES I—II

Charonina ventriculi (Jameson), silver impregnation, X 2600

1: General view from the left side
2: Optic section, of the right side of the anterior body part
3: Caudal zones
4: Zone of the buccal cilia and fibers, (view of the left dorsal side)
5: Buccal funnel of the left side
6: Kinetosomes beneath the zone of the ventral lip, right ventral side
7: Individual in division, division vacuoles are seen
8: Rudiment of the ciliary zone of the ventral lip; rudiments of the buccal ciliature and of the dorsal zone are seen as dark streaks
9: Rudiment of the ciliary zone of the ventral lip is on the right side of the ciliate body. The dark longitudinal streak marks the rudiment of the buccal ciliary zone on the left side
10: Advanced stage of division. Zone of buccal cilia (left dorsal side)
11: Advanced stage of division. Zone of the ventral lip cilia and buccal fibers (right dorsal side)



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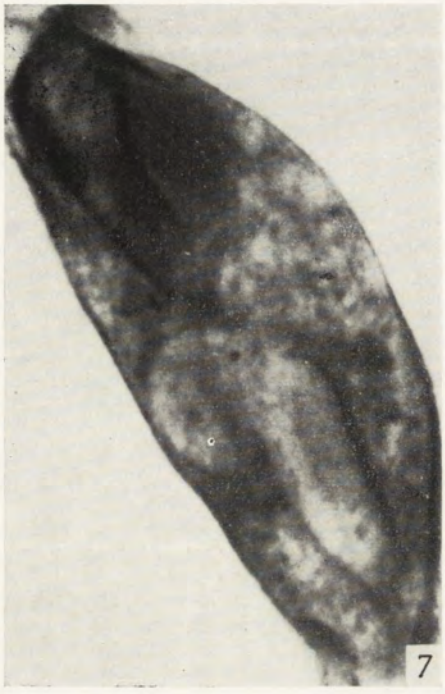

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Study on the family *Blepharocorythidae* Hsiung. III. *Raabena bella* gen. n., sp. n. from the intestine of the Indian elephant

Badania nad rodziną *Blepharocorythidae* Hsiung. III. *Raabena bella* gen. n., sp. n. z jelita słonia indyjskiego

Scarce information has been reported concerning the ciliates — parasites of the elephant intestine. A few species described belong to the families *Cycloposthiidae* and *Polydiniidae* (*Entodiniomorpha*). Buisson 1923 described *Prototapirella elephantis* from the intestine of the African elephant. Kofoid 1935 described *Polydinium mysoreum* and *Elephantophilus zeta* from the intestine of the Indian elephant. More recently, Latteur 1958 gave the description of *Thoracodinium vorax* from the intestine of the Indian elephant from the zoological garden in Anvers.

When examining the faeces of the Indian elephant, I had opportunity of finding the presence of a new ciliate. On account of its general morphological characters as well as of its morphogenesis, this form should be included into the family *Blepharocorythidae* Hsiung. Some of its characters however evoke the necessity to establish a new separate genus for it and the generic name *Raabena* is proposed [1].

The study of this new representative of *Blepharocorythidae,* the family which has been the object of my interest (Wolska 1966 a, 1966 b), may introduce some further data to our knowledge of the phylogenetic relations in this family.

In the material which becomes quicly decomposed, the ciliate remains sufficiently well preserved — the ciliature is unimpaired — and is suitable for description.

Material and methods

Material was taken from a female Indian elephant, *Elephas maximus* (8 years old) from the Zoological garden of Łódź [2]. The samples were fixed in 10% formalion. After filtration and rinsing in water, the ciliates were examined directly under these conditions. The study however is based mostly on silver impregnated material treated with the method described previously (Wolska 1966 a), or on preparations in wich the silver was replaced by gold.

[1] The generic name *Raabena* has been chosen in honor of Prof. dr. Zdzisław Raabe the Director of the Zoological Institute of the University of Warszawa, Member of the Polish Academy of Sciences.

[2] I wish to express my thanks to Direction of Zoological garden in Łódź for enabling me investigation.

Results

Raabena bella gen. n., sp. n.

The ciliate is flattened laterally. The body outline is more or less ovoid, the posterior end of the body being more narrow than the anterior one (Fig. 1 A, Pl. I 1—2). In the anterior part of the ventral side, protrudes a vacuole of an unknown character, similarly as in the genus *Blepharocorys* (W o l s k a 1966 a). Dimensions of the body: length 44—67 μ, width (or rather thickness) 27—36 μ. Buccal overture is broadly open on the left body side and leads to a funnel-shaped concavity which is slightly bent (Pl. I 1, 2) and reaches approximately as far as the half of the body length. The deep groove beneath the left ventral margin produces a sort of pocket in the anterior part of the buccal funnel. Above the buccal overture, on the left — dorsal side protrudes a small process (the frontal process).

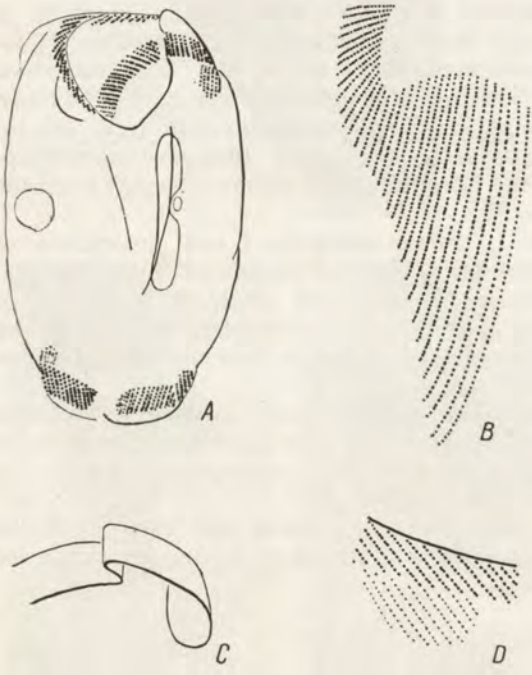

Fig. 1. *Raabena bella* gen. n., sp. n., A. General feature and pattern of somatic ciliature (scheme). B. Ciliature of vestibulum. C. Course of the ciliary zone on the frontal process and its transition to vestibulum. D. Sector of the ciliary zone of the ventral lip (right ventral side), delicate kineties on the territory of vacuole

The ciliature is composed of four zones of long cilia located on the anterior and posterior parts of the body (Fig. 1 A, Pl. I 1). Two zones at the posterior pole lie dorsally and ventrally as related to cytopyge. The ventral zone lies mainly on the left body side and only its small segment passes over to the right side. On the contrary, only a small segment of the dorsal zone encroaches upon the left side whereas its long segment lies on the right side.

One of the anterior zones runs along the right and ventral margin and partially along the left one of the buccal aperture. This corresponds to the zone of the ventral lip in the genus *Blepharocorys*. On the right-ventral side kineties

of this zone are prolonged on the territory of the vacuole. Here they are fained and sometimes they lose the connection with the zone of their origin (Fig. 1 D). The second anterior zone, similarly as in the genus *Blepharocorys,* begins at the base of the frontal process on the right side, passes over to the left side of the process, bends on its ventral margin and encroaches upon the right wall of the anterior part of buccal concavity (vestibulum?). The disposition of this zone on the frontal process as well as its transition to the buccal concavity are illustrated in Fig. 1 C. In its subsequent course this zone describes an arch on the right anterior wall of the concavity (Fig. 1 A), it broadens and is prolonged as far as the apex of the buccal funnel. In consequence, the right wall of the funnel becomes coated with longitudinal kineties (Pl. IV 11 b, 13) which densely cover a triangular field (Fig. 1 B). The buccal ciliary field is — in this way — a continuation of the somatic ciliary zone.

The anterior part of the buccal concavity, between the arched part of the ciliary zone and the margin of the overture, is lined with a delicate network of fibers (Pl. II 7). Pairs of short fibers run from the arched part of the zone and bend backwards, their free ends overlap tegularly. In this way they produce a uniform framing of the arched zone (Pl. II 5, 6). Near the margin of the buccal overture begin other, thicker fibers. These fibers split just near the right margin and run to meet the former fibers almost touching them. On the left ventral margin, where the groove called the pocket begins, the fibers penetrate into it and remain undivided along a rather long distance (Pl. III 9, IV 11 a). In the deep part of the pocket the fibers split and bend (Pl. III 8, 9), pass over to the right wall and then — as thin ones — reach the framing of the arched zone (partly seen also in the Pl. II 5).

The deep narrowed part of the funnel is strengthened by thick semicircular fibers which run along its dorsal and left side (Pl. I 2, IV 11 a, 13). In many cases it looks as if those fibers initiated of the last longest buccal kinety (the nearest the dorsal side). So it looks in the Pl. II 6. However possibly those fibers initiate earlier on the right side. In Pl. II 4 fibers on the right wall, are seen against the background of buccal kineties. One fiber which runs above the anterior ends of the buccal kineties is especially distinctly seen (the ends of kineties are the anterior ones if we neglect the fact that the direction of kineties is here reversed). The semicircular fibers terminate on the left wall at the strongly impregnated longitudinal streak (Pl. III 10, IV 11 a, 13). What is the impregnated structure is difficult to decide. Possibly it is a bundle of fibers which is formed of overlaping extermities of the semicircular fibers (Pl. III 10). Impregnated preparations fail to provide distinct images in this case. The streak seems to penetrate over the wall of the pocket but here the mutual relation of the fibers becomes complicated, the more so as a new fan-shaped fiber group appears (Pl. II 4 b). Where these fibers initiate and what is their relation to the longitudinal streak and to the semicircular fibers— this cannot be elucidated presently.

The elongated macronucleus lies usually at the middle of the body length, nearer the dorsal margin. Nevertheless it is sometimes shifted forwards or backwards. Its axis coincides with the long body axis of the ciliate. The round micronucleus lies in the concavity of the macronucleus at the half of its length (Fig. 1 A).

One contractile (?) vacuole is present on the ventral side more or less at the half of the body length. In cytoplasm numerous spherical or ovoid shining bodies are dispersed. They resemble those which are present in the "Konkre-

mentenvacuole" in *Paraisotrichidae* and in *Buetschliidae*.

The few dividing individuals observed indicate that the division occurs in the same way as in the genus *Blepharocorys* (W o l s k a 1966 a, 1966 b). The new ciliature arises in the vacuoles. Each of the four zones arise separately. Pl. I 3 and IV 12 show the formed fronto-buccal zone in its nearly definitive form, and the semicircular fibers.

Raabena gen. n., diagnosis

The ciliate flattened laterally. The extensive buccal overture on the left body side. Four ciliary zones. One zone on the so called ventral lip, another one is on the frontal process and penetrates into the buccal depression. Two other zones are on the posterior pole. The contractile(?) vacuole is on the ventral body side at the half of its lenght. Vacuole of unknown character lies in anterior part of the body.

Type — species: *Raabena bella* sp. n. Parasite of the Indian elephant intestine (*Elephas maximus* L.).

Discussion

The genus *Raabena* gen. n. should be included into the family *Blepharocorythidae* Hsiung because its body is elongated, flattened laterally, the ciliature is reduced to several zones in the anterior and posterior parts of the body; it has a frontal process, ciliated vestibulum, single contractile vacuole.

The type of morphogenesis is in *Raabena* the same as that which occurs in the genus *Blepharocorys*.

The family *Blepharocorythidae* was established by H s i u n g 1929 for the genera *Blepharocorys* Bundle and *Charon* Jameson (presently *Charonina* Strand). D o g i e l 1934 considered this genus as identical with *Blapharocorys* Bundle, W o l s k a 1966 b reestablished genus *Charonina* Strand. C h a v a r i a 1933 added here the new genus *Ochoterenaia* with one species *O. appendiculata*. S t r e l k o v 1939 after all included this species to the genus *Blepharocorys* and created a new genus *Charonnautes* for *Ch. equi* (Hsiung, 1930).

The genera *Ochoterenaia* and *Charonnautes* present only an insignificant component of the family and have not been studied with the method of silver impregnation. In describing the family *Blepharocorythidae* the attention should be in first place concentrated on the genus *Blepharocorys* which constitutes the bulk of the family and on the genus *Charonina*. The genus *Blepharocorys* with its numerous species which live in the intestine of *Equidae* (mostly in horse) and also in some rodents, has not been studied in a sufficient degree either. The extensive research with application of the electron microscope, silver impregnation and other methods have been initiated by G r a i n 1966. The study of a more restricted scope, concerning mostly the infraciliature, have been begun by me (W o l s k a 1966 a, 1966 b). Our data achieved as yet seem to be sufficient to compare (mostly concidering the infraciliature) *Raabena bella* gen. n., sp. n. with the species of the genus *Blepharocorys* Bundle and *Charonina* Strand.

Raabena bella gen. n., sp. n. has a more complet ciliature than the species of the genus *Blepharocorys*. On its posterior end exist two rather large ciliary zones. It should be recognized for that reason that *R. bella* presents a more primitive character of ciliature than do the species of the genus *Blepharocorys*, and is in this respect nearer *Charonina ventriculi* in which two ciliary zones exist as well (W o l s k a 1966 b), although they are smaller and more reduced.

In the representatives of the genus *Blepharocorys* and *Charonina* the ciliature of vestibulum has no connection with the somatic ciliature. This was stated by Grain 1966 and Wolska 1966 a. In division the ciliature of vestibulum arises independently of the somatic one (Wolska 1966 a, 1966 b). In *R. bella* the ciliature of vestibulum is the continuation of the somatic one and it arises in morphogenesis as continuation of the somatic zone. The buccal ciliature of *R. bella* has then character of vestibular ciliature which is characteristic of *Trichostomata*. It is more primitive than in the genus *Blepharocorys* and *Charonina* in which the buccal ciliature becomes independent of the somatic one and arises independently of it in ontogenesis.

When comparing the buccal ciliature of the representatives of the genus *Blepharocorys, Charonina ventriculi* and *R. bella,* it becomes evident that the most advanced is the evolution of ciliature in the species of the genus *Blepharocorys* where it is not only independent of the somatic one but is differentiated into two separate groups. It is more primitive in *Charonina ventriculi* being independent of the somatic one however not differentiated. The most primitive is the buccal ciliature in *R. bella* being abundant, undifferentiated and presenting the continuation of the somatic one.

Is really this type of vestibulum characteristic of *Trichostomata*? Are *Blapharocorythidae* to be considered as *Trichostomata*? Grain 1966 also put this question, leaving it with no answer till morphogenesis is studied.

The type of morphogenesis which occurs in *Blepharocorythidae* is not characteristic of *Trichostomata* (Wolska 1966 a, 1966 b) but of the higher groups of ciliates. The development of this evolutionary branch — *Blepharocorythidae* — had followed a quite specific trend. Had they branched off from *Trichostomata*? Or is it perhaps a specific evolution of parasitic *Gymnostomata*? Little alteration is needed to make the ciliature of the posterior end of *Didesmis* — which is composed of two adhering zones — quite similar to that of *Raabena*. The flattening of the body exists already in *Didesmis*. Grain 1966 stressed this character as exceptional in *Buetschliidae*. The only contractile vacuole in *Didesmis* has a similar position as in *Raabena*. A similar position has the Konkrementenvacuole in *Didesmis* as the vacuole with kinetosomes in *Raabena*. That vacuole is presumably (Wolska 1966 a) an atrophied Konkrementenvacuole. The anterior ciliary zone in *Didesmis* is really composed of two ones, which are formed separately in ontogenesis. Each of those zones might develop in a different manner in *Blepharocorythidae*. One of them remained on the surface, the second one wrapped round the frontal process (new structure), dived partly inside producing at first the vestibular ciliature of *Raabena;* in the subsequent development it differentiates into the somatic and buccal part in the genus *Charonina* and *Blepharocorys*. It schould be postulated that a leap in the development occurred — perhaps in connection with the atrophy of non — ciliated kinetosomes on the body wich exist in *Didesmis* — and caused that morphogenesis assumed the feature of restoring the ciliature entirely de novo without transmission of any rudiments of the parental ciliature to the opisthe.

Summary

A new ciliate genus: *Raabena bella* gen. n., sp. n. has been described from the intestine of the Indian elephant *Elephas maximus* L. This ciliate should be included into the family *Blepharocorythidae* Hsiung. The specific development of this family is discussed.

STRESZCZENIE

Autorka opisała nowy gatunek orzęska *Raabena bella* gen. n., sp. n. z jelita słonia indyjskiego *Elephans maximus* L. Orzęsek ten powinien być włączony do rodziny *Blepharocorythidae* Hsiung. Autorka omawia swoisty rozwój tej rodziny.

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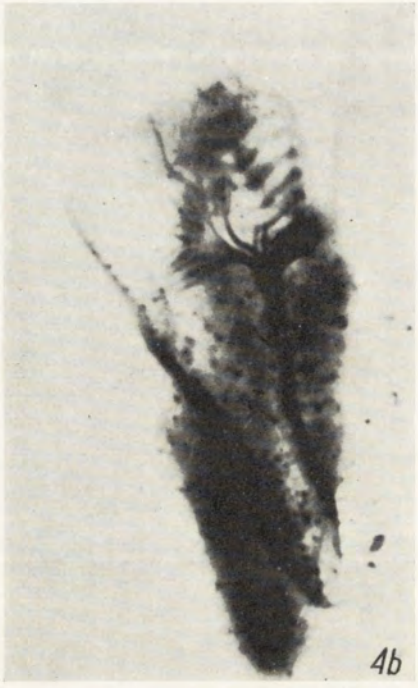
EXPLANATION OF PLATES I—IV

Raabena bella gen. n., sp. n.

1: General view, ciliary groups are impregnated
2: Semicircular fibers and fibers of the "pocket"
3: Division. a) semicircular fibers of the opisthe, optical section, b) ciliature of vestibulum of the opisthe another optical section
4: Vestibulum. a) view of the dorsal side. b) view of the ventral side, fan-shaped fibers
5: Isolated fronto-buccal zone, the zone of ventral lip and fibers coating vestibulum
6: Isolated fronto-buccal zone, fibers running from the arched part of the zone
7: Fibers of the anterior wall of vestibulum
8: Anterior part of the ciliate body showing the inside of the pocket; splitting of fibers is seen
9: View of the "pocket" fibers: single in the anterior segments and split in the deeper part
10: Isolated kineties of vestibulum, semicircular fibers and the longitudinal streak
11: Left ventral margin and interior of vestibulum. a) kineties of the ventral lip, fibers in the "pocket" and semicircular fibers are seen. b) vestibulum kineties on the right wall (the same specimen as in a) another optic section)
12: Fronto-buccal zone of the opisthe and semicircular fibers
13: Isolated kineties of vestibulum, semicircular fibers and the longitudinal streak
Photomicrographs of impregnated preparations.
Magnification: 1—3 1000 ×, 4—9 2000 ×, 7—13 2600 ×

M. Wolska auctor phot.



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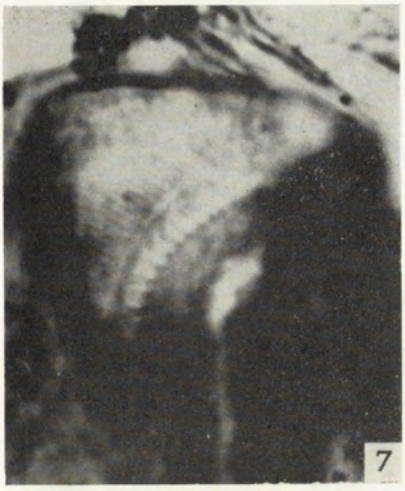

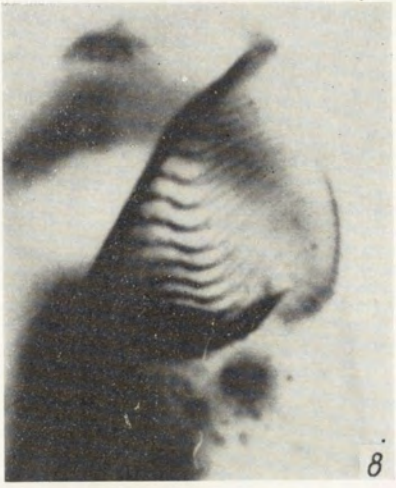

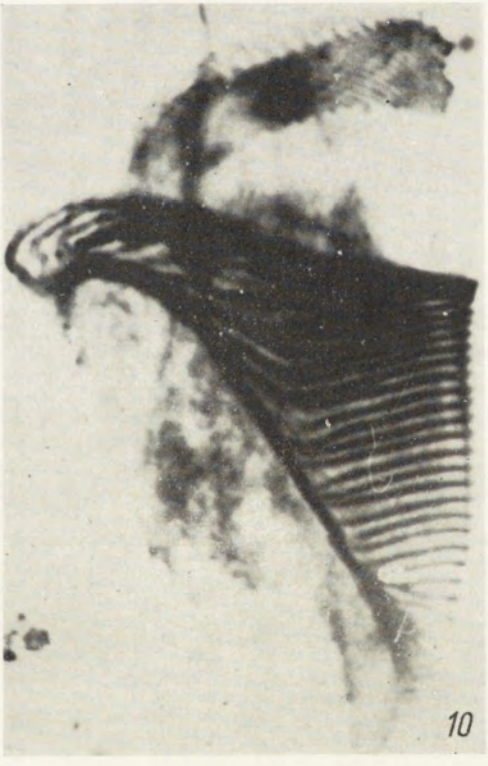

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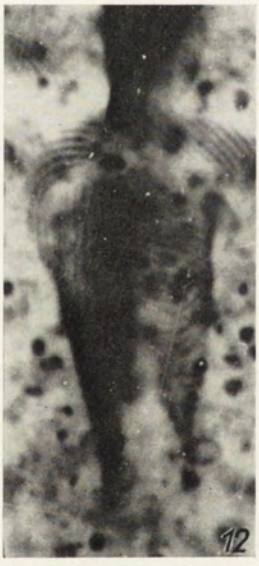

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Паразитические инфузории *(Peritricha, Urceolariidae)* некоторых рыб Камчатки

Parasitic Ciliates (*Peritricha, Urceolariidae*) of some fishes of the Kamchatka

При просмотре немногочисленных работ, посвященных паразитам рыб Камчатки, бросается в глаза полное или почти полное отсутствие в списке паразитов паразитических инфузорий. Лишь в одной работе (Ахмеров 1954) приводятся сведения о том, что в устье реки Камчатка и в Озере Ушка на колюшках (*Pungitius pungitius* и *Gasterosteus aculeatus*) были обнаружены *Trichodina domerguei* f. *latispina* (син. *T. domerguei* subsp. *domerguei*). Вследствие этого мы сочли целесообразным опубликовать те далеко не полные данные, которые были получены в результате обработки переданных нам для определения сборов разных лиц, работавших на Камчатке в разное время. Использованы сборы Л. С. Исакова за 1960 и 1962 гг., С. М. Коновалова за 1963 и 1965 гг. и собранные по нашей просьбе в 1965 г. А. В. Успенской инфузории с колюшек озера Дальнего. Всем этим товарищам мы приносим свою искреннюю благодарность.

Инфузории семейства *Urceolariidae* были обнаружены у 7 видов рыб:

1. *Oncorhynchus tschawytscha* (Walbaum) — чавыча (район Усть-Камчатска),
2. *Salmo mykiss* Walbaum — микижа (река Николка),
3. *Thymallus arcticus arcticus* (Pall.) — камчатский хариус (река Николка, Авья-ваям — зал. Корфа),
4. *Salvelinus leucomaenis* (Pall.) — кунджа (озеро Азабачье),
5. *S. alpinus* (L.) — голец озерно-речной, жилая форма (озеро Азабачье),
6. *Gasterosteus aculeatus* L. — трехиглая колюшка (озера Ближнее, Дальнее, Азабачье, река Паратунка),
7. *Pungitius pungitius* (L.) — девятиглая колюшка (озера Дальнее, Азабачье).

Во всех водоемах было вскрыто по 15 экземпляров рыб каждого вида, в озере Дальнем вскрыто несколько сот экземпляров колюшек обоих видов.

Было обнаружено 6 видов инфузорий:

1. *Tripartiella* (*Paratrichodina*) *incisa* Lom, 1959,
2. *Trichodina domerguei* subsp. *domerguei* (Wallengren, 1897),
3. *Trichodina tenuidens* Fauré-Fremiet, 1943,
4. *Trichodina gasterostei* sp. n.,

5. *Trichodina nigra* f. *kamchatika* forma nova,

6. *Trichodina* sp. (тип *T. nigra*).

Инфузорий на сухих мазках импрегнировали $AgNO_3$ по Клейну или фиксировали жидкостью Шаудинна с последующей окраской железным гематоксилином Гейденгайна по обычным методикам.

Tripartiella (Paratrichodina) incisa Lom, 1959 (Рис. 1, Табл. II 5).

Обнаружены на жабрах хариуса из реки Николки (заражено 27%) и Авья-ваям (47%). В нашем распоряжении были только препараты импрегнированные серебром.

Сравнительно мелкие инфузории с диаметром прикрепительного диска 22.5—30.0, диаметром венчика 19.5—30.0 μ. Число зубцов в венчике варьирует от 23 до 30, наиболее часто 27 и 26 зубцов ($M \pm m = 26.7 \pm 0.12$). Зубцы характерной для представителей рода *Tripartiella* формы: наружная сторона центральной конусовидной части зубцов имеет хорошо развитый направленный

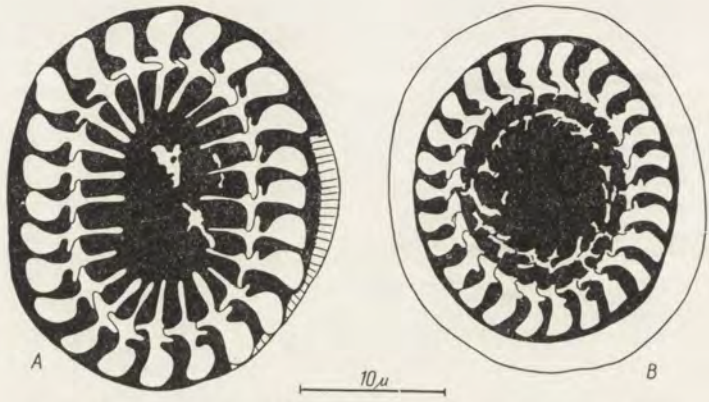

Рис. 1. *Tripartiella* (*Paratrichodina*) *incisa* (Lom, 1959). A. Прикрепительный диск. B. Реконструкция прикрепительного аппарата после деления. Импрегнация $AgNO_3$
Fig. 1. *Tripartiella* (*Paratrichodina*) *incisa* (Lom, 1959). A. The adhesive disk. B. Reconstruction of the adhesive disk after fission. Silver impregnation

вперед вырост. Наружные лопасти расширены и имеют выпуклый закругленный передний и слабо вогнутый или почти прямой задний края. Ближе к центральной части лопасть сужается и имеет вид тонкого стебля. Внутренние отростки (лучи) палочковидные, прямые, с закругленными или слабо заостренными концами. Длина наружных лопастей 3.0—4.5, чаще 4.5, внутренних отростков 3.0—4.5 μ. На каждый зубец приходится 6—8 полос прикрепительного диска. Всего промерено 153 экземпляра. Строение адоральной спирали нам рассмотреть не удалось.

По мнению Лома (Lom 1963), представители подрода *Paratrichodina*, в отличие от типичных *Tripartiella*, имеют лопасти со сравнительно тонким передним краем, который плохо импрегнируется на препаратах. Вследствие этого создается впечатление о наличии выемки на переднем краю лопасти и направленном вперед выросте наружной стороны центральной части зубца, то есть в действительности передний вырост не является настоящим (как у

Tripartiella, где он входит в выемку заднего края лопасти, которую у некоторых инфузорий не удается изобразить полностью.

Лом обнаружил представителей этого вида на жабрах *Nemachilus barbatulus*, *Phoxinus phoxinus*, *Rutilus rutilus*, *Gobio gobio* в водоемах Чехословакии.

Сравнение биометрических данных *T. incisa* с разных хозяев дано в Таблице 1.

Как следует из таблицы, инфузории с хариуса отличаются более крупными размерами прикрепительного аппарата, а также большей длиной лопастей и лучей.

Для водоемов Камчатки *Tripartiella incisa* описывается впервые, впервые в список хозяев этого вида включается хариус.

Таблица 1

Сравнение *Tripartiella incisa* с разных хозяев (измерения в μ)

Comparison of *Tripartiella incisa* from different hosts (measurments in μ)

	Наши данные Present author	Lom 1963			
	Thymallus arcticus	*Rutilus rutilus*	*Nemachilus barbatulus*	*Phoxinus phoxinus*	*Gobio gobio*
Диаметр тела Diameter of the body	—	28–33(30)	23–30(25)	18–27(23)	22–28(26)
Диаметр прикреп. Диска Diameter of the adhesive disk	22.5—30.0	20–27(23)	16–25(18)	18–27(19)	16–21(18)
Диаметр венчика Diameter of the denticulate ring	19.5—30.0	12–15(13)	10–15(12)	9–17(13)	8–11(9)
Длина лопасти Length of the blade	3.0—4.5(4.5)	3.0	2.5	3.3	2.8
Длина луча Length of the ray	3.0—4.5	2.2	1.9	2.5	1.8
Число зубцов Number of denticles	23—30(27,26)	21–30(24)	19–26(23)	19–29(24)	21–24(22)
Число полос Number of radial pins	6—8	5(6)	4–5	4–5	4

Trichodina domerguei subsp. *domerguei* (Wallengren, 1897) (Рис. 2).

Син. *T. domerguei* f. *latispina* Dogiel, 1940.

Широко распространенный в пресноводных и солоноватоводных водоемах Евразии вид. Обнаружен на жабрах, поверхности тела и плавниках *Gasterosteus aculeatus* и *Pungitius pungitius* в озерах Ближнем, Дальнем и Азабачьем.

Массовый материал был изучен на препаратах, импрегнированных серебром и окрашенных гематоксилином Гейденгайна.

Диаметр прикрепительного диска 42.0—69.0, венчика 37.5—63.0 μ. Венчик состоит из 20—31 зубцов, наиболее часто 24—26 зубцов. Длина наружной лопасти 6.0—10,5, внутреннего луча 4.5—9.0 μ. На каждый зубец приходится 8—12

полос прикрепительного диска. Ширина краевой мембраны 3.0—6.0 μ. Всего промерено около 500 экземпляров инфузорий.

Имея большой материал, собранный в 1965 г., мы смогли сравнить инфузорий с колюшек из двух озер — Дальнего и Азабачье. Первое расположено недалеко от Авачинской губы в восточной части полуострова Камчатка, тогда как второе — в центральной части полуострова (Таблица 2).

Рис. 2. *Trichodina domerguei* subsp. *domerguei* с жабер *Thymallus arcticus*. Импрегнация $AgNO_3$
Fig. 2. *Trichodina domerguei* subsp. *domerguei* from the gills of *Thymallus arcticus*. Silver impregnation

Таблица 2

Сравнение бисметрических данных *T. domerguei* subsp. *domerguei* из разных озер (измерение в μ)

Comparison of biometric data of *T. domerguei* subsp. *domerguei* from different lakes (measurements in μ)

	Оз. Дальнее Lake Dalnieye	Оз. Азабачье Lake Azabychye
Диаметр прикрепит. диска Diameter of the adhesive disk	42.0—69.0	42.0—63.0
Диаметр венчика Diameter of the denticulate ring	40.5—63.0	37.5—63.0
Длина лопасти Lenght of the blade	6.0—10.5	6.0—10.5
Длина луча Lenght of the ray	6.0—9.0	4.5—9.0
Число зубцов Number of denticles	23—31 ($M \pm m =$ $= 25.91 \pm 0.006$)	20—29 ($M \pm m =$ $= 23.63 \pm 0.12$)
Число полос Number of radial pins	8—12	8—12
Ширина краевой мембраны Width of the border membrane	3.0—6.0	—
Промерено экземпляров Number of specimens examined	186	162

Сравнение показывает, что инфузории из разных озер имели примерно одинаковые размеры и отличались лишь по числу зубцов в венчике, причем это различие оказалось статистически достоверным ($t = 19.0$) с надежностью, превышающей 99.9% (доверительный уровень $P > 99.9\%$).

В то же время инфузории с разных видов хозяев в одном и том же водоеме и с плавников и жабр одного и того же хозяина по этому признаку не имели существенных различий (Таблица 3 и 4).

Таблица 3

Сравнение числа зубцов *T. domerguei* subsp. *domerguei* из разных водоемов и с разных хозяев

Comparison of denticles number in *T. domerguei* subsp. *domerguei* from different waters and from different hosts

Озеро Lake	Хозяин Host	Промерено экземпляров Number of specimens examined	min-max	$M \pm m$	t
Озеро Дальнее	*G. aculeatus*	224	23—31	25.89±0.11	1.9
Lake Dalnieye	*P. pungitius*	30	24—28	25.93±0.19	
Озеро Азабачье	*P. pungitius*	100	21—28	23.68±0.13	0.5
Lake Azabachye	*G. aculeatus*	53	20—29	23.51±0.25	

Кроме *G. aculeatus* и *P. pungitius* один экземпляр *T. domerguei* subsp. *domerguei* был обнаружен на хариусе из реки Николка. Инфузория имела следующие размеры: диаметр прикрепительного диска 46.5, венчика 45.0 μ. Венчик состоял из 22 зубцов с одинаковыми по длине наружными и внутренними отростками (7.5 μ). На каждый зубец приходилось 10—12 полос (Рис. 2).

По-видимому, к этому же виду следует отнести и инфузорий с жабер и из носовых ямок *Oncorhynchus tschawytscha*, обнаруженных в 1960 г. в районе Усть-Камчатска.

Таблица 4

Сравнение числа зубцов у *T. domerguei* subsp. *domerguei* с жабер и плавников одного экземпляра *P. pungitius* (1962 г.)

Comparison of denticles number in *T. domerguei* subsp. *domerguei* from gills and fins of same specimen of *P. pungitius* (1962)

Локализация Localization	Промерено экземпляров Number of specimens examined	min-max	$M \pm m$	t
Жабры The gills	64	21—28	24.48±0.23	0.45
Плавники The fins	74	21—29	24.62±0.20	

Trichodina tenuidens Fauré-Fremiet, 1943 (Табл. I 1—2).

Син. *T. gracilis* Poljansky, 1955.

Вид, широко распространенный в пресноводных и морских водоемах Европы на жабрах, реже на поверхности тела трехиглой и девятииглой колюшки, обнаружен у тех же хозяев в обследованных реках и озерах Камчатки.

Сравнение *T. tenuidens* с жабер *Pungitius pungitius* и с поверхности тела и плавников *Gasterosteus aculeatus* проводится в Таблице 5.

Как видно из таблицы, инфузории с поверхности тела и плавников трехиглой колюшки отличаются большими размерами, по сравнению с инфузориями с жабер девятииглой колюшки.

В водоемах Камчатки *T. tenuidens* отмечается впервые.

Таблица 5

Сравнение *T. tenuidens* с разных хозяев и из разных водоемов (измерения в μ)
Comparison of *T. tenuidens* from different hosts and different waters (measurements in μ)

	P. pungitius жабры The gills	*G. aculeatus* плавники, поверхность тела The fins and body surface	
	оз. Азабачье Lake Azabachye	оз. Азабачье Lake Azabachye	оз. Дальнее Lake Dalnieye
Диаметр прикрепит. диска Diameter of the adhesive disk	33.0—48.0	45.0—57.0	42.0—67.5
Диаметр венчика Diameter of the denticulate ring	30.0—45.0	39.0—54.0	39.0—60.0
Длина лопасти Length of the blade	4.5—7.5	7.5—9.0	6.0—9.0
Длина луча Length of the ray	4.5—7.5	6.0—9.0	7.5—10.5
Число зубцов Number of denticles	24—31 (26, 27)	23—29	25—32 (26)
Число полос Number of radial pins	8—10	12 (6 пар—pairs)	8—12
Ширина краевой мембраны Width of the border membrane	—	—	3.0—6.0
Промерено экземпляров Number of specimens examined	57	11	16

Наряду с типичными *T. domerguei* subsp. *domerguei* и *T. tenuidens* на жабрах и поверхности тела обоих видов колюшек во всех обследованных водоемах встречались довольно крупные триходины (Табл. II 3—4), отличавшиеся рядом признаков, характерных для обоих видов, и большим числом зубцов в венчике. Подобно *T. domerguei*, у них крупные, но более узкие лопасти зубцов. Подобно *T. tenuidens*, как правило, внутренние лучи длиннее лопастей, палочковидные, прямые и слегка расширяющиеся к концу (у *T. domerguei*, как правило, лучи к концу слегка сужаются). Центральная часть прикрепительного диска на препаратах, импрегнированных серебром, имеет различный вид: та-

кой же, как у типичных вегетативных особей *T. domerguei*, с большим количеством аргентофильных участков или состоит из отдельных фрагментов. В редких случаях в центральной части диска вообще отсутствовали светлые неимпрегнируемые участки.

Возможно, что различное строение центральной части прикрепительного диска соответствует разным стадиям жизненного цикла инфузорий. Промерено 53 экземпляра. Диаметр тела 64.5—68.6, прикрепительного диска 46.5—73.5, венчика 42.0—70.1 μ. Венчик состоит из 28—37 (33, 31, 32) зубцов, полос 10—12, реже 8. Ширина краевой мембраны 3.0—6.0 μ. Сравнение биометрических данных описываемых инфузорий („крупные триходины") с *T. domerguei* subsp. *domerguei* и *T. tenuidens* дано в Таблице 6. Достоверность различий числа зубцов у трех сравниваемых форм приведена в Таблице 7.

Таблица 6

Сравнение биометрических данных „крупных триходин" с *T. domerguei* subsp. *domerguei* и *T. tenuidens* (измерения в μ)

Comparison of biometric data of "big trichodinids", *T. domerguei* subsp. *domerguei* and *T. tenuidens* (measurements in μ)

	„Крупные триходины" "Big trichodinids"	*T. domerguei* subsp. *domerguei*	*T. tenuidens*
Диаметр тела Diameter of the body	64.5—68.6	—	—
Диаметр прикрепит. диска Diameter of the adhesive disk	46.5—73.5	42.0—69.0	33.0—67.5
Диаметр венчика Diameter of the denticulate ring	42.0—70.1	37.5—63.0	30.0—60.0
Длина лопасти Length of the blade	6.0—10.5	6.0—10.5	4.5—7.5
Длина луча Length of the ray	7.5—12.0	4.5—9.0	4.5—10.5
Число зубцов Number of denticles	28—37 (33, 31, 32)	20—31 (24—26)	24—32 (26, 27)
Число полос Number of radial pins	8, 10—12	8—12	8—12
Ширина краевой мембраны Width of the border membrane	3.0—6.0	3.0—6.0	3.0—6.0

В совместной статье Лом и Штейн (Lom and Stein, 1966) отнесли этих инфузорий с большим числом зубцов к *T. tenuidens*.

Одновременно с *T. domerguei* и *T. tenuidens* на жабрах и поверхности тела *G. aculeatus* были обнаружены инфузории рода *Trichodina* по форме зубцов сходные с *T. tenuidens* и отнесенные в вышеупомянутой статье Лома и Штейн к аберрантной форме этого вида. От *T. tenuidens* они в первую очередь отличаются значительно более мелкими размерами и хорошо импрегнируемой центральной частью прикрепительного диска. Второй и очень существен-

Таблица 7

Достоверность различий числа зубцов у „крупных триходин" *T. domerguei* subsp. *domerguei* (оз. Дальнее) и *T. tenuidens*

Statistical significance of the difference of the number of denticles in "big trichodinids", *Trichodina domerguei* subsp. *domerguei* (from Lake Dalnieye) and *T. tenuidens*

Виды инфузорий Species of ciliates	Промерено экземпляров Number of specimens examined	min-max	$M \pm m$	t	P
T. domerguei subsp. *domerguei*	224	23—31	25.89±0.11	4.46	99.9%
T. tenuidens	82	24—32	26.87±0.19	15.8	99.9%
„Крупные триходины" "Big trichodinids"	70	28—37	32.49±0.30		

ной особенностью этих инфузорий является строение их адоральной спирали, которая, по-видимому, делает более двух оборотов (Рис. 3 B). В водоемах Камчатки эти инфузории встречались неоднократно. К сожалению, строение

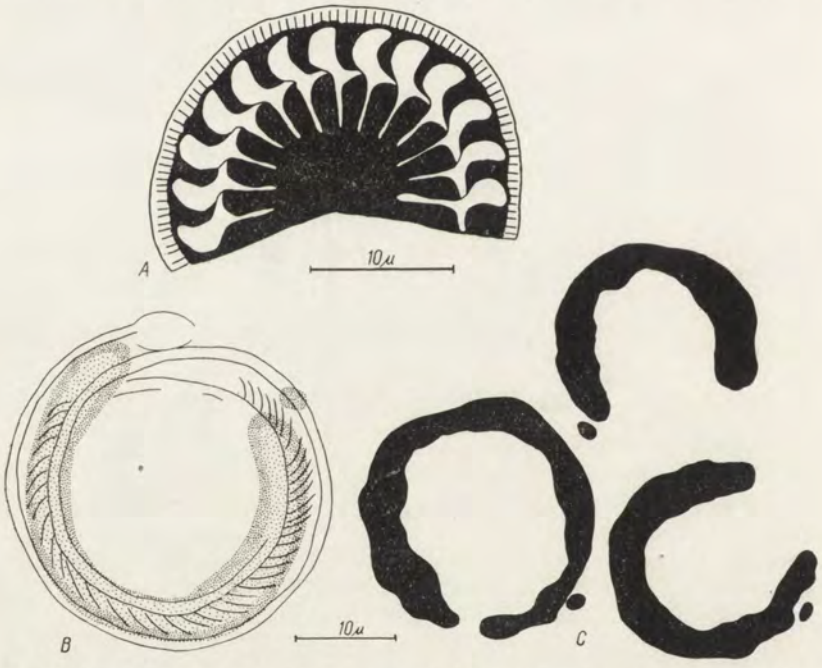

Рис. 3. *Trichodina gasterostei* sp. n. A. Прикрепительный диск (участок). B. Адоральная спираль. C. Ядерный аппарат, различное положение микронуклеуса. A. Импрегнация $AgNO_3$, B и C гематоксилин Гейденгайна.

Fig. 3. *Trichodina gasterostei* sp. n. A. The adhesive disk (fragment). B. The adoral zone. C. Nuclear apparatus. Different positions of micronucleus. A. Silver impregnation. B and C. Heidenhain's iron haematoxylin

адоральной спирали можно было рассмотреть лишь на препаратах, окрашенных железным гематоксилином. На мазках, импрегнированных серебром, были только прикрепительные диски. На основании особенностей строения прикрепительного диска и адоральной спирали мы считаем возможным описать с жабер и поверхности тела *Gasterosteus aculeatus* новый вид *Trichodina gasterostei* sp. n. К какому подроду (*Vauchomia* или *Paravauchomia*) следует отнести этот вид можно будет решить лишь после детального изучения строения адоральной спирали.

Trichodina gasterostei sp. n. (Рис. 3. Табл. II 6).

Жабры и поверхность тела *Gasterosteus aculeatus*. В озере Азабачье колюшки были заражены на 80%.

Таблица 8

Результаты измерений *T. gasterostei* sp. n. (измерения в μ)

Results of measurements of *T. gasterostei* sp. n. (measurements in μ)

	Гематоксилин гейденгайна Heidenhain's iron haematoxylin (1960)	Импрегнация $AgNO_3$ Silver impregnatin (1965)
Диаметр тела Diameter of the body	28.6—40.0	52.5 (1 экз. — 1 specimen)
Диаметр прикрепит. диска Diameter of the adhesive disk	21.5—41.5	21.0—33.0
Диаметр венчика Diameter of the denticulate ring	17.2—38.6	19.5—28.5
Длина лопасти Length of the blade	2.9—4.3	3.0—4.5 (3.0)
Длина луча Length of the ray	2.9—4.3	3.0—4.5 (4.5)
Число зубцов Number of denticles	20—27 (23, 24)	18—25 (23, 22)
Число полос Number of radial pins	6—10	6—8, 10 (6)
Диаметр макронуклеуса Diameter of macronucleus	20.0—25.7	—
Диаметр микронуклеуса Diameter of micronucleus	1.4—2.9	—
Отрезок "x" Distance "x"	5.7—11.4	—
Отрезок "y" Distance "y"	1.4—8.6	—
Адоральная спираль Adoral zone	>720°	—
Ширина краевой мембраны Width of the border membrane	—	1.5—3.0 (3.0)

Описывается по препаратам, фиксированным жидкостью Шаудинна и окрашенным гематоксилином Гейденгайна, и по мазкам, импрегнированным серебром по Клейну.

Сравнительно мелкие инфузории с зубцами характерной для рода *Trichodina* формы. Наружные лопасти слабо изогнутые, широкие, с закругленными или реже слегка заостренными концами. Внутренние лучи прямые или чуть изогнутые, палочковидные. Центральная часть прикрепительного диска равномерно импрегнируется серебром (Рис. 3 А).

Ядерный аппарат состоит из подковообразного макронуклеуса (Ма) и довольно крупного микронуклеуса (Mi), лежащего сбоку от Ма на разном расстоянии от его конца (Рис. 3 С).

Адоральная спираль совершает больше двух оборотов (> 720°) и ее средняя часть на препаратах покрыта довольно длинными ресничками, которые не удалось проследить по всей длине спирали (Рис.3 В). Ее диаметр примерно равен диаметру прикрепительного диска. Биометрические данные приведены в таблице 8.

Как видно из таблицы, инфузории, зафиксированные жидкостью Шаудинна и импрегнированные серебром, имели на препаратах примерно одни и те же размеры. Промерено 197 экземпляров.

По строению зубцов *T. gasterostei* имеет наибольшее сходство с *T. tenuidens*, отличаясь длиной адоральной спирали, значительно меньшими размерами, отсутствием аргентофобной центральной части прикрепительного диска.

Дифференциальный диагноз: Мелкие *Urceolariidae*, *Trichodininae*, с хорошо развитыми зубцами венчика типа *Trichodina*. Наружные лопасти расширенные и закругленные, внутренние лучи прямые или чуть изогнутые, палочковидные. Центральная часть прикрепительного диска равномерно импрегнируется серебром. На каждый зубец приходится 6—10 полос прикрепительного диска. Ядерный аппарат состоит из подковообразного Ма и округлого Mi, расположенного сбоку от Ма. Адоральная спираль делает больше двух оборотов, ее диаметр примерно равен диаметру прикрепительного диска.

Хозяин: *Gasterosteus aculeatus*.

Локализация: жабры, поверхность тела.

Местонахождение: водоемы Камчатки (озера Дальнее, Азабачье), Старый Петергоф (Ленниградская область).

Впервые с жабер рыб описываются инфузории рода *Trichodina*, характеризующиеся большой длиной адоральной спирали (> 720°).

Trichodina nigra forma *kamchatika* forma nova (Рис. 4. Табл. II 7—8).

Обнаружена на жабрах гольца, микижи и хариуса из реки Николка, Авья-ваям и озера Азабачье. Экстенсивность заражения разных хозяев, по сообщению С. М. Коновалова, была следующей: микижа из реки Николка — 20%, в озере Азабачье — 27%, озерно-речная форма гольца в Азабачьем — 35%, озерная форма — 13%. Хариус из Николки — 33%, в Авья-ваям — 13%, кунджа в Азабачьем — 6.6%.

Довольно крупные инфузории с массивным венчиком, напоминающим по форме наружных лопастей *T. domerguei* subsp. *domerguei*. Лопасти широкие, серповидно изогнутые, с более или менее закругленными вершинами. Внутренние отростки палочковидные, слегка изогнутые, примерно одинаковой ширины на всем протяжении, более тонкие у инфузорий с хариуса и микижи. Внутренний край центрального конуса зубца образует небольшой выступ перед внутренним отростком. Результаты измерениий приведены в таблице 9.

Из таблицы следует, что инфузории с гольца отличались меньшим числом зубцов в венчике и более мелкими размерами. На препаратах центральная часть прикрепительного диска импрегнируется серебром (темная).

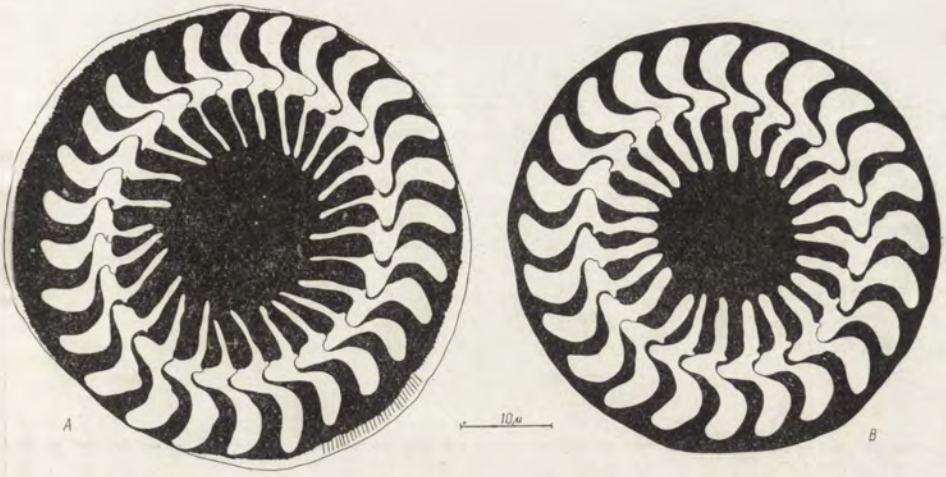

Рис. 4. *Trichodina nigra* f. *kamchatika* f. nova. Прикрепительный диск. А. Экземпляр с жабер *Salmo mykiss*. В. Экземпляр с жабер *Salvelinus alpinus*. Импрегнация $AgNO_3$.

Fig. 4. *Trichodina nigra* f. *kamchatika* f. nova. The adhesive disk. A. Specimen from the gills of *Salmo mykiss*. B. Specimen from the gills of *Salvelinus alpinus*. Silver impregnation

Таблица 9

Сравнение *T. nigra* f. *kamchatika* с разных хозяев (измерения в μ)

Comparison of *T. nigra* f. *kamchatika* from different hosts (measurements in μ)

	Salvelinus alpinus	*Salmo mykiss*	*Thymallus arcticus*
Диаметр прикрепит. диска Diameter of the adhesive disk	43.5—55.5	49.5—61.5	—
Диаметр венчика Diameter of the denticulate ring	39.0—49.5	45.0—54.0	48.0—52.5
Длина лопасти Length of the blade	6.0—9.0	7.5—9.0(7.5)	7.5—9.0
Длина луча Length of the ray	7.5—9.0	7.5—9.0	7.5—9.0
Число зубцов Number of denticles	23—28	26—30	27—29
Число полос Number of radial pins	12—14	12—14	14
Промерено экземпляров Number of specimens examined	22	7	3

Таблица 10

Сравнение *T. nigra* f. *kamchatika*, *T. fultoni*, *T. tumefaciens* и *T. nigra* (измерения в μ)
Comparison of *T. nigra* f. *kamchatika*, *T. tumefaciens* and *T. nigra* (measurements in μ)

	T. nigra f. *kamchatika*	*T. fultoni*	*T. tumefaciens*	*T. nigra*
	Наши данные Present author	Lom and Hoffman, 1964	Жуков, 1964; Davis, 1947	Lom, 1961
Диаметр прикрепит. диска Diameter of the adhesive disk	43.5—61.5	62—86	30—41 (29—38)	32.0—69.0
Диаметр венчика Diameter of the denticulate ring	39.0—54.0	41—58	18—27 (18—23)	20.0—39.0
Длина лопасти Length of the blade	6.0—9.0	6.5—7.5	3.6—4.2	4.5—7.0
Длина луча Length of the ray	7.5—9.0	5.5—6.0	2.1—3.6	5.0—9.0
Число зубцов Number of denticles	23—30	25—31 (27, 28)	22—27 (19—26, 21—25)	17—33 (21—23, 25—29)
Число полос Number of radial pins	12—14	12—14	8, реже — rarely 10 (7)	8—10
Хозяева Hosts	*Salvelinus alpinus*, *Salmo mykiss*, *Thymallus arcticus*	*Lepomis cyanellus*, *Micropterus salmoides*, *Rhinichthys atratulus*, *Tinca tinca*, *Nemachilus barbatuius*	*Thymallus arcticus*, *Cottus caganowskii* (*Cottus baiardii*?)	пресноводные рыбы fresh-water fishes
Местонахождение Locality	пресноводные водоемы Камчатки fresh-water reservoirs of Kamchatka	морские и пресноводные водоемы США, пресноводные водоемы Чехословакии sea and fresh-water reservoirs of USA and fresh-water of Czechoslovakia	водемы США, водоемы Чукотки water reservoirs of USA and Chukotka	пресноводные водоемы Чехословакий, СССР fresh-water of Czechoslovakia and USRR

Сравнивая триходин с гольца, микижи и хариуса с ранее описанными видами, мы пришли к выводу, что не можем полностью идентифицировать их ни с одним видом. Наибольшее сходство наблюдается с такими видами как *Trichodina fultoni* Davis, 1947, *T. tumefaciens* Davis, 1947 и *T. nigra* Lom, 1960. Сравним камчатских триходин с этими видами более детально. В таблице 10 сравниваются биометрические данные. В графе, посвященной *T. tumefaciens* цифры, стоящие в скобках, взяты из работы Дэвиса (Davis 1947).

С *T. fultoni* камчатских инфузорий сближает форма наружных лопастей и число зубцов в венчике, число полос прикрепительного диска. Отличаются они строением центральной части прикрепительного диска. На препаратах импрегнированных серебром у *T. fultoni* (Lom and Hoffman 1964) центральная часть диска светлая, как у представителей вида *T. domerguei* (*T. fultoni* — синоним *T. domerguei* f. *magna*), у камчатских инфузорий темная, как у *T. nigra*. У *T. fultoni* более короткие, по сравнению с лопастями, внутренние лучи, изогнуты примерно в том же направлении, что и лопасти. У камчатских инфузорий лучи примерно равны или длиннее лопастей, почти прямые. Диаметр розетки *T. fultoni* значительно больше, чем у камчатских инфузорий.

Trichodina tumefaciens Davis, 1947 обнаружил на Чукотке у *Thymallus arcticus* Жуков (1964). Кроме общих хозяев, камчатских триходин с этим видом сближает, судя по рисунку Жукова, некоторое сходство в форме зубцов и указание Дэвиса (Davis 1947) на то, что он у *T. tumefaciens* насчитывал 7 (может быть 7 пар?) полос прикрепительного диска. В то же время значительно более крупные общие размеры, более узкие наружные лопасти, постоянно большее число полос прикрепительного диска (12—14, у Жукова 8—10), иное соотношение лопастей и внутренних лучей — все это препятствует сближению камчатских триходин с *T. tumefaciens*.

В пользу сближения триходин с гольца, хариуса и микижи с *Trichodina nigra* Lom, 1960, говорит следующее: 1. характер импрегнации серебром центральной части прикрепительного диска, 2. форма наружных лопастей и внутренних лучей, 3. соотношение лопастей и лучей, 4. число зубцов в венчике. По размерам венчика и числу полос прикрепительного диска эти виды заметно отличаются (у *T. nigra* на один зубец приходится, как правило, 8—10 полос).

Учитывая все сказанное выше, мы сочли возможным включить инфузорий с гольца, хариуса и микижи в состав *T. nigra* Lom, 1960 в качестве отдельной формы впредь до уточнения их систематического положения.

Trichodina sp. (Табл. II 9).

На жабрах *Gasterosteus aculeatus* из озера Азабачье была обнаружена инфузория, напоминавшая по характеру импрегнации серебром центральной части прикрепительного диска инфузорий типа *T. nigra*. Она имела следующие размеры: диаметр прикрепительного диска 66.0 μ, венчика 57.0 μ, длина наружной лопасти 6.0 μ, внутреннего луча 9.0 μ. Венчик состоял из 35 зубцов, на каждый зубец приходилось 10 полос прикрепительного диска. Ширина краевой мембранн 4.5 μ.

Ввиду того, что представленный в статье материал является в какой-то мере случайным, мы не считаем возможным обсуждать в этой статье характерные черты фауны урцеоляриид с камчатских рыб и ограничимся лишь краткими выводами.

Как литературные данные, так и собственные наблюдения, с большой до-

лей вероятности позволяют предполагать, что *T. domerguei* subsp. *domerguei* (= *T. domerguei* f. *latispina*) относится к инфузориям с очень широким распространением. Этот вид многократно описывали как из пресных, так и морских водоемов Европы, Европейской и Азиатской частей Советского Союза, на острове Сахалин (Богданова и Штейн 1963) на Чукотке (Жуков 1964) и, наконец, на Камчатке. Чен Чи-лю (Chen Chih-leu 1963) описал *T. domerguei* subsp. *domerguei* с прудовых рыб Китая. Очень интересно было бы обнаружить представителей этого вида в бассейне Амура в СССР и в водоемах США.

На основании собственных наблюдений на Балтийском, Белом, Баренцовом, Японском морях, на многочисленных пресноводных водоемах Советского Союза, в том числе и камчатских, мы считаем *T. tenuidens* Fauré-Fremiet, 1943 (= *T. gracilis*) специфичным паразитом колюшек. Нам ни разу не удалось, в отличие от *T. domerguei* subsp. *domerguei*, зарегистрировать этих инфузорий на других хозяевах.

Представляет интерес обнаружение на хариусе *Tripartiella incisa*, впервые описанной Ломом (Lom 1959) с пресноводных рыб в Чехословакии. Позднее этот вид находили и в водоемах Европейской части СССР. В Азии он отмечается впервые.

Как нам кажется, большой интерес для систематики семейства представляет тот факт, что впервые у триходин, паразитирующих на жабрах, обнаружена многократно закрученная адоральная спираль. Очень важно было бы продолжить подробное изучение этого вида, как, впрочем, и всей фауны урцеолярид Камчатки.

Резюме

Изучали инфузорий семейства *Urceolariidae* с жабер и поверхности тела 7 видов рыб. Описываются 6 видов, относящихся к родам *Tripartiella* и *Trichodina*. *T. gasterostei* и *T. nigra* f. *kamchatika* являются новыми, *Tripartiella incisa*, *Trichodina domerguei* subsp. *domerguei*, *T. tenuidens* и *Trichodina* sp. впервые указываются для водоемов Камчатки.

SUMMARY

Ciliates of the family *Urceolariidae* from gills and skin of 7 fish species were studied. Six species *Tripartiella* (*Paratrichodina*) *incisa* Lom, 1959, *Trichodina domerguei* subsp. *domerguei* (Wallengren, 1897), *T. tenuidens* Fauré-Fremiet, 1943, *T. gasterostei* sp. n., *T. nigra* f. *kamchatika* f.n., *Trichodina* sp. (typ. *T. nigra*) are described.

Trichodina gasterostei sp. n.

Diagnosis: small *Urceolariidae*, *Trichodininae* with well developed denticulate ring of the *Trichodina* type. The outer blades broadened and rounded, the inner rays straight or slightly bent, rod-shaped. The central part of the adhesive disc is impregnated uniformly with silver. Each denticle coincides with 6—10 radial pins of the adhesive disc. Nuclear apparatus constitutes of a horse-shoe-shaped Ma and of a spherical Mi, located on the side of Ma. The adoral spiral winds more than two turns, its diameter is approx. the same as that of the adhesive disc. Fig. 3, Pl. II 6 Biometric data in Table 8.

Host: *Gasterosteus aculeatus.*

Localization: gills, body surface.

Locality of occurrence: waters of Kamchatka (lakes: Dalnieye, Azabachye), Old Peterhof (Leningrad district).

The ciliates of the genus *Trichodina* distinguished by the length of their adoral spiral (> 720°) are described first.

Trichodina nigra forma *kamchatika*, forma nova.

Fairly big ciliates with compact denticulata ring crown of a shape resembling the form of outer blades in *T. domerguei* subsp. *domerguei*. Blades broad, sickle--shaped, bent, with more or less rounded apexes. Inner rays: rod-shaped, slightly bent, approx. of the same width along their full lenght, thinner in the ciliates from *Thymallus arcticus* and *Salmo mykiss*. Inner margin of the central part of denticle forms a small protrusion in front of the inner rays. Fig. 4. Biometric data in the Tab. 9.

Found on gills of: *Salvelinus alpinus, Salmo mykiss* and *Thymallus arcticus* from the river Nikolka, Avia-vayam and the lake Azabachye.

Tripartiella incisa, Trichodina domerguei subsp. *domerguei, T. tenuidens* and *Trichodina* sp. are mentioned for the Kamchatka for the first time.

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1—2: *Trichodina tenuidens* с жабер *Pungitius pungitius* и *Gasterosteus aculeatus*
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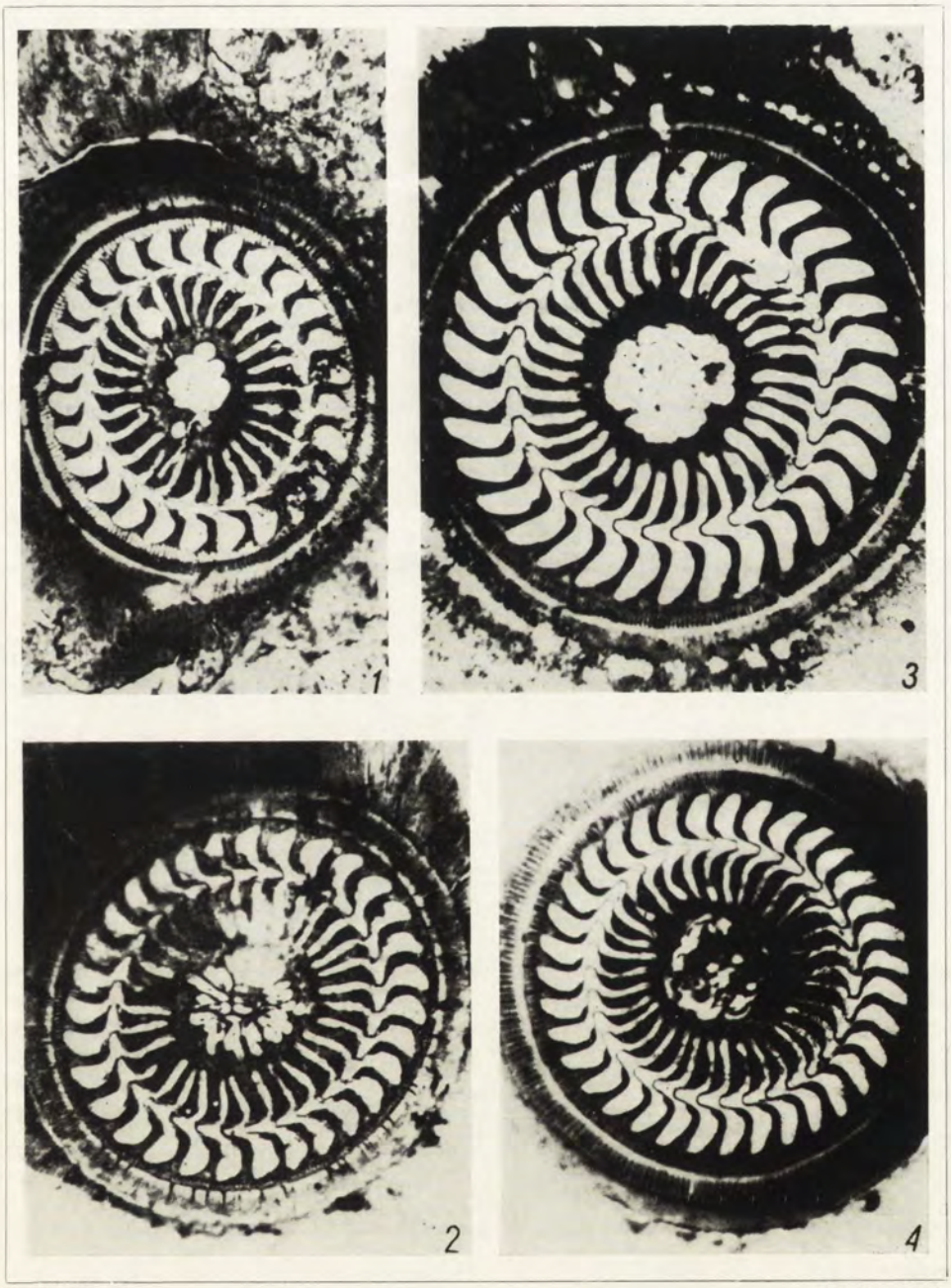
8: *T. nigra* f. *kamchatika* from gills of *Salvelinus alpinus*
9: *Trichodina* sp. from gills of *Gasterosteus aculeatus.*



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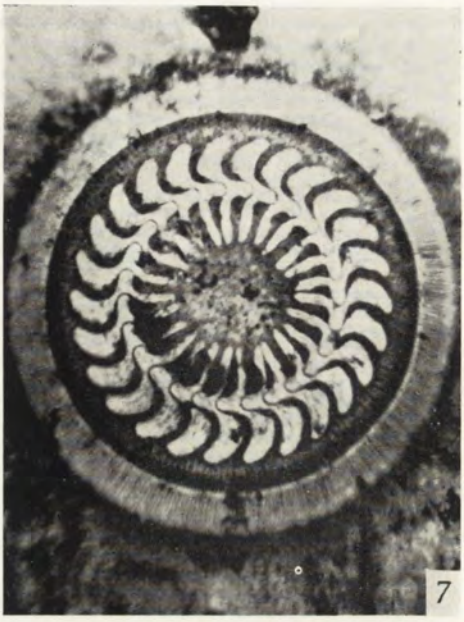
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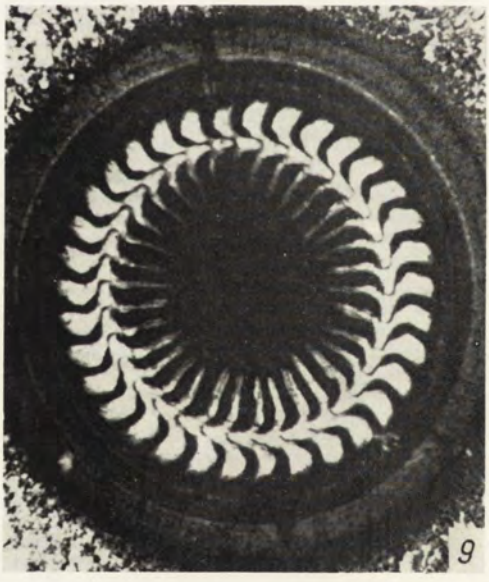
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Myxosporidia of fishes from the Zegrze Lake

Myxosporidia ryb Jeziora Zegrzyńskiego

Myxosporidia are a parasite group which has been very little investigated on the territory of Poland. The only extensive work on this subject is the study of Wegener 1909. In more recent publications these parasites are only mentioned (Kozicka 1951, 1953, 1959, E. Grabda, J. Grabda and Wierzbicki 1961).

Material and methods

The present study concerns *Myxosporidia* from gills of 9 species of fishes from the Zegrze Lake which is an artificial reservoir formed at the water dam constructed below the confluence of the rivers Bug and Narew above their common estuary into Vistula.

Cysts were studied on smear preparations. For fixation, the fluids of Schaudinn and of Carnoy, for staining — haematoxylin of Heidenhein or of Delafield were applied. Observation of living material was also performed. Since all the species of this order show a high variability, measurements of at least 30 spores — fresh and stained ones together — were executed. Fresh spores are characterized — as a rule — by bigger dimensions, especially of their polar capsules, their shell and filament spiral are more distinct. Keeping spores in distilled water for a prolonged time (several days) makes those structures much more distinct, whereas their living parts decompose.

In the description of the species investigated, attention was payed to the kind of sporoblasts which seems to be a diagnostic feature besides the commonly accepted criteria as: shape and dimensions of spores and of cysts, species of the host, tissue or organ in which the parasite lives.

The sequence of genera and species is based on the system of Schulman 1962.

Review of species

Myxosoma dujardini Thélohan, 1899 — *Scardinius erythrophthalmus* (L.), *Leuciscus idus* (L.), gills,

Myxosoma anurus (Cohn, 1895) — *Esox lucius* L., gills,

Myxobolus sandrae Reuss, 1906 — *Lucioperca lucioperca* (L.), operculum,

Myxobolus dispar Thélohan, 1895 — *Rutilus rutilus* (L.), *Carassius carassius* (L.), gills,

Myxobolus permagnus Wegener, 1909 — *Perca fluviatilis* L., gill arch,

Myxobolus cycloides Gurley, 1894 — *Rutilus rutilus* (L.), gills,

* *Myxobolus macrocapsularis* Reuss, 1906 — *Rutilus rutilus* (L.), gills,
Myxobolus ellipsoides Thélohan, 1892 — *Tinca tinca* (L.), gills,
Myxobolus exiguus Thélohan, 1895 — *Abramis brama* (L.) gills,
Myxobolus mülleri Bütschli, 1882 — *Abramis brama* (L.), *Leuciscus idus* (L.), *Rutilus rutilus* (L.), gills,
Myxobolus sp. — *Leuciscus idus* (L.), gills,
Henneguya lobosa (Cohn, 1895) — *Perca fluviatilis* L., gills,
Henneguya psorospermica Thélohan, 1895 — *Esox lucius* L., gills,
Thélohanellus pyriformis (Thélohan, 1892) — *Tinca tinca* (L.), gills.

Myxosoma dujardini Thélohan, 1899 (Fig. 1 A—F)

Vegetative stage: cysts of irregular shape, highly branched, morula-like, most frequently covering the whole gill lamella. Monosporous sporoblasts — sporoblasts transforming into one spore.

Spores: pear-shaped, their anterior end more or less narrowed, sometimes bent to one side. Polar capsules of an even or nearly even size. Measurements were taken of 40 spores originating from 4 cysts. Dimensions of spores: length 9.5—13 μ, breadth 5.3—8.3 μ, thickness 4—7.5 μ, polar capsules 4.3—7.3×1.3—2.8 μ, difference of lenght 0—1.1 μ. Filament, 38—45 μ in length, forms 8—10 coils of the spiral inside the polar capsule. Exceptionally in one spore of a 7.2 μ length, the filament measured 29 μ and produced 6 coils of the spiral. The spore shell is of a uniform thickness approx. of 0.4 μ except the anterior end where it reaches the thiskness of 1—1.3 μ, in the sutural edge 0.5—0.7 μ.

According to Kudo 1919, Dogiel 1932, Dyk, Dykova 1957, the typical form of *M. dujardini* is that with its anterior end bent asymetrically which was found by me only sporadicaly. The dimensions of spores cited above are, except the length of filament (70 μ according to Kudo 1919, Dogiel 1932, Schulman 1962) in agreement with those reported by former authors, they show however a more extensive range of variability.

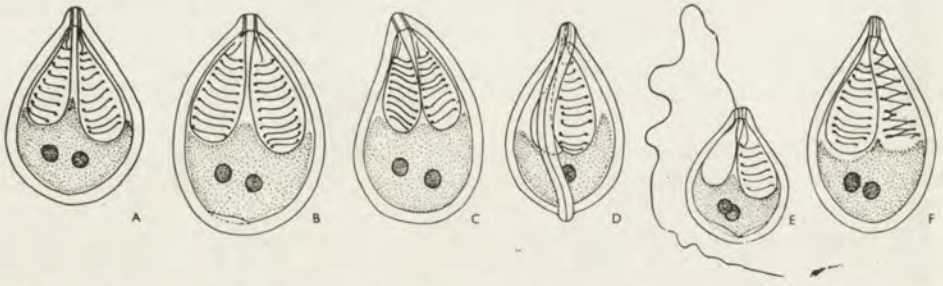

Fig. 1. *Myxosoma dujardini* Thélohan, 1899. A — E. Structure of the normal spores, F. abnormal one. 2500×

In the majority of spores, in their posterior part, folds were observed on each of shell valves. They overlap one upon another in the suture (Fig. 1 B, E). These structures were not reported in earlier studies.

Among the normal spores, some with an anomaly was found which was a result of disturbances in morphogenesis of one of the two polar capsules.

* — species found for the first time in Poland.

This spore had only one polar capsule with a filament inside coiled into a normal spiral, the other capsule was absent and the filament was coiled into a irregular spiral in the space bordered by the spore shell, sporoplasm and the polar capsule (Fig. 1 F).

Myxosoma anurus (Cohn, 1895) (Fig. 2 A—H)

Vegetative stage: cysts are oval, reniform, semi-discoidal or discoidal. They lie at the base or at the free end of the gill lamellae in a number varying from several to some tenths on one gill arch. The dimensions of cysts amount 0.14—0.5 mm. Cysts of *M. anurus* occur frequently beside the cysts of *Henneguya psorospermica*. Monosporous sporoblasts.

Spores: oval or ovoid in front view, lens-shaped in side view. Polar capsules long, narrow, parallel to one another, of equal or nearly equal size. Measurements were made of 80 spores from 12 cysts. Spore dimensions: length 9.8—

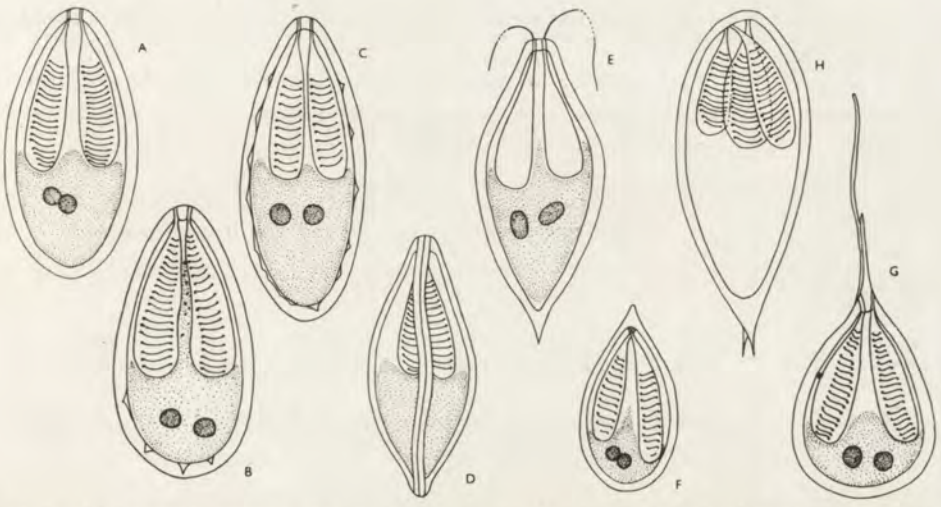

Fig. 2. *Myxosoma anurus* (Cohn, 1895). A — D. Structure of the normal spores, E — H. abnormal ones. 2500×

16 μ (most frequently 12—14 μ), breadth 5—7.8 μ, thickness 4—5.5 μ. Polar capsules 5.5—8.1×1—2.4 μ, difference of length 0—0.6 μ. Filament approx. 55 μ long forms a spiral of several coils and is disposed in the shape of letter S on account of the insignificant breadth of the polar capsule. Shell of the spore, of a uniform thickness 0.4—0.5 μ, is broader on the sutural edge 0.5—0.8 μ. Sometimes in the suture markings are seen which were not described as yet. The above measurements of spores indicate a much broader range of variability than it was reported by other authors. These differences may be involved by the fact that authors give only the dimensions which occur most frequently or their results are based on a number of measurements statistically not extensive.

In the cysts studied, abnormal spores were found and the anomalies were of two types: anomalies of shape and the developmental ones. The first type concerns the spores with shell outgrowths (Fig. 2 E—H) in their anterior or poster-

ior part. To the second type belong the spores with 3 polar capsules (Fig. 2 H) which is possibly the result of irregular division in the early stages of sporoblast formation.

D o g i e l 1932 suggests the identity of species *M. anurus* and *M. dujardini*.

Table 1
Comparison of spores of *Myxosoma anurus* and *M. dujardini*

	M. anurus	*M. dujardini*
Hosts	Representatives of fam. *Esocidae* (2 species) *Percidae* (1 species)	Representatives of fam. *Cyprinidae* (7 species)
Cysts	oval, discoidal, with smooth margins	branched, morula-like
Spore shape	a. oval, slightly narrowed in anterior part b. broadest in 1/2 of spore lenght c. narrow in side view, lenticular	as a rule highly narrowed in anterior part broadest below 1/2 of length occasionally at 1/2 pear-shaped in side view
Ratio $\frac{\text{length}}{\text{breadth}}$ of spore	1.7—2.6 (most frequent>2)	1.4—2 (most frequent 1.6—1.8)
Ratio $\frac{\text{length}}{\text{breadth}}$ of polar capsules	3.5—4.3	2.2—2.7
Structure of shell	sutural markings found occasionally, folds absent	sutural markings frequent, sutural folds frequent

As follows from my observations the above forms are separate species although the ranges of dimension of their spores coincide in great extent. The differences occurring in the dimensions of these two species are represented in Table 1.

Myxobolus sandrae Reuss, 1906 (Fig. 3 A—I)

Vegetative stage: cysts discoidal, oval with smooth edges, reaching up to 2 mm of diameter. Monosporous sporoblasts.

Spores: oval, nearly discoidal or ovoid (more narrow in their anterior part). The polar capsules of even or nearly even size. Measurements were made of 50 spores from 5 cysts. Dimensions of spores: length 6.8—10.6 μ, breadth 5.7—8.5 μ, thickness 3.8—6 μ. Polar capsules: 3.2—5.4 ×1.3—2.7 μ, difference of length 0—1 μ, most frequently 0.4 μ. Filament 30—35 μ long forms a spiral of 7—8 coils in the polar capsule. Spore shell of a uniform thickness 0.4 μ, in the sutural edge 0.6—1 μ. Sutural markings distinct, their number 5—8. Intercapsular appendix absent which is proved by observation of empty shells (Fig. 3 F). The above dimensions are in agreement with that reported by S c h ä f e r n a und J i r o v e c 1931 (for *M. luciopercae* syn. *M. sandrae*), D o-

giel 1932, Schulman 1962. The variability range of spores studied by me is broader. The only controversy concerns the length of filament which according to Schäferna und Jirovec 1931 amounts 60—67 μ.

The anomalous spores are characterized by the presence of posterior outgrowths of the shell (Fig. 3 H, I).

Myxobolus dispar Thélohan, 1895 (Fig. 4 A—H)

Vegetative stage: in *Rutilus rutilus* occur oval or discoidal cysts, at the base or in the mid-length of the gill lamellae. Dimensions of cysts up to 0.3 mm. In *Carassius carassius* occur oval or discoidal cysts on the gill arch, their dimensions 2—3 mm. Monosporous sporoblasts.

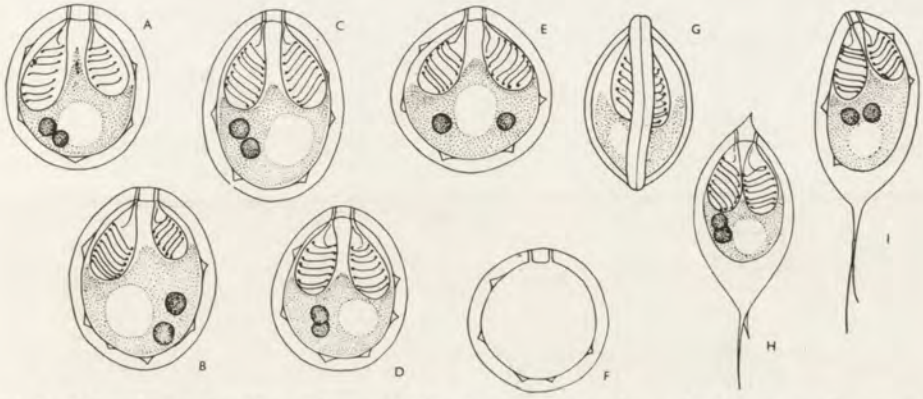

Fig. 3. *Myxobolus sandrae* Reuss, 1906. A — G. Structure of the normale spores, H — I. abnormal ones. 2500×

Spores: shape of spores various, oval, less frequently nearly discoidal asymmetrical in relation to the longitudinal axis of the spore, or narrowing in the anterior or posterior part. In different cysts various forms dominate in number. Polar capsules unequal in size. Thickness of the spore shell in the

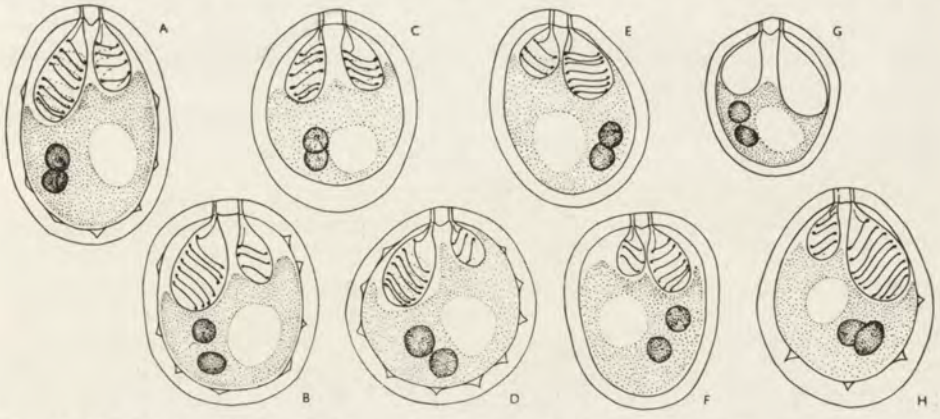

Fig. 4. *Myxobolus dispar* Télohan, 1895. A — H. Structure of the normal spores. 2500×

sutural edge amounts 0.6—0.9 μ, or sometimes thicker in its posterior part reaching 1.6 μ. The filament of the minor polar capsule, 13—19.5 μ in length forms 3—4 coils of the spiral, that of the major polar capsule being 23—37 μ long, forms 7—9 coils.

Table 2 comprising the comparison of dimensions of the spores in *M. dispar* from both hosts, is based on measurements of groups, each of them embracing 30 spores from 2 cysts taken from each host species. It follows from the above comparison (Tab. 2) that the spores of *M. dispar* from *R. rutilus* are slightly

Table 2

Dimensions of spores of *Myxobolus dispar* from different hosts in μ

Host	*R. rutilus*	*C. carassius*
Length of spore	8.4—12	10—14.3
Breadth of spore	7.1—9.5	7.9—10.2
Thickness of spore	5—6	6.6
Polar capsule major	3,6—5.1 × 1.9—3	3.7—5.7 × 1.8—3.2
Polar capsule minor	2.5—4.1 × 1.6—2.2	2.8—3.7 × 1.4—2.2
Differences of length of polar capsules	0.2—2.8	0.3—1.6
Differences of breadth of polar capsules	0.2—0.9	0.4—1.6

smaller than those from the gills of *C. carassius*. Similar results were obtained by Dogiel 1932 after comparison of spores of this species from the gills of *R. rutilus* with those from muscles of *R. rutilus* and *Leuciscus cephalus*. In my material, the dimensions of the polar capsules, as well as the differences in size of both capsules are smaller than those reported by former authors. The conformity of other dimensions, of shape of spores and the identicity of the host species allow to reckon the spores under study as *M. dispar*.

Myxobolus permagnus Wegener, 1909 (Fig. 5 A—G)

Vegetative stage: discoidal cyst, its diameter 1 mm. Monosporous sporoblast.

Spores: oval narrowing in their anterior part (in 65% of the general number of spores, Fig. 5 B, C), regularly oval (in 24%, Fig. 5 A), or nearly discoidal (in 11%, Fig. 5 D). Polar capsules pear-shaped opening near each other at the distance of 1—1.7 μ, their opposite ends diverge broadly. Spore shell is of a uniform thickness in the sutural edge and measures 0.9—1.3 μ, or is the thickest in its posterior part (1.1—2.2 μ) and the thinnest in the anterior part (0.7 μ). Shell thickness beyond the suture amounts 0.7 μ. Breadth of the sutural ridge is 1.3—1.6 μ. In the sutural plane markings are seen in the number

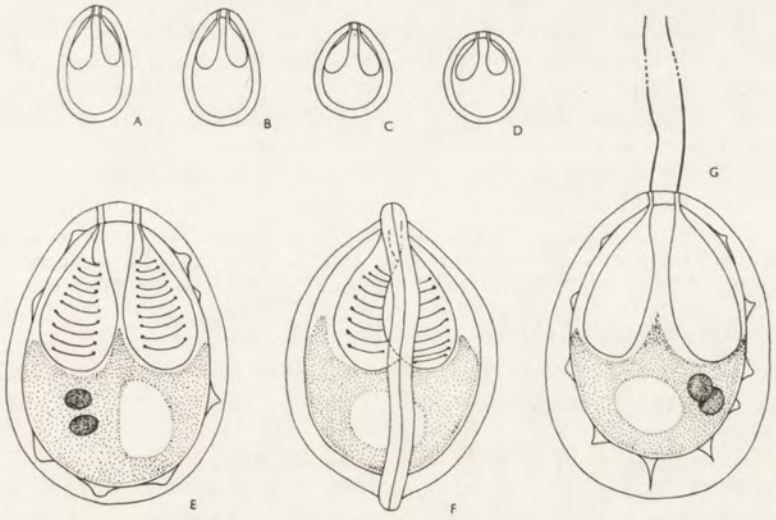

Fig. 5. *Myxobolus permagnus* Wegner, 1909. A — D. Variation in shape of the normal spores. E — G. Structure of the normal spores. A — D. 1250 ×, E — G. 2500 ×

Table 3

Dimensions of spores of *Myxobolus permagnus* according to different authors in μ

Author	Wegener 1909	Kudo 1919	Dogiel 1932	Schulman 1962	Present author
Length of spore	17—18	17—18	17—18	17—20	12—16.3
Breadth of spore	10—13	10—13	10—13	10—11.5	9—12
Thickness	—	—	—	—	9—10.8
Length of polar capsule	7—8	7—8	7—8	7—11.2	6—9.3
Breadth of polar capsule	3.5—4	3.5—4	—	4—4.5	2.1—4.6
Sutural markings	5—6	5—6	5	—	6—10
Filament	—	—	—	96—100	60—70

6—10. The intercapsular appendix is absent as well in the stained as in fresh spores, as in those kept in distilled water for 2 months. Schulman 1962 describes the intercapsular appendix as scarcely visible.

As follows from Table 3 and from the included drawings, the spores found by me and determined as belonging to *M. permagnus* differ from spores studied by other authors in length, in a higher variability of shape and in the length of filament. These differences might be the consequence of the fact that spores measured by me in the number of 50, taken from one cyst, may originate from a spore smaller than the average one for this species. The other dimensions, as well as the host species, shape and size of the cyst, are in accordance with the data reported by other authors.

Myxobolus cycloides Gurley, 1893 (Fig. 6 A—K)

Vegetative stage: cysts oval, in the mid-length of the gill lamellae. Dimensions of cysts 2.5×1 mm. Found once in the number of 2 cysts. Monosporous sporoblasts.

Spores: oval, often broader in their anterior part, or nearly discoidal rarely asymmetrical in respect to the long axis. In side-view or observed from capsular end symmetrical, oval. Polar capsules of an even or nearly even size, smaller or equal to half of the length of spore. The spore shell of uniform thick-

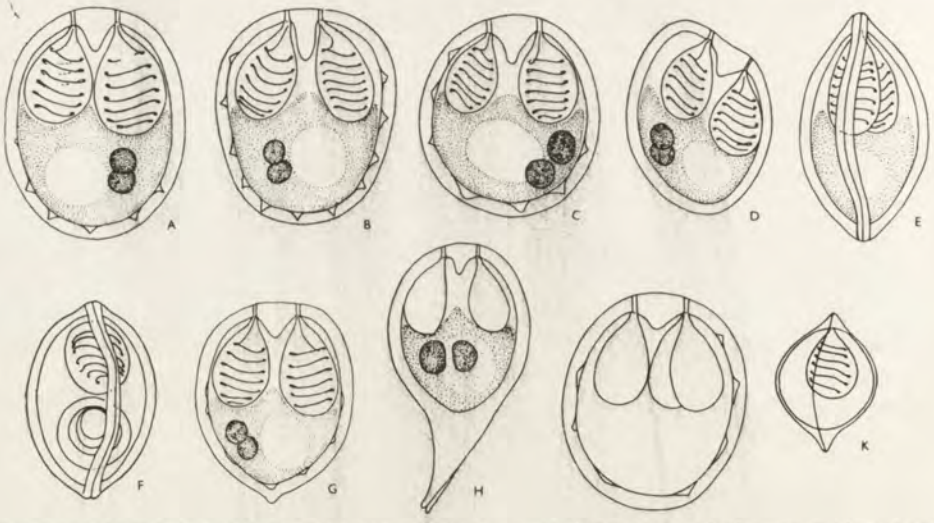

Fig. 6. *Myxobolus cycloides* Gurley, 1893. A — F. Structure of the normal spores, G — K. abnormal ones. 2500×

ness 0.5—0.6 μ in sutural plane 0.5—0.8 μ. Sutural markings distinct in number 6—11. The intercapsular appendix big, triangular, its length 0.7—2.2 μ, most frequently 1.4 μ. Filament forms a spiral of 6—7 coils in the polar capsule. The length of expelled filament amounts 30—54 μ. Measurements were made of 60 spores fixed and stained and of 10 fresh spores. Dimensions of spores: length 8.6—11.2 μ (fresh ones 10.2—12.2 μ), breadth 7.3—9.5 μ (fresh 7.8—10.4 μ), thickness 6.5 μ (fresh 6.8—6.9 μ), polar capsules 4—5×2.1—2.7 μ (fresh 4.2—5.8× ×2.6—4 μ).

The anomalies encountered are connected with the presence of the posterior shell outgrowth in the form of a scarcely marked structure or of two distinct tails (Fig. 6 G, H) or with an abnormal number of polar capsules (Fig. 6 I, K). In this case, the spore has one polar capsule, the shell being distinctly composed of two shell valves, or it has 3 polar capsules but only 2 exit canals in the spore shell.

The spores described above coincide in their dimensions with the spores of *M. cycloides* type A as described by W e g e n e r 1909, *M. cycloides*, L o m 1961, *M. mülleri* syn. *M. cycloides*, S c h u l m a n 1962. In my material, the spores of *M. mülleri* from *R. rutilus* differ from spores of *M. cycloides* of the same host in their thickness and in the size of the intercapsular appendix. Both values are higher in the spores of *M. cycloides*. According to L o m 1961 the intercapsular appendix is the biggest in *M. cycloides* when compared with the remaining species of the genus Myxobolus.

Myxobolus macrocapsularis Reuss, 1906 (Fig. 7 A—E)

Vegetative stage: cysts discoidal, their diameter 0.6—1.2 mm. Monosporous sporoblast.

Spores: ovoid with a distinctly narrowed anterior end, less frequently oval or discoidal. Polar capsules — as a rule — longer than half length of the spore, open near one another, with widely diverging opposite ends. The shell of the spore is of uniform thickness 0.5—0.6 μ and forms a small intercapsular appendix which cannot be measured. 36 spores from two cysts were measured. Dimensions of spores: length 7.4—10.4 μ, breadth 6.6—7.8 μ, thickness 5.5 μ, polar capsules 3.7—5.9×1.5—2.4 μ, differences of length 0—0.8 μ. Filament 50 μ long forms a spiral composed of 7—9 coils in the polar capsule.

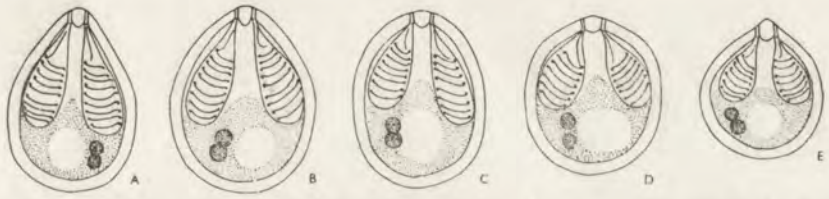

Fig. 7. *Myxobolus macrocapsularis* Reuss, 1906. A — E. Structure of the normal spores. 2500×

The length of spores and of the polar capsules measured by me is lower than those cited in the literature. It coincides however with the range of variability as reported by S c h u l m a n 1962 for this species.

Myxobolus ellipsoides Thélohan, 1892 (Fig. 8 A—K)

Vegetative stage: cysts oval, nearly discoidal or of irregular shape. Dimensions 0.6—2 mm. Monosporous sporoblast.

Spores: oval, with a slight constriction in the mid-length of spore, narrowing in the posterior or — less frequently — in anterior part of the spore, or nearly discoidal. Spores with a tapering posterior end were found very rarely (Fig. 8 K). Polar capsules of even or almost even size. Spore shell thick when compared with these structures in other species. Its thickness in the sutural plane fluctuates in different spores of the same cyst in the limits 0.8—1.7 μ,

being sometimes slightly thicker in its posterior part. Intercapsular appendix is — as a rule — not visible or very small, rarely distinct, big triangular of 1.2—1.5 μ in length (Fig. 8 C I,). Sutural markings are found very rarely. 75 spores of 7 cysts were measured. Dimensions of spores: length 12.3—18.4 μ, breadth 7.2—11.6 μ, thickness 7 μ, polar capsules 4.1—6.1×1.6—2.7 μ, differences of length 0—0.7 μ, ocasionally 3 μ (Fig. 8 I).

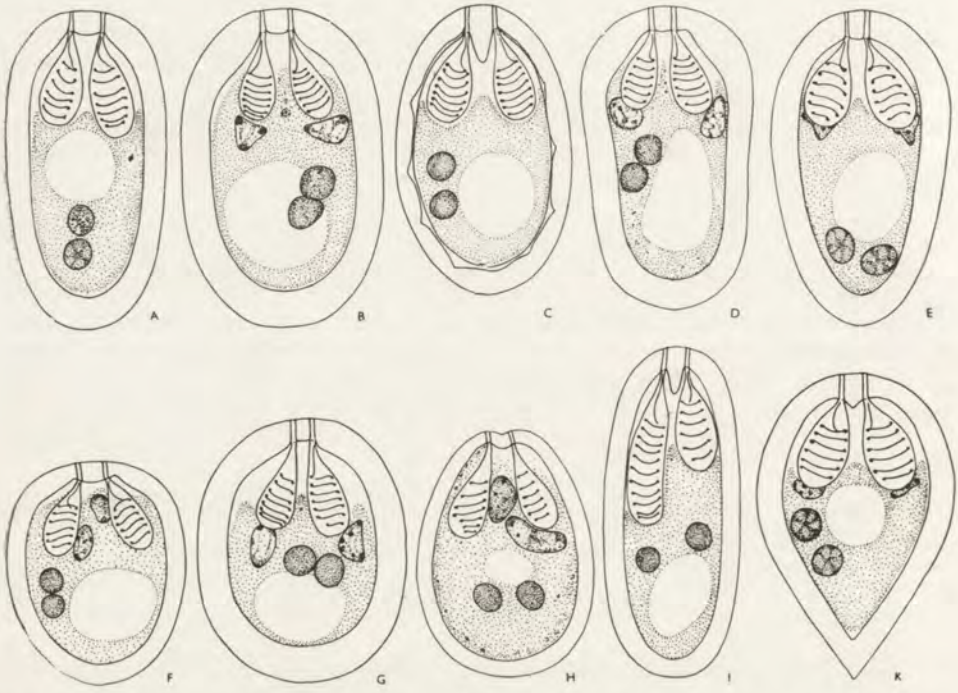

Fig. 8. *Myxobolus ellipsoides* Thélohan, 1892. A — K. Structure of the normal spores. 2500 ×

Myxobolus exiguus Thélohan, 1895 (Fig. 9 A—L)

Vegetative stage: cysts elongated with folded margins, occurring on the basal or median part of the gill lamellae or on their free ends. Their number amounts 1 up to 300 on one host individual. Dimensions of cysts 0.5—3.5 mm. Monosporous and disporous sporoblasts.

Spores: oval with a narrowed anterior end, regularly oval or nearly discoidal, rarely narrowed in their posterior part. In the majority of cysts, dominate spores narrowed at their anterior end (Fig. 9 A, B) in the others, the oval ones (Fig. 9 C, D). Polar capsules of even or almost even size, open near one another at the distance of 0.8—1.3 μ. The spore shell is 0.4—0.6 μ thick, slightly thicker (0.6—0.9 μ) in its sutural edge. The intercapsular appendix trapezoid-shaped, rectangular or triangular, its length 0.5—0.9 μ. Measurements of 200 spores were performed. Dimensions of spores: length 6.6—9.7 μ, breadth 5.3—7.1 μ, thickness 3—4.6 μ. Polar capsules 3.2—4.4×1.1—2.2 μ, length differences 0—0.5 μ. Filament 22—28 μ long forms a spiral composed of 5—7 coils.

Anomalous spores were found with outgrowths of shell in the anterior or posterior part of the spore (Fig. 9 G—L), as well as anomalies of developmental type (Fig. 9 K, L). The latter ones arose presumably from a pansporoblast with 4, instead of 2, cells determined for producing the spore shell and as result a spore is formed of a double character.

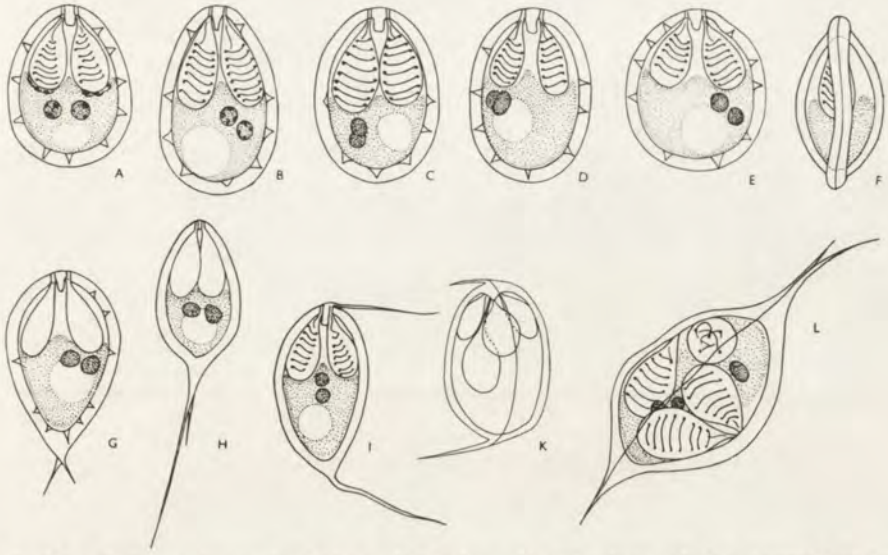

Fig. 9. *Myxobolus exiguus* Thélohan, 1895. A — F. Structure of the normal spores, G — L. abnormal ones. 2500×

Myxobolus mülleri Bütschli, 1882 (Fig. 10 A—N)

Vegetative stage: shape and dimensions of cysts are given in table 4. Monosporous and disporous sporoblasts.

Spores: broadly oval, broader in their anterior part, nearly discoidal, asymmetrical in relation to the long spore axis or narrowed in the anterior part of the spore. Broadly oval forms (Fig. 10 A) dominate in particular cysts, or those broader at their anterior part. (Fig. 10 D). Polar capsules of even or almost even size, distance of their openings amounts 1.3—1.9 μ. The spore shell is 0.5—0.9 μ thick in the sutural edge and forms 4—9 sutural markings. Intercapsular appendix is of a triangular, rectangular or trapezoidal shape. Measurements were made of 100 spores from 4 cysts found in *Leuciscus idus*, of 130 spores from 5 cysts from *Abramis brama* and of 30 spores from 1 cyst from *Rutilus rutilus*. The dimensions of spores originating from different hosts are compared in Table 4.

As follows from the presented table, spores of *M. mülleri* occurring in different hosts differ in the range of variability of particular dimensions. Those differences however may be due to the various number of spores measured from each of the species of fishes. Confronting the above with those reported by authors for spores of M. *mülleri*, it becomes clear that among the spores measured by me occur some spores of a minor breadth, thickness and of smaller polar capsules.

The abnormal spores were found in cysts from *L. idus* and *A. brama* (Fig.

10 K—N). A curious developmental anomaly presents a spore with 3 polar capsules, 3 shell appendices, it seems however that the shell is composed of only 2 shell valves. In sporoplasm of this spore, 2 sporoplasm nuclei and 3 nuclei near the polar capsules are seen after staining with orcein.

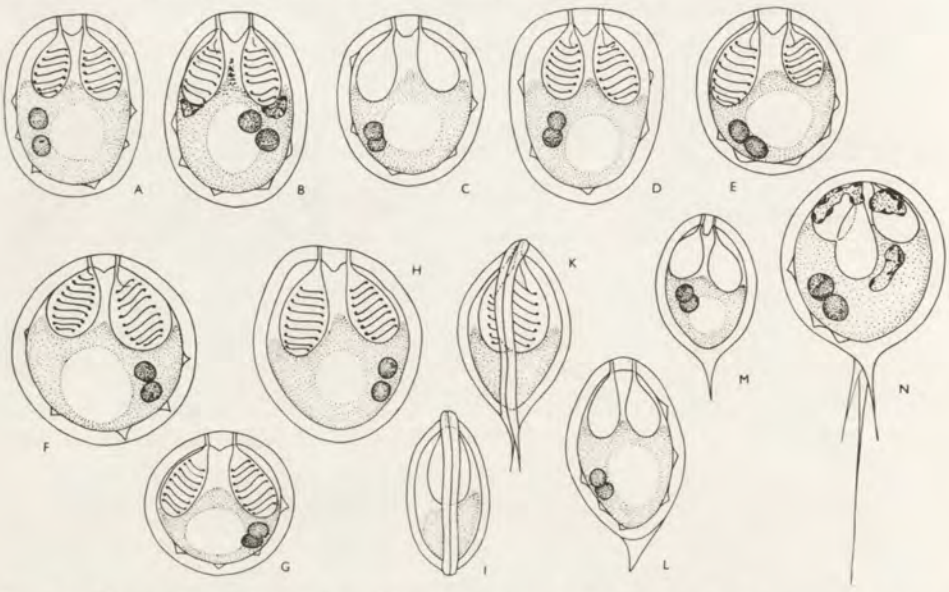

Fig. 10. *Myxobolus mülleri* Bütschli, 1882. A — I. Structure of the normal spores, K — N. abnormal ones. 2500×

Myxobolus sp. (Fig. 11 A—C)

Cysts were not observed. The spores studied originate from a control smear [1] from *Leuciscus idus*.

Spores: oval, nearly discoidal, occasionally asymmetrical in relation to the long spore axis. The polar capsules of even or nearly even size open near one another. Thickness of the spore shell in its sutural edge amounts 1—1.6 μ. Neither the sutural markings nor the intercapsular appendix were seen in preparations. Dimensions of spores: length 14.7—19.2 μ, breadth 11.1—15.1 μ, polar capsules 6.7—8.7×2.7—4 μ, differences of length amounted 0—1.3 μ. The above data fail to provide sufficient information for determining the species of spores studied; the vegetative stage was not observed, dimensions of filament and thickness of spores is unknown, the fresh spore shell was not studied which would allow to ascertain the presence or lack of the intercapsular appendix. Besides, the number of collected and measured spores (30) was too low for a more detailed study.

Henneguya lobosa (Cohn, 1895) (Fig. 12 A—E)

Vegetative stage: cysts oval, discoidal, lobular, of dimensions 0.3—1 mm, occurring as well at the basal as on median part as near the free end of the branchial lamellae. Monosporous and disporous sporoblasts.

[1] As the control smear, such a smear from the gill is considered, in which no *Myxosporidia* cysts were visible. Other smears were made of the cysts.

Table 4
Dimensions of spores of *Myxobolus mülleri* from different hosts in μ

Hosts	*L. idus*	*A. brama*	*R. rutilus*
Shape, size of cysts	oval, reniform, discoidal, 1–3,5 mm	oval, reniform, discoidal, 0.3–1 mm	oval, discoidal 0.05—0.9 mm
Length of spores	7.2—9.9	7.5—11	7.9—12
Breadth of spores	5.8—7.8	6—9.7	7.1—10
Thickness of spores	3.5—5.2	5	5
Length of polar capsules	2.7—4.6	3—5	3—5.6
Differences of length of polar capsules	0—0.8	0—0.9	0—0.5
Breadth of polar capsules	1.4—2.3	1.4—2.7	1.5—3.1
Length of filament and number of coils	25 6—8	35—43 6—9	? ?
Length of inter-capsular appendix	0.3—1.1	0.4—0.9	0.5—1
Sutural markings	4—11	4—9	7—10

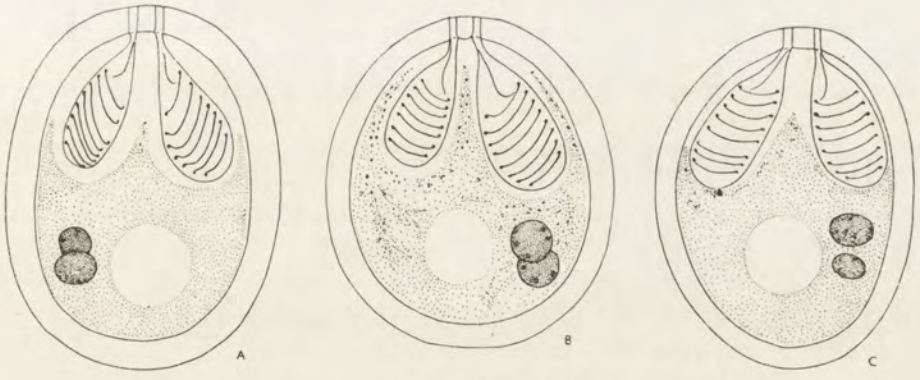

Fig. 11. *Myxobolus* sp. A — C. Structure of the normal spores. 2500×

Spores fusiform, their broader part coincides with the mid-length of the spore or is shifted anteriorly (Fig. 12 A). Polar capsules of even or almost even length, in a position parallel to one another. Differences of length 0—1.5 μ. The spore shell of uniform thickness 0.4—0.8 μ, prolonged in its posterior part so as to

form 2 tail appendices with a long base i.e. this part with which they join together — as it seems — only with their edge. As result of this disposition, posteriorly, behind the spore cavity, an empty space arises which might be of importance for infection, promoting the spores to float on water (Fig. 12 D). Tails of even or uneven length, differences reaching up to 11 μ.

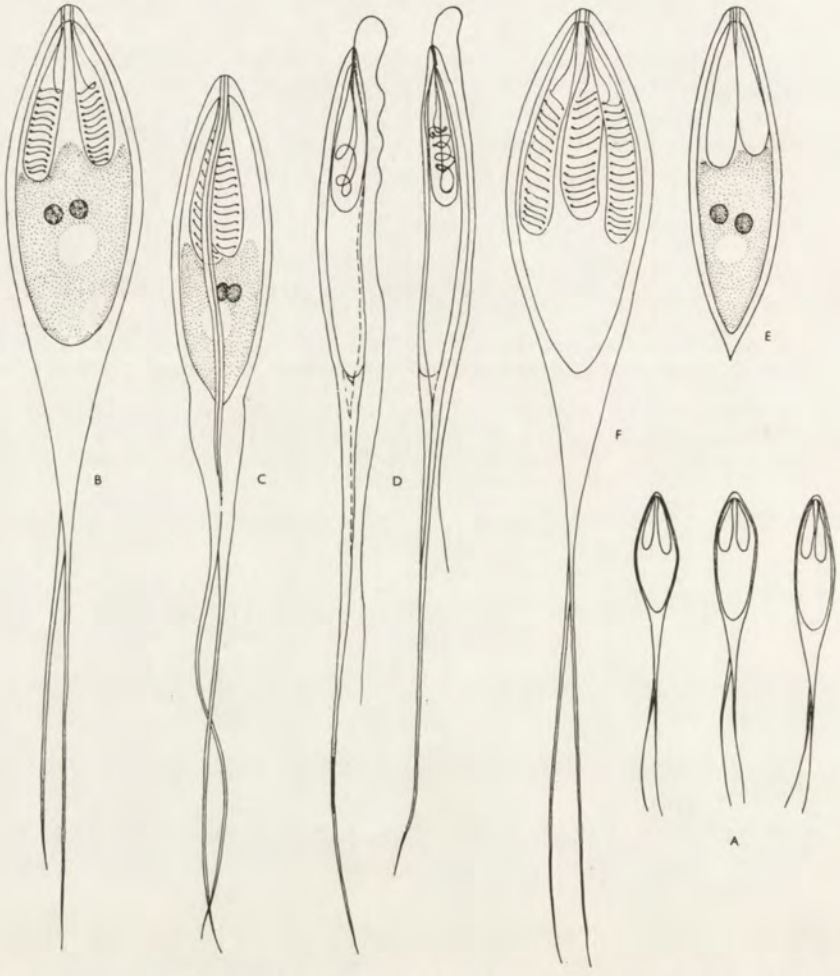

Fig. 12. *Henneguya lobosa* (Cohn, 1895). A. Variation in shape of the normal spores. B — D. Structure of the normal spores, E — F. abnormal ones. A. 1250 ×, B — F. 2500 ×

The spores under study show a coincidence of dimensions and of shape with data reported by other authors for *H. lobosa,* (Table 5) and differ only in occurrence in another host species. Considering however that many *Myxosporidia* species parasites of *Esox lucius* occur in *Perca fluviatilis* as well, it seems possible that *H. lobosa* may occur in both hosts.

Shape anomalies were found in the form of tailless spores (Fig. 12 E), and also one spore with an anomaly of developmental type was observed (Fig. 12 F), in which 3 polar capsules were present and only 2 openings in the spore shell were seen.

Henneguya psorospermica Thélohan, 1895 (Fig. 13 A—N)

Vegetative stage: cysts oval, reniform or nearly discoidal, occurring at the base of the branchial lamellae, occasionally at their free ends or at mid-length. Dimensions of cysts 0.3—2.5 mm. The observed intensity of infection: 2—30 cysts in one host individual.

The size of cysts seems to be related to the degree of development of the trophozoit and not to the intensity of infection of the host. Infection with 3 cysts of dimensions not exceeding 0.5 mm and with 11 cysts measuring about

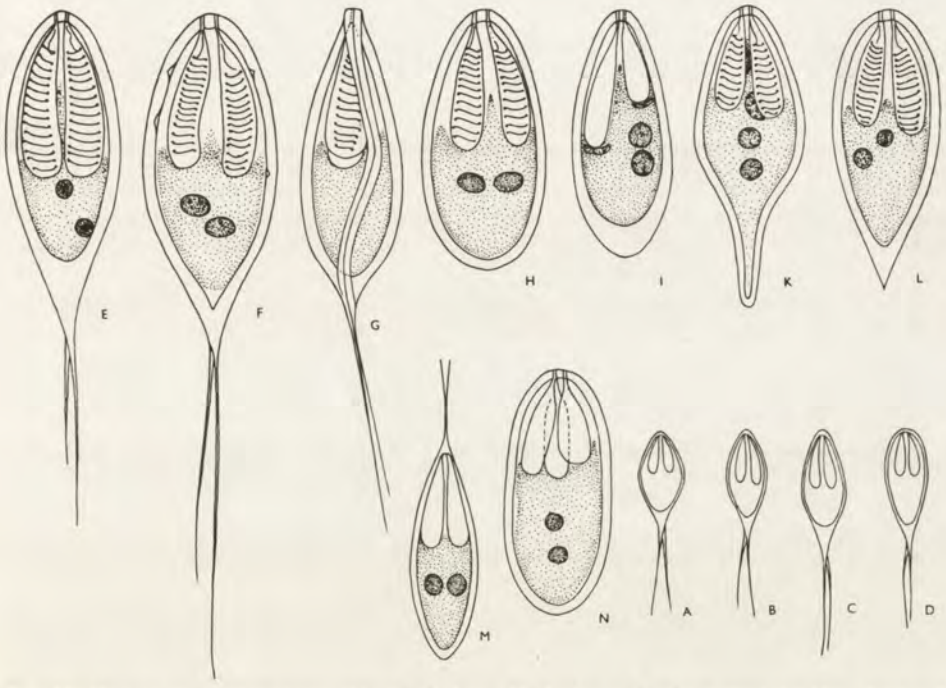

Fig. 13. *Henneguya psorospermica* Thélohan, 1895. A — D. Variation in the shape of normal spores. E — G. Structure of the normal spores, H — N. abnormal ones. A — D. 1250×, E — N. 2500×

1 mm was observed. The degree of development of trophozoit was studied only on smears. I stated that in small cysts (below 1 mm) a high number of vegetative and generative nuclei are present as well as of earlier stages of sporogenesis whereas the number of formed spores is small. In contrast to this, in big cysts (1—2.5 mm) the number of spores is high, and as to the earlier development stages only the late sporoblasts occur; vegetative nuclei are very scarce. These observations suggest that the propagation of trophozoit by means of nucleogony fails to extend on the whole period of sporogenesis. Consequently,

Ta

Dimensions of spores of *Henneguya lobosa*

Author	Cohn 1895 ac. Kudo 1919	Wegener 1909	Auerbach 1911 ac. Kudo 1919
Host	*E. lucius*	*E. lucius*	*E. lucius*
Length of spore	30—40	35—40	30
Length of spore cavity	11.5—15	13—15	—
Length of tails	22—28	20—25	—
Breadth of spores	5—6.5	5	4—6
Thickness of spore	—	—	—
Polar capsules	6.5—8×2—2.5	6—7×2.5—5	6
Filament	—	—	48—54

the cysts in which the free generative nuclei and early sporoblasts are not observed but only spores and some late stages of sporogenesis, would grow only in a trophic way. The size of cyst seems to be distinctly related to the degree of advancement of sporogenesis, therefore in the cysts of a diameter over 2 mm it would be almost entirely completed since the number of sporoblasts related to the general number of spores and sporoblasts amounts in them approx. 3%, in the cysts of dimensions 1.5 mm — 25%. These suggestions require control by examination of sections of a high number of cysts of different dimensions. Monosporous sporoblasts.

Spores fusiform. The diversity of forms is presented in Fig. 13 A—D. The most frequent is the form B, the least common one — D. The shape of spores as well as their dimensions show a high variability within one cyst — i.e. the variability of spores originating from one mother-spore for the trophozit — similarly as in the general mass of spores originating from different cysts of different host individuals. The polar capsules of even or almost even length lie parallel to one another. Differences in length of the polar capsules amount 0—1 μ. Thickness of the spore shell is 0.5—0.6 μ and in the sutural edge — 0.5—0.8 μ. The filament 45—100 μ long forms a spiral with 13—16 coils. 120 spores from 10 cysts were measured.

As follows from the comparison (Table 6) the spores examined in the present study are more narrow and their polar capsules are smaller, however the range of their variability coincides in some degree with the variability ranges which were reported earlier.

Besides the normal spores, i.e. spores with tails — which are typical for the genus *Henneguya* — tailless spores occur as well. Among these, spores are found with a shell rounded in its posterior part (Fig. 13 H), narrowed and sometimes thicker than in the remaining part of spore (Fig. 13 I), much more narrow and elongated (Fig. 13 K) and also narrowed and tapering (Fig. 13 L). The number of tailles spores in particular cysts is various and seems to be related to the tail length of normal spores. Considering the tail length in the group of normal

ble 5
according to different authors in μ

Dogiel 1932	Jirovec 1942	Lom 1961	Schulman 1962	Present author
E. lucius	*E. lucius*	*E. lucius*	*E. lucius*	*P. fluviatilis*
40—45	26	34—37	30—57	34.5—54.8
—	15	12—13	11.5—27	12.4—19.5
—	10—12	—	20—30	17.5—35.5
4—5	—	3—4	4—8	4.7—7.7
—	—	—	4—4.6	3.1—4.2
6—7	5—6×1.2—2	4×1.5	6—10×1.5—2.5	6.5—9.4×1.2—1.9
—	—	—	—	43—50

Table 6
Dimensions of spores of *Henneguya psorospermica* according to different authors in μ

Author	Cohn 1896 ac. Kudo 1919	Wegener 1909	Dogiel 1932	Baryševa, Bauer 1957	Lom 1961	Schulman 1962	Present author
Length of spore	29—38	35—38	29—40	29	36	—	12—14
Length of spore cavity	15—20	15	15—20	—	16	10—14	7.7—16.2
Length of tail	14—18	15—20	—	—	—	14—30	1—26.6
Breadth of spore	9—10	7—8	7—9 5—6	5.5	5—6	7—9	3.3—7.1
Thickness of spore	—	—	—	—	—	5—6	3.3—5.2
Polar capsules	8—11×2	8×2—3	8—11	7.6	8—9 × 2.2	7—11 × 2.3—3	4.3—7.9 × 1—1.9

spores, 3 types have been differentiated: short-tailed (tail is shorter than the half length of the spore cavity), middle-tailed (of a tail length not exceeding the length of the spore cavity), long-tailed (tail is longer than the spore cavity) (Table 7).

The percentage ratio has been calculated for cysts taken from different host individuals, based on measurements of 100—150 spores from each cyst.

As follows from the table, the higher the percentage of short-tailed spores, the higher the percentage of anomalous spores.

Besides the tailless spores, one spore was found with anterior outgrowths of the shell (Fig. 13 M), and another one with 3 polar capsules (Fig. 13 N).

Thelohanellus pyriformis (Thélohan, 1892) (Fig. 14 A—F)

Vegetative stage: cysts elongated with smooth or folded margins reaching up to 2 mm in length.

Spores: pear-shaped, often asymmetric in relation to the spore axis, their anterior end being bent towards one side. The polar capsule often shifted side-

Table 7

Percentage share of abnormal spores and of different types of normal spores of *Henneguya psorospermica* in cysts from different host individuals

Abnormal	1	1.2	3	11.4	39.6	48.8	69
short-tailed	3.1	8.4	15	14.2	55.2	38	31
middle-tailed	22.2	62.2	66	57.1	5	9.5	0
long-tailed	73.7	28.2	16	17.3	0.2	3.7	0

ways. Sporoplasm highly vacuolized. Measurements were made of 30 spores. Dimensions: length 15.6—19.4 μ, breadth 6.8—9.2 μ, thickness 6.3—7 μ, polar capsule 7—8.9×2.7—3.7 μ. Spore shell 0.4 μ thick, in the sutural edge 0.5—0.6 μ. Sutural markings absent. Filament measures approx. 200 μ in length and forms a spiral composed of 12—14 coils inside the capsule.

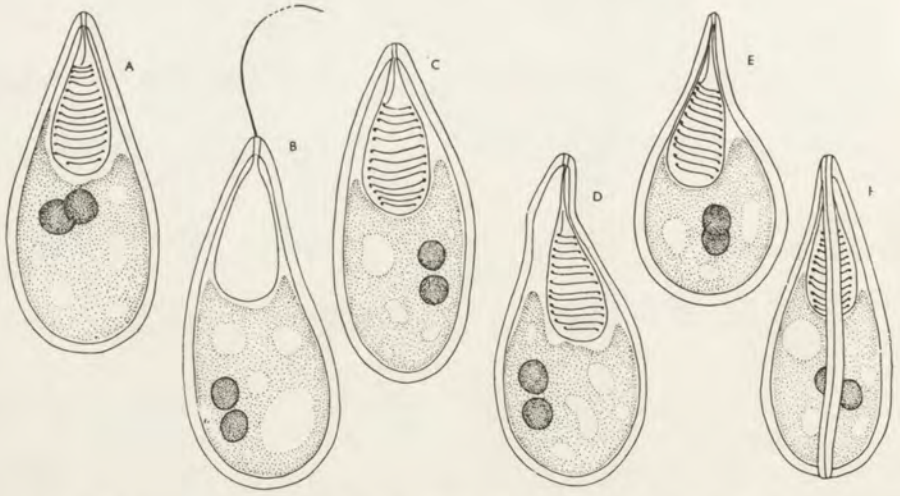

Fig. 14. *Thelohanellus pyriformis* (Thélohan, 1892). A — F. Structure of the normal spores. 2500×

Summary

The article contains the description of 14 species of *Myxosporidia* of the genera: *Myxosoma, Myxobolus, Henneguya* and *Thelohanellus* which are gill parasites of 9 species of fishes from the Zegrze Lake near Warsaw. Almost in all the species, the range of variability was ascertained which was higher or different from those reported in litterature. Attention was payed to the features of sporoblasts which may serve as a diagnostic character. Approximately in all the species studied, the presence of two types of anomalous spores was stated: 1. anomalies of shape and 2. developmental anomalies characterized by an increased or diminished number of polar capsules than that typical for the given species.

STRESZCZENIE

Praca zawiera opisy 14 gatunków *Myxosporidia* z rodzajów: *Myxosoma, Myxobolus, Henneguya* i *Thelohanellus* pasożytujących na skrzelach 9 gatunków ryb pochodzących z Jeziora Zegrzyńskiego. U wszystkich prawie gatunków stwierdzono większy lub inny zakres zmienności, niż podawany dotychczas w literaturze. Zwrócono uwagę na rodzaj sporoblastów, mogących służyć jako cecha diagnostyczna. Stwierdzono u prawie wszystkich badanych gatunków obecność spor anormalnych dwojakiego typu: anomalie kształtu i anomalie rozwojowe, charakteryzujące się większą lub mniejszą liczbą torebek biegunowych, niż typowa dla danego rodzaju.

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Formation des taxocénoses des *Testacea* dans le lac de Zegrze

Kształtowanie się taksocenoz *Testacea* w Jeziorze Zegrzyńskim

En 1961 et 1962 des recherches furent faites dans quelques petits bassins situés à l'endroit où le Bug se jette dans la Narew (Moraczewski 1965). Ces bassins se trouvaient dans la vallée des deux rivières. Dès moment où fut construit à Dębe le barrage de la Narew, le terrain des recherches a formé le fond du réservoir d'eau, c'est-à-dire du lac de Zegrze. En 1963—1964 de nouvelles recherches furent faites dans ce terrain (materiaux réunis par l'auteur dans les cadres des recherches de l'Institut de l'Économie de l'Eau). L'objectif de ces travaux était l'étude de la faune de *Testacea* de quelques petits bassins, qui formerent plus tard la base des recherches sur la faune habitant le nouveau lac, ainsi que sur le processus qui peupla le réservoir dans les premières années qui suivirent l'inondation. On a aussi examiné les populations de *Testacea* des petits bassins et du lac de barrage, en suivant leur développment dynamique durant deux ans. L'étude présente est la suite des recherches entreprises par l'auteur en 1957 sur les taxocénoses des *Testacea* provenant de biotopes aquatiques variés. Il semble qu'une connaissance exacte et une classification de ce type de populations de *Testacea* peut-être intéressante pour l'écologie des protozoaires et d'autres micro-organismes, qui constituent un des chaînons les plus importants d'alimentation de la biocénose aquatique. On signale de plus en plus souvent des observations écologiques concernant ce groupe d'organismes dans la littérature mondiale.

Il semble que le problème du peuplement d'un nouveau milieu par des organismes aquatiques est des plus intéressants. Ces processus ont été examinées dans d'autres groupes d'animaux. La faune de fond, et particulièrement les *Tentipedideae* ont été étudiés. Jusqu'à présent aucune étude sur le peuplement des nouveaux bassins n'a été faite concernant les Testacés. Ceeb 1950, Goorvitch 1961 et Štepanek 1954 ont uniquement donné des listes des espèces trouvées dans les réservoirs de barrage durant la première année de remplissage. Aucun des auteurs ne disposait cependant de matériaux récoltés sur le terrain du réservoir encore vide.

Le présent article serait donc une première tentative de détermination de la cadence et des sources de peuplement par les Testacés, qui constituent l'élément de base du microbenthos, groupe très variable et en même temps jouant un rôle decisif dans la destruction des sédiments de fond.

Description de réservoir

Le réservoir du barrage de Dębe est composé de trois parties (Fig. 1). Deux bras qui correspondent aux rivières Narew et Bug constituent les anciens lits de ces rivières, légèrement appronfondis et élargis. Le lac de Zegrze forme la partie centrale du réservoir et se trouve en aval de l'embouchûre du Bug dans la Narew. La troisième partie du réservoir, c'est le segment qui va de Zegrze au barrage de Dębe. Tout comme en amont c'est l'ancien lit de la Narew, mais environ trois fois plus large, et d'une profondeur qui atteint 6 à 8 mètres.

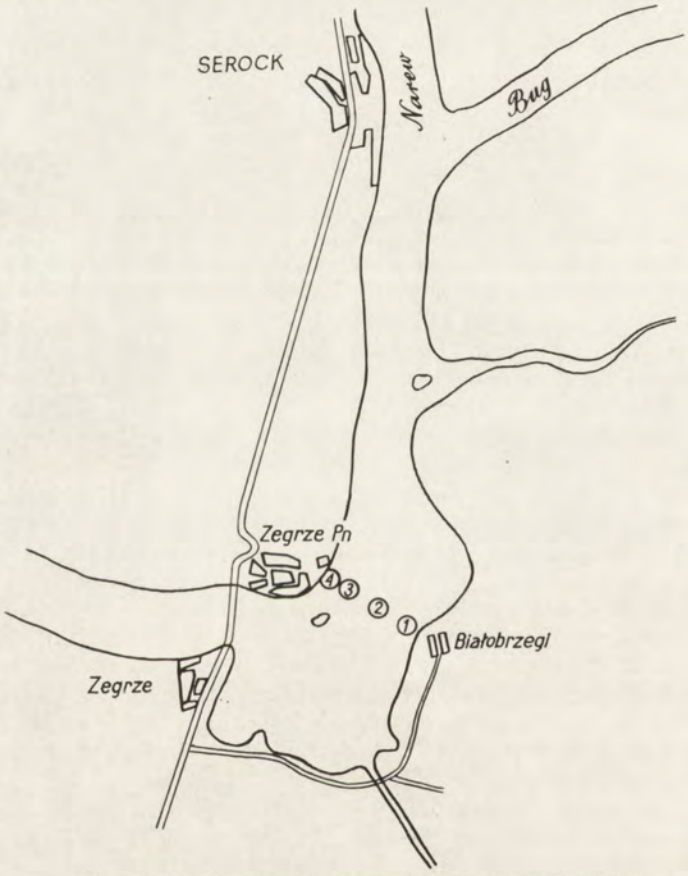

Fig. 1. Le lac Zegrze. 1 — station de recherche

Des observations de modèle et le relief du terrain environnant le bassin indiquent que c'est uniquement la partie centrale du réservoir du lac de Zegrze qui presente des conditions lacustres, c'est-à-dire que le conurant fluvial y a été réduit à zéro. Sur terran du lac l'eau forme deux endroits stagnants, que mobilisent uniquement les courants d'inondation. Les petits bassins examinés ont été trouvés sur le fond d'un de ces endroits.

Les observations physico-chimiques poursuivies ont démontré l'existence à certains endroits du lac, été comme hiver (sous la glace) d'une stratifications

thermique et d'oxygène. Cette stratification se forme seulement au cours d'une période de calme et de chaleur. La surface du réservoir est fortement agitée par les vagues et sa position n'est pas favorable à la formation de superficies obritées du vent. Ce phénomène ne pouvait donc jouer un rôle trop important. Au station 1, hiver comme été la température de la vase était toujours plus basse que cella de l'eau qui le recouvrait. Ce phénomène ne fut pas constaté aux endroits moins profonds.

Le fond du réservoir dans les bras du Bug et de la Narew est assez varié et la profondeur de l'eau, en dehors du lit de la rivière ne dépasse pas 3 m. La partie centrale du réservoir, le lac de Zegrze peut être divisée quant au relief du fond — en deux parties. La partie supérieure, depuis l'embouchûre de la rivière Rządza jusqu'à la localité de Białobrzegi a un fond très varié. C'est là que se trouvaient les petits lacs inondés de Białobrzegi et Ostrowiec et la profondeur du réservoir à cet endroit mesure environ 8 m. Soixante-dix pourcents environ de cette partie du lac atteignent 3 m de profondeur. La section profonde de la riviere depuis la localité de Białobrzegi jusqu'à la plage et le pont de la route à Zegrze a moins de 3 m de profondeur. La partie inférieure du réservoir, entre Zegrze et le barrage est plus profond, à certains endroits le fond affleure à la surface. Généralement parlant, la partie du réservoir d'une profondeur de 2.5 à 3 m présente le pourcent suivant:

Pułtusk — Serock — 30% environ,
Popowo — l'embouchûre de la Rządza — 60% environ,
l'embouchûre de la Rządza — le barrage — 8% environ.

Le caractère du sol et les inondations continues étaient cause que toute la vallée de la Narew et du Bug, dans les parties qui furent inondées par suite du barrage, était utilisée en majorité comme pâturages et près — 35%. Les forêts consituaient — 3.8%, les champs cultivés — 2%, les broussailles et les bords plantés d'osiers — 32.2%, les anciens lits de rivières et les mares— 27%.

La végétation qu'on trouve au bord de la Narew, du Bug et des petits bassins peut être considérée comme initiale pour le lac. Les espèces qui y poussent furent les premières à paraître parmi la flore du réservoir. Jusqu'à présent, les espèces suivantes ont paru dans l'enceinte du réservoir: le poivre d'eau, les nénuphars jaunes, la cornifle, l'élodée et diverses polygonacées. Ce furent avant tout les plantes à feuilles flottantes ou submergées qui s'y établirent les premières. A la fin de la deuxième année de l'existance du bassin, sur ses bords commença à apparaître le végétation de émergée.

Quatre points furent choisis sur le lac, disposés le long de la section depuis Białobrzegi jusqu'à Zegrze, dans le terrain où précédemment se trouvaient les petits bassins examinés.

Le point 1 — à environ 40 m du bord, à la limite de la localité de Białobrzegi. Le bord à cet endroit n'était pas endigué, en pente raide, sablonneux, les habitations rares. La profondeur de l'eau comportait 7 m environ. Ce poste se trouvait sur le terrain de l'ancien lac d'Ostrowiec. Le fond était recouvert de vase noire gélatineuse, bien macérée. On y a observé quelquefois une stratification thermique et d'oxygène sans grande importance.

Le point 2 — se trouvait à 200 m du bord, sur le lac. L'eau y avait 3 m de profondeur. A cet endroit croissait en assez grande quantité *Poligonum ambiguum*. Le fond était une ancienne prairie, qui au cours de recherches fut recouverte par une couche de vase d'environ 3 cm.

Jusqu'à la fin de l'année 1964, on distinguait parfaitement la limite entre la couche d'herbe et la vase qui la recouvrait.

Le point 3 — à 15 m environ du bord nord du lac. L'eau avait 0.5 m de profondeur. En 1964, on pouvait y remarquer l'apparition de quelques espèces végétales qui recouvraient le fond d'une couche assez touffue. Ces espèces étaient: *Poligonum ambiguum, Potamogeton* sp. et aussi des roseaux. Avant le remplissage du réservoir, cet endroit formait une petite colline sablonneuse. Au cours de l'année 1963 il fut recouvert d'une couche d'environ 4 cm de vase et au cours de l'année suivante d'une couche végétale.

Le point 4 — occupait un point sur l'ancien courant principal de la rivière Narew. L'eau y atteignait 5 m de profondeur. Le fond était sablonneux, recouvert d'une mince couche de vase. L'épaississement de cette couche au cours de 1964 indiquerait une stabilisation du fond.

Méthode

Les échantillons étaient prélevés sur le fond du réservoir à l'aide d'un appareil d'échantillonage de fond, modifié par Goorvitch et Ceeb (1958). La modification permettait le prélèvement d'une pièce circulaire du fond d'une surface de 8 cm^2. En même temps on prélèvait 1 cm d'eau d'au dessus du fond et 1 cm de pelogène. Après conservation, l'échantillon était couvert d'eau et dilué à un volume de 100 cm^3, et les Testacées comptés dans trois cuvettes (1 cm^3) à l'aide d'un microscope retourné. Le nombre d'individus était ensuite calcule par cm^2 de surface. Pour plus de com modité dans le tracé des courbes représentant les variantes du nombre d'individus, les logarithmes décimaux de ces nombres étaient calculés. Pour l'évaluation de la fidélités et de la constance on se servait de la méthode décrite par l'auteur dans les études précédentes Moraczewski (1962, 1963).

Il semble que la méthode de prélèvement des échantillons permet d'opérer une analyse assez exacte du dynamisme des populations de Testacés. Avant de procéder aux expériences, on fit certains essais en prélevant des échantillons de matériaux des couches profondes. Il fut constaté que 95% des espèces habitent la couche supérieure de la vase.

Description de la faune

Dans les station examinés du lac de Zegrze, 66 espèces ainsi que 17 formes et variétés de Testacés ont été trouvées, dont 9 étaient nouvelles dans la faune de Pologne (Table 1). Parmi la population de ces milieux, 67 espèces et formes furent trouvées dans les petits bassins, qui auparavant occupaient ces terrains, et 15 habitaient la Narew avant la construction du réservoir. Aucune des autres ne fut trouvée jusqu'à présent sur ce terrain, ce qui n'est pas une preuve de leur non-existence. Cette liste permet de supposer que la base de la faune de Testacés dans le réservoir est constituée par les petits bassins qui l'occupaient précédemment. Goorvitch 1961 est parvenu à la même conclusion en étudiant la composition générique du microbenthos des lacs de barrage. L'analyse de la composition de la faune d'après l'Écologie générale des Thecamoebiens de Charder a prouvé que les espèces classées comme aquatiques dominent (Fig. 2). Les espèces non-aquatiques, c'est-à-dire sphagniques, des mousses ou du sol constituent à peine 9.6% de toute la faune et peuvent être considérées à coup

sûr comme élément accidentel charrié par l'eau ou lavé des bords. La notion de l'espèce aquatique est très inexacte, puisque on y trouve tout autant les populations habitant le fond que le périphyton, et que les espèces habitant les eaux interstitielles du sol ou des mousses y sont incluses.

Il a été constaté que nombre d'espèces faisant partie de la faune des sphaignes ou des mousses, et trouvées dans les petits bassins de ces terrains se sont maintenues dans le réservoir du barrage. 62% des espèces des petits bassins sont passès dans le rèservoir.

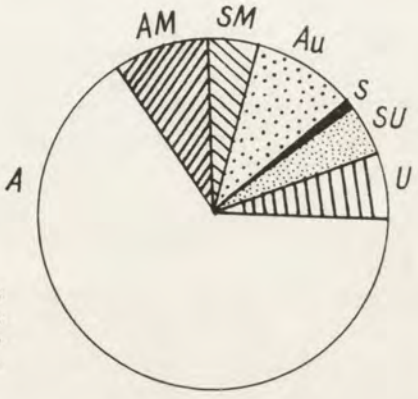

Fig. 2. Participation (en %) des differents groups ecologiques dans le faune des Testacés du lac Zegrze. Espèces: *A* — aquatique, *M* — mousse, *S* — sphaigne, *U* — ubiquiste, *Au* — autré

La détermination des caractères écologiques des espèces, nouvelles pour la Pologne a été élaborée en s'appuyant sur la littérature d'après un schéma appliqué déjà précédemment par l'auteur.

Arcella rotundata Playfair

Cette espèce se trouve dans les lacs (Schönborn 1962), les réservoirs de barrage (Štepanek 1959), les étangs (Štepanek 1963), les rivières (Golemansky 1963, Štepanek 1962, 1963, Chardez 1964), les marécages (Štepanek 1962), parmi les plantes aquatiques (Deflandre 1928, Bartoš 1954, Schönborn 1962, Golemansky 1963), sur le fond (Schönborn 1962, Štepanek 1962, 1959, Golemansky 1963) aussi bien que sur les mousses humides revétant les arbres (Chardez 1961). C'est donc une espèce lacustre, périphytonique.

Centropyxis gibba var. *inermis* Bartoš

D'après Chardez ce serait une espèce aquatique (1965). Bartoš (1964) l'a rencontrée dans des torrents sur des pierres, Štepanek en 1952 l'a trouvée dans la vase de petits bassins et en 1962 dans des tourbières. Cette espèce pourrait être paléophile.

Centropyxis marsupiformis var. *obesa* Deflandre

D'après Chardez 1965, c'est aussi une espèce aquatique. Deflandre 1929 a constaté sa présence dans des marécages et des étangs. Bartoš 1954 mentionne qu'on peut uniquement la trouver parmi les plantes. Les informations de la littérature à ce sujet sont assez pauvers, ce qui ne permet pas de formuler des conclusions quant à son habitat.

Table 1

Index des especes trouvées dans les stations etudies

Les stations	1963				1964			
	I	II	III	IV	I	II	III	IV
Difflugia globulosa	13.2 IV	21.7 V	7.2	25.0 V	22.0	22.0 V	38.0 V	42.0 V
Difflugia gramen var. *globularis*	11.4 IV	3.1	18.4 V	11.0 V	4.0	14.0 III	12.0	6.0
Difflugia oblonga var. *brevicolis*	1.6	0.6	2.4	2.1	2.0	4.0	8.0	6.0
Centropyxis ecornis	6.8 V	5.4	16.8 V	7.0	8.0	4.0	12.0	10.0
Difflugia acuminata	1.8	9.0	1.6	4.2	5.0	8.0	4.0	0.6
Difflugia oblonga	7.2	9.0 V	1.2	4.2	14.0 V	14.0 V	6.0 V	0.6
Centropyxis constricta	10.4 V	1.8	4.8	1.4	4.0	—	—	2.0
Difflugia oblonga var. *longicolis*	9.0	30.7 III	12.2 V	5.0	50.0 V	4.0	—	—
Centropyxis orbicularis	4.2	1.2	4.8	—	2.0	4.0	2.0	2.0
Cyphoderia ampula	1.2	0.6	—	0.7	2.0	2.0	—	20.0IV
Difflugia elegans	1.0	0.6	—	—	2.0	4.0	—	8.0
Difflugia corona	1.0	1.8	23.8 III	3.5	—	2.0	6.0	—
Cyclopyxis arcelloides	0.6	0.6	2.1	—	—	—	—	2.0
Difflugia oblonga var. *parva*	1.2	—	0.6	—	—	6.0	6.0	2.0
Euglypha loevis	1.6	1.8	0.6	—	1.0	—	—	6.0
Difflugia pristis	2.0	—	—	—	—	2.0	2.0	2.0
Centropyxis cassis	7.2	4.0	0.6	0.7	—	—	2.0	—
Arcella gibosa	1.8	0.6	—	—	2.0	2.0	—	—
Euglypha acanthophora	1.6	2.6	0.6	—	—	—	2.0	—
Arcella megastoma	0.6	1.5	2.8	2.8	2.0	—	—	—
**Euglypha rotundata*	0.6	—	0.6	—	2.0	—	—	—
Difflugia oblonga var. *angusticolis*	1.2	4.0	1.2	—	2.0	—	—	—
Difflugia oviformis	0.3	1.8	0.6	2.8	—	—	—	—
Difflugia fallax	2.5	2.6	3.0	1.4	—	—	—	—
Euglypha tuberculata	2.6	0.5	4.0	2.2	—	—	—	—
Difflugia lobostoma	2.4	—	2.2	2.1	—	—	—	—
Trinema enchelys	4.2	2.0	1.0	—	—	—	—	—
Arcella haemisphaerica	1.2	1.2	1.2	—	—	—	—	—
Arcella rotundata var. *aplana*	1.2	—	0.6	—	—	—	—	—
Weilesella eboracensis	1.0	—	0.5	—	—	—	—	—
Centropyxis plathystoma	0.6	1.0	—	—	—	—	—	—
Difflugia similion	0.6	1.2	—	—	—	—	—	—
Cucurbitella mespiliformis	0.6	4.0	0.6	—	—	—	—	—
Difflugia viscidicula	0.6	0.6	—	10.5 III	—	—	—	—
Difflugia lanceolata	0.6 I	—	—	—	—	—	—	—
Centropyxis discoides	0.6 I	—	—	—	—	—	—	—
Arcella vulgaris	0.6 I	—	—	—	—	—	—	—
**Trinema verrucosa*	0.6 I	—	—	—	—	—	—	—
**Euglypha aspera*	1.0 I	—	—	—	—	—	—	—
Trinema lineare	3.5 II	—	—	—	—	—	—	—
Difflugia oblonga var. *lacustris*	3.9 II	—	—	—	—	—	—	—
Arcella discoides var. *scuteliformis*	2.9 III	—	—	—	—	—	—	—

cont. tabl. 1

Les stations	1963				1964			
	I	II	III	IV	I	II	III	IV
Difflugia oblonga var. *claviformis*	1.2 I	—	—	—	—	—	—	—
Centropyxis aculeata	—	3.5	1.2	—	—	—	8.0	10.0
Trigonopyxis arcula	—	0.5	—	—	—	—	2.0	—
Difflugia acuminata var. *acaulis*	—	3.6	—	—	2.0	—	—	—
Centropyxis spinosa	—	0.6	3.0	—	—	—	—	—
Difflugia mica	—	2.0	—	—	—	—	—	—
Arcella gibbosa var. *loevis*	—	0.6	—	—	—	—	—	—
Pyxidicula cymbalum	—	0.6	—	—	—	—	—	—
Difflugia acuminata var. *laevanderi*	—	6.0	—	—	—	—	—	—
Difflugia urcoelata	—	—	3.0	2.1	2.0	—	20.0 V	2.0
Difflugia bidens	—	—	3.0	2.1	—	—	6.0	6.0
Pontigulasia bigibbosa	—	—	1.8	—	—	2.0	—	—
Difflugia scalpellum	—	—	0.6	—	6.0	—	—	—
**Difflugia dificilis*	—	—	3.6	3.5	—	—	—	—
Centropyxis kahli	—	—	10.8	—	2.0	—	—	—
Cyphoderia loevis	—	—	3.0	2.1	—	—	—	—
Pontigulasia spectabilis	—	—	3.0	0.7	—	—	—	—
**Contropyxis gibba* var. *inermis*	—	—	1.5 I	—	—	—	—	—
Euglypha cristata	—	—	0.5 I	—	—	—	—	—
Euglypha scutigera	—	—	3.5 I	—	—	—	—	—
Corynthion dubium	—	—	1.0 I	—	—	—	—	—
Plagiopyxis callida	—	—	0.5 I	—	—	—	—	—
Difflugia cilindricus	—	—	0.6 I	—	—	—	—	—
Corynthio pulchellum	—	—	3.0 I	—	—	—	—	—
Difflugia gramen	—	—	—	1.4	2.0	4.0	10.0	4.0
Cyphoderia trochus		—	—	2.8	2.0	2.0	2.0	6.0
Difflugia acuminata var. *curvata*	—	—	—	0.7	—	4.0	—	—
Difflugia avelana	—	—	—	1.4	—	—	—	—
**Arcella rotundata*	—	—	—	1.4	—	—	—	—
Difflugia amphora	—	—	—	0.7	—	—	—	—
Plagopyxis labiata	—	—	—	—	2.0	—	—	—
Tracheluglypha dentata	—	—	—	—	2.0	—	—	—
Cencropyxis gibba	—	—	—	—	2.0	—	—	—
Centropyxis plathystoma var. *armata*	—	—	—	—	—	2.0	—	—
Difflugia oblonga var. *kempni*	—	—	—	—	—	2.0	—	—
Centropyxis marsupiformis	—	—	—	—	—	—	6.0	—
**Centropyxis marsupiformis* var. *obesa*	—	—	—	—	—	—	6.0	—
Difflugia pulex	—	—	—	—	—	—	2.0	—
**Difflugia mamiliaris*	—	—	—	—	—	—	2.0	—
Pseudodifflugia gracilis	—	—	—	—	—	—	—	2.0
Leusquereusia modesta	—	—	—	—	—	—	—	2.0

* Le signe indique les especes nouvelles pour la Pologne.
Le chiffre arabe indique la quantité d'individus/cm².
Le chiffre romaine indique la classe de constance.

Difflugia dificilis Thomas

Espèce aquatique (Chardez 1965), rencontrée dans les petits bassins (Thomas 1955, Chardez 1964), dans le sapropèle (Chardez 1962). On peut supposer que c'est une espèce paléophile.

Difflugia mamiliaris Penard

Espèce aquatique (Chardez 1965), rencontrée dans les lacs (Grospietsch 1957) et les petits bassins (Chardez 1964). Cette espèce habite les bassins d'eau quelle que soit leur grandeur et leur profondeur.

Euglypha aspera Penard

Rencontrée le plus souvent dans les lacs (Penard 1902, Kourova 1925, Decloitre 1962, Grospietsch 1957), dans le sapropèle (Bartoš 1954). D'après ces données on peut la classer parmi les espèces lacustres paléophiles.

Euglypha rotunda Weiles

Cette espèce d'après Thomas 1960, Decloitre 1962 et Chardez 1965 se trouve le plus souvent dans le sol et les mousses, plus rarement dans les sphaignes.

Tracheleuglypha dentata (Vej.) Deflandre

Dans les petits bassins (Deflandre 1927), les tourbières (Opravilova 1960) et le sol (Bartoš 1954), dans les mousses (Cash 1955, Bartoš 1954, Schönborn 1962a) et les sphaignes (Chardez 1960, Penard 1962). On l'a trouvée aussi sur le fond d'un réservoir de barrage (Štepanek 1959). D'après Chardez 1965, c'est une espèce aqua-sphagnique.

Trinema verrucosa Franken

Malgré l'opinion de Chardez qui la considère comme une espèce aquatique (1965), on l'a rencontré aussi dans le sol (Thomas 1960), les sphaignes (Deflandre 1927), la mousse humide des bois (Štepanek 1962), les tourbières (Bartoš 1954). Il semble que c'est plutôt une espèce qui tient aux sphaignes ou aux mousses humides, rencontrée par hasard seulement dans de plus grands bassins aquatiques.

Les taxocénoses des Testacés du lac de Zegrze

Des tentatives de classification ont été entreprises pour les populations de Testacés en utilisant les matériaux collectionnés dans les stations choisis sur le lac de Zegrze. C'est ainsi qu'on a isolé une série de taxocénose en se servant des méthodes mentionnées plus haut.

La base de ce travaux reposait sur l'examen du développement des populations, leur dynamisme annuel et la comparaison des résultats d'observations de plusieurs années. Les matériaux furent rassemblés au cours de deux années. Entretemps, en hiver 1963—1964, une pollution du réservoir par suite d'écoulement de déchets de la sucrerie eut lieu, ce qui ne fut pas sans influencer le microbenthos c'est sans doute pour cette raison que les populations de 1964 ne sont pas la succession de celles de l'année précédente. La deuxième cause de cette situation serait que des masses importantes d'eau avaient été écoulées du réservoir, ce qui entraîna la circulation du pélogène constituant l'habitat des Testacés.

Première station — on a trouvé ici au cours de 1963—43 espèces. Table 1 présente les moyennes arihmétiques du nombre d'individus au cm² de fond pendant toute l'année. Les espèces dominantes étaient les suivantes: mai — *Trinema enchelis,* juin — *Centropyxis cassis,* juillet — *Difflugia oblonga,* août — *Difflugia globulosa,* septembre — *Difflugia gramen* var. *globularis.* En octobre on n'a pas noté d'espèce dominante distincte. Le changement continuel de domination indique qu'une taxocénose stable ne s'était pas développée. Ce n'est que l'année suivante que la structure de cette population se stabilise. Jusqu'à fin juin domine *Difflugia oblonga* var. *longicolis,* et ensuite, jusqu'en octobre *Difflugia globulosa.* D'après les moyennes de toute l'année on peut considérer la population de Testacés de la première station comme taxocénose pleinement développée. Ces données indiquent que l'espèce dominante en moyenne en 1963 était *Difflugia globulosa* — 13.2 individus au cm², et que les adominantes étaient *Difflugia gramen* var. *globularis* — 11.7 indiv./cm², et *Centropyxis constricta* — 10.4 indiv./cm². Dans ce poste, neuf espèces constatées pouvaient être considérées comme caractéristiques pour l'endroit.

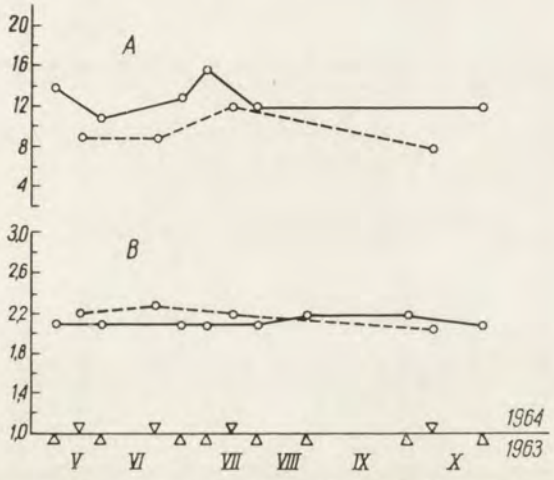

Fig. 3. Le nombre d'espèces (A) et le logarythm de nombre d'individus (B) en 1963 (ligne continue) et en 1964 (ligne pointillée) sur la station 1

Afin de mieux connaître le dynamisme de ces taxocénoses, au cours de 1963 et 1964, des diagrammes des variantes du nombre des espèces et des individus ont été élabores (exprimés en logarithmes). (Fig. 3, 7 A). Des oscillations insignifiantes du nombre des individus et des espèces, ainsi que le cours concordant des courbes pour ces deux années indiquent la stabilité de la population rencontrée à la station 1. Ce milieu est assez uniforme. Ces données sont confirmées par l'observation de la vase, ainsi que par l'histogramme, préparé sur la base de la fréquence des espèces particulières. D'après O d u m (1960), le milieu homogène est caractérisé par la plus grande quantité d'espèces appartenant à la 1re classe de constance, et la plus petite à la IVme, tandis que dans la Ve une augmentation insignifiante se fait observer. La taxocénose de Testacés mentionnée en 1963 répondait à ces conditions. Au cours de l'année suivante on a noté une certaine déviation. Il est possible qu'à la suite de changements physiques

du milieu, une nouvelle niche écologique s'est formée, qui troubla son uniformité.

Deuxième station-— on a pu y constater au cours de l'année 1963—37 espèces et en 1964 — 20 espèces de Testacés, dont douze étaient communes aux deux années. En 1963, les espèces dominantes étaient, en mai: *Difflugia oblonga* var. *longicolis,* et ensuite jusqu'à la fin de l'année *Difflugia globulosa.* L'année suivante jusqu'à fin juin — *Difflugia oblonga,* suivie de *Difflugia globulosa.* Sur la base des moyennes arithmétiques du nombre des individus au cm², et de la fréquence pour 1963, on a distingué l'espèces dominantes — *Difflugia globulosa* — 21.7 indiv./cm², et l'adominante — *Difflugia oblonga* var. *longicolis* — 30.7 indiv./cm². Cette dernière espéce a présenté une plus grande quantité d'individus au cm² que la dominante, mais ce fut un cas unique. La taxocénose de cette station possédait 4 espèces caractéristiques (Table 1). Tout autant le nombre d'espèces que celui des individus subissait des oscillations assez importantes au cours de l'année (surtout au printemps). Avec la fin de juin ces valeurs se stabilisent. Durant la deuxième année de l'existence du réservoir elles atteignent un certain équilibre. Ce milieu était homogène — de la vase entremêlée de *Poligonum ambiguum.* Ceci est aussi confirmé par l'analyse de la fréguence des espéces (Fig. 4, 7 B).

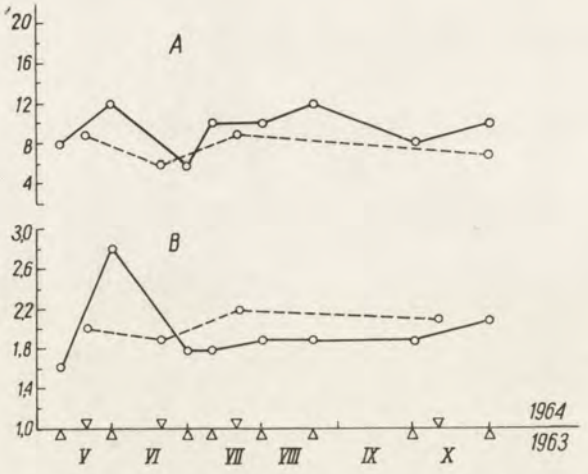

Fig. 4. Le nombre d'espèces et le logarythm de nombre d'individus sur la station 2 (pour description v. Fig. 3)

Troisième station — assez richement peuplée par les Testacés. Pendant la première année après le remplissage du réservoir, on y observe 45 espèces, pendant la deuxième — 27, dont 15 — communes aux deux années. Trente trois pourcents à peine des espèces de 1963 se retrouvent dans cette station et l'année suivante. La nouvelle population est composée à 45% d'espèces, non encore observées à cet endroit. La taxocénose en 1963 avait la structure suivante: dominante — en mai *Centropyxis ecornis,* juin — juillet *Difflugia oblonga* var. *longicolis,* août de nouveau *Centropyxis ecornis,* septembre *Difflugia corona,* octobre *Difflugia fallax.* En moyenne, la dominante de l'année était *Difflugia gramen* var. *globularis* 18.4 indiv./cm², les condominantes étaient *Difflugia*

corona 23.8 indiv./cm² (un cas seulement) et *Centropyxis ecornis* 16.8 indiv./cm². Sept espèces caractéristiques purent être distinguées.

La taxocénose de 1964 possédait une structure plus stable, en mai dominait *Difflugia urceolata*, et ensuite jusqu'à la fin de l'année *Difflugia globulosa*. En se référant aux moyennes, il convient de considérer aussi comme dominante *Difflugia globulosa*, et comme condominante *Difflugia oblonga*. Trois espèces caractéristiques ont pu être distinguées. Le nombre d'espèces et d'individus présentait des oscilations marquées, tout autant en 1963 qu'en 1964 (Fig. 5, 7 C). Malgré la couverture végétale du fond et une profondeur insignifiante, l'analyse

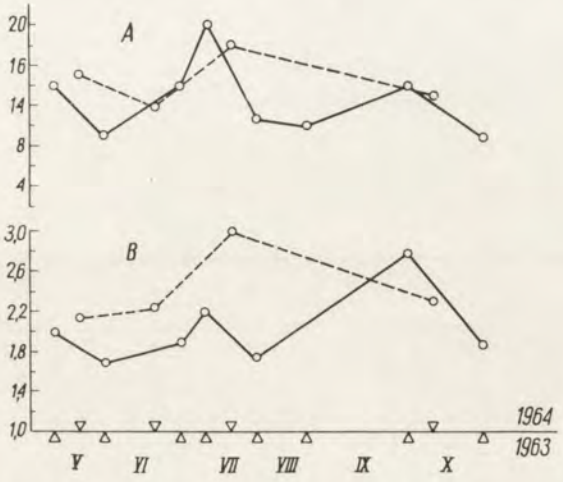

Fig. 5. Le nombre d'espèces et le logarythm de nombre d'individus sur la station 3 (pour description v. Fig. 3)

de fréquence des espèces indique l'homogénéite de ce milieu. On peut supposer que les différences mentionnées plus haut entre les taxocénoses des années particulières ainsi que les oscillations du nombre des espèces et des individus sont en relation directe avec la profondeur insignifiante de l'eau à cet endroit. Il en résulte toute une série de conséquences: la mise à nu du fond au cours du vidage du réservoir, l'écoulement de la vase pendant les inondations, un fort ensoleillement, un effet marqué des vagues. Tous ces facteurs exercent leur influence sur le microbenthos, et également sur la faune des Testacés.

Quatrième station — ancien lit de rivière couvert de sables mouvants ce qui a donné des conditions très spéciales, caractérisés par une faune très pauvre de Testacés. En 1963 on y a noté 29 espèces, et dans le courant de l'année suivante — 20, dont 11 provenaient de l'année prècédente; le reste était nouveau (45%). Jusqu'à la fin de 1963, on ne pouvait y distinguer une dominante. En août et septembre c'était *Difflugia globulosa* qui dominait. En mai 1964, la dominante était *Cyphoderia ampula*, une espèce très abondante dans la Narew (Moraczewski 1963). Depuis la fin de juin jusqu'en octobre dominait *Difflugia globulosa*. On peut déduire des moyennes la structure suivante de la taxocénose, caractéristique pour l'année 1963: dominante — *Difflugia globulosa* 25.0 indiv./cm², adominante *Difflugia gramen* var. *globularis* 11.0 indiv./cm² et

Difflugia viscicula 10.5 indiv./cm². Les espèces caractéristiques étaient *Arcella rotundata* 1.4 indiv./cm², et *Difflugia amphora* 0.7 indiv./cm².

En 1964, dans la taxocénose dominait aussi *Difflugia globulosa* 42.0 indiv./cm², l'adominante était *Cyphoderia ampula* 20.0 indiv./cm². Les espèces caractéristiques étaient *Pseudodifflugia gracilis* 2.0 indiv./cm², *Lesquereusia modesta* 2.0 indiv./cm².

Les courbes qui représentent le dynamisme de développement de la taxocénose en 1963 et 1964 sont antagonistes (Fig. 6, 7 D). En 1963, la taxocénose

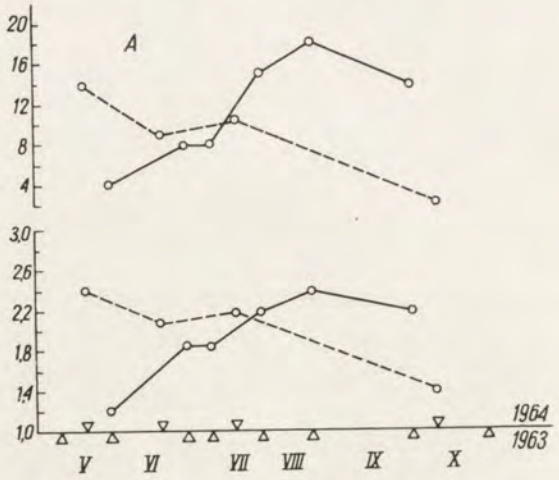

Fig. 6. Le nombre d'espèces et le logarythm de nombre d'individus sur la station 4 (pour description v. Fig. 3)

atteint son nombre maximum en septembre. Tout au contraire, durant l'année suivante, le plus grand nombre d'individus et d'espèces se place en juin. Ce phenomène pourrait trouver son explication dans des changements de courants ou dans d'autres facteurs hydrologiques, car comme l'ont confirmé les modeles examinés, la masse d'eau principale continue à suivre dans le lac la direction de l'ancien lit de la Narew.

En comparant sur le diagramme le cours des courbes de variation du nombre d'individus et des espèces au cours de l'année, on peut constater d'abord qu'il existe une corélation entre le nombre des espèces et des individus, et en second lieu — que le long de la section, c'est-à-dire de Białobrzegi à Zegrze les oscillations du nombre des espèces et des individus augmentent. A l'emplacement de la station 1 on n'observe pas dans le courant de l'année de changements visibles quanitatifs dans la taxocénose durant les deux premières années de l'existence du bassin. Ceci résulte sans aucun doute du caractère de ce milieu, et notamment de sa profondeur importante de changements infimes de la quantité d'oèygène, des petites oscillations de température. La stabilité des conditions et la taxocénose des Testacés, qui existaient préalablement au remplissage du réservoir sont cause que les changements qualitatifs dans son enceinte ne se répercutent pas sur sa composition quantitative.

Le deuxième station — deux fois moins profonde, située sur l'emplacement d'une ancienne prairie, a été, peuplée par une assez grande quantité d'espèces,

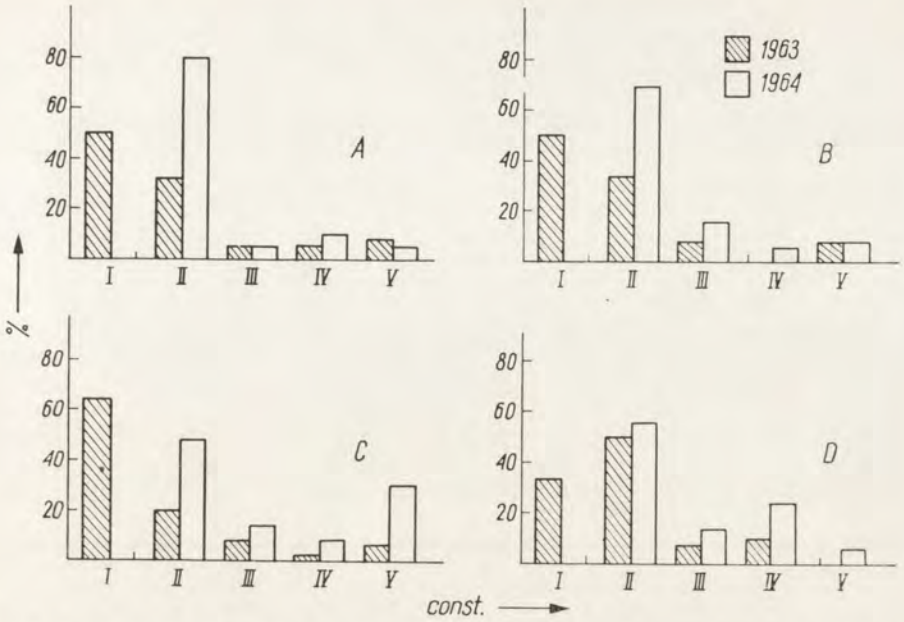

Fig. 7. Participation (en %) des classes des constances des espèces en taxocénoses des Testacés. A — station 1. B — station 2. C — station 3. D — station 4. *const.* — class de constance

probablement charriées par les eaux des rivières des petits bassins, situés dans la vallée inondée. Après une élimination préliminaire d'une série d'espèces (coude de la courbe en juillet) et l'établissement de la moyenne de densité du peuplement à 100 indiv./cm², la structure quantitative de la taxocénose se maintient à un niveau plus ou moins stable. La situation est analogue au cours de l'année suivante. On n'observe pas pourtant de peuplement aussi intense que dans la première année. Ceci provient du manque de sources abondantes de faune de Testacés, qui étaient representées par les petits bassins situés dans cette région. En se basant sur la section examinée on peut supposer que le fond du réservoir en 1963 dans cette région avait une densité de population d'environ 120 indiv./cm². C e e b 1958 — 75 indiv./cm², G o o r v i t c h 1961 — 60 indiv./cm². La formation d'un certain niveau stabilisé de population est aussi lié au substrat. Dans l'étude précédente (M o r a c z e w s k i 1965), on a traité d'une façon plus détaillée l'importance du substrat pour la faune des Testacés. Les remarques de l'auteur confirment les observations sur la composition spécifique et quantitative des taxocénoses dans le lac de Zegrze. Ceci se remarque en particulier dans la troisième station qui est rapidement envahi par les plantes et change son caractère de vase sablonneuse en celui de prairie sous-aquatique. G o o r v i t c h 1962 dans le réserwoir Kahowski distingue de nombreuses agglomérations de microbenthos, dont le psammophile et le poléophile pourraient être distingués dans le lac de Zegrze. Il semble que cela pourrait expliquer ces oscillations importantes tout autant du nombre d'espèces que du nombre d'individus. Les courbes de 1964 s'adaptent aux courbes de développement des microphytes de fond. La quatrième station était pleine de vase, ce qui favorisa un peuplement graduel

au cours de la première année entière, tandis que durant la deuxième année, pour des raisons inconnues la vase était graduellement entrainée par l'eau en même temps que les Testacés. Comme on le voit, le processus de peuplement du réservoir par les Testacés est très rapide. Le remplissage partiel d'eau du réservoir au début de l'hiver 1962/63, et l'inondation au printemps de 1963 favorisaient le lavage et la diffusion de Testacés des petits bassins. Comme on l'a remarqué au cours des investigations des petits bassins, la crue des eaux emportait de ces bassins d'assez grandes quantités de vase, en la redéposant sur les près. Cette vase aportée par les eaux d'inondation de règions assez distantes, est maintenant peut-être la source principale de la faune du microbenthos. Ce processus est favorisé par l'afflux violent de l'eau et de son écoulement beaucoup plus lent, comme cela eut lieu au moment du remplissage expérimental du réservoir. Un facteur assez important dans la cadence du peuplement est aussi la distance des petits bassins.

La comparaison des résultats de deux années indique que dans la majorité des postes une nouvelle taxocénose va se développer chaque année. Il semble que, du moment que la composition des agglomérations végétales (du substrat) sera stabilisée, de nouvelles taxocénoses vont se développer annuellement, si toutefois les effets resultant de violentes pollutions seront exclus.

Conclusions

1. Les petits bassins, formant autrefois le fond actuel du lac de Zegrze étaient peuplés par une faune très riche de Testacés, et dans beaucoup d'entre euè des taxocénoses purent être distinguées.
2. Au cours de la première année qui suivit le remplissage du réservoir, on a pu observer un rapide peuplement des nouveaux milieux et le développement de populations de Testacés.
3. La cadence du peuplement se trouvait en relation directe avec la position des anciens bassins, les conditions hydrologiques et le genre du substrat (sable, vase, végétation).
4. Au cours de la deuxième année on a pu observer dans la majorité des postes la formation de nouvelles, mais beaucoup plus stables taxocénoses de Testacés.
5. La formation des taxocénoses, renaissantes annuellement est liée, à l'envahissement du réservoir par les macrophytes et le développement de la faune qui règle le nombre de Testacés.

Résumé

On examine la dislocation des Testacés sur quelques stations du lac artificiel Zegrze au cours de deux années aprés le remplissage du réservoir. Dans le lac étudié on nota 83 espèces et variétès, dont 9 étaient nouvelles pour la faune de la Pologne. On constata, au cours de la première année qui suivit le remplissage du réservoir, un rapide peuplement des nouveaux milieux et développement des populations de Testacea. Au cours de la deuxième année on a pu observer la stabilisation des taxocénoses. La cadence du peuplement se trouvait en relation directe avec la position des anciens bassins et la genre du substrat.

STRESZCZENIE

Zbadano na kilku stanowiskach rozmieszczenia *Testacea* w sztucznym jeziorze w ciągu dwuch lat po jego wypełnieniu. Napotkano 83 gatunki i formy *Testacea*, z których 9 było nowych dla fauny Polski. Stwierdzono, że w pierwszym roku po wypełnieniu zbiornika nastąpiło intensywne zasiedlenie nowych środowisk przez *Testacea*. W ciągu drugiego roku obserwowano stabilizację taksocenoz. Tempo zasiedlania było związane z położeniem w stosunku do dawnych małych zbiorników i rodzajem podłoża.

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The behaviour of toxic trichocysts in the course of regeneration in *Dileptus cygnus* Clap. et Lachm.

Zachowanie się trichocyst w przebiegu regeneracji u *Dileptus cygnus* Clap. et Lachm.

The toxic trichocysts in ciliates are closely connected with the superficial layer of cytoplasm. This is associated with their aggressive-defensive functions. Their distribution in ectoplasm may be various: either regular as in *Paramecium,* or dominating on the anterior pole as in *Colpidium* (Cheissin and Mosevich 1962), or on the anterior pole only as in *Prorodon* (de Puytorac 1964), or their occurrence is just restricted to some precisely defined ectoplasm surface as the ventral side of proboscis in *Dileptus*. In ectoplasm, trichocyts are fixed by one end to the pellicle surface which was stated by Hayes 1938 for *Dileptus*.

The presence of trichocysts suspended in endoplasm was reported by many authors for various forms of *Holotricha* as e.g. in *Frontonia leucas* they were found by Wohlfarth-Bottermann 1953. In *Dileptus* trichocyts in endoplasm were described by Peschkowsky 1931 and Studitsky 1930. They may also be found in the photographs of the study of Dumont 1961. Studitsky 1930 obtained images interpreted as formation and degeneration of trichocyts in endoplasm of *Dileptus anser*. Trichocyts in *Dileptus* were studied most extensively by Dragesco 1952, 1962 a, 1963, by application of various methods. The more recent observations of this author proved, after application of electron microscopy, that the toxic trichocysts in *Dileptus* are of endoplasmic origin. Dragesco, Auderset et Baumann 1965 described the structure of trichocysts and the stages of their formation. A similar origin of trichocysts in other ciliates was decribed in *Paramecium* by Yusa 1963, and in *Frontonia* by Yusa 1965.

Formation of trichocysts was not related by the authors to any defined moment of the ciliate life cycle. Dragesco and others 1965 mentioned that they failed to find any special manner of trichocysts formation in the division processes. A question arises as to the character of trichocysts which are seen in endoplasm: whether they present a reserve stock of trichocysts or some immature stages on their way to the place of their location in ectoplasm. The possibility that trichocysts are accumulated as reserve organelles would account for the difficulty of finding the images of their formation. In this case formation of trichocysts might occur independently of the morphogenetic processes.

The study of regeneration in *Dileptus* was carried out by: Sokoloff 1924,

Vapenik 1927, Golińska and Doroszewski 1964, Golińska 1966. These studies failed to provide any information concerning the behaviour of trichocysts in the course of regeneration of the new proboscis. A high rate of the regeneration processes of *Dileptus* was noticed as well as the restitution of the possibility of exploding trichocysts in early stages of regeneration (Doroszewski 1961). The aim of the present investigations has been to follow the behaviour of trichocysts in the course of regeneration in *Dileptus cygnus*.

The authors express their thanks to dr. J. Dragesco for introducing them into the method of staining with protargol.

Material and methods

Cultures of *D. cygnus* were fed every day with a mixture of cultures of *Colpidium colpoda* Ehrbg. and *Tetrahymena pyriformis* Ehrbg. The ciliates used as nutrition for *Dileptus* were fed with a suspension of dried yolk. As medium of the culture, the Pringsheim's fluid was applied. Preparations were executed by means of the silver impregnation method using protargol following the procedure of Dragesco 1962 b. Unfortunately this method of staining proved to be not specific for trichocysts. Satisfactory results were obtained only in some preparations. For mass preparations as well individuals in division as those in the course of regeneration were used. For the study of regeneration, three series of preparations were executed: the first 0—30 min after operation, the second 30—60 min. after operation, and the third 60—90 min. after operation. Ciliates used for operation were starved for 24 hrs. prior the treatment. Operations were carried out free hand with a steel needle, under a low magnification (approx. 20×). Care was taken to cut a possibly highest number of ciliates within 30 min. in a drop of densified culture.

Results

Distribution of trichocysts in a morphostatic individual

On account of their location the trichocysts in *Dileptus* may be divided into two groups: trichocysts in ectoplasm and those immersed in endoplasm (Fig. 1 A). The ectoplasm trichocysts of a morphostatic individual are located only on proboscis, on the elongated convexity of its ventral side between the rows of the feeding cilia. Those trichocysts are disposed perpendicularly to the longitudinal axis of proboscis and to the surface of the pellicle, as described by Canella 1951 for related species. They are distributed rather regularly, from the cytostome (excluding the lip which encircles the cytostome) as far as the very tip of proboscis which is very long in this species. The number and the degree of agglomeration of trichocysts show very considerable individual variations (Pl. II 2, 3). They may be disposed in several rows or in one row only. When trichocysts are few, the equal space between the two neighbouring ones is observed along the whole length of proboscis.

The second group — trichocysts immersed in endoplasm — differ neither by their shape nor size from the ectoplasm trichocysts when stained with protargol and examined under the light microscope. They all appear as dark rods about 5.4 μ long, equally thick on their whole length. In the median body part, the number of trichocysts is the lowest, they are the most numerous on the oral and caudal body poles. In most cases the endoplasm trichocysts are oriented

at random, in all possible directions (Pl. I 4). This concerns also the trichocysts of the thicker part of proboscis which are not included into the ectoplasmic convexity. Somewhat more regular systems may be observed only in the pharyngeal region (Pl. I 1) as well as in the caudal body part (Pl. I 5). Trichocysts of the pharyngeal trichits region imitating by their pattern the shape of the cytopharyngeal basket, were observed frequently. It may be presumed that the

Fig. 1. Pattern of trichocysts in *Dileptus cygnus* A. Morphostatic individual. B. Fragment 30 m. after operation. C. Fragment 60—90 min. after operation

arrangement of trichocysts in the pharyngeal region is the result of their disposition along the trichits. A striking fact is that a considerable agglomeration of trichocysts was observed in the caudal part. This occurred so frequently that it should be recognized as a typical phenomenon. The arrangement of trichocysts is in this region rather regular along the long body axis. This arrangement is possibly effected by the shape of the body in the caudal region, especially in the distal part of the tail. Presumably the movements of endoplasm are slower in this region which may promote accumulation of trichocysts.

Except some few cases which were excluded from the study because of their non-specific staining result, trichocysts showed an amazing stability of dimensions. The mean 5.4 μ holds as well for trichocysts of starving as for those of fed individuals, as for those with numerous or scarce trichocysts. Even in dwarf specimens after a prolonged starvation, the dimensions of trichocysts

(but not their number) failed to deviate from the norm. Trichocysts distinctly smaller than the mean ones which could be looked upon as development stages were not clearly distinguished among the abundant inclusions of the ciliate body.

Accumulations of trichocysts in endoplasm were found rather frequently. Those accumulations were star-shaped (Pl. II 6) or — more frequently — formed irregular bundles composed of several to over ten components. Trichocysts in the accumulation were of a normal size. Those accumulations were found as frequently in the morphostatic as in regenerating forms. It should be stressed that the total number of trichocysts in different *Dileptus* individuals fluctuates in unexpected broad limits (a considerably intense accumulation is seen in Pl. II 7) from several tens to hundreds of units.

Behaviour of trichocysts in the course of division

In the course of division processes, formation of trichocysts or even occurrence of distinctly smaller ones were not observed which is in agreement with the observations of Dragesco and others 1965. Nevertheless formation of regular groups of trichocysts was observed in both division rudiments: in that of the proboscis armature (Pl. II 9) and of the cytopharyngeal complex (Pl. II 8) (Golińska and Doroszewski 1964).

In both rudiments the position of trichocysts was perpendicular to the body surface. Owing to convexity of the rudiment, the complex assumed a cone-shaped form. As the dorsal rudiment grows, a stripe of trichocysts is formed along one side of the ciliate body. This produces an impression as if in the course of this process still new trichocysts from the surrounding area were included into the rudiment of proboscis. Distinct agglomerations of trichocysts in the median part of the body were not observed in the early stages of division.

The phenomenon just described provides explanation of our previous observations (unpublished) on the material stained with hematoxylin where exploded trichocysts were seen in the early dorsal division rudiments.

Behaviour of trichocysts in regeneration

In the periods of time under study any signs of formation of trichocysts were not observed. The size of trichocysts in different systems failed to deviate from the mean one.

The previous study on *Dileptus* proved an unusually high rate of regeneration in this ciliate. For the individuals cut by half the selected time — up to 1.5 hr. — is the period of high advancement of regeneration processes with formation of both rudiments and, for individuals in which only the proboscis had been cut off, it is the time of full regeneration together with the restitution of capability of killing the prey.

Regeneration of the opimer

Parts of proboscis are cut off

Operation of this type does not induce the formation of specific regeneration rudiments. Regeneration consists in a gradual elongation of the proboscis remnants. The results obtained seem to indicate that the trichocysts from

endoplasm are wedged between the regular trichocysts of the proboscis remnant in various places (Pl. III 11) without preference of the apical or cytostomal part.

Proboscis and the cytopharyngeal complex are cut off

Into this group all fragments cut off approximately in the middle of the body and containing the nuclear apparatus have been included. Prior the formation of regeneration rudiments at the rounded anterior body end, an accumulation of trichocysts appears (Pl. III 12). This accumulation lies in endoplasm and is composed of trichocysts arranged in various directions, their number is different in separate groups. The images obtained seem to indicate the migration of trichocysts from the neighbouring regions of endoplasm — even from its caudal part — to the regeneration agglomeration. The size of the trichocyst agglomerations makes impossible considering this phenomenon as effect of shrinkage of the wound. Besides, in the case of an intense regeneration agglomeration, trichocysts are less numerous in the tail than normally. Groups of trichocysts were also found in the median part of the fragment before formation of the aggregation, as if they were "on the way" to their position. The aggregations of trichocysts appeared in preparations after 0—30 min, but in majority of fragments they appeared after 30—60 min.

Simultanously with the formation of the proboscis armature and of the cytopharyngeal complex, occurs the regulation of the form of the fragment: its antero-dorsal part protrudes much forwards, the whole anterior part of the fragment becomes flattened laterally. This process of shape regulation is closely connected with growth and development of the regeneration rudiments. The oblique comb on the anterior part of the fragment is occupied by the increased rudiment of the proboscis armature. This was the final stage (about 1.5 hr. after operation) of behaviour of trichocysts which has been observed. The first stages of formation of the dorsal rudiment are also the first signs of pushing forwards the dorsal part. At this stage the trichocysts still form a distinct accumulation (Pl. IV 16) and some of them penetrate into the rudiment, approach closely the pellicle and occupy a position perpendicular to its surface (Pl. IV 17) As the proboscis rudiment grows, still new trichocysts become included into the regular row in ectoplasm (Pl. IV 18—21). About that time, the trichocysts accumulation in endoplasm becomes gradually less discernable.

Posterior anuclear fragments

Sporadically anuclear fragments were found in preparations. Well developed regeneration accumulation of trichocysts was observed in those fragments (Pl. III 13). Unfortunately in preparations executed 60—90 min. after operation anuclear fragments were not found.

Regeneration of the promer

Behaviour of trichocysts in regeneration of the tail

For the experiments, big fragments containing the nuclear apparatus were chosen. It was stated that after the posterior part has been cut off and the fragment became rounded, an agglomeration of trichocysts (Pl. III 14) is being formed in the posterior part of promer. It resembles the agglomeration in the anterior part of opimers. However in the regeneration agglomeration of promers,

shifting of trichocysts to the surface of the pellicle was not observed. The process of disposition of trichocysts along the tail seems to occur simultaneously with narrowing of its posterior part. In the period studied, the dispersion of the posterior agglomeration of trichocysts was not observed either.

Posterior anuclear fragment

As a rule those fragments were small, and their posterior agglomeration of trichocysts was rather insignificant (Pl. III 15). The size of this agglomeration was in this case distinctly depending on the size of the fragment. All the fragments contained simultanously the normal number of trichocysts in the armature of proboscis. This indicates that the trichocysts fail to return from ectoplasm into endoplasm.

Discussion

The results of the present study fully confirm the concept of the endoplasmic origin of trichocysts. However any connection of trichocysts with kinetosomes (review — Grimstone 1961) was not stated. The observations suggest that trichocysts arise in endoplasm and migrate subsequently towards the periphery of the ciliate, here they seem to be arrested at one spot and included into the ectoplasm of proboscis. The fact of accumulation of trichocysts in the caudal part migration of trichocysts and their arrest in the caudal part owing to the shape of the ciliate body which may define their position. At any rate dispersed trichocysts have been stated in the whole endoplasm of the ciliate. This is possibly associated with the high utilization of those organelles in killing the prey (Dragesco 1962 a.) Consequently, a permanent accumulation of trichocysts and renewal of their reserve become indispensable. The fact that the trichocysts revealed were of the same dimensions and that their formation was not stated in the stage of a spherical vesicle (Dragesco and others 1965) seems to be due to their late visualization in the light microscope after treatment with protargol their appear only at a definite conclusive phase of their development.

In the course of division of *Dileptus,* disposition of trichocysts in the proboscis rudiment and in that of the cytopharyngeal basket was stated (Golińska and Doroszewski 1964). However our information concerning the behavious of trichocysts in division is only fragmentary.

In the course of regeneration, a rapid accumulation of trichocysts occurs in the anterior part. Accumulated trichocysts are all of the normal size and collect so rapidly that their formation de novo is little probable. It should be rather accepted that they derive of some reserve organelles which had been formed previously and collect near the lesion owing to some unknown reasons. The formation of the regeneration accumulation of trichocysts in the prosterior part of the promer as well, seems to speak in favour of the postulation that appearing of the regeneration accumulation is associated with the regeneration processes in general and not only with the regeneration of the oral apparatus. It seems doubtless that the subsequent disposition of trichocysts at the place of future proboscis is a sign of determination of this place and of initial regulation of the shape. Inclusion of trichocysts into the rudiment and their adhesion to the surface of the pelicle prove a very early determination of this property of ectoplasm in proboscis. This promotes a definite arrangement of trichocysts and their subsequent explosion.

The problem of accumulation of trichocysts in bundles and in rosettes — which was observed in some cases — remaines unexplained. Studitsky 1930 considers the accumulation of trichocysts in bundles as symptom of degeneration. However the possibility that they represent some stages of their formation cannot be excluded.

The role of trichocysts in regeneration is an example of formation of regenerating elements not in the place of their conclusive position. This indicates the role which is played in regeneration by structures already existing, as if prefabricated, produced previously as a result of normal physiological activity.

Soon after being placed in the cytoplasm of proboscis, trichocysts are capable of their normal activity, as proved by the observations of Doroszewski 1961. The author stated killing the prey already 40 min. after the operation of removing proboscis. This results have been elucidated by the occurrence of trichocysts accumulation which was stated in the present study. They may also be connected with the concept of Golińska 1966 who explains the permanent readiness for regeneration in *Dileptus* by the necessity of a rapid regeneration of trichocysts used in great number by the preying individual. The location of trichocysts in rows on the side of the future proboscis is a manifestation of an already advanced regeneration of the ciliate.

Following conclusions may be drawn from the presented results:

1. The endoplasmic origin of the trichocysts in *Dileptus* evokes no doubt.
2. In the course of regeneration, and accumulation of trichocysts is formed near the place of lesion. In the subsequent stage trichocysts occupy their position in the place where proboscis is formed and are ready for explosion.
3. Trichocysts of the proboscis rudiment originate of the reserve organelles dispersed in endoplasm and are not produced especially for a given rudiment.

Summary

The distribution of trichocysts was examined in the morphostatic, dividing and regenerating individuals of *Dileptus cygnus*. In morphostatic individuals, besides the typical trichocysts distributed in proboscis, their presence in considerable quantity was stated in endoplasm. This finding is in conformity with the observations of other authors. In dividing individual trichocysts are present in the place where the oral rudiments arise. In the first phase of regeneration, trichocysts collect in the anterior part of the posterior fragment. In the subsequent phases they are already distributed in the place where proboscis is being formed and take their normal position.

STRESZCZENIE

Zbadano rozkład trichocyst u *Dileptus cygnus* u osobników morfostatycznych, podziałowych i regenerujących. U osobników morfostatycznych stwierdzono, poza typowym rozmieszczeniem trichocyst w proboscis ich obecność w dużych ilościach w endoplazmie. Jest to zgodne z obserwacjami innych autorów. U osobnika podziałowego trichocysty są obecne w miejscu, gdzie tworzą się zawiązki oralne. W pierwszej fazie regeneracji trichocysty skupiają się w przedniej części tylnego fragmentu. W następnych fazach są już rozmieszczone w miejscu, gdzie tworzy się proboscis i zajmują swoją normalną pozycję.

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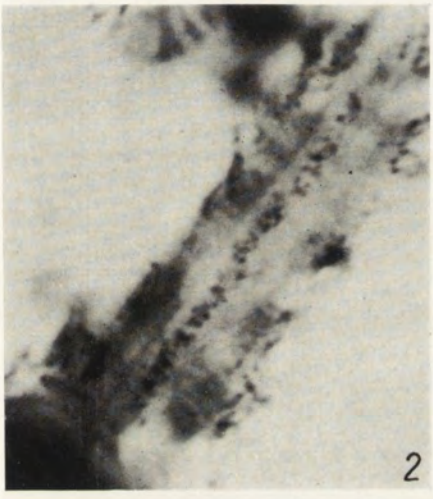

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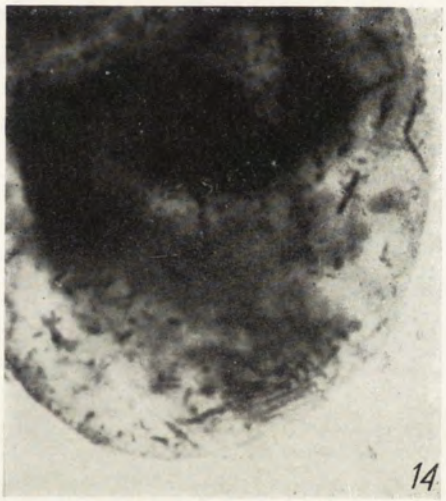

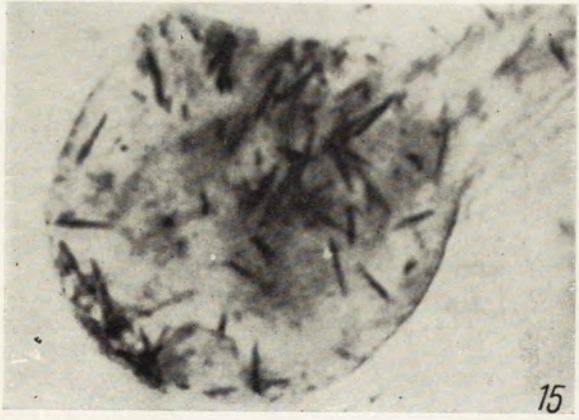

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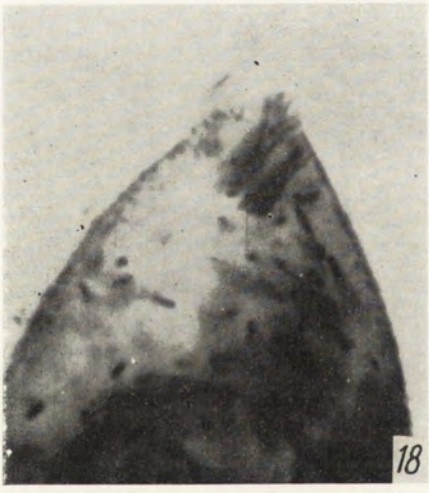
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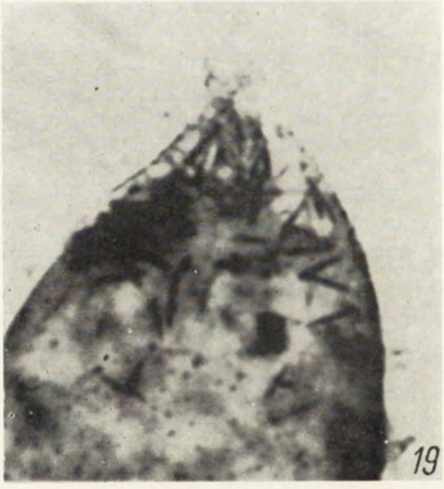
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EXPLANATION OF PLATES I—IV

Distribution of trichocysts in the morphostatic, dividing and regenerating individuals of *Dileptus cygnus*

1: System of trichocysts in proboscis in the region of the cytopharyngeal complex in a morphostatic individual
2 and 3: Differences in the trichocysts number in proboscis of morphostatic forms
4: Trichocysts in endoplasm. Morphostatic individual
5: Trichocysts located in the tail of the morphostatic individual
6: Star-shaped pattern of trichocysts in a regenerating individual
7: Agglomeration of trichocysts in endoplasm of morphostatic individual
8: Group of trichocysts near the rudiment of the cytopharyngeal complex during division
9: Disposition of trichocysts in the division rudiment of proboscis
10: Disposition of trichocysts in the proboscis of opistor a short time before fission of offsprings
11: Regeneration of proboscis broken near the cytostome
12: Agglomeration of trichocysts in the anterior part of opimer
13: Agglomeration of trichocysts in the anucleated opimer
14: Agglomeration of trichocysts in the posterior part of promer
15: A slight agglomeration in the posterior part of the anucleated promer
16—21: Consecutive images of location of trichocysts in the developing rudiment of proboscis

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The effect of certain salt solutions and osmotic stimuli on ciliary movement and food intake in *Paramecium caudatum*

Wpływ pewnych roztworów soli i czynników osmotycznych na ruch rzęsek i pobieranie pokarmu u *Paramecium caudatum*

Evidence suggests that food vacuole formation in *Paramecium* and other ciliate species is closely related with the function of somatic and peristomal cilia. According to Jennings 1910, food particles are carried into the cystome by currents of water created by beating cilia. Later Mast and Lashley 1916, Bozler 1924, Mast 1947 and Grębecki 1961 concurred in this view. However, Kitching 1938 observed that in some species of *Peritricha* the formation of the food vacuole continued despite the fact that the ciliary movement was blocked by agar.

Metalnikow 1912, Losina-Losinksy 1931, and Bragg 1936 indicated that *Paramecium* demonstrates differential selection of particles to be taken up and that it prefers nutritious or digestible substances over indigestible substances such as carmine, India ink, Sepia ink, etc. However, particles of powdered sulphur or glass rubbed into egg yolk or starch are taken up quite readily by *Paramecium* (Dembowski 1922). Brutkowska 1963 has recently shown that *Paramecium* forms food vacuoles filled with powdered glass particles in a medium containing no nutritious material. According to Mueller, Röhlich and Törö 1965, no selection between nutritious and non-nutritious particles could be revealed in *Paramecium multimicronucleatum* and *Tetrahymena pyriformis*.

It has commonly been seen that the intake of indigestible particles occurs after starvation. From the work of numerous authors it apprears that the acceptance of a particle is determined by a physical rather than chemical factor (cf. Mueller, Röhlich and Törö 1965).

Metalnikow 1912, 1914, 1915 found that the size and number of food vacuoles depend on the composition of the medium. For *Bursaria* Lund 1914, and for *Vorticella* Koltsov 1915 reported that both acids and alkalies produced a decrease of food vacuole formation. In *Colpidium* Mills 1931, and in *Paramecium* Lee 1942, and Brutkowska 1963 determined the pH optimum for the size and number of food vacuoles. In his work on *Peritricha* Kitching 1938 found that diluted sea-water exerts an inhibitory influence on food intake. On the other hand, Frisch 1939 showed that hypertonic solutions reduce the formation of the food vacuoles in *Paramecium*.

The action of inorganic salts upon food intake in *Paramecium* has been

investigated by several workers. Dogiel und Issakowa-Keo 1927 found that Ba^{2+}, Fe^{2+} and Mg^{2+} ions cause the food vacuoles to change in shape. Dogel, Issakowa-Keo i Strelkov 1928 reported that calcium and magnesium salts decrease the ingestion of food. According to Eisenberg-Hamburg 1932, the strontium salts were found to decrease the number of food vacuoles.

Seravin 1957 has made studies of the effect of various salts, acids, alkalies, urea and glucose on food vacuole formation in *Paramecium*. However, he paid more attention to adaptation processes than to an animal's direct reactivity to a chemical agent.

Eisenberg 1924 was able to demonstrate that when hypertonic solutions of glucose are added the separation of the food vacuole from the pharynx is very slow. According to Chejfec 1935, the number of food vacuoles decreases under such conditions.

Despite the common observation that the shape and size of the food vacuoles is determined by environmental factors, especially by the chemicals of the surrounding medium, the fact remains that there exist very few suitable demonstrations of an indirect influence of the medium agents on food intake, and no convincing data are published to indicate that a decrease or an increase in feeding behavior in paramecia may be secondary to the disturbance in ciliary movement resulting from the changes in the composition of the medium. Of interest in this regard is the recent work of Grębecki 1965 that offers reliable information on the correlation between the ciliary movement and the formation of the food vacuoles. In the present experiments, the effects of a number of movement-disturbing chemical agents were studied with respect to food intake in paramecia.

Material and method

Organisms and maintenance. The stock of *Paramecium caudatum* investigated was established in 1955 from a pond near Warszawa. The animals were grown in a letuce, monobacterial (*Aerobacter aerogenes*) infusion prepared acording to the method described by Sonneborn 1950, as modified in this laboratory (Sikora, 1966). Under these conditions, ca. 1.0 division per day occurred. The *Paramecium* stock was kept at room temperature. Also the experimental studies were made at room temperature. All observations were carried out on the individuals which grew well, displayed the required fission-rate, and showed normal locomotor activity.

Procedure. Chemical substaces used. The effect on food vacuole formation of the following substances was studied: 1) inorganic salts: potassium, sodium, calcium, magnesium, and barium chlorides, and 2) osmotically acting substances: urea and glucose. In Experiment 1, 10 mM, 20 mM and 30 mM concentrations of potassium, sodium, calcium, and magnesium chlorides were used. In Experiment 2, a combination of calcium and barium chlorides in the following proportions was used: 1.5 mM $CaCl_2$ plus 1.5 mM $BaCl_2$, 3.0 mM $CaCl_2$ plus 3.0 mM $BaCl_2$, and 4.0 mM $CaCl_2$ plus 4.0 mM $BaCl_2$. In Experiment 3, 50 mM, 80 mM, 120 mM, and 160 mM concentrations of urea, and 25 mM, 50 mM, 75 mM, and 110 mM concentrations of glucose were used. All the substances in the proportions used in this work were found to disturb the locomotion of the paramecia.

Carmine suspension. To render food vacuoles carmine suspension was used. 200 mg powdered carmine were stirred with 10 ml. of distilled water in a porcelain mortar, and filtered. The carmine suspension thus obtained contained particles of 0.1—0.5 micron in diameter.

Pretreatment. The animals were thoroughly washed and left for 1—3 hours in the buffer solution of pH = 7.10[1] (Dryl 1959), or distilled water, depending on the subsequent treatment. Samples of the *Paramecium* suspension were mixed with samples of carmin suspension in buffer solution or distilled water. For experimental studies, only were those animals used whose samples formed about 3 food vacuoles within 3 min., and about 7 vacuoles within 10 min.

Proper experiment. After pretreatment, the remaining individuals of the *Paramecium* suspension were divided into 2 groups: the experimental group and the control group. In each group, there were ca. 6 000—8 000 specimens in 1 ml. Experimental group: when the animals were treated with sodium, calcium, and magnesium chlorides buffer solution containing carmine was used; when were treated with a combination of calcium and barium chlorides, urea, and glucose distilled water containing carmine was used; finally, during treatment with potassium chloride, there was used a) buffer solution containing carmine, and b) distilled water containing carmine with or without 0.01 mM $CaCl_2$. The control group was not treated with chemical substances. It was placed in buffer solution or distilled water containing carmine particles only. For control group, the carmine suspension was mixed with buffer solution or distilled water in proportion 1 : 1. For experimental group, the carmine suspension was mixed, in proportion 1 : 1, with the solution containing the relevant chemical substance at a concentration 4 times stronger than the final concentration.

The animals of the experimental group were exposed to the action of the chemical substance for 3, 10 and 30 min. After each exposure, the animals were mixed with 10 mM $NiCl_2$ at 1:1 proportion to stop ciliary movement[2] (Brutkowska 1967). The number of food vacuoles was then counted in ca. 100 specimens, using a low-power light microscope. Only regularly shaped food vacuoles were considered. Similar procedure was repeated with samples of the control group, except that these samples were not exposed to the action of a chemical agent. Time measurements were taken with a stop-watch.

The proper experiment lasted approximately 3—6 hours. An abnormal food vacuole formation occurred in the control group when the animals' stay in buffer solution exceeded 6 hours. Abnormal vacuole formation was also found in the initial period after washing the animals with buffer solution.

Statistics. Results of statistical comparisons between the control group and the experimental group for all three exposure times and the concentrations used are summarized in Tables 1—5. The *p* values were obtained by two-tailed probabilities associated with differences between control and experimental groups, using the Kolmogorov-Smirnov two-sample test (Siegel 1956).

The author wishes to express her gratitude to Docent Dr. Kazimierz Zieliński for advice on statistics.

[1] The buffer solution of pH = 7·10 contains calcium chloride, sodium citrate and sodium phosphate.

[2] It has been found that when paramecia are mixed with appropriate dilutions of $NiCl_2$ a total inhibition of ciliary activity occurs with no sign of any other harm to the animals. In the opinion of the author, some $NiCl_2$ concentrations give the best of the known immobilization effects.

Results

Experiment 1 : The effect of potassium, sodium, calcium, and magnesium chlorides upon food vacuole formation and ciliary movement (Table 1)

Potassium chloride in buffer solution increased food vacuole formation above the control level. The greatest number of food vacuoles occurred with 10 mM concentration after 3 min., with 20 mM and 30 mM concencentrations after 10 min., and with 30 mM concentration after 30 min. The smallest number of food vacuoles (attaining the control level) was obtained with 10 mM after 30 min., and with 30 mM after 3 min.

All three concentrations used caused disturbances in movement. A continuous reversal of cilia was observed within the first 50 sec., 1—2 min., and 2—3 min. when the animals were exposed to 10 mM, 20 mM, and 30 mM concentrations, respectively. This was followed by a partial reversal (D r y l 1961 a, G r ę b e c k i 1965) which lasted for several minutes and was positively correlated with the concentration. Eventually a slow forward swimming occurred. It is to be noted that only during the continuous ciliary reversal moved the animals uniformly. At all the stages of partial reversal (backward swimming, rotation, avoiding reactivity, etc.) individual specimens exhibited various sorts of movements which appeared to be due to local changes in chemical reagent content and in the physiological state of the organism.

Sodium chloride in buffer solution. The results were similar to those obtained with potassium chloride. No clear-cut continuous reversal was seen but partial reversal did occur.

Table 1

Number of food vacuoles in *Paramecium caudatum* induced by potassium, sodium, calcium, and magnesium chlorides in buffer solution of pH 7.10

Measure		Time of exposure in minutes								
		3			10			30		
		Mean	Median	p	Mean	Median	p	Mean	Median	p
Control group										
		3.4	3	—	6.3	6	—	13.8	14	—
Experimental group										
KC	10	4.1	4	<0.001	7.2	8	<0.001	13.9	14	ns*
in mM	20	3.6	4	ns	8.0	8	<0.001	16.8	17	<0.001
	30	3.5	3	ns	8.0	8	<0.001	18.7	19	<0.001
NaCl	10	4.6	5	<0.001	7.4	7	<0.001	12.9	13	ns
in mM	20	4.6	4	<0.001	8.2	8	<0.001	15.8	16	<0.001
	30	4.0	4	<0.001	8.9	9	<0.001	17.9	17	<0.001
$CaCl_2$	10	1.7	2	0.001	3.6	4	<0.001	10.6	11	<0.001
in mM	20	2.8	3	ns	3.9	4	<0.001	7.5	7	<0.001
	30	3.3	3	ns	5.4	5	<0.001	6.1	6	<0.001
$MgCl_2$	10	2.3	2	<0.001	5.4	5	<0.001	7.6	8	<0.001
in mM	20	2.3	2	<0.001	3.1	3	<0.001	4.9	5	<0.001
	30	3.1	3	ns	3.2	3	<0.001	2.7	2	<0.001

* ns — not significant

Calcium chloride in buffer solution. This exerted an obvious inhibitory effect, reducing the formation of the food vacuoles below the control level. In contrast to potassium and sodium chlorides, the maximum number of food vacuoles was obtained with 10 mM concentration after 30 min., and with 30 mM concentration after 3 min., whereas the minimum number of vacuoles occurred in response to the 10 mM and 30 mM concentrations within 3 min. and 30 min., respectively.

Calcium chloride markedly increased forward swimming. Upon longer exposure to the 30 mM concentration, the organisms became flat and transparent.

Magnesium chloride in buffer solution. The effect of magnesium chloride was similar to that of calcium chloride but magnesium chloride appeared more toxic. Paramecia died if allowed to remain in a 30 mM $MgCl_2$ cencentration for 10 hours. With 20 mM concentration deformed food vacuoles were obtained. After 3 min. such vacuoles were seen in 50 per cent of specimens while they occurred in only 25 per cent specimens after 10 min; a complete recovery occurred within 30 min. Reversed courses of the vacuole formation process in 3-min. and 10-min. experiments may be due to the fact that a decreasing number of deformed food vacuoles occurred with time. As pointed out in the earlier section of this paper deformed food vacuoles were not considered.

Similarly to calcium chloride, magnesium chloride increased forward swimming. It also resulted in flattening of the organisms. This often occurred as early as after 10 min. of exposure to stronger concentrations. When paramecia were exposed to 30 mM concentration for 30 min. disturbances in contractile vacuole functioning was noted.

In conclusion, uni-and bivalent salts were found to exhibit opposed effects upon food vacuole formation in paramecia.

Table 2

Number of food vacuoles in *Paramecium caudatum* induced by KCl alone and KCl in combination with .01 mM $CaCl_2$

Measure		Time of exposure in minutes								
		3			10			30		
		Mean	Median	p	Mean	Median	p	Mean	Median	p
Control group										
		4.1	4	—	6.6	7	—	17.3	17	—
Experimental group										
		KCl alone								
KCl	10	2.6	3	<0.001	7.5	7	ns*	14.3	14	<0.001
in mM	20	1.5	1	<0.001	4.9	5	<0.001	12.5	12	<0.001
		KCl in combination with .01 mM $CaCl_2$								
KCl	10	4.4	5	<0.025	6.7	7	ns	16.9	17	ns
in mM	20	5.6	6	<0.001	9.6	9	<0.001	21.5	21	<0.001
	30	4.7	5	<0.001	7.4	7	ns	9.7	10	<0.001

* ns — not significant

Table 2 shows that calcium chloride neutralizes the effect of potassium chloride. Potassium chloride in distilled water was found to be very toxic. Observations could be carried out exclusively within 3 min. and 10 min. of exposure since the animals died if the exposure continued. Only single specimens survived a 30-min. exposure to a 30 mM concentration. With the exception of a slight increase in the number of food vocuoles with 10 mM concentration following a 3-min. exposure, a decrease of the number of vacuoles below the control level generally occurred. The early action of KCl induced a partial ciliary reversal.

The toxic effects of potassium chloride were found to be reduced when 0.01 mM calcium chloride was added. Only after an exposure of paramecia to 30 mM KCl plus 0.01 mM $CaCl_2$ concentrations over a period of 30 min. was the food vacuole formation decreased below the control level. Weaker KCl concentrations combined with 0.01 mM $CaCl_2$ increased the number of the food vacuoles above the control level. In the presence of Ca^{2+} cations, all KCl concentrations used caused a marked continuous reversal of cilia.

Experiment 2 : The combined effect of calcium and barium chlorides upon food vacuole formation and ciliary movement (Table 3)

The combination of calcium and barium chlorides had a decreasing effect upon food vacuole formation, except for the weakest concentration after 30 min. No appreciable correlation between the concentration and time of the action of this combination could be established.

As a consequence of the action of the calcium and barium chloride combination a periodic ciliary reversal (D r y l 1961 a) occurred that consisted of successive forward and backward swimming movements at intervals of 1—2 sec. Practically, the periodic reversal lasted indefinitely.

Table 3

Number of food vacuoles in *Paramecium caudatum* induced by a combination of $CaCl_2$ plus $BaCl_2$

Measure	Time of exposure in minutes								
	3			10			30		
	Mean	Median	*p*	Mean	Median	*p*	Mean	Median	*p*
Control group									
	3.5	4	—	8.2	8	—	15.1	15	—
Experimental group									
$CaCl_2+BaCl_2$ in mM									
1.5+1.5	2.5	3	<0.001	5.7	6	<0.001	16.9	17	<0.001
3.0+3.0	1.9	2	<0.001	3.4	3	<0.001	15.3	15	ns*
4.0+4.0	2.8	3	<0.001	5.4	6	<0.001	12.7	13	<0.001

* ns — not significant

Experiment 3 : Food vacuole formation and ciliary movement in response to the osmotic concentration produced by urea and glucose (Tables 4 and 5)

U r e a. With two exceptions (50 mM and 80 mM) urea in various concentrations decreased the food vacuole formation below the control level. However,

Table 4

Number of food vacuoles in *Paramecium caudatum* induced by various concentrations of urea solutions

Measure		Time of exsposure in minutes								
		3			10			30		
		Mean	Median	*p*	Mean	Median	*p*	Mean	Median	*p*
Control group										
		3.4	3	—	5.4	5	—	14.4	14	—
Experimental group										
urea in mM	50	3.4	4	ns*	4.5	4	ns	12.5	12	<0.001
	80	3.6	4	<0.050	5.1	5	ns	11.3	11	<0.001
	120	2.8	3	ns	2.9	3	<0.001	3.2	3	<0.001
	160	2.8	3	ns	2.6	3	<0.001	3.9	4	<0.001

* ns — not significant

even the highest concentration used (160 mM) did not abolish it entirely. In the urea solutions of great concentrations paramecia became shrivelled and flat and, eventually, died.

G l u c o s e. Somewhat similar results were obtained with glucose. The solutions in concentrations reaching the maximum value (110 mM) resulted in

Table 5

Number of food vacuoles in *Paramecium caudatum* induced by various concentrations of glucose solutions

Measure		Time of exsposure in minutes								
		3			10			30		
		Mean	Median	*p*	Mean	Median	*p*	Mean	Median	*p*
Control group										
		4.9	5	—	7.5	7	—	16.6	17	—
Experimental group										
glucose in mM	25	3.4	3	<0.001	8.5	8	<0.001	15.4	15	<0.001
	50	3.8	4	<0.001	9.2	9	<0.001	19.0	19	<0.005
	75	4.4	4	ns*	10.5	10	<0.001	21.5	21	<0.001
	110	5.0	5	ns	4.7	5	<0.001	4.9	5	<0.001

* ns — not significant

a considerable shrinkage of the body and death. Under lower concentrations and the lethal (110 mM) concentration after 3 min. food vacuole formation attained or exceeded the control level.

Both urea and glucose solutions in great concentrations led to a temporary accleration of forward swimming.

Discussion

The principal result of these experiments is that inorganic salts and osmotic stimuli which are found to interfere with ciliary movement affect the formation of the food vacuoles in *Paramecium caudatum*.

Information has been presented by several workers to show that ciliary movement is essential for food intake in paramecia. Recent studies and deductions of Grębecki 1965 are of special significance. They have the merit of showing that all sorts of ciliary reversals, from continuous reversal, backward swimming and rotation to the avoiding reactions, have a decidedly beneficial effect on food vacuole formation. On the other hand, Grębecki 1965 reports that active forward swimming does not promote food intake since during an active forward movement peristomal cilia exhibit an orientation that does not necessarily facilitate sweeping the suspended particles into the cytostome.

The present results lend credence to the Grębecki's view. Regardless of the fact that some concentrations of chemical agent solutions faciliatate ciliary reversal and some do not, there has been a consistent finding that continuous and partial reversals are associated with an increased food vacuole formation. The fact remains however that the paramecium is able to maintain the uptake of suspended particles until at least some sort of ciliary movement is retained. An abolition of food vacuole formation occurs when injurious or immobilizing agents are used (Dryl, Brutkowska and Sikora 1963, Brutkowska 1967).

Surprisingly little work has been done with paramecium in regard to the role of the salts of potassium, sodium, calcium, and magnesium as related to food vacuole formation. The information which is available is not comparable with that deriving from the foregoing experiments due to the fact that both design and certain technical details were specific. In somewhat similarly executed investigation, Seravin 1957 studied the effect of various chemicals on phagocytosis but he concentrated his attention on the change in the food vacuole formation after at least a 15-min. action of the chemical reagent. The present results clearly demonstrate that some difference in the effect of mono- and bivalent cations upon food vacuole formation often occurs as early as after a period of 3 min. Moreover, it has become apparent from the present paper that the effects of some salt solutions on food vacuole formation must be considered in terms of the exposure time.

Much has been written about K^+ ion vs. Ca^{2+} ion antagonism. It is well known that a KCl—$CaCl_2$ balance is an important factor in the proper functioning of contracticle and conductive tissues. Mast and Nadler 1926, and Oliphant 1938 reported that numerous chemical substances, especially potassium chloride, induce ciliary reversal in paramecia. Many theories have been presented to explain the reversal. It is the opinion of Kamada 1940 that reversal is caused by a decrease of Ca^{2+} ions in the medium. Kamada and Kinosita 1940 expressed the view that when Ca^{2+} ions are absent K^+ ions do not induce ciliary reversal. Also, Jahn 1962, and Grębecki 1964 believe that reversal in paramecia occurs in response to K^+ ions combined with Ca^{2+} ions. This agrees fully with the present findings showing that KCl alone lowers food vacuole formation while KCl in buffer solution containing calcium chloride, or KCl in combination with 0.01 mM $CaCl_2$ may enhance food vacuole formation above the control level. On the other hand, it has been shown (Brutkowska, unpubl. observations) that concentrations of calcium chloride which

are stronger than 5 mM exert a definite inhibitory effect on food vacuole formation.

Jahn 1962 stated that reversal depends on the level of calcification of the cell membrane. It is evident that in absorption processes, there is a clear-cut competition between K^+ ions and Ca^{2+} ions. An addition of K^+ ions to the environment causes a reduction of Ca^{2+} ions in the membrane, and this, in turn, results in ciliary reversal. Since, as pointed out earlier, all sorts of reversal in paramecia are associated with an increase in the formation of the food vacuoles, it is clear that the KCl—$CaCl_2$ combination used in Experiment 1 was, for the most part, a promoting factor.

With magnesium chloride, somewhat more complex effects were noted in that the paramecia formed food vacuoles which were irregular in shape. This observation suggests a severe injury inflicted upon the cytostome membrane and the internal contens of the animal. It is likely that magnesium chloride inhibits cytoplasmic contractions which are necessary for separation of the food vacuole.

The present findings are in general agreement with those obtained by Dogiel und Issakowa-Keo 1927, except for the fact that we were unable to show abnormally shaped food vacuoles in the presence of barium chloride. In our belief, the decreased number of food vacuoles obtained with some concentrations of solutions containing $BaCl_2$ is due to the toxicity of this compound rather than to the resulting periodic reversal.

In regard to osmotic factors it is worthwhile emphasizing that even the lethal concentrations of urea and glucose did not stop entirely the formation of the food vacuole. Eisenberg 1924 found that hypertonic glucose solutions prevented a full separation of the food vacuole from the cytostome, and held that this was due to viscous changes in the cytoplasm produced by dehydration. Somewhat similar interpretation was given by Chejfec 1935 who reported that under strong glucose concentrations paramecia decrease their food vacuole formation.

It appears likely that the some differential effect of urea and glucose upon food vacuole formation as indicated by the present results is due to differences in specific gravity of urea and glucose. Dryl 1961 b contended that specific gravity of a substance determines its penetration across the surface membrane. Although glucose can be taken up by paramecia, great concentrations of glucose produce dehydration associated with a definite lowering of the rate of food vacuole formation.

Summary

The effect on ciliary movement and the number of food vacuoles in *Paramecium caudatum* of various concentrations of inorganic salts and osmotic factors within 3 min., 10 min., and 30 min. was investigated.

Potassium, sodium, calcium, and magnesium chlorides were placed in buffer solution containing calcium chloride, sodium citrate, and sodium phosphate, together with carmine suspension. Under these conditions, K^+ and Na^+ cations induced ciliary reversal associated with an increase in the number of food vacuoles, whereas Ca^{2+} and Mg^{2+} cations accelerated forward swimming associated with a decrease of the number of food vacuoles. K^+ ions in distilled water, were found to be very toxic and caused a decrease of the number of food va-

cuoles, except for the weakest concentration (10 mM) within 3 min. An addition of Ca^{2+} ions induced an increase in food vacuole formation. In the presence of Ca^{2+} ions, K^+ ions caused a reversal of the cilia.

A combination of Ca^{2+} plus Ba^{2+} cations in distilled water caused periodic reversal and a decrease of food vacuole formation, except for the weakest concentration used (1.5 mM $CaCl_2$ plus 1.5 mM $BaCl_2$).

Depending on the concentration and exposure time, urea and glucose solutions decreased or increased vacuole formation. Even under lethal concentrations, there were formed some food vacuoles. Great concentrations of urea and glucose accelerated swimming.

These findings are consistent with the view that ciliary reversal has a beneficial effect on food vacuole formation. The decreased number of food vacuoles obtained with some concentrations of solutions containing $BaCl_2$ is due to the toxicity of this coumpound rather than to the resulting periodic reversal.

STRESZCZENIE

Badano wpływ różnych stężeń roztworów soli nieorganicznych i czynników osmotycznych na ruch rzęsek i liczbę tworzących się wodniczek pokarmowych u *Paramecium caudatum* w ciągu 3 min., 10 min. i 30 min.

Chlorki potasu, sodu, wapnia i magnezu rozcieńczano roztworem buforowym, zawierającym chlorek wapnia, cytrynian sodu i fosforan sodu, i mieszano z zawiesiną karminu. W takim środowisku kationy K^+ i Na^+ wywoływały rewersję ruchu rzęsek, której towarzyszyło zwiększenie się liczby wodniczek pokarmowych, natomiast kationy Ca^{2+} i Mg^{2+} przyśpieszały ruch postępowy pantofelków, z czym był związany spadek liczby tworzących się wodniczek. Jony K^+ w wodzie destylowanej wykazywały wielką toksyczność, powodowały zmniejszenie się liczby wodniczek pokarmowych, z wyjątkiem bardzo słabego stężenia (10 mM) działającego w ciągu 3 min. Dodatek jonów Ca^{2+} wzmagał tworzenie się wodniczek. W obecności jonów Ca^{2+} jony K^+ wywoływały rewersję ruchu rzęsek.

Mieszanina chlorku wapnia i baru w wodzie destylowanej wywoływała periodyczną rewersję ruchu rzęsek i spadek liczby wodniczek pokarmowych, z wyjątkiem najmniejszego stężenia (1.5 mM $CaCl_2$+1.5 mM $BaCl_2$).

Zależnie od stężenia i czasu ekspozycji, roztwory mocznika i glukozy wzmagały lub osłabiały proces tworzenia wodniczek pokarmowych. Jednakże nawet letalne stężenia nie znosiły całkowicie tego procesu. Duże stężenia mocznika i glukozy przyśpieszały ruch postępowy.

Powyższe dane potwierdzają pogląd, że rewersja ruchu rzęsek jest czynnikiem, który wzmaga proces tworzenia się wodniczek pokarmowych. Zmniejszenie się liczby wodniczek pokarmowych w niektórych stężeniach roztworów zawierających chlorek baru należy przypisać raczej toksyczności tego związku niż wywoływanej przez jego działanie rewersji periodycznej.

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The movements and responses of *Halteria grandinella*

Die Bewegungen und Reaktionen von *Halteria grandinella*

Little has been known of the significance of the different forms of movement available to the genus *Halteria (Oligotrichida, Ciliata)*. Thus Jahn and Bovee 1964 include the saltatory movement of certain ciliates with heavy body cirri (e.g., *Halteria, Cyclidium*) in their list of poorly understood movements. Szabó 1934 describes the typical jumping of *H. grandinella* as an escape movement. Tamar 1965 reports a second escape movement, rapid backward spiraling.

Only a minimum of information has also been obtained on the responses of *Halteria* to stimuli (Tamar 1965). Yet, because of the peculiar movements of *Halteria*, their loss of somatic ciliature with the exception of the equatorial groups of cirri, and the high development of their adoral membranelles, the possibility of interesting responses presents itself. This possibility appears enhanced by the suggested distinct evolution of the *Oligotrichida* from the much more highly-investigated *Hypotrichida*, although both orders are *Spirotricha* and originate from the *Heterotrichida* (Mackinnon and Hawes 1961).

In the present paper both the responses of *H. grandinella* to a series of stimuli, and the nature and function of the various movemens, as shown in these responses, are reported. An attempt is made, on the basis of the responses observed, to elucidate the relationship between the 2 escape movements, jumping and rapid backward spiraling.

Materials and methods

The experimental stock of *H. grandinella*, obtained from Deming Lake, Terre Haute, Indiana, has been previously described (Tamar 1965). It was cultured in a medium of 20 ml. bacteriologically-filtered lake water, one house fly (*Musca domestica*), and one drop of 0.1 N HCl (Tamar 1965).

Experimental temperatures varied between 20°—30°C. The chemical test solutions were determined within 3%, the acetic acid solutions being made volumetrically from glacial acetic acid. A potentiometer accurate to $\pm$0.05 pH units was employed in determining pH, and observations were made primarily with a binocular microscope, and also with a phase-contrast microscope.

A sample of the Deming Lake water used in making up lake water solutions and the culture medium contained 5.08 milliequivalents of total solids per liter (254 parts/million), of which 1.59 ME's/liter were cations. The water contained 122 parts Ca^{+2}/million and 81 parts Mg^{+2}/million, and had a pH of 7.65.

The detailed procedure of each experiment precedes its results.

Movements and definitions

H. gardinella exhibits 3 main forms of active movement, forward spiraling, rapid backward spiraling, and jumping, all of which are easily discriminated from the others. At times specimens under normal conditions also rotate in a slow movement in which the adoral membranelles seem to approach inactivity. This slow movement is interrupted by jumps or forward spiraling.

1 a. Forward spiraling: this forward movement is produced by the anterior 16 adoral membranelles (Pl. I 1). The organism spirals to its left in a spiral path easily noticed at lower magnifications. Frequently specimens did forward spiraling in a partial circle to remain within a favorable zone, or to go around an unfavorable solution. Forward spiraling is also referred to as normal forward spiraling.

b. Slow normal forward spiraling: A relatively slower forward spiraling. A spiral path can still easily be seen at lower magnifications.

c. Slow forward spiraling: a slow and reduced movement which is not seen to follow a spiral path. Spiraling of the organisms is not clearly discernable at 30× magnification with the binocular microscope, and they seem to be still or to be drifting about. Slow forward spiraling was not successfully differentiated from the slow movement sometimes observed under normal conditions.

d. Noticeable slow forward spiraling: as above, but movement is much clearer and faster, and the spiraling of the organisms is more easily discernable at 30× magnification.

2. Rapid backward spiraling: this backward movement is produced by the anterior 16 adoral membranelles, and the organism spirals to its left. It normally is much faster than forward spiraling and is seen to be in a straight line, without spirals, at 30× binocular magnification. However, under adverse conditions it may slow down and show a spiral path, and clearcut movement of this type may be referred to as slower backward spiraling, or may be included in the more general term backward spiraling. Rapid backward spiraling appears to be an unusually fast reversal.

In culture fluid an interval of rapid backward spiraling was generally followed, after only a motionless moment, by forward spiraling. The forward spiraling was usually at an obtuse angle to the line of rapid backward spiraling, but often the specimen turned around completely to continue by forward spiraling in the same direction.

3. Jumping: this short, rapid movement, performed by means of the somatic cirri, is always backward, or in the direction of the posterior of the organism. It generally has a curved component. Later forward motion is in a new direction. Jumping is performed on hitting an obstacle such as another specimen, the substrait, a fungal filament, or the surface. It is also frequent during forward motion without a cause being visible to the observer. On the other hand, specimens can be seen doing forward spiraling for a considerable period without jumping.

4. Periodic ciliary reversal (PCR): since rapid backward spiraling seems to be an unusually fast reversal, and is certainly homologous with ciliary reversal in other Protozoa, a periodic alternation between rapid backward spiraling or other backward spiraling and any form of forward spiraling will be termed PCR, or followed by these letters in parenthesis. This is in keeping with the use of this term by Dryl 1961, 1965 and Grębecki 1965 b for similar phenomena in other protozoans.

Observations

Osmotic pressure

A lake of culture fluid containing numerous *H. grandinella* on a slide was allowed to evaporate so that a white rim of precipitated salt appeared along its edge. If a drop of fresh culture fluid, bacti.-filtered lake water, or distilled water was now carefully placed into this culture fluid as a separate entity by micropipette, the *H. grandinella* concentrated in large numbers in the drop area (Pl. I 2). In the case of distilled water the organisms often left the area of the drop empty at first, and then, as diffusion took place, a circle of organisms moved inward and finally they concentrated in the drop area.

pH was eliminated as the sole factor responsible for concentration. In one experiment at uniform temperature culture fluid with an original pH of 7.75 had a pH of 8.45 after a long period of evaporation. When drops of distilled water brought to pH 8.69 or pH 8.53 with 0.1 N NaOH were now placed into this culture fluid, high concentrations of organisms formed in the drop areas. In 2 tests at 23°C, clear concentration in the distilled water drop area was noted as late as 22 and 23 minutes after drop placement.

Gathering in the area of a distilled water drop presented an excellent illustration of Jennings' (1906) explanation of "positive reactions" or concentrations on the basis of avoidance reactions. Most specimens which entered the drop area and then continued their forward spiraling into some portion of its periphery jumped backwards several times at short intervals from the more concentrated culture fluid, until their forward spiraling was directed back into the drop area. A single jump usually resulted in forward spiraling in a new direction, and sometimes was even followed by a complete reversal of direction. In some cases, on reaching the periphery, the path of forward spiraling became circular to go around a portion of the drop area. A few specimens seemed to slow down and almost stop their forward spiraling as they swam into the periphery. Rapid backward spiraling was never observed in or around the drop area!

As the specimens concentrated, the region of concentration slowly became increasingly reduced to a small circle in the center of the distilled water drop area. The *H. grandinella* then appeared to move with much greater speed in the area of concentration than beyond it. This was due to their colliding with each other much more frequently in their area of great density, resulting in a much higher rate of jumping. The high speed of the jumping movements produced the effect of very fast motion, but the rate of forward spiraling in the area of concentration was normal. The same phenomenon was observed in a drop of culture fluid placed in a $CaCl_2$ — lake water solution, and also around pieces of protein material.

When bacti.-filt. lake water was allowed to slowly evaporate over a lamp to 1/4 of its original volume, specimens later placed in it did not concentrate in a drop of distilled water, but did so after a short period of additional evaporation.

Protein material

In many cases, when culture fluid was placed on a slide, bits or small sheets of white, opaque material were included in the culture fluid. These plaques of detritus, perhaps originating from the fly (*Musca domestica*) included

in the culture medium, contained large numbers of bacteria, including bacilli and short, thick spirilla.

H. grandinella was often observed to show a strong "positive response" to this material. During one observation the concentration of specimens around a sunken piece of the material became sufficient for the specimens to appear to be moving faster at this location than elsewhere on the slide, as described under osmotic pressure.

In a number of tests the *H. grandinella* concentrated over plaques of the afore-mentioned material which had become fixed to the slide (Pl. I 3), or under plaques floating on the surface. These plaques presented no obstructions to movement which might have physically produced concentration.

Concentration was due to avoidance reactions, or positive phobotaxis, as described by Jennings 1906. No positive contact reaction, as reported for *Paramecium* by Jennings 1906, was observed.

KCl drops placed in culture fluid

Method

In order to determine the response of *H. grandinella* in the described culture medium to various concentrations of potassium choride (KCl), drops of bacteriologically-filtered lake water solutions of 1 molar, M/10, M/50, M/100, M/200, M/300, M/500, M/700 and M/1,000 KCl were placed with micropipettes in lakes of *Halteria*-rich culture fluid on slides. Small quantities of 0.1 N HCl or 0.1 N NaOH were added to the KCl solutions when necessary to bring them close to a neutral pH (most at 6.9). The culture solutions ranged between pH 6.9 and 7.5.

Control experiments were run using pH 6.9 and 7.1 bacti.-filt. lake water drops. A number of different KCl solutions and controls were tested consecutively in the same culture fluid throughout the experiment.

Results

When drops of the 1M—M/200 KCl solutions were placed in the culture fluid the *H. grandinella* clearly retreated, leaving a vacant area. This retreat may have been partly produced by the physical expansion of the drop, as well as by diffusion currents.

The organisms then re-entered the areas of the drops in the case of all KCl solutions, with the exception of the 1 molar solution.

The *H. grandinella* also quickly re-entered the control lake water drops, and formed the typical fast-moving concentrations in the centers of these drop areas which develop in response to more favorable lower osmotic pressures and lower pH.

Meaningful results for the stimulating efficiency of the KCl solutions could thus only be obtained by comparing the rate of return of the specimens into the KCl drop areas with the rate of return into the control lake water drop areas.

The rate of return into the KCl drop areas became increasingly rapid with lower concentration of the KCl solution, until it did not clearly differ from the rate of return into lake water drops at a concentration of M/500 KCl. The rate of return into M/700 KCl and M/1 000 KCl could not be distinguished from that into the control lake water.

At concentrations of M/50 KCl—M/500 KCl the *H. grandinella* formed rings

as they moved inwards, and later center concentrations, in which the organisms clearly moved more slowly than those in the surrounding culture fluid. This indicates an effect of these KCl solutions on motor activity. In some M/10 KCl tests a thick ring of organisms formed around the drop area.

In both some M/50 KCl and some M/100 KCl tests, a number of organisms were seen to do forward spiraling in a circular path around the drop area. This seems to indicate an optimum zone in which lower osmotic pressure and pH more than balanced the effects of the KCl.

From the above, it can be seen that no positive response to KCl as such could be noted with any solution, and above a concentration of M/500 the effect of the KCl was clearly negative.

Halteria caught by the expanding drops of the 1M—M/50 KCl solutions did rapid backward spiraling through the drop area. Some rapid backward spiraling was also observed in M/100 KCl during its expansion. There was little or no rapid backward spiraling through M/10—M/100 KCl drops after their period of rapid expansion, or at any time through M/200—M/1 000 KCl drops.

Although a few *H. grandinella* were killed on contact with drops of 1M KCl as these were placed, the passage of a rapid backward spiraling organism through a located drop of 1M KCl or any other KCl solution did not cause its death. Rapid backward spiraling organisms, whether "caught" by the expanding drop or caused to start rapid backward spiraling beyond the drop area by shaking the slide, after traversing the drop area stopped momentarily in the opposite periphery of the drop area, among other *H. grandinella*, and then started normal forward spiraling and jumping, or went on by faster forward spiraling.

H. grandinella on the periphery of the stronger KCl solutions remained outside the drop areas by jumping and normal forward spiraling (Pl. I 4, II 5—6). These movements were also used to remain within drops of culture fluid placed into M/50 and M/100 KCl solutions.

H. grandinella in KCl solutions

Method

A small drop of culture fluid containing many *H. grandinella* was mixed with 20—30× that volume of each of the reported bacti.-filt. lake water solutions of potassium chloride (KCl) on a slide. The KCl solutions were given pH's between 6.8—7.1, using drops of 0.1 N HCl and 0.1 N NaOH. Between 10—22 tests were carried out with each KCl solution. The values given for time intervals represent the total range observed from all the tests performed with a concentration of KCl. All stated times are in terms of the period elapsed since the start of the tests.

Results

1 Molar KCl. The *H. grandinella* died immediately on contact.

M/10 KCl. The organisms did rapid backward spiraling on entrance into the solution. Then all were inactive on the substrait between 3/4—1 minute, and died.

M/50 KCl. Rapid backward spiraling on entrance, stopped by all *H. grandinella* between $3^3/_4$—$8^1/_4$ mins. Then rapid backward spiraling was replaced by slower spiraling (Pl. II 7) and, by between 7—10 minutes, most specimens turned on their axis on the substrait or were inactive on the substrait. A few still did

slow spiraling until 10 minutes. A number of *H. grandinella* continued to turn on the substrait indefinitely (this was observed in one case at 95 minutes), and others remained inactive but alive. Very occasional slow forward spiraling was observed late in some tests.

In 2 specimens bursts of more rapid spiraling alternated with slower backward spiraling.

M/100 KCl. *H. grandinella* did rapid backward spiraling on entrance, but all stopped this between 3^{+}—7 minutes. Rapid backward spiraling was replaced by spiraling in place, and then by inactivity on the substrait on the part of many organisms. Some slow forward spiraling was observed in a test at 7 minutes, and in most tests this increased in amount as organisms recovered, and continued indefinitely (slow forward spiraling was observed at 93 minutes). In a few cases of PCR the slow forward spiraling was interrupted several times, at intervals by backward spiraling.

Jumping was observed to be inhibited during much of the slow forward spiraling in M/100 KCl (also T a m a r 1965).

M/200 KCl. Rapid backward spiraling on entrance, stopped by all or most *H. grandinella* between $^1/_2$—$2^1/_2$ minutes. Normally rapid backward spiraling was followed by slow forward spiraling continued indefinitely, with an increased rate of jumping appearing in most tests.

M/300 KCl. In most tests all or many *H. grandinella* did rapid backward spiraling on entrance, but a number of specimens did slow forward spiraling on entrance. All rapid backward spiraling stopped between a few moments to 1 minute after entrance.

Rapid backward spiraling was followed for the most part by slow forward spiraling and normal jumping, which both continued indefinitely. There was also noticeable slow forward spiraling observed later in a few tests, and inactivity on the substrait at varying times.

M/500 KCl. There was no rapid backward spiraling on entrance. Instead, most *H. grandinella* showed slow forward spiraling and normal jumping from the start, and continued this indefinitely. In a few tests the slow forward spiraling was of the noticeable type from the start, and in a few more it became noticeable. In a few other tests there was slow normal forward spiraling from the start, and it was observed eventually in most tests.

In comparative observations the rate of swimming in M/500 KCl was lower than that in M/700 KCl or that in culture fluid.

M/700 KCl. There was no rapid backward spiraling on entrance. In most tests the *H. grandinella* did fairly fast normal forward spiraling after the start, while in a few tests they did slow normal forward spiraling or slow forward spiraling with normal jumping.

Later in the tests, most specimens did normal forward spiraling, much of it fast, and a lesser number did slow normal forward spiraling. Noticeable slow forward spiraling was seen later in several tests.

In most comparative observations the speed of movement of *H. grandinella* in M/700 KCl was slower than that in the original culture fluid.

M/1 000 KCl. The *H. grandinella* only did normal forward spiraling and jumping. There may have been some slowing of the organisms' movements.

Summary of movements in KCl solutions

The above observations can be condensed into several main points on principal movements. The organisms die immediately in 1 molar KCl and after

a short period of rapid backward spiraling in M/10 KCl, and show close to normal forward spiraling in M/700 KCl and normal forward spiraling in M/1 000 KCl. Rapid backward spiraling has the longest duration in M/50 KCl, and then its duration decreases with increasing dilution until in M/300 KCl only many specimens do rapid backward spiraling in some tests, and r.b.sp. is always over within 1 minute. In M/50 KCl rapid backward spiraling is followed mainly by slower spiraling and then turning on the substrait and inactivity. Between M/100—M/300 KCl there is earlier, and also an increasing amount of slow forward spiraling with greater dilution and a decreased period of rapid backward spiraling. In M/300 KCl noticeable slow forward spiraling appears later in a few tests, and in M/500 KCl some noticeable slow forward spiraling and normal forward spiraling accompany slow forward spiraling from the start, and the faster movements increase in amount with time. These relationships are illustrated in Fig. 1.

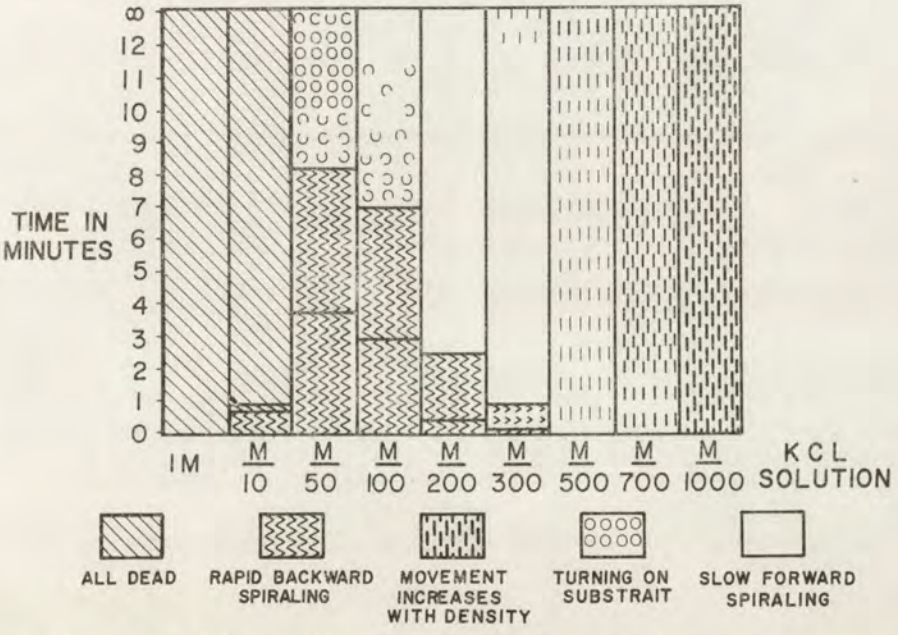

Fig. 1. Movements of *H. grandinella* in KCl solutions. The heavy lines at the top of and in the rapid backward spiraling pattern respectively indicate the longest and shortest periods which elapsed in a solution before all rapid backward spiraling stopped. In the M/300 column the rapid backward spiraling pattern is broken to indicate some slow forward spiraling from the start. In the turning on the substrait pattern the circles are left open when slow spiraling occurred at the same time, and there is increased spacing between the circles with more slow spiraling. The straight lines in the M/300 column represent noticeable slow forward spira'ing. In M/500 KCl there later was increasingly fast spiraling, and it approached normal speed after a time in M/700 KCl.

Inactivity on the substrait

In the M/100—M/500 KCl — lake water solutions varying numbers of *H. grandinella* became inactive on the substrait (some spiraled around their

axis in M/100 KCl). In M/700 KCl and in culture fluid an occasional organism also showed inactivity. In M/200 KCl some inactive specimens were observed to die. However, normally the inactive specimens recovered at widely varying times and swam off.

In M/100 KCl inactivity was observed at $2^1/_2$ minutes in 1 test, and almost all organisms were inactive bewteen 5—$7^1/_2$ minutes after start in 4 tests. In a few M/300 KCl tests many specimens became inactive from soon after mixing to $5^1/_2$ minutes, and periods of inactivity ranging from $1^1/_2$—46 minutes in length were observed.

In M/100 KCl recovery was seen between $11^1/_2$—30 minutes. In 1 test almost all specimens were inactive at 6 minutes, but half had recovered by 40 minutes.

Recovery was followed by slow forward spiraling sometimes interrupted by irregular bursts of rapid backward spiraling (PCR), or also by more extensive spiraling, in M/100 KCl. In M/200 KCl rising organisms did rapid backward spiraling. In M/500 KCl 2 inactive specimens disturbed with a glass needle at 99 minutes swam away slowly, but in M/700 KCl specimens so disturbed did rapid backward spiraling.

Two inactive organisms were observed at higher magnification in M/200 KCl. Their contractile vacuoles functioned, and they contracted and extended their adoral membranelles. The adoral membranelles of both also began to vibrate. After several more contractions of the adoral membranelles, these specimens rose up at different times.

A few inactive specimens left a sticky, translucent substance on the substrait as they swam off or were pushed with a glass needle. The heterotrich *Spirostomum* can attach itself to solids with mucus (Jennings 1906).

M/100—M/700 KCl solutions containing specimens were shaken at intervals, and this usually produced rapid backward spiraling in M/200—M/700 KCl. In M/100 KCl shaking more frequently caused only some spiraling, or resulted in no response. In M/50—M/700 KCl occasional rapid backward spiraling occurred naturally later in the tests.

H. grandinella in $CaCl_2$ solutions

Distilled water — $CaCl_2$ solutions

Method

H. grandinella in a small drop of culture fluid were mixed with 20—30× that volume of solutions of calcium chloride ($CaCl_2$) in distilled water. The $CaCl_2$ solutions had been neutralized with insignificant quantities of 0.1 N HCl and 0.1 N NaOH. Between 6 and 23 tests were performed with a solution.

Results

Organisms added to 1 molar $CaCl_2$ died instantly. In M/10 $CaCl_2$ some *H. grandinella* showed spurts of rapid backward spiraling alternating with slower backward spiraling. This was followed by continuous rapid backward spiraling and death. In some cases death was immediate.

In M/50 $CaCl_2$ in distilled water the backward spiraling of some specimens was interrupted by forward spiraling (PCR), a slower backward movement, or stopping (Pl. II 8). Rapid backward spiraling also alternated with forward spiral-

ing (PCR) or slower backward movement. *H. grandinella* died between $1^1/_4$—$3^1/_2$ minutes after the start in many tests, and in 1 test were observed to develop a shrunken shape (osmotic effect) before death.

In M/100 $CaCl_2$ in distilled water longer forward spiraling was briefly interrupted by backward spiraling (PCR), while in an equal number of cases longer periods of backward spiraling by individual specimens were briefly interrupted by forward spiraling. Some slow forward spiraling — backward spiraling PCR was also seen. In addition, forward spiraling was interrupted by spurts of rapid backward spiraling (PCR).

In both M/50 and M/100 $CaCl_2$ some specimens did only continuous forward spiraling, slow forward spiraling, or backward spiraling. Times of inactivity on the substrait varied, and jumping appeared to decrease with time.

Lake water — $CaCl_2$ solutions

A similar group of experiments was performed with solutions of $CaCl_2$ in bacteriologically-filtered lake water.

In M/50 $CaCl_2$ in lake water there was periodic alternation (PCR) between forward spiraling and rapid backward spiraling in most tests. In some tests with M/100 $CaCl_2$ there was also f.sp. — r.b.sp. PCR, or spurts of rapid backward spiraling were performed, while in other tests the organisms showed normal behavior. Shaking these solutions elicited rapid backward spiraling.

Mixtures of distilled water — M/10 $CaCl_2$ with culture fluid

A good amount of forward spiraling — rapid backward spiraling PCR was observed in mixtures of 1, $1^1/_2$, 2, and 3 parts of culture fluid (pH 7.0—8.3) to 1 part of a distilled water solution of M/10 $CaCl_2$ (pH 7.0). In these mixtures PCR was not long-lasting, and was followed by forward spiraling, smooth rapid backward spiraling, or death.

Summary of movements in $CaCl_2$ solutions

The outstanding and peculiar effect of $CaCl_2$ solutions thus was to produce periods of backward spiraling and rapid backward spiraling, alternating with forward spiraling, slow forward spiraling, slower backward movement, or momentary stopping.

In an additional experiment, specimens avoided a M/50 $CaCl_2$ drop by jumping and forward spiraling.

Acetic acid drops placed in culture fluid

Method

In order to study the responses of *H. grandinella* in the present culture fluid to acetic acid (CH_3COOH), bacti.-filt. lake water solutions of 0.1%—0.0005% acetic acid were placed by micropipette in lakes of culture fluid. Control tests with bacti.-filt. lake water brought to pH 7.0 by use of 0.1 N HCl and 0.1 N NaOH were interspersed among the acetic acid tests.

Results

At concentrations between 0.1%—0.01% some specimens died in the region of the acetic acid drop. Drops of 0.1%—0.005% acetic acid clearly caused the

H. grandinella to retreat by forward spiraling and jumping. A clearcut reaction to 0.001% acetic acid could not be identified in most tests. At 0.001% and 0.0005% acetic acid there were a few positive responses, but no consistent positive response to acetic acid was observed at any of the tested concentrations. At concentrations of 0.01% and less the specimens swam in a circular path around the periphery of the drop in a few tests, or formed a ring as they moved back toward the center of the drop area.

The responses to the pH 7 lake water controls were ambivalent. Specimens in culture fluid from many test tubes at first retreated from the drop area. However, in another series of tests, in which the culture fluids were lowered to pH 7, no difference was observed between the responses to culture fluid drops from the test tubes from which the *H. grandinella* originated and the responses to drops of pH 7 lake water. In a small group of tests specimens from older test tubes entered pH 7 culture fluid from new test tubes more rapidly than pH 7 culture fluid from their own test tube, and concentrated only in the new culture fluid.

It thus appears that the few positive responses to 0.001% and 0.0005% acetic acid, as well as circular swimming and ring formation around acetic acid drops, may have been produced by the lower osmotic pressure of the drop, or perhaps by other features of the lake water in the solutions or of the *H. grandinella*-containing culture fluid, such as the relative levels of metabolic waste products.

Acetic acid drops and reversal.

In an additional experiment drops of 1% (and also of 0.1%) acetic acid and drops of 1% (and also of 1 molar) KCl were alternately placed in lakes of culture fluid on slides. As the drop areas of KCl expanded, specimens did rapid backward spiraling through the KCl and were not killed by the KCl unless they stopped in it. As the acetic acid drop areas expanded, many specimens were killed if reached by the CH_3COOH. In almost all tests there was no rapid backward spiraling through the acetic acid. If rapid backward spiraling was initiated in another portion of the culture fluid lake, almost all specimens stopped on reaching the acetic acid, and some then even did rapid backward spiraling back toward their original location (a few specimens did rapid backward spiraling into the acetic acid during 1 test, and died).

When slides were shaken to stimulate rapid backward spiraling, again specimens did not do rapid backward spiraling into acetic acid as they did into and through KCl, but stopped at the edge of the acid. Thus *H. grandinella* ends its fast reversal on reaching the edge of a killing acid solution, but not on coming to the edge of a strong (even 1 molar) salt solution.

H. grandinella in acids

Method

In order to determine the effect of low pH, *H. grandinella* were observed in dilute acids.

In 16 tests, solutions of acetic acid in bacti.-filt. lake water were given a desired pH, about 0.5 units below the intended test pH, by adding lake water or acetic acid in lake water. Now a small drop of *Halteria*-rich culture fluid was placed into the well of a liquiloc culture slide, supplied by Misco Corp., Ann

Arbor, Michigan. Then 20—30× as great a volume of a solution of acetic acid was mixed with the drop. The pH was read after 1 minute. Controls indicated that the saturated KCl solution in the glass electrode of the pH meter did not leak out sufficiently to affect the *H. grandinella*. The culture fluid drops had a pH of 7.78—7.80, and the solutions produced by mixing them with a dilute acetic acid rose an average of 0.1 pH unit in 4 minutes.

In addition to a series of less accurate pH tests with acetic acids, experiments were performed with known percentages of acetic acid, made up with lake water given a neutral pH by means of 0.1 N HCl. Also, 43 tests were carried out with HCl- lake water solutions, using culture fluid drops of pH 7.58—7.79.

Results

1. Death point. In the more accurate 16 pH tests with acetic acid solutions, the death point lay in a pH range of 5.70—5.74, as measured shortly after mixing. The series of less accurate acetic acid experiments corroborated a death point in the pH 5.6—6.0 range. The specimens invariably died in tests with 0.1%—0.03% acetic acid in neutral lake water, and always became normal in 0.003%—0.0005% acetic acid. They died in most tests with 0.02% acetic acid, which had pH's below 5.6 before mixing.

In the HCl tests, the death point appeared to lie in the pH 5.0—5.6 range. This lower death point in HCl solutions may reflect the slower biological action of strong mineral acids, due to inferior penetration. Also, since HCl dissociates more completely than acetic acid and does not have the latter's buffering action, it may allow the pH to rise more rapidly.

2. Behavior. At pH 5.6 or less in acetic acid solutions rapid backward spiralling, slower backward spiraling, or PCR between rapid backward spiraling and forward spiraling were usually the only responses preceding death. As the pH became increasingly acid death ensued sooner, and there were increasingly shorter periods of rapid and slower backward spiraling.

A period of forward spiraling — rapid backward spiraling PCR was observed in many tests. In acetic acid tests above pH 5.6 or in HCl tests above pH 5, spurts of jumping and unusually slow forward spiraling were seen (Pl. III 9). If above these pH's rapid backward spiraling, or rapid backward spiraling — forward spiraling PCR took place, they normally preceded and/or were synchronous with spurts of jumping or slow forward spiraling. The last 2 movements in turn sometimes were interespersed with and then also followed rapid backward spiraling.

In a number of acetic acid and HCl tests, the specimens gathered at the surface of the test solution. This was probably due to the lower solubility of 0_2 in these solutions, as compared to that in pure culture fluid.

H. grandinella in distilled water

Method

In over 30 tests a drop of culture fluid containing numerous *H. grandinella* was mixed with 20—30× that volume of distilled water having a neutral pH.

Results

The makeup of the culture fluid from which the *H. grandinella* originated had a major effect on their responses. In a few cases specimens from some

highly-evaporated cricket cultures prepared for this experiment became instantly immobile on mixing with the distilled water. More frequently specimens first reacted by doing unusually much jumping or only jumping, by slow forward spiraling with some doing fairly fast normal forward spiraling, and by some spurts of backward spiraling. This was followed by rapid backward spiraling and backward spiraling, or by spurts of rapid backward spiraling usually alternating with stopping. In a few cases PCR between rapid backward spiraling and forward spiraling was observed. Later the organisms became inactive on the substrait and died.

In a large sample of tests with organisms from normal fly cultures rapid backward spiraling and backward spiraling began between $^1/_2$—1 minutes after the start of the test. There was no jumping during the period of backward spiraling. Most organisms were inactive on the substrait after $2^1/_2$ minutes.

Under normal conditions *H. grandinella* responds negatively to distilled water. If a culture fluid drop containing specimens was placed as a separate entity in distilled water they remained in the drop area by jumping back from its periphery.

Galvanotaxis

Method

Responses to direct electric current were studied with nonpolarizable 0.5 mils Ag — AgCl electrodes in miniature corkstoppered bottles. A bent 15 cm.-long glass tube of 4 mm. internal diameter, its tapered distal end plugged with cotton, extended between each bottle and the *Halteria*-containing culture fluid (pH7—8.3) on a slide. The bottles and glass tubes were filled with culture fluid from the culture being used, and their culture fluid was changed after every observation, as were the experimental *H. grandinella*. The cross-sectional area of the lake of culture fluid ranged around 0.1 cm^2, and the anode and cathode glass tubes were 2—5 cm. apart.

Voltages were provided by a 170 volt D.C. generator and power distribution board, and tests were performed between 0.005 mA, 2 volts, and 0.44 mA, 150 volts.

Results

Between 0.005—0.008 milliamperes (2 and 3 volts) the direction of swimming of the *H. grandinella* could not be clearly discerned. Some drifting to the cathode, due to slow forward spiraling, appeared at 0.011—0.013 mA (4 volts). There was a general drift toward the cathode at 0.014 mA (5 volts). Above 0.014 mA and to 0.44 mA (150 volts) there was slow forward spiraling, noticeable slow forward spiraling and also fast normal forward spiraling toward the cathode (Pl. III 10).

The forward spiraling toward the cathode was opposed by jumping, and also rapid backward spiraling, toward the anode. Jumping toward the anode was clearly observed between 0.070 mA (24 volts) and 0.44 mA (150 volts), or over most of the effective tested current range (Pl. III 11).

There was a higher than normal rate of jumping under electric stimulation, and in some tests bunches of organisms tended to maintain their position at a certain distance from the cathode by jumping. These observations suggest that oriented jumping was performed in opposition to movement toward the cathode,

rather than being only a consequence of the orientation of the anterior ends of the organisms toward the cathode.

Oriented rapid backward spiraling toward the anode occurred between 0.056 mA (20 volts) and 0.44 mA (150 volts).

The specimens often reached the cathode, and frequently also gathered at it. How near in a test specimens approached to either the cathode or the anode depended on their starting position.

Conclusions and discussion

Jumping is the escape movement employed on meeting physical obstacles, and the foregoing results indicate it is also used to remain within a more favorable solution. Thus *H. grandinella* stays at the periphery of fluid with an unfavorable osmotic pressure, outside areas of salt concentration or acidity, and with in the diffusion zone of nutrient material partly by jumping. Jumping also occurs only at higher pH's in acid solutions, and precedes rapid backward spiraling, which is then followed by death, in distilled water.

Rapid backward spiraling is therefore a response to stronger stimuli, or has "caught" in an expanding strong salt solution or on entering one. It alternates with forward spiraling movements in PCR, and precedes death in acids, distilled water, M/10 KCl and M/10 $CaCl_2$. It also precedes partial recovery in M/200 and M/300 KCl, and jumping appears during this recovery.

Rapid backward spiraling is therefore a response to stronger stimuli, or has a higher threshold, than jumping. The last is used when a small retreat, or no advance, is a sufficient response.

Rapid backward spiraling is apparently homologous with the ciliary reversal of other *Protozoa*. This view is strengthened by the fact that most ciliates spiral to their left both during forward spiraling and ciliary reversal (Ludwig 1929), this also being the direction of spiraling during forward spiraling and rapid backward spiraling in *H. grandinella* (Tamar 1965). Furthermore, rapid uniform temperature change and unlocalized shaking elicit rapid backward spiraling, as they do reversal in other ciliates (Jennings 1906). The fact that rapid backward spiraling has a higher speed than the usual ciliary reversal can be related to the higher development of the adoral membranelles in *Halteria*.

Jumping, on the other hand, evolved independently in the *Oligotrichida* and is linked with the development of equatorial somatic cirri. Since a short reversal is the basis of the avoidance reaction shown by lower ciliates in response to lower stimuli, it can be said that jumping has replaced reversal as a response to weaker stimuli in *Halteria*.

The evolution of a faster reversal, and the replacement of reversal by jumping as the escape reaction to a lower level of stimulation, could be of much benefit to an organism. Rapid backward spiraling, because of its high speed, might enable *Halteria* to escape predators, or pass through an occasional strong stimulating agent for a considerable distance without damage. However, such fast reversal could possibly represent a significant energy drain. The existence of only this escape movement might result in a high frequency of excessive response to minor stimuli. The short, although still rapid, jumping movement, on the other hand, appears better suited for frequent response to multiple minor physical and chemical stimuli.

Since usually jumping frequently interrupts forward spiraling and slow movement, even when no obstacles are visible, the possibility exists that even

small particles or minor concentration differences might elicit jumping. The fact that sometimes long periods of uninterrupted forward spiraling can be observed enhances this possibility.

Specimens placed in M/500 KCl, and many in M/300 KCl, did slow forward spiraling immediately after entrance into these solutions. Thus in *H. grandinella* more dilute KCl solutions can reduce motor activity without a previous period of fast reversal. This suggests that the slow forward spiraling following reversal in M/200 KCl, etc. is actually the consequence of some accommodation. That accommodation during K reversal may result in reduced motor activity is made more plausible by the finding of Jahn 1962 that in Kamada and Kinosita's 1940 experiments with *Paramecium* there was adaptation to K. It resulted in shorter K reversal. The maximum duration of ciliary reversal attained with K^+ — containing test solutions was always less for paramecia adapted to solutions containing higher K^+ concentrations.

Dryl 1965 reports a 10—15 minute period of K reversal, followed by almost complete stoppage of movement caused by strong inhibition of motor activity, in *Stylonychia*.

Grębecki 1964 (note 1) agrees with Oliphant 1938 that increased K concentration can only increase the duration of reversal. He believes that no K concentration can produce a maximal duration of reversal. The present results corroborate this view. The longest reversal in *H. grandinella* was produced by M/50 KCl, and in the next higher concentration, M/10, reversal was cut short only by death.

Czarska 1964 states that Ca accelerates protozoan movement, while K slows it down as to cause reversal, i.e. its action is inhibitory. This description of the action of K satisfactorily explains the reduced motor activity of slow forward spiraling. However, it is difficult to think of the unusual, rapid reversal of *H. grandinella* in terms of inhibition. In *Stentor* the membranelles are inhibited at the same time that the body cilia are reversed (Sleigh 1962), suggesting a critical difference in sensitivity of the 2 types of structures. The present KCl results with only the adoral membranelles show 2 qualitatively different levels of response, determined by both concentration and time. Perhaps partial depolarization of the adoral membranelles of *H. grandinella* by KCl results in reduced motor activity (inhibition), while more complete depolarization, beyond a critical threshold, produces a full response, reversal. The studies of Jahn 1961, Naitoh 1958, and Okajima 1953 on the relationship between membrane potential and reversal are well-known.

The considerable inactivity in KCl solutions is probably also a result of inhibition.

As is predictable from the concept of the stomato-caudal gradient (Grębecki 1965 a), a higher concentration of KCl is required to inhibit jumping than is necessary to inhibit the adoral membranelles and produce slow forward spiraling.

It is likely that the higher concentrations of KCl exerted an osmotic effect on the experimental organisms. However, forward spiraling, although slowed (partly due to rising pH?), remained normal in highly-evaporated or even drying culture fluid.

Considerable PCR, and some continuous reversal, appeared in the distilled water and lake water solutions of $CaCl_2$, as well as in the culture fluid — M/10 $CaCl_2$ mixtures. These results seem to corroborate the finding of Grębecki

1965 b that the loss of Ca^{2+} is not the only basis for ciliary reversal. However, in the present work, unlike Grębecki's, there was no previous decalcification, and there was no addition of ions able to compete for adsorption sites with Ca^{2+}, as were added by Dryl 1965. The present results are surprising, since the addition of Ca does not affect the normal movement of *Paramecium* (Grębecki 1965 b). According to Jahn 1962, it is probable that at very high concentrations of divalent ions mutual ionic competition will cause most or all of the binding to be monovalent. Perhaps in the present research a smaller amount of monovalent binding of Ca^{2+} was sufficient to produce PCR.

Like most ciliates, in a direct electric current *H. grandinella* turns its anterior to the cathode, swims toward the cathode, and in stronger currents does reversal toward the anode. However, only the anterior circle of adoral membranelles (and to a small extent perhaps the oral membranelles) are available for swimming. The organisms can therefore swim toward the cathode essentially only by normal movement of the adoral membranelles. Does this necessary absence of anterior reversal contradict the Ludloff phenomenon? Cathodal movement consists primarily of slow forward spiraling. This suggests that only a general inhibition is produced, instead of general fast reversal, due to low sensitivity of the adoral membranelles to electric stimulation. Párducz 1963 found that a minimal current does not cause the anterior cilia of *Paramecium* to reverse, but only causes them to beat approximately caudally, probably with reduced effectiveness. Jahn 1961 assumes a threshold degree of depolarization of the normal membrane potential or of current density causes reversal. The adoral membranelles could have a higher threshold to electric stimulation than the anterior somatic cilia of other ciliates, and might only undergo partial depolarization during much of the exposure to the tested current strengths. Such a higher threshold would have antecedents, since Párducz 1959 states that the peristomial ciliature represents the conservative element of protozoan ciliature, and has the tendency to maintain its normal beating against all stimuli. The above explanation would correlate well with the high threshold of the adoral membranelles to other stimulation (lower stimuli generally elicit only jumping). It would also relate well with their having a low level of response to KCl, termed by Grębecki 1963 the chemical cathelectrotonus. *H. grandinella* is only inhibited in M/500 KCl (less than 122 parts Ca^{2+} and 81 of Mg^{2+}/million). However, its periods of reversal could not be shown to be shorter than those to be expected with *Paramecium* in equivalent solutions by referring to the researches of Grębecki 1964, Oliphant 1942 and 1938, and Mast and Nadler 1926.

Over most of the tested current range specimens did fast reversal toward the anode, after periods of slow forward spiraling to the cathode. These scattered reversals may have been due to an increasing depolarization with time, and may reflect individual differences in sensitivity. Both reversal and jumping toward the anode may also have resulted from the accumulation of cations in the culture fluid at the cathode (where bases too would form).

Perhaps *H. grandinella* in a direct current originally turns its anterior toward the cathode because of more vigorous normal movement of the adoral membranelles on the side toward the anode.

It is interesting that in *H. grandinella* the normal movements of the 2 groups of motor organelles, the adoral membranelles and the somatic cirri, located in different body regions, oppose each other. This is true in the natural environ-

ment, and, with the limitation of inhibition of the adoral membranelles, also in an electric current. The stimulation of jumping is the stimulation of a normal movement, not of a reversal.

Since the extent of inhibition and reversal on the cathodal surface of *H. grandinella* is easily determined because of the anterior localization of all the swimming organelles, this could be an experimental species of choice for measurement of the processes at the cathodal surface. In *H. grandinella* even extensive reversal ceases shortly after the end of the excitatory process, since recovery begins anteriorly. There is no balance between anterior and posterior cilia as in *Paramecium*, in which reversal and recovery involves 5 steps (P á r d u c z 1959).

Summary

H. grandinella reacted to large differences in osmotic pressure and concentrated near protein material. Only reduced movement appeared in dilute KCl, and followed fast reversal in more concentrated KCl. Considerable periodic ciliary reversal was produced by $CaCl_2$, and less by acids and distilled water. In acetic acid death occurred between pH 5.70—5.74. Slow cathodal galvanotaxis was opposed by jumping and reversal. Inhibition is differentiated from reversal. Jumping has replaced reversal as a response to weaker stimuli, and an unusually high threshold is suggested for the fast reversal of the adoral membranelles.

ZUSAMMENFASSUNG

H. grandinella reagierte zu grossen Unterschieden im osmotischen Druck, und konzentrierte sich um einen proteinischen Stoff. Nur herabgesetzte Bewegung zeigte sich im verdünnten KCl, und folgte dem schnellen Rückwärtsschwimmen im mehr konzentrierten KCl. Beträchtliches periodisches Rückwärtsschwimmen wurde von $CaCl_2$ hervorgerufen, und wenigeres von Säuren und destilliertem Wasser. Der Todespunkt in Essigsäure lag zwischen pH 5.70—5.74. Der langsamen kathodischen Galvanotaxis sind Springen und Rückwärtsschwimmen entgegengetreten. Hemmung wird vom Rückwärtsschwimmen unterschieden. Springen hat Rückwärtsschwimmen als eine Antwort auf schwächere Reize ersetzt, und ein ungewöhnlich hoher Schwellenwert wird für das schnelle Rückwärtsschwimmen der Adoralmembranellen angedeutet.

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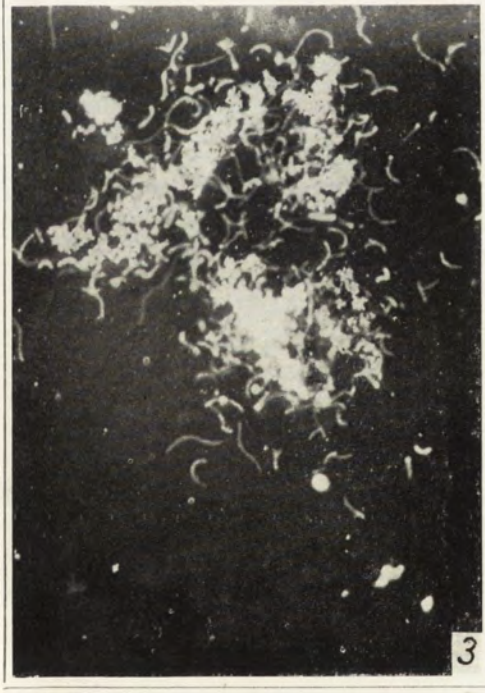
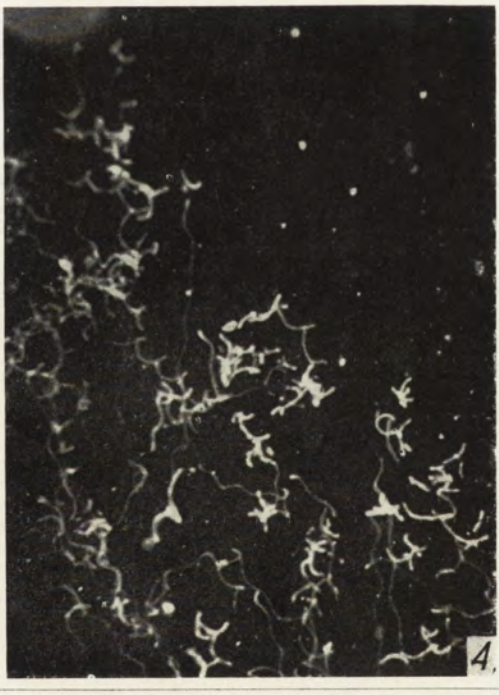
EXPLANATION OF PLATES I—III

1: Side-view of forward spiraling *H. grandinella*, showing whirling adoral membranelles and one group of 4 somatic cirri. Phase contrast, 500×
2: Concentration in drop of distilled water placed in drying culture fluid
3: Concentration over protein material adhering to slide
4: Backward jumping and forward spiraling at edge of 1 M KCl drop
5: Backward jumping and forward spiraling at edge of 1 M KCl, 30×
6: Backward jumping at edge of 1 M KCl, 30×
7: Rapid backward spiraling (reversal) in M/50 KCl. One specimen, to right of center, is doing slower backward spiraling, and others are becoming slower
8: Forward spiraling, followed by spurts of rapid backward spiraling (reversal) interrupted by stopping, in M/50 $CaCl_2$
9: Repetitive jumping in dilute acetic acid, 30×. Note the undulating line of jumps
10: Forward spiraling toward a bare Ag-AgCl cathode.
11: Jumping toward, and some forward spiraling away from, a bare Ag—AgCl anode
All photomicrographs, except first, with binocular microscope.
At 10×, unless stated otherwise. Tri-X film, Diafine developer.
Printed by Audio-Visual Center, Indiana State University.





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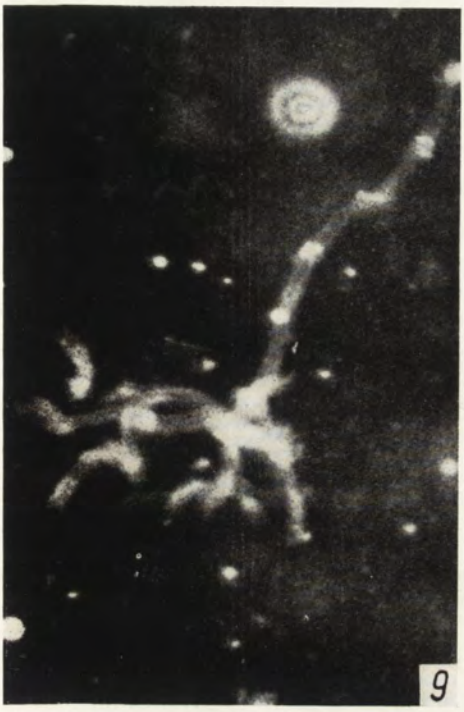

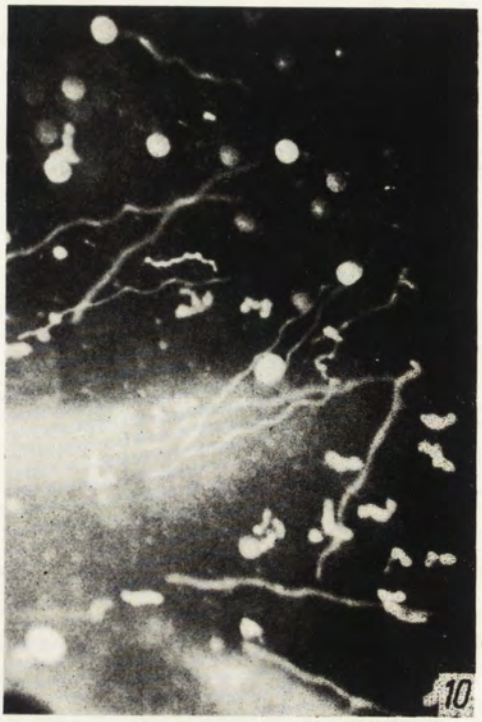

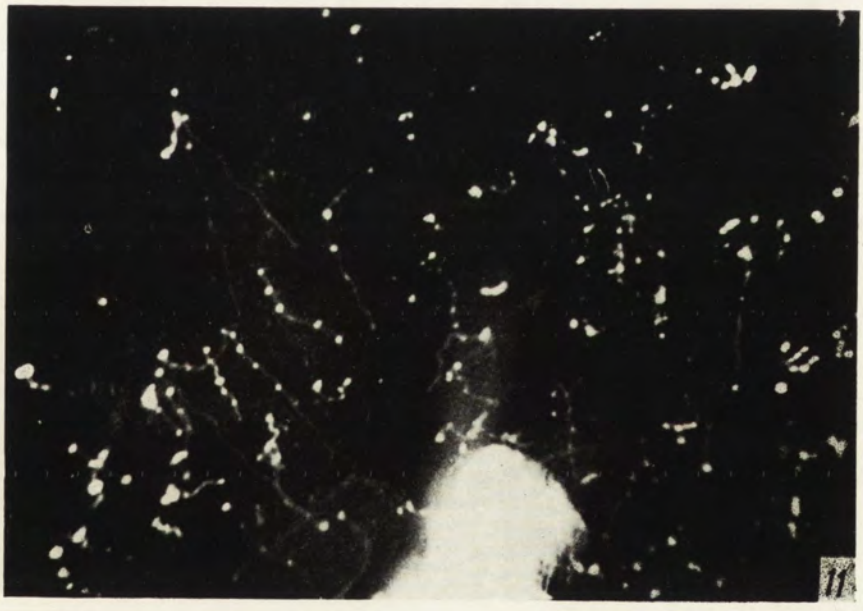

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Some observations on the inversion of spiralling in *Paramecium caudatum*

Obserwacje nad inwersją spiralizacji u *Paramecium caudatum*

Under the conditions hampering the work of ciliary apparatus in *Paramecium* of the *"aurelia"* group, occurs the inversion of spiralling from the normal left (FLS) to the right one (FRS). The change of FLS to FRS is evoked by the increase of the medium viscosity (Alverdes 1922, Ludwig and Schlicksupp 1951), by nickel ions (Párducz 1962, Grębecki, Kuźnicki, Mikołajczyk 1966) and by the treatment with homologous antiserum (Kuźnicki and Sikora 1966). Therefore this phenomenon seems to be independent of the factor evoking it which suggests that in *P. aurelia, P. caudatum* and *P. multimicronucleatum,* FRS is a more effective form of forward movement than FLS.

Under the action of nickel ions, inversion of spiralling occurs in two manners: 1. FLS → looping → FRS, after application of relatively low concentrations, 2. FLS → repeated ciliary reversal (CCR) → FRS, in concentrations evoking the immobilization of ciliates in a rather short time (4—12 min.) — Grębecki, Kuźnicki, Mikołajczyk 1966.

P. aurelia placed in solutions of homologous antiserum show exclusively the second type of sequence, before immobilization however FRS changes recurrently into CCR and CCR into FRS (Kuźnicki and Sikora 1966).

It may be concluded from these facts that the cause of different ways of changing FLS for FRS is of a mechanical nature, i.e. it depends on the intensity of factor which hampers the forward movement of the ciliate. This problem has not been analyzed till now from this point of view. As it seems, in this case the most univocal answer could be given by the results of experiments with a factor which exerts an action of physical nature on the ciliary apparatus. Considering this, studies were carried out on the ways of change of FLS for FRS after the transfer of ciliates into methyl cellulose solutions of different density. The influence of gradual and abrupt fall of temperature on the motory reactions of *Paramecium* was also analyzed.

The effect of increasing viscosity of the medium

Experiments were carried out on a clone of *Paramecium caudatum* cultivated on milk nutrient. Prior the experiment ciliates were rinsed in a 1.0 mM $CaCl_2$ solution and collected from the geotactic column. This operation was repeated 3 times so as to dilute the culture medium to 1000×. The methyl cellulose solutions were also prepared in 1.0 mM solution of $CaCl_2$. The liquid

Fig. 1. FLS and typical motory responses of *P. caudatum* after the transfer into methyl cellulose solutions of various density, A. FLS, B. Looping with no inversion of spiralling, C. Inversion of spiralling of the type FLS → looping → FRS, D. Inversion of spiralling of the type FLS → CCR → FRS, E. Sequence of motory reactions FLS → CCR → FRS → CCR... with a shortened phase of FRS movement

with ciliates was mixed with solutions of methyl cellulose in the ratio 1 : 1 and the whole sample was subsequently stirred rapidly and carefully. Observations were performed 1 min. after the ciliates were placed in the solution of methyl cellulose. All the experiments were carried out in the temperature of 20±1°C.

The movement reactions of *P. caudatum* were studied in methyl cellulose solutions of a concentration 0.05—1.5%. The behaviour of ciliates was observed in a stereoscopic miscroscope and, for control, the method of photographic registration of movement (D r y l 1958) was also applied.

P. caudatum introduced into a methyl cellulose solution show different movement reactions depending on concentration of the compound. Their most characteristic forms have been illustrated in Fig 1.

The ciliates introduced into 0.05—0.1% methyl cellulose solutions keep their left spiralling, however the majority of individuals has changed the character of their path. Then spiralling disappears, and paramecia describe loops. After having left the loops, they continue their normal movement of the FLS type (Fig. 1 B). A few minutes later, the tendency to form loops disappears, the paths of ciliates straighten and are similar to those in the calcium medium (Fig. 1 A).

Beginning with the concentrations 0.1%, the methyl cellulose solutions evoke the inversion of spiralling FLS for FRS in separate individuals. In proportion of the increase of concentration this process augments and in concentration 0.5% it concerns 100% of individuals in a sample. The transition from FLS to FRS fails to differ in its character from that described for the nickel ions (P á r d u c z 1962, G r ę b e c k i, K u ź n i c k i, M i k o ł a j c z y k 1966). The ciliate entering the loop with a movement of FLS type, ceases spiralling on the arch, and after having left it, begins the right spiralling (Fig. 1 C). At concentrations above 0.5%, this way of inversion of spiralling disappears in favour of a sequence of the type FLS → CCR → FRS. Ciliates respond uniformly to the transfer to 1.0% methyl cellulose solution. A few seconds-long ciliary reversal (CCR) occurs, followed by a forward swimming with right spiralling (FRS). FRS changes repeatedly to CCR, and CCR to FRS (Fig. 1 D).

Ciliates respond in a similar way to methyl cellulose solutions higher than 1%, their FRS phase however becomes gradually more reduced in favour of CCR (Fig. 1 E).

In 1% solutions of methyl cellulose the motor responses of *P. caudatum* show a parallelism with the behaviour of *P. aurelia* after the treatment with homologous antiserum (K u ź n i c k i and S i k o r a 1966).

The effect of gradual and abrupt fall of temperature

The study of the effect of gradual and abrupt fall of temperature on the motor responses of *P. caudatum* were carried out in a thermostatic chamber of the air temperature +3°C.

The methods of preparation experimental samples and of observations were the same as in the case of experiments with methyl cellulose. The behaviour of ciliates after a gradual change of temperature was observed for 40 min. The initial medium temperature was +20°C and the final one +3°C.

Up to +5°C no changes were observed in the character of path and in direction of spiralling except slowing down of the movement. At the temperature of +4.5°C separate paramecia begin to describe loops not followed by any change of spiralling in FRS, similarly as it occurs in the methyl cellulose solutions 0.05 up to 0.1% (Fig. 1 B). At the change of temperature from +4.5 to +3°C

this type of response embraces 98—100% of individuals in a sample. Separate individuals (0—2%) may change the direction of spiralling FLS for FRS at this range of temperature. Inversion of spiralling occurs over a typical loop as illustrated in Fig. 1 C.

The experiments with the abrupt change of temperature were modified in this way that ciliates in their medium at temp. +20°C were mixed with 1 mM $CaCl_2$ solution of temperature 0°C in a proportion which resulted in the sample temperature +3°C (±0.5%). In these experiments the motory response of ciliates is uniform. A few seconds long ciliary reversal appears (CCR) which is left by paramecia with the FRS movement.

Discussion

Observations of motor responses in *P. caudatum* after placing them into the methyl cellulose solutions of different density, give an answer to the question what is the cause of different ways of the inversion of spiralling. The way of transition FLS → FRS depends on the intensity of the factor hampering the work of cilia.

In the case when the action upon the ciliary apparatus is of a mechanical nature — as it takes place after transferring the ciliates into methyl cellulose solutions — the interdependence of the reaction type and the degree of hampering may be expressed quantitatively in physical values. At a relative increase of medium viscosity up to 1.3 centipoises (0.1% methyl cellulose concentration), the unic form of response remains looping not followed by the inversion of spiralling. This type of response is fully reversible and all the individuals return to their movement FLS. Looping involving the change of spiralling occurs in the limits 1.3—3.5 centipoises of the relative increase of viscosity (0.1—0.5%). The above limits embrace also individual differences in the behaviour of ciliates under the conditions of increased medium viscosity (after a viscosity increase of 3.5 centipoises, 100% of individuals exhibit the change of FLS for FRS).

At the increase of relative medium viscosity in the limits 3.5—50 centipoises (0.5—1.0%) this type of inversion of spiralling disappears in favour of the sequence FLS → CCR → FRS. Ciliates, when transferred to a medium of a relative viscosity $>$ 5.0 centipoises ($>$ 1.0%) respond uniformly according the sequence FLS → CCR → FRS → CCR...).

Under the influence of factors acting selectively on the ciliary apparatus (nickel nois, homologous antiserum) the scope of hampering the work of cilium, cannot be determined quantitatively in physical values. Nevertheless the parallelism of the ways of transition from FLS to FRS indicates that the cause is in both cases the same. Some other evidences suggest that the motor responses under the influence of nickel cannot be reduced to a "calcium" mechanism (competitive release of calcium ions by nickel ions from the cortical layers of the ciliate). Those evidences have been presented in the article of Grębecki, Kuźnicki, Mikołajczyk 1966.

The observations on the effect of gradual and abrupt fall of temperature upon the motor responses of *P. caudatum* proved that the inversion of spiralling under the influence of this factor depends not on its absolute value but on the manner of its application.

The observations of motor responses in *P. caudatum* in 4—6 mM solutions of chloral hydrate for 48 hrs. help to elucidate these facts. Under these condi-

tions, immobilization of 80% of individuals is gained (Grębecki and Kuźnicki 1961, Kuźnicki 1963). In this case immobilizatino occurs as a result of breaking of the cilia at the level of axosome (Kennedy 1966). The first effect of the chloral hydrate action is a gradual fall of the rate of the ciliate movement. After 14—16 hrs. in separate individuals the first symptoms of breaking of the cilia are observed. This process increases slowly and only after 48 hrs. the effect of a complete or nearly complete loss of cilia is accomplished (Kuźnicki 1963). The motor responses of *P. caudatum* until the time of immobilization are similar to their behaviour during a gradual fall of temperature. In the first place, the inversion of spiralling is never observed under the influence of chloral hydrate (Kuźnicki, unpublished). It should be concluded therefore that the obligatory condition of the change of FLS to FRS is a rather quick action of the inducing factor. A very slow and gradual decrease of ciliary activeness excludes the inversion of spiralling (chloral hydrate) or reduces it to the cathegory of exceptional phenomena (gradual fall of temperature). After abrupt changes of temperature, the transit FLS → CCR → FRS takes place immediately, i.e. in a phase when the motory apparatus has no time to adjust its activeness.

The results presented above together with the facts described previously (Alverdes 1922, Ludwig and Schlicksupp 1951, Párducz 1962, Kuźnicki and Sikora 1966, Grębecki, Kuźnicki, Mikołajczyk 1966) indicate that as well the inversion of spiralling as the manner of transition of FLS to FRS are not depending in any specific manner on the factor evoking them but are the consequence of hampering the work of the ciliary apparatus. This induces the authors to put forward a hypothesis of mechanical factors sharing in the regulation of the ciliary apparatus in *P. aurelia, P. caudatum* and *P. multimicronucleatum.*

This hypothesis is based on the following points: 1. The effectiveness of the ciliary work is independent of the angle of beat and of the rate at which it works (Pigoń and Szarski 1955). Consequently if the body structure is twisted clockwise ciliates of the above species overcome the less medium resistance swimming with their posterior end forwards (CCR). In the forward movement, FRS (consistent with the body twisting) is the more effective manner of motion than FLS (opposite to the direction of the ciliate body twist). From the point of view of hydrodynamic effectiveness, the following range is postulated: FLS < FRS < CCR. 2. Ciliary apparatus is a system endowed with autoregulation properties, its essential function is to secure the maximal effectiveness of swimming. Under the conditions of a hampered work of ciliary apparatus an automatic compensation occurs consisting in the change of manner of swimming from a form hydrodynamically less effective (FLS) for some forms hydrodynamically more effective (FRS, CCR). It is postulated as well, in agreement with the suggestion of Sleigh 1965, that in *Paramecium* the metachronal coordination is mechanical.

Summary

Motory responses of *P. caudatum* were studied after placing the ciliates in methyl cellulose solutions of various density as well as under the influence of a gradual and abrupt fall of temperature. The obtained results and the facts described previously, allow a hypothesis of mechanical regulation of the ciliary

apparatus in paramecia of the *"aurelia"* group. If the structure of ciliates is considered, from the point of view of hydrodynamics the effectiveness of swimming is the following: FLS < FRS < CCR. Under the conditions of hampered work of the ciliary apparatus, in the individuals of the above species an automatic compensation occurs consisting in the change of manner of swimming: from FLS (forward movement with left spiralling) for the forms hydrodynamically more effective, FRS (forward movement with right spiralling), or CCR (continous ciliary reversal).

STRESZCZENIE

Badano reakcje ruchowe *P. caudatum* po przeniesieniu do roztworów metylocelulozy o różnej gęstości oraz pod wpływem stopniowego i szokowego obniżania temperatury. Na podstawie porównawczej analizy uzyskanych wyników z faktami opisanymi uprzednio, wysunięto dla gatunków *Paramecium* z grupy *„aurelia"* hipotezę mechanicznej regulacji aparatu rzęskowego. Jeśli uwzględni się budowę orzęsków, z punktu widzenia efektywności pływania FLS < FRS < CCR. W warunkach utrudnionej pracy aparatu rzęskowego zachodzi u osobników w/w gatunków automatyczna kompensacja, polegająca na zmianie sposobu pływania z FLS (ruch do przodu ze spiralizacją lewoskrętną) na formy bardziej efektywnie hydrodynamicznie: FRS (ruch do przodu ze spiralizacją prawoskrętną) lub CCR (ciągła rewersja rzęskowa).

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Right spiralling induced in *Paramecium* by Ni ions and the hydrodynamics of the spiral movement

Prawoskrętna spiralizacja wywołana u *Paramecium* przez jony Ni i hydrodynamika spiralizacji ruchu

It is well established from the times of Bullington 1925 and 1930 that all the species of *Paramecium* belonging to the "aurelia group" (*P. caudatum*, *P. aurelia* and *P. multimicronucleatum*) rotate always to the left, when swimming forwards or backwards, which results in describing a spiral (more exactly — a helix) twinning in the anti-clockwise manner. The ciliary mechanism of this constant left spiralling has been elucidated in most details by Párducz 1956 who gave firm evidence that during the forward movement the metachronal waves are oriented along the lines NW—SE and cilia beat effectively to SE, whereas in the backward movement the waves assume a NE—SW pattern and the effective ciliary stroke is directed to NE, that is to say in both cases cilia beat with an oblique deviation to the right [1].

The right (i.e. the clockwise) spiralling during the forward movement in *Paramecium caudatum* was at first observed by Alverdes 1922 in the viscous media and in some lethal solutions where it precedes death. The inversion of spiralling as a mass long-lasting phenomenon was described and carfully examined by Párducz 1962 in *P. multimicronucleatum* pretreated by Ni ions. Recently the forward movement with the right rotation was described in *P. caudatum* exposed to an increase of viscosity or a decrease of temperature (Grębecki, Kuźnicki, Mikołajczyk 1966) and in *P. aurelia* exposed to the homologous antiserum (Kuźnicki and Sikora 1966).

The right spiralling induced by Ni ions seems to be the most suitable for a further analysis being the most regular. On the other hand, its study is very promising in this respect, that Ni ions are also known to block gradually the effective work of ciliary apparatus and induce the state of immobilization in different ciliates (Gelei 1935, Tartar 1950, Thomas 1953, Kuźnicki 1963, de Puytorac, Andrivon et Serre 1963, and Seravin 1963).

The aim of the present study was: 1. to confirm and re-analyse the findings of Párducz 1962 on the induction of right spiralling by Ni^{2+}, 2. to compare the development and the ionic conditions of the right spiralling with those of the ciliary reversal which is the common motory response of ciliates following

[1] All the terms indicating the direction, such as right, clockwise, NW—SE etc., refer to the situation of an observer who is looking at the upper surface of *Paramecium* from the posterior end of the ciliate body.

the excitation, 3. to examine paramecia forced to the right spiralling or immobilized by Ni^{2+} as to their ability of producing the normal motory responses under stimulation, 4. to discuss the hydrodynamical conditions of the right and left spiralling.

Material for this study was *P. caudatum* kept in the milk cultures and *P. aurelia* fed on *Aerobacter aerogenes*. Before the experimental paramecia were rinsed and transferred by geotaxis into the initial experimental medium. The solutions tested were mixed with the sample in the equal volume ratio. Nickel was applied in the form of chloride. The movement of ciliates was registered photographically by the method of Dryl 1958. The ciliary pattern was examined in fixed and stained specimens following Párducz 1952 after the modification of Grębecki 1964 b.

The following abbreviations, as suggested in a review by Dryl and Grębecki 1966, are to be used in the text: FLS (forward left spiralling) for the normal movement, FRS (forward right spiralling) for the inversed spiral movement, CCR (continuous ciliary reversal) for the backward movement with the left spiralling.

Stages of the nickel action

The aim of the first experimental series was to confirm the findings of Párducz 1962 concerning the behaviour of the animal itself as well as of its ciliary apparatus under the influence of relatively low concentrations of Ni^{2+}, those which are said to induce the inversion of spiralling to the right.

Character of movement

As it is well known from different reports, the rate of appearance of the effect of various noxious agents, especially of heavy metal ions, is strongly dependent on the amount of calcium in the medium. This holds also true in the case of Ni^{2+}. Moreover, the effectiveness of Ni^{2+} seems also to be related to some, not defined as yet, culture conditions and as result the quantitative data recorded may vary in some extent from day to day. For this reason the motory behaviour described in this chapter should be related not to any precisely defined amount of nickel but rather to some broader ranges of its concentration. Nevertheless, the appearance and the sequence of all the motory responses proved to be unambiguous, irrespective of the slight changes in the absolute intensity of the acting factor. As to the medium conditions, the observations were carried out in the culture medium, distilled water, tap water, and different solutions of calcium chloride. The ranges of Ni concentration given below concern the experiments in which paramecia (*P. caudatum*) were rinsed three times in distilled water as to dilute the initial culture medium 1000 times.

The concentration of 0.005 mM of $NiCl_2$ proved to be very suitable to evoke the right spiralling (FRS). During the first 2—4 min. after introducing Ni ions into the medium paramecia continue to swim normally (FLS), as demonstrated by the Pl. I 1, but their speed gradually decreases and the rotary component of the movement becomes distinctly reduced. Finally they cease to rotate at all and follow the paths forming broad loops. This kind of movement has been called "arching" by Párducz 1962 and is defined as "looping" by the present authors. Looping is visible in a mass sample in the Pl. I 2 and a higher magnification of a single loop is given by the Pl. I 3a. Some other specimens in the same sample may exhibit repeatedly the short ciliary reversals, as seen in the Pl. I 2

and 3b. Under the conditions in question as well looping as the short CCR response are both transitory stage preceding the forward right spiralling movement (FRS). This kind of movement appears a few min. later in all the specimens and may last, in the concentration tested, from about 10 min. up to 1 hr. or even more. The paths followed by paramecia during FRS (Pl. I 4) are quite similar to those followed during FLS, and therefore both types of the forward movement may be distinguished by no other means than the observation of the direction of rotation under a binocular microscope. Under the conditions in question the self-renormalization may occur, that is to say paramecia finally return from FRS to FLS. Looping is also a transitory stage of the recovery.

In the lower concentrations of $NiCl_2$ the nickel action is delayed, i.e. the initial lag period of FLS lasts longer, looping — and consequently also FRS — appear later. On the other hand, the recovery begins sooner. It follows from this that with further decrease of the Ni concentration below 0.005 mM the FRS becomes gradually shorter. Finally, in the solutions of about 0.001 mM it disappears completely and a period of transitory looping remains the only symptom of the Ni action. It is worth to be noted that in this low range of concentration (below 0.005 mM) CCR fails to occur at all and looping is the only transition possible from FLS to FRS.

When the Ni concentration is raised up over 0.005 mM the initial lag period of FLS becomes gradually shorter, that is to say the transition to FRS appears sooner. The transition itself gradually changes its character, namely looping becomes much less pronounced and the short repeated CCR responses (looking as a serial avoiding) replace it. In spite of the sooner onset of FRS, its duration is not prolonged but even shortened, because it becomes interrupted by the immobilization. Before immobilization the right spiralling slows down, the animals sink to the bottom where they move crawling without any rotation. Then they enter the immobilization state in the meaning of Kuźnicki 1963, i.e. they manifest some shifts on the spot but they cannot cover a distance longer than their own length (Pl. I 5). The present authors suggest to call this kind of immobilization the physiological immobilization. Subsequently, the shifts become much more limited (Pl. I 6) and the stage follows which will be called the traumatic immobilization, because (as stated in the electron microscope by Pitelka and Párducz 1965) in this phase nickel causes the loss of cilia and disturbes the superficial alveolar pattern.

The interference of immobilization and the gradual shortening of FRS in the increasing nickel chloride concentrations concludes at about 0.1 mM in the complete disappearance of the right spiralling. In such solutions, immediately after addition of the Ni^{2+} (i.e. without the lag period of FLS) paramecia show such short CCR responses that they result only in stopping without any true withdrawal, and occurring at so short intervals that they give the impression of a "jolting movement" (word of Párducz 1962). Jolting is not followed by FRS but, at once, by crawling and both subsequent stages of immobilization.

The experimental series described above, as well as the parallel series carried out on *Paramecium caudatum* and *P. aurelia* in different media, confirm in full extent the discovery of right spiralling (FRS) as a regular mass phenomenon which was made by Párducz 1962 on *P. multimicronucleatum*. Also the appearance and sequence of other motory responses accompanying the induction of FRS, agree in both studies. The subsequent stages of the nickel action upon the motory behaviour of *Paramecium* should be arrayed as follows:

FLS → looping and/or CCR → FRS → physiologic immobilization → traumatic immobilization. Some stages may be prolonged or shortened or even disappear, at all, depending on the concentration of Ni^{2+} applied and on the other medium conditions.

Ciliary patterns

Rapid fixation with osmic acid and staining the cilia with haematoxyline were effected following the technique of Párducz 1954 with the modification of Grębecki 1964 b. Paramecia of both species under study were fixed at different stages of the nickel action.

Nothing new has been revealed in the individuals fixed in the transitory period between FLS and FRS. In the case of short repeated CCR responses, some specimens exhibited the normal pattern of metachronal waves, and some others the waves typical for the ciliary reversal. In the case of looping the ciliary pattern was similar to that of normal movement, however the waves seemed to be less numerous and slightly more longitudinal.

Our results as to the ciliary pattern during FRS differ from those of Párducz 1962. Párducz reported that the pattern of ciliary waves is disintegrated in most individuals, but he found in a few specimens the NE—SW waves (i.e. perpendicular to the NW—SE waves characteristic of FLS). In fact, the orientation of ciliary stroke in such waves would correspond perfectly to the right spiralling, that is to say to the clockwise rotation of the body. However, in our fixed preparations in which the ciliary pattern has been preserved in the major part of individuals, the NE—SW waves were found only occasionally, in a few specimens. In the samples in which 100% of individuals manifested a well pronounced FRS at the moment of fixation, and after staining, over 50% beared the clearly discernable waves, these waves were as a rule longitudinal, that is to say nearly parallel to the body axis. Consequently, the authors see the necessity to recognize the longitudinal waves as characteristic of FRS, although the postulation of Párducz 1962 offerred a more simple mechanical explanation of the origin of the right spiralling. As it is demonstrated by the photomicrographs the longitudinal waves are found during FRS both in *P. aurelia* (Pl. VII 46—48) and *P. caudatum* (Pl. VII 49—51).

The picture of the ciliary cloth of paramecia fixed during the physiologic immobilization, is very difficult to be understood. All the body cilia are inclined nearly at the same angle only slightly twisting from one end of the body to another. This may mean as well a synchrony of ciliary beating as a presence of extremely long waves which exceed the length of the body. During the traumatic immobilization, the fixed individuals reveal the gradual loss of cilia.

Motory responses during right spiralling

Effect of calcium and decalcification

The effect of calcium ions was tested on *Paramecium caudatum* (only a number of control experiments were carried out on *P. aurelia*, and the results proved to be consistent in both species).

When calcium has been added to the experimental medium prior to introduction of nickel ions, the general effect is a delay of appearance of all the nickel action symptoms. This had already been reported by Kuźnicki 1963 as well as by de Puytorac, Andrivon et Serre 1963 in the case of immobiliza-

tion. The same holds also true in respect to the appearance of the right spiralling.

In the first series of experiments the low concentrations of $NiCl_2$ were applied (0.005 mM and below) which cannot evoke the immobilization, i.e. those which induce only FRS followed by the recovery and return to FLS. The effect of these nickel solutions was tested in the presence of different concentrations of $CaCl_2$, varying from 1 up to 32 mM. It may be stated in general that, under such conditions, the raise of the external Ca concentration clearly delays the appearance of FRS and, on the other hand, it advances the reappearance of FLS, that is to say the duration of the right spiralling becomes much shorter. Eventually, the FRS response may disapear completely when higher concentrations of calcium chloride are used.

In the second experimental series stronger concentrations of $NiCl_2$ were tested ($>$ 0.01 mM), those in which FRS is not followed by a return to the normal swimming but by immobilization. In this case, raising of the external Ca concentration delays also slightly the onset of FRS, but it delays much more distinctly the immobilization. As result, FRS lasts clearly longer, and eventually — in higher concentrations of calcium chloride — it may even conclude in the recovery, instead of immobilization.

The results are rather similar when the concentration of Ca^{2+} is raised not prior to the application of Ni^{2+}, but following it, that is to say in the course of FRS. When FRS has been induced by the lower concentrations of $NiCl_2$ addition of Ca reduces its duration, and when FRS has been evoked by stronger solutions of nickel chloride — raising of the Ca concentration delays the onset of immobilization.

Shortening of FRS in the presence of an excessive amount of $CaCl_2$ in the medium seems at first sight to be similar to the commonly known shortening of CCR by the external calcium, which had been extensively studied by former authors (Kamada and Kinosita 1940, Jahn 1962, Grębecki 1964 a). It might appear that calcium exerts the same renormalizing effect on paramecia which manifest the right spiralling as on those which undergo the ciliary reversal. However, a very significant difference of the mode of Ca action in both cases contradicts such a conclusion. As commonly known, addition of a sufficient amount of Ca^{2+} interrupts CCR instantly bringing paramecia back to the normal movement. This never occurs in the case of FRS. The right spiralling changes for the left one slowly and gradually, even if calcium chloride in concentration as high as 50 mM has been applied. The immediate effect of calcium is only a general acceleration of rotation, without any change in its direction.

Another significant difference between the mechanism of FRS and of CCR is revealed when the action of decalcifying agents is tested. As known from the former studies, EDTA added to the medium in low concentration evokes the ciliary reversal, but in high concentration — on the contrary — it makes CCR impossible at all, or interrupts this response if it has been induced previously. For instance, in *P. caudatum* incubated in a medium containing 1 mM of $CaCl_2$, a clear ciliary reversal is obtained by introducing 1—5 mM EDTA and the reversal becomes completely inhibited by 10 mM or more of this agent (Grębecki 1965 b). An analogous inhibition of FRS has never been obtained. In paramecia treated by 0.005 mM $NiCl_2$ solution (in the presence of 1 mM $CaCl_2$) the right spiralling persisted unchanged even after addition of 10 mM of EDTA. When EDTA had been used in the concentration as high as 25 mM, only a short-

-lasting ciliary reversal was manifested but this was also followed by return to the right spiralling.

Summarizing the results reported above, it should be stressed, that a significant difference between the ciliary reversal and the right spiralling consists in this fact that CCR may be easily interrupted as well by Ca addition as by decalcification and it seems to be directly related to a definite level of the Ca content (G r ę b e c k i 1965 b), whereas FRS persists in spite of very abrupt changes of the amount of Ca in either direction and its direct dependence on the behaviour of calcium ions cannot be established.

Effect of potassium and barium

In the subsequent series of experiments the right spiralling paramecia were investigated as to their ability to manifest the ciliary reversal. CCR was induced by the ion the most effective in this respect, i.e. by K^+. Initially potassium chloride solutions in different concentrations (varying from 1 up to 64 mM) were tested on paramecia which manifested FRS induced by 0.005 mM $NiCl_2$ (in the presence of 1 mM $CaCl_2$), and the immediate motor response of animals was recorded.

Introducing of 1 and 2 mM of KCl proved to be without any clearly marked effect, that is to say paramecia continued the forward swimming with rotation to the right (FRS). When 3 mM solution had been used, the character of movement was altered: paramecia swam still forwards without any symptoms of ciliary reversal but the right spiralling ceased, being substituted by looping i.e. they manifested this kind of movement which is typical of the transition between FRS and FLS. As a matter of fact, with a little more potassium added to the medium (4 mM KCl) the right spiralling changes for the left one, i.e. for the normal movement (FLS). It should be pointed out that in the right spiralling paramecia the normal movement evoked by potassium ions is rather slow, lasts for a very short time, and could be induced only by a strictly defined concentration of KCl. Raise of external potassium concentration to 5 mM KCl was already sufficient to induce the first symptoms of ciliary reversal, i.e. the circling movement commonly called the partial ciliary reversal (PaCR). All higher concentrations of potassium chloride (from 6 up to 64 mM) evoke immediately the continuous ciliary reversal (CCR). Its duration rises with the increase of the K^+ amount added to the medium.

The right spiralling paramecia in which CCR has been evoked by a concentrated solution of potassium chloride, recover after a certain time the forward movement with the rotation to the right (FRS).

The aim of the next experiment was to record photographically all the transitory stages between CCR and FRS. In this purpose a sample of *P. caudatum* in the medium containing 0.5 mM $CaCl_2$ had been initially treated with 0.005 mM $NiCl_2$. About 5 min. later, when all the specimens manifested the well pronounced FRS, 16 mM KCl was added to the medium, which resulted in the appearance of CCR. Then 10 sec. dark field exposures were continuously taken at 5 sec. intervals, until the animals recovered the FRS type of movement (i.e. for 15 min.). Selected pictures are given in the Pl. II (mass samples) and the Pl. III (single specimens).

Initially, paramecia manifest a very well pronounced CCR, swimming rapidly backwards (Pl. II 7 and III 13). The pictures taken 60 sec. later, show that the rate of backward swimming decreases distinctly and the paths followed by para-

mecia become clearly spiralized (Pl. II 8 and III 14). Subsequently, the helicoidal character of the paths becomes extremely well pronounced (Pl. III 15). At this stage paramecium swims still backwards, but its body axis is strongly deviated from the resultant direction of movement, being even almost perpendicular to it. Gradually this deviation is still increased, and — as a result — the coils of the spiral become more narrow and the backward swimming slows down: finally spiralling transforms into a circling movement performed by the animal at one spot (Pl. III 16—17). In the picture taken from the mass sample between 105—115 sec. of experiment (Pl. II 9), many narrow helicoidal paths typical of the late CCR are simultaneously seen with the small discs representing the circling movement (PaCR).

Subsequently, the circling movement transforms into spiralling again, that is the animal starts to swim forwards (Pl. III 18—19). Its path is also strongly helicoidal as in the late CCR, although the spiral is distinctly broader (Pl. III 20). At this stage, in spite of the change in the direction of locomotion, still no change in its rotation occurs: it remains anti-clockwise (left) as it was during CCR. In other words, CCR has changed — through the intermediary stage of PaCR — into the normal forward movement with left spiralling (FLS). However, the FLS lasts for a very short time and soon loops arise (Pl. III 21) which anounce the return of the right spiralling. The mass picture taken 4 min. after the beginning of experiment (Pl. II 10) shows the strongly helicoidal FLS paths together with the looping which simultaneously sets on in other specimens. The loops described by paramecia at this stage are often very striking owing to their regular shape (Pl. III 22—24). After the period of looping the animals change the direction of their rotation, that is to say they recover the right spiralling. Typical paths followed during the transition of the loop into FRS are presented in the Pl. III 28—29. Sometimes the stage of looping is so short that all the transition from FLS through the loop into FRS may be recorded during a single exposure (Pl. III 25—27). The mass pictures taken 6 min. (Pl. II 11) and 14 min (Pl. II 12) after the start of experiment demonstrate also the loops and their gradual transition into the right spiralling simultaneously.

It may be stated in general, basing as well on the immediate effect of different concentrations of K^+ on the right spiralling paramecia as on the course of the recovery from CCR to FRS, that all the motor responses taken in consideration could be arranged in the following sequence: CCR → PaCr → FLS → looping → FRS. In other words, the normal movement (FLS) appears to be an intermediary mode of locomotion between the continuous ciliary reversal (CCR) and the forward right spiralling (FRS), or rather — properly speaking — the ciliary reversal and the right spiralling are two opposite deviations from the normal movement.

Besides the CCR response induced by potassium ions, another kind of ciliary reversal can also be evoked in the right spiralling paramecia, namely the periodic ciliary reversal (PCR). In the present research it was induced, as in the study of D r y l 1961, by barium chloride. FRS had been previously evoked by 0.005 mM $NiCl_2$ in the presence of 1 mM $CaCl_2$, and thereafter 1, 2 or 5 mM of $BaCl_2$ was introduced into the medium. In all those cases the well pronounced PCR was obtained. The difference between PCR manifested by normal left spiralling paramecia and the PCR exhibited by the animals spiralling to the right, is diagrammatically explained in the Fig. 1. In the first case PCR consists in fact of a rapid sequence of responses: FLS — CCR — FLS — CCR... As a con-

sequence, only the direction of the progressive component of movement changes alternately, not the direction of rotation. In the second case, PCR represents the following sequence: FRS — CCR — FRS — CCR..., what means that as well the progressive component of movement as the rotary one change both their direction simultaneously.

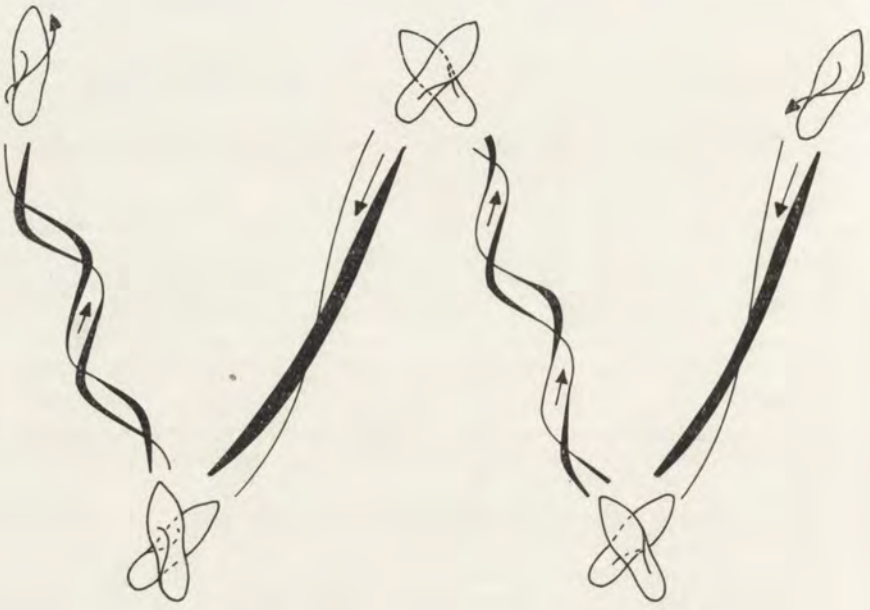

Fig. 1. Diagrammatic representation of the periodic ciliary reversal induced by Ba ions in paramecia spiralling to the right. PCR consists in this case of alternating phases: FRS—CCR—FRS—CCR... i.e. not only the direction of progressive movement alternates but also the direction of the rotary one

Effect of electric stimulation

The ability of the right spiralling paramecia to manifest the normal ciliary reversal under the influence of K and Ba ions allows to expect the possibility of evoking in them also the normal galvanotactic reactions. In fact, in the direct current they swim regularly towards the cathode. Pl. IV 30 presents the movement of the right spiralling specimens before making the current, and the Pl. IV 31 the movement in the same sample 5 sec. after closing the circuit (4 mA/cm^2). Paramecia swimming towards the cathode rotate still to the right. The galvanotactic treshold seems to be considerably higher in the right spiralling paramecia as compared with the normal ones, but no quantitative data can be reported in this respect.

In the normal paramecia, i.e. during FLS, the most typical response to the alternating current is the oriented movement perpendicular to the lines of the electric field (Pl. IV 32). Paramecia exhibiting the CCR swim in most cases in parallel to the lines of the field, that is to say they move in both opposite directions: towards the electrodes (Pl. IV 33). The behaviour of specimens pretreated with $NiCl_2$ is presented in the next pictures. Before the onset of FRS they be-

have as untreated paramecia, i.e. they swim transversally across the field (Pl. IV 34). Later on when spiralling to the right, the same paramecia start to swim in parallel, towards the electrodes (Pl. IV 35). That means that the animals manifesting FRS react to the alternating current in a similar manner as those which exhibit CCR. It should be pointed out that the same picture shows a very low sensibility to the electric stimulation in the specimens which undergo the stage of looping.

Motory responses during immobilization

Effect of calcium and decalcification

A number of specimens of *P. caudatum* were treated with $NiCl_2$ solutions (0.01—0.05 mM) in the presence of calcium ions (1 mM $CaCl_2$), and their responses to the changing external Ca concentration were studied when they entered the stage of physiological immobilization (cf. p. 391). As it has been pointed out above, at this stage paramecium cannot effectively swim forwards or rotate but its motor apparatus seems unimpaired and cilia continue their work.

The raise of the external Ca concentration (without any alteration in the Ni content) from the initial level of 1 mM $CaCl_2$ up to 2, 4, 8, 16, 32, and 64 mM has no visible immediate effect on the behaviour of immobilized specimens. The locomotion is still inhibited and the animals may only perform some limited shifts at the spot which are in general characteristic of the physiological immobilization (Kuźnicki 1963).

A quite different picture is provided by decalcification of paramecia during their physiological immobilization. The immobilization state had been induced in the same way as in the former experiment. Thereafter, the Ca ions were chelated by means of EDTA-Na salt which was introduced into the medium in concentrations varying from 0.1 up to 5 mM. The 1 mM solution of EDTA proved to be the most effective. Instantly after introduction of EDTA, the immobilized specimens start to swim. They move backwards (CCR), or forwards with the clockwise rotation (FRS), that is to say they recover this kind of movement which is the stage preceding the physiological immobilization. In the range of lower concentrations of EDTA (0.1—1 mM) the same but less marked effect could be observed. In the stronger EDTA solutions (2—5 mM) paramecia die very soon (probably because of the increased membrane permeability which promotes the rapid entrance of Ni^+ inside the cell).

The recovery of locomotion in the immobilized specimens after their moderate decalcification by 1 mM of EDTA, may be of essential importance as to the mechanism of the action of Ni ions. However, the rather low specificity of EDTA to calcium and its ability to chelate in certain degree also other bivalent ions may evoke some doubts for interpretation. It could be eventually suggested that the results reported above are not due to decalcification but to the direct removal of Ni^{2+} from the surface structures of paramecium. To avoid this ambiguity the experiments were repeated with applying ethylene glycol bis (-amino--ethylether)-N,N ¹-tetraacetic acid (EGTA), instead of EDTA. EGTA is known as showing a very high specificity to the Ca ions. It has been proved that EGTA exerts the same effect on the Ni-immobilized specimens, i.e. it promotes the reappearance of locomotion, and the concentration of 1 mM is also the most effective one in this respect.

The Pl. V presents an experiment with EGTA performed in the manner which enables its photographical recording. The first picture (Pl. V 36) shows a sample of paramecia in the stage of physiological immobilization: only few specimens which retained still their locomotory ability are scattered at random over the field. Thereafter, a small drop of the solution containing 1 mM of EGTA had been put in the center of this area, and the next picture (Pl. V 37) was taken. As clearly seen, the locomotion immediately reappears in all specimens lying in the round area where EGTA was added. 15 sec. later, the picture is rather similar, and the effect seems even to be more pronounced because the diffusing agent affects a larger number of specimens (Pl. V 38). After 2 min. a further diffusion of EGTA and the corresponding fall of its concentration efaces the phenomenon (PL. V 39).

The experiments with EGTA allow to conclude that, really, the gradual decalcification is the factor which promotes the forward movement in paramecia which had been previously introduced into the state of physiological immobilization by the Ni-treatment.

Effect of potassium and barium

As well the effect of K ions as this of Ba ions is strongly dependent on the degree of the physiological immobilization induced previously.

In the first period of immobilization, introducing of KCl solutions into the medium may induce the ciliary reversal, which depends on the concentration applied (1 up to 64 mM solutions were tested). At this stage paramecia react also to the Ba ions. As commonly known, they induce PCR in normal specimens. In those which are in the condition of a slight physiological immobilization, introduction of $BaCl_2$ (1—10 mM) into the medium induces a series of regular short withdrawals, that is to say it evokes the PCR response devoid of the forward phase but consisting only of the periodical backward phases.

In the subsequent period, when the immobilization advances gradually, the motor responses to K^+ and Ba^{2+} turn also to be much less pronounced. Potassium ions induce only a strong rotation of the body without its backward or forward shift. Ba-induced withdrawals become very short and finally it is impossible to distinguish them from the "vibrations" manifested by Ni-immobilized paramecia without any additional ionic effect.

During the traumatic immobilization, i.e. when the ciliary apparatus becomes destroyed, no motory response may be induced by K or by Ba ions.

It may be concluded in general that, during the physiological immobilization and especially in its initial phase, both ions which are known to be the most effective depolarizers of the cell membrane in paramecium, evoke still motory responses of the ciliary apparatus. In this respect their effect is quite opposite to that of calcium but similar to that of decalcification, which supports the view expressed by the authors before (Grębecki 1965 b and Kuźnicki 1966) that the depolarizing ions release the bound calcium from the cell surface of paramecium.

Effect of electric stimulation

The effect of electric current on paramecia subjected to the physiological immobilization by $NiCl_2$, is presented by the Pl. VI. The first picture (Pl. VI 40) shows a sample before making the current and the next one (Pl. VI 41) — the same specimens after closing the circuit of direct current (10 mA/cm^2). Para-

mecia start to move instantly after the current has been made. They swim forwards with the clockwise rotation (FRS) towards the cathode, however their orientation in the field is much less precise than in the normal specimens. In most cases the oblique galvanotaxis is manifested, similar to that which has been reported before by Dryl 1963 and Grębecki 1963. After opening the circuit, paramecia fail to stop instantly but their locomotion slows down and disappears gradually.

The alternating current promotes also the locomotion of paramecia undergoing the physiological immobilization (Pl. VI 42—43). When the immobilization becomes deeper (Pl. VI 44) the same current exerts a less pronounced effect (Pl. VI 45). The movement promoted in the immobilized specimens by alternating current seems not to be oriented in respect to the electric field. In the case of AC stimulation, the locomotion reappears instantly after the circuit has been closed and it disappears only gradually after its opening.

The findings concerning the electric stimulation seem to corroborate the results reported for the ionic effects. As commonly known, the cathodal current strongly depolarizes the cell membrane in *Protozoa* and probably releases calcium from its binding sites (Jahn 1962) It seems reasonable to postulate therefore that the recovery of movement in the DC field is induced by the cathodal stimulation and is analogous to the recovery of locomotion reported in this study under the effect of decalcification and of depolarizing ions. At present nothing could be concluded as to the mechanism of recovery in the AC field since even its effect on the normal paramecia is as yet not satisfactory elucidated.

Discussion

The first problem which arises in relation to the action of nickel ions on the motory behaviour of *Paramecium* is the question whether the induced responses depend on the same mechanism of membrane depolarization and Ca release which underlies all the other known reactions of the ciliary apparatus.

No doubt, any additional amount of calcium in the medium restrains the Ni action. However there exists an important difference between the protective action which exerts Ca in the case of Ni action and that which it exerts in the case of any depolarizing agent. In the latter case the Ca effect is immediate, that is to say calcium does not delay the effect of a depolarizer but instantly makes it impossible (in an adequate concentration ratio), and — inversely — a sufficient amount of Ca added may interrupt at once any motory response induced previously. No such immediate effect is exerted by Ca in the case of Ni action. Calcium interference is long-lasting, i.e. it can only delay the Ni effect or promote the recovery. This difference may be explained by the double role which is played by Ca^{2+} in the cell membrane: 1. hyperpolarization of the membrane (Kinosita, Dryl and Naitoh 1964), and 2. increase of its ohmic resistance, decrease of permeability, and raise of its mechanical resistance. It is evident that the re-polarization is responsible for the immediate Ca effect in the most motory responses, and apparently the reasons mentioned as 2. explain the prolonged Ca effect in the case of Ni action. In other words, Ca only delays the entrance of Ni into the cell and reinforces the membrane structure. This point of view finds a support in the electron microscope findings of Pitelka and Párducz 1965 which prove that Ni impairs the cell membrane in *Paramecium*.

A further significant difference between the right spiralling induced by Ni and the ciliary reversal induced by K or other depolarizers consists in the fact that the lag period between the addition of acting ion and the motory response evoked lasts in the case of ciliary reversal 22—36 miliseconds (K i n o s i t a, M u r a k a m i and Y a s u d a 1965) and in the case of FRS it should be measured in seconds or even minutes.

All the reasons mentioned above, namely: 1. very slow development of FRS, 2. very slow Ca counter-action, 3. impairment of the membrane as a morphological symptom, prove that FRS and other motory responses accompanying it (looping) are not induced by Ni acting on the cell membrane itself but by Ni penetrating into the cell interior.

This last conclusion justifies the hypothesis that Ni may affect directly the contractile fibrils of cilia by masking the SH groups in the site of contraction. However the attempt to establish an analogy between the Ni action and the effect of such agents as MIA, PCMB and Mersalyl gave no expected results (G r ę b e c k i, K u ź n i c k i and S k o n i e c z n a — unpublished).

Although the influence of Ni upon the cell interior remains unknown, the fact that it is not exerted on the membrane itself and the lag period lasts up to several minutes, prove that FRS is not a true active motory response and Ni^{2+} is not a stimulus in the proper meaning. The link stimulus-reaction, even in the unicellular organisms, involves the cell membrane: its excitation, change of its electrical parameters, release of Ca which will evoke the reaction of contractile structures in miliseconds. This means that the case of Ni action cannot be interpreted in the terms of excitation. FRS develops rather step-by-step as a mechanical after-effect of the gradual damaging of some cellular structures.

This last conclusion inclines to discuss the mechanical, or rather hydrodynamical, conditions of FRS, that is to say the relation between the direction of the effective ciliary stroke, the body shape, and the spiralling movement. All the species in which FRS has been studied (*Paramecium caudatum, P. aurelia* and *P. multimicronucleatum*) have the body twisted in the clockwise direction, i.e. in the form of a right convoluted screw. It is an amazing paradox (pointed out already by L u d w i g 1932) that this structural spiral does not correspond to the functional spiral, that of movement. During the normal forwrad movement (FLS) paramecium spiralizes to the left, in the anti-clockwise manner. This is effected by cilia which form the NW—SE waves, i.e. their effective stroke is perpendicular to the line of the morphological twist of the body. This means that under normal conditions the ciliate swims in this way which is the most difficult, "against the principles of hydrodynamics".

It might be postulated therefore that a certain degree of impairment of the motory apparatus makes the cilia (it remains unknown by what mechanism) to assume another direction of the effective stroke, such a one which would be "conform to the priciples of hydrodynamics", that is to say cilia start to work in the manner which is easier and more effective. In fact, during FRS the spiral of the movement is clockwise and agrees with the clockwise twisting of the body.

What direction of the effective ciliary stroke is necessary to promote the forward right spiralling (FRS)? P á r d u c z 1962 postulated the oblique stroke nearly corresponding to the direction of morphological spiral, and consequently — the NE—SW metachronal waves. However, in this study the NE—SW

waves were found only occasionally, and the longitudinal (N—S) waves proved to be typical for FRS.

Let us consider at first the hydrodynamical conditions of the normal forward movement spiralling to the left (FLS). As it was revealed at first by Párducz 1956 and confirmed subsequently in other studies, during FLS the metachronal waves are oriented in the NW—SE direction and the effective stroke of each cilium is directed to SE (Fig. 2 A). This means that the driving

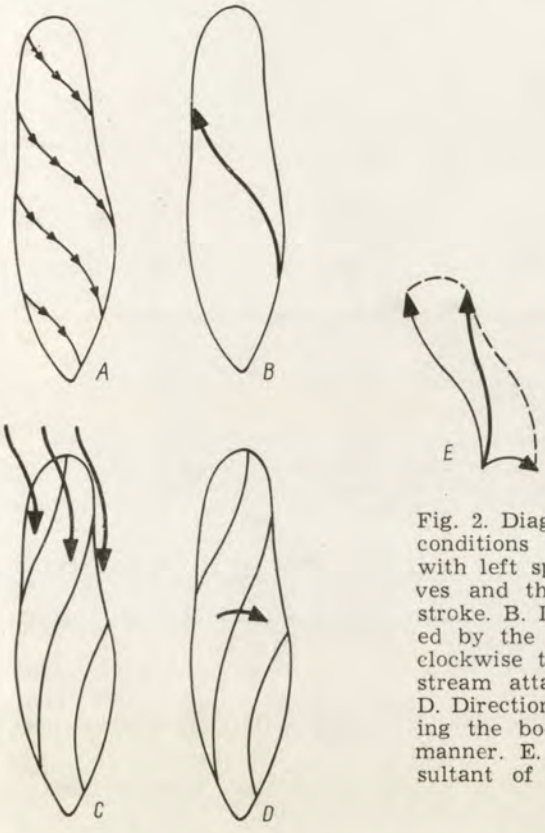

Fig. 2. Diagram explaining the hydrodynamical conditions of the normal forward movement with left spiralling (FLS). A. Metachronal waves and the direction of the effective ciliary stroke. B. Direction of the driving force exerted by the ciliary apparatus. C. Morphological clockwise twisting of the body and the water stream attacking the surface from the front. D. Direction of the hydrodynamical force rotating the body, as a turbine, in the clockwise manner. E. Tentative extrapolation of the resultant of ciliary driving force (B) and the turbine effect (D)

force of the motory apparatus is oriented as indicated in the Fig. 2 B and it should result in a forward movement with the left, anti-clockwise spiralling. Now, the resistance of medium is to be additionally taken in consideration. The water stream moving in respect to the body and the clockwise morphological twisting of the body itself are represented in the Fig. 2 C. It seems obvious that under such conditions paramecium should manifest a tendency to behave like a screw-form water turbine which would be forced to rotate to the right (Fig. 2 D). We can represent both components, the driving force of cilia and the turbine effect, in the form of vectors and find the resultant form of movement (Fig. 2 E). It should be concluded that during the normal movement (FLS) the left spiralling is slightly less pronounced than it would result only from the angle of the ciliary stroke. Such a conclusion seems very plausible to everybody

who has compared the registered paths of FLS with the ciliary pattern in fixed specimens.

A further consideration of the vector components (Fig. 3) indicates what should occur when the ciliary driving force has become reduced. At some definite degree of its reduction (Fig. 3 B) the resultant indicates the straight forward movement without spiralling. It may be postulated that just this is the case of looping without any rotation described in this study as the first symptom of Ni action, and elsewhere (Grębecki, Kuźnicki, Mikołajczyk 1966) after a gradual fall of temperature [2]. A further reduction of the driving force of ciliature should result in spiralling to the right (Fig. 3 C). Probably this is

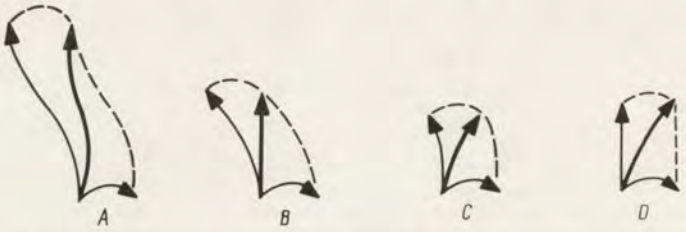

Fig. 3. Transformations of the resultant as expected when the ciliary driving force becomes gradually reduced. A. The initial situation (cf. Fig. 2 E). B. First degree of reduction leading to a transitory lack of the resultant rotation. C. A further reduction leading to the inversion of the resultant rotation. D. Effect of the additional change of the angle of ciliary stroke as involved by the appearance of longitudinal metachronal waves

the case of the clockwise rotation preceding death of paramecia exposed to the high concentrations of the heavy metal ions, phenomenon described at first by Alverdes 1922. May be, the onset of FRS in Ni solutions is also due to the same mechanism, as well as FRS evoked by a fall of temperature (Grębecki, Kuźnicki, Mikołajczyk 1966) or by a homologous antiserum treatment (Kuźnicki and Sikora 1966). However, it was stated in this resaerch that during the Ni-induced FRS, the metachronal waves assume a longitudinal orientation, that is to say the angle of ciliary stroke changes itself [3]. Such a situation is represented in the form of vectors in the Fig. 3 D which proves that the result is a still more pronounced clockwise spiralling.

It may be stated in general that taking into account the hydrodynamical turbine effect as a component of the forward movement gives evidence that

[2] A radially symmetrical ciliate should move, in the absence of spiralling, straigth forwards, but the functional ciliary lines in *Paramecium* follow an asymmetrical stomato-caudal gradient (Grębecki 1965 a) which results in describing the loops.

[3] It is not clear what is the mechanism of the change of oblique waves into the longitudinal ones. A tentative explanation may be eventually based on the theory of Sleigh 1965 that the ciliary co-ordination in *Paramecium* is mechanical one, effected by the water stream resulting from the movement. The onset of right spiralling due to the turbine effect changes the angle at which water stream attacks the ciliated surface, and it might be imagined therefore that this re-arranges the metachronal waves into a more longitudinal pattern, and the after-effect would be a further reinforcement of FRS. This would represent an obvious case of the positive feed-back.

the NE—SW metachronal waves, postulated by Párducz 1962, are not necessary to bring about the right spiralling. Paramecium is forced to rotate clockwise with the longitudinal (N—S) waves as found this study, and even with the normal NW—SE waves if the driving force of cilia becomes distinctly reduced.

It seems rather obvious that there exists a possibility to obtain the same result in the opposite way — by increasing the turbine effect instead of decreasing the ciliary driving force. The turbine effect should increase with increase of the general resistance of medium, i.e. with its viscosity (the force of ciliary beating does not change in viscous media, as proved by Pigoń and Szarski

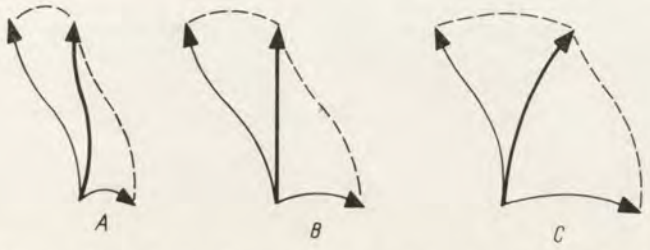

Fig. 4. Transformations of the resultant as expected when the turbine effect is gradually increasing. A. The initial situation (cf. Fig. 2 E). B. First degree of increasing leading to a transitory lack of the resultant rotation. C. A further increase leading to the inversion of the resultant rotation

1955) [4]. The changes of spiralling expected in the viscous media are represented in the Fig. 4. The normal movement with the left rotation (Fig. 4 A) should be substituted at a certain degree of viscosity by swimming without rotation (Fig. 4 B) i.e. by looping, and in a still more viscous medium by rotation to the right (Fig. 4 C) i.e. by FRS. That is just what had been in fact observed by Alverdes 1922 and recently analysed in details by the present authors (Grębecki, Kuźnicki, Mikołajczyk 1966).

The considerations presented above seem to provide a common explanation of all known examples of the forward right spiralling movement (FRS) in paramecium and of the transition from FLS to FRS through looping, as well evoked by Ni-treatment, as by the premortal effects of heavy metals, by the low temperature, by homologous antisera and by increased viscosity of medium.

Discussion of the role of the hydrodynamical turbine effect in both types of the forward movement, the left spiralling (FLS) and the right spiralling (FRS), should be completed by a consideration of its role in the ciliary reversal (CCR) which is in fact the backward left spiralling movement.

The diagram representing the hydrodynamical conditions of CCR is given in the Fig. 5. According to Párducz 1956 and later authors the metachronal waves in CCR assume a NE—SW pattern and each cilium beats effectively to the NE direction (Fig 5 A). As result, the driving force of the ciliature is directed as shown in the Fig. 5 B and it brings about the backward movement with an anti-clockwise (left) spiralling. The water stream which, in this case, attacks the body from its posterior end, and the screw-like clockwise twisting of the

[4] The turbine effect should relatively increase in spite of the reduced speed of the ciliary work because it depends on the total surface of the body (cilia+pellicle).

body itself, are presented in the Fig. 5 C. Under such conditions the screw-form water turbine would be forced to rotate to the left (Fig. 5 D). This means that in CCR both components: the driving force of ciliary apparatus and the turbine effect are anti-clockwise (Fig. 5 E). It should be postulated in consequence that during the ciliary reversal paramecium should spiralize more than it would result from the angle of ciliary stroke only. Indeed, paramecia in CCR manifest a very vigourous spiralling, in spite of this fact that the metachronal waves are commonly only slightly oblique.

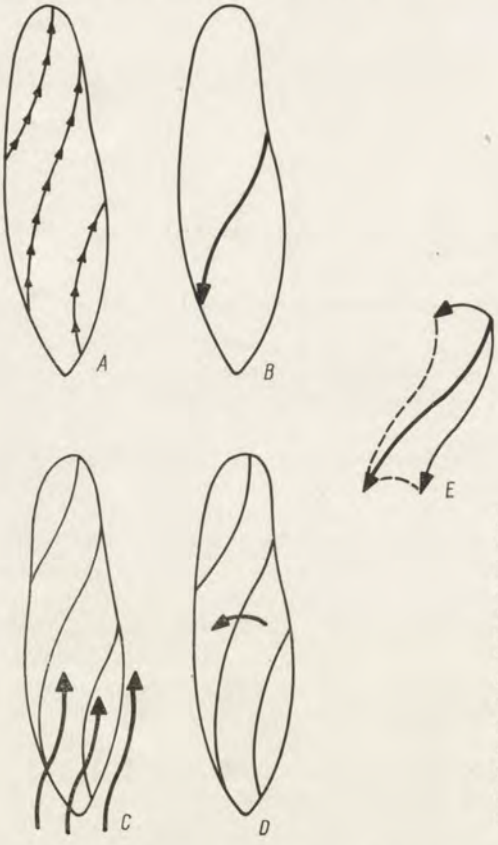

Fig. 5. Diagram explaining the hydrodynamical conditions of the backward movement with left spiralling (CCR). A. Metachronal waves and the direction of the effective ciliary stroke. B. Direction of the driving force exerted by the ciliary apparatus. C. Morphological clockwise twisting of the body and the water stream attacking the surface from behind. D. Direction of the hydrodynamical force rotating the body, as a turbine, in the anti-clockwise manner. E. Tentative extrapolation of the resultant of ciliary driving force (B) and the turbine effect (D)

If we compare the relations between both components of movement represented in the form of vectors in the Figs. 2—5 it becomes clear that CCR is the most effective type of movement, FLS the most difficult, and FRS occupies an intermediate position. This conclusion, theoretically substantiated here, has already been drawn empirically by the present authors elsewhere (Grębecki, Kuźnicki, Mikołajczyk 1966).

The highest hydrodynamical effectiveness of CCR may account for a transitory appearance of this response before FRS when the inducing agent acts very sharply. Under such conditions paramecium has no time to effect the gradual transition to FRS by looping, and instead — it may only manifest alternately both kinds of movement in which the driving force of cilia and the

turbine effect corroborate one another, that is to say CCR and FRS, until the full stabilization of FRS takes place. As a matter of fact, the short repeated CCR responses are the characteristic form preceding FRS in the high concentrations of methyl cellulose (Grębecki, Kuźnicki, Mikołajczyk 1966) of homologous antiserum (Kuźnicki and Sikora 1966), and of Ni ions (Párducz 1962 and this study).

Finally, the differentiated hydrodynamical effectiveness of the three principal types of movement: CCR > FRS > FLS, explains the ability of paramecium to manifest different motory responses in the subsequent stages of the Ni action.

The first phase is the exclusion of the possibility of the normal left spiralling forward movement (FLS) as the most difficult. However FRS and CCR are still possible. In consequence, paramecium swims forwards with the right spiralling and — as it was proved in this study — it can also manifest CCR under the influence of K^+, Ba^{2+}, EGTA and electric current. Only FLS cannot be induced (even when it appears as a transition from CCR to FRS, it is very short and clearly defective).

Further weakening of the ciliary apparatus by the Ni action makes also FRS impossible. Only CCR remains possible as the kind of movement easiest from the point of view of hydrodynamics. However, CCR cannot be manifested by a resting cel, without its excitation. As result, the ciliates cease to move at all and the stage of physiological immobilization sets on. In fact, at this stage paramecium cannot move forwards but it manifests very short spontaneous withdrawals (Kuźnicki 1963) and the continuous ciliary (CCR) may be still induced by K^+, Ba^{2+}, EGTA and the electric stimuli, as it was put in evidence in this study.

Finally, even CCR becomes excluded and the stage of the traumatic immobilization sets on, at which no motory response is possible.

It should be concluded in general that Ni ions do not promote any active responses by means of the cell excitation but they attack directly the motory mechanism and gradually confine the effectiveness of its work, and — as a consequence — they exclude step-by-step all the kinds of movement strictly in such an order that this type of locomotion disappears at first which is the most difficult for the hydrodynamical reasons, and this one which is the most easy disappears at last.

Summary

Ni^{2+} induces in *P. caudatum* and *P. aurelia* the right spiralling during forward movement (FRS), which is preceded by looping and/or short CCR, and may be followed by immobilization. Metachronal waves in FRS are longitudinal and the clockwise spiralling is due to a turbine effect resulting from the clockwise twisting of the body. This morphological twist makes the normal forward movement with left spiralling (FLS) the most difficult, the ciliary reversal (CCR) the most easy, and FRS intermediate from the point of view of hydrodynamics. Ni^{2+} does not induce any active response by excitation but it gradually affects directly the cilia. The weakening of ciliary apparatus excludes step-by-step the types of movement in the order corresponding to their hydrodynamical effectiveness. In the earlier phase only FLS is excluded, and therefore paramecia swim by FRS and may manifest CCR, in the later one FRS becomes also

impossible and paramecia are immobilized but CCR still may be induced in them under stimulation. At last, after excluding even CCR, the immobilization becomes complete.

STRESZCZENIE

Ni^{2+} wywołuje u *P. caudatum* i *P. aurelia* prawoskrętną spiralizację podczas ruchu do przodu (FRS); zjawisko to poprzedza opisywanie pętli lub krótkie rewersje (CCR), a po nim następuje immobilizacja. Fale metachronalne przy FRS są wzdłużne a spiralizacja zgodna z ruchem wskazówek zegara jest spowodowana przez efekt turbinowy, który wynika z tego właśnie kierunku skręcenia ciała. To skręcenie morfologiczne powoduje, że normalny ruch do przodu z lewoskrętną spiralizacją (FLS) jest najtrudniejszy z punktu widzenia hydrodynamiki, rewersja rzęskowa (CCR) jest najłatwiejsza, a FRS zajmuje pozycję pośrednią. Ni^{2+} nie wywołuje aktywnej reakcji w drodze pobudzenia, lecz stopniowo bezpośrednio atakuje rzęski. Osłabienie aparatu rzęskowego kolejno wyklucza poszczególne typy ruchu w porządku odpowiadającym ich hydrodynamicznej efektywności. We wcześniejszej fazie wykluczony jest tylko FLS, wobec czego pantofelki pływają na sposób FRS i mogą wykazywać CCR, a w fazie późniejszej również FRS staje się niemożliwy i pantofelki zostają immobilizowane, lecz wciąż jeszcze można wywołać u nich CCR pod wpływem drażnienia. Wreszcie po wykluczeniu nawet CCR, immobilizacja staje się zupełna.

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EXPLANATION OF PLATES I—VII

Phases of the Ni action

1: Paths of the normal forward movement with left spiralling (FLS) as manifested by *Paramecium caudatum* in distilled water in the first minute after addition of $NiCl_2$ (0.005 mM)
2: The same sample 4 min. later, during the transition from left spiralling (FLS) to the right one (FRS)
3: Paths typical for the transition from FLS to FRS, under a higher magnification (3a — a loop, 3b — a series of CCR responses)
4: The same sample 10 min. after addition of $NiCl_2$, exhibiting a fully developed FRS
5: Another sample 10 min. after addition of 0.02 mM $NiCl_2$, undergoing the physiologic immobilization
6: The same sample 30 min. later, in the stage of traumatic immobilization

Recovery from CCR to FRS

7: A mass sample pretreated with $NiCl_2$ 0.005 mM as to induce FRS, and thereafter treated by KCl 16 mM as to induce CCR. The picture taken immediately after addition of KCl, during the well pronounced ciliary reversal
8: The same sample 1 min. later. The speed of CCR decreases
9: The same sample at the end of the 2nd min. after addition of KCl. The paths of CCR become extremely spiralized and in many specimens already the circling movement (PaCR) appears seen in the form of small discs
10: Th same at the end of the 4th min. after addition of KCl. Transitory stage of a defective, strongly spiralized forward left spiralling movement (FLS). Looping sets on simultaneously
11: The same at the end of the 6th min. after addition of KCl. Looping becomes the most common type of movement
12: The same in the 14th min. after addition of KCl. Looping is substituted by forward right spiralling (FRS)
13: High magnification of a CCR path recorded instantly after addition of KCl
14: A CCR path recorded 1 min. later
15: Strongly spiralized CCR path (end of the 2nd min.)
16—17: Strongly spiralized CCR paths concluding in the circling movement (the discs)
18: The disc of circling movement as a transition between CCR and FLS
19: The disc of circling movement transforming into a path of strongly spiralized transitory FLS movement (end of the 4th min.)
20: Another path of the transitory forward left spiralling
21: Transformation of the strongly spiralized FLS movement into a loop
22—24: Examples of the regular loops (5-6th min.)
25—27: Short looping as a transition from FLS to FRS
28—29: Loops transforming into the forward right spiralling (14th min.)

Effect of electric current during FRS

30: Right spiralling paramecia before making the current
31: Movement oriented to the cathode in the same sample 5 sec. after closing the circuit
32: Effect of the alternating current on normal paramecia (FLS)
33: Effect of the alternating current on paramecia swimming beckwards (K-induced CCR)
34: Effect of alternating current on paramecia pretreated with 0.005 mM $NiCl_2$, before the onset of first symptoms of Ni action
35: Effect of alternating current on the same sample after the development of FRS

Effect of EGTA during the physiologic immobilization

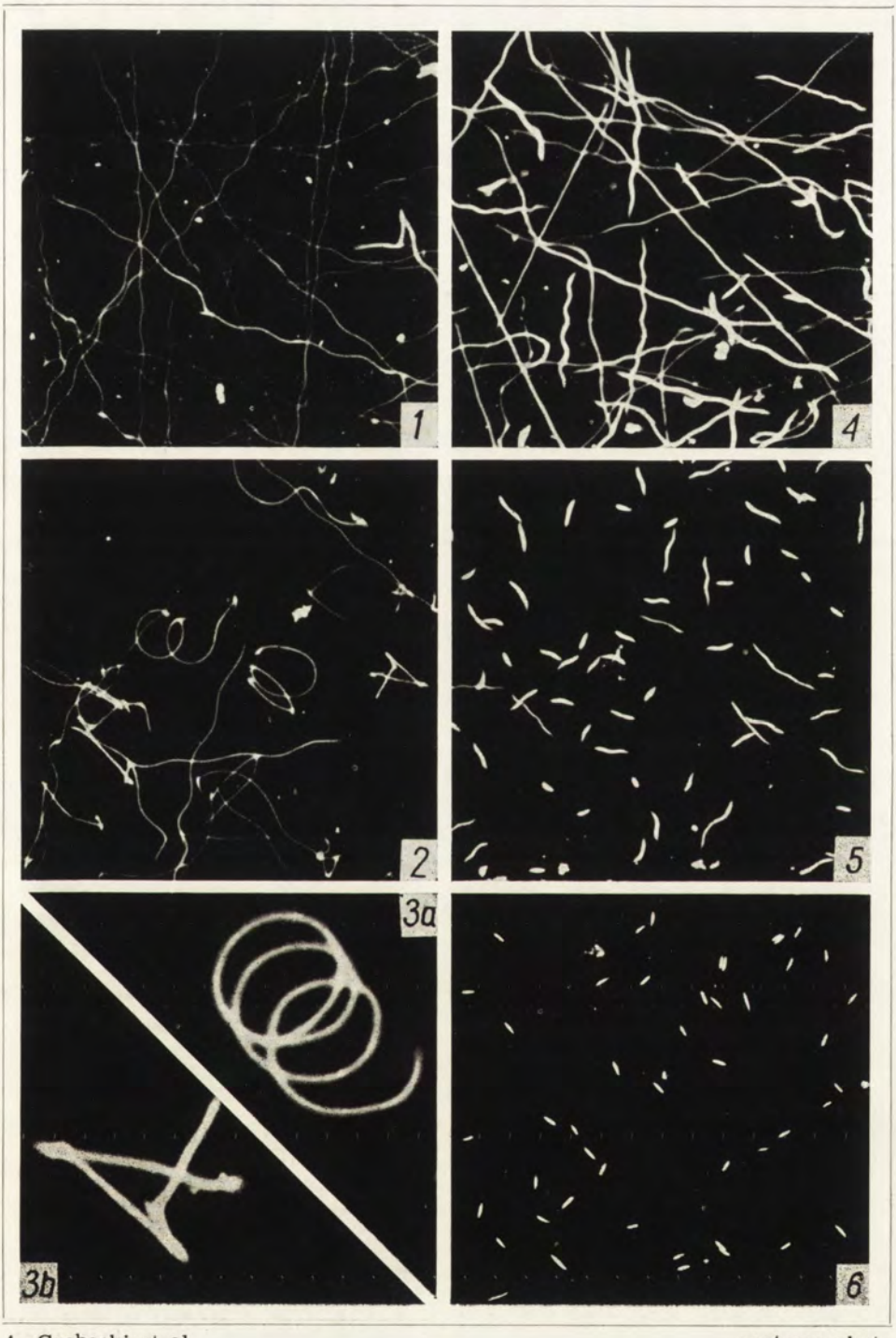
36: A sample showing the physiologic immobilization induced by 0.02 mM $NiCl_2$
37: The same sample immediately after addition of 1 mM EGTA in the centre of field. Paramecia start to move
38: Increasing of the phenomenon in the same sample 15 sec. later
39: Effacement of the phenomenon after 2 min.

Effect of electric current during the physiologic immobilization

40: A sample showing the physiologic immobilization induced by 0.02 mM $NiCl_2$
41: The same sample after closing the circuit of direct current
42: Another sample in the state of physiologic immobilization
43: The same sample after closing the circuit of alternating current
44: Another sample showing a very strong physiologic immobilization, approaching the state of traumatic immobilization
45: The same sample after closing the circuit of alternating current

Ciliary patterns of FRS

46—48: Metachronal longitudinal waves in *P. aurelia* fixed during the forward right spiralling movement induced by 0.005 mM $NiCl_2$
49—51: Metachronal longitudinal waves in *P. caudatum* fixed during the forward right spiralling movement induced by 0.005 mM $NiCl_2$



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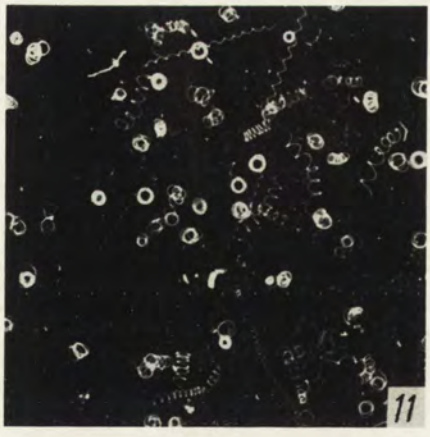

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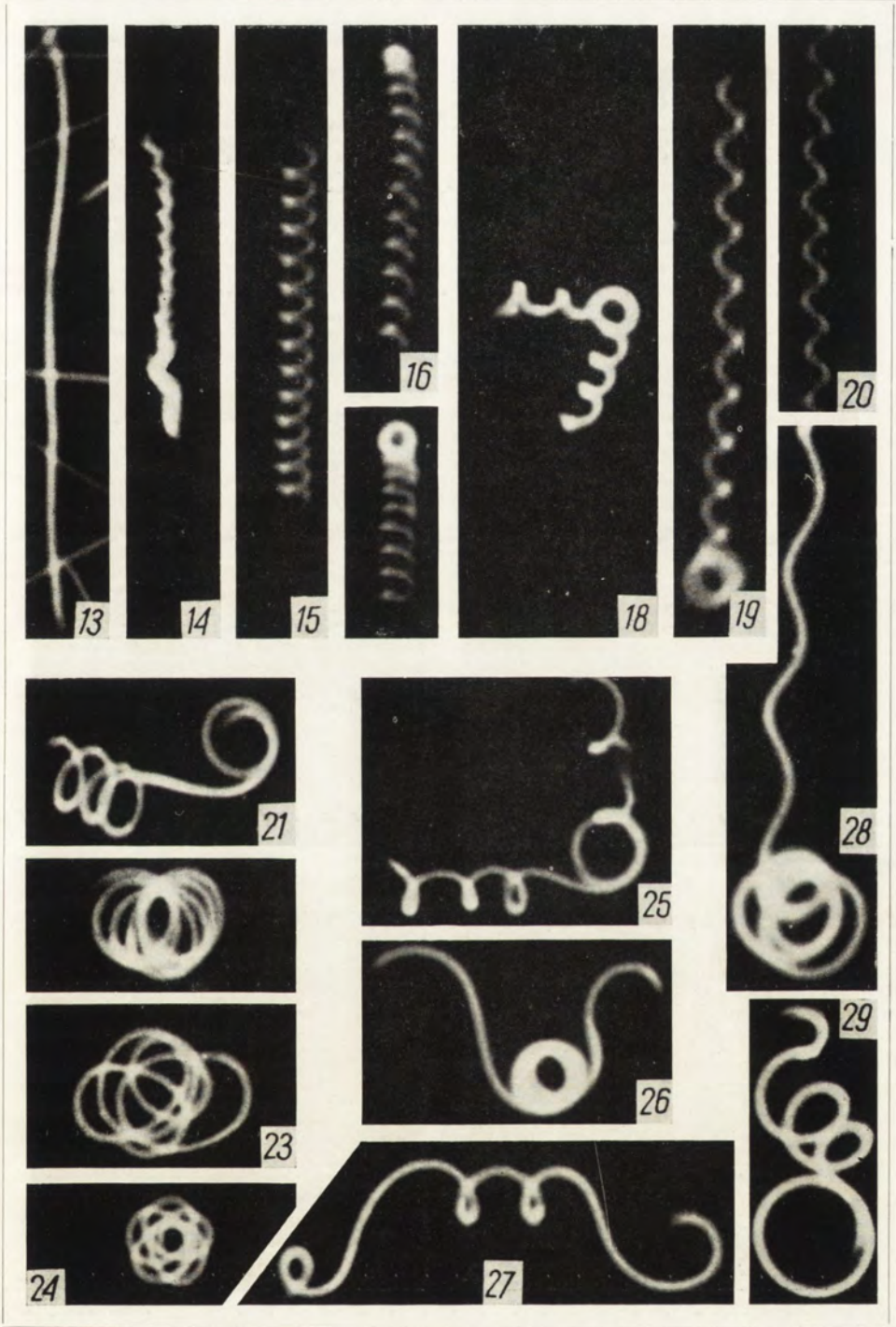

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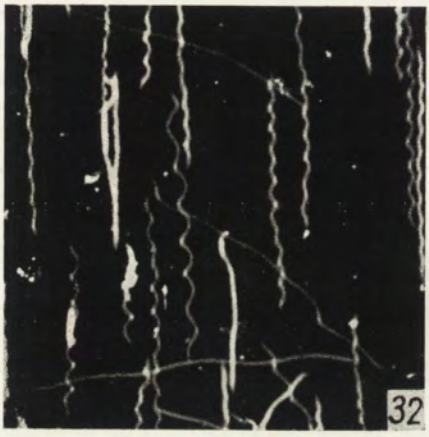

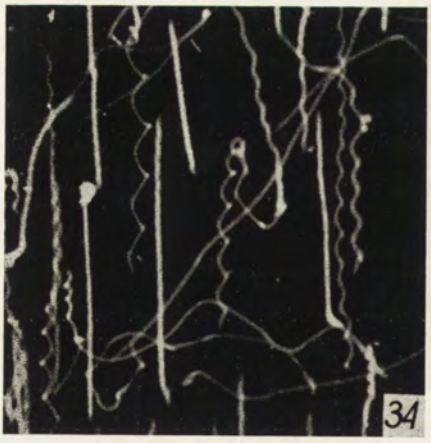

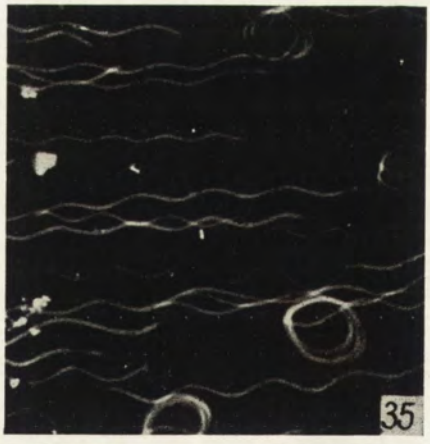

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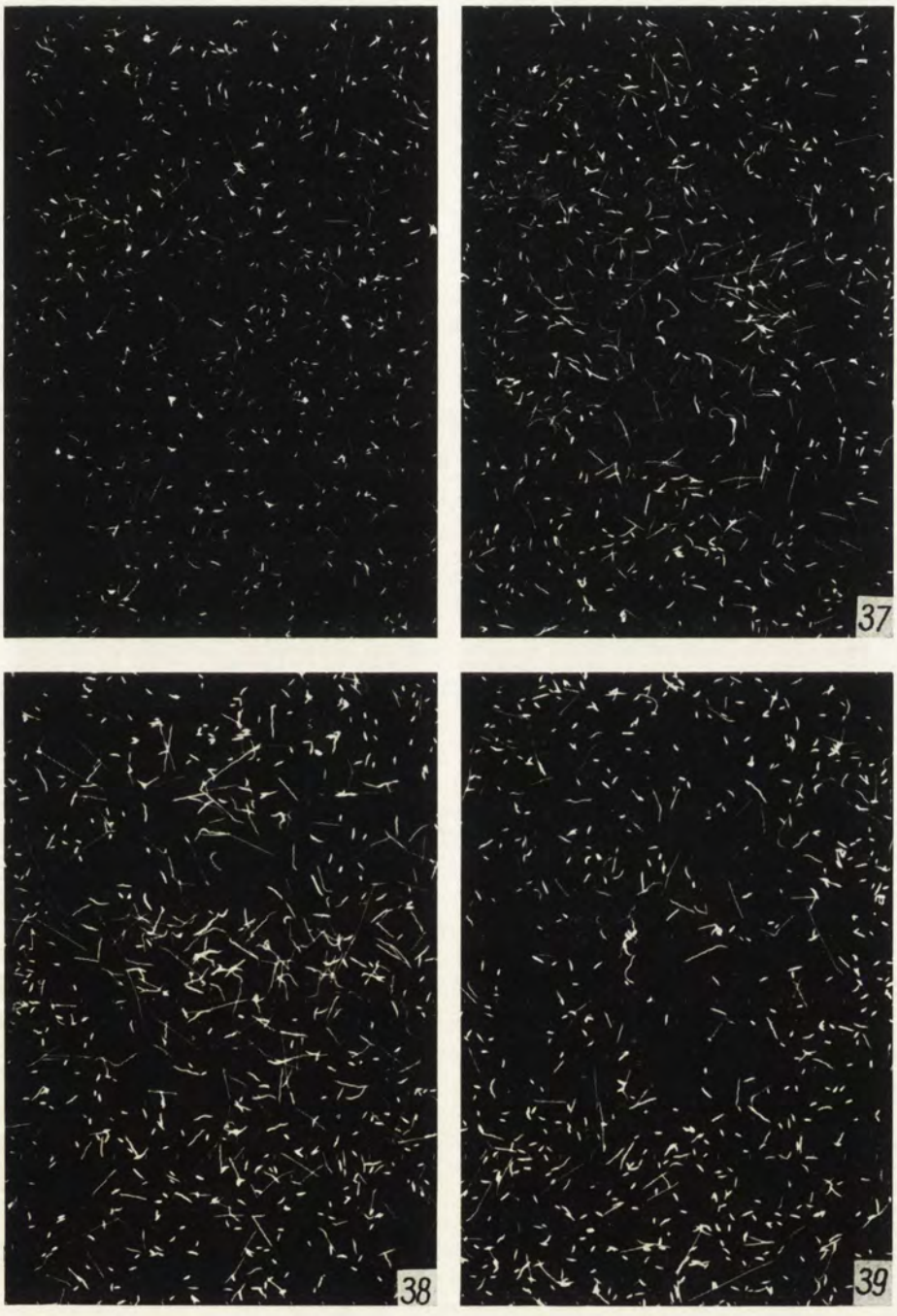

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